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Effect of the Trimethoxy Group

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EFFECT OF THE TRIMETHOXY GROUP

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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 tripelennamine, 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 tripelennamine 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 potentiating effect of the parent compounds.

LD_{50} studies indicated that the trimethoxy derivatives are less toxic than the parent compounds.

The preliminary behavioral studies (rolling roller and inclined plane tests) indicated that the C.N.S. activity of these compounds is other than on the cerebral cortical areas of the brain.

TABLE OF CONTENTS

page

ACKNOWLEDGMENT ii

ABSTRACT iii

LIST OF TABLES v

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 2

III. EXPERIMENTAL PROCEDURE 11

Green Activity

Sleeping Time

LD_{50} Studies

Preliminary Behavioral Studies
Rolling Roller
Inclined Plane

IV. RESULTS 15

V. DISCUSSION 23

VI. SUMMARY AND CONCLUSIONS 26

VII. REFERENCES 28

LIST OF TABLES

Table		Page
1.	Comparative Effects of Experimental Compounds on Normal Gross Activity in Mice by the Actophotometer	16
2.	Comparative Effects of Experimental Compounds on Gross 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 LD_{50} of Experimental and Parent Compounds in Mice	20
6.	The Effects of the Experimental Compounds in Mice Subjected to the Rolling Roller	21
7.	The Effects of the Experimental Compounds in Mice Subjected to the Inclined Plane	22

I

INTRODUCTION

Diphenhydramine and tripelennamine are well known and widely used antihistaminics. 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 mechanism of their action on the C.N.S. is still obscure.

The fact that reserpine, mescaline 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 expect 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.

II

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 Bovet et al. (1933) who demonstrated this action by the use of certain phenolic ethers.

Edlbacher (1937) demonstrated that although certain amino acids, histidine, cysteine and arginine possess histamine inhibiting activity, this action is too weak to be of therapeutic use.

Since Bovet, many investigators have tried to synthesize new antihistaminics. As a result, pyramisamine (Halpern, 1942), pyrilamine (Bovet & Halpern, 1944), diphenhydramine (Loew et al., 1945) and tripelennamine (Mayer et al., 1945) have been produced.

Diphenhydramine was first reported as an antihistaminic agent by Loew et al. (1945). This group was testing seventeen synthesized compounds of the type Ph_2CHOR, and measuring 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 aminophylline.

Many workers such as Wells et al. (1945), Loew 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. (1945) were testing eleven compounds of the general formula $R_1R_2NCH_2CH_2N(R_3)_2$ on the isolated strips of guinea pig intestine and found that the compound of R_1 = pyridyl, R_2 = benzyl, and R_3 = methyl (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 antianaphylactic actions of diphenhydramine and tripelennamine have been studied by numerous workers, Loew et al. (1945), Mayer (1946) and Winter (1947) on bronchoconstriction, Sherrod et al. (1947) and Loew (1947) on smooth muscle spasm, Loew et al. (1946), Yonkman et al. (1946), March et al. (1947), and Sherrod et al. (1947) on vasodepressant effects, and Arbesman (1946), Friedlaender et al. (1946) and Beck (1947) on wheal formation and anaphylactic reaction.

Marassi-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-ulcer agents.

Diphenhydramine and tripelennamine 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 dullness, lassitude, drowsiness, sleepiness, and motor incoordination.

Curtis et al. (1945) calculated the LD_{50} 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 ataxia with recovery in one to two hours.

Loew et al. (1946) observed no evidence of drowsiness in dogs which received diphenhydramine (10 mg/kg) subcutaneously and Winter et al. (1948) obtained similar results with similar doses in mice, rats, dogs, guinea pigs, and monkeys.

Mayer et al. (1946) observed no drowsiness resulting from low doses of tripelennamine, but did report excitability and convulsions with toxic doses given by intravenous injection. Similar results were obtained by Winter (1948) in rats and mice as well as dogs by intraperitoneal, oral and subcutaneous administration of tripelennamine.

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 tripelennamine is also reported by Beck (1947). He mentioned many untoward side reactions

that occurred in 20 to 25% of cases treated with tripelennamine 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 tripelennamine.

McCavack 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 man 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.

Glaser (1953) showed that diphenhydramine was effective against seasickness. The hyoscine like property of the antihistamine determined its essential action on motion sickness.

Baird 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 200 mg/kg produced convulsions and a mortality of 80%.

The excitation of the C.N.S. by tripelennamine and diphenhydramine in clinical studies has been reported by Feinberg et al.

(1947), Henderson et al. (1947) and Churchill et al. (1949).

Iwasaki (1957) determined the subcutaneous LD_{50} in mice of diphenhydramine (147.8 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 more frequently they exert a sedative action, and they produce somnolence presumably due to cortical depression.

The most important actions of diphenhydramine and tripelennamine 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 (1948) found that the potentiating effect of diphenhydramine upon the sedative action of the barbiturates was much greater than that of tripelennamine and both diphenhydramine and tripelennamine prolong 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 40% by diphenhydramine. He concluded that whatever potentially sedative effect antihistaminic

drugs might have in animals it was masked by a co-existing excitatory effect, and that, in low doses, the two effects might cancel each other, and in high doses, the excitatory effect predominated.

Winter et al. (1951) reported that antihistaminic drugs produced muscular incoordination and evidences of cerebral depression in trained animals, resulting in prolongation of the climbing time on a vertical rope or even complete failure, and pointed out a very different order of relative effectiveness of the antihistamines on potentiation of barbiturates than had previously been reported (Winter, 1948). Whereas previously, it was reported that tripelennamine only slightly potentiated the hypnotic effect of barbiturates in mice while diphenhydramine showed a relatively marked potentiation, their results were in the reversed order. Tripelennamine had a much more pronounced effect upon the performance of the trained rats. They suggested that different higher centers were involved in the two tests. Their experiment seemed to demonstrate that the antihistaminic drugs were depressants of the higher centers of the C.N.S. The general behavior of the animals, as well as the fact that the effect could be antagonized by caffeine and amphetamine, suggested that the site of action was probably in the cerebral cortex.

Tanaka (1952) studied the change of electroencephalogram (E.E.G.) by diphenhydramine in man and rabbit. He found that the E.E.G. became similar to patterns characteristic of those at onset of sleep in man, and that it was changed differently in different individuals depending on the normal pattern of the subject and it showed central excitement and corresponding changes in the rabbit. On the

other hand, in his experiment, 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. (1952) reported that sodium pentobarbital increased the intraperitoneal LD_{99} 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 aborted 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.

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

Baird 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.

Solovév (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.

Sherman'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.

Tookes and his coworker (1957) reported that some hyperactivity was observed with effective doses of diphenhydramine (26 - 60 mg/kg) and that the ED_{50} 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 ataxia.

Chen (1958) studied two 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.

Onizuka (1958) reported that intravenous injection of diphenhydramine caused uneasiness 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 urethan. 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 aminohexane, chloropromazine, and procaine.

Maxwell 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.

Martelli (1961) reported that tripelennamine inhibited the catabolism of pentobarbital in vivo or in vitro.

Gilmore et al. (1960) and Sieé (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 dialkylaminoethyl 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 drugs. 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.

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* Metro Industries, Mineola, 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 (50 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.

LD_{50} 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.

Preliminary Behavioral Studies

Rolling Roller

The rolling roller used in this experiment was a modified apparatus from Dunham and Miya's rolling roller. The rolling roller apparatus employs a 115 v., 60 cycle, a.c., Bird Electric Kymograph, 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, 13 inches long and 5/8 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 masonite 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 3 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 3 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 P value of 0.05.

Determinations of the LD_{50} 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 1

COMPARATIVE EFFECTS OF EXPERIMENTAL COMPOUNDS ON NORMAL GROSS ACTIVITY IN MICE BY THE ACTOPHOTOMETER*

Compound	Dose mg/kg	% Change from normal	Compound	Dose mg/kg	% Change from normal
B-HCl**	12.16	66 ↓ 18 ↓ 54 ↓	TMB-HCl***	16.0	98 ↓ 54 ↓ 92 ↓
	24.32	18 ↑ 143 ↑ 99 ↓ 2 ↑ 27 ↓ 3 ↑		32.0	82 ↓ 80 ↓ 91 ↓ 93 ↓ 46 ↓ 36 ↓
PBZ-HCl**		-- -- --	TMPBZ-HCl***	17.6	53 ↓ 80 ↓ 64 ↓
	22.2	63 ↓ 37 ↓ 72 ↓		26.4	93 ↓ 93 ↓ 89 ↓

* ; Metro Industries, Mineola, L. I., N. Y.

** ; Diphenhydramine hydrochloride

*** ; Trimethoxy diphenhydramine hydrochloride

** ; Tripelennamine hydrochloride

*** ; Trimethoxy tripelennamine hydrochloride

TABLE 2

COMPARATIVE EFFECTS OF EXPERIMENTAL COMPOUNDS

ON GROSS ACTIVITY IN MICE INFLUENCED BY

AMPHETAMINE HYDROCHLORIDE BY THE ACTOPHOTOMETER*

Comp.	Dose mg/kg	% Change from normal
Amph-HCl+	5.0	120 ↑
		156 ↑
		184 ↑
		113 ↑
	5.0	62 ↑
		289 ↑
		83 ↑
	10.0	61 ↑
		119 ↑
		89 ↑
Amph-HCl+	5.0	362 ↑
		174 ↑
		115 ↑
		103 ↑
D-HCl++ & Amph-HCl+	12.16	62 ↑
		148 ↑
		42 ↑
		96 ↑
	24.32	78 ↑
		74 ↑
		107 ↑
	24.32	183 ↑
		127 ↑
		78 ↑
PBZ-HCl** & Amph-HCl+	14.8	--
		192 ↑
		91 ↑
		68 ↑
TMB3-HCl+++ & Amph-HCl+	16.0	189 ↑
		136 ↑
		108 ↑
		238 ↑
	32.0	200 ↑
		57 ↑
		12 ↓
	32.0	69 ↑
		130 ↑
		107 ↑
TMPBZ-HCl*** & Amph-HCl+	17.6	79 ↑
		119 ↑
		78 ↑
		155 ↑

* ; Metro Industries, Mineola, L. I., N.Y.

\+ ; Amphetamine hydrochloride

\++ ; Diphenhydramine hydrochloride

\+++ ; Trimethoxy diphenhydramine hydrochloride

** ; Tripelennamine hydrochloride

*** ; Trimethoxy tripelennamine hydrochloride

TABLE 3

COMPARATIVE EFFECTS OF EXPERIMENTAL COMPOUNDS

ON SODIUM PENTOBARBITAL* SLEEPING TIME IN MICE

CONTROL			EXPERIMENTAL		
Expt. No.	No. of Animals	Sleeping Time (min.) Mean ± S.D.	Expt. No.	No. of Animals	Sleeping Time (min.) Mean ± S.D.
1	10	13.7 ± 4.2	B-HCl++ 1	10	46.9 ± 21.0[s]
2	7	23.0 ± 13.7	2	10	42.1 ± 8.8[s]
3	9	20.3 ± 7.9	3	10	48.7 ± 12.9[s]
4	7	12.5 ± 3.4	4	9	27.3 ± 7.3[s]
5	10	33.0 ± 17.0	TMB-HCl+++ 5	10	78.4 ± 33.4[s]
6	7	30.8 ± 8.0	6	10	68.9 ± 22.4[s]
7	10	33.6 ± 18.3	7	10	79.9 ± 25.5[s]
8	10	66.2 ± 23.5	8	10	114.0 ± 37.0[s]
9	9	14.1 ± 3.0	PBZ-HCl** 9	10	33.3 ± 8.5[s]
10	8	25.5 ± 14.6	10	10	26.0 ± 16.0
11	8	15.2 ± 6.8	11	9	19.9 ± 9.6[s]
12	9	39.0 ± 16.4	TMPBZ-HCl*** 12	10	28.0 ± 19.5[ns]
13	10	32.3 ± 10.3	13	10	42.1 ± 12.3[ns]
14	8	41.0 ± 13.0	14	9	46.6 ± 26.8[ns]
15	10	56.8 ± 19.9	15	6	51.3 ± 14.2[ns]

* ; Sodium pentobarbital — 30 mg/kg IP

++ ; Diphenhydramine hydrochloride — 25 mg/kg IP

+++ ; Trimethoxy diphenhydramine hydrochloride — 32 mg/kg IP

** ; Tripelennamine hydrochloride — 14.8 mg/kg IP

*** ; Trimethoxy tripelennamine hydrochloride — 17.6 mg/kg IP

s ; significant at 0.05 level

ns ; not significant

TABLE 4

COMPARATIVE EFFECTS OF EXPERIMENTAL COMPOUNDS

ON SODIUM PENTOBARBITAL* SLEEPING TIME IN MICE+

WITH ALL COMPOUNDS TESTED DURING SAME TIME PERIOD

Expt. No.	Control Mean ± S.D.	B-HCl** Mean ± S.D.	TMB-HCl*** Mean ± S.D.	PBZ-HCl** Mean ± S.D.	TMPBZ-HCl*** Mean ± S.D.
1	52.0 ± 8.7	100 ± 17.3	88.0 ± 24.0	70.2 ± 25.3	60.3 ± 13.0
2	29.0 ± 10.7	51 ± 17.0	43.0 ± 19.3	40.0 ± 9.8	38.0 ± 2.8

* ; Sodium pentobarbital 50.0 mg/kg IP

** ; Diphenhydramine hydrochloride 25.0 mg/kg IP

*** ; Trimethoxy diphenhydramine hydrochloride 32.0 mg/kg IP

** ; Tripelennamine hydrochloride 14.8 mg/kg IP

*** ; Trimethoxy tripelennamine hydrochloride 17.6 mg/kg IP

\+ ; 6 animals for each determination

TABLE 5

COMPARISON OF LD_{50} OF EXPERIMENTAL

AND PARENT COMPOUNDS IN MICE

Compound	LD_{50}	Compound	LD_{50}
D-HCl++	75 mg/kg+ 70 - 80	PBZ-HCl**	79 mg/kg* 64 - 76
TMD-HCl+++	142 mg/kg* 135 - 149	TMPBZ-HCl***	155 mg/kg* 146 - 164

++ ; Diphenhydramine hydrochloride

+++ ; Trimethoxy diphenhydramine hydrochloride

** ; Tripelennamine hydrochloride

*** ; Trimethoxy tripelennamine 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 mg/kg	No. of Animals	No. of Animals Falling within 3 minutes *
B-HCl++	12.16	9	3
	24.32	9	2
TMB-HCl+++	16.0	9	2
	32.0	9	1
PEL-HCl**	14.8	9	1
	29.6	9	2
TMPEL-HCl***	17.6	9	2
	35.2	9	0

++ ; Diphenhydramine hydrochloride

+++ ; Trimethoxy diphenhydramine hydrochloride

** ; Tripelennamine hydrochloride

*** ; Trimethoxy tripelennamine hydrochloride

TABLE 7

THE EFFECTS OF THE EXPERIMENTAL COMPOUNDS

IN MICE SUBJECTED TO THE INCLINED PLANE

Compound	Dose mg/kg	5 min.	30 min.	60 min.
B-HCl++	12.16	ND	ND	ND
	24.32	ND	ND	ND
TMB-HCl+++	16.0	ND	ND	ND
	32.0	ND	ND	ND
PBZ-HCl**	14.8	ND	ND	ND
	29.6	ND	ND	ND
TMPBZ-HCl***	17.6	ND	ND	ND
	35.2	ND	ND	ND

++ ; Diphenhydramine hydrochloride

+++ ; Trimethoxy diphenhydramine hydrochloride

** ; Tripelennamine hydrochloride

*** ; Trimethoxy tripelennamine hydrochloride

ND ; Normal descent

V

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 in activity 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 tripelennamine 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 trimethoxy diphenhydramine, 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 LD_{50} 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 clonic 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 ptosis similar to that observed during reserpine sedation.

Results from the preliminary behavioral studies by the

rolling roller 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 tripelennamine and trimethoxy tripelennamine showed depressant effect with the trimethoxy tripelennamine 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 tripelennamine potentiated the effect of sodium pentobarbital sleeping time whereas trimethoxy tripelennamine produced no apparent effect.
4. The LD_{50} 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.

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