EFFECTS OF ACUTE AND CHRONIC DIPHENHYDRAMINE ADMINISTRATION ON METHAQUALONE DISPOSITION

A Thesis
Submitted to the College of Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of
Master of Science in the
College of Pharmacy

by
Kwok Wai Leung, B.S.P.
Saskatoon, Saskatchewan
(c) 1982, K.W. Leung
To My Parents
The author has agreed that the library, University of Saskatchewan, may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor or professors who supervised the thesis work recorded herein or, in their absence, by the Head of the Department or the Dean of the College in which the thesis work was done. It is understood that due recognition will be given to the author of this thesis and to the University of Saskatchewan in any use of the material in this thesis. Copying or publication or any other use of the thesis for financial gain without approval by the University of Saskatchewan and the author's written permission is prohibited.

Requests for permission to copy or to make other use of material in this thesis in whole or in part should be addressed to:

Dean of the College of Pharmacy
University of Saskatchewan
Saskatoon, Saskatchewan S7N 0WO
Canada.
ACKNOWLEDGEMENTS

Sincere appreciation is expressed to Dr. K.W. Hindmarsh and Dr. S.M. Wallace whose guidance, encouragement and invaluable criticism made the completion of this thesis possible.

Appreciation is extended to Ms. S. So for typing this manuscript.

Thanks is expressed to the University of Saskatchewan for financial support.

To the Faculty, Staff, graduate students of College of Pharmacy and many individuals who offered their assistance and moral support, I express my sincere gratitude.
ABSTRACT

Because of the frequent prescribing of diphenhydramine by physicians for treating allergy and cold, interaction of the antihistamine with other drugs is a concern. Diphenhydramine has been reported to inhibit drug metabolism on acute administration (Kato, et al., 1964; LeGatt et al., 1980) and stimulate enzyme activities following chronic treatment (Kato et al., 1964; Conney et al., 1960). On the contrary, there are studies reporting a lack of the acute inhibitory effect (Hindmarsh et al., 1983) and chronic inductive effect (Hunninghake and Azarnoff, 1969) of diphenhydramine on drug metabolism.

To clarify these discrepancies of diphenhydramine on drug metabolism, acute and chronic diphenhydramine-methaqualone interactions were examined in the present investigation. Methaqualone blood levels were monitored (analyzed by gas-liquid chromatography) to reflect any effects of diphenhydramine on methaqualone metabolism.

To investigate the acute effect of diphenhydramine on methaqualone metabolism, methaqualone (40 mg/kg) alone orally (Control group) or in combination with diphenhydramine (5 mg/kg) either orally (Oral-diphenhydramine group) or intraperitoneally (IP-diphenhydramine group) was administered to rats. A comparison of the effect of the two routes of administration of diphenhydramine on methaqualone metabolism was to verify the previous investigation that diphenhydramine inhibits the intestinal metabolism of methaqualone (LeGatt et al., 1980). Methaqualone blood levels were significantly greater in the oral diphenhydramine group and the IP-diphenhydramine group than the control group.
at 60 minutes and 120 minutes after dosing. Methaqualone blood levels were also significantly greater in the oral-diphenhydramine than the IP-diphenhydramine group at 30 and 60 minutes after dosing. In addition, time required to achieve the maximum methaqualone blood concentration ($t_{\text{max}}$) is 30 minutes in the control group and 60 minutes in both diphenhydramine treated groups.

Both oral and intraperitoneal administration of diphenhydramine caused changes in blood levels of orally administered methaqualone. Diphenhydramine given orally had a greater effect on methaqualone blood levels than diphenhydramine given intraperitoneally. The data suggested that the gastrointestinal tract is a site of diphenhydramine-methaqualone interaction. However, the changes in methaqualone blood levels caused by intraperitoneally administered diphenhydramine indicate that mechanisms other than inhibition of intestinal methaqualone metabolism are also involved. The delay in $t_{\text{max}}$ observed in diphenhydramine treated groups suggests that other possible mechanisms of interaction may include an interference with methaqualone absorption by diphenhydramine (both oral and intraperitoneal routes), presumably by its centrally mediated anticholinergic properties, and possibly a dose-dependent inhibition of hepatic methaqualone metabolism by diphenhydramine.

To have a better understanding of the chronic inductive effect of diphenhydramine, the induction activity of the antihistamine was examined in rats utilizing different dosages (25 mg/kg once daily, 25 mg/kg twice daily, 25 mg/kg three times daily, 50 mg/kg three times daily), routes of administration (oral and intraperitoneal), dosage schedule (a schedule with equally spaced 8-hour dosage interval, a schedule with a 16-hour overnight interruption in the interval) and ages of animals (Wistar rats weighing approximately 40 g, 100 g, 250 g).
Rats were pretreated with diphenhydramine using the dosing variables mentioned above. After diphenhydramine pretreatment, rats were then given either methaqualone, zoxazolamine or hexobarbital. Enzyme induction was determined by monitoring methaqualone blood levels, or by measuring zoxazolamine paralysis time and hexobarbital hypnosis time.

Enzyme induction occurred when diphenhydramine was given at a dosage of 50 mg/kg orally or 25 mg/kg intraperitoneally every eight hours to young animals (weighing 100 g). The results showed that the enzyme induction activity of diphenhydramine was influenced by the dosage regimen. Dosing parameters which favour enzyme induction were: a multiple daily administration of the antihistamine, intraperitoneal route of administration (which provides a larger fraction of a dose to the liver than the oral route) and equally spaced dosage intervals (which provide more consistent drug levels than irregular dosage intervals). The data agree with the previous observation that maintaining a sufficiently high drug level in the liver for a period of time is an important requirement for enzyme induction (Rammer, 1969). Thus the dosing variables (regimen) could determine the induction activity of diphenhydramine by affecting the hepatic drug levels.

The result of this present study suggests that diphenhydramine is a weak inducer. The induction effect of the antihistamine may not be clinically significant.

The present investigation demonstrated an acute inhibitory effect and a chronic inductive effect of diphenhydramine on drug metabolism. These activities of diphenhydramine are influenced by many pharmacodynamic and pharmacokinetic factors of the drug (e.g. anticholinergic effect, varied bioavailability by different
routes of administration, short biological half-life). The result of a drug interaction with diphenhydramine is hence difficult to predict.
TABLE OF CONTENTS

ACKNOWLEDGEMENT ........................................... iii
ABSTRACT .................................................. iv
LIST OF TABLES ........................................... xii
LIST OF FIGURES ........................................... xiii
I. INTRODUCTION ........................................... 1
   1. Diphenhydramine ..................................... 1
      1.1 History ......................................... 1
      1.2 Therapeutic Use ................................ 4
      1.3 Mechanism of Action ........................... 8
      1.4 Absorption and Disposition .................. 13
      1.5 Toxicity ....................................... 15
      1.6 Drug Interactions ............................. 19
   2. Microsomal Enzyme Induction ..................... 20
      2.1 Properties of Enzyme Inducers ............... 20
      2.2 The Consequences of Enzyme Induction .... 22
      2.3 Morphological and biochemical changes
          Resulting from Enzyme Induction ............ 24
      2.4 Mechanism of Enzyme Induction ............. 27
   3. Present Investigation: ............................ 29
II. EXPERIMENTAL .......................................... 31
   1. Animal Studies ................................... 31
      1.1 Handling of Animals .......................... 31
      1.2 Administration of Drugs ..................... 31
      1.3 Acute Administration of Diphenhydramine.. 31
          1.3.1 Preparation of Solutions .............. 31
          1.3.2 Drug Administration .................. 32
      1.4 Chronic Administration of
          Diphenhydramine ............................ 33
1.4.1 Preparation of Solutions ................. 33
  1.4.1.1 For Oral Use .......................... 33
  1.4.1.2 For Intraperitoneal Use .......... 33
1.4.2 Drug Administration .......................... 33
  1.4.2.1 Estimation of Potency of Diphenhydramine as an Enzyme Inducer ......... 33
  1.4.2.2 Enzyme Induction Activity of Diphenhydramine in Young Animals .............. 34
  1.4.2.3 Effect of Diphenhydramine Routes of Administration on Metabolism .......... 36
  1.4.2.4 Effect of Diphenhydramine Dosage on Metabolism .................. 36
  1.4.2.5 Enzyme Induction Activity of Diphenhydramine in Mature Animals .......... 37
2. Analytical Procedure for Methaqualone ............... 37
  2.1 Extraction from Blood ...................... 37
  2.2 Gas-Liquid Chromatographic Analysis of Methaqualone ................................ 39
  2.3 Construction of Calibration Curves .......... 39
  2.4 Extraction Efficiency ...................... 40
3. Data Analysis .................................. 41

III. RESULTS AND DISCUSSION ........................ 43
  1. Analytical Procedure ................................ 43
    1.1 Gas-Liquid Chromatographic Analysis ........... 43
    1.2 Extraction .................................. 45
    1.3 Calibration Curves .......................... 48
  2. Animal Studies ............................... 50
    2.1 Animal Model .............................. 50
2.2 The Effect of Acute Administration of Diphenhydramine on the Metabolism of Methaqualone ........................................ 50

2.3 The Effect of Chronic Administration of Diphenhydramine on the Metabolism of Methaqualone ................................. 61

2.3.1 Effect of Chronic Oral Administration of Diphenhydramine (at a once-daily dosage) on the Methaqualone Blood Levels in Mature Rats (approximately 250 g)........ 62

2.3.2 Effect of Chronic Oral Administration of Diphenhydramine (at a twice-daily dosage) on the Methaqualone Blood Levels in Young Rats (approximately 100 g)........ 64

2.3.3 Effect of Chronic Intraperitoneal Administration of Diphenhydramine (at a twice-daily dosage) on the Pharmacological Activities of Zoxazolamine and Hexobarbital in Young Rats (approximately 40 g)..... 64

2.3.4 Effect of Chronic Oral and Intraperitoneal Administration of Diphenhydramine (at a dosage of 25 mg/kg three times daily) on the Methaqualone Blood Levels in Young Rats (approximately 100 g)..... 69

2.3.5 Effect of Chronic Oral and Intraperitoneal Administration of Diphenhydramine (at dosage of 25 mg/kg or 50 mg/kg three times a day) on the Methaqualone Blood Levels in Young Rats (approximately 100 g)........ 70

2.3.6 Effect of Chronic Administration of Diphenhydramine (at a dosage of 25 mg/kg intraperitoneally or 50 mg/kg orally three times a day) on the Methaqualone Blood Levels in Mature Rats (approximately 250 g).... 73

2.3.7 Diphenhydramine as an Enzyme Inducer ........................................ 73

2.3.8 The Combined Effect of the Acute and Chronic Administration of Diphenhydramine on Methaqualone Blood Levels .......................... 79
IV. SUMMARY AND CONCLUSION ........................................... 82
V. REFERENCES ............................................................. 86
VI. APPENDICES ............................................................ 94
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Recovery of Added Methaqualone and Codeine from Rat Blood as Determined by Gas-Liquid Chromatographic Assay</td>
<td>49</td>
</tr>
<tr>
<td>II</td>
<td>Effect of Diphenhydramine Administration on Methaqualone Blood Levels at Various Times after Dosing</td>
<td>56</td>
</tr>
<tr>
<td>III</td>
<td>Effect of Chronic Oral Administration of Phenobarbital and Diphenhydramine on the Blood Levels of Methaqualone in Mature Rats (approximately 250 g)</td>
<td>63</td>
</tr>
<tr>
<td>IV</td>
<td>Effect of Chronic Oral Administration of Phenobarbital and Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)</td>
<td>65</td>
</tr>
<tr>
<td>V</td>
<td>Effect of Chronic Intraperitoneal Administration of Various Drugs on the Duration of Zoxazolamine Paralysis and Hexobarbital Hypnosis in Young Rats (approximately 40 g)</td>
<td>67</td>
</tr>
<tr>
<td>VI</td>
<td>Effect of Different Routes of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)</td>
<td>71</td>
</tr>
<tr>
<td>VII</td>
<td>Effect of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)</td>
<td>72</td>
</tr>
<tr>
<td>VIII</td>
<td>Effect of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Mature Rats (approximately 250 g)</td>
<td>74</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Binding of Histamine to its Receptor</td>
<td>10</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Postulated Pathway of Diphenhydramine Metabolism in the Rhesus Monkey (Drach and Howell, 1968)</td>
<td>16</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Gas Chromatogram of Control Blood (----) and Blood Spiked with Methaqualone (I) and Codeine (II) (______)</td>
<td>46</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Calibration Curve for Methaqualone Determination</td>
<td>51</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Methaqualone Blood Levels (± S.D.) for Groups of Rats Receiving Methaqualone Plus Diphenhydramine Orally (●●); Methaqualone Orally Plus Diphenhydramine Intraperitoneal (■■); Methaqualone Alone Orally (▲▲)</td>
<td>54</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

1. Diphenhydramine

1.1. History

As early as 1933, two French investigators, Fourneau and Bovet, observed that the compound 2-(N-piperidinomethyl)-1, 4-benzodioxane (1) protected animals from bronchial spasm caused by inhalation of histamine (Burger, 1970).

\[
\text{CH}_2\text{N}
\]

(1)

In the same year, Fourneau synthesized two more ether type compounds \( \beta-(5\text{-isopropyl-2 methyl phenoxyethyl}) \) diethylamine (2) and its dimethyl homolog (3) which were also active against histamine.

Although these compounds were too toxic to have any clinical value, this discovery initiated an interest in synthesis and investigation of related antihistaminic agents. Structure-activity relationships of \( \text{RR}'\text{NCH}_2\text{CH}_2\text{NR}'' \), the ethylenediamines (4) and \( \text{ROCH}_2\text{CH}_2\text{NR}'' \), the aminoalkylethers (5) were investigated (Burger, 1970). \( R \) was phenyl or substituted phenyl, \( R' \) was alkyl or aralkyl, and \( R'' \) a smaller alkyl group.

Thereafter, the development of a clinically useful antihistamine became more promising.
The first antihistamine to be used clinically was phenbenzamine (Antergan®) (6). Modification of phenbenzamine resulted in the product pyrilamine maleate (Neo-antergan®) (7) (Goodman and Gilman, 1980).

Pyrilamine maleate produced fewer side effects and remains one of the most effective antihistamines.

While most of these developments took place in France, investigations with aminoalkyl ethers were concurrently underway in United States. Diphenhydramine (8) was the first widely used drug in this series, (Goodman and Gilman, 1980).
Clinically, diphenhydramine is $\beta$-dimethyl aminoethyl benzhydryl ether, synthesized by condensing dimethaminoethanol in the presence of sodium carbonate (Burger, 1970).

Diphenhydramine is a highly effective antihistamine, which is 16 times more potent than papaverine, and 33 times more potent than aminophylline in protecting guinea pigs from inhaled histamine. Diphenhydramine is still one of the most extensively prescribed antihistamines.

1.2 Therapeutic Use

Diphenhydramine, in the form of the hydrochloride salt, is mainly indicated for various allergic diseases. Because of central properties it also has other clinical uses. Marketed as Benadryl® (Parke Davis and Co.), this histamine antagonist is beneficial in arresting allergic disorders for which histamine plays a major role in precipitating the symptoms. However, since antihistamines do not antagonize the root cause of various allergic diseases, i.e. antigen-antibody reaction, there are limitations for their use in the different types of hypersensitivity reactions.

Diphenhydramine is ineffective against other autocoids released during the allergic reaction. The efficacy of diphenhydramine in counteracting hypersensitivity reactions will therefore vary, depending on the degree to which the symptoms are due to histamine.
Thus the protection varies markedly in different tissues. For example, in bronchial asthma, where other bronchial constrictors such as SRS-A are also involved, diphenhydramine only offers partial relief. Diphenhydramine is slow in combating severe attacks of systemic anaphylaxis and angioedema involving the larynx. Physiological antagonists such as epinephrine, isoproterenol and theophylline are much more effective in these conditions because they not only provide immediate relief of the symptoms caused by histamine and other autocoids, but also reverse the undesirable effects rather than merely reducing them (Goodman and Gilman, 1980).

Acute exudative types of allergy such as seasonal rhinitis (hay fever, pollinosis) are by far most responsive to diphenhydramine treatment (Gates and Ceccarelli, 1972). Most patients obtain relief from symptoms such as sneezing, rhinorrhea and itching of eyes, nose and throat. However, chronic nasal congestion resulting from prolonged exposure to the offending allergen is not improved by diphenhydramine ingestion. The antihistamine has no value in treating perennial (vasomotor) rhinitis.

Diphenhydramine has long been included in the therapy of other non-allergic respiratory diseases such as the common cold and cough. Despite popular belief, the drug has no significant effect on the common cold. Its anticholinergic activity may have some benefit in controlling rhinorrhea and does give some relief especially if the viral infection is aggravated by allergic conditions (Saporiti and Marino, 1963). The antitussive activity of diphenhydramine has been demonstrated in animals (Wax et al., 1962) and man (Lilienfield et al., 1976). In chronic cough related to bronchitis, diphenhydramine significantly reduces the frequency of cough. Preasthmatic cough in
children is also controlled by diphenhydramine. It has been suggested this antitussive activity of diphenhydramine is due to direct action and not because of its sedative effect (Lilienfield et al., 1976).

Diphenhydramine is also effective in treating certain allergic dermatoses (Goodman and Gilman, 1980). Acute urticaria, another example of an exudative type of allergy, is particularly responsive to diphenhydramine therapy, although edema and erythema are not as well controlled as the itching in this condition. Chronic urticaria only responds moderately. Other conditions such as atopic dermatitis and contact dermatitis can be treated fairly well by topical administration of the compound.

Diphenhydramine plays only a limited role in the treatment of systemic allergies. The drug should not be the first choice in treating patients with a severe systemic anaphylactic attack. The antihistamine is useful in prophylaxis of allergic response to contrast media (Millbern and Bell, 1979). However, once the reaction takes place, diphenhydramine is of no value. Although urticarial and edematous lesions of serum sickness and blood transfusion reactions of non-hemolytic and non-pyrogenic types respond well to diphenhydramine therapy, often large doses or combination therapy with other medications are required to treat and control severe cases.

Many central activities of diphenhydramine which were once thought to be undesirable side effects are now considered clinically beneficial. By far the most common therapeutic application of these central effects is the prophylaxis and treatment of motion sickness (Krogh, 1982; Goodman and Gilman, 1980; Foye, 1974). Although dimenhydrinate (e.g. Dramamine®), a chlorotherealine salt of diphenhydramine, is usually employed for this purpose, the hydrochloride salt (e.g. Benadryl®) is equally active (Burger, 1970). Postoperative nausea and vomiting,
nausea of pregnancy and vestibular disturbances such as Meniere's disease and vertigo are effectively controlled. The drug also gives some relief to symptoms of drug-induced Parkinsonism and extrapyramidal reactions (Patton, 1975). All of these therapeutic properties are probably due to the anticholinergic activity of diphenhydramine. Sedation, the most common disturbing side effect, has been recently explored for its potential therapeutic usefulness. Diphenhydramine may be a safe sleeping aid in treating pediatric sleep disorder (Russo et al., 1976). The hypnotic efficacy has also been examined in adults although it is by no means as effective as barbiturates and may only have limited value in selected patients (Teutsch et al., 1974). With its central nervous system (CNS) depressant property, diphenhydramine can be a useful minor tranquilizer in psychiatrically disturbed prepubescent children (Korein et al., 1971). However, the clinical effect of diphenhydramine appears to be age-dependent. After the age of 10, children begin to respond like adults in that the drug produces drowsiness before it reduces anxiety and hyperactivity.

Diphenhydramine has other minor properties whose therapeutic value has yet to be determined. Its local anesthetic activity may be helpful in counteracting pain and itching of the skin and mucous membrane (Goodman and Gilman, 1980). However, the risk of producing allergic dermatitis with local application of the antihistamine must be recognized. Patients having hyperammonemia, a fatal condition associated with hepatic failure or portosystemic shunt with encephalopathy, may receive some relief from diphenhydramine administration. Diphenhydramine has been found to reduce the blood level of ammonia and decrease the mortality from 12% in dogs with glycine-produced hyperammonemia (Darin et al., 1972). Diphenhydramine has been shown in vitro and in vivo (mice) to have direct antibacterial
activity against a large number of Gram positive and Gram negative bacteria (Dastidar and Saha, 1976).

In summary, diphenhydramine possesses such a diversity of activities with a wide range of therapeutic uses that it is one of the most extensively prescribed drugs. With its many potential therapeutic applications diphenhydramine will continue to be the subject of many clinical investigations.

1.3 Mechanism of Action

Diphenhydramine (8) exerts antihistaminic activity by acting as a competitive antagonist of histamine at the hypothetical histamine receptor. This antagonistic activity of diphenhydramine is dependent on its structural similarity to histamine (9).
To be a histamine antagonist, the compound must be a nitrogenous base. Its aliphatic amine side chain resembling that of histamine plays an important role in competing for the receptors while the cyclic ring is essential for the antihistaminic activity (Melville, 1973).

Existing as a histamonium ion at physiologic pH, histamine binds to the receptor by a strong electrostatic attraction between the nitrogen of the imidazole group of the receptor and the protonated nitrogen of the histamonium ion (Silva, 1969). The dipole-dipole interaction between the imidazole group of histamine and the amide group of the receptor further facilitates the attachment (Korolkovas and Burckhalter, 1976; see Fig. 1). As diphenhydramine also has a positively charged protonated tertiary nitrogen, it can strongly bind to the negatively charged nitrogen of imidazole group of the receptor. Dipole-dipole interaction is also possible between the ether oxygen of diphenhydramine and the amide group of the receptor. Furthermore, the two bulky aromatic rings of diphenhydramine form van der Waals and hydrogen bonding with non-specific sites of the receptor, thus further strengthening the binding (Korolkovas and Burckhalter, 1976).

It is important to recognize the two different types of histamine receptors in the body, namely \( H_1 \) and \( H_2 \) receptors. All classical antihistamines, including diphenhydramine, block the effect of histamine by occupying the \( H_1 \) receptor without themselves initiating a response. They are ineffective against the activities of histamine mediated by \( H_2 \) receptors. The difference between \( H_1 \) and \( H_2 \) receptors is the internitrogen distance within the receptor.
Figure 1: Binding of Histamine to its Receptor (Korolkovas and Burckhalter, 1976)
Histamine probably exists in two distinct confirmations in order to initiate different biological responses. The spatial variation in the H₂ receptors prevents the antihistamine from antagonizing the responses mediated by H₂ receptors. As illustrated in Fig. 1, the distance between the two nitrogens in the H₁ receptor is 4.55 Å while the distance in the H₂ receptor is 3.60 Å (Korolkovas and Burckhalter, 1976).

Thus, diphenhydramine can protect the H₁-receptor-rich tissues from the action of histamine but has no effect on H₂-receptor-rich tissues. The protection has been demonstrated in most smooth muscles in which H₁ receptors predominate. Diphenhydramine antagonizes the increased histamine-induced capillary permeability and edema formation, opposes the constrictor action of the autocoid on respiratory smooth muscles, and inhibits both vasoconstrictor and vasodilator effects of histamine within vasculature (Goodman and Gilman, 1980). Apparently, H₂ as well as H₁ receptors are both present in the vascular tree because a combination of H₁ and H₂ blocking drugs are more effective in controlling activities of histamine in the vascular tree. On the other hand, the H₂-mediated gastric secretion is not inhibited by diphenhydramine. Similarly, the effect of histamine on rat heart and rat uterus (where the H₂ receptors predominate) is influenced little by diphenhydramine.

There is no evidence the antihistamine destroys histamine in vitro or in vivo (Melville, 1973). Although high concentrations of antihistamine do inhibit histamine release in vitro, diphenhydramine probably does not work by this mechanism in vivo. The concentrations used in in vitro studies are much higher than those likely to be produced with protective doses of the antihistamine in vivo (Foye, 1974).

The mechanism of diphenhydramine-initiated central action is not fully understood. Histaminergic nerves may be present in
the brain. However, the antihistaminic activity and the central effect are not parallel. Fairgold and Berry (1972) demonstrated that strikingly similar electrographic effects were induced by both the levorotatory form and the racemic mixture of chlorpheniramine while the ability of these compounds to antagonize histamine differed substantially. This finding suggested that the central effect of antihistamines may not be related to antihistaminic activity.

Of all the centrally mediated activities of diphenhydramine, antimotion sickness action has been studied most extensively. Electrophysiological recordings in laboratory animals reveal that the locus of the antimotion sickness action of diphenhydramine is the region of the vestibular nucleus (Jaju and Wang, 1971). Instead of having an effect on the vestibular midbrain "integrative vomiting center" or the medullary chemoreceptive trigger zone the drug appears to block excitatory labyrinthine impulses at the cholinergic synapses in the vestibular nuclei. Similar electrophysiological readings have been obtained with low doses of more potent antimotion sickness agents such as scopolamine (Jaju and Wang, 1971). Additionally, promethazaine, which has more potent anticholinergic activity, is a superior antihistamine for combating motion sickness (Goodman and Gilman, 1980).

The mechanism of action in management of Parkinsonism and extrapyramidal reactions is attributable to both anticholinergic and antihistaminic effects of diphenhydramine (Patni and Dandiya, 1972). Diphenhydramine decreases the resulting elevated brain levels of histamine and acetylcholine after a perphenazine-induced catatonia (Chopra and Dandiya, 1975). However, these studies are in contrast to previous findings indicating the central effect is not
related to antihistaminic properties of the drug (Fairgold and Berry, 1972). More research is needed to clarify the mechanism of the central activity.

1.4 Absorption and Disposition

Diphenhydramine is readily absorbed from the gastrointestinal tract. On oral administration, antihistaminic activity is seen within 15 to 30 minutes with peak activity occurring within 1 to 2 hours. The duration of action is approximately 4 to 6 hours. It is not known whether the drug is metabolized in the gastrointestinal tract. However, a large first pass effect is evident, with about 50% of the drug being metabolized by the liver before it reaches the systemic circulation (Albert et al., 1975).

From the blood, diphenhydramine is rapidly transported to various tissues. Animal studies show that a large fraction of intravenously administered diphenhydramine is removed from the bloodstream in less than one minute, being retained by cellular elements in the tissues (Drach et al., 1970). Peak concentrations in most tissues are observed in 1 to 2 hours after administration of the drug (Glazko et al., 1949). When diphenhydramine is given parenterally (intraperitoneally and intravenously) or subcutaneously, the highest concentrations are found in lung tissue, followed by spleen, kidney, brain, liver and muscle. Oral administration results in high levels in the liver and spleen (Glazko et al., 1949).

The major sites of metabolism are the liver, kidney and heart. Impaired hepatic function results in prolonged action of diphenhydramine. The major metabolites found in animal plasma and urine studies are diphenylmethoxyacetic acid and its glutamine conjugate (in rhesus monkey) or glycine conjugate (in dog) (Drach...
et al., 1970). The carboxylic acid, together with the conjugates, account for nearly two-thirds of the urinary metabolites. Other minor metabolites consist of basic mono- and di-demethylated derivatives and N-oxide products. Little of the drug is excreted unchanged in urine. A postulated pathway for diphenhydramine metabolism in the rhesus monkey is shown in Fig. 2 (Drach and Howell, 1968).

The same metabolites have been detected in human plasma and urine (Chang et al., 1974). Species difference in the metabolism of diphenhydramine is evident. Diphenylmethoxyacetic acid, the major metabolite in humans and monkeys and dogs, is not found in rat urine (Drach et al., 1970).

Within 24 hours after administration the urinary excretion of total diphenhydramine metabolites represent about 64% of the dose (acute studies) in human volunteers but only 49% after multiple doses (Glazko et al., 1974). Following the administration of 14C-labelled diphenhydramine to rats, approximately 50% of the dose is recovered in the urine within 24 hours (Glazko, 1949). The remainder is found in the feces, strongly suggesting extensive biliary excretion. Mass balance studies on routes of excretion with radio-labelled drug in human subjects have yet to be done.

Diphenhydramine has a rather short biological half-life in animals and humans. Estimated half-lives are 1 hour in the rat (Drach et al., 1970) and 7 hours in man (Glazko et al., 1974). The major metabolite, diphenylmethoxyacetic acid, binds strongly to plasma protein (Drach et al., 1968). Binding affinity is 40-fold greater than the parent compound. The metabolite thus has a longer plasma half-life of about 12 hours. The effect of the strong protein binding characteristic of this metabolite on the distribution of drug,
1.5 Toxicity

Diphenhydramine rarely exhibits serious side effects in therapeutic doses. Although adverse effects will often subside with continued therapy, withdrawal of the drug is necessary if the patient is too disturbed by the effects.

By far the most common side effect of diphenhydramine is sedation. While this effect can be considered as therapeutic, it can interfere with daytime activities and impair alertness (Goodman and Gilman, 1980; Krogh, 1982). Other undesirable central effects are dizziness, tinnitus, lassitude, incoordination, fatigue, blurred vision, diplopia, euphoria, nervousness, insomnia, and tremors (Jaattela et al., 1971).

Side effects involving the ingestive system are frequently experienced by patients taking the drug. Complaints include loss of appetite, nausea, vomiting, epigastric distress, and constipation or diarrhea (Krogh, 1982; Goodman and Gilman, 1980).

Many side effects of diphenhydramine are due to the anticholinergic properties of the antihistamine. These side effects include dryness of mouth, throat and respiratory passages, sometimes inducing cough, urinary retention, pupil dilatation and blurred vision.

Allergic reactions can be induced by diphenhydramine especially when the drug is applied topically. Contact dermatitis and photoallergic dermatitis are not uncommon (Shelly and Bennett,
Figure 2: Postulated Pathway of Diphenhydramine Metabolism in the Rhesus Monkey (Drach and Howell, 1968). The (diphenylmethoxy) moiety is denoted by R.
The diphenhydramine photoallergy appears to be different from most other forms of photoallergies in that it is elicited by ultraviolet light in the 290-320 nm range instead of the more usual range of 320-400 nm (Horio, 1976; Emmett, 1974).

Other unusual reactions of the antihistamine include acute dystonia (Lavenstein and Cantor, 1976), facial dyskinesia (Brait and Zagerman, 1977), leukopenia and agranulocytosis. Fortunately, these complications are very rare. Palpitation, hypotension, headache, tightness of chest, tingling, heaviness and weakness of the hands are other minor side effects resulting from diphenhydramine therapy (Goodman and Gilman, 1980).

Using the rat as a model, Liu and Lin (1970) demonstrated a highly significant retardation of salivary gland and body growth in immature animals taking diphenhydramine. However, the drug causes only a slight but insignificant decrease in salivary gland and body weight in adult rats. The significance of such an effect in humans has not yet been evaluated.

The effect of diphenhydramine on reproduction has also been studied. Rats treated with diphenhydramine daily for short periods during mating or early pregnancy have a diminished fertility rate (Shelesnyak and Davis, 1955). High doses of diphenhydramine (200 mg/kg/day) are reported to be embryotoxic in rats (Naranjo and de Naranjo, 1968). However, Schardein (1971) found no teratogenic effects of diphenhydramine in rats and rabbits even when the drug was given during the period of organogenesis. An association between the occurrence of cleft
palate and maternal diphenhydramine intake during pregnancy has been postulated in a retrospective study of human subjects (Saxen, 1974). Withdrawal symptoms (such as generalized tremulousness and diarrhea) in a newborn infant related to maternal use of diphenhydramine have also been reported (Parkin, 1974). In general practice today, diphenhydramine is not contraindicated in pregnancy. However more studies are required to establish whether the drug is teratogenic or not.

An overdose of diphenhydramine is serious especially in young children. Many deaths of children under three years of age have been reported after accidental ingestion of diphenhydramine (Aaron, 1953; Davies and Hunt, 1949; Reyes-Jacang and Wenzel, 1969; Wyngaarden and Seeves, 1951). Toxic symptoms are not necessarily dose related. Both CNS depressant and stimulant properties (such as convulsion seen in toxic dosage) of diphenhydramine constitute its greatest danger in severely poisoned patients. In small children, the dominant effect is stimulation. Initially, symptoms such as excitement, ataxia, incoordination, athetosis are seen. Muscular twitching will progress to convulsion. Fixed dilated pupils, flushed face and fever are common in pediatric patients. Terminally, deepening coma with cardiorespiratory collapse will lead to death, usually within 2 to 18 hours after ingestion. In adults, there is a cycle of depression, followed by stimulation and then depression. Lethargy and even coma are followed by convulsion, cardiorespiratory collapse and death.
1.6 Drug Interactions

Since diphenhydramine is usually prescribed for long term control of allergy and is found in many cold remedies for self medication, drug interactions resulting from the concurrent use of diphenhydramine with other drugs is probable.

The combination effect of diphenhydramine with other CNS depressants such as alcohol has received much attention. Such combinations have an additive effect and seriously impair mental performance such as cognitive thinking, information process rate and attention (Burns and Moskowitz, 1980). The concurrent use of diphenhydramine with a barbiturate is a dangerous combination (Davis and Hunt, 1949). It is not advisable to use a barbiturate to control the convulsions observed in diphenhydramine poisoning.

Diphenhydramine, with its anticholinergic properties, can slow gastrointestinal motility. Diphenhydramine delays gastric emptying and intestinal transit of phenol red in the rat (Feldman and Putcha, 1977). The slowing of gastrointestinal motility seems to influence the absorption of many drugs administered concurrently with diphenhydramine. The antihistamine is shown to inhibit the gastrointestinal absorption of p-aminosalicylate in rats and humans (Lavigne and Marchand, 1972) and acetaminophen in rabbits (Imamura et al., 1981).

Diphenhydramine has a biphasic effect on the metabolism of many drugs (Kato et al., 1964), i.e. an acute inhibitory effect and a chronic inductive effect. Hindmarsh et al. (1979) demonstrated
a competitive type of inhibition of methaqualone metabolism by diphenhydramine in rat liver homogenate. *In vivo* studies suggested that the inhibition of intestinal metabolism of methaqualone might be the major mechanism of interaction responsible for the increased blood and brain levels of methaqualone after concurrent oral administration with diphenhydramine (LeGatt *et al.*, 1980). On chronic administration to rats, diphenhydramine induced metabolism of meprobamate, pentobarbital (*Kato* *et al.*, 1964), zoxazolamine (*Conney* *et al.*, 1960), and its own metabolism (*Burns* *et al.*, 1965). However, significant drug interactions due to the enzyme induction properties of diphenhydramine have not been reported clinically.

2. Microsomal Enzyme Induction

2.1 Properties of Enzyme Inducers

Microsomal monooxygenase enzyme activity is stimulated by many exogenous compounds. An increase in concentration of enzyme protein appears in association with this increased metabolic activity. The process is referred to as enzyme induction. Brown *et al.* (1954) first observed the enzyme induction phenomenon. Since then, enzyme induction in liver microsomes had been investigated extensively. Potent inducers such as barbiturates, and polycyclic aromatic hydrocarbons have been identified. Today, more than two hundred compounds, with various pharmacological activities and chemical structures, are known to stimulate microsomal enzyme activity.

In general, there is no structure-activity relationship observed in the inducing agents. Steric requirements for induction, however, are evident within groups of structurally related compounds. Studies with polycyclic aromatic hydrocarbons reveal that coplanar hydrocarbons are more potent inducers than
noncoplanar, with the optimal size for activity ranging from 75 to 150 $R$ (Arcos et al., 1961).

Although the inducing agents do not have any apparent specificities for their enzyme induction activity, they do have some common characteristics that suggest important criteria for screening potential inducers. Most enzyme inducers are soluble in lipid at physiological pH and have a prolonged biological half-life. Consequently, compounds with low lipid solubility generally lack the inductive effect. Repeated administration of lipid soluble compounds with short biological half-lives may induce drug metabolism. Maintaining a high concentration of the compound in the liver for a period of time appears to be an important requirement for enzyme induction (Rammer, 1969).

At least two groups of inducers have been identified. The first group, also known as Type I inducers, consists of drugs such as phenobarbital and many insecticides. The compounds in this group are similar in that they stimulate many hepatic metabolic pathways such as oxidation and reduction, glucuronide formation and de-esterification (Conney, 1967). The second group, the Type II inducers, is composed mostly of polycyclic aromatic hydrocarbons, e.g. 3-methylcholanthrene and 3, 4-benzpyrene. The compounds in this group induce the metabolism of only a limited number of substrates (Conney, 1967; Mannering, 1968).

Although it is difficult to generalize the time course of induction for different compounds, in general, the Type II inducers cause enzyme induction at a faster rate than the Type I inducers. Rats must be given phenobarbital daily for at least three days before maximal increase of enzyme activity is observed (Conney et al., 1960). In contrast, enzyme activity in rats treated with
polycyclic aromatic hydrocarbons is more than doubled within 3 to 6 hours with maximal increases occurring in 24 hours (Conney et al., 1956). Inducers in the same group do not necessarily have the same rate of induction. For example, upon repeated administration of chlordane, a potent Type I inducer, a recognizable effect is not apparent within three days and maximal increases in enzyme activity are only reached in 1 to 2 weeks (Hart et al., 1963). On the other hand, polycyclic aromatic hydrocarbons stimulate aryl hydrocarbon hydroxylase activity in vitro after a lag of 35 minutes although they can cause maximal increases in 24 hours (Gelboin, 1971).

2.2 The Consequences of Enzyme Induction

The result of enzyme induction is an accelerated biotransformation of compounds upon which the induced enzyme acts. When the duration and intensity of the action of compound is largely dependent on its rate of metabolism by the liver microsomal enzymes, enzyme induction has a significant influence on its pharmacological activities. For example, enzyme induction accelerates the formation of the inactive metabolites of zoxazolamine, meprobamate, diphenylhydantoin and several barbiturates. The duration of action of these compounds is shortened (Conney, 1967). In contrast induction increases the duration of action of compounds such as the dimethoxy ester of benzotriazine dithiophosphoric acid (Guthion®) and octamethyl pyrophosphoramide (Schradan®) since their metabolites are actually responsible for their pharmacological activities in the body. Drugs such as barbital are not metabolized and hence not affected by enzyme induction.

Enzyme induction can increase or decrease the intensity of drug action. A 100% mortality in rats receiving a dose of 150 mg
zoxazolamine versus 0% mortality in the rats protected by a single
dose of 3-methycholanthrene 24 hours before the dose of zoxazolamine
is a striking example of how enzyme induction can markedly decrease
the toxicity of a drug (Burns et al., 1965). On the other hand,
when the rats are treated with inducers such as phenaglycadol or
thiopental, the Schradan®-induced mortality rate increases from 6% to 80% (Kato, 1961).

Chronic administration of many drugs may induce their own
metabolism and alter their pharmacological activity. Examples are
phenylbutazone, chlorcyclizine, probenecid (Burns et al., 1965) and
barbiturates such as hexobarbital, pentobarbital and phenobarbital
(Rammer, 1962). Because of these pharmacological consequences, the
effects of enzyme induction should be taken into consideration in
multiple drug therapy or in chronic toxicity studies.

Since the metabolism of many endogenous compounds also
requires monooxygenase systems, the possible physiologic changes
resulting from enzyme induction should not be overlooked. Indeed,
it has been reported that several structurally unrelated drugs,
such as phenobarbital, diphenylhydantoin, chlorcyclizine and
phenylbutazone, can stimulate the hydroxylation of many steroid
hormones in laboratory animals (Conney, 1967). The anesthetic
action of large doses of progesterone decreases as progesterone
hydroxylase activity increases in phenobarbital-pretreated animals
(Kuntzman et al., 1965). The action of estradiol or estrone on the
uterus is decreased by the administration of the inducers of
steroid-hydroxylating enzymes (Levin et al., 1967). Changes in
lipid and bilirubin metabolism are also observed in animals with
induced enzyme activity. Phenobarbital causes a 4-fold increase
in the conversion of acetate to cholesterol in liver slices of golden
hamsters although accumulation of cholesterol in the liver and plasma is not detected in in vivo studies (Jones and Armstrong, 1965). Rats, treated with phenobarbital, show a 7-fold increase in the incorporation of glycine into bilirubin and increased rate of bile pigment excretion (Schmid et al., 1966). Despite all these well documented reports on the influence of enzyme induction on the metabolism of endogenous compounds, the significance of such effects in humans has not been determined.

2.3 Morphological and Biochemical Changes Resulting from Enzyme Induction

The most obvious morphological change due to enzyme induction is a marked increase in liver weight. Compounds like phenobarbital exert an anabolic effect on the liver resulting in 20 to 40% increase in microsomal protein per gram of the liver (Conney et al., 1960; Conney and Gilman, 1963; Rammer and Merker, 1963). In contrast, polycyclic hydrocarbons such as 3-methylcholanthrene have no such anabolic effect on microsomal protein, but do stimulate liver growth by increasing the synthesis of total liver protein (Arcos et al., 1961; Conney and Gilman, 1963).

Marked proliferation of smooth endoplasmic reticulum (SER) is observed in phenobarbital pretreated animals while 3-methylcholanthrene has a slight effect on SER proliferation (Rammer and Merker, 1963; Fouts and Roger, 1965). In both cases, rough-surfaced endoplasmic reticulum (RER) is little affected. Subfractionation experiments seem to suggest that polycyclic aromatic hydrocarbons stimulate the production of enzyme-containing vesicles different from those in control or phenobarbital treated animals (Murphy et al., 1969). Fouts (1961) indicated that drug-metabolism enzymes in the liver microsomes were concentrated in the SER, whereas other studies
either suggested that the enzymes were equally distributed between SER and RER (Orrenius and Ernster, 1964) or that the drug was metabolized at the same rate by the two types of membranes (Grams et al., 1967). No direct or conclusive correlation can be drawn between the hepatic morphological changes and increased drug metabolizing ability.

Biochemical changes produced by enzyme induction include increases in NADPH-oxidase, NADPH-cytochrome C reductase, NADPH-cytochrome P₄₅₀ reductase and cytochrome P₄₅₀ (Orrenius, 1965; Ernster and Orrenius, 1965; Peters, 1973; Koudstaal and Hardonk, 1969; Mannering, 1968). Most inducing agents have fairly specific actions on monooxygenase systems. Other microsomal enzymes are infrequently affected. For instance, phenobarbital can stimulate the activities of glucose 6-phosphate dehydrogenase and isocitric acid dehydrogenase without inducing changes in glucose 6-phosphatase and cytochrome b₅ (Ernster and Orrenius, 1965; Koudstaal and Hardonk, 1969; Orrenius et al., 1969). Phenobarbital does not affect most mitochondrial cytochromes (Orrenius et al., 1969; Schmid et al., 1966).

The difference between Type I and Type II inducers may be explained by the ability of the former to stimulate the metabolism of a wide range of substrates as compared to the limited activity of the latter. Sladek and Mannering (1969) suggested that phenobarbital and 3-methylcholanthrene stimulated different forms of cytochrome P₄₅₀. Observed alterations of $K_m$ and $K_s$ together with $V_{max}$ indicate a quantitative as well as a qualitative difference in the phenobarbital and polycyclic aromatic hydrocarbon inductions (Guarino et al., 1969; Alvares et al., 1971; Gurtoo et al., 1968).

Furthermore, it is observed that the maximum UV absorption
of the reduced cytochrome P_{450}-CO complex from 3-methylcholanthrene-treated animal shifts from 450 nm (as usually observed in control and phenobarbital-treated animals) to 446-448 nm (Alvares et al., 1967; Hildebrandt et al., 1968). This change in spectral properties of cytochrome P_{450} strongly suggests the existence of two different forms of the enzyme. To differentiate these two hemoproteins, the normal or phenobarbital-induced cytochrome is designated as P_{450}, while the polycyclic hydrocarbon-induced one is called P_{448}, P_{446}, or P_{1-450}.

Cytochrome P_{450} and P_{448} differ in their binding capacity for substrates. Phenobarbital can increase the metabolism of both Type I compounds such as hexobarbital and Type II compounds such as aniline while polycyclic hydrocarbons can only induce the metabolism of Type II compounds. Thus a deficiency of Type I binding sites in cytochrome P_{448} is hypothesized (Kato et al., 1969; Shoeman et al., 1969).

Many studies indicate that formation of cytochrome P_{448} is a direct combination of polycyclic hydrocarbons to the cytochrome P_{450}, presumably at the Type I binding sites (Schenkman et al., 1969; Fisher and Spencer, 1974). The combination seems to be sex hormone-dependent. Protein synthesis inhibitors such as actinomycin D can prevent the increase of cytochrome P_{448} in benzantracene-treated mature female rats, but not in immature animals (Fisher and Spencer, 1974). Investigators postulate that while benzanthracene can bind directly to cytochrome P_{450}, sex hormones stabilize the existing enzymes and prevent them from conversion except during or soon after the synthesis of the enzymes. These observations can partially explain the fact that younger animals are more susceptible to enzyme induction.
2.4 Mechanism of Enzyme Induction

Although sex hormones have an influence on the production of cytochrome $P_{448}$, the stimulation of hepatic microsomal enzymes, in general, does not require hormones of the testes and ovary, or of other glands such as the pituitary, adrenal or thyroid (Shimazu, 1965; Conney 1967; Orrenius et al., 1965). Enzyme induction can be demonstrated in isolated prefused livers and cell culture (Juchau et al., 1965; Gelboin et al., 1972; Nebut and Gelboin, 1968; Wattenberg and Leong, 1966). This suggests induction takes place in vivo in the liver at the cellular level.

Kinetic studies indicate that Michaelis constants for the enzymes do not change while the maximum velocity for the reactions are increased by phenobarbital treatment (Rubin et al., 1964; Netter and Seidel, 1964; Gillette, 1963; Rammer, 1962). Thus the enzyme induction cannot be interpreted as an altered affinity of the enzymes for the substrates.

An increase in synthesis of enzyme protein appears responsible for the increased activity of microsomal enzyme systems. The hypothesis is strongly supported by the counteracting activities of protein synthesis inhibitors, such as ethionine, puromycin and actinomycin D, on the enzyme inducing effect of phenobarbital and 3-methylcholanthrene (Conney, 1967).

Since these protein synthesis inhibitors work by different mechanisms, the level at which enzyme induction occurs has yet to be determined. Ethionine blocks the protein synthesis by replacing methionine in S-adenosylmethionine and prevents ATP synthesis (Villa-Trevino et al., 1963), which in turn inhibits amino acid activation, messenger RNA or transfer RNA synthesis (Gelboin, 1971). Puromycin specifically blocks the transfer of soluble RNA-bound amino acids
into microsomal protein (Yarmolinsky and de la Haba, 1959). Actinomycin D inhibits the DNA-directed synthesis of RNA by binding to DNA (Reich et al., 1961).

Studies using radio-labelled amino acids show that phenobarbital and 3-methylcholanthrene specifically increase the incorporation of a number of amino acids in microsomal preparations. Other cell fractions such as nuclei, mitochondria and cell supernatant are not affected (Kato et al., 1965; Gelboin and Sokoloff, 1961). The observation seems to suggest that transfer of RNA and formation of protein are the mechanisms of enzyme induction. Further studies suggest that increases in both the number of the active microsomal binding sites (for messenger RNA) and the microsomal messenger RNA content are responsible for the enzyme induction activity of phenobarbital and 3-methylcholanthrene (Gelboin, 1964).

Although increased protein synthesis seems to play an important role in the induction caused by phenobarbital and 3-methylcholanthrene, considerable evidence contradicts this hypothesis for the polycyclic aromatic hydrocarbons. In contrast to phenobarbital, 3-methylcholanthrene does not increase phenylalanine incorporation in hepatic microsomes from adrenalectomized or hypophysectomized rats (Jondorf et al., 1966).

Other possible mechanisms have not yet been thoroughly explored. For example, the increase of microsomal protein could be the result of the enzymes being stabilized by the inducers (Shuster and Jick, 1966; Kuriyama et al., 1969). Inducers are also known to increase phospholipid turnover, which in turn induces both smooth and rough endoplasmic reticulum proliferation (Orrenius and Ericson, 1966). Inducers may also influence heme biosynthesis resulting in the stimulation of cytochrome P450 and
monooxygenase systems (Baron and Tephly, 1969).

3. Present Investigation

Because diphenhydramine is readily available to the general public in many cold remedies and is usually administered chronically to control allergic symptoms, the antihistamine may be used concurrently with other drugs on many occasions. The acute and chronic effects of diphenhydramine on the action of other drugs warrants investigation.

After acute administration, diphenhydramine decreases the absorption of p-aminosalicylate in rats and humans (Lavigne and Marchand, 1972) and acetaminophen in rabbits (Imamura et al., 1981). In contrast, the antihistamine causes an increase in the blood levels and brain levels of methaqualine in rats (Hindmarsh et al., 1979; LeGatt et al., 1980). Chronically, diphenhydramine induces the metabolism of zoxazolamine (Conney et al., 1960), meprobamate, pentobarbital (Kato et al., 1964) and its own metabolism (Burns et al., 1965). In contrast to these findings, dogs treated with diphenhydramine for 14 days do not show a shortened plasma half-life of warfarin (Hunninghake and Azarnoff, 1968).

The present study was carried out to verify some of the acute and chronic effects of diphenhydramine. Since diphenhydramine is combined with methaqualone in commercial drug preparations (Mandrax®, Lancesomnal®, Mattholdorm®), it is of interest to determine if diphenhydramine would increase the sedative-hypnotic effect of methaqualone (as drug manufacturers had promoted) on acute administration and/or diminish the effectiveness of the hypnotic on chronic administration. To this end, the effect of acute
and chronic administration of diphenhydramine on methaqualone
blood levels were determined. Specifically the following experiments
were conducted:

1) To verify if the site of interaction following acute
administration of diphenhydramine and methaqualone is
the gastrointestinal tract (LaGatt et al., 1980), rats
were administered an oral dose of methaqualone, or an
oral dose of methaqualone in combination with an oral
dose of diphenhydramine, or an oral dose of methaqualone
in combination with an intraperitoneal dose of diphenhydramine.
Blood levels of methaqualone in the three groups of rats
were compared. It was thought if acute interaction of
the two drugs occurred in the gastrointestinal tract, the
intraperitoneally administered diphenhydramine would have
a minimal effect on orally administered methaqualone.

2) To demonstrate the effect of chronic administration of
diphenhydramine on drug metabolism, rats were pretreated
with diphenhydramine (employing different dosages, dosage
schedules and routes of administration) for a period of
time (3 to 6 days) prior to the administration of a single
oral dose of methaqualone. Blood levels of methaqualone
were monitored to determine if enzyme induction had
occurred.

From the data collected, an attempt was made to elucidate a more
complete picture of diphenhydramine interactions with methaqualone.
II. EXPERIMENTAL

1. Animal Studies

1.1 Handling of Animals

All animals were conditioned to the laboratory environment for at least one week prior to any treatment in order to offset the effect on the animals of transportation and change of environment. Each animal was housed individually in a stainless steel metabolic cage. Light in the laboratory was simulated as a 12 hour diurnal cycle.

For the acute study, animals were fasted 18 hours prior to the administration of drugs. For the chronic study, all animals were given food and water ad libitum during the course of diphenhydramine pretreatment. The animals were then fasted 18 hours prior to the administration of methaqualone, zoxazolamine or hexobarbital.

1.2 Administration of Drugs

Oral doses of the drugs were administered using a disposable plastic syringe fitted with a stainless steel stomach tube. Intraperitoneal doses were administered using a disposable plastic syringe fitted with sterile disposable needle (0.50 x 16 mm).

1.3 Acute Administration of Diphenhydramine

1.3.1 Preparation of Solutions

With the aid of a Vortex mixer, methaqualone hydrochloride was dissolved in propylene glycol to yield a concentration of 10 mg/ml. For oral administration, a solution of methaqualone hydrochloride (10 mg) and diphenhydramine hydrochloride (1.25 mg) was prepared by dissolving both drugs in 1 ml of propylene glycol. Diphenhydramine hydrochloride
(2.5 mg) was dissolved in normal saline (1 ml) for intraperitoneal injections.

1.3.2 Drug Administration

In order to verify whether the intestine was a possible site of interaction between methaqualone and diphenhydramine, the two drugs were administered concurrently by two different methods to adult male albino rats (Wistar strain) weighing approximately 250 g (246 ± 11 g).

Seventy-five rats were divided into three groups and treated as follows. Group I (25 animals) was given a single oral dose of methaqualone (40 mg/kg) and an intraperitoneal injection of diphenhydramine (5 mg/kg). Group II (25 animals) was given a single oral dose of methaqualone (40 mg/kg) and diphenhydramine (5 mg/kg). This group of rats also received an intraperitoneal injection of normal saline to ensure similar animal manipulation in each group. A control group (25 animals) received a single dose of methaqualone (40 mg/kg) orally and an intraperitoneal dose of normal saline. Five animals (in each group) were sacrificed by decapitation at specified times after dosing (30, 60, 120, 240 or 360 minutes). Individual blood samples were collected in heparinized beakers and transferred to heparinized culture tubes for storage. The blood samples were frozen (-10°C) until analyzed. Methaqualone blood levels, following the oral administration of methaqualone alone, or in combination with diphenhydramine (administered by either oral or intraperitoneal routes), were measured in the five rats at each of the five sampling times by a gas liquid chromatographic method.
1.4 Chronic Administration of Diphenhydramine

1.4.1 Preparation of Solution

1.4.1.1 For Oral Use

Solutions of diphenhydramine for oral administration were prepared by dissolving 5 mg of diphenhydramine hydrochloride in 1 ml of distilled water. Sodium phenobarbital was dissolved in distilled water to produce a concentration of 10 mg/ml. Methaqualone base was dissolved in propylene glycol (10 mg/ml).

1.4.1.2 For Intraperitoneal Use

For intraperitoneal administration, drugs were dissolved in normal saline instead of distilled water to maintain physiologic pH and osmotic pressure in the peritoneal cavity. A higher concentration than that for oral administration was required so the volume of injection would not exceed 0.35 ml for large rats (100 g and 250 g) and 0.15 ml for small rats (40 g). Diphenhydramine hydrochloride (10 mg) was dissolved in 1 ml of normal saline. Sodium phenobarbital was dissolved in normal saline to yield a concentration of 10 mg/ml. Sodium hexobarbital was dissolved in normal saline to produce a concentration of 60 mg/ml. Zoxazolamine solutions were prepared by adding 3.6 ml of 1N hydrochloric acid to 300 mg of zoxazolamine and diluting to 15 ml with normal saline (to give a concentration of 20 mg/ml). 3, 4-Benzpyrene was dissolved in corn oil to produce a concentration of 6 mg/ml.

1.4.2 Drug Administration

1.4.2.1 Estimation of Potency of Diphenhydramine as an Enzyme Inducer

As a preliminary experiment to estimate the potency of diphenhydramine as an enzyme inducer, the antihistamine was compared with the potent inducer, phenobarbital.
Eight adult male albino rats (Wistar strain) each weighing approximately 250 g (254 ± 8 g) were divided into three groups. Rats in group I (4 animals) were given an oral dose of diphenhydramine (25 mg/kg) once daily (8:00 am) for either 3 days (2 animals) or 6 days (2 animals). An oral dose of phenobarbital (60 mg/kg) was given to group II (2 animals) once daily (8:00 am) for 3 days as the reference group. The control group (2 animals) was given distilled water once daily (at 8:00 am) by the oral route for 3 days.

On the 4th or 7th Day, all rats received a single oral dose of methaqualone (40 mg/kg) (at 8:00 am). Each animal was sacrificed by decapitation at 75 minutes after dosing. The blood was collected in heparinized beakers and transferred to heparinized culture tubes for storage. Blood samples were frozen at -10°C until analyzed. Samples were usually extracted and analyzed within 48 hours.

1.4.2.2 Enzyme Induction Activity of Diphenhydramine in Young Animals

a) To demonstrate the enzyme induction activity of diphenhydramine in younger animals, male albino rats (Wistar strain) weighing approximately 100 g (103 ± 7 g) were used.

Twelve rats were divided into three groups. Group I (6 animals) was given diphenhydramine (25 mg/kg) orally twice a day (8:00 am and 4:00 pm). Group II (3 animals) was given an oral dose of phenobarbital once daily (8:00 am) and a dose of distilled water at 4:00 pm. This was to ensure the same amount of animal manipulation in each group. The control group (3 animals) received distilled water twice daily (8:00 am and 4:00 pm). The dosage regimen was continued for 6 days.
On day 7, methaqualone was administered (8:00 am) and blood samples collected as described above.

b) The following experiment is a repetition of the work of Conney et al. (1960), which indicated diphenhydramine was an enzyme inducer.

Eighty infant male Wistar rats weighing approximately 40 g (41 ± 6 g) were divided into six groups and dosed as follows. Group I (14 animals) received diphenhydramine (25 mg/kg) intraperitoneally twice a day (at 8:00 am and 4:00 pm). Group II (14 rats) received diphenhydramine (12.5 mg/kg) intraperitoneally twice a day (at 8:00 am and 4:00 pm). Group III (14 rats) was given phenobarbital (37.5 mg/kg) intraperitoneally twice a day (at 8:00 am and 4:00 pm). These three groups were treated for 4 days. Group IV (12 rats) was given only one intraperitoneal dose of 3,4-benzpyrene (25 mg/kg) 24 hours before the administration of zoxazolamine or hexobarbital.

There were two control groups in this study. Control group I (14 rats) was given an intraperitoneal dose of normal saline twice daily for 4 days. Control group II (12 rats) corresponding to the 3,4-benzpyrene-pretreated group was given one dose of corn oil (the solvent used for 3,4-benzpyrene) 24 hours before the administration of zoxazolamine or hexobarbital.

On day 5, half of the rats in each treatment group received an intraperitoneal dose of zoxazolamine (100 mg/kg) and the other half received hexobarbital (125 mg/kg). The duration of paralysis (zoxazolamine) or hypnosis (hexobarbital) was determined by measuring the time required for the animals to regain their rightening reflex, which was monitored by two persons.
1.4.2.3 Effect of Diphenhydramine Routes of Administration on Metabolism

To determine whether routes of administration (oral or intraperitoneal) affect the enzyme induction activity of diphenhydramine, the drug was administered by both routes.

Sixteen male albino rats (Wistar strain) weighing approximately 100 g (106 ± 4 g) were divided into four groups and treated as follows. Group I (4 animals) received an oral dose of diphenhydramine (25 mg/kg) three times a day (8:00 am, 12:00 noon and 4:00 pm). Group II (4 animals) was given diphenhydramine (25 mg/kg) intraperitoneally three times a day (8:00 am, 12:00 noon and 4:00 pm). Group III (4 animals) was treated with phenobarbital (60 mg/kg) orally once a day. A control group (4 animals) received distilled water orally three times a day (8:00 am, 12:00 noon and 4:00 pm). This dosage schedule was continued for 6 days.

On the morning of the 7th day (8:00 am), all rats received an oral dose of methaqualone (40 mg/kg). The rats were sacrificed by decapitation at 75 minutes after dosing. Blood samples were collected in heparinized beakers and transferred into heparinized culture tubes for storage. Samples were frozen at -10°C until analyzed.

1.4.2.4 Effect of Diphenhydramine Dosage on Metabolism

To examine the effect of drug dosage on the enzyme induction activity of diphenhydramine, two different dosages of diphenhydramine were administered by the different routes of administration.

Twenty male albino rats (Wistar strain) weighing approximately 100 g (102 ± 5 g) were divided into five groups. Group I (4 animals) was given diphenhydramine (25 mg/kg) orally three times a day (8:00 am., 4:00 pm and 12:00 midnight). Group II (4 animals) received intraperitoneal injections of diphenhydramine (25 mg/kg) three times a day (8:00 am.,
4:00 pm and 12:00 midnight). Group III (4 animals) was given diphenhydramine (50 mg/kg) orally three times a day (8:00 am, 4:00 pm and 12:00 midnight). Group IV (4 animals) was given diphenhydramine (50 mg/kg) intraperitoneally three times a day (8:00 am, 4:00 pm and 12:00 midnight). The control group (4 animals) was given distilled water three times a day. All rats were treated for 6 days. Methaqualone was administered and blood samples collected as described above in Section 1.4.2.1.

1.4.2.5 Enzyme Induction Activity of Diphenhydramine in Mature Animals

To examine the effect of age on the enzyme induction activity of diphenhydramine, the dosage regimens yielding enzyme induction in young animals (approximately 100 g) were administered to mature rats.

Sixteen adult male albino rats of the Wistar strain weighing approximately 250 g (256 ± 9 g) were divided into four groups. Group I was given diphenhydramine (50 mg/kg) orally three times a day at 8:00 am, 4:00 pm and 12:00 midnight. Group II received an intraperitoneal injection of diphenhydramine (25 mg/kg) three times a day (8:00 am, 4:00 pm and 12:00 midnight). Group III was given phenobarbital (60 mg/kg) orally once a day. The control group received oral doses of distilled water three times a day (8:00 am, 4:00 pm and 12:00 midnight). Each group consisted of four animals. All rats were treated for 6 days. Methaqualone was administered and blood samples collected as described in Section 1.4.2.1.

2. Analytical Procedure for Methaqualone Determination

2.1 Extraction from Blood
Procedure 1

Distilled water (200 μl) was added to aliquots of blood (200 μl) containing methaqualone and codeine (an internal standard). The diluted blood sample was basified with one drop of 10N NaOH and extracted twice with 1 ml of 1-chlorobutane using a Multi-purpose Rotator® (speed setting of 10 for 15 minutes). The mixture was centrifuged at 350 g for 10 minutes to separate the organic layer from the aqueous layer. The organic layer was transferred to a Reacti-Vial® (1 ml) and evaporated under a gentle flow of nitrogen in a Thermolyne® Dri-Bath at 85°C. The combined residues of the blood extract were taken up into methanol (20 μl). Aliquots of 2 μl were injected into the gas-liquid chromatograph (GLC) for analysis.

Procedure 2

The procedure was similar to procedure 1 with the exception that the extraction solvent was a mixture of dichloromethane (DCM) and ether (11:14) instead of 1-chlorobutane.

Neither procedure 1 nor 2 produced good and consistent recoveries of methaqualone. These procedures were therefore modified (see procedure 3).

Procedure 3

The blood sample (200 μl containing methaqualone and the internal standard) was diluted with saturated ammonium carbonate solution (400 μl). The diluted blood sample was then extracted twice with DCM : ether (11:14; 4 ml) using a Multi-purpose Rotator (speed setting of 10 for 15 minutes). The mixture was centrifuged at 350 g (10 minutes). The organic layers were pooled, transferred to culture tubes and evaporated to dryness at 55°C in a Thermolyne® Dri-Bath under a gentle flow of nitrogen. The residue was dissolved
in methanol (500 ml) by mixing with the solvent twice for 30 seconds (utilizing Vortex-Genie®). The solution was transferred to a Reacti-Vial® (1 ml) and the methanol evaporated to dryness under a gentle flow of nitrogen at 85°C. The residue was then taken up into 20 μl of methanol for injection into the GLC.

2.2 Gas-Liquid Chromatographic Analysis of Methaqualone

Methaqualone was analyzed using a Hewlett-Packard Model 5750B Gas Chromatograph. A stainless steel column (1.2 m x 3.1 mm o.d.) packed with 3% OV-101 on Chromosorb W (high performance, 80/100 mesh) was chosen for the analysis. The gas chromatographic operating conditions employed were: injection port and detector temperature, 300°C; column oven temperature, 190°C; flow rate of carrier gas (nitrogen), 50 ml/min. Hydrogen and compressed air flow rates were adjusted to give maximum response.

Several compounds (imipramine, chlorpromazine, procaine, chlordiazepoxide, and codeine) were tested as possible internal standards. The compounds (10 μg) were dissolved in a small amount of methanol. The methanol was evaporated. A blood sample containing methaqualone was added to the residue and the contents mixed for 30 seconds. Methaqualone and the internal standard were extracted as described in Procedure 3 of Section 2.1. Using the GLC conditions described above, each sample was chromatographed and evaluated. The internal standard was chosen for extractability and suitable chromatographic properties. Codeine was chosen as the most appropriate internal standard.

2.3 Construction of Calibration Curves

Calibration curves were prepared for the determination of methaqualone concentrations in blood. Varying amounts of methaqualone (0.2, 0.4, 0.8, 1.6, 3.2 or 6.4 μg) and codeine
phosphate (10 µg) were added as methanolic solutions to individual culture tubes. The solvent was evaporated to dryness. Drug-free blood (200 µl) was added to each tube to produce a methaqualone concentration of 1, 2, 4, 8, 16 or 32 µg/ml. The contents of each tube were mixed for 30 seconds, extracted and analyzed by GLC as previously described (Section 2.1). Calibration curves were constructed by plotting the peak height ratios (methaqualone to the internal standard, codeine) against known methaqualone concentrations. A triplicate determination at each concentration was performed to ensure reproducibility. Samples for the determination of calibration curves were extracted on the same day as the unknown blood samples were analyzed.

2.4 Extraction Efficiency

To investigate the extraction efficiency of methaqualone from blood, three sets of peak height ratios (methaqualone to codeine) were determined and compared.

Aliquots (200 µl) of drug-free blood were extracted as previously described in procedure 3 of section 2.1. Varying amounts of methaqualone (0.4 µg and 4 µg) and codeine (10 µg) were then added to the dried residue of the blood extracts. The samples were analyzed by GLC. The peak height ratios of methaqualone to codeine represent a 100% recovery of methaqualone at the blood concentrations of 2 µg/ml and 20 µg/ml. The reciprocals of the peak height ratio (i.e. codeine to methaqualone) represent a 100% recovery of codeine.

A second set of peak height ratios was produced by extracting aliquots of blood samples containing methaqualone alone (0.4 µg/ and 4 µg/200 µl of blood. Codeine (10 µg) was
added as an external standard to the dried residue of the blood extracts. The extraction efficiency of methaqualone was determined by comparing this set of peak height ratios (methaqualone to codeine) with the set of peak height ratios which represented a 100% methaqualone recovery.

A third set of peak height ratios (codeine to methaqualone) was determined by adding methaqualone (0.4 μg and 4 μg) as an external standard to the dried residue of the blood extracts which contained codeine alone (10 μg/200 μl of blood before extraction). By comparing this set of peak height ratios with that representing a 100% codeine recovery, the consistency of codeine extraction was confirmed.

3. Data Analysis

The data in the present investigation were analyzed statistically by one-way analysis of variance (AOV) and the Newman-Kauls test. A value of p less than 0.05 was considered statistically significant. Statistical calculations were performed using the Texas Instruments model 59 calculator and the Applied Statistics Solid State Software TM module.

The area under the blood concentration (methaqualone) versus time curve was estimated by the trapezoidal rule (equation 1):

\[ \int_{t_0}^{t_1} C_p(t) \, dt = \frac{C_0 + C_1}{2} (t_1 - t_0) \]  

Where \( C_0 \) and \( C_1 \) are the blood concentration at the time \( t_0 \) and \( t_1 \) respectively.
The slope and intercept of calibration curves were calculated by least-square linear regression using a Texas Instruments calculator model 55.
III. RESULTS AND DISCUSSION

1. Analytical Procedures

1.1 Gas-Liquid Chromatographic Analysis (GLC)

Of the many methods reported for the qualitative and quantitative determination of methaqualone and its metabolites in biological fluid, GLC was chosen for the present investigation. Due to its sensitivity and specificity, GLC is frequently used for quantitation of methaqualone (Christensen and Holfort, 1975; Bailey and Jatlow, 1973; Anweiler et al., 1976; Douglas and Shahinian, 1973; Frederichsen, 1978; Mitchard and Williams, 1972; Gravey and Jain, 1974; Chin and Fastlich, 1974; Horning et al., 1975; Mule et al., 1978; Hindmarsh et al., 1977; Wilson et al., 1978; Kazyak et al., 1979; Ericsson and Danielsson, 1977; Kazyak et al., 1977; Permisohn et al., 1976; Bonnichsen et al., 1972; Bonnichsen et al., 1974; Burnett et al., 1976; Peat and Finkle, 1980; Stillwell et al., 1975; Delong et al., 1976; Evenson and Lensmeyer, 1974; Aivan et al., 1973; Morris et al., 1972; Bonnichsen et al., 1975).

Other methods such as radioimmunoassay (RIA) and thin-layer chromatography (TLC) have been used extensively for qualitative screening programs (Rock and Moore, 1976; Budd et al., 1980; Jain et al., 1975; Keqpler et al., 1977; Allen et al., 1970; Berman et al., 1975; Kokaki et al., 1973; Helger et al., 1978; Burnett et al., 1969; Sleeman et al., 1975; Goudie and Burnett, 1971). RIA is extremely useful as a sensitive screening procedure. Concentrations as low as 2 μg/liter can be detected in urine by this method (Bost et al., 1976). However, because the RIA antibody is not specific and cross reacts with methaqualone metabolites, the procedure is not suitable for specific quantitation of the drug.
TLC can separate methaqualone and its major metabolites without interference from other drugs and endogenous compounds in biological fluids. The distinctive spot pattern produced by the methaqualone metabolites minimizes false positive results. The drawback of TLC is its lack of sensitivity. A comparison of GLC with RIA and TLC, for detecting methaqualone, showed that TLC produced 0.4% false positive results but 38.4% false negative results while RIA produced only 1.2% false positive results and 2.1% false negative results (Mule et al., 1978). Although quantitative procedures combining TLC and mass spectrometry have been used, TLC alone is not suitable for quantitative determinations of methaqualone and its metabolites (McReynolds et al., 1975).

Methaqualone and its metabolites have also been quantitated by spectrophotometric, colorimetric, spectrofluorometric and ultraviolet procedures (Douglas and Shahinian, 1973; Pirl et al., 1972; Stoll et al., 1978; Delong et al., 1976; Smyth et al., 1973). Due to high blank values and the similarity in the absorbance spectra of methaqualone and its metabolites, spectrophotometric and ultraviolet procedures lack specificity. Colorimetric and spectrofluorometric procedures require extensive sample purification. The procedures are therefore less accurate than GLC.

The comparison of methods for the quantitation of methaqualone shows that GLC is a preferred method for sensitive and accurate determinations. The GLC method developed by Hindmarsh et al. (1979) was employed for the quantitation of methaqualone. In the present study, using a relatively non-polar 3% OV-101 GLC column, temperatures of 300°C, 190°C and 300°C for the injection port, column oven, and detector respectively, a single sharp peak for methaqualone with a
retention time of 2.8 minutes was seen. The methaqualone peak was adequately separated from endogenous materials present in blood (Fig. 3).

The extraction efficiency and chromatographic properties of several other compounds were tested to determine their suitability as an internal standard for methaqualone quantitation. Chlordiazepoxide showed more than one chromatographic peak whether extracted or injected directly onto the GC column. Peaks for procaine and imipramine were not well resolved from that of methaqualone. Lowering the column temperature did not improve their resolution. Both chlorpromazine and codeine gave single sharp peaks which were well separated from the peaks of methaqualone and other endogenous material of the blood. The retention times of chlorpromazine and codeine were 8.5 and 5.5 minutes respectively. However, after repeated injections onto the GC column, the chlorpromazine peak broadened and the peak height varied. Although codeine also produced inconsistent peak heights after the first few injections, the inconsistency was corrected by loading the column, i.e. by injecting a large amount of codeine before any samples were analyzed. Codeine was found to be the most suitable internal standard for this study.

1.2 Extraction

To ensure accurate quantitative analysis of methaqualone, the drug had to be extracted efficiently from the biological fluid and separated from any endogenous components which could interfere with the detection and the subsequent quantitation. Many methods have been developed for the extraction of methaqualone from plasma
Figure 3: Gas Chromatogram of Control Blood (-----) and Blood Spiked with Methaqualone (I) (2 μg) and Codeine (II) (10 μg) (-----)
and serum (Morris et al., 1972; Alvan et al., 1973; Alvan et al.,
1974; Nyak et al., 1974; Delong et al., 1976; White et al., 1976).
Since previous studies on methaqualone-diphenhydramine interaction
used whole blood for analysis (Hindmarsh et al., 1979), whole blood
was also used in this study as a comparison of results
between the present study and previous studies would then be
possible.

A technique for the extraction of methaqualone from whole
blood was recently reported by Hindmarsh et al., (1979). Blood
samples were diluted with distilled water, basified with NaOH and
then extracted with 1-chlorobutane. The addition of distilled
water prior to basifying the blood prevented emulsion formation.
Dilution of proteins present in plasma or lysis of red blood cells
by the hypotonic solution may have contributed to the prevention
of emulsion formation (Ganong, 1971). Although methaqualone could
also be extracted from acidic medium, Cravey and Jain (1974) found
that alkaline extractions produced a 'cleaner' extract.
Hence alkaline extraction was used.

The method developed by Hindmarsh and associates (1979)
was employed initially. However, recovery of methaqualone was not
consistent. The inconsistency of methaqualone recovery might be
explained by the small volume of blood samples (200 μl) and solvent
used. Even a minor emulsion formed in the interface between the
aqueous and the organic layers could result in a large variation in the
recovery of the drug. To improve the recovery of methaqualone, other
solvents were tested. Although dichloromethane (DCM) has been used
for the extraction of methaqualone (Allen et al., 1970; Murata and
Yamamoto, 1970; Horning et al., 1974: Ericsson and Danielsson, 1977), DCM, with a density greater than 1, often became contaminated with aqueous residue during separation of the two layers. A mixture of ether:DCM (14:11) produced a density less than 1 and was satisfactory as an extraction solvent (Midha et al., 1973). To optimize conditions for extraction, a larger volume (4 ml) of the DCM:ether (11:14) mixture was added. An internal standard (codeine) was used to compensate for any irregularity in the recovery of methaqualone due to emulsion formation. To minimize the problem of emulsion formation, ammonium carbonate was used to basify the blood samples instead of sodium hydroxide. The emulsion formed using ammonium carbonate was easily broken by centrifugation. In addition, the ammonium salt also exerted a "salting out" effect to improve the extraction efficiency of methaqualone. To ensure a consistent and reproducible extraction, a measured amount of saturated solution of ammonium carbonate (300 µl) was added to each sample. All extractions were performed in culture tubes with Teflon® lined caps to prevent contamination by exogenous material from rubber liners.

The extraction efficiency of methaqualone from blood was determined at the concentrations of 2 µg/ml and 20 µg/ml. The mean recovery of methaqualone from blood was 81.08% ± 4.52 (Table I). To ensure the validity of the interpretation of the data, the consistency of codeine recovery was determined. The mean recovery of codeine from blood was 80.24% ± 3.34 (Table I).

1.3 Calibration curves

A plot of peak height ratios (methaqualone versus codeine) against methaqualone concentration was linear (R², coefficient of determination, was 0.998) over the range of 1 to
<table>
<thead>
<tr>
<th>Methaqualone Blood Concentration</th>
<th>Number of Determination</th>
<th>% Methaqualone Recovery (using codeine as external standard)</th>
<th>% Codeine Recovery (using methaqualone as external standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 µg/ml</td>
<td>3</td>
<td>77.56 ±3.49</td>
<td>80.01 ±4.95</td>
</tr>
<tr>
<td>20 µg/ml</td>
<td>3</td>
<td>84.68 ±1.02</td>
<td>80.45 ±1.85</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td></td>
<td>81.08 ±4.52</td>
<td>80.24 ±3.34</td>
</tr>
</tbody>
</table>
32 µg of methaqualone/ml blood (Fig. 4). Calibration curves were constructed on each day of analysis in order to circumvent the problem of day-to-day variation in the extraction and GLC analytical procedures.

2 Animal Studies

2.1 Animal Model

Wistar rats were chosen for the study, since this species has been reported to exhibit a higher activity of drug metabolizing enzymes than Buffalo and Sprague-Dawley rats (Kato et al., 1970). Male rats were selected because the liver microsomal fraction of the male rat is reported to oxidize a number of drug substrates including strychnine, pentobarbital, and morphine more efficiently than the corresponding liver fraction of the female rat (Mazel, 1972). In addition, the four day estrus cycle of the mature female rat affects enzymatic activity (Mazel, 1972).

2.2 The effect of Acute Administration of Diphenhydramine on the Metabolism of Methaqualone.

Concurrent use of diphenhydramine and methaqualone has been claimed to increase the sedative-hypnotic effect of the latter. In vitro (Hindmarsh et al., 1978) and in vivo (LeGatt et al., 1980) studies have been conducted to investigate the possible interaction between the two drugs and to determine the mechanism of interaction.

The present investigation of the acute effects of a diphenhydramine-methaqualone interaction was designed as a supplementary study to the in vivo studies conducted by Hindmarsh et al., (1979) and LeGatt et al (1980). In their studies, the two
Figure 4: Calibration Curve for Methaqualone Determination.
Each point represents the mean ± standard deviation of 3 samples.
drugs were given concurrently by the oral, intraperitoneal or intravenous routes of administration. The results suggested that inhibition of intestinal metabolism of the methaqualone by diphenhydramine contributed to an increase in methaqualone blood and brain levels after co-administration of the two drugs (Hindmarsh et al., 1979 LeGatt et al., 1980). To test the hypothesis that the site of interaction was in fact the gastrointestinal tract, the present study was designed to compare the effect of oral and intraperitoneal administration of diphenhydramine on an oral dose of methaqualone. If the site of interaction is the gastrointestinal tract, an intraperitoneal dose of diphenhydramine should have a minimal effect on blood levels of methaqualone.

Male albino Wistar rats (approximately 250 g) were divided into three groups. On group received methaqualone and diphenhydramine concomitantly by the oral route (designated as the oral-diphenhydramine group). A second group received an oral dose of methaqualone and an intraperitoneal dose of diphenhydramine (designated as the IP-diphenhydramine group). The third group, employed as the control group, received methaqualone alone orally. Blood was collected from the animals at specific time intervals, 30, 60, 120, 240 and 360 minutes after dosing. Five rats were used in each group for each group for each collection time.

The blood levels of methaqualone were determined by gas-liquid chromatography (GLC). The area under the blood level
curve (AUC) of methaqualone for each group (Fig. 5) was estimated by the trapezoidal rule. Since blood samples were not collected as sequential blood samples from each rat but as individual samples for each collection time, the AUC was calculated from the averaged blood level at each collection time for each group of rats. Therefore, only one AUC was obtained for each group and statistical comparison of AUC was not possible.

The AUC_{360} for the oral-diphenhydramine group, the IP-diphenhydramine group and the control group were 4510 µg min/ml, 3612 µg min/ml and 2887 µg/ min/ml respectively. In a previous in vivo study (LeGatt et al., 1980), the AUC_{360} of methaqualone in rats treated with a methaqualone-diphenhydramine combination orally and methaqualone alone orally were 4982 µg min/ml and 2895 µg min/ml respectively (calculated by the trapezoidal rule from the data of the work of LeGatt et al. (1980).

To gain a better statistical perspective on the difference in methaqualone blood levels among the three treatment groups, the blood levels of methaqualone at each collection time were compared by one way analysis of variance (AOV) and the Newman-Keuls test. Differences in the blood levels of methaqualone were seen among the three treatment groups at the first three collection times (Table II). At 30 minutes, methaqualone blood levels in the oral-diphenhydramine group (19.18±3.00 µg/ml) were significantly higher than in the IP-diphenhydramine group (13.04± 1.66 µg/ml).
Figure 5: Methaqualone Blood Levels (± S. D.) for Groups of Rats Receiving Methaqualone Plus Diphenhydramine Orally (●●●●); Methaqualone Orally Plus Diphenhydramine Intraperitoneally (■■); Methaqualone Alone Orally (▲▲▲▲)

Each point represents the mean ± standard deviation of 5 samples.
The control group (15.86±2.63 µg/ml), with a mean methaqualone blood level between the other two groups, was not statistically different from either group. At 60 minutes, all three groups were significantly different from each other. Methaqualone blood levels were highest in the oral-diphenhydramine group (24.02±2.66 µg/ml) and lowest in the control group (12.92±1.66 µg/ml). At 120 minutes, the oral-diphenhydramine group (16.10±1.42 µg/ml) and the IP-diphenhydramine group (14.16±1.61 µg/ml) had similar methaqualone blood levels and were both higher than the control (8.65±1.58 µg/ml). Mean blood levels in the three groups at 240 and 360 minutes were not significantly different.

Diphenhydramine whether given orally or intraperitoneally increased methaqualone blood levels at 60 and 120 minutes after dosing (Table II). The data lend support to the previous findings that co-administration of diphenhydramine and methaqualone increased the blood levels of the latter (Hindmarsh et al., 1979). In addition, methaqualone blood levels were higher in the rats treated with oral doses of diphenhydramine than those receiving intraperitoneal doses of the antihistamine at 30 and 60 minutes (Table II). In contrast to oral administration, diphenhydramine given intraperitoneally would bypass the gastrointestinal tract and would not inhibit any intestinal metabolism. This higher methaqualone blood levels seen in the oral-diphenhydramine group support the hypothesis that the site of interaction of the two drugs is in fact the gastrointestinal tract (LeGatt et al., 1980). However, methaqualone blood levels in the rats receiving diphenhydramine intraperitoneally were also significantly higher than in the control group at 60 and 120 minutes (Table II). Hence the involvement of mechanisms other than the inhibition of
TABLE II  Effect of Diphenhydramine Administration on Methaqualone Blood Levels at Various Times after Dosing

<table>
<thead>
<tr>
<th>Time after Dosing</th>
<th>Methaqualone Blood Levels (µg/ml)</th>
<th>Methaqualone Blood Levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment (oral)</td>
<td>Treatment (oral)</td>
</tr>
<tr>
<td></td>
<td>Methaqualone (oral)</td>
<td>Methaqualone (intraperitoneal)</td>
</tr>
<tr>
<td>30 min.</td>
<td>19.18 ± 3.00</td>
<td>13.04 ± 1.66</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>60 min.</td>
<td>24.02 ± 2.66</td>
<td>18.02 ± 1.81</td>
</tr>
<tr>
<td>120 min.</td>
<td>16.10 ± 1.42</td>
<td>14.16 ± 1.61</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>240 min.</td>
<td>10.06 ± 2.32</td>
<td>7.60 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>360 min.</td>
<td>3.29 ± 1.22</td>
<td>3.20 ± 1.19</td>
</tr>
</tbody>
</table>

1. Lines join the blood levels which are not significantly different (p>0.05, AOV and Newman-Keuls test)
intestinal methaqualone metabolism is suggested.

In addition to the different methaqualone blood levels at various time intervals after dosing, the time for achieving the maximum methaqualone blood level ($t_{\text{max}}$) was also different between the control group and the diphenhydramine-treated groups (Fig. 5). The $t_{\text{max}}$ was at 30 minutes after dosing in the control group and at 60 minutes in both oral-diphenhydramine and IP-diphenhydramine groups. Since individual rats were used for each collection time, the difference in $t_{\text{max}}$ might be due to inter-animal variation and the significance of the difference in $t_{\text{max}}$ could not be determined. A similar shift in $t_{\text{max}}$ was also observed in the previous diphenhydramine-methaqualone interaction studies by LeGatt et al.(1980).

Diphenhydramine interferes with the absorption of other drugs. A marked decrease in gastric emptying and an increase in intestinal transit time in rats receiving diphenhydramine either orally or intraperitoneally are documented (Feldman and Putcha, 1977). Diphenhydramine, when given intraperitoneally, decreases the absorption of p-aminosalicylate in rats and humans (Lavigne and Marchand, 1973). Orally administered diphenhydramine is also shown to decrease the maximum blood concentration ($C_{\text{max}}$) of acetaminophen and cause a delay of $t_{\text{max}}$ in three out of the six diphenhydramine-treated rabbits (Imamura et al., 1981). The interference in absorption of other drugs by diphenhydramine is probably due to the anticholinergic effect of the antihistamine.

The time required to achieve the maximum blood concentration is a function of absorption and elimination rate constant. The $t_{\text{max}}$ may increase because of a slowing of absorption and/or an increase in the elimination half-life (e.g. as with inhibition of metabolism). Competitive inhibition of methaqualone metabolism by diphenhydramine
has been demonstrated in vitro in rat liver homogenate (Hindmarsh et al., 1978), thus suggesting the elimination half-life might increase in vivo. As the half-lives of methaqualone among the different groups could not be compared in the present study*, whether the oral and the intraperitoneal routes of diphenhydramine administration inhibited the hepatic metabolism of methaqualone could not be determined. However in an in vivo study, LeGatt et al., (1980) could not detect a decrease in systemic clearance or an increase in half-life when methaqualone was administered intravenously in combination with diphenhydramine. The authors suggested that the inability of diphenhydramine to inhibit methaqualone metabolism was probably due to an insufficient hepatic level of diphenhydramine after the 5 mg/kg intravenous dose of the antihistamine. The theory was substantiated by other investigations in which relatively low levels of diphenhydramine were found in liver tissue when compared with brain, lung, heart, spleen and plasma of rats administered diphenhydramine intravenously (Wagner, 1975; Glazko et al., 1949). As with the intravenous administration, diphenhydramine given intraperitoneally at a dose of 5 mg/kg does not appear to achieve a sufficiently high concentration in the liver to produce a metabolic interaction. Blood level profiles of methaqualone after intraperitoneal administration of the drug alone or in combination with diphenhydramine were similar and the terminal phase of the profiles appeared to be parallel (LeGatt et al., 1980). Thus any difference of $t_{\text{max}}$ seen in the

*Because individual rats were used for each collection time, only one average value of half-life of methaqualone could be obtained for each group of animals, and the half-lives could not be compared statistically.
present investigation is likely due to a change in the absorption rate constant for methaqualone rather than a change in the elimination rate constant.

The dose of diphenhydramine will determine the concentration of the drug at the possible sites of interaction, whether the gastrointestinal tract, the liver (for the inhibition of methaqualone metabolism) and/or the CNS (for the inhibition of drug absorption by the centrally mediated anticholinergic effect). The interaction between diphenhydramine and methaqualone may be a dose-dependent process. In a human study (Hindmarsh et al., 1983), volunteers were given diphenhydramine (25 mg) and methaqualone (250 mg) concurrently. In a separate experiment, one subject also received a 50 mg dose of diphenhydramine. Most subjects did not show a change in the elimination half-life of methaqualone. In the aforementioned subject, the terminal half-life for methaqualone increased from 25 hours (methaqualone alone) to 38 hours (methaqualone plus 25 mg of diphenhydramine) and 66.4 hours (Methaqualone plus 50 mg of diphenhydramine), suggesting a dose-dependent inhibition of methaqualone metabolism by diphenhydramine. However, the corrected AUC of methaqualone (for changes in half-life) in the subject decreased with increasing dosages of diphenhydramine. These results suggested a dose dependent decrease in methaqualone absorption by diphenhydramine. Lavigne and Marchand (1973) also demonstrated this dose-dependent inhibition or p-aminosalicylate absorption by diphenhydramine in rats. A dose of 15 mg/kg or less of diphenhydramine injected intraperitoneally produced lower p-aminosalicylate blood levels in the test groups than in the control groups up to 60 minutes after dosing. At a dose of 10 mg/kg of diphenhydramine, the lowering of p-aminosalicylate blood levels could only be detected for 30 minutes, and at a dose of 2 mg/kg for only 10 minutes after the
administration of the drug.

The methaqualone-diphenhydramine interaction thus appears to be a complex one. The mechanisms of interaction may involve inhibition of methaqualone metabolism by diphenhydramine in the gastrointestinal tract and in the liver, and the slowing of methaqualone absorption due to a diphenhydramine-induced decrease in gastrointestinal motility. In addition, the interaction may be a concentration-dependent process. The outcome of the interaction, therefore, can be a net result of the different mechanisms of interaction which augment or antagonize each other. For example, both the inhibition of intestinal and hepatic metabolism of methaqualone by diphenhydramine could increase the blood levels of the hypnotic. On the other hand, the anticholinergic effect of diphenhydramine could slow the absorption of methaqualone. Slowed absorption could possibly decrease the maximum blood concentration ($C_{\text{max}}$).

The complex nature of the interaction may account for the varied and unpredictable findings of methaqualone-diphenhydramine interaction studies. The recent human study administering a combination of methaqualone (250 mg) and diphenhydramine (25 mg) reported no increase of methaqualone blood level except in one subject who also received a double dose of diphenhydramine (50 mg) (Hindmarsh et al., 1983). Animal studies utilizing a similar dosage ratio of the two drugs (40 mg/kg of methaqualone to 5 mg/kg of diphenhydramine) observed an increase in methaqualone blood levels (LeGatt et al., 1980). However, on a dose per body weight basis, the dosage of diphenhydramine used in animal studies was much higher than in the human study (5 mg/kg in rats versus an average of 0.4 mg/kg in man). The different dosage regimens may explain the discrepancy between the animal and human studies.

The present study lends support to the previous hypothesis that diphenhydramine inhibits the intestinal
metabolism of methaqualone. Other mechanisms of interaction are also suggested. Inhibition of hepatic metabolism of methaqualone by diphenhydramine is possible, but did not occur at the dosage employed in the present study. The increased methaqualone blood levels are mainly due to the inhibition of intestinal methaqualone metabolism by diphenhydramine. More studies are required for confirmation of the mechanisms of methaqualone-diphenhydramine interaction. Further investigations should utilize higher dosages of diphenhydramine and different routes of administration.

To minimize inter-animal variation blood profiles should be constructed by sequential blood sampling method (i.e. blood samples collected from the same animal for one blood profile). More pharmacokinetic information may help clarify the mechanisms of interaction between the two drugs and the significance of the interaction in clinical practice.

2.3 The Effect of Chronic Administration of Diphenhydramine on the Metabolism of Methaqualone

In contrast to its inhibitory effect on drug metabolism following acute administration, diphenhydramine has been reported to stimulate drug metabolism after chronic administration. The antihistamine has been reported to induce metabolism of zoxazolamine (Conney et al., 1960), pentobarbital, meprobamate (Kato et al., 1963) and its own metabolism (Burns et al., 1965). However, diphenhydramine is reported to have no effect on the plasma half-life of warfarin (Hunninghake and Azarnoff, 1968). This discrepancy may be partially explained by the dosage regimens of diphenhydramine. Hunninghake and Azarnoff (1968) administered 15 mg/kg of diphenhydramine while the other studies used a dosage
of 25 mg/kg. In an attempt to determine whether the stimulatory effect of chronic diphenhydramine administration is dose-dependent, three preliminary experiments using different dosages, routes of administration and age of animals were conducted.

2.3.1. Effect of Chronic Oral Administration of Diphenhydramine (at a once-daily dosage) on Methaqualone Blood Levels in Mature Rats (approximately 250 g)

The purpose of this experiment was to estimate the potency of diphenhydramine as an enzyme inducer. A potent inducer, phenobarbital, was chosen as the reference for comparison.

Male albino Wistar rats (approximately 250 g) were divided into groups for treatment. Because phenobarbital produces a maximal increase of enzyme activity in three or four days (Conney et al., 1960), a three-day treatment schedule either with phenobarbital or diphenhydramine was designed. One group of rats was given a six-day treatment with diphenhydramine to determine if the antihistamine might take a longer time to induce enzyme activity. A control group was given distilled water instead of drug during the pretreatment period. An oral dose of methaqualone was given to each rat at the end of the pretreatment period. Methaqualone blood levels (at 75 min) were then determined by GLC.

The mean blood levels of methaqualone for the reference group (phenobarbital-pretreated), the test groups (diphenhydramine-pretreated) of the three-day and six-day treatments and the control group (given distilled water) were 12.5 µg/ml, 18.5 µg/ml, 19.0 µg/ml and 19.5 µg/ml respectively (Table III). The reference group (phenobarbital pretreated) had a significantly lower methaqualone blood level when compared to the other groups. Methaqualone blood levels in the control group and
### TABLE III  Effect of Chronic Oral Administration of Phenobarbital and Diphenhydramine on the Blood Levels of Methaqualone in Mature Rats (approximately 250 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Blood Level (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>20.43 18.64</td>
</tr>
<tr>
<td>Phenobarbital&lt;sup&gt;2&lt;/sup&gt;</td>
<td>60 mg/kg</td>
<td>12.50 12.50</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg</td>
<td>17.02 20.03</td>
</tr>
<tr>
<td>Diphenhydramine (for 6 days)</td>
<td>25 mg/kg</td>
<td>18.05 20.04</td>
</tr>
</tbody>
</table>

1. All treatments were given once daily for 3 days. One group of rats was treated with diphenhydramine for 6 days. Methaqualone (40 mg/kg) was given orally 24 hours after the pretreatment.

2. Value is significantly different from the control (p<0.05).
in rats treated with diphenhydramine for the three and six days were not significantly different from one another.

2.3.2 Effect of Chronic Oral Administration of Diphenhydramine (at a twice-daily dosage) on the Methaqualone Blood Levels in Young Rats (approximately 100 g)

Young animals are often used to screen potential inducers since the inducing effect is more pronounced than in mature animals (Mathur et al., 1975). To duplicate the conditions commonly employed in chronic studies with diphenhydramine, the preliminary study was repeated using young rats (approximately 100 g) and diphenhydramine was administered at a dosage of 25 mg/kg twice daily.

The mean blood level of methaqualone in the reference group (Phenobarbital-pretreated) was significantly lower than the control group (Table IV). Although the daily dose of diphenhydramine was double in this second experiment, enzyme induction by diphenhydramine could not be demonstrated. These data did not agree with the previous findings that diphenhydramine at this dose (25 mg/kg twice a day) was an inducer (Conney et al., 1960; Kato et al., 1963).

2.3.3 Effect of Chronic Intraperitoneal Administration of Diphenhydramine (at a twice-daily dosage) on the Pharmacological Activities of Zoxazolamine and Hexobarbital in Young Rats (approximately 40 g)

Because diphenhydramine (25 mg/kg twice daily) did not induce enzyme activity (see 2.3.2), an experiment similar to Conney's study (1960) was undertaken. However, the strain of rats (Holtzman) used in their study was not available. Instead, rats of the Wistar strain were used in order to be consistent with the other experiments carried out in the present investigation.
TABLE IV  Effect of Chronic Oral Administration\(^1\) of Phenobarbital and Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g).

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Blood Levels (µg/ml)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>16.25 ± 0.74</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>60 mg/kg</td>
<td>11.37 ± 0.82(^3)</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg</td>
<td>17.79 ± 1.51</td>
</tr>
</tbody>
</table>

1. Diphenhydramine was given twice daily and phenobarbital once daily for 6 days. Methaqualone (40 mg/kg) was given orally on the 7th day.

2. Values are mean ± standard deviation for 3 to 6 animals.

3. Value is significantly different from the control (p < 0.05).
Young Wistar rats (approximately 40 g) were given the various drugs intraperitoneally twice a day. All dosages for the drugs (phenobarbital, 37.5 mg/kg, 3,4-benzpyrene 25 mg/kg and diphenhydramine 25 mg/kg) were the same as those administered in Conney's study except for an additional smaller dosage of diphenhydramine (12.5 mg/kg twice daily) introduced to examine the enzyme induction activity of diphenhydramine at a lower dosage.

The results of the present experiment and Conney's study are included in Table V. The effects of chronic administration of phenobarbital and 3,4-benzpyrene on pharmacological activities of zoxazolamine and hexobarbital were similar to those obtained by Conney and associates. Both phenobarbital and 3,4-benzpyrene reduced the duration of zoxazolamine paralysis significantly, but only phenobarbital induced the metabolism of hexobarbital. 3,4-Benzpyrene significantly increased the duration of hexobarbital hypnosis.

Diphenhydramine at the dosages of 25 mg/kg twice daily and 12.5 mg/kg twice daily did not affect the zoxazolamine paralysis time or the hexobarbital hypnosis time in the test group compared to the control group. Although the zoxazolamine paralysis time decreased from 121 minutes in the control group to 106 minutes and 107 minutes in the test groups, and the hexobarbital hypnosis time from 84 minutes to 75 minutes and 73 minutes respectively, the differences were not statistically significant.

In these preliminary experiments, phenobarbital was used as a reference inducer. Administration of this potent inducer provided comparative information. Rats treated with phenobarbital for 3 days and 6 days had mean methaqualone blood levels of 12.5 µg/ml and 11.4 µg/ml respectively (Tables III, IV). Thus maximum induction by phenobarbital occurred in three days.
Table V: Effect of Chronic Intraperitoneal Administration of Various Drugs on the Duration of Zoxazolamine Paralysis and Hexobarbital Hypnosis in Young Rats (approximately 40 g).

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Duration of Zoxazolamine Paralysis in Minutes</th>
<th>Duration of Hexobarbital Hypnosis in Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Present Study</td>
<td>Conney's Study (1960)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>121 ± 8</td>
<td>730 ± 251</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>37.5 mg/kg (Twice daily)</td>
<td>72 ± 13³</td>
<td>102 ± 47⁴</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg (Twice daily)</td>
<td>107 ± 23</td>
<td>307 ± 90⁴</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>12.5 mg/kg (Twice daily)</td>
<td>106 ± 18</td>
<td>-</td>
</tr>
<tr>
<td>Control for 3,4-benzpyrene</td>
<td></td>
<td>133 ± 8</td>
<td>-</td>
</tr>
<tr>
<td>3,4-benzpyrene</td>
<td>25 mg/kg</td>
<td>23 ± 5³</td>
<td>17 ± 7⁴</td>
</tr>
</tbody>
</table>

1. All drugs except 3, 4-benzpyrene were given twice daily for 4 days. On the 5th day, zoxazolamine (100 mg/kg) or hexobarbital (120 mg/kg) were given intraperitoneally. Only one dose of 3, 4-benzpyrene was given 24 hours before the administration of zoxazolamine or hexobarbital.

2. For the present study, values are mean ± standard deviation for 4 to 7 animals.

3. Value is significantly different from the control (p<0.05).

4. Value is significantly different from the control (p<0.05) in Conney's Study (1960).
The once-daily dosing of diphenhydramine administered to mature animals was a test of the potency of diphenhydramine as an inducer. Phenobarbital, a potent inducer, was able to induce drug metabolism under these conditions while diphenhydramine was not an inducer under the same experimental conditions. It has been documented that potent inducers stimulate drug metabolism in both old and young animals while weak inducers only affect young animals (Mathur et al., 1975). If diphenhydramine is an inducer, it is not as potent as phenobarbital.

The lack of enzyme induction by diphenhydramine administration twice daily to young rats receiving methaqualone, zoxazolamine or hexobarbital was difficult to interpret. These experiments were basically repetitions of the chronic studies done previously with diphenhydramine, (Kato et al., 1963; Burns et al., 1965; Conney et al., 1960), which showed that diphenhydramine at a dosage of 25 mg/kg twice daily was an inducer.

The only difference between the present experiment and Conney's work was the strain of rats used, Holtzman rats, (Conney et al., 1960) versus Wistar rats (present study). Species variations in enzyme induction activity have been reported (Alvares et al., 1970). The effect of strain variation on enzyme induction activity has also been documented (Furner et al., 1969). A comparison of data between the study of Conney and the present study seemed to suggest the two strains of rats are different in terms of their response to diphenhydramine (Table V). The duration of drug action in Wistar rats was much shorter than in Holtzman rats. The control group of Holtzman rats had a mean zoxazolamine paralysis time of 730 minutes while Wistar rats were paralyzed for only 121 minutes. Likewise, the duration of hexobarbital hypnosis in the Holtzman and Wistar rats were 216 minutes.
and 84 minutes respectively. However, the difference between the two strains of rats became less pronounced after enzyme activity was induced. After pretreatment with phenobarbital, the zoxazolamine paralysis time decreased from 730 minutes to 102 minutes in Holtzman rats and from 121 minutes to 72 minutes in Wistar rats. The duration of action of zoxazolamine and hexobarbital were sometimes shorter in Holtzman rats than in Wistar rats after enzyme induction. For example, after pretreatment with phenobarbital, the duration of hexobarbital hypnosis in Holtzman rats and Wistar rats were 11 minutes and 20 minutes respectively; and the duration of zoxazolamine paralysis was 17 minutes in Holtzman rats and 23 minutes in Wistar rats pretreated with 3, 4-benzpyrene. These data seem to suggest Holtzman rats metabolize zoxazolamine and hexobarbital more slowly and are more susceptible to enzyme induction.

The result of these experiments did not provide any conclusive evidence as to diphenhydramine's ability to induce liver enzymes. To determine if the dosage regimen influenced the induction activity of diphenhydramine, additional experiments were carried out. Since diphenhydramine was probably a weak inducer and the inductive effect was more pronounced in young animals, young male Wistar rats were used.

2.3.4 Effect of Chronic Oral and Intraperitoneal Administration of Diphenhydramine (at a dosage of 25 mg/kg three times daily) on the Blood Levels of Methaqualone in Young Rats (approximately 100 g).

Since rats treated with the twice-daily dosage regimen of diphenhydramine did not show any sign of increased enzyme activity, a three-time daily dosing regimen was initiated. This paralleled the recommended dosing regimen in clinical situations. For convenience, the diphenhydramine was administered at 8:00 a.m.
12:00 noon and 4:00 p.m. Diphenhydramine (25 mg/kg three times a day for 6 days) did not induce the metabolism of methaqualone (Table VI). The mean methaqualone blood level for rats treated with oral diphenhydramine (17.68 \( \mu g/ml \)) was not significantly different from the control (17.97 \( \mu g/ml \)). The group receiving intraperitoneal diphenhydramine showed slightly decreased, but not statistically different, methaqualone blood levels (15.85 \( \mu g/ml \)).

2.3.5 Effect of Chronic Oral and Intraperitoneal Administration of Diphenhydramine (at a dosage of 25 mg/kg or 50 mg/kg three times a day) on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)

Since the 16-hour overnight interval might have affected the induction activity of diphenhydramine (see discussion in Section 2.3.7), the three-times-daily dosing schedule was modified to give equal time intervals between each dose of diphenhydramine. Diphenhydramine was administered orally or intraperitoneally at 8:00 a.m., 4:00 p.m. and 12:00 midnight. A dosage of 25 mg/kg orally or intraperitoneally was compared with a dosage of 50 mg/kg orally or intraperitoneally 50 mg/kg.

The change in dosage interval significantly affected the activity of diphenhydramine as an inducer (Table VII). The intraperitoneal treatment of 25 mg/kg three times a day (at 8:00 a.m., 12:00 noon and 4:00 p.m.), which did not cause enzyme induction previously, produced significantly lower methaqualone blood levels (12.50 \( \mu g/ml \)) when the doses are administered at 8:00 a.m., 4:00 p.m. and 12:00 midnight. However, rats given diphenhydramine orally (25 mg/kg) still failed to induce drug metabolism with both dosage regimens. The rats had decreased methaqualone blood levels but these were not significantly different from the control (14.90 vs
TABLE VI  Effect of Different Routes of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Levels ($\mu g/ml$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>17.91 ± 1.81</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>60 mg/kg</td>
<td>11.23 ± 0.78&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diphenhydramine (orally)</td>
<td>25 mg/kg</td>
<td>17.68 ± 2.00</td>
</tr>
<tr>
<td>Diphenhydramine (intraperitoneally)</td>
<td>25 mg/kg</td>
<td>15.85 ± 0.61</td>
</tr>
</tbody>
</table>

1. Diphenhydramine was given either orally or intraperitoneally three times daily at 8:00 a.m., 12:00 noon and 4:00 p.m. for 6 days. Phenobarbital was given orally once daily for 6 days. On the 7th day, methaqualone (40 mg/kg) was given orally.

2. Values are mean ± standard deviation for 4 animals.

3. Value is significantly different from the control (p < 0.05).
TABLE VII  Effect of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>16.92 ± 2.26</td>
</tr>
<tr>
<td>Diphenhydramine (orally)</td>
<td>25 mg/kg</td>
<td>14.90 ± 1.82</td>
</tr>
<tr>
<td>Diphenhydramine (orally)</td>
<td>50 mg/kg</td>
<td>12.28 ± 1.04</td>
</tr>
<tr>
<td>Diphenhydramine (intraperitoneally)</td>
<td>25 mg/kg</td>
<td>12.50 ± 0.62</td>
</tr>
</tbody>
</table>

1. Diphenhydramine at a dosage of 25 mg/kg or 50 mg/kg was given either orally or intraperitoneally three times a day at 8:00 a.m., 4:00 p.m. and 12:00 midnight for 6 days. On the 7th day, methaqualone (40 mg/kg) was given orally.

2. Values are mean ± standard deviation of 4 animals.

3. Value is significantly different from the control (p < 0.05).
16.91 µg/ml). Rats treated with 50 mg/kg of diphenhydramine orally three times a day showed signs of induction. The mean blood level of methaqualone in this group, 12.28 µg/ml, was similar to the mean blood level in rats treated with phenobarbital (Table VI). At 50 mg/kg, diphenhydramine administered intraperitoneally caused the animals to become restless and irritable. Two animals died within 2 days of treatment. The intraperitoneal administration of diphenhydramine at a dose of 50 mg/kg three times daily was considered too high for chronic administration.

2.3.6 Effect of Chronic Administration of Diphenhydramine (at a dosage 25 mg/kg intraperitoneally or 50 mg/kg orally three times a day) on the Blood Levels of Methaqualone in Mature Rats (approximately 250 g).

The dosage which had induced metabolism in young animals did not increase enzyme activities in mature rats (Table VIII). The methaqualone blood levels in rats given diphenhydramine orally were similar to those of the control group. Although rats receiving diphenhydramine intraperitoneally had a slight decrease in methaqualone blood level, the difference was not significant. Thus the enzyme induction activity of diphenhydramine is different in young and mature animals.

2.3.7 Diphenhydramine as an Enzyme Inducer

Enzyme induction activity of diphenhydramine was determined by examining whether pretreatment with diphenhydramine resulted in a decrease in methaqualone blood level.

Enzyme induction occurred only when diphenhydramine was administered intraperitoneally in a dose of 25 mg/kg or orally in a dose of 50 mg/kg to young animals at equally spaced intervals over 24 hours (8:00 a.m., 4:00 p.m. and 12:00 midnight). The conditions
### TABLE VIII  Effect of Chronic Administration\(^1\) of Diphenhydramine on the Blood Levels of Methaqualone in Mature Rats (approximately 250 g).

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Levels ((\mu g/ml))(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>18.33 ± 1.84</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>60 mg/kg</td>
<td>12.18 ± 0.90(^3)</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>50 mg/kg</td>
<td>18.78 ± 0.93</td>
</tr>
<tr>
<td>(orally)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg</td>
<td>16.53 ± 1.19</td>
</tr>
<tr>
<td>(intraperitoneally)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Diphenhydramine was given either orally (50 mg/kg) or intraperitoneally (25 mg/kg) three times a day at 8:00 a.m., 4:00 p.m. and 12:00 midnight for 6 days. Phenobarbital was given orally once a day for 6 days. On the 7th day, methaqualone (40 mg/kg) was given orally.

2. Values are mean ± standard deviation for 4 animals.

3. Value is significantly different from the control (\(p < 0.05\)).
employed in the present study indicated that many factors affect the inductive activity of diphenhydramine. Dosage, routes of administration, age, of the animals and even the dosing intervals affected the outcome of the chronic studies.

The requirement of multiple dosing for enzyme induction by diphenhydramine may be due to a very short biological half-life of the drug. The estimated biological half-life of diphenhydramine in rats is 1 hour (Drach et al., 1974). Most inducers are lipid soluble at physiological pH and have a prolonged biological half-life (Remmer, 1969). Generally, lipid soluble compounds with short biological half-lives only induce drug metabolism on repeated administration. These observations suggest the importance of maintaining a high hepatic concentration of the inducing agent for activity. The short biological half-life of diphenhydramine may result in a low level of active drug in the liver. Consequently, multiple administration of a high dosage of the antihistamine is required to induce drug metabolism.

Because of the conditions required for diphenhydramine to induce enzyme activity, one may question whether or not the drug is a clinically significant enzyme inducer. To answer the question, the dosage required to produce enzyme induction and the usual clinical dosage are compared. Normal dosage of diphenhydramine in man is 25 - 50 mg three to four times a day. On a mg/kg body weight basis, the dosage is about 0.36 to 0.71 mg/kg in an average man weighting 70 kg. The dosages used in animal studies (25 - 50 mg/kg) were much higher than that used in humans. However, extrapolation of the animal study to human subjects must be interpreted carefully. The pharmacokinetics of diphenhydramine in rats and humans are significantly different. The estimated biological half-life of diphenhydramine in rats is 1 hour while it is about 6-7 hours in humans (Glazko et al., 1974).
Such a variation in half-life can create significant differences in response to the drug between rat and man. Although it is difficult to compare the dosage required to produce enzyme induction in man and animal, a comparison of the dosage used in rats to produce an antihistaminic response may be of interest. The antihistaminic dose of diphenhydramine in rats is 10 mg/kg (Bastian and Clements, 1961) while it is 50 mg for a man with an average weight of 70 kg (0.714 mg/kg). Considering the requirement of 50 mg/kg of diphenhydramine three times a day to stimulate drug metabolism, i.e. a dosage much higher than that required to produce antihistaminic response, it appears that the enzyme induction activity of the antihistamine is probably not significant in clinical situations.

Routes of administration also affect the activity of diphenhydramine as an enzyme inducer. In addition to oral doses, the intraperitoneal route of administration was also included in the present investigation. Although this route is rarely used in clinical practice, most of the previous studies on enzyme induction activity of diphenhydramine employed this route of administration. In both oral and intraperitoneal routes of administration, drugs are absorbed into the portal circulation and transported to the liver before circulating to other parts of the body. With the intraperitoneal route, drugs bypass the gastrointestinal tract. For drugs that are destroyed in the acidic environment of the stomach, largely metabolized in the gastrointestinal tract, or poorly absorbed, the fraction of active drug reaching the liver would be decreased.

The data obtained in the present study suggested the possibility of different drug levels (blood or tissue) achieved by the two routes of administration. The intraperitoneal route required a lower dosage of diphenhydramine (25 mg/kg) to exert its inductive
effect than the oral route (50 mg/kg) in young animals (Table VII). Furthermore, the intraperitoneal injections of diphenhydramine at 50 mg/kg appeared to cause acute toxicity in rats while administration of the same dosage by the oral route did not induce any immediate adverse affects but did induce enzyme activity.

Other findings also lend support to the above observations. The LD₅₀ of diphenhydramine in rats is 500 mg/kg (Sachs, 1948) when given orally and 82 mg/kg when given intraperitoneally (Loew, 1947). This 6-fold difference in LD₅₀ suggested that the fraction of the dose which reaches the liver intact by the oral route is substantially less than by intraperitoneal route, and thus affect the induction activity of the drug.

An important findings in this chronic study was the effect of dosage interval on the enzyme induction activity of diphenhydramine. Doses of 25 mg/kg of diphenhydramine either orally or intraperitoneally were given three times a day in two different schedules, 8:00 a.m., 12:00 noon and 4:00 p.m. (Schedule I and 8:00 a.m., 4:00 p.m. and 12:00 midnight (Schedule II). In Schedule I the first two dosing intervals were 4 hours and the overnight interval was 16 hours. Whether diphenhydramine was given by oral or intraperitoneal routes it did not significantly alter methaqualone blood levels when compared to the control (Table VI). In Schedule II, with equal dosage intervals (8 hours), intraperitoneally administered diphenhydramine did produce significantly lower methaqualone blood levels (Table VII). Orally administered diphenhydramine (25 mg/kg) failed to induce enzyme activity. However, oral administration of 50 mg/kg of diphenhydramine every 8 hours (Schedule II) gave significantly lower methaqualone blood levels when compared to the control. The major difference between the two dosage schedules was the large gap between the last
dose of the day and the first dose of the following day in Schedule I. Since diphenhydramine has such a short biological half-life in rats (1 hour), the 16 hour overnight interval might provide sufficient time for the rats to recover from any effect diphenhydramine exerted before the first dose of the next day was administered. At a dosage of 25 mg/kg, the orally administered diphenhydramine did not appear to produce sufficient hepatic levels. Consequently, the dosage interval did not have any effect on the enzyme induction activity of diphenhydramine. On the other hand, at a dosage of 25 mg/kg administered intraperitoneally, more diphenhydramine might be available to the liver than by the oral route of administration, and provide sufficient hepatic levels to induce drug metabolism. The dosage schedule therefore had an effect on the induction activity of intraperitoneally administered diphenhydramine.

As with most weak inducers, the induction activity of diphenhydramine was dependent on the age of animals tested. Diphenhydramine (at a dosage of 25 mg/kg intraperitoneally or 50 mg/kg orally every 8 hours) could induce metabolism in young rats but failed to do so in mature animals (Table VIII). The reasons for the different response to weak inducers in old and young animals are not known. For some types of inducers, it has been suggested that sex-hormones may play a role (Fisher and Spencer, 1974). Type II inducers (exemplified by 3,4-benzpyrene) stimulate the formation of a modified form of the terminal oxidase, cytochrome P\textsubscript{450}, presumably by binding to the Type I binding sites of the enzyme. The modified oxidase is designated as P\textsubscript{448}. Researchers have suggested that sex hormones stabilize the existing enzyme and prevent the conversion except
during or soon after the synthesis of the enzyme.

The mechanism by which diphenhydramine produces a differential response to the induction activity of the drug in different age groups of animals may not be the same as 3, 4-benzpyrene. With a chemical structure different from that of the polycyclic aromatic hydrocarbons, diphenhydramine is not likely a Type II inducer. Diphenhydramine is probably a Type I inducer as this group of inducers is comprised of compounds with varying chemical structures.

From the results of these experiments, diphenhydramine appears to be a weak inducer. Its effect is dependent on many parameters including dosage, dosage schedule, routes of administration, age of the animals and possibly strain (Furner et al., 1969) and species (Alvares et al., 1970) of animals. With so many factors contributing to the inductive activity of diphenhydramine, it is not surprising to find contradictory results in the different studies on this subject. Despite many animals studies reporting the enzyme induction activity of diphenhydramine, drug interactions involving this effect of the antihistamine are probably not clinically significant. As recommended by the American Pharmaceutical Association in their publication Evaluation of Drug Interactions (Ascione, 1976), no additional precaution other than other those normally employed are necessary when warfarin and diphenhydramine or other related antihistamines are used concurrently.

2.3.8 The Combined Effect of the Acute and Chronic Administration of Diphenhydramine on Methaqualone Blood Levels

Enzyme induction activity of diphenhydramine was determined by monitoring methaqualone blood levels in the control
groups and the diphenhydramine-pretreated groups. Methaqualone blood levels were determined from blood samples collected at 75 minutes after dosing. Lower blood levels in diphenhydramine-pretreated groups would indicate that enzyme induction had occurred. In contrast, in the acute study, methaqualone blood levels at 75 minutes were higher in rats treated with diphenhydramine-methaqualone in combination than in those treated with methaqualone alone (Fig. 5). Thus one may question whether the acute effect of diphenhydramine would interfere with the interpretation of the chronic study.

In the chronic study, methaqualone was given at least 8 hours after diphenhydramine pretreatment. Since diphenhydramine is readily absorbed in the gastrointestinal tract, the intestinal interaction between diphenhydramine and methaqualone seen in the acute study would be minimal. In addition, diphenhydramine has a very short biological half-life in rats (1 hour). When methaqualone was administered to the rats, the majority of the diphenhydramine dose was probably already metabolized or excreted.

In the present investigation, the acute inhibitory effect and the chronic inductive effect of diphenhydramine on drug disposition were studied independently in two phases. If methaqualone was given concurrently with the last dose of diphenhydramine at the end of the pretreatment period, a combined effect of acute and chronic administration of diphenhydramine might have complicated the interpretation of the chronic study. However such combined effect of diphenhydramine may be of interest and significance. More studies are necessary for detail investigations of the combined effect of diphenhydramine. For example, methaqualone blood profiles should be constructed after diphenhydramine pretreatment instead of a one-point-
determination (at 75 minutes after dosing). More pharmacokinetic information (such as AUC, the half-life of methaqualone) would help to determine the relative importance of the acute inhibitory effect of diphenhydramine after chronic administration of the antihistamine.
IV. SUMMARY AND CONCLUSIONS

1. Acute Administration of Diphenhydramine on Methaqualone Metabolism

To verify the hypothesis that diphenhydramine inhibits the intestinal metabolism of methaqualone, rats were divided into three groups and drugs administered as follows:

a. Concurrent oral administration of diphenhydramine (5 mg/kg) and methaqualone (40 mg/kg) (Oral-diphenhydramine group)

b. Concurrent administration of diphenhydramine (5 mg/kg) intraperitoneally and methaqualone (40 mg/kg) orally (IP-Diphenhydramine group).

c. Oral administration of methaqualone (40 mg/kg) alone (Control group)

The blood levels of methaqualone were analyzed using gas-liquid chromatography. The effects of oral and intraperitoneal administration of diphenhydramine on the blood levels of orally administered methaqualone were compared. Significant differences in methaqualone blood levels were noted between the:

i. Oral-diphenhydramine group and the control group at 60 minutes after dosing (24.02 μg/ml vs 12.92 μg/ml) and at 120 minutes (16.10 μg/ml vs 8.65 μg/ml).

ii. Oral-diphenhydramine group and IP-diphenhydramine group at 30 minutes (19.18 μg/ml vs 13.04 μg/ml) and at 60 minutes (24.02 μg/ml vs 18.02 μg/ml).

iii. IP-diphenhydramine group and the control group at 60 minutes (18.02 μg/ml vs 12.92 μg/ml) and 120 minutes (14.16 μg/ml vs 8.65 μg/ml).
The area under the curve for the oral-diphenhydramine group, the IP-diphenhydramine group and the control group were 4510 µg min/ml, 3612 µg min/ml and 2887 µg min/ml respectively. In addition, the time for achieving maximum methaqualone blood concentration ($t_{\text{max}}$) was at 30 minutes after dosing in the control group and 60 minutes in diphenhydramine-treated group (both oral and intraperitoneal routes administration).

Diphenhydramine, whether given orally or intraperitoneally, caused changes in blood levels and $t_{\text{max}}$ of orally administered methaqualone. Diphenhydramine given orally had a greater effect on methaqualone blood levels than diphenhydramine given intraperitoneally. The data suggests that more than one mechanism is involved---inhibition of intestinal metabolism and centrally mediated inhibition of absorption.

Diphenhydramine has been reported to interfere with the absorption of other drugs by its centrally mediated anticholinergic properties (Lavigne and Marchand, 1973; Imamura et al., 1981). A slowing of methaqualone absorption by diphenhydramine may also be responsible in the changes in methaqualone blood levels and $t_{\text{max}}$.

A delay in $t_{\text{max}}$ can also be a result of a decrease in elimination rate. Inhibition of methaqualone metabolism by diphenhydramine in vitro in liver homogenate has been shown (Hindmarsh et al., 1978). Although inhibition of hepatic methaqualone metabolism by diphenhydramine has not been demonstrated in vivo animal studies (LeGatt et al., 1980), a dose-dependent inhibition of methaqualone metabolism in liver is suggested in a human study (Hindmarsh et al., 1983). However, there is no indication of changes in methaqualone
biological half-life in the present study and the previous animal study (LeGatt et al., 1980) employing the same dosages (5 mg/kg). The administration of 5 mg/kg of diphenhydramine may not provide sufficient drug levels in the liver for inhibition.

The diphenhydramine-methaqualone interaction, thus, appears to be a complex process.

2. Chronic Administration of Diphenhydramine on Methaqualone Metabolism

Because diphenhydramine has been reported to be an enzyme inducer (Kato et al., 1964; Conney et al., 1960) when administered chronically induction activity of diphenhydramine was examined in rats utilizing different dosages, routes of administration, dosage schedules and ages of the animals. The following experiments were conducted:

i. Rats weighing 250 g were given diphenhydramine 25 mg/kg orally once a day.

ii. Rats weighing 100 g were given diphenhydramine 25 mg/kg orally twice a day.

iii. Rats weighing 40 g were given diphenhydramine 25 mg/kg intraperitoneally twice a day.

iv. Rats weighing 100 g were given diphenhydramine 25 mg/kg either orally or intraperitoneally three times a day at 8:00 a.m., 12:00 noon and 4:00 p.m.

v. Rats weighing 100 g were given diphenhydramine 25 mg/kg either orally or intraperitoneally, or 50 mg/kg orally three times a day at 8:00 a.m., 4:00 p.m. and 12:00 midnight.

vi. Rats weighing 250 g were given diphenhydramine either 50 mg/kg orally or 25 mg/kg intraperitoneally three times a day at
8:00 a.m., 4:00 p.m. and 12:00 midnight.

Rats were pretreated with diphenhydramine using the dosage regimens mentioned above. After diphenhydramine pretreatment, rats were then given either methaqualone, zoxazolamine or hexobarbital. Enzyme induction was determined by monitoring methaqualone blood levels, or by measuring zoxazolamine paralysis time and hexobarbital hypnosis time.

Enzyme induction occurred when diphenhydramine was given at a dosage of 50 mg/kg orally or 25 mg/kg intraperitoneally every eight hours to young animals (weighing 100 g). The results suggest diphenhydramine is a weak inducer. The enzyme induction activity of the antihistamine was influenced by the dosing regimens. Maintaining a sufficiently high drug level in the liver appears to be an important requirement for enzyme induction. Due to the very short biological half-life (1 hour) of diphenhydramine in rats, more than one dose of the drug per day was required to induce enzyme activity. A smaller dosage of the drug was required for induction by the intraperitoneal route (than orally). An equally spaced dosage interval was necessary before any induction was seen. An interruption in the interval probably provided sufficient time for the rats to recover from any slight inductive effect.

The data of this present study suggested that diphenhydramine was a weak inducer. However, the induction effect of the antihistamine may not be clinically significant.
V. REFERENCES


Fairgold, C L and Berry, C A, Neuropharmacol, 11 : 491 (1972).


Korein, J, Fish, B and Shapiro, T, Arch Gen Psychiat, 24 : 36 (1971)


Lavigne, J and Marchand, C, Clin Pharmacol. and Ther, 14 : 404 (1972)


Shimazu, T, Gann, 56 : 143 (1965).


VI. APPENDICES

APPENDIX I

Materials and Supplies

Ammonium Carbonate (British Drug House (Canada) Ltd., Toronto, Ontario).

3, 4-Benzpyrene (Aldrich Chemical Co., Milwaukee, Wisconsin).

Chlorpromazine (Smith, Kline and French Co., Philadelphia).

Codeine phosphate powder (Lymans Ltd., Montreal, Quebec).

Diphenhydramine hydrochloride powder (Parke Davis and Co., Brockville, Ontario).


Hexobarbital sodium (British Drug House (Canada) Ltd. Toronto).

Imipramine (Geigy Pharmaceuticals - Division of Ciba-Geigy Canada Ltd., Dorval, Quebec).

Methaqualone (base) powder (Roussel Canada Ltd., Montreal, Quebec).

Methaqualone hydrochloride powder (Boots Pure Drug Co., Nottingham, England and Charles E. Frosst and Co., Montreal, Quebec).

Needles (0.50 x 16 mm) (Sherwood Medical Industries Inc. Deland, Florida, U.S.A.).


Pasteur pipettes, 229 mm. disposable (Scientific Products - Division of American Hospital Supply Corporation, McGaw Park, Illinois).

Phenobarbital sodium (British Drug Houses (Canada) Ltd. Toronto).

Procaine (Allen and Hanburys, Toronto, Canada).

Rat Metabolism units with urine-faces separators (Hoeltge Inc. Cincinnati, Ohio, U.S.A.).

Reacti-Vials, 1 ml (Pierce Chemical Co., Rockford, Illinois).


Solvents

1 - Chlorobutane (J. T. Baker Chemical Co., Phillipsberg, New Jersey) distilled prior to use.

Corn oil (Mazola, Canada Starch Co. Inc. Toronto).

Dichloromethane (distilled in glass), (Caledon Laboratories, Georgetown, Ontario).

Ethyl Ether (anhydrous, reagent grade) (Caledon Laboratories, Georgetown, Ontario), distilled prior to use.

Hydrochloric acid, reagent grade (Fisher Scientific Co. Ltd., Fair Lawn, New Jersey).

Methanol (distilled in glass) (BDH Chemicals, Toronto, Ontario).

APPENDIX II

Apparatus:


Hewlett Packard Model 5750B Research Gas Chromatograph equipped with a hydrogen flame ionization detector (FID) (Hewlett Packard Co., Avondale, Pennsylvania). The Chromatographic columns were 1.2 m x 3.1 mm o.d. stainless steel, packed with 3% OV - 101 on 80/100 mesh, high performance Chromosorb W (Chromatographic Specialties, Brockville, Ontario). Chromatograms were recorded utilizing a Hewlett-packard Model 7127A Strip Chart recorder.

LEC/B-60 Ultra Centrifuge (Damon/IEC Division, Needham Heights, Massachusetts).


Multi-purpose Rotator Model 151(Scientific Industries, Inc., Bohemia, N.Y.).

Thermolyne Dri-Bath (Thermolyne Corporation, Dubuque, Iowa).

Vortex-Genie (Fisher Scientific Co. Ltd., Montreal, Quebec).
APPENDIX III

Raw Data

Raw Data for Table I

E - external
I - internal
MTQ - methaqualone

I. Blank blood (200 μl)

<table>
<thead>
<tr>
<th>Peak Height Ratio</th>
<th>MTQ (E)</th>
<th>Codeine (E)</th>
<th>MTQ (E)</th>
<th>Codeine (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 μg (2 μg/ml)</td>
<td>0.22</td>
<td>10 μg</td>
<td>4 μg (20 μg/ml)</td>
<td>10 μg</td>
</tr>
<tr>
<td>(a)</td>
<td>0.22</td>
<td>(a)</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>0.22</td>
<td>(b)</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>0.25</td>
<td>(c)</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.23</td>
<td>Average</td>
<td>1.24</td>
<td></td>
</tr>
</tbody>
</table>

II. Blood (200 μl) + Codeine (E) (10 μg)

<table>
<thead>
<tr>
<th>Peak Height Ratio</th>
<th>MTQ (I)</th>
<th>2 μg/ml</th>
<th>MTQ (I)</th>
<th>20 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td></td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td>0.19</td>
<td>(a)</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>(b)</td>
<td>0.17</td>
<td>(b)</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>0.18</td>
<td>(c)</td>
<td>1.05</td>
</tr>
</tbody>
</table>
## I. Blank blood (200 μl)

<table>
<thead>
<tr>
<th>Peak Height Ratio Codeine/MTQ</th>
<th>(1) MTQ (E) 0.4 μg</th>
<th>Codeine (E) 10 μg</th>
<th>(2) MTQ (E) 4 μg</th>
<th>Codeine (E) 10 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>4.54</td>
<td>(a)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>4.54</td>
<td>(b)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>4.07</td>
<td>(c)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>4.37</td>
<td>Average</td>
<td>0.81</td>
<td></td>
</tr>
</tbody>
</table>

## II. Blood (200 μl) + Codeine (I) (10 μg)

<table>
<thead>
<tr>
<th>Peak Height Ratio Codeine/MTQ</th>
<th>(1) MTQ (E) 0.4 μg</th>
<th>2 μg/ml</th>
<th>(2) MTQ (E) 4 μg</th>
<th>20 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>3.45</td>
<td>(a)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>3.75</td>
<td>(b)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>3.33</td>
<td>(c)</td>
<td>0.64</td>
<td></td>
</tr>
</tbody>
</table>
Raw Data for Table II


<table>
<thead>
<tr>
<th>Time after Dosing</th>
<th>Methaqualone Blood Level (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methaqualone (Oral) +</td>
</tr>
<tr>
<td></td>
<td>Diphenhydramine (Oral)</td>
</tr>
<tr>
<td>30 min.</td>
<td>16.0, 17.6, 16.8, 23.5, 22.0</td>
</tr>
<tr>
<td>60 min.</td>
<td>24.5, 22.8, 19.6, 26.0, 27.2</td>
</tr>
<tr>
<td>120 min.</td>
<td>14.0, 18.4, 15.6, 16.0, 16.5</td>
</tr>
<tr>
<td>240 min.</td>
<td>7.6, 9.6, 7.6, 13.0, 12.5</td>
</tr>
<tr>
<td>360 min.</td>
<td>2.0, 3.4, 1.8, 4.5, 4.8</td>
</tr>
</tbody>
</table>
Raw Data for Table IV

Effect of Chronic Oral Administration of Phenobarbital and Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Blood Levels (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>15.25, 17.0, 16.5</td>
</tr>
<tr>
<td>Phenobarbital 60 mg/kg</td>
<td></td>
<td>10.6, 11.0, 12.5</td>
</tr>
<tr>
<td>Diphenhydramine 25 mg/kg</td>
<td></td>
<td>16.5, 15.25, 17.75, 19.25, 18.5, 19.5</td>
</tr>
</tbody>
</table>
Raw Data for Table V

Effect of Chronic Intraperitoneal Administration of Various Drugs on the Duration of Zoxazolamine Paralysis and Hexobarbital Hypnosis in Young Rats (approximately 40 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Duration of Zoxazolamine Paralysis (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>125, 106, 120, 123, 131</td>
</tr>
<tr>
<td>Phenobarbital 37.5 mg/kg (Twice daily)</td>
<td></td>
<td>61, 60, 65, 91, 84</td>
</tr>
<tr>
<td>Diphenhydramine 25 mg/kg (Twice daily)</td>
<td></td>
<td>102, 164, 96, 92, 88, 119, 96</td>
</tr>
<tr>
<td>Diphenhydramine 12.5 mg/kg (Twice daily)</td>
<td></td>
<td>88, 110, 107, 105, 87, 140</td>
</tr>
<tr>
<td>Control for 128, 142, 126, 125, 143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, 4-benzpyrene 25 mg/kg</td>
<td></td>
<td>31, 19, 22, 20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Duration of Hexobarbital Hypnosis (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>90, 73, 76, 86, 77, 94, 93</td>
</tr>
<tr>
<td>Phenobarbital 37.5 mg/kg (Twice daily)</td>
<td></td>
<td>22, 21, 20, 19</td>
</tr>
<tr>
<td>Diphenhydramine 25 mg/kg (Twice daily)</td>
<td></td>
<td>75, 87, 66, 65</td>
</tr>
<tr>
<td>Diphenhydramine 12.5 mg/kg (Twice daily)</td>
<td></td>
<td>84, 66, 80, 89, 58</td>
</tr>
<tr>
<td>Control for 61, 64, 123, 70, 83, 60, 62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, 4-benzpyrene 25 mg/kg</td>
<td></td>
<td>101, 126, 86, 122, 116</td>
</tr>
</tbody>
</table>
### Raw Data for Table VI

Effect of Different Routes of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>16.4, 17.0, 20.5</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>60 mg/kg</td>
<td>10.3, 11.2, 12.2</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg</td>
<td>15.0, 20.4, 18.5, 16.8</td>
</tr>
<tr>
<td>(Orally)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg</td>
<td>15.0, 16.6, 15.6, 16.2</td>
</tr>
<tr>
<td>(Intraperitoneally)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Raw Data for Table VII

Effect of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Young Rats (approximately 100 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>dose</th>
<th>Methaqualone Levels (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>19.0, 17.25, 13.15, 18.25,</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg</td>
<td>15.6, 17.5, 13.75, 12.75,</td>
</tr>
<tr>
<td>(Orally)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>50 mg/kg</td>
<td>12.25, 10.6, 13.0, 13.25,</td>
</tr>
<tr>
<td>(Orally)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>25 mg/kg</td>
<td>12.5, 12.0, 13.5, 12.0</td>
</tr>
<tr>
<td>(Intraperitoneally)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Raw Data for Table VIII

Effect of Chronic Administration of Diphenhydramine on the Blood Levels of Methaqualone in Mature Rats (approximately 250 g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Methaqualone Blood Levels (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>17.8, 15.6, 19.4, 20.5</td>
</tr>
<tr>
<td>Phenobarbital 60 mg/kg</td>
<td></td>
<td>20, 18.7, 17.4, 19.0</td>
</tr>
<tr>
<td>Diphenhydramine 50 mg/kg (Orally)</td>
<td></td>
<td>15.5, 16.7, 15.5, 18.4</td>
</tr>
<tr>
<td>Diphenhydramine 25 mg/kg (Intraperitoneally)</td>
<td></td>
<td>12.5, 11.2, 13.5, 11.5</td>
</tr>
</tbody>
</table>