INTERACTIONS OF FORMAMIDINES WITH THE PLATELET SEROTONIN UPTAKE SYSTEM

A Dissertation

Presented to

the Faculty of the Graduate School

University of Missouri - Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Terry L. Johnson

Dr. Charles O. Knowles Dissertation Supervisor

December 1981

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. Charles O. Knowles for his invaluable guidance, suggestions, and insight throughout the course of this study and the preparation of this manuscript.

Thanks are also extended to Dr. G. Michael Chippendale of the Department of Entomology and to Dr. Gary D. Osweiler of the Department of Veterinary Anatomy-Physiology for reading this thesis, to Dr. Keith H. Byington of the Department of Pharmacology for his interest and suggestions during this study, and to Dr. Jerry A. White and Dr. Merton F. Brown of the Electron Microscope Facility for the scanning electron microscopy work included in this thesis.

A personal feeling of gratitude goes to the author's wife, Maggie, for her encouragement and understanding throughout this endeavor.

N18238

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

An important aspect of toxicology is the determination of the actions of pesticidal compounds. In addition to providing information on the mechanisms of toxicity such studies also provide knowledge of the physiology and biochemistry of affected target and non-target species, and can lead to the future development of effective and selective pesticides. Some types of pesticides, such as organophosphate insecticides, have a well defined mode of action. However, information about the actions of members of other classes of compounds remains obscure. The formamidines are one such group.

The forerunner of the formamidines is chlordimeform or N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine. Chlordimeform, which is sold under the brands Galecron® and Fundal®, is active against insects and acarines and is especially toxic to those that are resistant to organophosphates. Chlordimeform and other formamidines are known to interact with physiologic processes involving biogenic amines in both arthropods and mammals. Since biogenic amines include important neurotransmitters and neurohormones, interference with their formation, degradation, or function could have serious

physiological consequences. For example, an essential regulatory function at aminergic synapses and receptor sites is the uptake of neuroactive biogenic amines. Interference with amine uptake at these sites could result in abnormal physiological and behavioral actions.

Rat blood platelets provide an easily accessible model for the study of amine uptake mechanisms. Thus, it was used to investigate the influence of chlordimeform and other formamidines on the uptake of serotonin or 5-hydroxytryptamine (5-HT). The specific objectives of this investigation were:

1. To determine whether chlordimeform inhibited the uptake of 5-HT by rat blood platelets,
2. To determine the relationship between formamidine structure and 5-HT uptake inhibitory potency,
3. To study the mechanism by which formamidines inhibit 5-HT uptake using chlordimeform as a model compound, and
4. To determine the effect of formamidines on the release of 5-HT from platelets.

CHAPTER II

REVIEW OF LITERATURE

Formamidines are one of the more recently developed classes of agricultural chemicals. They possess a broad spectrum of activity which includes bactericidal, nematocidal, herbicidal, fungicidal, insecticidal, and acaricidal effects (Benezet et al., 1978). The formamidines that show activity as insecticides and acaricides are generally substituted aryl formamidines. Of this group of compounds the most widely used for insect and acarine control is chlordimeform (Figure 1). Extensive study of this compound and its formamidine metabolites, N'-(4-chloro-o-tolyl)-N-methylformamidine (demethylchlordimeform) and N'-(4-chloro-o-tolyl)formamidine (didemethylchlordimeform), has provided the majority of information about the mode of action of formamidine insecticides and acaricides. This section will consist of a review of the toxicology of chlordimeform and its formamidine metabolites since formamidines as a class have been thoroughly reviewed (Hollingworth, 1976; Knowles, 1976; Knowles, 1982; Matsumura and Beeman, 1976).

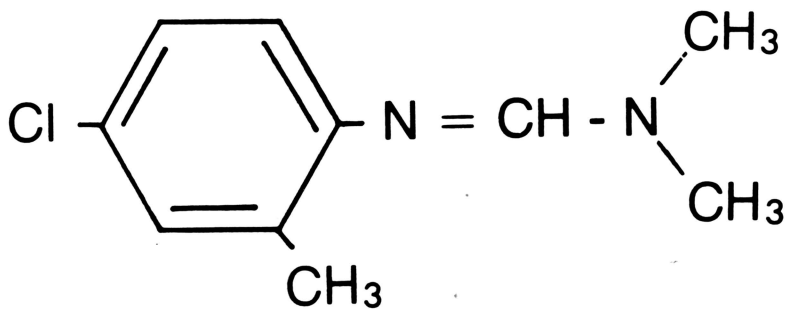


Figure 1. Structural formula of chlordimeform.

STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF CHLORDIMEFORM

Chlordimeform or N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine (molecular weight, 196.7) has a melting point of 35°C and a boiling point of 163 to 164°C at 14.0 mm of mercury (Anonymous, 1970). It exists as the E-form or trans configuration in both base and salt forms (Knowles, 1976). Chlordimeform is a base of medium strength with a pK_a of 7.2 and forms salt crystals in strong acids (Knowles, 1976). At 20°C its vapor pressure is 3.5×10^{-4} mm of mercury, and its volatility is 4.0 mg/m^3 of air. Solubility of the base is greater than 20% in most organic solvents, and it is scarcely soluble in water. Hydrolysis is slow in acid medium. Chlordimeform is hydrolyzed to 4'-chloro-o-formotoluidide and then to 4-chloro-o-toluidine (Anonymous, 1970). As the hydrochloride salt, chlordimeform is more than 50% soluble in water and greater than 30% soluble in methanol. Hydrolytic decomposition of this compound increases with pH above pH 5.0 (Anonymous, 1970).

METABOLISM OF CHLORDIMEFORM

The metabolic fate of radiolabled chlordimeform in insects, acarines, microorganisms, plants, and mammals was recently reviewed by Knowles (1976). A summary is given in Figure 2. The metabolism of chlordimeform to its monodeme-

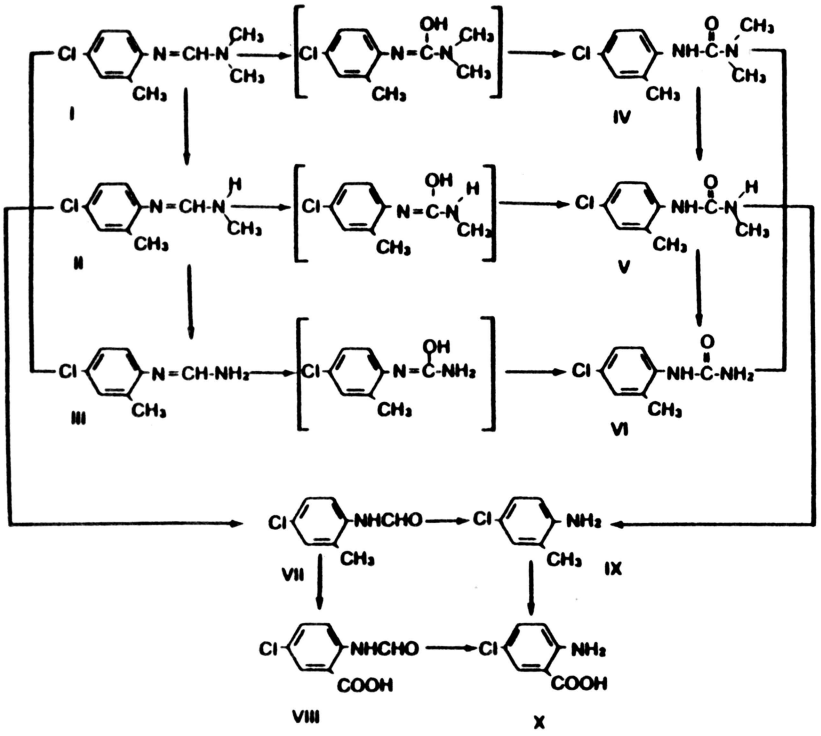


Figure 2. Proposed metabolic paths for chlordimeform (I) metabolism in rats and mice. Reprinted with permission from C.O. Knowles and H.J. Benezet, Journal of Agricultural and Food Chemistry 25, 1022-1026 (1977). Copyright 1977 American Chemical Society.

thylated (II) and didemethylated (III) formamidine metabolites and their subsequent breakdown to 4'-chloro-o-formotoluidide (VII) and 4-chloro-o-toluidine (IX) were qualitatively similar in most organisms tested (Knowles, 1976). The following additional metabolites were detected in some cases: N-formyl-5-chloroanthranilic acid (VIII), 5-chloroanthranilic acid (X), 1,1-dimethyl-3-(4-chloro-o-tolyl)urea (IV), 1-methyl-3-(4-chloro-o-tolyl)urea (V), 3-(4-chloro-o-tolyl)urea (VI) and N-(2-methyl-4-chloro-phenyl)-D-glucosylamine (Knowles, 1976; Knowles and Benezet, 1977; Sen Gupta and Knowles, 1969).

TOXICITY OF CHLORDIMEFORM - DIRECT LETHALITY

Direct lethality is defined herein as death attributed to a causal agent and occurring in a relatively short time span. Chlordimeform, and formamidines in general, are directly lethal to all life stages of mites and ticks, to the eggs and early instars of some Lepidoptera, and to some Homoptera (Knowles, 1976; Knowles, 1982).

In mammals, chlordimeform had an acute oral LD_{50} value of 250 mg/kg (base) and 335 mg/kg (salt) for rats and 625 mg/kg (base) for rabbits (Knowles, 1976). Demethylchlordimeform had an LD_{50} around 125 mg/kg following subcutaneous injection of rats, and didemethylchlordimeform gave a LD_{50}

of about 30 mg/kg subcutaneously injected into rats (Benezet et al., 1978).

Relation of formamidine structure to toxicity

The effect of structure on the toxicity of chlordimeform and other formamidines was examined in acarines, insects, and mammals. This subject was thoroughly reviewed recently by Knowles (1982). It was apparent from these studies that certain substituents on the formamidine moiety increased biological activity. For example, Chang and Knowles (1977) studied the toxicity of one-hundred and four aryl formamidines to twospotted spider mites, Tetranychus urticae Koch, and Knowles and Roulston (1973) studied the toxicity of many of the same compounds to southern cattle ticks, Boophilus microplus (Canestrini). They concluded that the moiety shown in Figure 3 is essential for direct lethality; R being halogen or methyl and R^1 being hydrogen or lower alkyl. In insects, demethylchlordimeform, like chlordimeform, was toxic to eggs and early instars of the Asiatic rice stem borer, Chilo suppressalis (Walker) (Dittrich and Loncarevic, 1971). Similarly, in rats, demethylchlordimeform and didemethylchlordimeform are more toxic than the parent compound (Benezet et al., 1978).

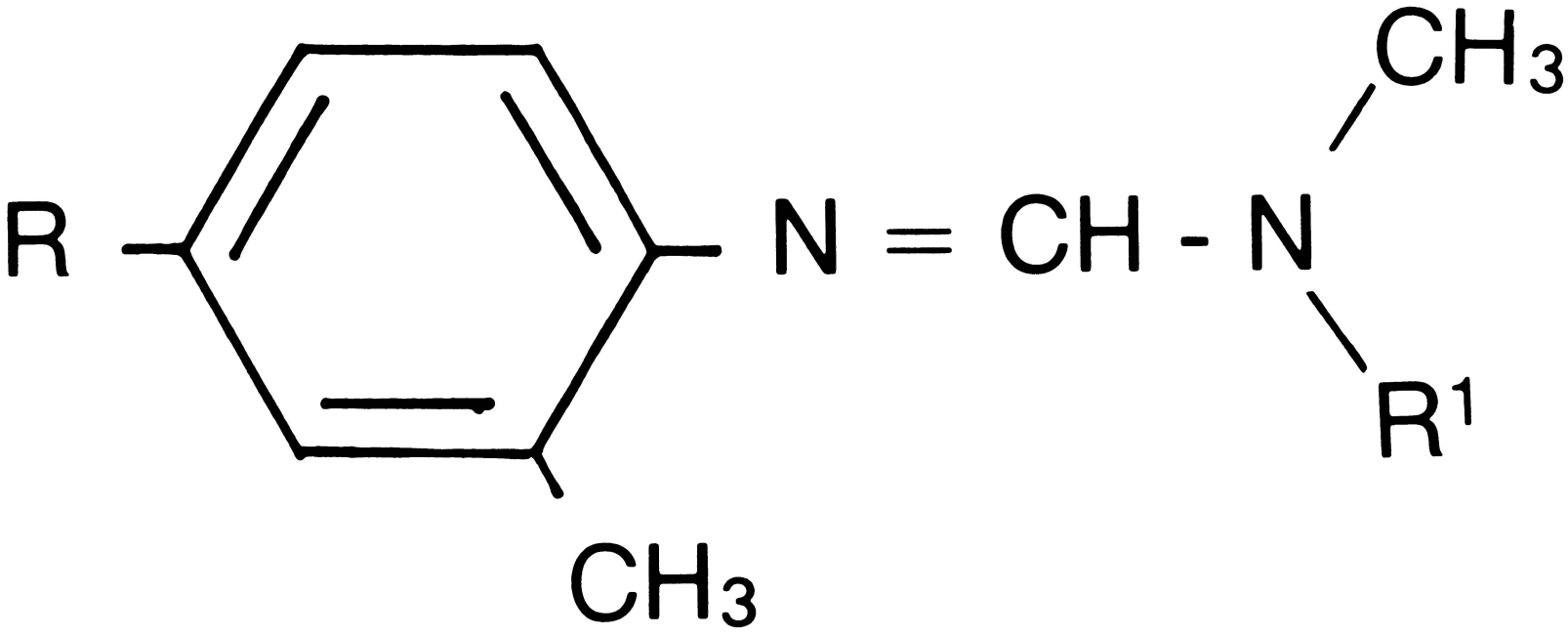


Figure 3. Formamidine moiety essential for toxicity to mites and ticks.

Influence of N-demethylation on toxicity

Several studies support the suggestion that in some cases demethylchlordimeform is the toxic component of chlordimeform (Knowles, 1982). In rats treated with chlordimeform or its formamidine metabolites, there was a latent period prior to the appearance of poisoning symptoms. A decrease in this latent period of toxicity was observed that correlated with the degree of N-demethylation of chlordimeform (Benezet et al., 1978). This suggested a probable metabolic activation by N-demethylation (Benezet et al., 1978).

The interaction of chlordimeform with classical insecticide synergists produced antagonism of toxicity in the southern cattle tick. Demethylchlordimeform in association with certain of these compounds, however, produced synergism of toxicity (Knowles and Roulston, 1972). These data indicate that in the tick chlordimeform had to be activated to demethylchlordimeform for toxicity. In insects Dittrich and Loncarevic (1971) suggested that demethylchlordimeform was the major component of chlordimeform-treated rice plants that killed the rice stem borer.

TOXICITY OF CHLORDIMEFORM - INDIRECT LETHALITY

Indirect lethality is defined herein as aberrant or

uncharacteristic behavior of an organism attributed to a causal agent that will ultimately lead to death of the individual or a population. Several indirectly lethal effects were apparent with chlordimeform. The southern cattle tick, the three host lone star tick, Amblyoma americanum Linnaeus, the brown dog tick, Rhipicephalus sanguineus (Latreille) and the wood tick, Dermacentor andersoni Stiles, upon chlordimeform treatment became hyperactive, detached and eventually fell from their host (Gladney et al., 1974; Roulston et al., 1971; Stone and Knowles, 1973; Stone et al., 1974). Three species of mites, the twospotted spider mite, the carmine mite, Tetranychus cinnabarinus (Boisduval), and the Kanzawa spider mite, Tetranychus kanzawi Kishida, treated with chlordimeform or placed on treated plants demonstrated "walkoff" and "spindown" (Gemrich et al., 1976a; Gemrich et al., 1976b; Ikeyama and Maekawa, 1973). Doane and Dunbar (1973) found that the elm spanworm, Ennomos subsignarius (Huber), also dropped off treated foliage.

Chlordimeform caused decreased feeding in mites, several species of Lepidoptera and some species of Homoptera (Hirano et al., 1972; Hirata and Sogawa, 1977; Ikeyama and Maekawa, 1973; Lund et al., 1979b; Watanabe and Fukami, 1977). It is not clear whether chlordimeform induced antifeeding, anorexia, or repellancy (Knowles, 1982). However, Beeman and Matsumura

(1978b) asserted that chlordimeform was anorexigenic in the American cockroach, Periplaneta americana (Linnaeus).

Chlordimeform has a pronounced effect on fecundity of acarines and insects. In engorged female ticks, chlordimeform inhibited egg deposition and caused production of infertile eggs (Knowles and Roulston, 1973). One formamidine that produced this effect was not directly lethal to ticks or mites and thus demonstrated that the effect on fecundity was not related to direct lethality (Knowles, 1982). A reduced fecundity was also noted in several mite species, a weevil, and an aphid species (Ikeyama and Maekawa, 1973). The cotton bollworm, Heliothis zea (Boddie), showed a marked reduction in egg deposition and viability when chlordimeform-treated females were caged with untreated males (Phillips, 1971). Interference with mating was also noted in chlordimeform treated bollworm moths; the pairs were unable to separate following copulation (Phillips, 1971). Tobacco hornworm moths, Manduca sexta (Linnaeus), showed hyperreflexia, uncoordination, and increased wing beating in mating behavior when treated with chlordimeform (Lund et al., 1979b).

There were several manifestations of indirect lethality induced by chlordimeform in mammals. Chlordimeform and some other formamidines produced hyperphagia in rats following intraperitoneal injection of low doses. At high doses,

however, anorexia ensued accompanied by hyperactivity, hyperreactivity, and stereotyped behavior (Pfister et al., 1978a). Olsen et al. (1978) showed that rats prenatally exposed to chlordimeform exhibited unusual swimming behavior. Postnatally treated offspring demonstrated uncoordinated and unusual behavior characterized by swimming with their noses under the water (Olsen et al., 1978). Benezet et al. (1978) found that chlordimeform and demethylchlordimeform extended ethanol-induced sleep time in mice. Didemethylchlordimeform, a potent central nervous stimulator, had the opposite effect (Benezet et al., 1978).

MODE OF ACTION OF CHLORDIMEFORM

The mode of action of chlordimeform is as yet undetermined though several biochemical lesions have been observed. Unlike the carbamates and organophosphates, chlordimeform is not a cholinesterase inhibitor (Dittrich, 1966). Involvement of biogenic amine regulatory mechanisms is strongly suggested by existing information on chlordimeform interaction with amine systems (Benezet et al., 1978; Knowles and Aziz, 1974).

The inhibition of monoamine oxidase (MAO) by chlordimeform in cattle ticks was first suggested by Knowles and Roulston (1972). This was confirmed in vitro by Atkinson et al. (1974) and in vivo by Holden and Hadfield (1975). In

both studies, MAO inhibition was not related to chlordimeform lethality (Knowles, 1982). Inhibition of MAO was also reported in the mold mite, Tyrophagus putrescentiae (Schrank) (Beeman and Matsumura, 1978a).

The inhibition of MAO in mammals by chlordimeform was demonstrated in rat liver preparations by Aziz and Knowles (1973) and Beeman and Matsumura (1973). Benezet and Knowles (1976) found that chlordimeform inhibition of rat brain MAO was competitive and reversible with several biogenic amine substrates. This effect has been corroborated by studies in rat brain, liver, and serum (Bainova et al., 1979; Kadir and Knowles, 1981; Kaloyanova et al., 1978; Maitre et al., 1978; Neumann and Voss, 1977) and in mice (Hollingworth et al., 1979).

Other enzymes have been reported as subject to chlordimeform inhibition. Based on its ability to inhibit the oxidation of carbaryl in vivo in cattle ticks, chlordimeform was postulated to inhibit a mixed function oxidase (Shuntner and Thompson, 1976). Allais et al. (1979) reported chlordimeform inhibition of N-acetyl transferase (NAT) in vivo in the migratory locust, Locusta migratoria Linnaeus. They attempted to correlate chlordimeform-induced paralysis with NAT inhibition. Chlordimeform inhibited the metabolism of tryptamine in whole mold mites in vitro, possibly by enzyme inhibition (Matsumura and Beeman, 1976). Maitre et al. (1978) found

that chlordimeform altered catecholamine and serotonin or 5-hydroxytryptamine (5-HT) metabolism in rats, also suspecting enzyme inhibition. Chlordimeform was also reported to inhibit prostaglandin synthesis in rats (Yim et al., 1978). The fact that chlordimeform increased ethanol-induced sleep time in mice suggested an interaction of chlordimeform and an ethanol-metabolizing enzyme (Benezet et al., 1978). Knowles and Benezet (1979) showed that intraperitoneal injection of chlordimeform into mice inhibited ethanol metabolism to carbon dioxide in vivo and that in mouse liver preparations chlordimeform prevented ethanol and acetate converstion to carbon dioxide.

Possibly related to MAO inhibition is the chlordimeform-induced accumulation of biogenic amines in various tissues (Benezet et al., 1978). Rats treated with chlordimeform and demethylchlordimeform had a decreased ability to deaminate tyramine and tryptamine and showed an accumulation of 5-HT and dopamine in brain tissue (Benezet et al., 1978). Chlordimeform treated rats also accumulated norepinephrine (Beeman and Matsumura, 1973).

Robinson et al. (1975) showed that chlordimeform lethality to rats was not reduced by blockade of serotonergic receptors with methylsergide or blockade of α-adrenergic receptors with phentolamine or depletion of tissue stores of biogenic

amines by reserpine. A subsequent study by Robinson and Smith (1977) demonstrated no reduction in chlordimeform lethality to rats when 5-HT and norepinephrine stores were depleted with p-chlorophenylalanine or DL- ∝ -methyl-p-tyrosine, respectively. Also, pretreatment of rats with a directly acting ∝ -adrenergic receptor agonist, phenylephrine, resulted in no increased in chlordimeform lethality to rats. From these data Robinson and Smith (1977) concluded that MAO inhibition by chlordimeform was not a factor in lethality to rats. While chlordimeform inhibition of MAO may not contribute to lethality in mammals and may not be the primary lesion in acarines, the resultant increase in biogenic amines following chlordimeform treatment is probably of physiological significance (Knowles, 1982).

Chlordimeform was reported to uncouple oxidative phosphorylation in mitochondria of the rat liver (Abo-Khatwa and Hollingworth, 1973). Uncoupling of oxidative pophorylation was also demonstrated in the adult German cockroach, Blattella germanica (Linnaeus) and eggs of the Egyptian cotton leaf worm, Spodoptera littoralis (Boisduval), though the effect was not as pronounced as that in rat liver (Abo Khatwa and Hollingworth, 1972; Kotter, 1978).

Chlordimeform and several other formamidines were not active in blocking -adrenergic receptors as evidenced by

their lack of effectiveness as blockers of norepinephrine binding in rat cardiac microsomes (Knowles and Aziz, 1974).

Blockade of neuromuscular transmission by chlordimeform was studied by Zelenski et al. (1978). Using vascular smooth muscle in rabbit aortic strips they found that chlordimeform antagonized contraction induced by vasoactive agents such as potassium, histamine, 5-HT, and norepinephrine. An increase in the efflux of calcium from the media-intimal layer was noted with no effect on calcium uptake (Zelenski et al., 1978). Demethylchlordimeform was, however, a partial agonist for α -adrenergic receptors in rabbit central ear artery and antagonized contractions induced by vasoactive agents (Robinson, 1979; Robinson and Bittle, 1979). Pento et al. (1979) found that chlordimeform treated rats showed decreased plasma calcium, decreased duodenal calcium transport, and decreased body weight. Emran et al. (1980) found that demethylchlordimeform strongly inhibited calcium evoked secretion of catecholamines from isolated bovine adrenals and suggested that blockade of calcium influx is important to formamidine toxicity.

Chlordimeform effects on neurotransmission have centered around proposed octopaminergic systems. Lund et al. (1979a, 1979b) suggested a relationship of antifeeding in tobacco hornworm larvae and motor stimulation via central, noncho-

linergic synapses. Hollingworth and Murdock (1980) and Murdock and Hollingworth (1980) used octopamine-stimulated flashing in the firefly, Photinus pyralis Linnaeus, to show a similar effect elicited by chlordimeform and demethyl-chlordimeform. They suggested that these compounds acted postsynaptically with membrane bound receptors. Furthermore, they found that stimulation of light organ adenylate cyclase by octopamine and demethylchlordimeform was of the same order of magnitude (Hollingworth and Murdock, 1980).

Using an identified octopaminergic neuron of the grasshopper, Schistocerca americana gregaria (Dirsch), Evans and Gee (1980) showed that chlordimeform and demethylchlordimeform mimic the action of octopamine at the neuromuscular junction. This is a novel effect for a pesticide (Evans and Gee, 1980).

The structural similarity of chlordimeform to lidocaine led Chinn et al. (1977) to attribute the effects of chlordimeform on the cardiovascular and central nervous system to anesthetic-like action. Noting the similar effects of chlordimeform and local anesthetics on inhibition of catecholamine release in adrenomedullary tissue, Emran et al. (1980) came to the same conclusion. Lund et al. (1979c) suggested that this type of action could account for the acute lethal effect of chlordimeform through cardiovascular collapse in mammals.

The mode of action of chlordimeform seems to be complex and multifaceted. Formamidines exhibit direct and indirect lethality to acarines, insects, and mammals in a manner that depends upon both concentration and structure. In the case of chlordimeform it may be one or both of its demethylated formamidine metabolites that actually elicits the characteristic reactions associated with chlordimeform poisoning.

BLOOD PLATELETS AND CHLORDIMEFORM

The blood platelet-5-HT uptake system provided an unique model for the study of chlordimeform. This system is not a common one for the study of pesticides. The only references to pesticide-platelet 5-HT interactions concern the effect of nicotine on 5-HT and catecholamine release (Schievelbein and Schirren, 1964; Schievelbein and Werle, 1962a,b; Schievelbein and Zitzelsberger, 1964). For this reason a general discussion of the platelet 5-HT system is appropriate to put the present research results into perspective.

BLOOD PLATELETS AND THE 5-HT UPTAKE SYSTEM

Mammalian blood platelets have gained a great deal of support as a valid models for monoaminergic neurons and monoamine uptake systems (Drummond, 1976; Pletscher, 1978; Sneddon,

1973). Platelet affinity for the uptake, storage, and release of 5-HT and similarity to serotonergic neurons make it an easily obtainable model system (Pletscher, 1978; Von Hahn, 1980). The effect of serotonergically active chemicals may be examined in the 5-HT/platelet model (Pletscher, 1978). This section will consist of some general considerations of platelet structure and function and 5-HT interactions with platelets.

STRUCTURE AND FUNCTION OF PLATELETS

Platelets are formed from megakaryocytes. The circulating platelet count is about 2×10^8/ml in human blood and 1×10^9/ml in rats. Turnover is about 3.5×10^7/ml/day in humans (Gordon and Milner, 1976). The platelet is usually discoid in shape with a complex canalicular system. Human platelets range in diameter from 1 to 5 μ (Maupin, 1969). Two types of granules are present: alpha granules, probably containing lysosomal enzymes, and "very dense bodies," containing ATP complexed 5-HT (Gordon and Milner, 1976). Mitochondria and glycogen granules in platelets indicate that glycolysis and aerobic respiration occur (Gordon and Milner, 1976).

The function most closely associated with platelets is their role in blood clotting and wound healing. Platelets are suspected of carrying or releasing essential chemical

factors needed for blood clot formation, healing, and inflamation (Gordon and Milner, 1976). They are implicated in the pathological conditions associated with thrombosis, atherosclerosis, and transplant rejection (Gordon and Milner, 1976). Platelets also possess pinocytotic and phagocytotic abilities. This suggests a role as a scavenger for many particulate and chemical substances (Maupin, 1969).

Platelets manifest several characteristic reactions to many different biological agents. Active transport of 5-HT, dopamine, some amino acids, adenine and adenosine occurs in the platelets of most species. Adhesion, which is stimulated by collagen, and aggregation, due to platelet-platelet interaction, function in the clotting and wound healing prossesses. Secretion of 5-HT, ADP, and calcium from storage granules is involved in several processes including clotting, inflamation and atherosclerosis (Gordon and Milner, 1976).

5-HT INTERACTION WITH PLATELETS

Most 5-HT contained in circulating platelets originates in the enterochromaffin cells of the gastrointestinal tract (Drummond, 1976). Following its release into the bloodstream, 5-HT is taken up and stored by platelets.

Two sites of 5-HT uptake are present in platelets: the cytoplasmic membrane and the intracellular storage organelles.

Active uptake at the cytoplasmic membrane is an energy-dependent, temperature-sensitive, carrier-mediated transport mechanism. In a widely accepted model the carrier is postulated to possess a sodium ion receptor in addition to the 5-HT site. After attachment, 5-HT and sodium are transported across the membrane followed by detachment of the sodium from the carrier due to the relatively high potassium concentration inside the platelet. The detachment of sodium changes the conformation of the carrier and thereby releases 5-HT into the cell. Sodium is subsequently eliminated from the platelet by the sodium pump (Pletscher, 1978). The initial rate of 5-HT uptake follows Michaelis-Menten kinetics and, in rats, has Km values of 0.6 to 8 μM with a Vmax of 0.45 nmol/10^8 platelets/minute (Gordon and Overman, 1976). The effect of uptake into storage granules does not have a pronounced influence upon the kinetics over short, time periods (Pletscher, 1978). Passive transport across the platelet cytoplasmic membrane is significant only at high 5-HT concentrations and is not subject to inhibition by drugs that block receptors (Sneddon, 1973).

Active uptake of 5-HT across the cytoplasmic membrane is inhibited in a dose-dependent manner by metabolic inhibitors such as cyanide, ouabain, and dinitrophenol. These compounds probably decrease energy production needed to fuel carrier

activity or the sodium pump (Okuda and Nemerson, 1971; Pletscher, 1978). Interference with active 5-HT uptake at the membrane level is effected by several classes of drugs including tricyclic antidepressants, neuroleptics, phenyl- and indole-alkylamines, and α -and β -adrenergic receptor blockers (Pletscher, 1978; Sneddon, 1973). The most widely studied of these drugs is the tricyclic antidepressants, such as imipramine. As a class these compounds exhibited an IC_{50} of about $10^{-7}M$ (Tuomisto, 1974). Imipramine was found to bind to the substrate site of the 5-HT carrier in a competitive manner (Talvenheimo et al., 1979). Most compounds that are active inhibitors of 5-HT uptake at the cytoplasmic membrane of platelets also show inhibition of 5-HT and other biogenic amine uptake in nervous tissue, such as rat brain synaptosomes (Pletscher, 1978).

Uptake of 5-HT into storage granules is less understood than is uptake across the cytoplasmic membrane. The prevailing explanation of granular storage implicates ATP. Due to osmotic conditions, 5-HT stored in high concentrations in the granule would have to be complexed with another molecule. ATP is known to bind to 5-HT in an electrostatic manner in a 1 to 2 ratio (Berneis et al., 1969). Since platelet granules are rich in ATP it was proposed that 5-HT passively or actively enters the granule, forms complexes with ATP, and perhaps

calcium and magnesium, and is retained there by size exclusion (Pletscher, 1978). Granular uptake of 5-HT is inhibited by reserpine with little effect on cytoplasmic membrane uptake. The mode of action of reserpine is as yet undetermined (Laubscher and Pletscher, 1979; Sneddon, 1973).

Release of 5-HT by platelets is a significant part of their function in pathophysiological conditions. The release of 5-HT is induced by various agents including thrombin, vasopressin and collagen. Release of 5-HT by these agents is thought to occur due to stimulation of the cytoplasmic membrane followed by fusion of the storage granules with the membrane and subsequent exocytosis (Pletscher, 1978).

Another form of 5-HT release from platelet storage granules is elicited by reserpine and some other drugs. In this situation, liberation of 5-HT is not by exocytosis but by displacement from the granule to cytoplasm and extracellular space (Pletscher, 1978). Phenyl- and indolyl-alkylamines such as amphetamine and tyramine also induce 5-HT release though higher concentrations than with reserpine are required. Many other compounds cause 5-HT release including methylene blue, mepacrine, nicotine, quinidine and high concentrations of tricyclic antidepressants. Release by some of these compounds may be due to non-specific effects such as membrane damage (Pletscher, 1978).

The uptake, storage, and release of 5-HT by platelets are very similar to that of presynaptic serotonergic neurons. The two types of platelet membrane receptors, one for 5-HT uptake and one for shape change prior to aggregation, may be analogous to presynaptic uptake and regulatory receptors. No analogy has been found in the platelet for postsynaptic receptors (Pletscher, 1978; Sneddon, 1973).

The uptake of 5-HT by platelets is subject to inhibition at several sites including the plasma membrane and storage granules. A variety of neuroactive compounds are known to cause inhibition of 5-HT uptake. Since formamidines have neuroactive properties and interact with biogenic amines, it is plausible that they may have a significant effect on 5-HT uptake by rat platelets.

CHAPTER III

METHODS AND MATERIALS

COMPOUNDS

The properties and sources of Compounds 1 to 93, 97 to 103, and 108 to 112 of Tables I, II, and III were given in Chang and Knowles (1977). Compounds 94 to 96 and 104 to 107 of Tables II and III were obtained from The Upjohn Company, Kalamazoo, Michigan. Formparanate (UC-34096) was supplied by Union Carbide Corporation, South Charleston, West Virginia, and formetanate was obtained from NOR-AM Agricultural Products, Incorporated, Woodstock, Illinois. Chlormethiuron (C-9140) was supplied by CIBA Agrochemical Company, Vero Beach, Florida; nimidane (AC-84633) was obtained from American Cyanamid Company, Princeton, New Jersey. Ouabain, 2,4-dinitrophenol, reserpine, and imipramine were supplied by Sigma Chemical Company, St. Louis, Missouri.

Chlordimeform-^{14}C radiolabled in the ring (specific activity 5.03 mCi/mmol) was provided by CIBA-GEIGY Corporation, Greensboro, North Carolina. This compound was purified by thin layer chromatography (TLC) immediately before use. The solvent system used was that of Chang and Knowles (1977). Radiolabled 5-HT-2-^{14}C binoxalate (specific activity 48.3

mCi/mmol) was purchased from New England Nuclear, Boston, Massachusetts.

PLATELET ISOLATION

Blood was obtained from male albino rats (Sprague-Dawley strain; 250-300g) by cardiac puncture following light ether anaesthesia. It was collected in a 10ml plastic syringe containing 3.8% sodium citrate solution to yield a 9:1 blood-anticoagulant mixture. The mixture was centrifuged at 200xg for 15 minutes to yield platelet-rich plasma. Platelet concentration was determined by counting a diluted aliquot of platelet-rich plasma on a Neubauer Hemocytometer. Platelet-rich plasma was adjusted to the desired concentration of platelets with incomplete Tyrode-albumin solution at pH 7.4 (Loonen and Soudijn, 1979). For uptake experiments, platelet concentration averaged 1 to $2x10^7$ platelets/ml. Other experiments were performed with higher platelet concentrations as noted in the RESULTS section.

5-HT UPTAKE INHIBITION STUDIES

For 5-HT-^{14}C uptake inhibition studies, 0.2ml of platelet-rich plasma and 0.1ml of a Tyrode-albumin solution of the potential inhibitor at the desired concentration were incubated in 1.5ml Nalgene® centrifuge tubes at 37°C for 15

minutes in a Dubnoff Metabolic Shaker (Precision Scientific Company, Chicago, Illinois). For water insoluble compounds, the inhibitor was dissolved in acetone, placed in the reaction tube, and the acetone allowed to evaporate. Following the addition of 0.1ml of Tyrode-albumin solution and vortex mixing, 0.2ml of platelet-rich plasma was added and the incubation proceeded as described above. The uptake reaction was initiated upon addition to each tube of enough 5-HT-^{14}C to yield a final concentration of 4.8×10^{-7}M. Uptake was allowed to proceed for 10 minutes with shaking. The reaction was terminated by addition of 0.5ml of ice-cold Tyrode's solution containing 0.2% EDTA. The tube contents were centrifuged immediately at 2000xg for 10 minutes at 4^{o}C. The supernatant was removed and the inside of the tubes were wiped with tissue paper (Loonen and Soudijn, 1979).

Analysis of platelet associated 5-HT-^{14}C was accomplished by addition of 0.2ml of 0.4M perchloric acid to each tube. The tube contents were shaken, frozen and thawed twice, and centrifuged at 2000xg for 4 minutes (modified from Loonen and Soudijn, 1979). Duplicate 50 μl aliquots of the supernatant were radioassayed by liquid scintillation counting in a Beckman LS-7500 scintillation counter (Beckman Instruments, Incorporated, Fullerton, California). Scintillation cocktail for these assays consisted of 10ml of a mixture of toluene

(1.0L) and methylcellosolve (1.0L) plus 2,5-diphenyloxazole (10g) and 1,4-bis-2-(4-methyl-5-phenyloxazole)(0.6g).

Each experiment consisted of appropriate controls and an assay of imipramine ($1x10^{-5}M$) as a reference inhibitor. Data are represented as the mean of at least three experiments consisting of at least three assays of each compound $\pm$ SEM.

In uptake studies using reserpinized platelets, the following procedure was used. After isolation and dilution of platelet-rich plasma, the sample was divided in half. Reserpine in 0.6% acetic acid was added to one sample to yield a final concentration of $5x10^{-7}M$; the same volume of acetic acid was added to the other sample. The samples were incubated at 37°C for 30 minutes with shaking (Laubscher and Pletscher, 1979). Uptake inhibition experiments were then carried out as described above using chlordimeform or imipramine with reserpinized and non-reserpinized platelets.

CHLORDIMEFORM UPTAKE STUDIES

Samples of platelet-rich plasma were either cooled to near 0°C or warmed to 37°C for 20 minutes. Incubation was started by addition of 0.13 μCi chlordimeform-^{14}C. The incubations were carried out with constant gentle shaking. Aliquots of platelet-rich plasma were taken at several intervals and centrifuged at 2000xg for 10 minutes at 4°C. Dupli-

cate samples of the supernatant were radioassayed as described above.

Some sample aliquots were separated into pellet and supernatant fractions and extracted with ethyl acetate. Platelet pellets were extracted 3 times with 1 ml of ethyl acetate, and supernatant fractions were extracted 3 times with 2 ml of ethyl acetate. The respective extracts were combined, and their volumes were reduced under nitrogen. They were subsequently analyzed by TLC to determine chlordimeform degradation. The TLC solvent system was that of Chang and Knowles (1977). Samples of chlordimeform-^{14}C incubated with Tyrode-albumin solution alone were also analyzed for degradation products.

5-HT RELEASE STUDIES

Samples of platelet-rich plasma were incubated with 1 μCi of 5-HT-^{14}C/ml for 10 minutes at 37^{o}C to allow maximum uptake of the radioisotope. This was followed by centrifugation at 2000xg for 10 minutes. After removal of the supernatant, the platelet pellet was washed with ice-cold Tyrode's solution and resuspended in Tyrode-albumin solution. Following equilibration at 37^{o}C for 10 minutes, aliquots of the suspension were radioassayed to determine platelet 5-HT-^{14}C concentration. Release of 5-HT-^{14}C was measured by incubating

0.2 ml of ^{14}C-loaded platelet suspension with 0.1 ml of potential releaser compound made to desired concentration in Tyrode-albumin solution, or for controls Tyrode-albumin solution only, in a 1.5 ml Nalgene centrifuge tubles. The reaction was stopped at selected time intervals by immersion of tubes into ice and immediate centrifugation. Centrifugation and radioassay procedures were identical to those used for 5-HT uptake experiments. To determine the effect of bivalent cations on induced release the same experiment was carried out with the addition of Ca^{++} and Mg^{++} to the Tyrode-albumin solution used for control and compound solutions.

NATURE OF UPTAKE INHIBITION

In order to determine whether the inhibition of 5-HT uptake was competitive or non-competitive, 5-HT-^{14}C uptake inhibition data were analyzed by the method of Lineweaver and Burk (1934). For these experiments, 0.2 ml of platelet-rich plasma was incubated with 0.1 ml of chlordimeform-Tyrode-albumin solution of desired concentration for 15 minutes at 37^{o}C with shaking. Incubation was continued for 2 minutes following addition of 0.1 ml of 5-HT-^{14}C. Final 5-HT-^{14}C concentrations ranged from 0.94×10^{-7}M to 9.0×10^{-7}M. The reaction was stopped by the addition of 0.5 ml of Tyrode-EDTA. Centrifugation and radioassay were identical to that of the

previous 5-HT-^{14}C assays. This procedure was modified from Loonen and Soudijn (1979) and Stahl and Meltzer (1978).

EFFECT OF PROTEIN BINDING

The effect of binding of chlordimeform to media protein was examined indirectly. Platelet-rich plasma was centrifuged at 2000xg for 10 minutes, and the supernatant was removed. The platelet pellet was resuspended in incomplete Tyrode's solution to yield the desired concentration. Chlordimeform inhibitor solutions (10^{-4}M, final concentration) were prepared using Tyrode-Albumin solutions of varying albumin concentration (0% to 1.75% albumin). The assay was then conducted as in the previously described 5-HT-^{14}C uptake inhibition studies. The difference in inhibitory capacity was used as an index of protein binding.

ELECTRON MICROSCOPY

Platelet samples for scanning electron microscopy were isolated as previously described and resuspended in Tyrode solution. Incubation of platelets with or without chlordimeform treatment was carried out at 37^{o}C for 15 minutes. The samples were then centrifuged at 2000xg for 10 minutes and the supernatant was removed. The platelets were fixed by addition of 1% glutaraldehyde in 0.1 M phosphate buffer and

subsequently prepared for scanning electron microscopy by the method of Nishio et al. (1979). The samples were examined with a Joel JSM-35 Scanning Microscope.

CHAPTER IV

RESULTS

INHIBITION OF 5-HT UPTAKE BY CHLORDIMEFORM

Chlordimeform and its two formamidine metabolites, demethylchlordimeform and didemethylchlordimeform, inhibited platelet 5-HT-^{14}C uptake in a concentration dependent manner (Figure 4). The three compounds exhibited only slight 5-HT uptake inhibition at concentrations of $1x10^{-7}$M to $1x10^{-5}$M. The greatest differences in inhibitory capacity among the three compounds occurred at $1x10^{-4}$M (100 μM). At this concentration, chlordimeform gave 23.8% inhibition, demethylchlordimeform gave 42.8% inhibition, and didemethychlordimeform gave 59.6% inhibition. Inhibition at $1x10^{-3}$M and $1x10^{-2}$M was nearly complete for the three compounds. The pI_{50} values for chlordimeform, demethylchlordimeform, and didemethylchlordimeform was calculated to be 3.91, 4.22, and 4.40, respectively.

RELATIONSHIP OF FORMAMIDINE STRUCTURE TO 5-HT UPTAKE INHIBITION

The relationship of formamidine structure to inhibition

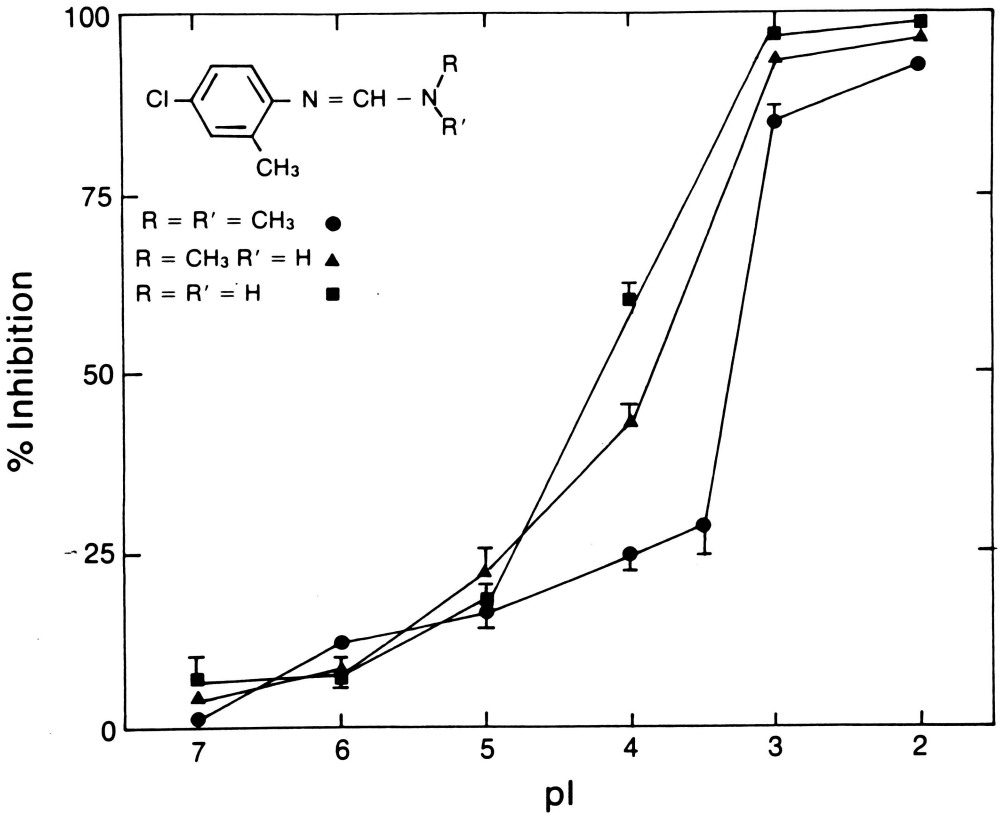


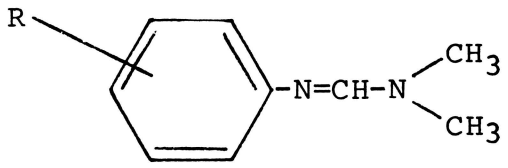
Figure 4. Inhibition of rat blood platelet uptake of 5-HT-^{14}C by chlordimeform (circle), demethylchlordimeform (triangle), and didemethylchlordimeform (square). pI is the negative logarithm of the molar inhibitor concentration. The data represent the mean of at least three experiments consisting of at least three assays of each compound. Bars represent standard error of the mean; where it was smaller than the height of the symbol the bar was omitted.

of 5-HT-^{14}C uptake by rat platelets was examined at an inhibitor concentration of 100 μM. Table I shows the percentage inhibition of uptake and amount of activity relative to chlordimeform for substituted aryl dimethylformamidines. Of the compounds having one ring substituent, the most active was the 4-n-butyl derivative (Compound 17). The 4-ethyl derivative (Compound 16) and the 3-methoxy derivative (Compound 19) showed moderately high activity, while the unsubstituted (Compound 1), 3-chloro (Compound 6), and 3-trifluoromethyl (Compound 25) derivatives had only slightly more activity than did chlordimeform. Increased activity associated with a specific ring substituent was not exhibited. However, substitution at the 2-position on the ring always yielded the lowest relative activity for each derivative group.

The most active of the di-substituted aryl dimethylformamidines were the 3,4-dichloro (Compound 31), 3,4-dimethyl (Compound 40), 3,5-dimethyl (Compound 41), 2,5-dimethoxy (Compound 43), 3-chloro, 4-methyl (Compound 51), 2-methyl, 3-nitro (Compound 55), and 2-methoxy, 5-methyl (Compound 59) derivatives, all demonstrating activities greater than 130. Compounds containing the nitro function showed little inhibitory activity with the exception of the 2-methyl, 3-nitro derivative (Compound 55). Poly-substituted compounds showed

TABLE I

INHIBITION OF RAT BLOOD PLATELET 5-HT-^{14}C UPTAKE BY N'-ARYL-N,N-DIMETYLFORMAMIDINES[a]



Compound Number	R	% Inhibition at 100 μM(±SEM)	Relative Activity[b]
1	H	26.9(3.1)	113
2	F-2	6.7(1.6)	28
3	F-3	19.7(1.6)	83
4	F-4	21.6(0.1)	91
5	Cl-2	11.7(1.2)	49
6	Cl-3	28.0(0.3)	118
7	Cl-4	21.5(3.7)	90
8	Br-2	10.3(0.5)	43
9	Br-4	20.3(2.0)	85
10	I-2	2.9(3.1)	12
11	I-3	22.7(5.0)	95
12	I-4	17.3(4.2)	73
13	Me-2	2.2(3.6)	9
14	Me-4	23.5(2.0)	99
15	Et-2	19.4(3.3)	82
16	Et-4	36.1(1.7)	152
17	n-Bu-4	49.0(4.0)	206

TABLE I (Continued)

Compound Number	R	% Inhibition at 100 μM($\pm$SEM)	Relative Activity[b]
18	MeO-2	21.7(0.6)	91
19	MeO-3	37.7(2.6)	158
20	MeO-4	22.4(1.8)	94
21	NO_2-2	3.8(3.9)	16
22	NO_2-3	14.5(2.4)	61
23	NO_2-4	8.4(1.5)	35
24	CF_3-2	13.5(2.5)	57
25	CF_3-3	26.7(2.2)	112
26	F-2,4	11.0(2.4)	46
27	Cl-2,3	15.7(1.8)	66
28	Cl-2,4	9.8(1.5)	41
29	Cl-2,5	12.8(2.3)	54
30	Cl-2,6	1.4(1.1)	6
31	Cl-3,4	37.4(3.3)	157
32	Cl-3,5	14.7(2.5)	62
33	Br-2,4	13.2(1.4)	55
34	Br-2,5	17.8(3.3)	75
35	Br-2,6	14.3(2.4)	60
36	Me-2,3	23.1(0.4)	97
37	Me-2,4	24.3(2.3)	102
38	Me-2,5	23.2(3.5)	97

TABLE I (Continued)

Compound Number	R	% Inhibition at 100 μM(±SEM)	Relative Activity[b]
39	Me-2,6	8.2(4.4)	34
40	Me-3,4	39.8(5.4)	167
41	Me-3,5	47.8(2.2)	201
42	MeO-2,4	25.4(1.4)	107
43	MeO-2,5	31.4(2.2)	132
44	MeO-3,4	13.0(1.8)	55
45	MeO-3,5	13.6(2.7)	57
46	NO_2-2,4	7.8(4.3)	33
47	F-2;Me-4	7.5(2.8)	32
48	Cl-2;Me-4	2.7(3.6)	11
49	Cl-2;Me-6	9.1(1.6)	38
50	Cl-3;Me-2	23.0(1.3)	97
51	Cl-3;Me-4	31.2(1.8)	131
52	Cl-4;Me-2	23.8(1.8)	100
53	Cl-5;Me-2	23.0(0.7)	97
54	Br-4;Me-2	9.9(3.1)	42
55	Me-2;NO_2-3	37.5(3.9)	158
56	Me-2;NO_2-5	18.1(4.0)	76
57	Me-2;NO_2-6	6.6(1.6)	28
58	Me-4;NO_2-2	5.8(2.0)	24
59	MeO-2;Me-5	42.9(2.1)	180

TABLE I (Continued)

Compound Number	R	% Inhibition at 100 μM (±SEM)	Relative Activity[b]
60	MeO-4;Me-2	10.4(4.7)	44
61	MeO-2;NO_2-4	-0.2(1.2)	
62	MeO-4;NO_2-2	2.1(2.3)	9
63	Cl-2;NO_2-4	-4.6(0.6)	
64	Cl-2;NO_2-5	3.4(3.6)	14
65	Cl-4;NO_2-2	-5.1(3.5)	
66	Cl-4;NO_2-3	16.4(1.0)	69
67	Cl-2;CF_3-5	9.4(1.2)	39
68	(-OCH_2O-)-3,4	20.1(2.9)	84
69	Cl-2,4,5	-1.2(1.3)	
70	Cl-2,4,6	5.4(4.9)	23
71	Me-2,4,6	15.1(5.2)	63
72	MeO-3,4,5	7.0(2.6)	29
73	MeO-2,4;Cl-5	29.9(3.6)	126
74	MeO-2,5;NO_2-2	10.2(1.4)	43
75	Me-4,5;NO_2-2	11.3(2.3)	47
76	Cl-2,4;NO_2-6	6.7(0.9)	28
77	Cl-2,5;NO_2-4	2.4(1.0)	10
78	Cl-2,6;NO_2-4	2.7(1.0)	11
79	Cl-4,5;NO_2-2	6.7(0.7)	28
80	I-2,6;NO_2-4	17.1(2.2)	72

TABLE I (Continued)

Compound Number	R	% Inhibition at 100 μM (±SEM)	Relative Activity[b]
81	F-2,3,5,6	10.1(0.8)	42
82	F-2,3,4,5,6	13.4(1.0)	56

[a]Abbreviations: Me=methyl, Et=ethyl, Bu=butyl.

[b]Chlordimeform(52)=100.

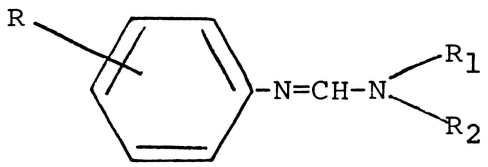
relatively little activity with the exception of the 2,4-dimethoxy, 5-chloro derivative (Compound 73) which had a relative activity of 126.

The inhibition of 5-HT-^{14}C uptake by substituted aryl dialkyl- and monoalkylformamidines is given in Table II. Dialkyl substitution at the amino nitrogen yielded no greater activity than chlordimeform when the ring substituents were 4-chloro, 2-methyl (Compounds 83, 84, and 85). A relative activity of 129 was observed with 2,4-dimethyl ring substituted diethylformamidine derivative (Compound 86). Moderate to high activity was found in monoalkyl derivatives with 4-chloro, 2-methyl or 2,4-dimethyl ring substituents (Compounds 87 to 94). The mono-_iso_propyl derivative (Compound 89) gave the highest relative activity of 234, while the 2,4-dimethyl ring substituted monoethyl derivative (Compound 94) showed an activity of 213.

Table III shows the percentage inhibition of 5-HT-^{14}C uptake by miscellaneous formamidine compounds at a concentration of 100 M. Compounds showing activity greater than chlordimeform were the thiophenyl derivatives of 4-chloro, 2-methyl (Compound 98) and 2,4-dimethyl (Compound 99) ring substituted _N_-methylformamidines; their relative activities were 140 and 157, respectively. Didemethylchlordimeform (Compound 101) had a relative activity of 250, and it was

TABLE II

INHIBITION OF RAT BLOOD PLATELET
5-HT-^{14}C UPTAKE BY OTHER
N'-ARYL-N,N-DIALKYL AND MONOALKYLFORMAMIDINES[a]



Compound Number	R	R_1	R_2	% Inhibition at 100 μM (±SEM)	Relative Activity[b]
83	Cl-4;Me-2	Et	Et	6.3(1.7)	26
84	Cl-4;Me-2	n-Pr	n-Pr	10.2(1.5)	43
85	Cl-4;Me-2	i-Pr	i-Pr	18.7(2.3)	79
86	Me-2,4	Et	Et	30.8(2.3)	129
87[c]	Cl-4;Me-2	Me	H	42.8(1.8)	180
88	Cl-4;Me-2	Et	H	22.0(1.8)	92
89	Cl-4;Me-2	i-Pr	H	55.6(0.9)	234
90	Cl-4;Me-2	n-Bu	H	43.8(4.5)	184
91	Cl-4;Me-2	i-Bu	H	36.8(3.0)	155
92	Cl-4;Me-2	s-Bu	H	27.2(3.0)	114
93	Cl-4;Me-2	t-Bu	H	39.4(3.3)	166
94[d]	Me-2,4	Me	H	50.7(1.7)	213
95	Cl-2,4	Me	H	10.7(3.6)	45
96	Cl-2;Me-4	Me	H	18.0(3.8)	76

[a]Abbreviations: Me=methyl, Et=ethyl, Pr=Propyl, Bu=butyl.
[b]Chlordimeform (52) = 100.
[c]Demethylchlordimeform.
[d]BTS - 27271.

TABLE III

INHIBITION OF RAT BLOOD PLATELET
5-HT-^{14}C UPTAKE BY
MISCELLANEOUS FORMAMIDINES[a]

Compound Number	Compound	% Inhibition at 100 μM(±SEM)	Relative Activity[b]
97[c]	Cl-4,Me-2-Ph-N=CH-N(Me)(CH_2SMe)	7.2(3.2)	30
98[d]	Cl-4,Me-2-Ph-N=CH-N(Me)(Sph)	33.3(2.0)	140
99[e]	Me-2,4-Ph-N=CH-N(Me)(SPh)	37.4(3.7)	157
100[f]	Cl-4,Me-2-Ph-N=CH-N(Me)($SCCl_3$)	7.9(1.1)	33
101[g]	Cl-4,Me-2-Ph-N=CH-NH_2	59.6(2.6)	250
102[h]	(Cl-4,Me-2-Ph-N=CH-)$_2$NMe	8.0(6.6)	34
103[i]	(Me-2,4-Ph-N=CH-)$_2$NMe	17.8(5.6)	75
104[j]	(Cl-2,4-Ph-N=CH-NMe)$_2$S	8.9(1.3)	37
105[k]	(Cl-4,Me-2-Ph-N=CH-NMe)$_2$S	17.2(1.8)	72
106[l]	(Cl-2,Me-4-Ph-N=CH-NMe)$_2$S	16.6(3.8)	70
107[m]	(Me-2,4-Ph-N=CH-NMe)$_2$S	34.2(1.6)	144
108	Cl-4,Me-2-Ph-N=CH-cyclohexylidene	19.4(1.0)	82
109	Cl-4,Me-2-Ph-N=CH-piperidino	20.0(4.3)	84
110	Cl-4,Me-2-Ph-N=CH-tetrahydropyridine	8.5(2.3)	36
111	Cl-4,Me-2-Ph-N=CH-methylmorpholino	14.7(3.2)	62

TABLE III (Continued)

Compound Number	Compound	% Inhibition at 100 μM(±SEM)	Relative Activity[b]
112	Cl-4,Me-2-Ph-N=CH-dimethylmorpholino	22.8(2.0)	96

[a]Abbreviations: Me=methyl, Ph=phenyl.

[b]Chlordimeform (52) = 100.

[c]Hokupanon[R].

[d]U-42558.

[e]U-42564.

[f]U-42662.

[g]Didemethylchlordimeform.

[h]BTS-23376.

[i]Amitraz.

[j]U-54074.

[k]U-46506.

[l]U-54076.

[m]U-44193.

the most potent formamidine tested. A thiobisformamidine (Compound 107) also showed a moderately high relative activity of 144.

Inhibition of 5-HT-^{14}C uptake by miscellaneous compounds is given in Table IV. These compounds were included due to their structural similarity to formamidines or as reference platelet-5-HT uptake inhibitors. Ouabain, a known uptake inhibitor, had a relative activity of 241. A classical 5-HT uptake inhibitor, imipramine, had an activity of 405 at 100 μM.

Some of the more active 5-HT-^{14}C uptake inhibitors were assayed over a range of concentrations to determine their pI_{50} values. Table V gives the slopes of the potency curves and the IC_{50} and pI_{50} values of these compounds.

EFFECT OF RESERPINE ON FORMAMIDINE INHIBITION OF 5-HT UPTAKE

To examine the role of platelet storage vesicles in formamidine 5-HT uptake inhibition, platelets pre-treated with reserpine were used in the assay of chlordimeform inhibition of 5-HT-^{14}C uptake (Figure 5). There was no appreciable difference in the effect of chlordimeform on the uptake of 5-HT-^{14}C in normal platelets and those pre-treated with reserpine.

TABLE IV

INHIBITION OF RAT BLOOD PLATELET 5-HT-^{14}C
BY MISCELLANEOUS COMPOUNDS[a]

Compound	% Inhibition at 100 μM (±SEM)	Relative Activity[b]
Chlormethiuron, Cl-4,Me-2-Ph-NHC(S)NMe_2	4.2(1.8)	18
Nimidane, Cl-4,Me-2-Ph-N=C(S)CH_2(S)	9.6(1.1)	40
Formetanate, MeNHC(0)0-(3-N=$CHNMe_2$Ph)	13.2(3.9)	55
Formparanate, MeNHC(0)0-(3-Me-4-N=$CHNMe_2$Ph)	18.9(0.5)	79
Ouabain	57.3(3.5)	241
2,4-Dinitrophenol	5.6(0.8)	24
Imipramine	96.4(0.3)	405

[a]Abbreviations: Me=methyl, Ph=phenyl.

[b]Chlordimeform (52) = 100.

TABLE V

POTENCY OF FORMAMIDINES AS INHIBITORS OF RAT BLOOD PLATELET 5-HT-^{14}C UPTAKE[a]

Compound Number	Slope	IC_{50}(M)	pI_{50}[b]
17	21.2	5.37×10^{-5}	4.27
37	18.7	1.25×10^{-4}	3.90
39	19.6	2.75×10^{-4}	3.56
40	19.3	5.37×10^{-5}	4.27
41	19.5	5.50×10^{-5}	4.26
52	19.6	1.22×10^{-4}	3.91
87	21.3	5.82×10^{-5}	4.23
88	17.7	4.29×10^{-4}	3.91
89	20.5	6.31×10^{-5}	4.20
90	20.2	8.15×10^{-5}	4.09
91	19.2	8.35×10^{-5}	4.08
92	18.2	1.09×10^{-4}	3.96
93	20.4	6.67×10^{-5}	4.18
94	21.8	7.24×10^{-5}	4.14
98	15.3	4.18×10^{-4}	3.38
99	18.2	2.25×10^{-4}	3.65
101	21.7	3.97×10^{-5}	4.40

[a]Calculated by regression analysis.

[b]pI_{50} is the negative logarithm of the molar concentration which gave 50% inhibition of 5-HT-^{14}C uptake.

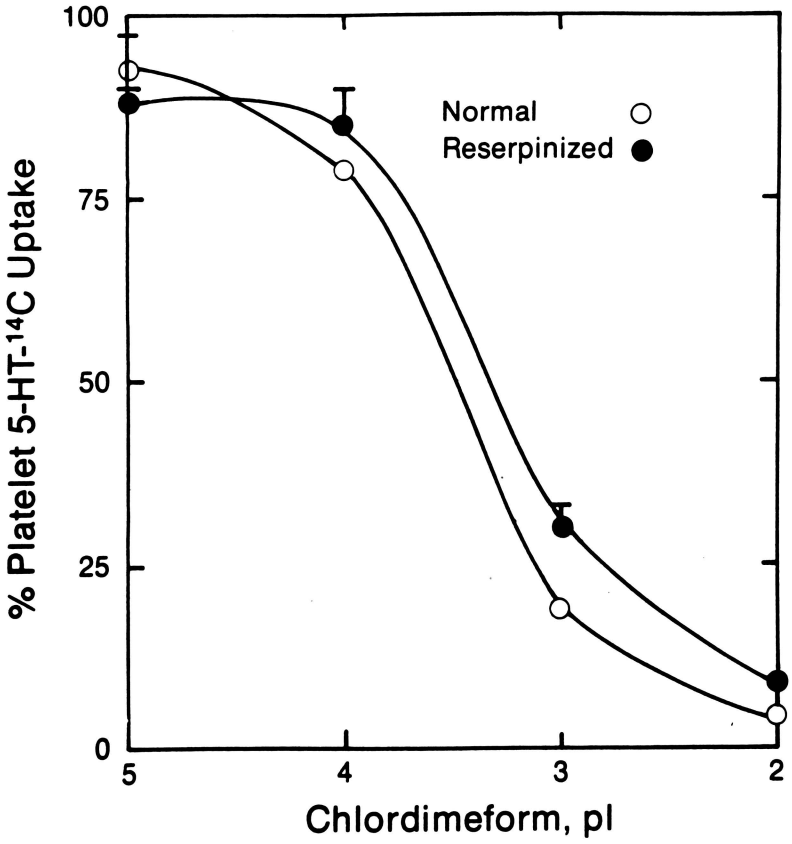


Figure 5. Inhibition of 5-HT-^{14}C uptake by chlordimeform in reserpine-treated and normal rat blood platelets.

NATURE OF 5-HT UPTAKE INHIBITION

From a Lineweaver-Burk plot (Figure 6), it was determined that inhibition of platelet 5-HT-^{14}C uptake was of a mixed competitive-non-competitive nature at chlordimeform concentrations of $5x10^{-4}$M and $1x10^{-4}$M.

CHLORDIMEFORM UPTAKE BY PLATELETS

The uptake of chlordimeform-^{14}C by platelets was measured at 0°C and 37°C over a two hour period. Figure 7 shows the concentration of chlordimeform-^{14}C associated with the platelets at selected intervals. The maximum concentration of chlordimeform-^{14}C in platelets at 0°C was about 17 pmoles, while at 37°C the maximum was about 112 pmoles. Analysis of the nature of the radiocarbon following 15 minutes incubation of chlordimeform-^{14}C with platelets yielded the results given in Table VI. These data showed that degradation of chlordimeform to 4-chloro-o-formotoluidide in the Tyrode-albumin media was 18.7% in the control and 16.2% in treatment samples. The majority of the radioactivity recovered from the platelet fraction was the parent compound.

The effect of protein concentration on the availability of chlordimeform for inhibition of uptake is shown in Figure 8. At 0% protein (protein-free media) inhibition of 5-HT-^{14}C uptake by 100 M chlordimeform was 38.7%. Using this

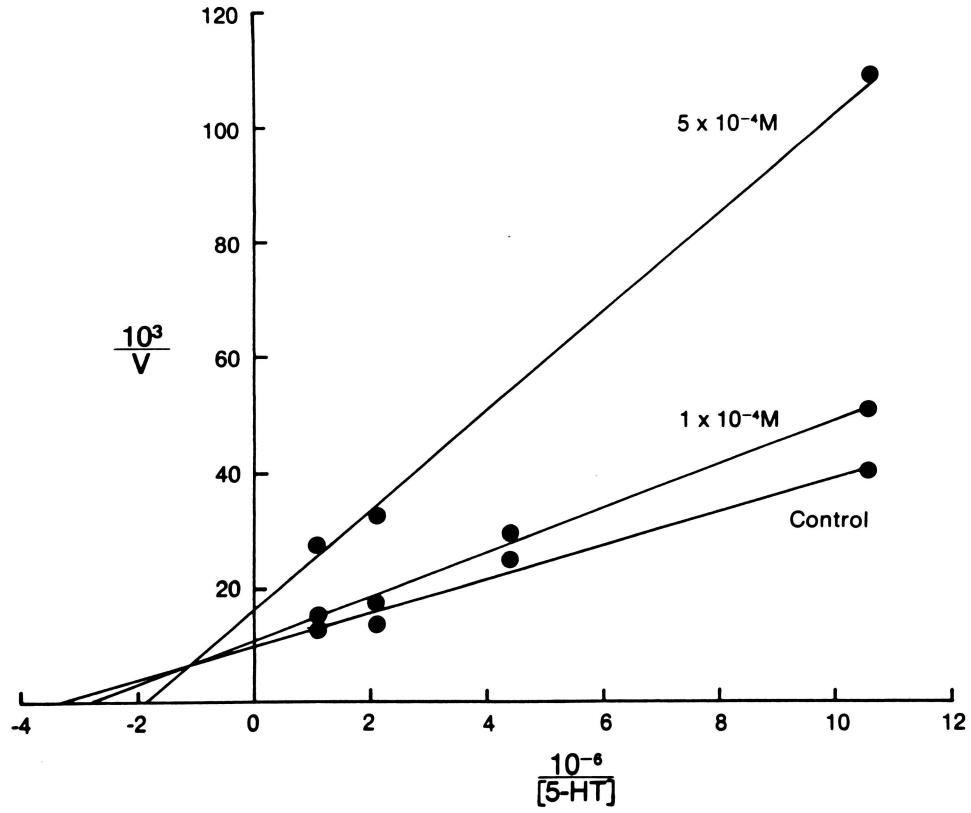


Figure 6. Lineweaver-Burk plot of chlordimeform inhibition of rat blood platelet 5-HT-^{14}C uptake. V is expressed as pmoles of 5-HT-^{14}C taken up by 2x10^8 platelets in 2 minutes. Lines determined by regression analysis.

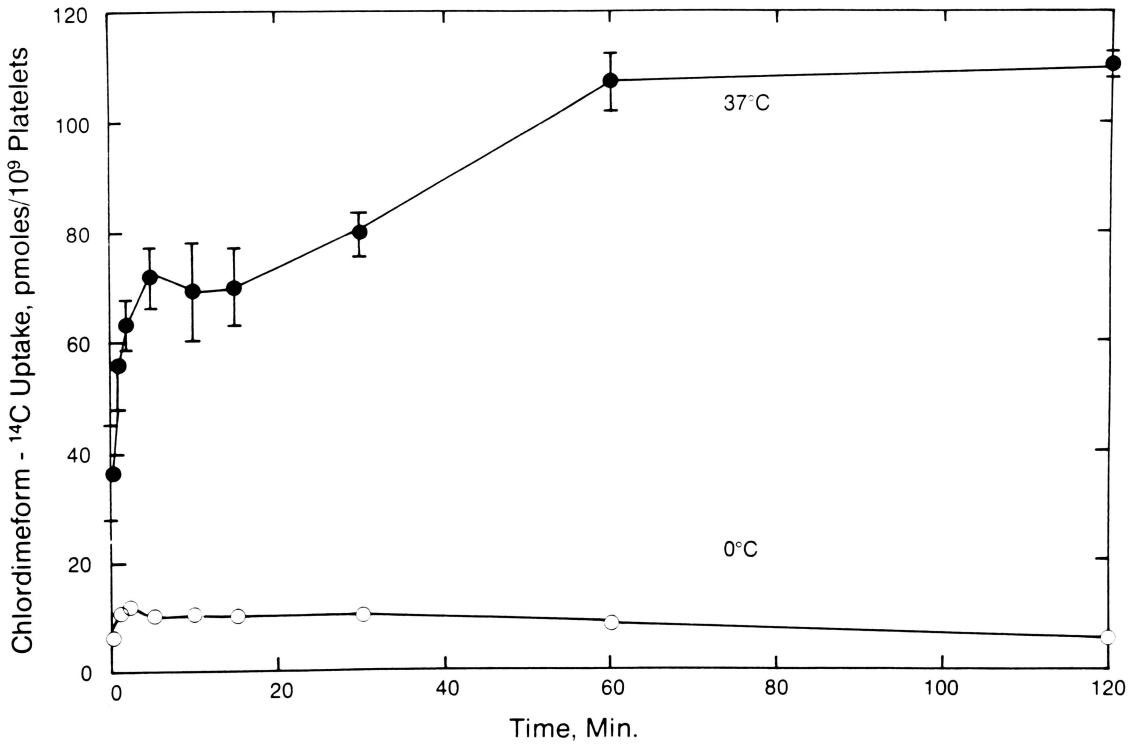


Figure 7. Influence of time and temperature on chlordimeform-^{14}C uptake by rat blood platelets. Initial concentration of chlordimeform-^{14}C at 37°C = 3.13×10^{-6}M; at 0°C = 1.1×10^{-6}M.

TABLE VI

NATURE AND RELATIVE CONCENTRATION OF RADIOCARBON FOLLOWING INCUBATION OF CHLORDIMEFORM-^{14}C WITH RAT BLOOD PLATELETS[a]

Compound	% Radioactivity		
		Treatment	
	Control[c]	Media	Platelets
Chlordimeform	78.8	80.2	1.6
Demethylchlordimeform	0.9	0.8	0.1
Didemethylchlordimeform	0.2	0.1	0.1
4-Chloro-o-formotoluidide	18.7	16.2	0.1
4-Chloro-o-toluidine	1.1	0.7	0.1
Origin	0.3	0.2	0.1

[a]Chlordimeform-^{14}C was incubated in Tyrode-albumin with or without platelets at 37°C for 15 minutes. Platelets were separated from media by centrifugation followed by ethyl acetate extraction of the two fractions. Data represent percentages of ethyl acetate extractable radiocarbon in the assay system; recovery of applied radioactivity was >90% in the control and treatment systems.

[b]Identified by TLC cochromatography with standards.

[c]Tyrode-albumin without platelets.

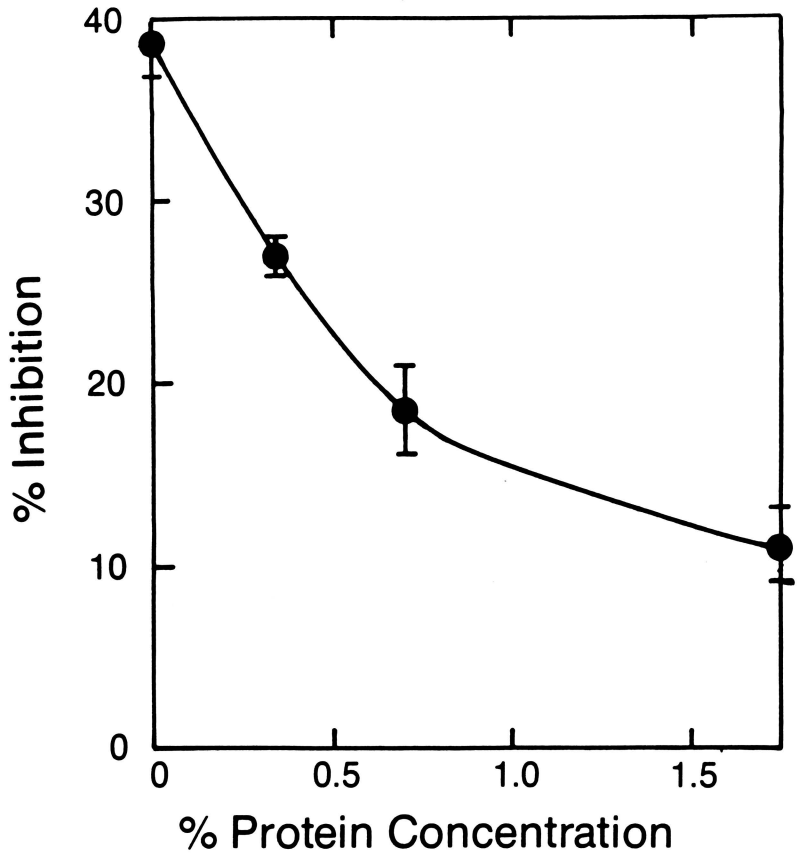


Figure 8. Influence of protein concentration on the inhibition of rat blood platelet 5-HT-^{14}C uptake by chlordimeform. Percentage protein concentration represents the percentage bovine serum albumin contained in the Tyrode's solution in which chlordimeform was dissolved.

value as an index for 0% protein-bound chlordimeform the amount of protein binding at selected protein concentrations was calculated. At 0.35% protein, the concentration used in all uptake assays, the inhibition of 5-HT uptake was 26.9%, representing 30.5% protein-bound chlordimeform.

RELEASE OF PLATELET 5-HT BY FORMAMIDINES

The ability of formamidines to induce release of endogenous 5-HT from platelets pre-loaded with 5-HT-^{14}C was found to be concentration and time dependent. Figure 9 shows the effect of chlordimeform at concentrations of 0 to 10^{-3}M. The most significant differences in releasing ability between successive concentrations was seen at $1x10^{-4}$M and $5x10^{-4}$M. At 30 minutes the difference in 5-HT release at these concentrations was almost 50%. Demethylchlordimeform-induced release had less significant differences between concentrations than chlordimeform, but all concentrations of demethylchlordimeform gave greater 5-HT release than did chlordimeform (Figure 10). At 30 minutes $1x10^{-4}$M demethylchlordimeform released about 60% of the platelet 5-HT-^{14}C content. Figure 11 depicts the release of 5-HT-^{14}C by didemethylchlordimeform. At 30 minutes this compound caused almost 25% release at $1x10^{-6}$M and about 50% release at $1x10^{-4}$M. All these formamidines showed greater than 80% release of 5-HT-^{14}C in 30

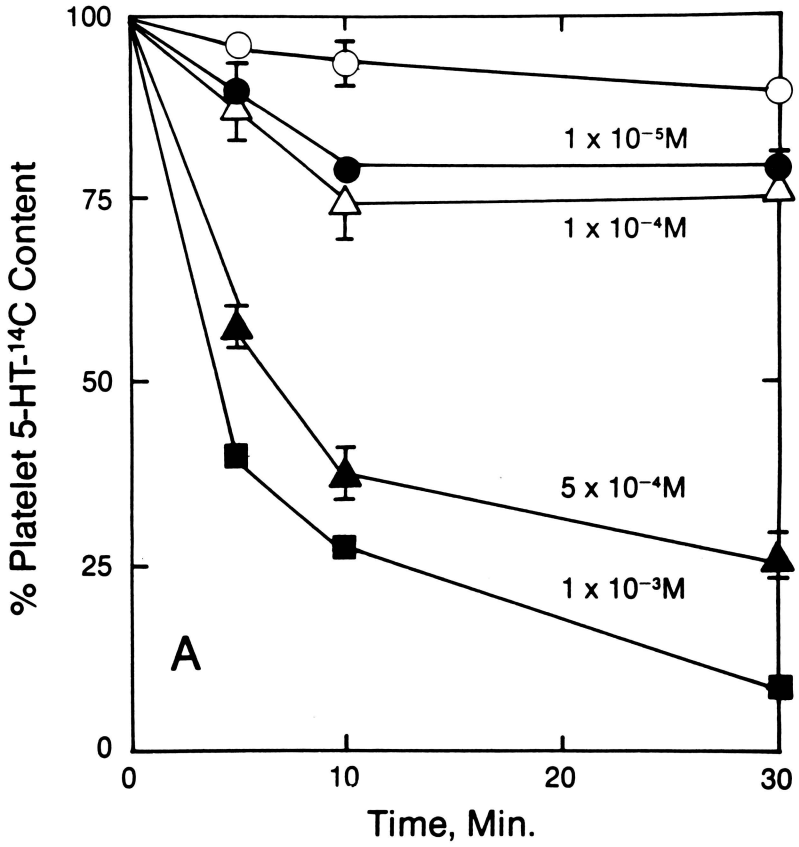


Figure 9. Release of rat blood platelet 5-HT-^{14}C by chlordimeform. Platelet 5-HT-^{14}C content expressed as percentage of 5-HT-^{14}C in 2x10^{7} platelets before the addition of chlordimeform.

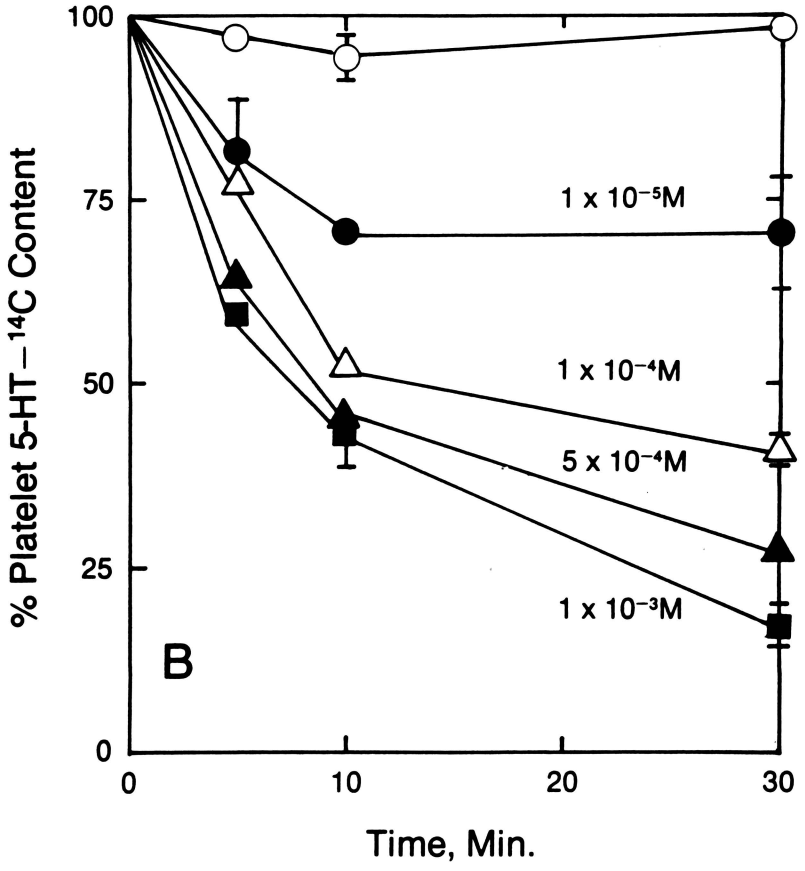


Figure 10. Release of rat blood platelet 5-HT-^{14}C by demethylchlordimeform. 5-HT-^{14}C content expressed as percentage of 5-HT-^{14}C in $5x10^7$ platelets before the addition of demethylchlordimeform.

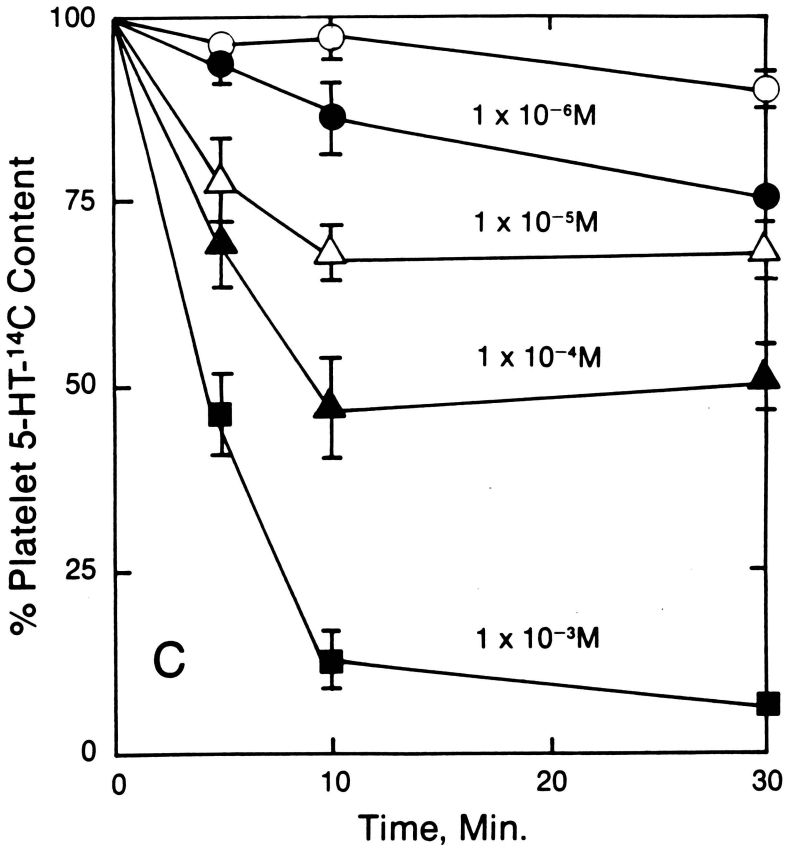


Figure 11. Release of rat blood platelet 5-HT-^{14}C by didemethylchlordimeform. Platelet 5-HT-^{14}C content expressed as percentage of 5-HT-^{14}C in $2x10^7$ platelets before the addition of didemethylchlordimeform.

minutes at $1x10^{-3}M$. The chlordimeform metabolite 4-chloro-o-formotoluidide was also assayed for releasing ability. Though not depicted this compound showed no significant 5-HT release activity.

Addition of Ca^{++} and Mg^{++} to the 5-HT-^{14}C release assay system gave no significantly different results than experiments done without these cations.

SCANNING ELECTRON MICROSCOPY

Scanning electron photomicrographs of platelets treated with selected concentrations of chlordimeform showed that membrane integrity was most probably preserved in these experiments. Figure 12 is a photomicrograph of a control platelet. This was representative of most of the cells viewed. Several pseudopods were seen extending from a relatively smooth surfaced platelet. A photomicrograph of a platelet treated with $10^{-4}M$ chlordimeform is shown in Figure 13. These platelets generally had fewer pseudopods than control platelets. The treated platelets also possessed more convoluted surfaces than did the controls and were about one half the size. Also apparent in this photomicrograph were two canalicular openings appearing as black holes in the platelet membrane. Figure 14 shows a platelet treated with $10^{-3}M$ chlordimeform. These cells had only a few short

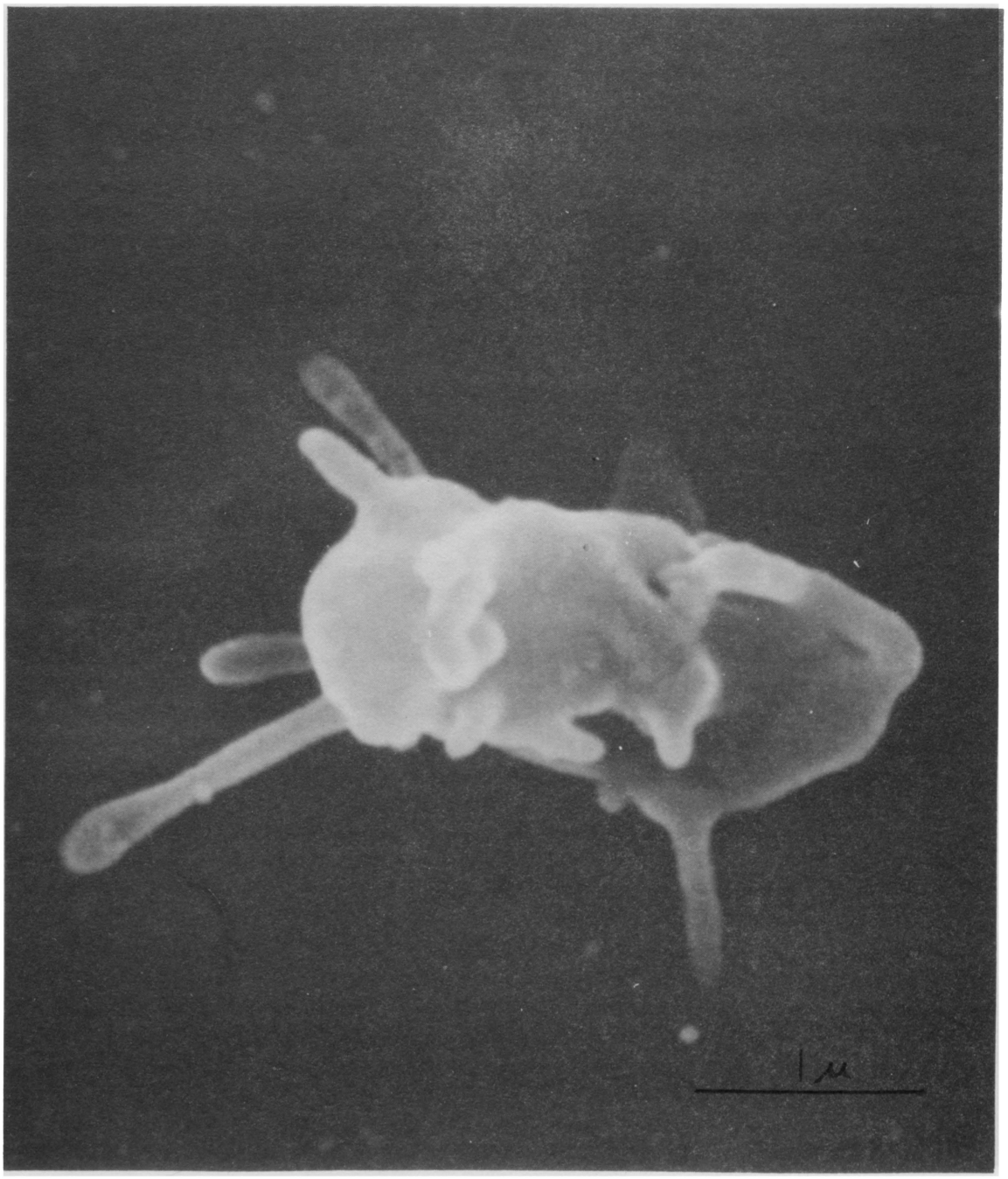


Figure 12. Scanning electron photomicrograph of control platelet.

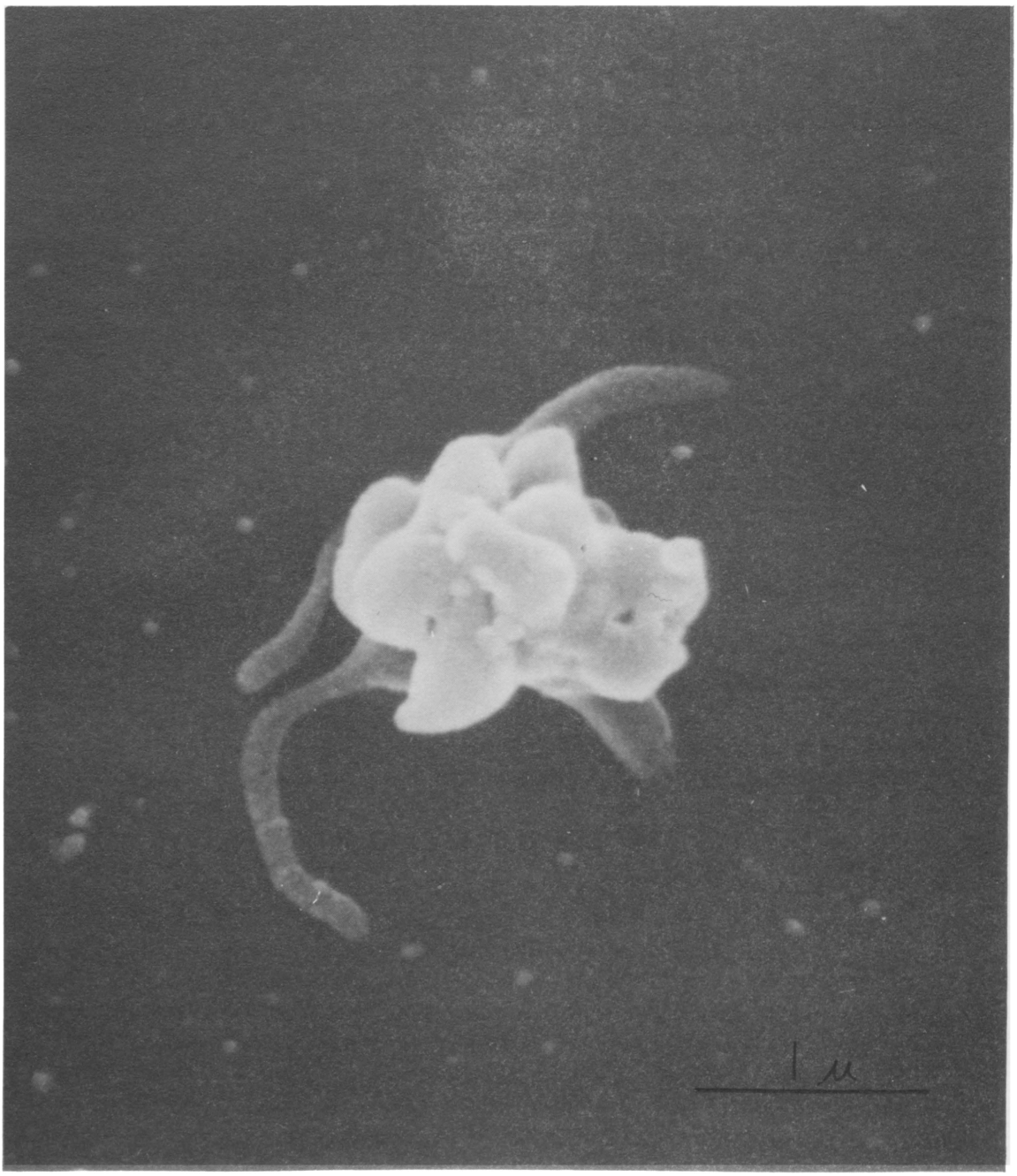


Figure 13. Scanning electron photomicrograph of a platelet treated with 10^{-4}M chlordimeform.

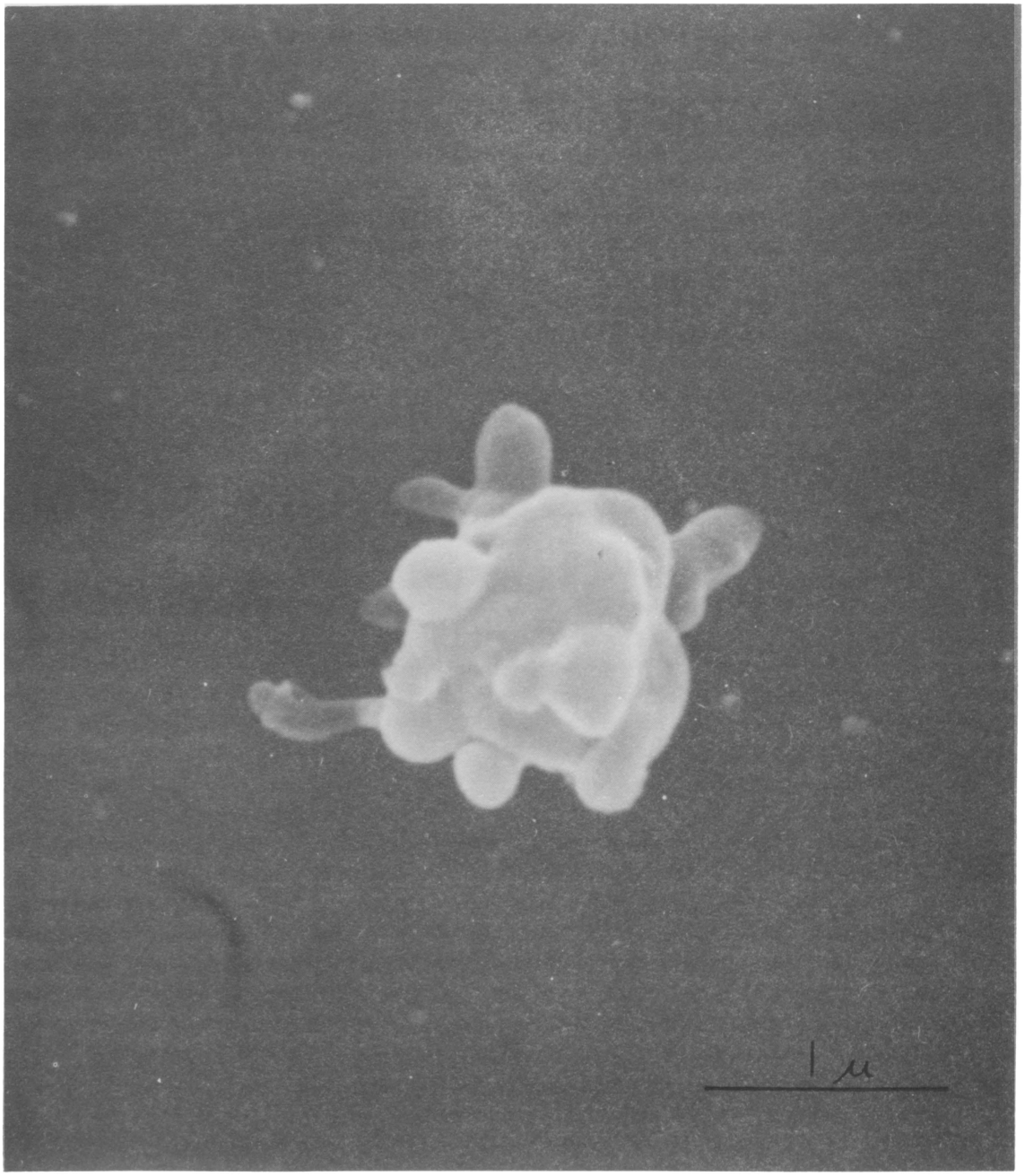


Figure 14. Scanning electron photomicrograph of a platelet treated with 10^{-3}M chlordimeform.

pseudopods and an extremely convoluted surface. They tended to be relatively spherical in shape.

CHAPTER V

DISCUSSION

The effect of chlordimeform and its two formamidine metabolites on the 5-HT uptake system of rat platelets was studied as a model for formamidine interaction with biogenic amine uptake systems. The results reported herein indicate that chlordimeform, demethylchlordimeform, and didemethylchlordimeform inhibit the uptake of 5-HT by platelets in a concentration-dependent, structurally-related manner. N-Demethylation of chlordimeform increased its inhibitory potency such that didemethylchlordimeform was two and one-half times as active as the parent. This type of relationship was also observed with respect to toxicity when rats were treated with these compounds (Benezet et al., 1978).

The classical 5-HT uptake inhibitor imipramine showed almost the opposite effect of N-demethylation on inhibitory potency. In this case, imipramine (the dimethyl compound) was the most potent inhibitor followed by didemethylimipramine and monodemethylimipramine (Pletscher, 1978). Another obvious difference between formamidine uptake inhibitors and imipramine was the order of magnitude of the IC_{50} values. Didemethylchlordimeform, the most potent formamidine tested,

had an IC_{50} of 3.97×10^{-5}M, while that for imipramine was about 1×10^{-7}M (Pletscher, 1978; Sneddon, 1973).

In addition to demethylchlordimeform, several other N'-(4-chloro-o-tolyl)-N-monoalkylformamidines showed greater uptake inhibitory activity than did chlordimeform. N-Monoalkyl substitutions that increased potency were: iso-propyl (Compound 89) > n-butyl (Compound 90) > methyl (Compound 87) tert-butyl (Compound 93) > iso-butyl (Compound 91) > sec-butyl (Compound 92) > ethyl (Compound 88). After studying inhibition of human platelet 5-HT by β-phenethylamine derivatives, Richter and Smith (1974) concluded that increased inhibitory potency was gained by N-substitution with methyl and ethyl. They further suggested that the lipophilic nature of lower alkyls contributed to the inhibitory potency of these compounds. Correlation of 5-HT uptake pI_{50} values of N'-(4-chloro-o-tolyl)-N-monoalkylformamidines with Taft's polor effect constant (σ^*) with Taft's steric factor (E_s) for the respective substituents failed to yield any conclusive information on lipophilic or steric effects.

Substitution of the aryl moiety of aryl dimethylformamidines with substiuents other than 4-chloro, 2-methyl (chlordimeform) did not, in general, increase inhibitory potency over that of chlordimeform. The significant exceptions to this were the 4-ethyl derivative (Compound 16), the 4-n-butyl

derivative (Compound 17), the 3-methoxy derivative (Compound 19), the 3,4-dichloro derivative (Compound 31), the 3,4-dimethyl derivative (Compound 40), the 3,5-dimethyl derivative (Compound 41), the 2,5-methoxy derivative (Compound 43), the 3-chloro, 4-methyl derivative (Compound 51) and the 2-methoxy, 5-methyl derivative (Compound 59). The significance of a particular aryl ring substituent and position was not apparent, though the most active compounds contained alkyl, methoxy, and chloro substituents. Compounds substituted at the 2 or 2,6 ring positions nearly always gave the lowest relative activity for their respective derivative groups. Thus these positions are probably the least favorable substituent configuration for 5-HT uptake inhibition by substituted aryl formamidines.

In order to elucidate whether the site of platelet 5-HT uptake inhibition by chlordimeform was at the plasma membrane, storage vesicle, or elsewhere, experiments were conducted to determine the nature of inhibition, the effect of reserpine, and the platelet uptake of chlordimeform. Lineweaver-Burk analysis of chlordimeform inhibition of 5-HT uptake by rat blood platelets showed a mixed competitive-noncompetitive nature. It was reported that 5-HT inhibitors that act at the plasma membrane do so in a competitive manner (Pletscher, 1978). Imipramine, for example, exerted its inhibitory

action at the plasma membrane by binding to the 5-HT carrier receptor site competitively (Talvenheimo et al., 1979).

Reserpine has been found to abolish uptake of 5-HT by storage vesicles without affecting plasma membrane uptake (Laubscher and Pletscher, 1979). Chlordimeform inhibition of 5-HT uptake was only slightly decreased in reserpinized platelets. This indicated that the major site of chlordimeform 5-HT inhibition was not the same as that of reserpine, but may still involve storage vesicles (Laubscher and Pletscher, 1979). The fact that chlordimeform-^{14}C was taken up by platelets substantiates the possibility of intercellular activity by this formamidine.

Chlordimeform and its two formamidine metabolites caused release of platelet 5-HT in a concentration-dependent, structurally-related manner. As with uptake inhibition by these three compounds, didimethylchlordimeform was more potent than demethylchlordimeform which was more potent than chlordimeform. Compared to the uptake inhibition activities at the same concentrations the 5-HT releasing ability was greater for chlordimeform and demethylchlordimeform but less for didemethylchlordimeform.

The effect of 5-HT release upon measurement of 5-HT uptake inhibition was determined to be not significant due to the relatively small levels of endogenous 5-HT (Drummond,

1976; Maupin, 1969). That is, if all endogenous 5-HT was released into the 5-HT-^{14}C pool, dilution of that pool would be only 10%. Therefore, in the uptake inhibition assay of $1x10^{-4}$M chlordimeform the 5-HT-^{14}C pool was, theoretically, diluted by 2.9% with endogenous 5-HT.

From the data reported herein, it is evident that formamidines inhibited platelet uptake of 5-HT and released endogenous 5-HT in a concentration-dependent, structurally-related manner. What remained unclear was whether these compounds ellicit their effect by interaction with the platelet plasma membrane, the storage vesicles, a cointeraction with both membrane and vesicles, or another site not considered in the present study. A variety of compounds including α and β receptor blockers, neuroactive drugs, and biogenic amines inhibited uptake of 5-HT at high inhibitor concentrations (Pletscher, 1978). These compounds were determined to act in a non-competitive manner due to nonspecific effects on the platelet. Nonspecific effects included metabolic inhibition, ion distribution imbalance, and membrane disruption (Drummond, 1976). Scanning electron photomicrographs of control and chlordimeform treated platelets showed that although they underwent shape-change the treated platelets maintained membrane integrity even with the $1x10^{-3}$M treatment. The extrusion of psuedopods was characteristic of

resting and pre-aggregating platelets (Rodman, 1971). Rough handling during isolation, washing and resuspension of the platelet may have caused greater than normal pseudopod formution. The fact that platelets treated with $1x10^{-3}M$ chlordimeform had the ability to change shape and extrude psuedopods was evidence that the compound at that concentration was not toxic to the cells.

The uptake of chlordimeform-^{14}C by platelets and the release of platelet 5-HT by chlordimeform and its two formamidine metabolites suggested that platelet 5-HT storage vesicles are the site of formamidine action.

CHAPTER VI

SUMMARY

Formamidines are one of the recently developed classes of agricultural chemicals having both insecticidal and acaricidal activity. This study was undertaken to investigate the interaction of substituted aryl formamidines with a model serotonin uptake system, the rat blood platelet. Using chlordimeform and its two formamidine metabolites as representative formamidines inhibition of platelet serotonin uptake, site of inhibition, and serotonin release by platelets were studied. The results are summarized below:

1. Chlordimeform and its two demethylated formamidine metabolites inhibited rat blood platelet serotonin-^{14}C uptake in a concentration-dependent, structurally-related manner. Chlordimeform had a pI_{50} of 3.91, demethylchlordimeform had a pI_{50} of 4.23, and didemethylchlordimeform had a pI_{50} of 4.40.

2. The relationship of structure of 112 formamidines to serotonin-^{14}C uptake inhibitory activity was determined. The following substituents in the aryl moiety of aryl dimethylformamidines gave significantly more inhibitory activity than chlordimeform: 4-ethyl; 4-n-butyl; 3-methoxy; 3,4-chloro;

3,4-methyl; 3,5-methyl; 2-methyl, 3-nitro; and 2-methoxy, 5-methyl. Substitution at the amino nitrogen of 4-chloro-2-methylphenyl substituted formamidines with monomethyl, mono-isopropyl, mono-n-butyl, mono-iso-butyl, and mono-tert-butyl, and hydrogen yielded significantly more uptake inhibition than did chlordimeform. This was also the case with 2,4-dimethyl ring substituted compounds having monomethyl and methyl, thiophenyl substituents at the amino nitrogen.

3. Inhibition of serotonin-^{14}C uptake by chlordimeform was of a mixed competitive-noncompetitive nature, indicating that it is probably not an inhibitor of the serotonin receptor at the plasma membrane.

4. Blockade of platelet storage vesicles by reserpine did not significantly alter the effect of chlordimeform on serotonin-^{14}C uptake inhibition. This suggests that the sites of reserpine and chlordimeform activity are probably not the same.

5. Chlordimeform-^{14}C was taken up by rat blood platelets in a time-and-temperature-dependent manner. The majority of platelet associated radiocarbon was determined to be chlordimeform.

6. Chlordimeform exhibited about 30% non-specific protein binding. This type of binding may inactivate chlordimeform such that its molar inhibitory potency is significantly

decreased.

7. Chlordimeform and its two formamidine metabolites caused release of platelet serotonin-^{14}C in a concentration-dependent, structurally-related manner. Chlordimeform was slightly more potent in its capacity as a serotonin releaser than as an uptake inhibitor.

8. Electron microscopy showed that increasing concentrations of chlordimeform changed platelet shape and size but did not alter the physical integrity of the platelet plasma membrane. The extrusion of pseudopods by the treated platelets suggested a lack of chlordimeform toxicity to the cells at the concentrations tested.

9. The uptake of chlordimeform-^{14}C by platelets and the release of platelet serotonin by chlordimeform and its two formamidine metabolites suggested that platelet serotonin storage vesicles are the site of formamidine action.

10. These studies demonstrated that chlordimeform, two of its formamidine metabolites, and other formamidines have specific effects on rat blood platelets. Similar effects, especially serotonin uptake inhibition, have been associated with significant physiological and behavioral dissorders in mammals. Further, the inhibition of serotonin uptake by formamidines suggests the possible inhibition of serotonin reuptake at serotenergic presynaptic neurons. Even more

importantly, however, may be the consequences of 5-HT release by formamidines at these same sites. That is, if an organism is treated with a potent formamidine pesticide there may be neuronal release of a neuroactive biogenic amine. This amine in turn could cause abnormal physiologic or behavioral functions that may ultimately lead to death.

L I T E R A T U R E C I T E D

LITERATURE CITED

Abo-Khatwa, N. and R. M. Hollingworth. 1972. Chlordimeform: the relation of mitochondrial uncoupling to toxicity in the German cockroach. Life Sci. 2. Biochem. Gen. Mol. Biol. 11:1181-1190.

Abo-Khatwa, N. and R. M. Hollingworth. 1973. Chlordimeform: uncoupling activity against rat liver mitochondria. Pest. Biochem. Physiol. 3:358-369.

Allais, J.-P., D. Gripois, B. Moreteau and F. Ramade. 1979. Ezymatic N-acetylation of tryptamine by brain homogenates of Locusta migratoria before and after intoxication by chlordimeform or lindane. Experientia 35:1358-1359.

Anonymous, 1970. NOR-AM Technical Information Bulletin DM 9-18.

Atkinson, P. W., K. C. Binnington and W. J. Roulston. 1974. High monoamine oxidase activity in the tick Boophilus microplus, and inhibition by chlordimeform and related pesticides. J. Aust. Entomol. Soc. 13:207-210.

Aziz, S. A. and C. O. Knowles. 1973. Inhibition of monoamine oxidase by the pesticide chlordimeform and related compounds. Nature. 242:417-418.

Bainova, A., Z. Zaprianov and F. Kaloyanova-Simeonova. 1979. Effect of pesticides on the activity of monoamine oxidase in rats. Arh. Hig. Rada. Toksikol. 30 suppl.: 531-535.

Beeman, R. W. and F. Matsumura. 1973. Chlordimeform: a pesticide acting upon amine regulatory mechanisms. Nature 242:273-274.

Beeman, R. W. and F. Matsumura. 1978a. Formamidine pesticides - actions in insects and acarines. pp. 179-188. In Pesticides and Venom Neurotoxicity. D. L. Shankland, R. M. Hollingsworth, and T. Smyth, Jr., eds. Plenum Press. New York.

Beeman, R. W. and F. Matsumura. 1978b. Anorectic effect of chlordimeform in the American cockroach. J. Econ. Entomol. 71:859-861.

Benezet, H. J., K.-M. Chang and C. O. Knowles. 1978. Formamidine pesticides - metabolic aspects of neurotoxicity. pp. 189-206. In Pesticides and Verom Neurotoxicity. D. L. Shankland, R. M. Hollingsworth, and T. Smyth, Jr., eds. Plenum Press. New York.

Benezet, H. J. and C. O. Knowles. 1976. Inhibition of rat brain monoamine oxidase by formamidines and related compounds. Neuropharmacol. 15:369-373.

Berneis, K. H., A. Pletscher and M. Da Prada. 1969. Micelle formation between 5-hydroxytryptamine and adenosine triphosphate in platelet storage organelles. Science 165: 913-914.

Chang, K.-M. and C. O. Knowles. 1977. Formamidine acaricides. Toxicity and metabolism studies with twospotted spider mites, Tetranychus urticae Koch. J. Agric. Food Chem. 25:493-501.

Chinn, C., A. E. Lund and G. K. W. Yim. 1977. The central actions of lidocaine and a pesticide, chlordimeform. Neuropharmacol. 16:867-871.

Dittrich, V. 1966. N-(2-methyl-4-chlorophenyl)-N',N'-dimethylformidine (C-8514/Schering 36268) evaluated as an acaricide. J. Econ. Entomol. 59:889-893.

Dittrich, V. and A. Loncarevic. 1971. New insecticides for Asiatic rice borer control in paddy rice. J. Econ. Entomol. 64:1225-1229.

Doane, C. C. and D. M. Dunbar. 1973. Field evaluation of insecticides against the gypsy moth and the elm spanworm and repellent action of chlordimeform. J. Econ. Entomol. 66:1187-1189.

Drummond, A. H. 1976. Interactions of blood platelets with biogenic amines: uptake, stimulation and receptor binding. pp. 203-239. In Platelets in Biology and Pathology. J. L. Gordon, ed. Elsevier/North-Holland Biomedical Press. Amsterdam.

Emran, A., N. M. Shanbaky and J. L. Borowitz. 1980. Blockade of adrenal catecholamine release by chlordimeform and its metabolites. Bull. Environm. Contam. Toxicol. 25: 197-202.

Evans, P. D. and Gee, J. D. 1980. Actions of formamidine pesticides on octopamine receptors. Nature 287:60-62.

Gemrich, E.G., G. Kangars and V. L. Rizzo. 1976a. Insecticidal and miticidal activity of arylthioformamidines. J. Agric. Food Chem. 24:593-595.

Gemrich, E. G., B. L. Lee, M. L. Tripp and E. VandeStreek. 1976b. Relationship between formamidine structure and insecticidal, miticidal, and ovicidal activity. J. Econ. Entomol. 69:301-306.

Gladney, W. J., S. E. Ernst and R. D. Drummond. 1974. Chlordimeform: a detachment-stimulating chemical for three-host ticks. J. Med. Entomol. 11:569-572.

Gordon, J. L. and A. J. Milner. 1976. Blood platelets as multifunctional cells. pp. 3-22. In Platelets in Biology and Pathology. J. L. Gordon, ed. Elsevier/ North-Holland Biomedical Press, Amsterdam.

Gordon, J. L. and H. J. Overman. 1976. Transport of 5-hydroxytryptamine by rat and human platelets. Brit. J. Pharmacol. 58:300P-301P.

Hirano, T., H. Kawaski, H. Shinohara and T. Kitagaki. 1972. Studies on some biological activities of N-(2-methyl-4-chlorphenyl)-N'-N'-dimethylformamidine (Galecron) to the rice stem borer Chilo suppressalis Walker. Botyu-Kagaku 37:135-141.

Hirata, M. and K. Sogawa. 1976. Antifeeding activity of chlordimeform for plant-sucking insects. Appl. Ent. Zool. 11:94-99.

Holden, J. S. and J. R. Hadfield. 1975. Chlordimeform and its effect on monoamine oxidase activity in the cattle tick, Boophilus microplus. Experientia 31:1015-1017.

Hollingworth, R. M. 1976. Chemistry, biological activity, and uses of formamidine pesticides. Environ. Health Persp. 14:57-69.

Hollingworth, R. M., J. Leister and G. Ghali. 1979. Mode of action of formamidine pesticides: an evaluation of monoamine oxidase as the target. Chem.-Biol. Interactions 24:35-49.

Hollingworth, R. M. and L. L. Murdock. 1980. Formamidine pesticides: octopamine-like actions in a firefly. Science 208:74-76.

Ikeyama, M. and S. Maekawa. 1973. Development of Spanone for the control of rice stem borers. Japan Pesticide Information, No. 14: 19-22.

Kadir, H. A. and C. O. Knowles. 1981. Inhibition of rat brain monoamine oxidase by insecticides, acaricides, and related compounds. Gen. Pharmacol. 12:239-247.

Kaloyanova, F., Z. Zapryanov and A. I. Baynova. 1978. Influence of the insecticide chlordimeform on the activity of the monoamine oxidase of albino rats. Dokl. Bolg. Akad. Nauk 31:491-493.

Knowles, C. O. 1976. Chemistry and toxicology of quinoxaline, organotin, organofluorine, and formamidine acaricides. Environ. Health Persp. 14:93-102.

Knowles, C. O. 1982. Structure activity relationships among amidine acaricides and insecticides. In Insecticidal Modes of Action. J. R. Coats, ed. Academic Press. New York. (In press).

Knowles, C. O. and S. A. Aziz. 1974. Interaction of formamidines with compounds of the biogenic amine system. pp. 92-99. In Machanism of Pesticide Action. G. K. Kohn, ed. ACS Symposium Series No. 2.

Knowles, C. O. and H. J. Benezet. 1977. Mammalian metabolism of chlordimeform. Formation of metabolites containing the urea moiety. J. Agric. Food Chem. 25:1022-1026.

Knowles, C. O. and H. J. Benezet. 1979. Inhibition of ethanol and acetate metabolism in mice by chlordimeform and related compounds. Gen. Pharmacol. 10:499-503.

Knowles, C. O. and W. J. Roulston. 1972. Antagonism of chlordimeform toxicity to the cattle tick Boophilus microplus by piperonyl butoxide. J. Aust. Entomol. Soc. 11:349-350.

Knowles, C. O. and W. J. Roulston. 1973. Toxicity to Boophilus microplus of formamidine acaricides and related compounds, and modification of toxicity by certain insecticide synergists. J. Econ. Entomol. 66:1245-1251.

Kotter, C. 1978. Effects of chlordimeform on mitochondria from eggs of Spodoptera littoralis. Pest. Biochem. Physiol. 9:263-267.

Laubscher, A. and A. Pletscher. 1979. Uptake of 5-hydroxy-tryptamine in blood platelets and its inhibition by drugs: role of plasma membrane and granular storage. J. Pharm. Pharmacol. 31:284-289.

Lineweaver, A. and D. Burk. 1934. The determination of enzyme dissociation constants. J. Amer. Chem. Soc. 56:658-666.

Loonen, A. J. M. and W. Soudijn. 1979. Effects of halopemide, a new psychotropic agent, on the uptake of serotonim by blood platelets. Arch. Int. Pharmacodyn. Ther. 237: 267-274.

Lund, A. E., R. M. Hollingworth and L. L. Murdock. 1979a. Formamidine pesticides: a novel mechanism of action in lepidopterous larvae. pp. 465-469. In Advances in Pesticide Science, Part 3. H. Geissbuhler, ed. Pergamon Press. Oxford.

Lund, A. E., R. M. Hollingworth and D. L. Shankland. 1979b. Chlordimeform: plant protection by a sublethal, non-cholinergic action or the central nervous system. Pest. Biochem. Physiol. 11:117-128.

Lund, A. E., R. M. Hollingworth and G. K. W. Yim. 1979c. The comparative neurotoxicity of formamidine pesticides. pp. 119-137. In Neurotoxicology of Insecticides and Pheromones. T. Narahashi, ed. Plenum Press. New York.

Maitre, L., A. Felner, P. Waldmeier and W. Kehr. 1978. Monoamine oxidase inhibition in brain and liver of rats treated with chlordimeform. J. Agric. Food Chem. 26: 442-446.

Matsumura, F. and R. W. Beeman. 1976. Biochemical and physiological effects of chlordimeform. Environ. Health Persp. 14:71-82.

Maupin, B. 1969. Blood platelets in man and animals. Vol. 1. Pergamon Press. Oxford. 541 pp.

Murdock, L. L. and R. M. Hollingworth. 1980. Octopamine-like actions of formamidines in the firefly light organ.

pp. 415-422. In Insect Neurobiology and Pesticide Action (Neurotox 79). Society of Chemical Industry. London.

Neumann, R. and G. Voss. 1977. MAO inhibition, an unlikely mode of action for chlordimeform. Experientia 33:23-24.

Nishio, H., T. Segawa and H. Takagi. 1979. Effects of concanavalin A on 5-hydroxytryptamine uptake by rabbit blood platelets and on their ultrastructure. Br. J. Pharmacol. 65:557-563.

Okuda, M. and Y. Nemerson. 1971. Transport of serotonin by blood platelets: a pump-leak system. Amer. J. Physiol. 220:283-288.

Olson, K. L., G. M. Boush and F. Matsumura. 1978. Behavioral effects of perinatal exposure of chlordimeform in rats. Bull. Environm. Contam. Toxicol. 20:760-768.

Pento, J. T., C. P. Robinson, J. A. Rieger and F. A. Horton. 1979. The influence of chlordimeform on calcium and glucose homeostasis in the rat. Res. Comm. Chem. Path. Pharmacol. 24:127-142.

Pfister, W. R., R. M. Hollingworth and G. K. W. Yim. 1978. Increased feeding in rats treated with chlordimeform and related formamidines: a new class of appetite stimulants. Psychopharmacol. 60:47-51.

Phillips, J. R. 1971. Bollworm control with chlorphenamidine. Arkansas Farm Res. 20:9.

Pletscher, A. 1978. Platelets as models for monoaminergic neurons. pp. 49-101. In Essays in Neurochemistry and Neuropharmacology. M.S.H. Youdim, ed. John Wiley and Sons Ltd. London.

Richter, A. and S. E. Smith. 1974. Inhibition of human platelet 5-hydroxytryptamine uptake by β-phenethylamine derivatives. J. Pharmac. Pharmacol. 26:763-770.

Robinson, C. P. 1979. Effects of U-40481 and formetanate on the isolated rabbit central ear artery. Pest. Biochem. Physiol. 12:109-116.

Robinson, C. P. and I. Bittle. 1979. Vascular effects of demethylchlordimeform, a metabolite of chlordimeform.

Pest. Biochem. Physiol. 11:46-55.

Robinson, C. P. and P. W. Smith. 1977. Lack of involvement of monoamine oxidase inhibition in the lethality of acute poisoning by chlordimeform. J. Toxicol. Environm. Health 3:565-568.

Robinson, C. P., P. W. Smith, J. D. Zelenski and B. R. Endecott. 1975. Lack of an effect of interference with amine mechanisms on the lethality of chlordimeform in the rat. Toxicol. Appl. Pharmacol. 33:380-383.

Rodman, N. F. 1971. The morphologic basis of platelet function. pp. 55-70. In The Platelet. K. M. Brinkhous, R. W. Shermer, and F. K. Mostofi, eds. Williams and Wilkins Co. Baltimore.

Roulston, W. J., R. H. Wharton, H. J. Schnitzerling, R. W. Sutherst and N. D. Sullivan. 1971. Mixtures of chlorphenamidine with other acaricides for the control of organophosphorus-resistant strains of the cattle tick Boophilus microplus. Aust. Vet. J. 47:521-528.

Schick, P. K. and B. P. Yu. 1973. Methylene blue-induced serotonin release in human platelets. J. Lab. Clin. Med. 82:546-553.

Schievelbein, H. and V. Schirren. 1964. Abschwachung der Toxizitat von Nicotin durch Erhohung der Thrombozytenzahl. Experientia 20:423-433.

Schievebein, H. and E. Werle. 1962a. Freisetzung von 5-HT durch Nicotin. Psychopharmacol. 3:35-43.

Schievelbein, H. and E. Werle. 1962b. Uber die Freisetzung von Serotonin durch Lobelin. Dtsch. Med. Wschr. 87:2023-2026.

Schievelbein, H. and B. Zitzelsberger. 1964. Vergleichende Untersuchungen zum Tranport von Histamin, 5-Hydroxytryptamin und Catecholaminen:I.-Freisetzung von biogenen Aminen. Med. Exp. 11:239-246.

Sen Gupta, A. K. and C. O. Knowles. 1969. Metabolism of N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine in apple seedlings. J. Agric. Food Chem. 17:595-600.

Sneddon, J. M. 1973. Blood platelets as a model for monoamine-containing neurones. pp. 151-198. In Progress in Neurobiology, Vol. 1, part 2. G. A. Kerkut and J. W. Phillips, eds. Pergamon. Oxford.

Stahl, S. M. and H. Y. Meltzer. 1978. A kinetic and pharmacologic analysis of 5-hydroxytryptamine transport by human platelets and platelet storage granules: comparison with central serotonergic neurons. J. Pharmacol. Exp. Ther. 205:118-132.

Stone, B. F., P. W. Atkinson and C.O. Knowles. 1974. Formamidine structure and detachment in the cattle tick Boophilus microplus. Pest. Biochem. Physiol. 4:407-416.

Stone, B. F. and C. O. Knowles. 1973. A laboratory method of evaluation of chemicals causing detachment of the cattle tick Boophilus microplus. J. Aust. Entomol. Soc. 12:165-172.

Talvenheimo, J., P. J. Nelson and G. Rudnick. 1979. Mechanism of imipramine inhibition of platelet 5-hyroxytryptamine transport. J. Biol. Chem. 254:4631-4635.

Tuomisto, J. 1974. A new modification for studying 5-HT uptake by blood platelets: a reevaluation of tricyclic antidepressants as uptake inhibitors. J. Pharm. Pharmacol. 26:92-100.

Von Hahn, H. P., C. G. Honegger and A. Pletscher. 1980. Similar kinetic characteristics of 5-hydroxytryptamine binding in blood platelets and brain membranes of rats. Neurosci. Letters 20:319-322.

Watanabe, H. and J. Fukami. 1977. Stimulating action of chlordimeform and desmethylchlordimeform on motor discharges of armyworm, Leucania separata Walker (Lepidoptera/Noctuidae). J. Pest. Sci. 2:297-301.

Yim, G. K. W., M. P. Holsapple, W. R. Pfister and R. M. Hollingworth. 1978. Prostaglandin synthesis inhibited by formamidine pesticides. Life Sci. 23:2509-2516.

Zelenski, J. D., C. P. Robinson and J. T. Pento. 1978. Effect of chlordimeform on vascular smooth muscle. Pest. Biochem. Physiol. 11:278-286.

VITA

Terry Johnson was born January 16, 1949, in Rockford, Illinois. Following graduation from the public school system in Rockford in 1967 he attended Northern Illinois University. His course of study was interupted by a tour of duty in the U.S. Army. Terry returned to college at Lincoln University at Jefferson City, Missouri where he graduated with a B.S. degree in Biology in 1977. He joined the Entomology Department at the University of Missouri in 1977 and obtained his M.S. degree in Entomology in 1978. Terry remained in that department to pursue the degree of Doctor of Philosophy.

The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

INTERACTIONS OF

FORMAMIDINES WITH THE

PLATELET SEROTONIN

UPTAKE SYSTEM

presented by Terry L. Johnson

a candidate for the degree of Doctor of Philosophy

and hereby certify that in their opinion it is worthy of acceptance.

Charles Knowles
G. M. Chippendale
Gary D. Osweiler
Keith H. Byington
Thomas R Yonke
Signature redacted for proection of privacy

University of Missouri - Columbia
ELL N18238
QL463.Y1981 J936
010-003717277

QL
463
.Y1981
J936
N18238

BOUND BY HNM

University Libraries
University of Missouri

Digitization Information Page

Local identifier	Johnson1981

Source information

Format	Book
Content type	Text with image
Source ID	010-003717277
Notes	Printed single-sided.

Capture information

Date captured	
Scanner manufacturer	Zeutschel
Scanner model	OS 15000
Scanning system software	Omniscan v.12.8 SR3 (2689) 64 bit
Optical resolution	600 dpi
Color settings	8
File types	tiff

Derivatives - Access copy

Compression	Tiff: LZW
Editing software	Adobe Photoshop CC
Resolution	600 dpi
Color	gray scale
File types	pdf created from tiffs
Notes	Images cropped, brightened, straightened, and canvased 8.5 x 11 in. Blank pages not included.