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PHARMACOLOGICAL MODIFICATION OF TOXIC EFFECTS OF
IMIPRAMINE IN THE RAT

A Thesis
Presented to the
School of Graduate Studies
University of Ottawa

by

DAVID C. CARPENTER
in partial fulfilment of requirements
for the degree of
Master of Science
September, 1975

DEDICATED TO.

My Mother
and Marlene,
and in loving memory of
Jackie Réniers.

ACKNOWLEDGEMENTS

Numerous individuals have contributed their goodwill, inspiration and truly dedicated labours to the undertaking of this investigation. To them I extend my sincere gratitude.

I should like to express my profound appreciation especially to:

Dr. R. Singhal and
Dr. B. Coldwell:

for so kindly giving me the opportunity to conduct this study.

Dr. P. Hrdina:

for his enthusiastic supervision and inspirational guidance.

Dr. A. Beaubien:

for his meticulous guidance, constant encouragement and unflagging patience.

Dr. M. MacConaill:

for his invaluable advice.

Mr. L. Watts and
Staff:

for their expert surgical preparation.

Miss L. Mathieu:

for her excellent technical assistance.

My Mother and
Mrs. J. Rankin:

for their patient typing of the manuscript.

Mr. A. Peterkin:

for his painstaking proof-reading.

The Medical Communications Services:

for expert preparation and photography of the figures.

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GENERAL INTRODUCTION

Tricyclic antidepressants are but the most recent development in the long, and as yet unfulfilled, search for a drug that is completely effective and safe in the treatment of endogenous depression. At first, only certain features of depression, e.g., insomnia, anxiety and agitation, and so forth, could be treated, using such drugs as barbiturates, chloral hydrate or bromides. Then, in the late nineteen-thirties, amphetamines were introduced for the symptomatic relief of mild depressive states (11). Unfortunately, these central nervous system (CNS) stimulants had only a transient effect on depression and sometimes resulted in addiction (94). In 1938-40, electroconvulsive therapy (ECT) proved useful in the treatment of many severe depressions (90). However, ECT has the disadvantage of inherent dangers and psychorganic side effects (e.g., loss of memory). Moreover, the efficacy of ECT tends to diminish with repetition (94). In the late nineteen-fifties, the monoamine oxidase (MAO) inhibitors and the tricyclic antidepressant, imipramine, were introduced almost simultaneously.

Certain observations linked, on the one hand, the antidepressant and euphoriant effect of the MAO inhibitor, iproniazid, with a large increase in the brain levels

of serotonin and noradrenaline and, on the other hand, the depressant effect of reserpine with a pronounced decrease in the brain levels of these mediators. This led Kline, in 1957 (192), to suggest that depression could be due to an imbalance in brain neurochemical transmitters. This idea was expressed by Schildkraut (150a) as the "catecholamine hypothesis of effective disorders," which proposes that, some, if not all, depressions are associated with an absolute or relative deficiency of catecholamines, particularly noradrenaline, at functionally important adrenergic receptor sites in the brain. Elation, on the other hand, may be associated with an excess of such amines.

Since the side effects of the MAO inhibitors were so numerous and dangerous, their clinical use gradually diminished in favour of imipramine, which had been first introduced for treatment of depressive states by Kuhn (94). This drug has been shown to produce remissions in 60% to 70% of cases and is hailed as being the broadest spectrum and best tolerated of the antidepressant agents (46). In keeping with the "catecholamine hypothesis" of endogenous depression, it has been postulated that the action of imipramine as an antidepressant may be linked to its action of blocking the reuptake of catecholamines and serotonin (77).

As the usefulness of imipramine as a specific antidepressant drug became apparent, there followed the synthesis and investigation of a number of its congeners collectively known as tricyclic antidepressants, including desipramine, trimipramine, amitriptyline, nortriptyline, protriptyline, doxepin and others. The chemical structure, dosage, pharmacology, clinical effects and toxicity of these compounds are so similar that they are commonly considered together (177). Since the early nineteen-sixties, the use of these drugs has increased steadily. For example, under the National Health Service in Great Britain, whereas in 1962, the combined total of prescriptions written for amitriptyline and imipramine was about 900,000, in 1965, this figure surpassed 2,000,000.

The widespread use of these drugs to treat depressives, many of whom by definition have suicidal tendencies, makes these drugs likely to be taken in overdose. Not surprisingly then, along with the increase in the use of these drugs, has been an increase in occurrence of intentional and accidental poisonings. For example, the intensive care unit of one Australian hospital reports that, of the poisonings treated over the years 1968 and 1969, 12% involved overdose with tricyclic antidepressants, but by 1972, this figure had increased to 20% (56).

As reported to poison control centres across Canada, the number of poisonings due to imipramine and amitriptyline almost doubled in two years, from 272 in 1970, to 495 in 1972. During the same period, the total number of reported poisonings from all agents, including among others, household chemicals and "street drugs," increased from 12,274 to 18,241 (an increase of less than 50%).

But, it is not just suicide attempts that account for overdoses of tricyclic antidepressants. After the introduction, in the mid-nineteen-sixties, of the use of these drugs for childhood enuresis, the rate of poisonings in children also began to climb rapidly. A recent report (63) concerning cases of poisoning treated at a particular children's hospital indicates that, of 60 children admitted between January, 1966, and July, 1973 - a period of seven-and-a-half years - half were admitted in the last year-and-a-half, and 60% of these patients had been prescribed tricyclic antidepressants for nocturnal enuresis.

In severe poisoning with imipramine-like antidepressants, the triad of coma, convulsions and cardiac disturbances is the cardinal feature. Ultimately, the most critical aspect of this "unholy trinity" is cardiac function. In a survey of 97 cases of poisoning with tricyclic

antidepressants, conducted by Hall in 1970 (68), it is noted that in all the fatal cases in which the ECG was monitored, cardiac abnormalities were observed.

Evidence that extracardiac load may increase the cardiotoxicity of tricyclic antidepressants has been demonstrated by Cairncross and Gershon (27), who found that smaller doses of imipramine were required for cardiotoxic effects in dogs rendered hypertensive. In poisoning with imipramine-like agents, seizure activity is a source of added strain on a heart whose function is already severely impaired. For this reason, a number of authors have stressed the importance of controlling the convulsions (177), (61). At present, such treatment is mainly symptomatic, being aimed at reducing convulsions without depressing key physiological parameters. In recent years, the most frequently used drugs to treat tricyclic antidepressant-induced convulsions include diazepam (142), (208), (53), (84), (56), (63), barbiturates (177), (100), (84) and diphenylhydantoin (24), (141), (65), (132).

As yet, however, no definitive experimental work has been done to establish the superiority of any one of these compounds, not only in relieving convulsions, but also in causing the least exacerbation of the other adverse

effects occurring after an overdose of tricyclic antidepressants. In view of this, an attempt is made to answer the following questions:

(1) Of the anticonvulsants, diazepam, phenobarbital and dilantin, which is most efficacious in preventing imipramine-induced convulsions in rats?

(2) What effect does a toxic dose of imipramine have on respiration, blood pressure, heart rate and body temperature in the unanaesthetized rat?

(3) If given in effective (therapeutic) doses, does the anticonvulsant found most useful in preventing imipramine-induced convulsions adversely affect blood pressure, heart rate, or body temperature, when administered in conjunction with toxic doses of imipramine?

It is hoped that the answers to these questions might eventually contribute to the clinical management of tricyclic antidepressant poisoning.

I. LITERATURE REVIEW

A. PHARMACOLOGY OF TRICYCLIC ANTIDEPRESSANTS

1. Potentialiation of Adrenergic Mechanisms

Probably the most important pharmacological effect of the tricyclic antidepressants, and imipramine in particular, is their ability to potentiate adrenergic mechanisms. This may occur both peripherally and centrally. In 1959, Sigg showed that imipramine (but not the phenothiazines) potentiates the actions of catecholamines on the cat nictitating membrane (161). Similar potentiation of the effect of noradrenaline by tricyclic antidepressants was found in the isolated vas deferens of the rat (191), (18), though this effect appears to be biphasic, with concentrations above 10^{-7} M antagonizing the response (201). Other peripheral effects of catecholamines which have been found to be potentiated by imipramine include the response in isolated guinea pig tracheal "chains" to noradrenaline (52) and inhibition of transmission in the superior cervical ganglion of the cat by noradrenaline (102).

In the cardiovascular system, although they do not appear to have direct effect, the tricyclic antidepressants have been found to enhance and prolong a number of responses due to exogenous noradrenaline. Among these are the vaso-pressor responses in such animals as the dog (26), (85), (86),

the cat (161), (154), the rat (150) and the rabbit (111), (42), (194). The tricyclics also potentiate the positive inotropic and chronotropic effects that sympathomimetic amines have on isolated heart preparations (15), (10), (36), (118).

The effects of endogenously released noradrenaline also are potentiated, as seen in the cat nictitating membrane (145), (26) and hypogastric-nerve urinary bladder preparation (164). In contrast, imipramine inhibits blood pressure rises produced by the indirectly acting vaso-pressor amines, such as tyramine and amphetamine (161). This effect is believed to be due to the inhibition of the uptake of these latter amines by sympathetic nerve endings (20).

Centrally, tricyclic antidepressants again appear to enhance adrenergic mechanisms. In fact, this effect is thought to be of prime importance in the antidepressant action of this class of drugs. For several reasons, depressive states have been associated with a brain amine deficiency (151). Several pharmacological studies have shown a relationship between the effect of some psychotropic drugs on behaviour and their effect on brain monoamines. For example, the tranquilizer, reserpine, which is known to reduce levels of stored serotonin and noradrenaline in the

brain, can produce depression in people on long-term anti-hypertensive therapy (70). Much evidence for the potentiation of central catecholamine actions by tricyclic antidepressants is based on studies using reserpinized animals, where it has been found that imipramine and desipramine can reverse the sedation induced by reserpine in these animals (181). Other studies have shown that the tricyclic antidepressants increase or prolong many physiological and behavioural effects of amphetamine and methamphetamine, such as enhanced self-stimulation (178), motor hyperactivity (69), (55) and hyperthermia (79), in rats.

To explain this potentiation of adrenergic mechanisms, Sigg (161) proposed that the tricyclic antidepressants may act by sensitizing adrenergic receptors. However, a more likely explanation for the potentiation of both exogenous and endogenous noradrenaline is that the tricyclics inhibit the neuronal uptake of noradrenaline. Evidence for this mechanism, acting both peripherally and centrally, has been amassed. The breakthrough occurred when Axelrod (8) and Hertting et al (73) demonstrated the inhibition of uptake of exogenous noradrenaline by imipramine at the peripheral nerve ending. A similar effect apparently also occurs in the central nervous system, since Dengler and Titus (37) found that in vitro imipramine,

desipramine (DMI) and amitriptyline all inhibited uptake of ^3H -noradrenaline into rat brain slices. This was confirmed in vivo by Glowinski and Axelrod (62) when uptake of ^3H -noradrenaline injected into rat brain ventricles was inhibited by these same drugs, but not by chlorpromazine. Later workers reported similar results (152), (55). In addition, Persson and Walleck (130) found that protriptyline also inhibited dopamine uptake in mouse brain. The order of potency of the tricyclic antidepressants' inhibition of noradrenaline uptake reveals that the secondary amines (e.g., DMI) are more effective than the tertiary ones (e.g., imipramine) (30).

The tricyclics may further act by increasing the rate of synthesis and turnover of brain catecholamines. This has been found for intracisternally administered ^3H -noradrenaline in rats chronically dosed with imipramine (152). Also, though tricyclic antidepressants do not prevent depletion of brain amines by reserpine, desipramine has been found to retard the rate of noradrenaline disappearance after reserpine (103). This delay in depletion could be explained by an increase in rate of noradrenaline synthesis after administration of desipramine (120), coupled with impairment of reuptake through the neuronal membrane.

The effect of the tricyclics on synthesis and reuptake in the brain implies a greater quantity of catecholamines available in the synaptic cleft to interact with adrenergic receptors.

2. Effect on the Serotonergic System

The tricyclic antidepressants enhance several peripheral effects of serotonin (5-HT), such as contractions of the cat nictitating membrane (67), (165) and isolated guinea pig intestine (161). A similar situation exists in the central nervous system, where the hyperthermic and sedative effects of 5-hydroxytryptophan (the precursor of serotonin) and of 5-HT were shown to be potentiated by tricyclic antidepressants (162), (98). Also, the ability of imipramine to produce fatal hyperpyrexia in rabbits pretreated with MAO inhibitors appears to be due to an exaggerated 5-HT response (64).

Again, as with noradrenaline, the tricyclics may exert their effect by inhibition of serotonin uptake. They have been demonstrated to inhibit the uptake of circulating serotonin by platelets (175), (7). In the central nervous system, Carlsson (29), among others, showed that tricyclic antidepressants block serotonin uptake in mouse cerebral slices, and Kannengiesser et al (83) demonstrated a blockade of 5-HT uptake in rat brain synaptosome preparation. A number

of researchers have noticed that the tertiary amines, such as imipramine and amitriptyline, are superior in blocking serotonin uptake, while the secondary amines, such as desipramine and nortriptyline, are more effective in blocking the uptake of noradrenaline (29), (194), (167), (83), (159). However, some of the pharmacological effects of serotonin, such as urinary bladder contraction in dogs (66), rat paw edema (186) and increased capillary permeability (116) have been shown to be diminished by tricyclic antidepressants. Imipramine-like compounds have thus: 1) a potentiating effect on serotonin (block of uptake); and, 2) an anti-serotonin effect in other cases.

3. Anticholinergic Effect

Another important pharmacological effect which tends to act physiologically in the same direction as the adrenergic potentiation is the anticholinergic effect of imipramine. Apparent anticholinergic action manifests itself clinically, during tricyclic antidepressant therapy, in the frequent atropine-like side effects, such as tachycardia, dry mouth, mydriasis, blurring of vision, constipation, and the appearance of latent glaucoma (161). Also, the hallucinations and delirium sometimes seen in tricyclic antidepressant poisoning very much resemble the toxic effects of atropine (50), (169).

Experimentally, Sigg (161) has shown that the administration of small doses of imipramine in cats diminishes bradycardia induced by stimulation of the severed vagus. He also found that imipramine reduced pilocarpine salivation and blocked the effect of acetylcholine on isolated intestine. The effect of carbamylcholine on the isolated fundus of rat stomach was also antagonized in competitive fashion by tricyclic antidepressants (5). In mice, a weak mydriatic effect occurs following tricyclic administration (69) and in dogs pretreated with atropine and neostigmine, amitriptyline completely prevented the acetylcholine-induced adrenomedullary secretion of catecholamines into the adrenal vein (106).

A central anticholinergic effect of tricyclic antidepressants is also in evidence. Imipramine has an effect similar to atropine on the EEG of normal subjects (50), and Benešová (17) reported that the EEG arousal produced by either physostigmine or nicotine was diminished by tricyclic antidepressants. She also found a correlation between the anticholinergic action and the clinical antidepressant activity of these compounds.

Reserpine produces a parasympathomimetic syndrome and toxicities which are enhanced by physostigmine, DFP and carbamylcholine (99). These facts led Sulser (181) to

suggest that the antireserpine properties of tricyclic antidepressants may be due to an anticholinergic effect. There has been controversy over this idea (98) but Hrdina and Ling (75) demonstrated that the tricyclic antidepressants do influence brain levels of acetylcholine, since they found that desipramine, when administered with reserpine, blocked the usual increase in levels of 'bound' acetylcholine seen after reserpine treatment alone. Desipramine, when administered alone, lowered the level of 'bound' acetylcholine. They proposed that this desipramine effect could be due to the drug directly influencing the uptake of choline and storage of acetylcholine and/or interfering with synthesis and release of acetylcholine.

The finding that the rigidity of parkinsonism, as well as the depression which sometimes accompanies this condition, are alleviated by imipramine and desipramine, is another point supporting the possibility that these drugs have central anticholinergic properties (12). Conversely, it has been shown that increasing central acetylcholine levels with the cholinesterase inhibitor, physostigmine, antagonizes manic symptoms and results in lethargy, drained feelings, slowed thoughts and motor retardation (78). This is significant, since physostigmine has been found to antagonize many of the effects of tricyclic antidepressants.

The extent to which the anticholinergic effect plays a role in the therapeutic action of the tricyclics is still unclear. It has been suggested that the central anticholinergic activity can be explained in terms of action via adrenergic mechanisms that may inhibit central parasympathetic outflow (162).

4. Antihistaminic Effect

The tricyclic antidepressants were first investigated pharmacologically as possible antihistaminic agents. Since their structure is closely related to that of certain antihistamines, such as promethazine, it is not surprising that they should show some antihistaminic activity. Antihistaminic effects of tricyclic antidepressants have been demonstrated both in vitro (39), (196) and in vivo (113), (111).

5. Other Effects

Certain other pharmacological effects of the imipramine-like drugs include an anorexigenic effect (147), and in animals, a slight sedative effect (196). Similar to the phenothiazines, tricyclic antidepressants possess local anaesthetic activity (57), (201). In addition, minor analgesic activity has been observed in experimental animals (125), (57), (186).

B. TOXICOLOGY OF TRICYCLIC ANTIDEPRESSANTS

1. Toxicity and Symptoms of Overdosing with Tricyclic Antidepressants

The exact amount of drug ingested in accidental poisoning cases often cannot be established. Available reports nevertheless indicate that, in adults, the lowest fatal amount of imipramine was 500 to 750 mg. and the highest non-fatal amount was 5,376 mg. (68). Thus, in adults, an amount scarcely four times greater than the usual therapeutic dose of 150 mg/day may be potentially fatal. In children, ingestion of less than 10 mg/kg may precipitate severe symptoms, and if the dose exceeds 20 mg/kg, severe symptoms are inevitable. The smallest fatal dose recorded is 32 mg/kg and the largest dose survived is 112 mg/kg (177).

The time of onset of symptoms after taking a toxic dose of imipramine usually ranges from one-half to three hours post-ingestion, though Giles (61) reported that one of his patients who had ingested an unknown amount of imipramine was asymptomatic until six hours following ingestion.

Poisoning may be classified as mild or severe. The symptoms of mild poisoning include, among others, drowsiness, restlessness, ataxia, flushing of the face, dry mouth, urinary retention, hallucinations, vomiting, mydriasis, nystagmus, hyperreflexia, sinus tachycardia,

and sometimes hypertension. In severe poisoning, symptoms progress to convulsions, hypotension, coma, ECG abnormalities and cardiorespiratory arrest.

The above symptoms fit into the categories of:

- a) peripheral atropine-like disturbances (e.g., dry mouth, urinary retention); b) cardiovascular disturbances; and,
- c) central nervous system (CNS) disturbances. Since the cardiovascular and the CNS manifestations (especially the convulsions) of tricyclic antidepressant poisoning can be life threatening, they merit separate consideration.

2. Clinical and Pharmacological Aspects of Cardiovascular Toxicity

a) ECG Effects

Cardiovascular side-effects already were observed in early therapeutic use of tricyclic antidepressants (94). In severe poisoning they occur invariably. With the exception of lithium, the ECG changes seen in tricyclic antidepressant poisoning are unique among the psychotropic drugs and are sometimes used by clinicians to diagnose the tricyclic antidepressant poisoning. Arrhythmias and abnormalities that appear in ECG recordings include: disturbances in repolarization manifested as changes in the configuration of the T wave and S-T segment (2), (155), (41), disturbances in intraventricular conduction, especially

right branch block (170), (27), AV conduction problems (e.g., increase in P-R interval) and ventricular extrasystoles (60). Changes in haemodynamic parameters reported in clinical literature include tachycardia (in mild poisoning or in early stages of severe poisoning) (41), (13), (33), (27), transient hypertension (95), (163), (202) and severe hypotension (15), (126), (65).

b) Haemodynamics

(i) Blood Pressure: The haemodynamic effects of tricyclic antidepressants in experimental animals are well documented. In general, very small doses (0.1 - 0.5 mg/kg i.v.) produce slight increases in blood pressure in experimental animals (163), (153), whereas larger doses produce a decrease in blood pressure whose duration and degree is dose-dependent (163), (27), (54). Schmitt (153), in an exhaustive study on the effects of imipramine on a number of cardiovascular parameters, found that in dogs, after doses of 1 and 2 mg/kg i.v., a slight drop in blood pressure occurred. A dose of 4 mg/kg produced a profound vasodepressor response. More recently, Elonen et al (42) found that in the rabbit, intracaval injections of 2.5 mg/kg of protriptyline, amitriptyline, doxepin and nortriptyline, produced a significant drop in blood pressure, the effect peaking between 45 seconds and 3 minutes

from the beginning of injection. Similar results were reported by Laddu and Somani (95), using infusion of desmethyylimipramine (DMI, 0.5 mg/kg/min) in dogs. An initial slight increase in blood pressure was followed by a vasodepressor effect. Interestingly, in work done on dogs by Sigg (163) and by Cairncross and Gershon (27), it was found that imipramine, in doses from 1 - 2.5 mg/kg i.v., produced a biphasic or even triphasic response of blood pressure, that is, a decrease, then an increase, followed sometimes by a decrease.

(ii) Peripheral Resistance: Attempts have been made to establish the cause of the changes in blood pressure produced by tricyclic antidepressants. Blood pressure changes can be caused by: changes in peripheral resistance, alterations in cardiac output, or both. In animal experiments only, Schmitt (153) actually recorded changes in total peripheral resistance. In anaesthetized dogs, injected intravenously with 4 mg/kg of imipramine, he observed a decrease in peripheral resistance of more than 20%.

Other authors measured the haemodynamic effects of tricyclics in animals made hypertensive by artificially increasing their peripheral resistance. Cairncross and Gershon (27) treated one group of dogs acutely with angiotensin, which increases peripheral vascular tone and results

in hypertension associated with bradycardia. Imipramine did not antagonize the hypertensive effect of angiotensin in these animals. In another group of dogs, hypertension was induced by bilateral denervation of the carotid sinus region. This neurogenic hypertension is associated with maximal vasoconstriction and tachycardia. In these preparations, however, imipramine was found to lower the hypertension. In renal hypertensive dogs, imipramine in doses up to 3 mg/kg i.v. had no effect on the elevated blood pressure (163).

As mentioned earlier, the tricyclics potentiate the pressor response to noradrenaline, most likely by blocking the uptake of this amine by sympathetic nerve endings. However, the quantitative relationship between the inhibition of noradrenaline uptake and the potentiation of tissue responses to noradrenaline is far from clear. While Bonaccorsi and Hrdina (20) felt that inhibition of uptake could be involved in the potentiation of noradrenaline by the tricyclics, the apparent adrenolytic effect of high doses may involve other mechanisms. Using isolated, perfused rat artery, they observed a biphasic effect of DMI on the noradrenaline-induced constriction, reminiscent of the biphasic effect seen on blood pressure. These authors also showed that DMI inhibited the response of vascular smooth

muscle to potassium-rich, depolarizing solution and antagonized the contractile effect of calcium ions. These effects also could be involved in action of tricyclic antidepressants on peripheral resistance.

*(iii) Cardiac Output: There is evidence from both clinical and pharmacological studies, of changes in cardiac output as a result of toxic doses of tricyclic antidepressants. In dogs, Schmitt (153) found a 25% decrease in cardiac output after i.v. injection of 4 mg/kg of imipramine, as did Sigg (163) in earlier experiments. The factors involved in changes of cardiac output, however, are somewhat more complicated than those involved in peripheral resistance changes. The cardiac output is directly dependent on the heart rate and stroke volume. The stroke volume, itself, is dependent on such factors as circulating blood volume, venous return and the contractile properties of the heart.

a. Heart Rate: A number of workers investigated the effect of tricyclic antidepressants on the heart rate changes in in vivo experiments. However, not all of the results of these studies are in agreement. After i.v. administration of imipramine to dogs, Cairncross and Gershon (27), using low doses (from 0.5 to 1.5 mg/kg) observed a transient increase, whereas Schmitt (153) noted

no change in heart rate. With a dose of 5 mg/kg i.v. in angiotensin-treated, hypertensive animals, Cairncross and Gershon (27) found a large increase of about 25% in heart rate, which they attributed to the blockade of vagally-induced reflex bradycardia. In a dog heart-lung preparation, Kaumann (85) found that DMI (2.5 mg/kg i.v.) increased the heart rate, from control values of 160 beats/min, to 212 beats/min at peak effect. On the other hand, in anaesthetized dogs, Schmitt (153) observed a 25% decrease in heart rate after a 4 mg/kg dose of imipramine i.v.

More recently, Elonen et al (42) administered 2.5 mg/kg of amitriptyline, nortriptyline, protriptyline and doxepin intracavally to rabbits and found that all the drugs produced a transient increase in heart rate of 20% to 30%. This paralleled the time course of the decrease in blood pressure noted earlier, that is, the heart rate peaked three minutes after start of injection. They therefore attributed the increase in heart rate to reflex tachycardia. In the same series of experiments, intracaval infusion of noradrenaline to rabbits resulted in a usual pressor response, accompanied by a simultaneous slowing of the heart rate by almost 30%. Amitriptyline, nortriptyline, protriptyline and doxepin, injected during the noradrenaline

infusion, restored the heart rate and lowered the blood pressure. This effect, supposedly, was due to blocking of vagally-induced reflex bradycardia. However, if the tricyclic antidepressant drugs were given thirty minutes prior to the noradrenaline infusion, they did not abolish the reflex bradycardia associated with the noradrenaline pressor responses (42).

The direct effect of tricyclics on the heart rate without the interference of reflexes was, perhaps, best demonstrated by Mundo et al (118). They monitored the effect of various tricyclic antidepressants on the rate of spontaneously beating, isolated rat atria. The atria were exposed to baths containing various tricyclics in concentrations from 10^{-7} to 10^{-5} M. At concentrations of 10^{-7} and 10^{-6} M, DMI increased the atrial rate, while at a concentration of 10^{-5} M all the drugs examined produced a marked fall in spontaneous atrial rate.

b. Contractility: Using Langendorf's perfused, isolated dog hearts, Cairncross and Gershon (27) found that low concentrations of imipramine (in the range of 100 - 300 $\mu\text{g/l}$) produced a positive, while concentrations above 1 mg/l gave a negative, inotropic effect. This observation was confirmed in numerous subsequent experiments, using different animal species (36), (95), (153).

Contractility of stimulated, isolated rat ventricles was found also to be depressed when the ventricles were exposed to a 12.5 $\mu\text{g/ml}$ solution concentration of imipramine (136). The effect of the drug progressed slowly over the course of five minutes' exposure and the recovery after washout was slow. Sodium salts of pyruvate or lactate, added to the bath after imipramine washout, had little effect on recovery. In contrast, sodium bicarbonate gave a rapid recuperation of contractile force. In a more recent study, Davis and McNeil (36) used spontaneously beating guinea pig right atria to test the inotropic action of several drugs, including DMI, imipramine, tyramine, cocaine and certain antihistamines. DMI at concentrations greater than 10^{-8} M, and imipramine at concentrations greater than 3×10^{-8} M, had positive inotropic effects. A dose-dependent biphasic effect of tricyclic antidepressants on contractility was seen also in whole animal experiments. In experiments performed on bilaterally vagotomized dogs by Laddu and Somani (95), a DMI solution (0.5 mg/kg/min) was infused intravenously. When the infusion was begun, there was an immediate increase in myocardial contractile force, which gradually subsided over the next five to ten minutes of infusion. There was then a gradual depression of myocardial contractile force.

This experiment showed very well the transition from positive inotropic effect with low doses to negative inotropic effect as the dose builds up. It is important to note that in this experiment, the depression of the myocardium was at all times greater than the decrease in blood pressure. Thus, when the contractile force had reached nearly zero, the blood pressure ranged from 40 to 70 mm Hg.

Imipramine, in doses greater than 3 mg/kg i.v., has been shown by Schmitt et al (153), to depress all parameters of cardiac contractility in dogs; this effect on the myocardium was explained as being due to depression of the vasomotor centre.

(iv) Summary: Experimental evidence reviewed above indicates that tricyclic antidepressants appear to have a dose-dependent biphasic effect on haemodynamics. While the known anticholinergic activity of the tricyclics may contribute to their cardiovascular effects, small doses of tricyclics seem also to act by an adrenergic potentiating effect. This potentiation probably occurs through inhibition of noradrenaline uptake. For example Elonen (42) has shown that a definite relationship exists between rank ordering of several tricyclics as to their ability to block ³H-noradrenaline uptake and to their noradrenaline potentiating effect in the rabbit heart.

At the depressant doses of drug, the decrease in blood pressure seen experimentally seems to be secondary to myocardial depression, although a direct peripheral vasodilator effect still cannot be ruled out. While depression of the vasomotor centre has been suggested, a direct action on the myocardium seems to be the most likely cause of the severe hypotension and disturbances in conductivity and repolarization seen in poisoning with tricyclics.

3. Clinical Signs of Central Nervous System Toxicity

A variety of symptoms resulting from toxic effects upon the central nervous system (CNS) appear in the clinical picture of acute poisoning with tricyclic antidepressants. Disturbances usually begin with agitation, confusion, restlessness and irritability (146), (24), (169), (144), (157). This is followed by, or alternates with, drowsiness and ataxia (58), (61), (100), (45). The symptoms may then progress to what Slovis (169) has described as a hyperactive or hypertonic coma, where unconsciousness is interrupted by periods of seizure activity (58), (139), (177), (24), (84), (65), (172). Finally, "deep flaccid coma" may develop, and at this stage, life threatening cardiorespiratory depression is often seen (169), (65). Changes in body temperature of unknown origin also frequently occur, either

as hyperpyrexia (58), (2), (61), (53), (65), or hypothermia (126), (142). Whether the body temperature rises or falls, seems to be dependent upon the occurrence of convulsions. In all the cases where hyperpyrexia was present, the patients had had generalized convulsions (53), (58), (61). In contrast, no convulsions had occurred in those patients where a decrease in body temperature was recorded (142).

Convulsions are frequently seen in severe poisoning and constitute the most dangerous CNS effect of tricyclic antidepressant poisoning, both because they add to the stress on the heart and because of the possibility of secondary cerebral damage (53). Other central nervous system effects of tricyclic antidepressant poisoning may include elevated cerebrospinal fluid pressure (61), striking nystagmus (61), (45), fixed and dilated pupils - most likely a peripheral anticholinergic effect (100), (177) - and hyperacusis (169).

C. CLINICAL MANAGEMENT OF IMIPRAMINE POISONING

Since there is no true specific antidote, treatment of imipramine poisoning is symptomatic. The pharmacotherapy is directed mainly at the cardiovascular and central nervous system manifestations of the poisoning.

1. Pharmacotherapy of Toxic Effects in the Cardiovascular System

As discussed earlier, in the initial stages of poisoning with tricyclic antidepressants hypertension may occur, while profound hypotension frequently supervenes in the later stages of severe poisoning. Although some authors report on the successful use of sympathomimetic agents in elevating the low blood pressure (129), (157), most often attempts at treating the hypotension with vasopressor agents (e.g., methoxamine, phenylephrine, metaraminol, angiotensin) meet with failure (58), (129), (202). Several authors (177), (146), (68), (84) caution against the use of these drugs in imipramine poisoning, since Cairncross and Gershon (27) showed that a rise in blood pressure in this condition worsens the cardiac symptoms of poisoning, and can induce cardiac arrhythmias. Solutions, such as albumin, have been infused, both as plasma expanders and to provide more binding sites for the excess amount of circulating drug (129), (24).

Since the hypotension probably is secondary to the direct toxic effect of tricyclic antidepressants on the heart, better results could be expected from counteracting the action of the tricyclics on the heart itself. These direct toxic effects, as noted before, consist mainly of a reduced cardiac excitability and contractility, and of cardiac arrhythmias. To counteract the decreased excitability and contractility, a number of authors have recommended the use of isoproterenol for its positive inotropic and chronotropic effects (170), (146), (180), (31). However, Teitelbaum (184) has warned that the use of isoproterenol in poisoning with tricyclics may be dangerous, since plasma concentrations of catecholamines in this condition are already elevated and the drug may accentuate the seizures and lead to heart failure. Moreover, Hall (68) pointed out that the increase in heart rate brought about by isoproterenol could result in fatal ventricular fibrillation.

In dogs intoxicated with DMI, Laddu and Somani (95) were able to restore cardiac contractility to near normal levels by intravenous injection of ouabain. This led them to believe that digitalis glycosides would be quite effective in antagonizing cardiodepression due to tricyclic antidepressants. Clinically, cardiac glycosides

have sometimes been recommended or used if congestive heart failure occurs as a result of imipramine poisoning (58), (180), (24), (31), (157). Steel et al (177), however, pointed out that, in the presence of AV block with ventricular extrasystoles (often seen in poisoning with tricyclics), the use of cardiac glycosides would be contraindicated. Similarly, Slovis et al (169) maintain that the potential dangers of cardiac glycosides, in this condition, outweigh the gains.

Arrhythmias which occur in poisoning with tricyclic antidepressants have been treated with antiarrhythmic agents, such as propranolol and local anaesthetics. The original rationale for the use of propranolol was to counteract the adrenergic dominance demonstrated by Sigg (161) to occur as a result of tricyclic poisoning (176). In experiments with dogs intoxicated with DMI, propranolol restored normal sinus rhythm, but concomitantly depressed the myocardial contractile force (95). Other experiments (36) showed that propranolol blocked the positive inotropic effect seen with low doses of imipramine or DMI on isolated guinea pig atria. Clinically, propranolol has been used successfully to treat the tricyclics-induced arrhythmias (208), (157).

It is not clear at present whether propranolol's effectiveness in this condition is due to its β -receptor

blocking action or to a quinidine-like antiarrhythmic action. Unfortunately, propranolol also has a myocardial depressant and hypotensive effect of its own, and in tricyclic antidepressant poisoning there is a danger that these could cause further cardiac deterioration and severe hypotension (95), (84). But, if ventricular fibrillation which, untreated, is usually lethal, occurs in poisoning with tricyclics, propranolol has been considered the drug of choice (157). Practolol, which apparently acts more selectively on the cardiac β -receptors, has been employed without causing hypotension and therefore is considered by some to be superior to propranolol (24), (56).

The local anaesthetic, lidocaine, has also been used to revert arrhythmias in poisoning with tricyclics (142), and appears to be especially effective against ventricular arrhythmias (208). Diphenylhydantoin has been used, as well, for the same purpose (84).

A group of drugs which has been described as a "specific" antidote to the effects of tricyclic antidepressant poisoning, are the anticholinesterases (124), (121). Pyridostigmine was found to correct the ECG changes in rabbits and to greatly reduce the lethality in mice due to tricyclic antidepressants (139), (124). It should be mentioned that both pyridostigmine and

neostigmine are quaternary amines and do not cross the blood-brain barrier. In humans, both these agents have been shown to counteract the peripheral effects of tricyclic antidepressant poisoning, including cardiac arrhythmias and supraventricular tachycardia (139), (100), (13), (142), (208), (84). Physostigmine, a tertiary amine which does cross the blood-brain barrier, has also been shown to have a beneficial effect on tricyclic-induced cardiac arrhythmias (45), (169). In fact, Slovis (169) claims that early administration of physostigmine may even prevent the occurrence of cardiac arrhythmias.

Finally, the monovalent cations K^+ and Na^+ have been found useful in the therapy of tricyclic antidepressant poisoning. Potassium supplementation is frequently indicated in this condition (202). Penny (129) used KCl to improve the imipramine-induced tachycardia. He felt that the potassium ions might act beneficially by decreasing imipramine's demonstrated inhibition of $Na^+ - K^+$ ATPase (183). A number of sodium salts were shown to restore the decreased excitability in isolated rat ventricles poisoned with imipramine (136). It is of interest that sodium bicarbonate was able to restore not only the altered excitability, but also the contractility in this isolated preparation. Clinically,

sodium bicarbonate administered as an intravenous infusion was reported to be valuable in reverting the cardiac arrhythmias (184) and correcting the metabolic acidosis which may occur in poisoning with tricyclic antidepressants (24), (84).

2. Pharmacotherapy of Toxic Effects on the Central Nervous System

The treatment of the central nervous system (CNS) effects of poisoning with tricyclic antidepressants is mainly centred on controlling convulsions. The first drugs commonly used for this purpose were barbiturates (3), (2), (58) and paraldehyde (61). Rasmussen (139) recommended using phenobarbital as a basic drug, with further administrations of pentobarbitone as required. Several authors reported on the successful use of barbiturates in controlling convulsions (100), (84). However, other workers have found that the barbiturates potentiated the depressant effects of tricyclics on respiration and have asserted that a drug, such as paraldehyde, which would not add to the respiratory depression, would be preferable to suppress convulsions in poisoning with tricyclics (177), (208).

As mentioned previously, diphenylhydantoin has been used to treat the cardiac arrhythmias

associated with tricyclic antidepressant poisoning. This drug has also been used for its anticonvulsant properties (141), (65), and some authors claim that it has the advantage of being effective against both tricyclic-induced cardiac, and CNS, toxic effects (24), (133).

The anticonvulsant presently being most often used and widely acclaimed as a drug of choice in the treatment of seizures in tricyclic antidepressant poisoning, is diazepam (142), (208), (53), (84), (56), (63), which is thought not to worsen the existing respiratory depression (208). However, there are views to the contrary (157).

The biggest advance in the treatment of tricyclic antidepressant poisoning has been the change from using the quaternary ammonium anticholinesterases, such as neostigmine, to using the tertiary amine, physostigmine. This drug does cross the blood-brain barrier and is thus suitable for treatment of both the peripheral and the central nervous system symptoms of poisoning. Falètta (45) was the first to use physostigmine to treat a case of amitriptyline poisoning. He reported that the drug, administered intravenously in a dose of 2 mg. to 4 mg., acted promptly and dramatically. Physostigmine has been found effective against the entire spectrum of

central nervous system effects of poisoning with tricyclics, including delirium (71), coma (169), (144), (172), and choreoathetosis and myoclonus (25). The reversal of coma following administration of physostigmine is important, since it helps in avoiding the use of an endotracheal tube and possible subsequent complication, such as aspiration pneumonia. However, the action of physostigmine is short-lived and most authors (25), (45), (144) describe having to repeat the administration of the drug several times during the course of recovery. Also, this "antidote" is not without dangers of its own, and Postlethwaite (133) warns of the possibility of oversalivation, vomiting, convulsions and bradycardia, which may occur following physostigmine treatment. In view of this, its use should be reserved for the treatment of severe cases of poisoning with tricyclic antidepressants.

3. Systemic Elimination of Tricyclic Antidepressants

In an attempt to remove the offending drug from the body more rapidly, both haemodialysis and peritoneal dialysis have been tried (142), (53), (65), (4), as has forced diuresis (142). Although some authors feel that these techniques are beneficial, no evidence has been presented to indicate that they are effective in

removing significant amounts of drug in cases of poisoning. Peritoneal dialysis and haemodialysis are theoretically and practically of no value, since the tricyclic antidepressants are rapidly absorbed and firmly tissue-bound (139), (177), (146), (129), (84), (109). Also, since the excretion of tricyclics is slow and independent of urinary flow, forced diuresis is pointless, and intravenous administration of large volumes of fluids may be dangerous if the myocardium is severely depressed (177), (208), (169), (24), (141), (63).

The only method of elimination which may be worthwhile trying is continuous, gastric lavage, since imipramine is known to undergo gastro-enteral circulation (129), (208), (157), (63). Since cardiac arrhythmias and seizure activity occur almost always in severe poisoning with tricyclic antidepressants, constant ECG monitoring and control of convulsions are essential. From the reports reviewed, it appears that it is not always possible, at present, to tell from one patient to the next which antiarrhythmic or anticonvulsant agent is going to be effective. Often, therefore, more than one of these agents must be administered. The anticholinesterases, and particularly physostigmine, are emerging as one of the most powerful tools in the



treatment of poisoning with tricyclic antidepressants. Unfortunately, dangerous side effects of these drugs dictate that they be used only in treatment of severe antidepressant overdose. The question of the optimal treatment of the victims of such poisoning is still not clear and requires further investigation.

II. EXPERIMENTAL PART

A. PHARMACOLOGICAL MODIFICATION OF
IMIPRAMINE-INDUCED CONVULSIONS

1. Introduction and Aims

The importance of controlling the seizures which invariably occur in severe cases of poisoning with tricyclic antidepressants has been emphasized, since convulsions put added stress on an already weakened heart function (61), (177). In fact, the primary cause of death in poisoning with these drugs has sometimes been attributed to central nervous system exhaustion, as a consequence of prolonged convulsions (122). Lack of knowledge of the most effective and safe drug to be used in this condition has often led to polypharmacy, worsening the patient's chances of survival (157), (65), (141). Since no definitive experimental study has been carried out, measuring the effectiveness of various anticonvulsants in poisoning due to tricyclic antidepressants, this work is aimed at determining which of the anticonvulsants, diazepam, phenobarbital or diphenylhydantoin, is the most efficacious in reducing imipramine-induced convulsions and mortality in rats.

2. Materials and Methods

a) Animals

Male Wistar rats were obtained from Woodlyn Farms (Guelph, Ontario) at least one week prior to experimentation. The animals were housed in colony cages and maintained on an unrestricted diet of pellet food. Weight range of rats at the time of experiments was 180 - 235 g.

b) Drugs

All the drugs were dissolved in an appropriate vehicle and administered by intraperitoneal injection in a standard volume of 3.5 ml/kg. Imipramine hydrochloride (Ciba-Geigy Canada Ltd.), phenobarbitone sodium (British Drug Houses Canada Ltd.) and pentylenetetrazol (Knoll Pharmaceutical Co.) were dissolved in 0.9% saline solution. Sodium diphenylhydantoin (Parke, Davis and Co.) was dissolved in a solution composed of 0.9% saline and 0.01N NaOH. Diazepam (Hoffman-La Roche Ltd.) was dissolved in a solution composed of 0.9% saline and 0.0186 N HCl. The pH's of the above solutions were as follows: saline, 7.25; imipramine (32.0 mg/ml), 5.4; diphenylhydantoin (57.1 mg/ml), 11.7 (the pH of the vehicle alone was 11.75); phenobarbitone sodium (34.3 mg/ml), 9.0; and diazepam (0.29 mg/ml), 2.1 (the pH of the vehicle alone was 1.8).

c) Determination of CD₅₀ and LD₅₀ of Imipramine

(i) Assessment of Seizure Activity: To help describe imipramine-induced seizures, in a preliminary experiment a comparison was made between these and the convulsions produced by pentylenetetrazol. Simultaneously, two groups of rats were given either a convulsive dose (90 mg/kg i.p.) of pentylenetetrazol or a toxic dose (112 mg/kg) of imipramine and carefully observed for occurrence, duration and pattern of seizures.

For the purposes of this study, seizure activity was defined as a burst of repetitive myoclonic jerks, involving at least the head and forelimbs, and consisting of not less than 3 jerks in rapid succession.

(ii) Experimental Protocol: The CD₅₀ and LD₅₀ of imipramine were determined by using an 8 x 8 Latin square design. The order in which animals were treated was pseudorandomized, to ensure that the content of the various solutions used was unknown to the experimenters until after the data had been collected. The doses of imipramine administered ranged from 50 to 112 mg/kg. Physiological saline was injected intraperitoneally 20 minutes prior to imipramine administration to simulate the anticonvulsant injections, which were to be given in subsequent experiments.

The animals were kept in separate cages to avoid effects due to aggregation (96). Occurrence of convulsions (as described above) was recorded and the time to convulsions and time to death were measured from the time of imipramine injection. The animals were observed for a period of 150 minutes.

(iii) Analysis of Data: Theoretical dose-response curves were generated by logit analysis according to Waud, Part I (198). Equation 4.35, from Finney (51), was used to obtain the 95% confidence limits. For potency ratio determination, the ratio $\pm$ S.E. of the dose-response curves for lethality-to-convulsions was calculated according to the technique of Waud, Part IV (198).

d) Determination of Anticonvulsant Effectiveness

(i) Experimental Sequence: Rats were pretreated with various doses of anticonvulsant agents or with the appropriate drug vehicle, 20 minutes prior to an LD₉₀ of imipramine, administered intraperitoneally. To avoid differences due to order of treatment, the animals were pseudorandomized within blocks containing one representative from each group. In each case, the experimenter was not informed of the contents of the various dosing solutions that were used. Occurrence and time to convulsions, and time to death were recorded as described in c)ii).

(ii) Analysis of Data: To determine if the anti-convulsant substance was effective in preventing imipramine-induced seizures or death, the proportion of animals responding in the vehicle control group was compared with the proportion responding in each of the groups given the higher doses of anticonvulsant, by using Chi-square testing, formula 8.10.3, Snedecor and Cochran (1971). If the anti-convulsant effect was judged significant (Chi-square probability, 0.01), a theoretical dose-response curve was generated as follows:

(1) The proportion of animals responding in the absence of an effective dose of anticonvulsant (P_c) was determined from the minimum value of the series P_0 , $(P_0 + P_1)/2$, $(P_0 + P_1 + P_2)/3$, $(P_0 + P_1 + \dots + P_i)/(i + 1)$ $P_0 + P_1 + \dots + P_h)/(h + 1)$ where P_0 was the proportion of animals responding in the vehicle control group, P_i was the proportion responding for the i^{th} dose of the anticonvulsant, and P_h was the proportion responding for the highest dose of the anticonvulsant.

(2) The value $N_i P_c$ (where N_i = the total number of animals in the i^{th} group) was subtracted from the number of animals responding and from N_i for each group (including the vehicle control group where $N_i = N_0$). The vehicle control group was assigned a dose value 10^{-3} times smaller than the

lowest dose of anticonvulsant used, and the data obtained after subtracting the contribution due to P_c was used to generate a dose-response curve by the logit method of Waud (198).

The 95% confidence limits were generated from Finney's equation 4.35 (51). Potency ratio estimation was done according to Waud, Part IV (198).

3. Results

a) Comparison of Metrazol and Imipramine-Induced Seizures

Rats given pentylenetetrazol (90 mg/kg i.p.) displayed extremely violent, continuous convulsions within 45 seconds of injection. The body was curled forward and myoclonus of all four limbs was observed.

Imipramine-treated rats showed no seizure activity until more than five minutes after injection. Prior to the occurrence of convulsions, the animals exhibited a crouched posture, with ataxia or immobility. When seizures ensued, often only the forelimbs and head were involved, while the hindlimbs were spread out behind. Opisthotonos, with the tail curved over the animal's back, was frequently observed. In contrast to the continuous seizure activity seen in the pentylenetetrazol-injected animals, convulsions in the

imipramine-treated rats came in bursts, each lasting less than a minute. The same pattern of convulsions was seen in rats given lower doses of imipramine.

b) Convulsive Activity and Lethality of Imipramine

Fig. 1 shows the dose-response relationship for convulsive activity and lethality due to administration of imipramine. The data are expressed in the form of log dose-response curves, as determined from logit analysis. The CD_{50} of imipramine was calculated to be 64.7 mg/kg, with 95% confidence limits of 59.3 and 70.0. The LD_{50} was found to be only slightly higher - 85.8 mg/kg - with 95% confidence limits of 78.4 and 95.3. The two curves can be considered to be parallel, since their slopes (11.0 ± 2.7 / for convulsions, and 8.23 ± 1.99 for lethality) were not significantly different. The potency ratio between the assay curves for imipramine lethality and convulsive activity, calculated by using Waud's (198) technique, was 1.25, with 95% confidence limits of 1.15 and 1.36. The LD_{90} of imipramine was calculated from the theoretical dose-response curves to be 112 mg/kg (95% confidence limits = 100 and 149). This dose of imipramine was used in subsequent experiments to test the activity of anticonvulsant agents studied.

The median time to convulsions was found to be 7.5 min ($P_{25} = 6.5$, and $P_{75} = 9.5$). As expected from

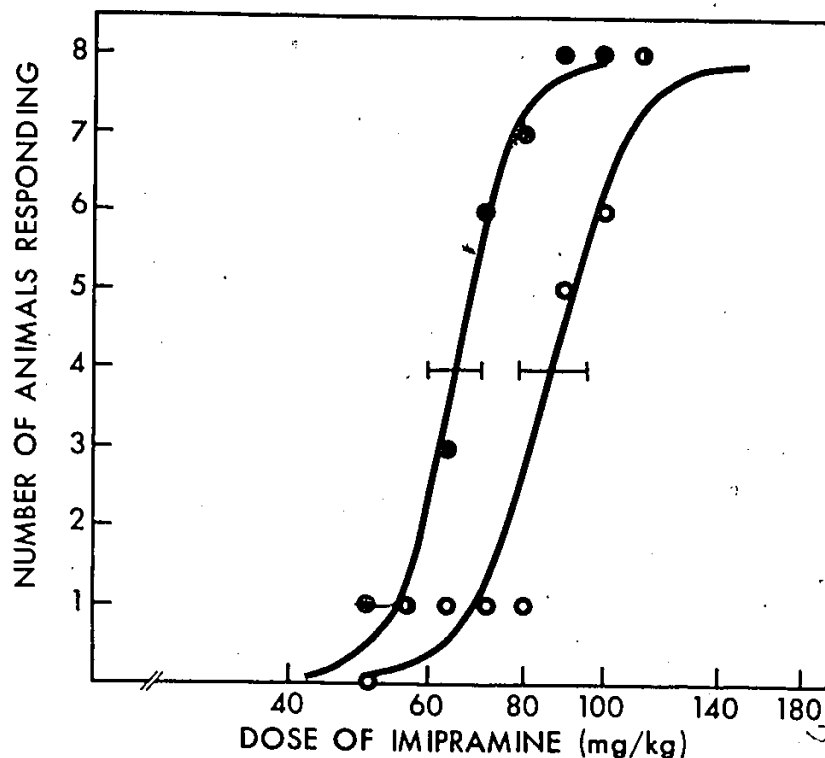


Fig. 1:

Dose response curves for imipramine-induced convulsions (●) and death (○). On the ordinate, the number of animals responding, on the abscissa (log scale), the dose of imipramine (mg/kg) injected intraperitoneally. The symbols represent experimentally obtained values, and the solid lines have been calculated by logit analysis (198). The horizontal bars represent the 95% confidence limits, eqn 4.35 Finney (51), of the ED₅₀.

pharmacokinetic considerations, the animals receiving higher doses tended to convulse sooner than those given lower doses of imipramine. The median time to death was 17.5 min ($P_{25} = 12.5$, and $P_{75} = 32.5$), and no meaningful relationship between the dose and the time to death was observed.

c) Modification of Convulsive Activity and Lethality of Imipramine by Anticonvulsants

(i) Imipramine-Induced Seizures: The ability of phenobarbital, diphenylhydantoin and diazepam to prevent the imipramine-induced seizures in rats was tested by administering varying doses of these anticonvulsants 20 minutes prior to an LD_{90} (112 mg/kg) of imipramine. The results are shown in Fig. 2. Chi-square tests, comparing the appropriate vehicle control group with the higher doses of anticonvulsant substances, showed that both diazepam and phenobarbital significantly protected the animals against imipramine-induced seizures ($p < 0.005$). Since virtually no protection against imipramine-induced convulsions was observed for animals pretreated with diphenylhydantoin, theoretical dose-response curves were generated (see Materials and Methods, c, iii) only for diazepam and phenobarbital. The PD_{50} value for diazepam was calculated to be 0.32 mg/kg (95% confidence limits = 0.05 and 0.70) and for phenobarbital,

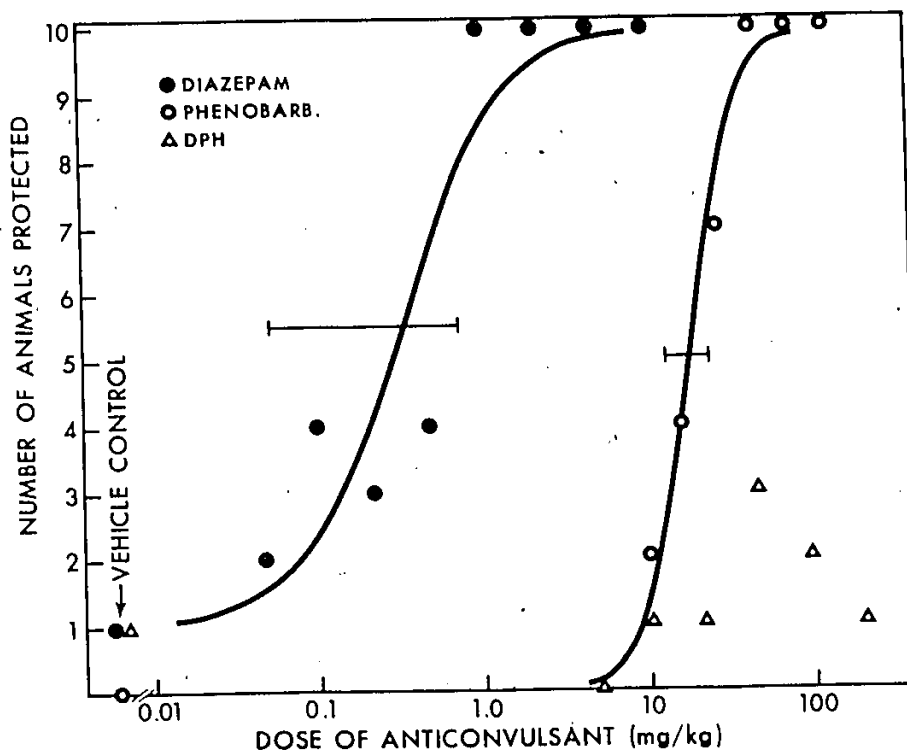


Fig. 2:

Protection against imipramine-induced convulsions achieved by administration of diazepam (●), phenobarbital (○) or diphenylhydantoin (Δ), 20 min prior to imipramine (112 mg/kg i.p.). On the ordinate, the number of animals protected, on the abscissa (log scale), the dose of the anticonvulsants (mg/kg). Theoretical dose-response curves were generated only for the data obtained with diazepam and phenobarbital.

17.6 mg/kg (95% confidence limits = 12.5 and 23.3). The potency ratio calculated by Waud's technique (198) was 32.2 (confidence limits = 15.5 and 67.2). The median time to convulsions was 9 min ($P_{25} = 7$, and $P_{75} = 11$) for animals pretreated with diazepam (including vehicle control animals), 8.5 min ($P_{25} = 6.5$, and $P_{75} = 11.5$) for the phenobarbital-pretreated rats and 8.5 min ($P_{25} = 6.5$, and $P_{75} = 9.5$) for rats pretreated with diphenylhydantoin. No obvious relationship between the time to convulsions and the dose of anticonvulsant agents was observed.

(ii) Imipramine-Induced Lethality: The anticonvulsants studied in these experiments (diazepam, phenobarbital and diphenylhydantoin) did not show any significant protection against imipramine-induced mortality (Table 1). In fact, both phenobarbital and diphenylhydantoin at higher dose levels seemed to enhance the imipramine lethality, but these effects were not significant by Chi-square testing. The median time to death was 22.5 min ($P_{25} = 12.5$, and $P_{75} = 37.5$) in diazepam-pretreated rats (including vehicle control groups), 17.5 min ($P_{25} = 12.5$, and $P_{75} = 27.5$) in animals pretreated with phenobarbital and 21 min ($P_{25} = 17$, and $P_{75} = 23$) in the group given diphenylhydantoin. Again, no meaningful relationship between the time to death and the dose of the anticonvulsant was observed for any of the substances tested.

Table 1

EFFECT OF ANTICONVULSANTS ON IMIPRAMINE-INDUCED LETHALITY

Diazepam		Phenobarbital		Diphenylhydantoin	
Dose (mg/kg)	Number of Deaths	Dose (mg/kg)	Number of Deaths	Dose (mg/kg)	Number of Deaths
Saline	10	Saline	5	Saline	9
Vehicle	6			Vehicle	6
0.05	8	10	6	5	9
0.1	7	16	4	10	6
0.2	8	27	4	21	6
0.5	4	44	5	45	9
1.0	6	73	9	95	9
2.2	5	120	9	200	10
4.7	6				
10.0	7				

The anticonvulsants or the corresponding vehicles were administered 20 min prior to imipramine (112 mg/kg i.p.). Each group consisted of 10 animals. No significant difference was detected by Chi-square testing between any group given a dose of anticonvulsant and the corresponding vehicle group.

d) Summary

Imipramine-induced seizure activity in rats consisted of bursts of myoclonus, mainly involving the head and forepaws. Opisthotonos, with the tail arched over the back, frequently was observed. After dosing, before convulsions occurred (5 - 10 minutes after injection) the rats usually remained crouched and immobile.

Diphenylhydantoin (5 - 200 mg/kg i.p.) was found to be completely ineffective in preventing seizures induced by an LD₉₀ dose of imipramine. In contrast, both diazepam and phenobarbital, against an LD₉₀ dose of imipramine, significantly suppressed the imipramine-induced convulsions. The PD₅₀ of diazepam, against an LD₉₀ dose of imipramine, was calculated to be 0.32 mg/kg (95% confidence limits = 0.05 - 0.70) and that of phenobarbital, 17.8 mg/kg (95% confidence limits = 12.5 and 23.3). None of these anticonvulsants caused a reduction in imipramine-induced mortality.

4. Discussion

These studies were carried out to establish which of the anticonvulsants, diphenylhydantoin, diazepam or phenobarbital, used clinically in poisoning with tricyclic antidepressants, is most efficacious in reducing imipramine-induced convulsions and mortality in rats. Knowledge of which anticonvulsant is best in blocking

imipramine-induced convulsions could avoid much of the dangerous polypharmacy seen in the treatment of these poisoning cases. Dosing was carried out by intraperitoneal rather than by oral route, because it was found in preliminary experiments that oral administration of imipramine inhibited gastric emptying, thereby blocking absorption and delaying the build-up of convulsive levels of imipramine. No doubt, in human poisoning cases where the drug is ingested, individual variation in drug absorption would result in a much less abrupt dose-response curve.

Clinically, depending upon its appearance, the convulsive activity seen in imipramine poisoning has been described by various authors as tonic-clonic seizures (177), (139), spasms (84), myoclonus of all limbs (172), status epilepticus (141), grand mal (56), (65) or just convulsions (146), (13), (129), (208), (53). Opisthotonos is also sometimes associated with the seizures (58), (61). Little experimental work has been done to characterize tricyclic antidepressant-induced seizures. Imipramine in large doses (30 - 50 mg/kg i.v.) has been shown to evoke convulsant spikes in rhinencephalic structures (195) and in the amygdala, hippocampus and other non-cortical areas in the rabbit (179).

As yet, few explanations of the convulsive activity of the tricyclic antidepressants have been ventured.

Recently, Burks et al (25) have hypothesized that the myoclonus observed in imipramine poisoning may be a result of increased serotonin levels at the synapse, due to a block of serotonin uptake produced by tricyclic antidepressants, since Klawans et al (88) found that large doses of 5-hydroxytryptamine, which increases brain serotonin levels, produced myoclonic movements.

The imipramine-induced seizures seen in the present experiments could best be described as myoclonic. Ataxia seen prior to convulsions in rats given high doses of imipramine may correspond to the ataxia and drowsiness seen in human poison victims prior to seizures (208), (177), (58). The steepness of the dose-response curves for imipramine indicates that a negligible fraction of the population was tolerant to the convulsive or lethal effects of imipramine. Since the dose ratio of lethality to convulsions for imipramine was found to be only 1.25, the occurrence of seizures is probably a very good indication that a poisoned patient is in critical danger.

An LD₉₀ dose of imipramine was used to test the effectiveness of the anticonvulsants, since this would not only determine whether or not the substance was successful in blocking seizures, but also give some idea as to whether or not it would reduce mortality. In these studies, the

anticonvulsants, diphenylhydantoin and diazepam, were not dissolved in the commercially used vehicles containing propylene glycol and alcohol, since these solvents may possess anticonvulsant properties in themselves.

Diphenylhydantoin has been used successfully in the long term treatment of seizures of the grand mal, focal and psychomotor varieties (22), (203). Its use for the prevention of tricyclic-induced seizures has also been advocated (133), though no good clinical or laboratory evidence exists to support this advice. In our experiments, diphenylhydantoin was found to be completely ineffective in preventing imipramine seizures in rats. In fact, there was indication that this drug might actually enhance imipramine's lethal effects perhaps by adding to the depressed state already brought about by the tricyclic drug.

The mechanism of diphenylhydantoin's anticonvulsant action may involve changes in ion flux across the nerve membrane (76). It has also been shown that the cerebellum is a necessary substrate for at least part of the anticonvulsant effect of diphenylhydantoin (81), (82), and even its effect of causing folic acid deficiency has been postulated to be involved in its anticonvulsant properties (140). Experimentally, diphenylhydantoin has been found ineffective in blocking pentylenetetrazol and other

chemically-induced seizures (189), (92), (44), nor does it raise electroshock seizure threshold unless it has been lowered from normal (190). In addition, Wise and Chinerman (204) demonstrated that diphenylhydantoin, in contrast to diazepam and phenobarbital, is ineffective against electrically kindled amygdaloid seizures in rats. This anticonvulsant is effective, however, in blocking the tonic phase of maximal electroshock seizure (205). The predominant effect of diphenylhydantoin appears to be its anti-spreading activity, attributed to its ability to block post-tetanic potentiation (206).

The ineffectiveness of diphenylhydantoin in blocking seizures due to imipramine is not surprising, since it is also without effect in infantile febrile convulsions (112) and the seizures associated with barbiturate withdrawal (43). Furthermore, diphenylhydantoin seems to be relatively ineffective against clonic seizures (166). In view of the results of these experiments, the use of diphenylhydantoin as an anticonvulsant in case of tricyclic antidepressant overdose cannot be recommended.

Phenobarbital, when compared to other barbiturates, has exceptionally strong anticonvulsant properties and is more specific than other barbiturates in raising the threshold for electrical stimulation in various brain

structures (40). It has also been shown to be as effective as trimethadione in protecting against pentylene-tetrazol-induced seizures (115). Its mode of action in blocking convulsions is unclear, but the limbic system appears to be especially prone to the depressant effects of various barbiturates (40). Clinically, phenobarbital is employed in the therapy of grand mal and various cortical focal seizures (189). It has also been used to treat tricyclic antidepressant-induced seizures (2), (139), (84). Our experiments suggest that phenobarbital is effective for this purpose.

A more recently developed drug now used as an anticonvulsant is the anxiolytic, diazepam. It has been found to be useful in the control of infantile myoclonic seizures (200), seizures of tetanus (199), (49) and it has been described as the drug of first choice in control of status epilepticus (59), (119), (101), (16). Currently, this drug is also being used to treat tricyclic antidepressant-induced seizures.

Diazepam has been said to have "a generalized depressant action upon epileptogenic structures throughout the brain" (72), and in particular, it is thought to act by depressant action on the limbic system (173). Electrophysiologically, diazepam has been found effective in

inhibiting maximal electroshock seizure (and increasing both electroshock and metrazol seizure thresholds in mice) (182).

Moreover, both strychnine and pentylenetetrazol-induced convulsions in mice are blocked by diazepam (137). The similarity between diazepam and diphenylhydantoin in stereochemical structure, as revealed by crystallographic studies, has been noted by Schussler (156), who suggested that both compounds may be able to bind with the same receptors in the central nervous system. The fact that both augment discharge in cerebellar Purkinje cells has been postulated as being the key to their anticonvulsant action (80), (81). However, the difference in the effectiveness of these two drugs in various tests of anticonvulsant activity would indicate a considerable dissimilarity in their mechanisms of action.

The results of experiments presented in Section 3.c) show that both diazepam and phenobarbital are fully effective in blocking the occurrence of seizures in imipramine poisoning. Clinical reports on the use of these two anticonvulsants in cases of poisoning with tricyclic antidepressants are in good agreement with our findings (139), (100), (208), (24). With both diazepam and phenobarbital, however, the prevention of seizures appeared to have no effect on the lethal properties of imipramine. This would

seem to indicate that control of seizures alone is not necessarily life saving, in itself, and other therapy may be required in addition to anticonvulsants.

Because of the extreme steepness of the dose-response curve of imipramine (Fig. 1), even a small uncontrolled variation from experiment to experiment could alter the values of CD_{50} or LD_{50} to a marked degree. This might explain, in the experiment testing the anticonvulsant effect of phenobarbital (Table 1), why an LD_{90} dose of imipramine caused only 50% mortality in the saline control group. The fact that the LD_{50} of imipramine was only 33% higher than the CD_{50} could explain the clinical observation that patients with seizures during imipramine poisoning are in critical condition (208), (177). It also should be noted that the vehicle control groups for both diazepam and diphenylhydantoin show an apparent (but not significant) decrease in mortality. The most likely explanation for this effect is that the extreme pH values of the two vehicles could have altered the peritoneal haemodynamics and hence the absorption of the challenging drug.

Evidence indicates that diazepam and phenobarbital have in common a depressant action on the limbic system, including the amygdala (173), (40). If convulsant activity due to imipramine originates in these structures, as it

would appear from the work of Steiner et al (179), this could explain the effectiveness of both phenobarbital and diazepam, and the ineffectiveness of diphenylhydantoin, in blocking imipramine-induced convulsions. On the other hand, since diphenylhydantoin has been found to act mainly by an anti-spreading action (206), the observed inability of this drug to prevent imipramine-induced convulsions suggests that such seizures do not depend on spreading for their development.

B. SAFETY OF ANTICONVULSANTS IN TREATMENT OF EXPERIMENTAL
IMIPRAMINE POISONING

1. Introduction and Aims

Although diazepam and phenobarbital, as indicated in Part A of this thesis, were found to be effective in suppressing imipramine-induced convulsions in rats, this does not necessarily mean that they are safe drugs to use in treatment of poisoning with tricyclic antidepressants. Severe disturbances in a number of physiological functions, including cardiovascular, respiratory, and thermoregulatory, which were shown to be associated with overdose of tricyclic antidepressants, necessitate great caution in administering other drugs that could worsen the patient's condition in some unforeseen manner. With this in mind, the effects of diazepam and phenobarbital on blood pressure, heart rate and body temperature of imipramine-poisoned rats were examined to investigate the relative safety of these drugs in poisoning with tricyclic antidepressants. The effects of imipramine on respiration rate and oxygen consumption were also examined, since experimental data on these important parameters is lacking.

2. Materials and Methods

a) Animals

Male Wistar rats were obtained from Woodlyn Farms (Guelph, Ontario) at least one week prior to experimentation. The animals were housed in colony cages and maintained on an unrestricted diet of pellet food. Weight range at the time of experimentation was 180 g. to 235 g.

b) Drugs

Solutions of imipramine hydrochloride, diazepam and sodium phenobarbital were prepared as described in Part A. The vehicle used for diazepam was a 0.9% saline solution brought to a pH of 1.73 with 1.0 N HCl. The dose of imipramine used in these experiments was 50 mg/kg. This corresponded closely to the CD_5 of imipramine (49.5 mg/kg), as calculated from the data obtained in experiments described in Part A. Any animals that convulsed following administration of the above dose of imipramine were rejected.

The anticonvulsants, phenobarbital and diazepam, were given in doses corresponding to PD_{90} against convulsions induced by an LD_{90} dose of imipramine, and calculated to be 40 mg/kg for phenobarbital sodium and 1.8 mg/kg for diazepam. All drugs were administered by intraperitoneal injection in a standard volume of 3.5 ml/kg.

c) Protocol for Cardiovascular Experiments

(i) Animal Preparation: To measure blood pressure and heart rate, the left carotid artery of each rat was cannulated under halothane anaesthesia with a PE50 Intramedic polyethylene tubing (Clay-Adams) according to the method of Popovic and Popovic (132). An exception to this technique was that the cannula was inserted only a distance of about 3 cm into the carotid artery, so that it did not reach the aorta. The cannula was exteriorized through the skin of the back of the rat, at a point between the scapulae. Before sealing, the cannulae were filled with physiological saline containing heparin (1,000 USP units/ml) to maintain patency. The animals were starved overnight, and the morning of experimentation the cannulae were again flushed with physiological saline containing heparin.

(ii) Method of Recording: Blood pressure and heart rate were recorded by attaching the exteriorized carotid cannula to a pressure transducer (P23AA, P23Dc, Statham Instruments, Inc.) via an extension cannula (PE90, Clay-Adams) 50 cm in length. The transducers were coupled to a Grass Model 5D polygraph.

(iii) Experimental Sequence: Matched controls were used in each experiment. An initial control reading was made immediately before drug injection. The animals

were then given an intraperitoneal injection of imipramine and readings of blood pressure or heart rate were taken at 5, 10 and 15 minutes following the injection. Immediately after the 15-minute reading, the anticonvulsant drug or the corresponding vehicle was administered. Readings continued to be taken every 5 minutes for the next 15 minutes, after which the readings were taken every 10 minutes for an additional 60 minutes. The blood pressure was recorded continuously during the 90-minute experimental period. The chart speed was 0.25 mm/sec, except for the readings at the above-mentioned time intervals, when the chart speed was increased for 1 minute to 5 mm/sec.

(iv) Analysis of Data: Heart rate was determined by counting the number of beats recorded in a 5-second interval at each recording time and multiplying by 12, to give a figure in beats/min. Systolic and diastolic blood pressure were read directly from the polygraph recording. The sensitivity was calibrated so that 1 cm deflection corresponded to 50 mm Hg. Since the pressure transducers were mounted approximately 16 cm above the animals' heart level, all direct readings of blood pressure were corrected by a factor of 11.8 mm. The mean blood pressure was then calculated according to the formula:

$$\frac{\text{systolic pressure} + \text{diastolic pressure}}{2} + 11.8.$$

Each experimental animal was used as its own control, and changes in blood pressure and heart rate were expressed in percentages taking the initial reading (prior to imipramine dosing) as 100%. Absolute initial values are given in legends to respective figures and absolute values from all experiments are contained in Appendix A. The number of animals used in various experimental groups is indicated in the legends to figures, as well as in Appendix A. The effect of treatment was evaluated, using a two-tailed Student's t-test for unpaired data.

As an alternate method to test for differences between control and treated groups, slope analysis of the time-series data obtained just prior to, and at several time intervals following drug administration, was used. This was accomplished as follows:

(1) The time-series data from each group of animals were tested to determine the length of time for which linearity was preserved. The regression slope of the data (using time as the abscissa) obtained just prior to (t_0) and at the first time interval (t_1) following drug administration was determined and compared with the regression slope of the data obtained at each successive pair of consecutive points (i.e., the slope of t_0-t_1 , was compared with that of t_1-t_2 , then with t_2-t_3 , etc.). As soon

as the slope of $t_i - t_{i+1}$, was found to be significantly different ($p < 0.05$) the process was terminated and the data from $t_0 - t_n$, inclusive, were taken to belong to the same straight line.

(2) The group with the shortest "straight" line segment, as obtained from (1), determined the time interval over which the slope of the data from the treatment group was compared with the slope of the data from the control group. For example, if the "linear" portion of the control group extended from t_0 to t_5 , and the "linear" portion of the treated group from t_0 to t_3 , the slopes from both groups would be compared from times t_0 to t_3 .

(3) Steps (1) and (2) were repeated for the remaining time points by re-assigning t_0 to the last time point used in step (2). In our example, t_3 would be the new t_0 . This process was repeated until the data from all the time points were analysed. Regression slopes were compared by using the formulae numbered 18G in Documenta Geigy (38). An example of slope analysis is given in Appendix C.

d) Protocol for the Measurement of Rectal Temperature

(i) Animal Preparation: Animals were starved overnight prior to the experiment.

(ii) Method of Recording: Rectal temperature was recorded by inserting a thermal probe (constructed from a thermistor, GB 32J2, Fenwal, Mass.) a distance of 6 cm into the rectum. The probe was secured to the tail with tape so that it remained in place for the duration of the experiment. Rectal temperature was read from a tele-thermometer (Model 46 TU, Yellow Springs Instruments Co.).

(iii) Experimental Sequence: Matched controls were used in each experiment. An initial control reading was made before imipramine was injected intraperitoneally. Measurements of rectal temperature were made at 5, 10, and 15 minutes following the dosing. When the effect of two drugs was being tested, the second drug was injected immediately after the 15-minute reading. Then, further readings were made at 20, 25, and 30 minutes, and then every 10 minutes until 90 minutes after imipramine administration.

(iv) Analysis of Data: Data were corrected according to the calibration factor of each probe and converted from Fahrenheit to Celsius scale. The effect of treatment was evaluated as in c)(iv).

e) Protocol for the Measurement of Respiratory Function

(i) Animal Preparation: The animals were starved overnight before the experiment.



(ii) Method of Recording: For recording of oxygen consumption, each rat was placed in a plexiglass chamber, constructed with a water jacket enclosing all sides (see Fig. 3). The water was cycled through a constant temperature circulator (Type K2R MGW Lauda Ltd., W. Germany) which could be set to the desired water temperature. After a period of equilibration (about 20 minutes) the system could maintain temperature inside the chamber at any desired level, which in these experiments was 21°C. The inside volume of the chamber was 2.8 L.

Once the lid was sealed against a rubber "O" ring, oxygen was supplied to the inside of the chamber from a polyethylene bag containing pure oxygen at atmospheric pressure. As the animal respired, consuming oxygen and producing carbon dioxide, the latter was absorbed into a 20% KOH solution (100 ml) placed at the bottom of the chamber. (The rat rested on a stainless steel mesh over the KOH). Water vapour, CO₂, O₂ and temperature were equilibrated within about 5 minutes.

The changes in resistance to the gas flow in the narrow bore tubing connecting the oxygen supply bag with the chamber, caused by small pressure fluctuation with each breath, enabled the respiratory rate to be monitored by a pressure transducer (P23 Dc Statham Instruments, Inc.).

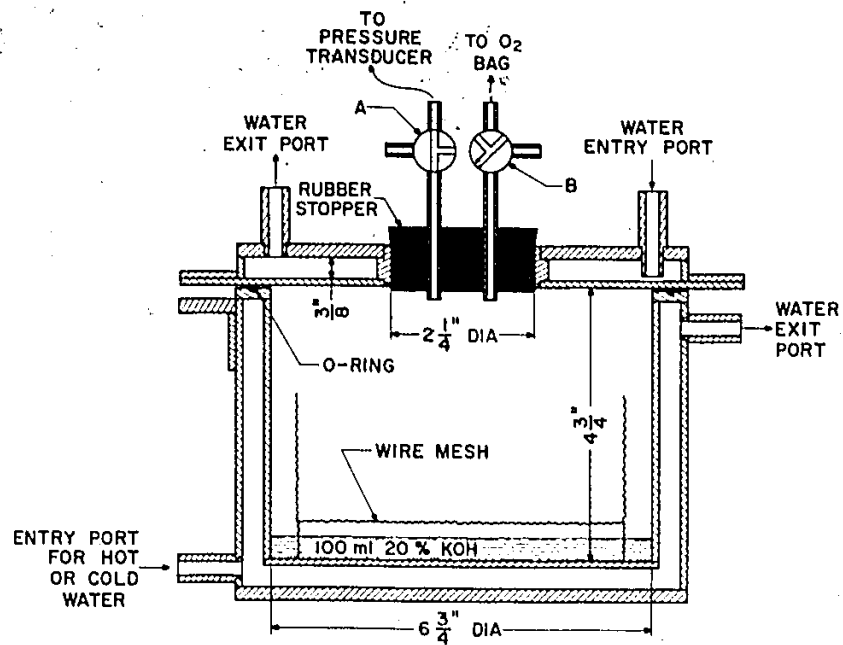


Fig. 3:

Diagrammatic representation of temperature-controlled respiration chamber used to measure oxygen consumption and respiration rate.

Oxygen consumption was calculated from the pressure change which occurred within the chamber after the valve to the oxygen bag was sealed off.

(iii) Experimental Sequence: In these experiments, the effect of imipramine on respiration rate and oxygen consumption was investigated. Saline-injected controls and imipramine-treated animals were run alternately. An initial control reading was taken before drug injection while the animal was in a resting state. The animal then was removed from the chamber and given an intraperitoneal injection of either saline or imipramine. The animal was returned to the chamber, which was then re-sealed and re-equilibrated. Readings of respiration rate and oxygen consumption were taken at 10, 20, 25 and 30 minutes, and then every 10 minutes until the end of the experiment (90 minutes after the drug administration).

(iv) Analysis of Data: The change in pressure (Δp), recorded while the valve to the oxygen bag was closed, was proportional to the volume of oxygen removed. This change in pressure was converted into oxygen consumption according to the following formula:

$$\begin{aligned} O_2 \text{ (ml/min)} &= \frac{\Delta p}{P} \times V \times \frac{1}{\Delta t} \times \frac{P}{760} \times \frac{273}{T} \\ &= \frac{\Delta p \times V \times 273}{\Delta t \times 760 \times T} \end{aligned}$$

where P is the barometric pressure (mm Hg), V is the chamber volume (ml) minus the rat's weight (g) and 100 ml for the KOH, T is the chamber temperature ($^{\circ}\text{K}$) and Δt is the time (min) over which Δp occurred. The factors $P/760$ and $273/T$ bring the volume of O_2 to standard pressure and temperature conditions. Since P conveniently cancels out, there is no need to keep track of fluctuations in barometric pressure. To remove variability due to differences in body weight, the O_2 consumption value was further divided by the animal's weight raised to the power 0.73 (135). Data was evaluated for treatment effect as described in Section c)(iv).

3. Results

a) Effect of Imipramine on Cardiovascular Parameters

The time course of changes in blood pressure of rats administered imipramine (50 mg/kg i.p. - CD_5) in comparison to that of the control group is shown in Fig. 4. The data are expressed in percentages, (taking the initial values (before imipramine or saline injection) as 100%. Blood pressure of the imipramine-treated group showed, within 10 minutes after injection of imipramine, a statistically significant drop to 80 - 85% of initial values. This effect lasted for the duration of the experiment.

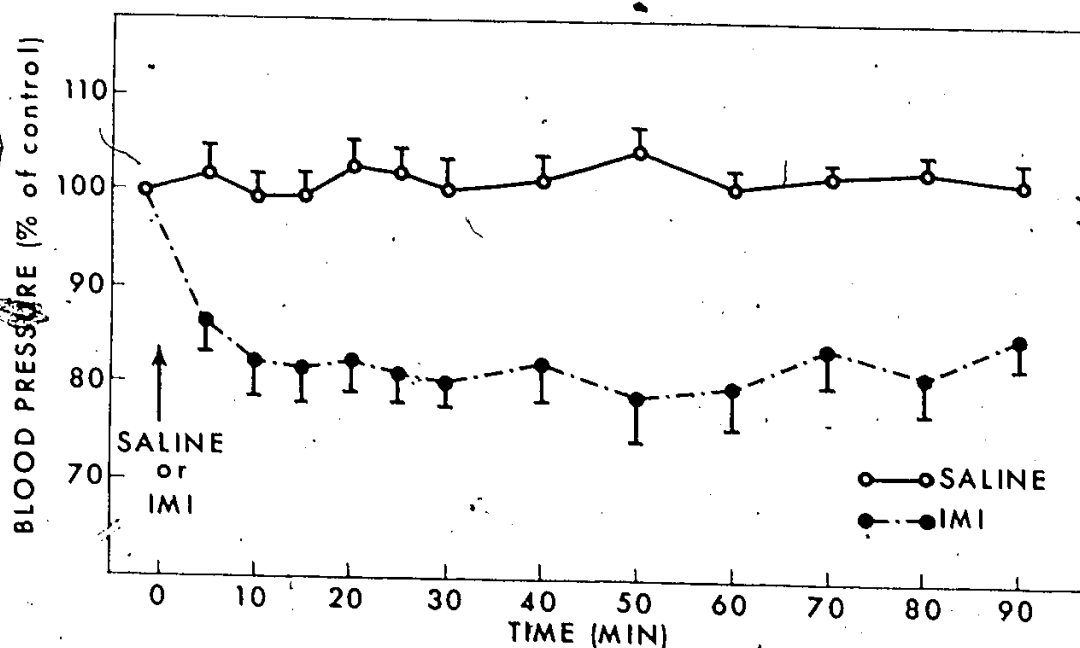


Fig. 4:

Effect of imipramine on blood pressure. Groups were injected i.p. at time 0 with either saline $\circ-\circ$ ($n=11$), or imipramine (50 mg/kg) $\bullet-\bullet$ ($n=10$). Blood pressure was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Blood pressure is expressed as a percentage of the control (or initial) values which were, for the saline group, 92.6 ± 1.3 mm Hg and for the imipramine group, 99.5 ± 2.5 mm Hg. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

All the values from the group of animals treated with imipramine, from 5 minutes on, are significantly different from the corresponding saline values ($p < 0.005$).

The administration of imipramine produced, at 5 minutes post-injection, an initial transient, non-significant increase in heart rate (Fig. 5). This was followed by a steady decrease in the heart rate of the imipramine-treated rats, becoming statistically significant within 20 minutes after the injection. At peak effect, 60 minutes after injection, the heart rate dropped almost 20% from the initial value. A very gradual return toward the normal rate then occurred, which was still incomplete at the end of the experiment. Analysis of slope confirmed the difference in blood pressure and heart rate between the control and treated groups, as analysed by Student's t-test.

b) Modification by Anticonvulsants

(i) Effect of Phenobarbital: Phenobarbital sodium (40 mg/kg i.p.), administered alone, had no significant effect on either blood pressure (Fig. 6) or heart rate (Fig. 7) of experimental animals.

Blood pressure, in the group given imipramine (50 mg/kg) and phenobarbital, did not differ from that of the group given imipramine and saline (Fig. 8). All of the post-imipramine-injection values for both the imipramine + saline and the imipramine + phenobarbital groups differed significantly from the values of saline-injected controls, with the exception of the 5-minute value of the imipramine and saline-injected group.

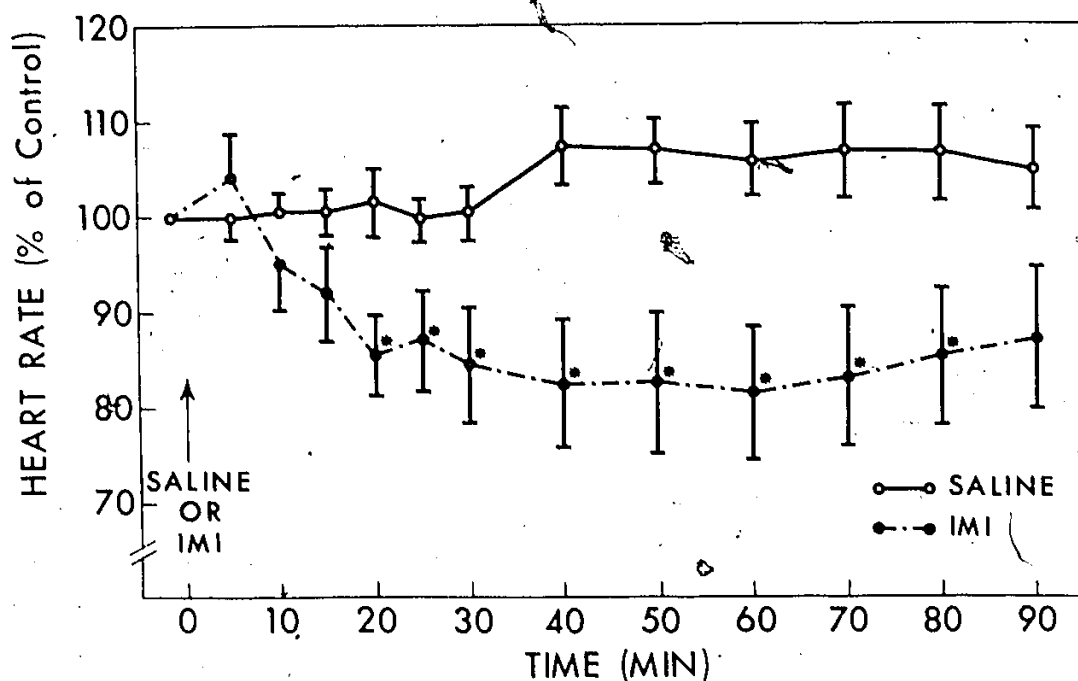


Fig. 5:

Effect of imipramine on heart rate. Groups were injected i.p. at time 0 with either saline $\circ-\circ$ ($n=10$), or imipramine (50 mg/kg) $\bullet-\bullet$ ($n=11$). Heart rate was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Heart rate is expressed as a percentage of the control (or initial) values which were, for the saline group, 343.1 ± 8.2 beats/min and for the imipramine group, 350.2 ± 10.2 beats/min. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

* A statistically significant difference when compared to animals given saline ($p < 0.05$).

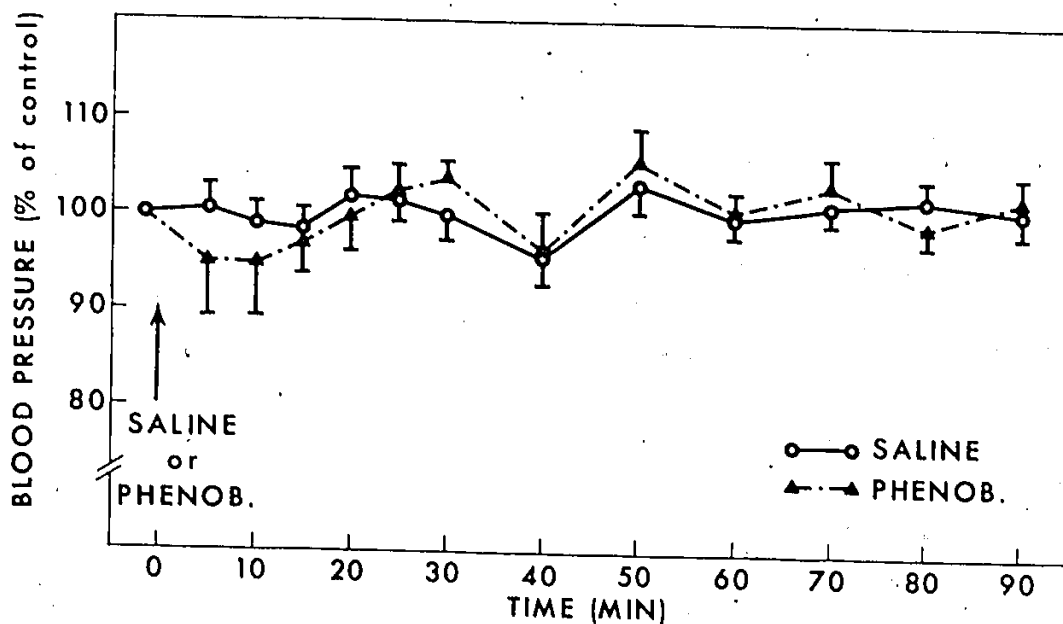


Fig. 6:

Effect of phenobarbital on blood pressure. Groups were injected i.p. at time 0 with either saline $\circ-\circ$ ($n=13$), or phenobarbital sodium (50 mg/kg) $\triangle--\triangle$ ($n=6$). Blood pressure was recorded every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Blood pressure is expressed as a percentage of the control (or initial) values which were, for the saline group, 92.9 ± 1.2 mm Hg and for the phenobarbital group, 91.3 ± 3.8 mm Hg. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

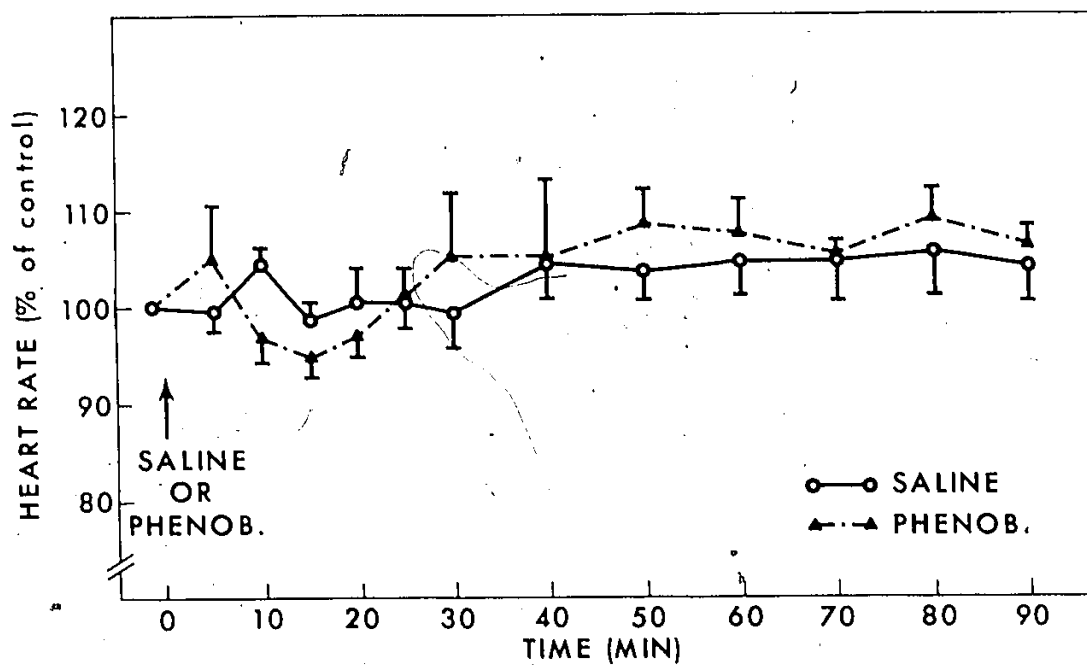


Fig. 7:

Effect of phenobarbital on heart rate. Groups were injected i.p. at time 0 with either saline $\circ-\circ$ ($n=13$), or phenobarbital sodium (40 mg/kg) $\triangle-\triangle$ ($n=6$). Heart rate was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Heart rate is expressed as a percentage of the control (or initial) values which were, for the saline group, 340.2 ± 7.2 beats/min and for the phenobarbital group, 317.0 ± 8.5 beats/min. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

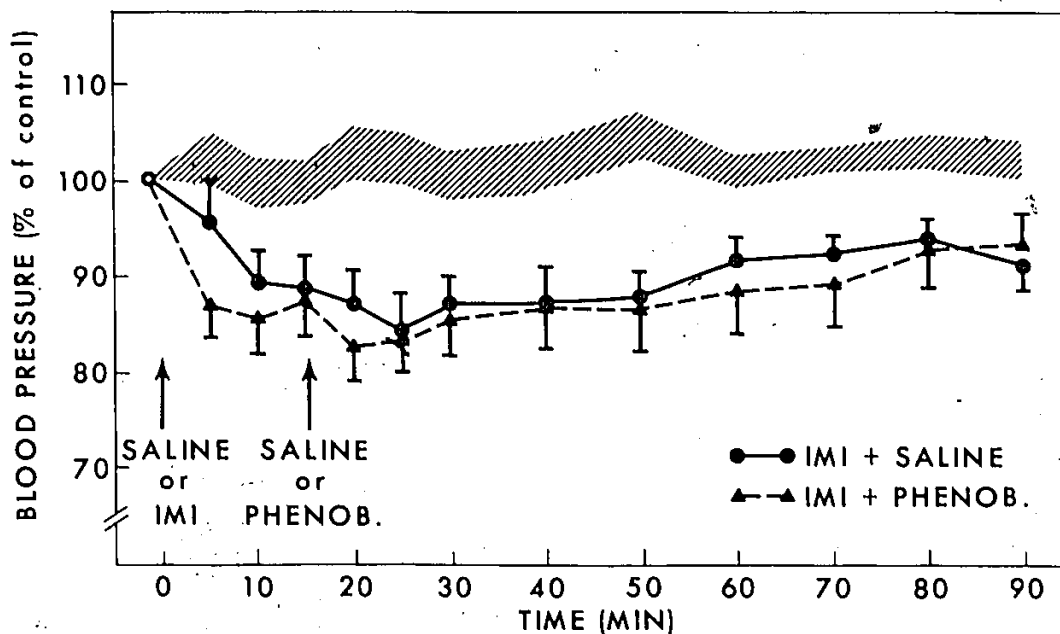


Fig. 8:

Effect of imipramine + phenobarbital on blood pressure. Groups were injected i.p. with either saline at time 0 (hatched area) (n=11), or imipramine (50 mg/kg) at time 0 and saline after 15 minutes (solid line with circles) (n=12), or imipramine (50 mg/kg) at time 0 and phenobarbital (40 mg/kg) after 15 minutes (dashed line with triangles) (n=14). Blood pressure was recorded every 5 minutes for the first 30 minutes, and then every 10 minutes for the final 60 minutes. Blood pressure is expressed as a percentage of the control (or initial) values which were, for the saline group, 92.6 ± 1.3 mm Hg; for the imipramine + saline group, 104.2 ± 3.2 mm Hg and for the imipramine + phenobarbital group, 97.5 ± 2.8 mm Hg. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

All of the post-injection values for both of the imipramine-treated groups showed statistically significant difference when compared to the animals given saline ($p < 0.05$), with the exception of the 5-minute value in the imipramine + saline curve.

As expected, administration of imipramine alone (Fig. 9) again resulted in a decrease of heart rate, which at 15, 25, 40 and 50 minutes showed a statistically significant difference when compared to the group injected with saline ($p < 0.05$). These significances, as determined by Student's t-test, were confirmed by analysis of slope. The heart rate values in the imipramine + phenobarbital group remained close to the values seen in the imipramine + saline group until after 40 minutes, when they began to climb back to the range of initial values. None of the data points on this curve showed a statistically significant difference when compared to either those of the curve for saline alone or those of the curve for imipramine + saline.

(ii) Effect of Diazepam: The blood pressure (Fig. 10) and heart rate (Fig. 11) of animals given diazepam (1.8 mg/kg i.p.) showed no statistically significant difference from those of animals given the drug-vehicle, though blood pressure values tended to be lower in animals treated with the anticonvulsant.

In this series of experiments, the blood pressure of animals given imipramine alone decreased within 15 minutes to about 85% of control values and tended to stay within this range for the rest of the experimental period (Fig. 12). Diazepam, given 15 minutes after

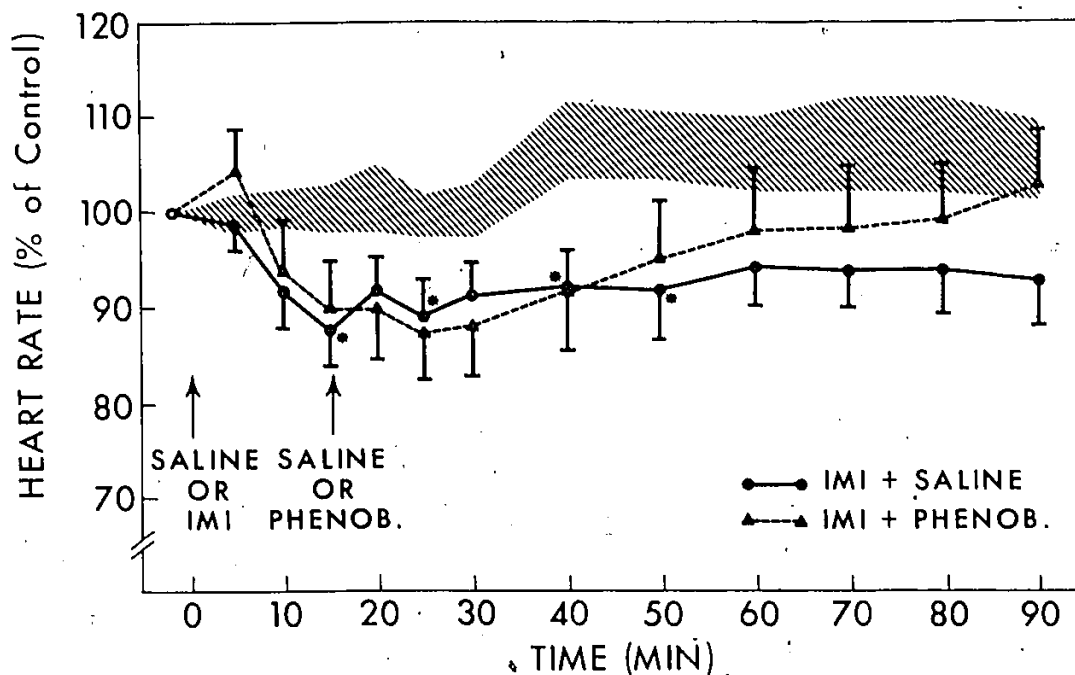


Fig. 9:

Effect of imipramine + phenobarbital on heart rate. Groups were injected i.p. with either saline at time 0 (hatched area) (n=11), or imipramine (50 mg/kg) at time 0 and saline after 15 minutes (solid line with circles) (n=12), or imipramine (50 mg/kg) at time 0 and phenobarbital (40 mg/kg) after 15 minutes (dashed line with triangles) (n=14). Heart rate was measured every 5 minutes for the first 30 minutes, and then every 10 minutes for the final 60 minutes. Heart rate is expressed as a percentage of the control (or initial) values (beats/min) which were, for the saline group, 343.1 ± 8.2 ; for the imipramine + saline group, 379.5 ± 14.7 and for the imipramine + phenobarbital group, 384.9 ± 11.3 . Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

* A statistically significant difference when compared to animals given saline ($p < 0.05$).

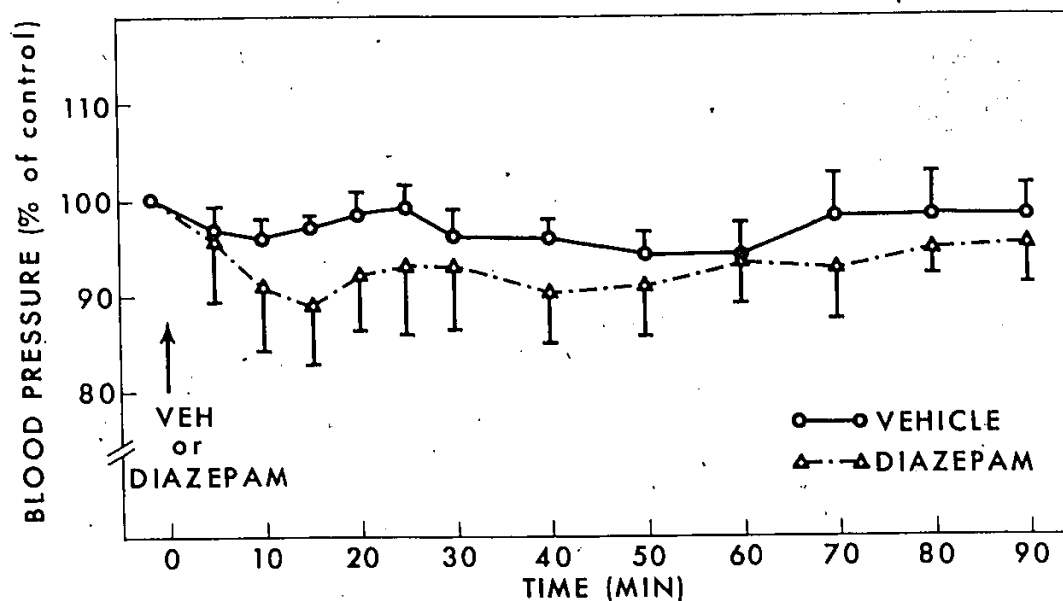


Fig. 10.

Effect of diazepam on blood pressure. Groups were injected i.p. at time 0 with either diazepam drug-vehicle $\circ-\circ$ ($n=6$), or diazepam (1.8 mg/kg) $\Delta--\Delta$ ($n=6$). Blood pressure was recorded every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Blood pressure is expressed as a percentage of the control (or initial) values which were, for the vehicle group, 99.6 ± 9.5 mm Hg and for the diazepam group, 99.3 ± 9.2 mm Hg. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

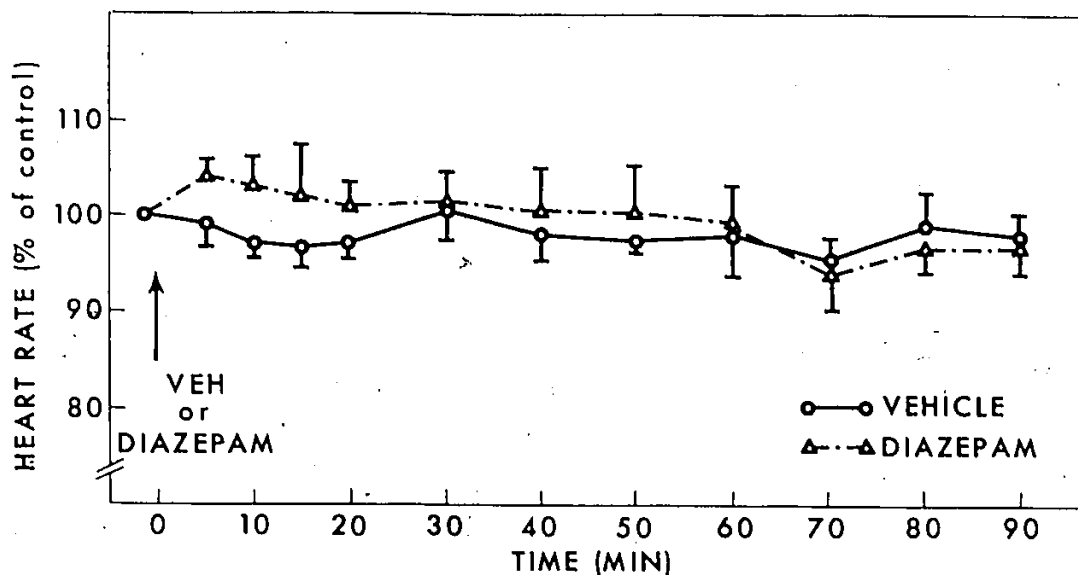


Fig. 11:

Effect of diazepam on heart rate. Groups were injected i.p. at time 0 with either diazepam drug-vehicle o—o (n=10), or diazepam (1.8 mg/kg) △---△ (n=6). Heart rate was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Heart rate is expressed as a percentage of the control (or initial) values which were, for the vehicle group, 330 ± 5.1 beats/min and for the diazepam group, 351 ± 14.8 beats/min. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

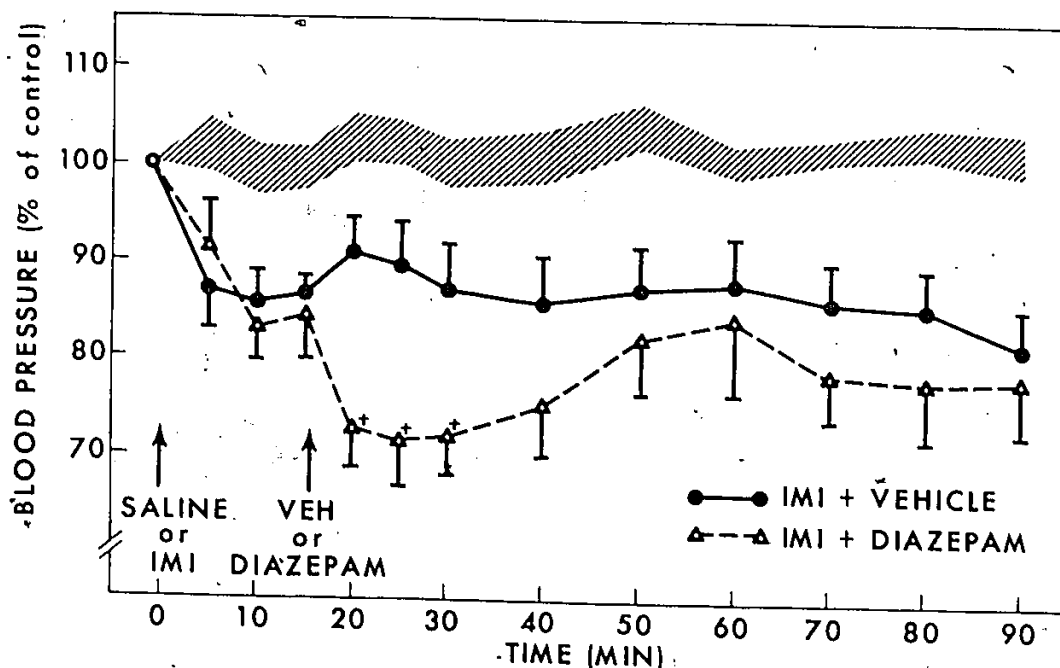


Fig. 12:

Effect of imipramine + diazepam on blood pressure. Groups were injected i.p. with either saline at time 0 (//////) (n=11), or imipramine (50 mg/kg) at time 0 and diazepam vehicle after 15 minutes (●—●) (n=10), or imipramine (50 mg/kg) at time 0 and diazepam (1.8 mg/kg) after 15 minutes (Δ—Δ) (n=11). Blood pressure was recorded every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Blood pressure is expressed as a percentage of the control (or initial) values which were, for the saline group, 92.6 ± 1.3 mm Hg; for the imipramine + vehicle group, 93.1 ± 2.3 mm Hg and for the imipramine + diazepam group, 100.9 ± 3.0 mm Hg. Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

+ A statistically significant difference when compared to the animals given imipramine + vehicle ($p < 0.05$). All the values from 5 minutes on, in both the imipramine + vehicle and imipramine + diazepam groups, are statistically different from the corresponding values in animals treated with saline ($p < 0.05$).

imipramine, caused a further sharp decrease in blood pressure to a low of about 72% of saline values 10 minutes after injection. From 5 minutes on, the blood pressure in both experimental groups showed a statistically significant difference when compared to that of the saline-injected group. At the 20, 25 and 30-minute readings, the values of the imipramine + diazepam group showed a statistically significant decrease when compared to the corresponding values of the imipramine + vehicle group by Student's t-test. However, slope analysis, conducted as described in the Materials and Methods, 2.c)(iv), failed to reveal any significant differences in blood pressure between the imipramine + diazepam and the imipramine + vehicle groups.

The imipramine-treated animals again showed an initial transient increase in heart rate followed by a progressive decrease, which from 40 to 80 minutes showed a statistically significant difference when compared to the mean values in animals given saline alone ($p < 0.05$). In contrast, the heart rate of animals receiving diazepam 15 minutes after the imipramine injection failed to continue decreasing and virtually remained within the range of values seen in control, saline-injected animals. Diazepam thus appeared to block the decrease in heart rate produced by imipramine. The mean values of the imipramine +

diazepam group differed significantly from the corresponding values of the imipramine + vehicle group at the 40, 50 and 70-minute time intervals (Fig. 13). These differences were confirmed by analysis of slope.

(iii) Summary: Imipramine (50 mg/kg i.p.) depressed both blood pressure and heart rate, although an initial transient increase in heart rate was observed in most experiments. Neither phenobarbital nor diazepam, administered alone (in doses corresponding to PD_{90} against imipramine-induced convulsions), significantly affected the cardiovascular parameters studied, though diazepam showed a tendency to lower the blood pressure. Phenobarbital, administered in the above doses 15 minutes after imipramine, had no immediate effect on the imipramine-induced depression of heart rate and blood pressure. However, after 15 minutes a trend to return to initial heart rate was seen in this group. When diazepam was given 15 minutes after imipramine, the usual imipramine-induced decrease in heart rate was antagonized, but the hypotensive effect of imipramine was transiently enhanced. This latter effect was demonstrated by t-test analysis but was not confirmed by slope analysis.

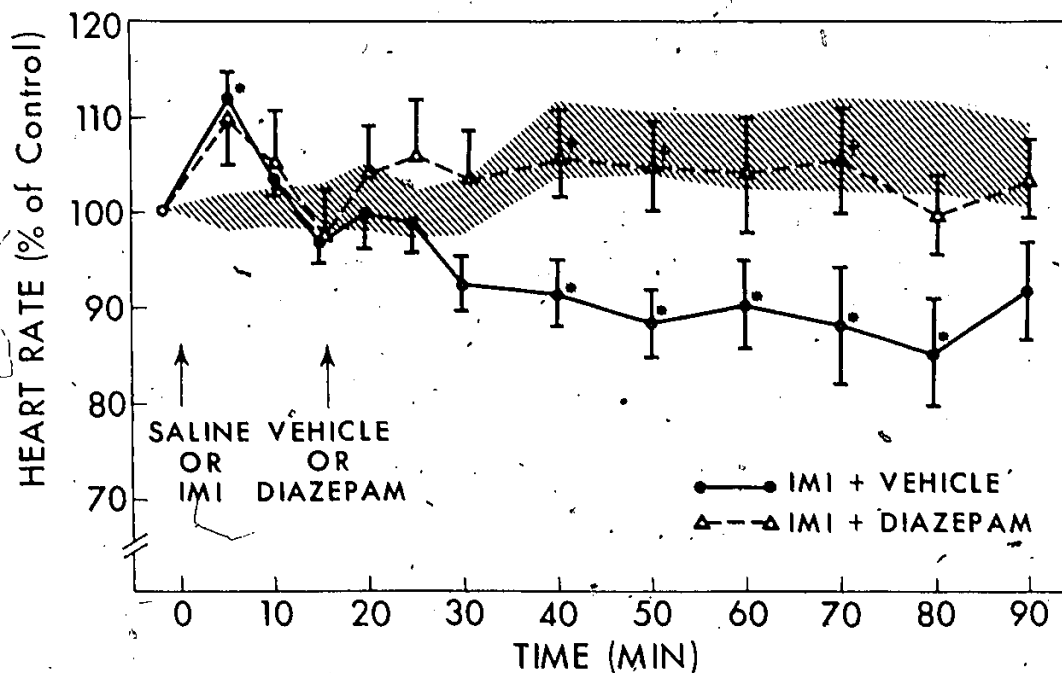


Fig. 13.

Effect of imipramine + diazepam on heart rate. Groups were injected i.p. with either saline at time 0 (hatched area) (n=11), or imipramine (50 mg/kg) at time 0 and diazepam vehicle after 15 minutes (●—●) (n=10), or imipramine (50 mg/kg) at time 0 and diazepam (1.8 mg/kg) after 15 minutes (△--△) (n=11). Heart rate was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Heart rate is expressed as a percentage of the control (or initial) values which were, for the saline group, 343.1 ± 8.2 ; for the imipramine + vehicle group, 324.6 ± 7.2 and for the imipramine + diazepam group, 342 ± 11.4 . Points represent the mean values $\pm$ S.E.M. (See Appendix A for absolute values).

* A statistically significant difference when compared to the animals given saline ($p < 0.05$).

+ A statistically significant difference when compared to the animals given imipramine + vehicle ($p < 0.05$).

c) Effect of Imipramine on Body Temperature

Saline-injected control animals showed an initial rise in body temperature, followed by a slight drop during the course of the experiment (Fig. 14). In animals treated with imipramine, the rectal temperature, after an initial slight increase, decreased steadily at a rate of about $0.023^{\circ}\text{C}/\text{min}$ and by the end of the 90-minute experimental period dropped to $35.8 \pm 0.2^{\circ}\text{C}$. The mean values from 60 minutes onward showed a difference that was statistically significant when compared to values of the saline-treated control group. Slope analysis confirmed a significant difference between the two groups ($p < 0.001$).

d) Modification by Anticonvulsants

(i) Effect of Phenobarbital: Phenobarbital (40 mg/kg i.p.) alone produced a decrease in rectal temperature similar to that seen after injection of imipramine. Mean body temperature, from 30 minutes after injection, showed a statistically significant difference when compared to that of the group given saline (Fig. 15). This was confirmed by analysis of slope.

The temperature changes seen after injection of imipramine, followed by saline, were the same as seen after injection of imipramine alone (see Fig. 14). In the animals given imipramine, followed by phenobarbital (Fig. 16),

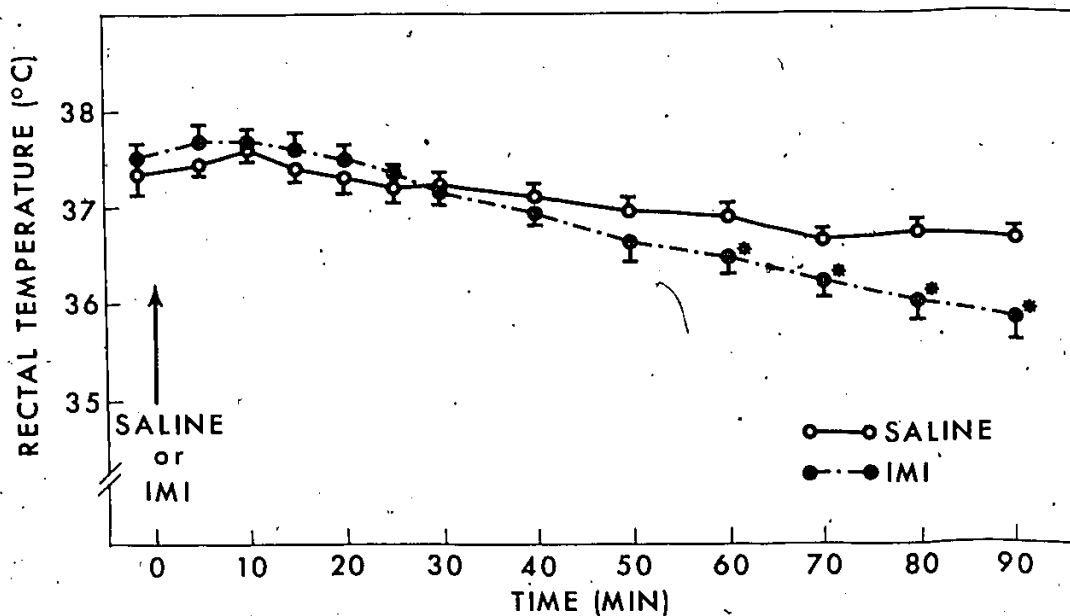


Fig. 14:

Effect of imipramine on rectal temperature. Groups were injected i.p. at time 0 with either saline $\circ$ — $\circ$ (n=10), or imipramine, (50 mg/kg) $\bullet$ — $\bullet$ (n=10). Rectal temperature was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the next 60 minutes. Rectal temperature is expressed in degrees centigrade. Points represent the mean values $\pm$ S.E.M.

* A statistically significant difference when compared to animals given saline ($p < 0.05$).

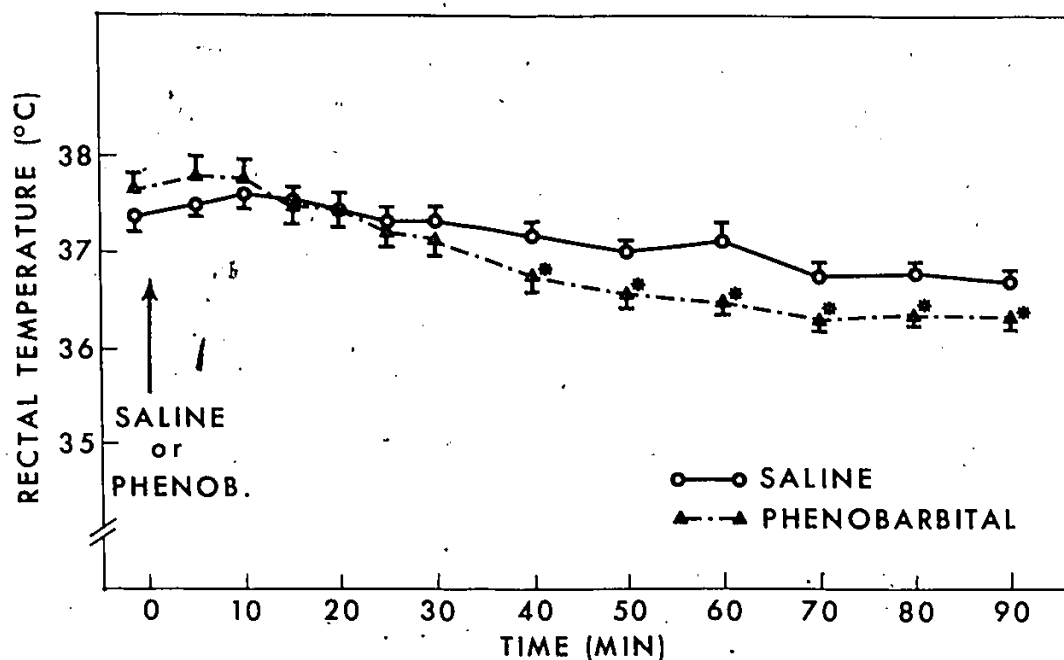


Fig. 15:

Effect of phenobarbital on rectal temperature. Groups were injected i.p. at time 0 with either saline $\circ$ — $\circ$ ($n=13$), or phenobarbital sodium (40 mg/kg) $\blacktriangle$ — $\blacktriangle$ ($n=6$). Rectal temperature was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the next 60 minutes. Rectal temperature is expressed in degrees centigrade. Points represent the mean values $\pm$ S.E.M.

* A statistically significant difference when compared to animals given saline.

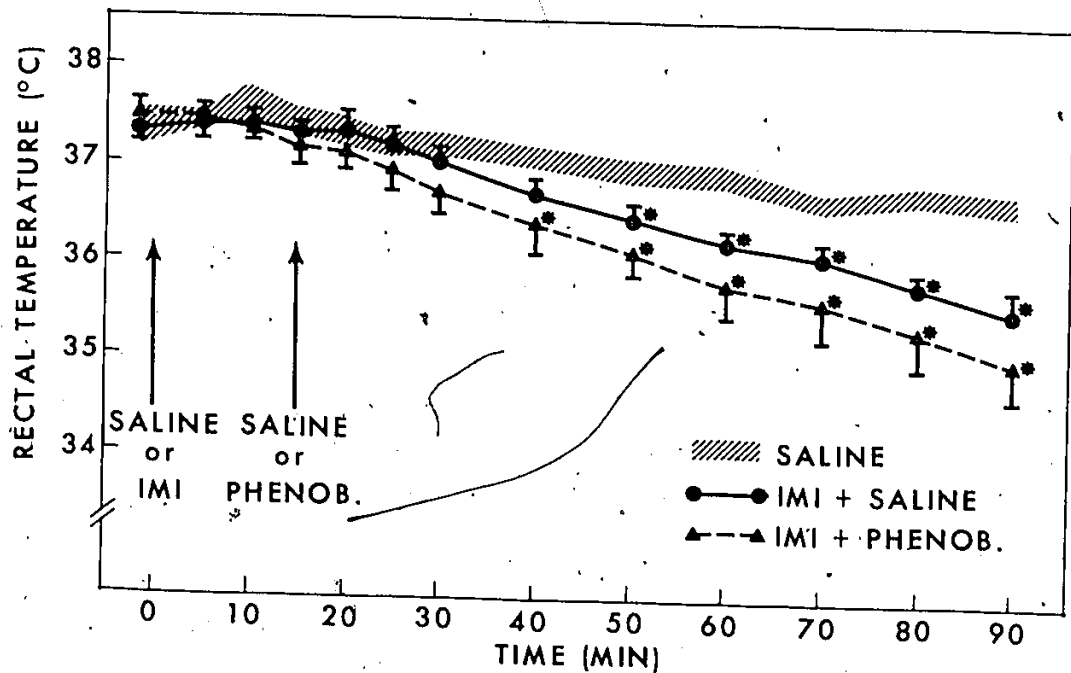


Fig. 16:

Effect of imipramine + phenobarbital on rectal temperature. Groups were injected i.p. with either saline at time 0 (hatched area) (n=10), or imipramine (50 mg/kg) at time 0 and saline after 15 minutes (solid line with circles) (n=10), or imipramine (50 mg/kg) at time 0 and phenobarbital sodium (40 mg/kg) after 15 minutes (dashed line with triangles) (n=10). Rectal temperature was measured every 5 minutes for the first 30 minutes, and then every 10 minutes for the final 60 minutes. Rectal temperature is expressed in degrees centigrade. Points represent the mean values $\pm$ S.E.M.

* A statistically significant difference when compared to animals given saline ($p < 0.05$).

body temperature continued to decrease, and by 90 minutes after the imipramine injection (75 minutes after the phenobarbital injection), had reached $35.0 \pm 0.4^{\circ}\text{C}$. (Initial body temperature for this group was $37.5 \pm 0.02^{\circ}\text{C}$). In this group, the mean values from 40 minutes onward differed significantly from the values of the saline-injected controls ($p < 0.05$). No points on this curve showed statistically significant difference when compared to the corresponding values of the imipramine + saline group. However, the drop in body temperature in the imipramine + phenobarbital group achieved significance, with respect to saline-treated control, 10 minutes earlier than in the imipramine + saline group. The rate of decline in body temperature for the imipramine + saline and the imipramine + phenobarbital groups was about the same ($0.025^{\circ}\text{C}/\text{min}$ and $0.028^{\circ}\text{C}/\text{min}$, respectively). Slope analysis also failed to show a significant difference between these two groups.

(ii) Effect of Diazepam: Rectal temperature in the animals given the vehicle only followed very much the pattern seen in animals given saline alone, namely, it displayed a very slight initial increase followed by a gradual decline throughout the experiment. The rectal temperature in animals given diazepam showed no statistically significant difference when compared to that in control animals given vehicle only (Fig. 17).

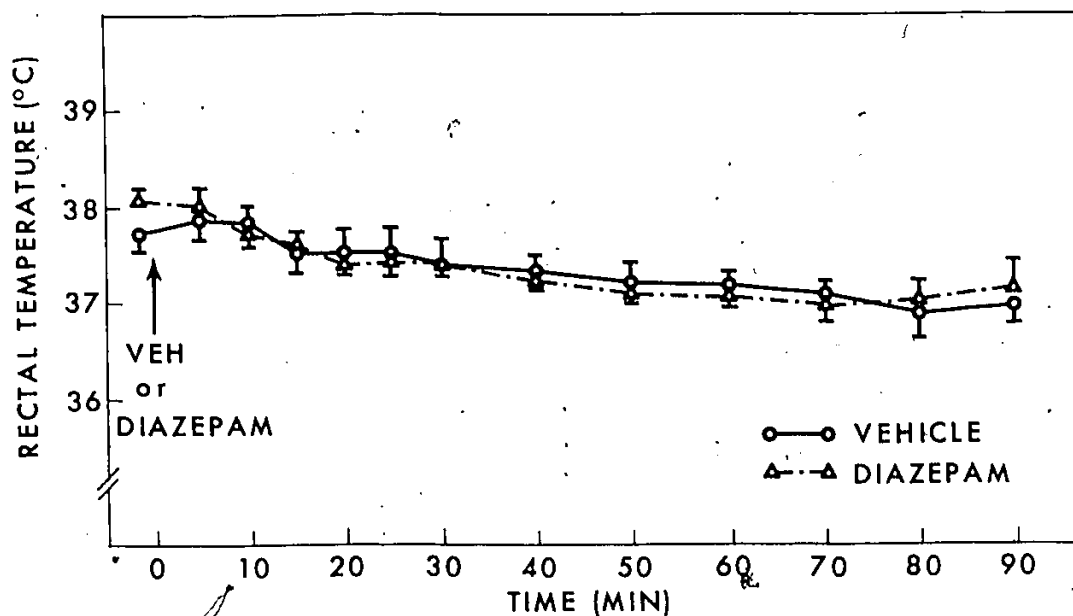


Fig. 17:

Effect of diazepam on rectal temperature. Groups were injected i.p. at time 0 with either diazepam drug-vehicle ○—○ (n=6), or diazepam (1.8 mg/kg) △--△ (n=6). Rectal temperature was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Rectal temperature is expressed in degrees centigrade. Points represent the mean values $\pm$ S.E.M.

Changes in the rectal temperature of animals given imipramine, followed by the vehicle of diazepam (Fig. 18), were similar to those seen with imipramine alone (see Fig. 14) or with the imipramine + saline combination (see Fig. 16). The mean values in this group were from 60 minutes onwards significantly lower than those observed in saline-injected controls. When imipramine-pretreated animals were given diazepam, the decreases in rectal temperature were pronounced and from 50 minutes onwards the mean values were significantly lower than those of the imipramine + vehicle group (Fig. 18). The mean rectal temperatures of the imipramine + diazepam group were from 40 minutes onwards also significantly different from saline-injected controls. Analysis of slope revealed that the body temperature curve of the imipramine + diazepam group differed significantly (i.e., had a more negative slope) from that of the imipramine + vehicle group, from 15 minutes onward.

(iii) Summary: In these experiments, imipramine (50 mg/kg i.p.) was found to cause a gradual decline in the rectal temperature of rats, which became significantly different from saline-injected controls at about 50 to 60 minutes after the injection. Diazepam (1.8 mg/kg i.p.) or phenobarbital (40 mg/kg i.p.) injected alone produced no significant effects on body temperature when compared to

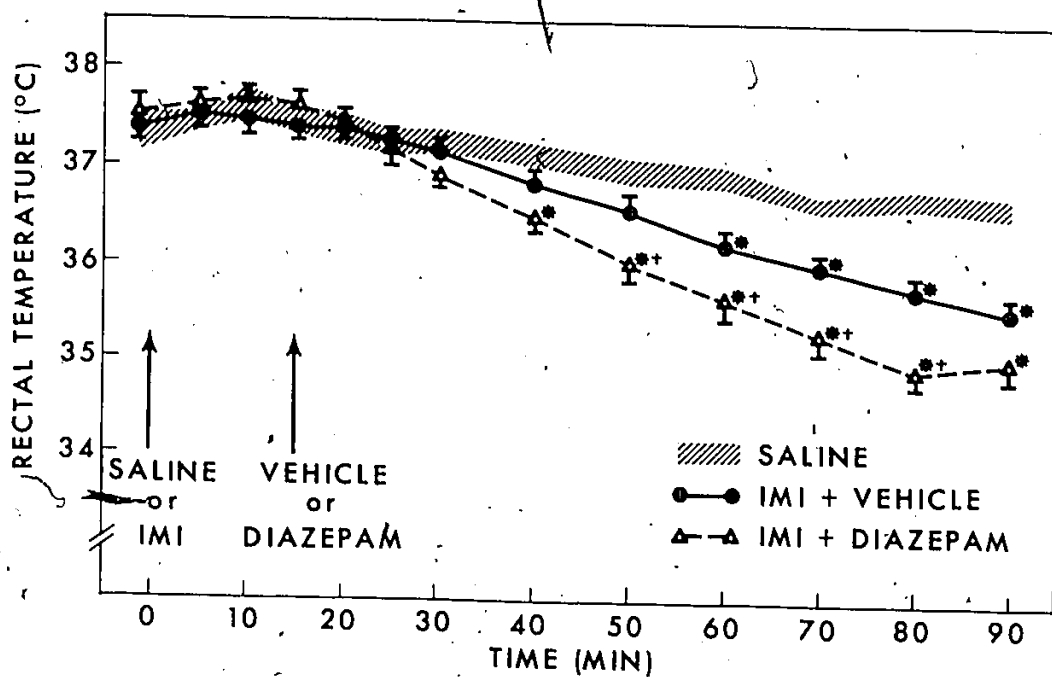


Fig. 18:

Effect of imipramine + diazepam on rectal temperature. Groups were injected i.p. with either saline at time 0 (n=10), or imipramine (50 mg/kg) at time 0 and diazepam drug-vehicle after 15 minutes (n=14), or imipramine (50 mg/kg) at time 0 and diazepam (1.8 mg/kg) after 15 minutes (n=14). Rectal temperature was measured every 5 minutes from 0 to 30 minutes, and then every 10 minutes for the final 60 minutes. Rectal temperature is expressed in degrees centigrade. Points represent the mean values $\pm$ S.E.M.

* A statistically significant difference when compared to animals given saline ($p < 0.05$).

+ A statistically significant difference when compared to animals given imipramine + vehicle ($p < 0.05$).

respective controls. Diazepam given 15 minutes after imipramine enhanced the hypothermic effect of the antidepressant drug. On the other hand, treatment with phenobarbital did not significantly alter the hypothermic response of rats to imipramine.

e) Effect of Imipramine on Respiration Rate and Oxygen Consumption.

Respiration rate in animals given saline alone remained fairly constant throughout the 90-minute observation period (Fig. 19). Imipramine administration (50 mg/kg i.p.) caused a marked initial increase in respiration rate up to almost 50% above that noted in controls. This effect lasted for about 60 minutes, at which time the respiration rate of imipramine-treated animals returned to the range of control values. Student's t-test showed this effect of imipramine to be significant within the period extending from 10 to 40 minutes after drug injection. Slope analysis confirmed a significant treatment effect.

On the other hand, according to statistical analysis by Student's t-test, imipramine failed to exert a significant effect on oxygen consumption when compared to saline injection (Fig. 20). However, slope analysis has revealed that the oxygen consumption in the imipramine-treated rats was significantly depressed (i.e., the slope

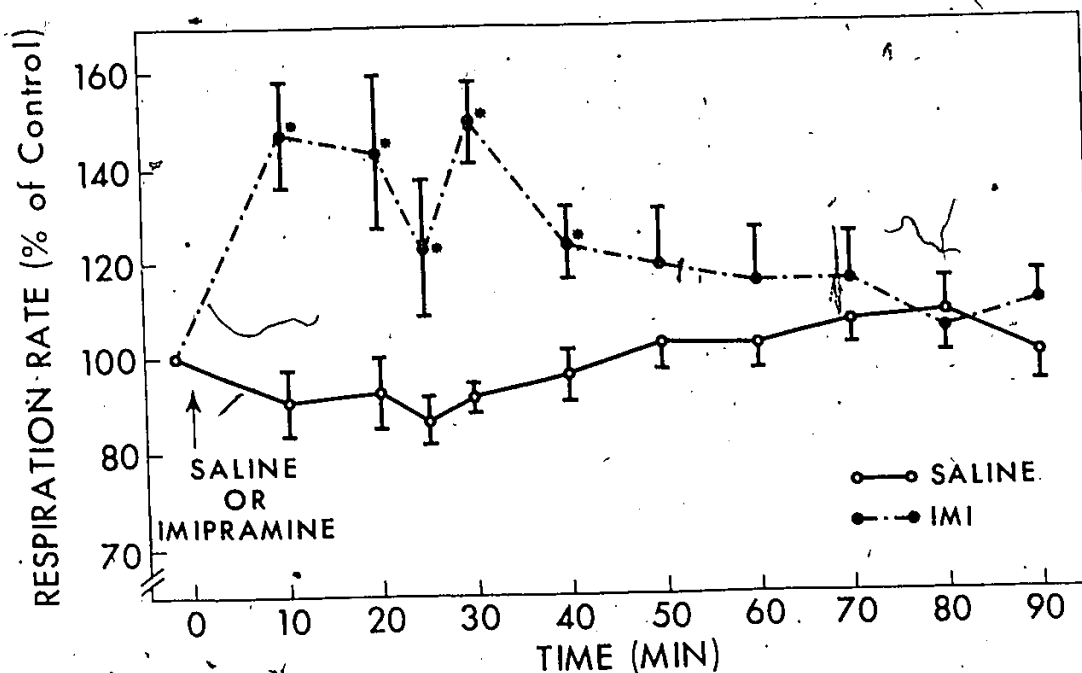


Fig. 19:

Effect of imipramine on respiration rate. Groups were injected i.p. at time 0 with either saline $\circ$ — $\circ$ ($n=7$) or imipramine (50 mg/kg) $\bullet$ — $\bullet$ ($n=7$). Respiration rate was measured for 90 minutes following dosing. The values obtained are expressed as a percentage of the control (or initial) values which were, for the saline group, 90.0 ± 4.1 breaths/min and for the imipramine group, 90.1 ± 6.3 breaths/min. Points represent the mean values $\pm$ S.E.M. (See Appendix B for absolute values).

* A statistically significant difference when compared to animals given saline ($p < 0.05$).

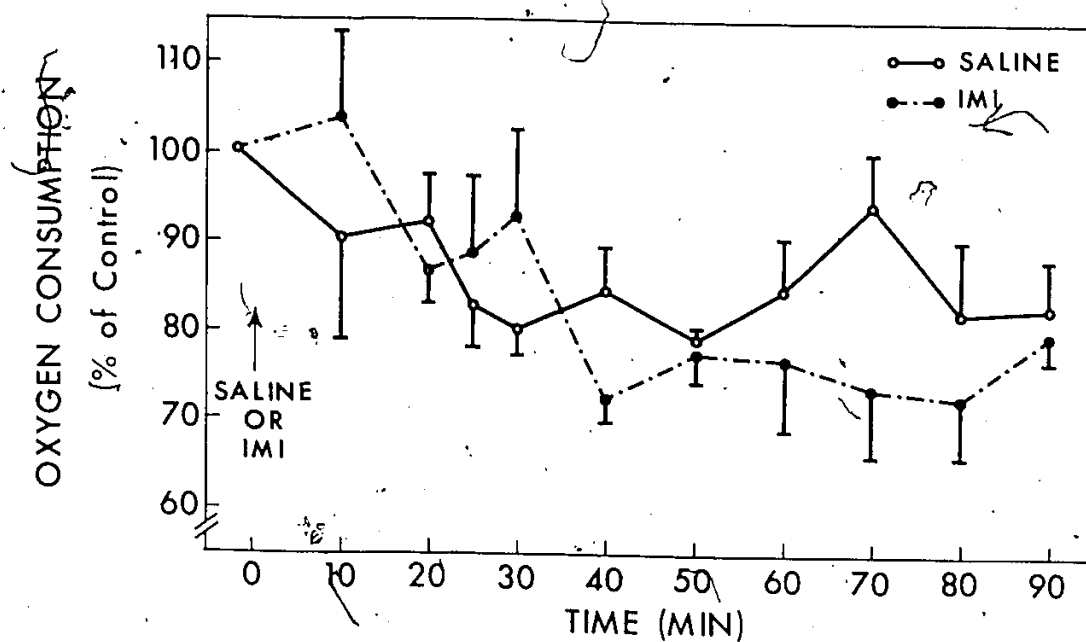


Fig. 20:

Effect of imipramine on oxygen consumption. Groups were injected i.p. at time 0 with either saline $\circ$ — $\circ$ ($n=7$) or imipramine (50 mg/kg) $\bullet$ — $\bullet$ ($n=7$). Oxygen consumption was measured for 90 minutes following dosing and measured in ml/min/kg $^{0.73}$. The values obtained are expressed as a percentage of the control (or initial) values which were, for the saline group, 16.9 ± 0.9 ml/min/kg $^{0.73}$ and for the imipramine group, 16.2 ± 0.8 ml/min/kg $^{0.73}$. Points represent the mean values $\pm$ S.E.M. (See Appendix B for absolute values).

of the data curve of this group was more negative than that of controls, $p < 0.05$) when compared to saline-injected controls.

To summarize then, imipramine caused a significant increase in respiration rate. A reduction in oxygen consumption was demonstrated by slope analysis, but not by Student's t -test analysis.

4. Discussion

The experiments described in Part A demonstrated that both diazepam and phenobarbital were effective in preventing imipramine-induced convulsions in rats. This finding, however, does not necessarily mean that they are safe drugs to use in patients poisoned with tricyclic antidepressants. Severe cardiovascular and respiratory disturbances associated with overdose of tricyclic antidepressants necessitate the use of extreme caution to avoid administering other drugs that could worsen the patient's condition. Lombroso (101) has pointed out that patients in convulsive conditions, such as status epilepticus, are especially susceptible to the depressant effects of anticonvulsant drugs, and this may also be the case during convulsions in imipramine poisoning. For these reasons, we have investigated the question whether diazepam or phenobarbital, in doses which

antagonized imipramine-induced convulsions, would alter basic cardiovascular and respiratory parameters and the body temperature of experimental animals when given alone, or following the administration of a toxic dose of imipramine.

Since convulsions would obviously interfere with the physiological parameters measured, a sub-convulsive (but still toxic) dose of imipramine (equivalent to CD_5) was used in these experiments. Animals which had convulsions with this dose of imipramine were not included in final evaluation of the results. Furthermore, since hormonal changes during the oestrous cycle have been shown to alter convulsion susceptibility in rats (207), only male animals were used.

A considerable amount of work on the effect of imipramine on the cardiovascular system in various animal species has been carried out, as reviewed in the first part of this thesis. Unlike most experiments to investigate the drug-induced changes of blood pressure in the whole animal, the technique used in the present work permitted measurement of blood pressure and heart rate without the influence of anaesthesia, which was found not only to considerably modify drug effects on the heart, but also to influence regulatory reflexes (14).

The fall in blood pressure, seen in our experiments following injection of imipramine, parallels the observations made earlier by other workers in dogs (27), (163), (153) and rabbits (42). Hypotension has also been frequently reported in clinical accounts of poisoning with tricyclic antidepressants (157), (141), (65), (63). The imipramine-induced reduction in heart rate which was observed is also in agreement with the findings of some of the previous studies (153), (27), (118).

In micro-electrode studies with rabbit atria, Matsuo (108) demonstrated that a 10 $\mu\text{g/ml}$ bath concentration of imipramine depressed action potential amplitude and progressively slowed the rhythm. In isolated rat ventricular strips, imipramine (12.5 $\mu\text{g/ml}$) was shown to directly depress contractility and to prolong the absolute refractory period (136). Using both guinea pig and rat ventricular slices, Auclair (6) found that imipramine (12.5 $\mu\text{g/ml}$) diminished the resting potential, slowed depolarization and elevated the excitation threshold. From this and other evidence already reviewed, there are several possibilities to explain the cardiovascular effects of imipramine observed in these experiments. The fall in blood pressure could be due to a decrease in peripheral resistance, as a result of 2° blockade (20) or an antagonism of the contractile effect

of calcium ions in the vascular smooth muscle (20), (74a). Most evidence, however, supports the possibility that the fall in blood pressure is due to a direct depressant effect on the myocardium (108), (136), (6). This argument could also be used to explain the decrease in heart rate seen after administration of imipramine. The initial increase in heart rate which occurred after imipramine dosing could be compensation for the falling blood pressure. A more likely explanation is that this transient increase in heart rate occurs when only small amounts of the drug are absorbed and acting. This would be reminiscent of the transient increases in heart rate seen by other authors in response to low doses of imipramine (27).

The importance of controlling body temperature to avoid additional stress in poisoning with tricyclic antidepressants has been emphasized by Sueblinvong et al (180). Laboratory investigation has shown that 30 mg/kg i.p. imipramine in mice leaves body temperature unaffected (160), while in guinea pigs 100 mg/kg s.c. caused hypothermia (57). In rats, 20 mg/kg i.p. gave a very slight decrease in body temperature (186).

Our finding of an approximately 2°C decline in body temperature of rats, over 90 minutes following 50 mg/kg i.p. imipramine, is thus in agreement with the above reports.

As mentioned earlier, both hyper (2), (53), (65) and hypothermia (126), (142) are seen in patients poisoned with tricyclic antidepressants.

The mechanism of imipramine's effect on body temperature is not clear. Evidence of central action was presented by Cooper (38a), who showed that imipramine, when injected intraventricularly, caused a drop in body temperature in cats, but produced an increase when injected in rabbits. Both effects could be explained by potentiation of central adrenergic mechanisms due to the blockade of the uptake of endogenously released noradrenaline by imipramine. It has been shown that species differences exist in the effect of intraventricularly injected noradrenaline. In cats it causes a drop (48), while in rabbits, a rise in body temperature (143). Noradrenaline also causes a drop in body temperature in the rat (99a). Therefore, potentiation of central adrenergic mechanisms could lead to a drop in body temperature. The slight hypothermia seen after imipramine could also be caused by the peripheral vasodilation effect that this drug has been shown to possess (20), (153). A similar mechanism was suggested by Kirkpatrick and Lomax (87) to explain the hypothermic effect of atropine methylnitrate in the rat. Although the drop in body

temperature observed in the present study was not very large, along with a drop in blood pressure and heart rate it may be an aggravating factor in imipramine poisoning (185).

A number of authors reported a depression of respiration in poisoning with tricyclic antidepressants, particularly following the administration of anticonvulsants (65), (169). Our results show that in the rat there is a definite increase in respiration rate, within 10 minutes after injection of imipramine. The relatively large standard errors seen in our data on the respiration rate of the imipramine-treated animals may reflect the fact that these animals tended to be more restless than the saline-injected controls. The increase in respiration rate observed in present experiments could merely be a reflection of the cardiovascular depression produced by imipramine, and secondary to increased PCO_2 in the blood, resulting from a drop in cardiac output. Respiratory alkalosis has, indeed, been reported in some clinical cases of imipramine poisoning (185); in others however, no changes were found (188). A similar argument could be used to explain any decrease in oxygen consumption, since the amount of oxygen available to the tissues was likely reduced as a result of decreased blood flow.

Although the slope analysis indicates that imipramine has a significant tendency to depress oxygen consumption,

analysis of data by Student's t-test failed to reveal a significant difference from the control group. The results are therefore inconclusive.

The main aim of this series of experiments was to examine whether administration of the two anticonvulsants used, phenobarbital and diazepam, would alter the imipramine-induced changes in cardiovascular, respiratory or thermoregulatory function. The anticonvulsants were administered 15 minutes after imipramine, since preliminary studies showed that the effects of imipramine, at least on blood pressure, were clearly evident by this time. Our results demonstrate that phenobarbital alone had no significant effect on heart rate or blood pressure, nor did the phenobarbital appear to alter the cardiovascular effects of a toxic dose of imipramine. Hypotension has been reported with pentobarbital used in anaesthetic doses (40 mg/kg i.p.) in rats (23) or following overdose in man (91). However, barbiturates taken orally in doses producing only sedation and sleep do not significantly affect the circulation (93).

The administration of phenobarbital alone resulted in a decrease in body temperature equivalent to that seen with imipramine. While barbiturates in anaesthetic doses (e.g., 35 mg/kg pentobarbital i.v. in the dog) (175a) have been shown to cause hypothermia, and mild decreases in body

temperature have been reported with sedative or hypnotic doses, this effect, "usually does not exceed that observed under similar conditions of inactivity in the absence of drugs" (110). It is of interest that the anticonvulsant did not significantly potentiate the imipramine-induced hypothermia. The effect on respiratory function of phenobarbital, following a toxic dose of imipramine, has yet to be studied. Such an investigation would be useful, since as mentioned in the review of the literature, a number of authors feel that barbiturates may contribute to respiratory depression in tricyclic antidepressant poisoning (177), (208), (3), (139), (58). As far as can be ascertained from these studies, phenobarbital is a useful drug in the treatment of imipramine-induced convulsions, since in rats an effective anticonvulsant dose did not augment the effects of imipramine on such important physiological parameters as heart rate, blood pressure, or body temperature.

Recently, a number of clinical papers have mentioned the successful control of imipramine-induced convulsions with diazepam (53), (56), (63), (84). This minor tranquilizer has been shown experimentally and clinically to have anticonvulsant properties and is claimed by some to cause little or no depression of vital functions (49), (174), (208). In our experiments, diazepam was administered

in acidified saline instead of its normal solvent, propylene glycol, since this latter substance, itself, may profoundly affect physiological functions. Indeed, Sharer et al (158) found in controlled experiments in cats that a large proportion (2/3) of changes seen after administration of diazepam in propylene glycol were due to the solvent.

In the present work, diazepam alone (1.8 mg/kg i.p.) showed no significant effect on either blood pressure or heart rate, though it had the tendency to lower blood pressure. Rapid injection of diazepam has been shown to produce dose-dependent decreases in blood pressure in dogs (21) and cats (32). Abel et al (1), however, found that diazepam (0.25 mg/kg), injected into the coronary circulation of dogs, had a positive inotropic effect. They concluded that diazepam augmented myocardial contractility by increasing coronary blood flow.

In clinical use of diazepam to treat status epilepticus, hypotension has been noted (134), (128), (149), (16), but it is not always clear what other anticonvulsants may have been used unsuccessfully prior to diazepam administration. In our experiments, diazepam given 15 minutes following imipramine dosing, did transiently potentiate the hypotensive effect of imipramine, and this may be of clinical significance in cases of imipramine poisoning with severe

hypotension. It is interesting to note that in imipramine + diazepam-treated animals the heart rate remained near control levels compared to the drop seen in animals not given diazepam. This may represent a reflex action compensating for the increased hypotension following diazepam administration. However, the observations of Chai and Wang (32) that diazepam lowers blood pressure by depressing hypothalamic and medullary responses would tend to preclude this possibility.

Diazepam given alone had no effect on body temperature of rats, but when given after imipramine, it potentiated the antidepressant-induced hypothermia. This effect could be relevant in an imipramine poisoning situation, since a fall in body temperature can reduce cardiac function (185). In this connection, it is of interest that diazepam given to women in labour, fifteen hours or less prior to delivery, impaired metabolic response to cold stress in the newborn (127).

Diazepam's effect on respiratory function in imipramine poisoning remains to be directly investigated. Intravenous administration of diazepam produces a moderate degree of hypoventilation in mice (107). Several authors have reported diazepam-induced respiratory depression in

treating status epilepticus (59), (134), (97), (16) and Thong (187) says that respiratory arrest is a potential hazard of diazepam therapy.

The cited advantage of diazepam in control of imipramine-induced convulsions is its apparent lack of depressant effects on physiological functions. The results of these experiments contradict this assumption, since diazepam was found to enhance the depression in body temperature, and most probably also, the depression in blood pressure caused by toxic doses of imipramine in rats.

III. SUMMARY AND CONCLUSION

The aim of this study was to determine which of the anticonvulsants used clinically in poisoning with tricyclic antidepressants, diphenylhydantoin, diazepam or phenobarbital was most efficacious and safe in preventing imipramine-induced convulsions in rats. The effect of imipramine on respiratory function was also investigated.

First, the dose-response curves for convulsive and lethal effects of imipramine were established. The CD_{50} was found to be 64.7 mg/kg and the LD_{50} , 78.4 mg/kg. Diphenylhydantoin, phenobarbital and diazepam were then tested for their ability to prevent convulsions induced by an LD_{90} of imipramine. Phenobarbital (PD_{50} , 17.6 mg/kg) and diazepam (PD_{50} , 0.32 mg/kg), but not diphenylhydantoin, were found to be effective in blocking the imipramine-induced convulsions. None of the anticonvulsant drugs tested significantly affected mortality caused by imipramine.

To investigate the safety of the anticonvulsants found effective in suppressing the imipramine-induced convulsions, the effect of these drugs alone, or in combination with imipramine, on important physiological parameters (heart rate, blood pressure and rectal temperature), was examined in unanaesthetized rats. Administration of a toxic dose of imipramine alone resulted in moderate, but significant, decreases of blood pressure, heart rate and rectal

temperature. In addition, neither phenobarbital nor diazepam, given alone, significantly affected blood pressure, heart rate or rectal temperature of experimental animals. When diazepam was given 15 minutes after administration of imipramine, the imipramine-induced decrease in heart rate was antagonized, but the hypotensive effect of the tricyclic antidepressants was transiently increased. The hypothermic effect of imipramine was significantly enhanced by diazepam. Administration of an effective anticonvulsant dose of phenobarbital did not significantly affect the imipramine-induced changes in any of the physiological parameters studied. Imipramine was found to transiently increase the respiration rate, and there was also some evidence of decreased oxygen consumption in rats given a toxic dose of this tricyclic antidepressant.

Thus, although both diazepam and phenobarbital were effective in blocking seizures due to a toxic dose of imipramine, phenobarbital was found to have a lesser effect on the vital functions studied, and therefore can be considered to be safer than diazepam in treatment of poisoning with imipramine-like compounds. The finding that diphenylhydantoin was completely ineffective in preventing imipramine-induced convulsions in rats, suggests that this drug may not have any value as an anticonvulsant in the therapy

of poisoning with tricyclic antidepressants. The fact that neither phenobarbital nor diazepam significantly decreased the imipramine-induced mortality indicates that, in severe imipramine poisoning, control of seizures alone is not necessarily life saving. Further supportive therapy is therefore mandatory for the survival of a patient with severe imipramine poisoning.

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V. APPENDICES

Table 1.

The Effect of Imipramine
on
Blood Pressure (mm Hg)

Time	Saline (n=11)	Imipramine (50 mg/kg) (n=11)	t values
C	92.6 ± 1.3 ^a	99.5 ± 2.5*	2.545
5 mins	94.4 ± 3.4	86.3 ± 3.3	1.722
10 mins	92.3 ± 2.8	82.5 ± 4.3	1.959
15 mins	92.4 ± 2.7	81.8 ± 3.9*	2.305
20 mins	95.3 ± 2.7	82.5 ± 3.6*	2.882
25 mins	94.7 ± 2.6	81.2 ± 3.5*	3.149
30 mins	93.2 ± 2.7	80.3 ± 3.2*	3.072
40 mins	94.1 ± 2.6	82.3 ± 4.3*	2.400
50 mins	97.2 ± 2.9	79.2 ± 5.0*	3.179
60 mins	93.6 ± 2.1	79.9 ± 4.3*	2.936
70 mins	94.7 ± 1.5	84.0 ± 3.9*	2.670
80 mins	95.3 ± 1.8	85.9 ± 3.6*	2.401
90 mins	94.3 ± 2.5	85.5 ± 3.7	1.991

C Initial values taken 2 mins. prior to dosing.

a Mean ± S.E.M.

* Statistically significant difference ($p < 0.05$).

Table 2.

The Effect of Imipramine
on
Heart Rate (Beats/Min)

Time	Saline (n=11)	Imipramine (50 mg/kg) (n=11)	t values
C	343.1 ± 8.2 ^a	350.2 ± 10.2	0.541
5 mins	342.0 ± 10.9	366.6 ± 13.5	1.431
10 mins	344.7 ± 10.0	335.4 ± 11.6	0.614
15 mins	345.2 ± 13.7	325.2 ± 11.9	1.093
20 mins	348.5 ± 14.5	302.0 ± 10.2*	2.576
25 mins	343.6 ± 13.5	306.6 ± 13.6	1.929
30 mins	344.2 ± 12.5	297.0 ± 15.9*	2.351
40 mins	367.6 ± 13.8	289.2 ± 18.0*	3.496
50 mins	367.6 ± 16.1	289.8 ± 20.8*	2.990
60 mins	362.7 ± 16.6	286.2 ± 19.9*	2.974
70 mins	362.7 ± 17.5	291.0 ± 21.6*	2.598
80 mins	366.0 ± 18.5	300.6 ± 22.7*	2.247
90 mins	360.5 ± 16.6	306.0 ± 23.6	1.920

C Initial values taken 2 mins. prior to dosing.

a Mean ± S.E.M.

* Statistically significant difference (p<0.05).

Table 3.

The Effect of Phenobarbital
on
Blood Pressure (mm Hg)

Time	Saline (n=13)	Phenobarbital (40 mg/kg) (n=6)	t values
0	92.9 ± 1.2 ^a	91.3 ± 3.8	0.517
5 mins	93.5 ± 2.9	86.6 ± 6.7	1.110
10 mins	92.1 ± 2.4	86.6 ± 6.7	0.966
15 mins	91.4 ± 2.6	89.1 ± 5.9	0.412
20 mins	94.1 ± 2.7	91.6 ± 5.7	0.453
25 mins	94.1 ± 2.4	93.3 ± 4.8	0.157
30 mins	92.5 ± 2.6	94.5 ± 4.6	0.409
40 mins	93.5 ± 2.4	92.3 ± 4.9	0.242
50 mins	95.7 ± 3.0	96.6 ± 5.2	0.164
60 mins	92.5 ± 2.2	91.8 ± 5.1	0.140
70 mins	93.6 ± 1.7	92.3 ± 4.8	0.325
80 mins	94.1 ± 2.0	90.8 ± 4.8	0.755
90 mins	93.3 ± 2.5	93.0 ± 4.6	0.069

C Initial values taken 2 mins. prior to dosing.
a Mean ± S.E.M.

Table 4.

The Effect of Phenobarbital
on
Heart Rate (Beats/Min)

Time	Saline (n=13)	Phenobarbital (40 mg/kg) (n=6)	t values
C	340.2 ± 7.2 ^a	317.0 ± 8.5	1.908
5 mins	338.8 ± 9.6	332.7 ± 16.7	0.339
10 mins	338.3 ± 9.7	308.0 ± 15.2	1.722
15 mins	331.1 ± 8.7	302.0 ± 13.9	1.866
20 mins	343.5 ± 14.2	309.0 ± 13.1	1.551
25 mins	343.3 ± 16.7	324.0 ± 17.6	0.742
30 mins	339.0 ± 12.6	335.0 ± 27.3	0.154
40 mins	354.0 ± 13.5	333.0 ± 29.3	0.748
50 mins	353.5 ± 15.6	345.0 ± 19.7	0.329
60 mins	357.5 ± 16.0	342.0 ± 19.6	0.582
70 mins	356.5 ± 17.2	333.0 ± 14.7	0.882
80 mins	360.5 ± 17.8	347.0 ± 18.7	0.472
90 mins	355.5 ± 16.0	338.0 ± 11.3	0.723

C Initial values taken 2 mins. prior to dosing.
a Mean ± S.E.M.

Table 5.

The Effect of Imipramine + Phenobarbital
on
Blood Pressure (mm Hg)

Time	IMI. + Sal. (50 mg/kg) (n=12)	IMI. + Phenob. (50 mg/kg) (40 mg/kg) (n=11)	t values
C	104.2 ± 3.2 ^a	97.5 ± 2.8	1.589
5 mins	99.7 ± 5.8	84.1 ± 2.5*	2.626
10 mins	92.6 ± 4.8	83.0 ± 3.3	1.685
15 mins	92.5 ± 4.5	84.4 ± 4.0	1.341
20 mins	91.1 ± 4.7	80.1 ± 2.9*	2.068
25 mins	88.1 ± 5.5	80.6 ± 2.6	1.304
30 mins	90.9 ± 4.6	82.4 ± 2.6	1.670
40 mins	91.5 ± 5.0	83.8 ± 3.2	1.374
50 mins	91.4 ± 4.0	83.4 ± 2.9	1.666
60 mins	95.0 ± 3.3	85.2 ± 3.2*	2.106
70 mins	96.2 ± 3.6	85.6 ± 2.9*	2.333
80 mins	97.3 ± 3.7	89.1 ± 2.7	1.842
90 mins	94.5 ± 3.0	88.3 ± 2.3	1.645

C Initial values taken 2 mins. prior to dosing.

a Mean ± S.E.M.

* Statistically significant difference (p<0.05).

Table 6.

The Effect of Imipramine + Phenobarbital
on
Heart Rate (Beats/Min)

Time	IMI. + Sal. (50 mg/kg) (n=12)	IMI. + Phenob. (50 mg/kg) (40 mg/kg) (n=14)	t values
0	379.5 ± 14.7 ^a	384.9 ± 11.3	0.293
5 mins	377.5 ± 21.1	399.0 ± 13.4	0.884
10 mins	350.0 ± 21.5	354.0 ± 15.1	0.154
15 mins	338.7 ± 21.8	345.0 ± 16.3	0.233
20 mins	349.0 ± 19.1	342.0 ± 15.6	0.286
25 mins	340.0 ± 20.9	330.4 ± 14.3	0.388
30 mins	346.5 ± 19.8	334.7 ± 15.1	0.480
40 mins	342.6 ± 16.3	345.9 ± 17.7	0.330
50 mins	344.2 ± 20.2	359.1 ± 17.4	0.563
60 mins	355.1 ± 16.6	368.6 ± 18.6	0.524
70 mins	354.0 ± 19.3	370.7 ± 18.4	0.621
80 mins	357.3 ± 23.0	375.0 ± 16.8	0.637
90 mins	350.7 ± 20.2	385.4 ± 15.3	1.395

C Initial values taken 2 mins. prior to dosing.
a Mean ± S.E.M.

APPENDIX A

Table 7.

The Effect of Diazepam
on
Blood Pressure (mm Hg)

Time	Vehicle (n=6)	Diazepam (1.8 mg/kg) (n=6)	t values
C	99.6 ± 9.5 ^a	99.3 ± 9.2	0.025
5 mins	95.5 ± 7.1	94.5 ± 9.8	0.083
10 mins	95.0 ± 8.7	90.0 ± 9.7	0.384
15 mins	96.8 ± 9.7	88.6 ± 10.6	0.569
20 mins	97.8 ± 9.5	91.0 ± 9.3	0.513
25 mins	98.6 ± 9.7	92.6 ± 11.4	0.401
30 mins	96.1 ± 10.5	92.6 ± 11.0	0.230
40 mins	95.8 ± 10.4	90.1 ± 11.2	0.371
50 mins	93.3 ± 8.3	90.5 ± 10.6	0.211
60 mins	93.8 ± 11.5	93.0 ± 10.5	0.053
70 mins	97.3 ± 10.8	92.5 ± 10.7	0.318
80 mins	97.8 ± 11.0	94.1 ± 9.5	0.252
90 mins	98.0 ± 10.6	95.1 ± 10.7	0.188

C Initial values taken 2 mins. prior to dosing.
a Mean ± S.E.M.

Table 8.

The Effect of Diazepam
on
Heart Rate (Beats/Min)

Time	Vehicle (n=6)	Diazepam (1.8 mg/kg) (n=6)	t values
C	330.0 ± 5.1 ^a	351.0 ± 14.8	1.337
5 mins	327.0 ± 9.3	366.0 ± 18.5	1.883
10 mins	321.0 ± 6.5	362.0 ± 21.1	1.856
15 mins	316.8 ± 6.1	345.6 ± 18.0	1.518
20 mins	322.0 ± 7.7	354.0 ± 15.3	1.865
25 mins	332.0 ± 12.6	354.0 ± 20.0	0.931
30 mins	324.0 ± 10.3	353.0 ± 21.9	1.197
40 mins	330.0 ± 14.8	364.0 ± 30.7	0.999
50 mins	322.0 ± 6.5	353.0 ± 28.9	1.046
60 mins	324.0 ± 11.6	349.0 ± 23.9	0.940
70 mins	315.0 ± 6.5	332.0 ± 23.9	0.685
80 mins	326.0 ± 10.4	340.0 ± 21.9	0.578
90 mins	322.5 ± 5.1	342.0 ± 19.0	0.808

C Initial values taken 2 mins. prior to dosing.
a Mean ± S.E.M.

Table 9.

The Effect of Imipramine + Diazepam
on
Blood Pressure (mm Hg)

Time	IMI. + Veh. (50 mg/kg) (n=10)	IMI. + Diaz. (50 mg/kg) (1.8 mg/kg) (n=9)	t values
0	93.1 ± 2.3 ^a	100.9 ± 3.0	2.094
5 mins	80.2 ± 2.6	89.2 ± 4.0	1.978
10 mins	79.1 ± 2.2	83.7 ± 3.5	1.121
15 mins	80.1 ± 1.9	85.2 ± 5.2	0.964
20 mins	84.1 ± 2.2	72.8 ± 3.5*	2.774
25 mins	82.4 ± 2.9	71.4 ± 4.0*	2.300
30 mins	80.2 ± 3.4	72.0 ± 3.5	1.670
40 mins	79.4 ± 3.3	74.9 ± 4.5	0.818
50 mins	80.9 ± 3.0	82.1 ± 4.7	0.224
60 mins	81.2 ± 3.1	83.5 ± 5.7	0.359
70 mins	80.2 ± 3.0	80.1 ± 4.4	0.037
80 mins	79.8 ± 3.0	79.2 ± 5.3	0.106
90 mins	80.5 ± 2.7	79.7 ± 4.9	0.145

C Initial values taken 2 mins. prior to dosing.

a Mean ± S.E.M.

* Statistically significant difference (p<0.05).

Table 10.

The Effect of Imipramine + Diazepam
on
Heart Rate (Beats/Min)

Time	IMI. (50 mg/kg) (n=10) + Veh.	IMI. (50 mg/kg) + Diaz. (1.8 mg/kg) (n=11)	t values
C	324.6 ± 7.5 ^a	342.0 ± 11.4	1.244
5 mins	362.4 ± 5.9	373.1 ± 12.7	0.738
10 mins	336.6 ± 5.8	355.1 ± 15.9	1.051
15 mins	314.4 ± 5.8	330.0 ± 12.7	1.118
20 mins	324.0 ± 10.8	352.9 ± 13.1	1.683
25 mins	319.8 ± 5.0	359.5 ± 16.1*	2.260
30 mins	299.4 ± 6.2	352.9 ± 15.7*	3.050
40 mins	296.4 ± 7.4	361.1 ± 16.0*	3.554
50 mins	285.6 ± 7.9	356.2 ± 14.4*	4.168
60 mins	291.6 ± 13.0	351.8 ± 15.0*	3.009
70 mins	286.2 ± 17.8	367.2 ± 17.7*	3.231
80 mins	277.8 ± 15.6	346.8 ± 13.0*	3.397
90 mins	297.6 ± 15.9	358.8 ± 11.0*	3.163

C Initial values taken 2 mins. prior to dosing.

a Mean ± S.E.M.

* Statistically significant difference (p<0.05).

Table 1.

The Effect of Imipramine
on
Oxygen Consumption

Time	Saline (n=7)	Imipramine (50 mg/kg) (n=7)	t values
C	16.3 ± 0.9 ^a	16.2 ± 0.8	0.106
10 mins	14.2 ± 1.1	16.6 ± 1.3	1.379
20 mins	14.9 ± 0.8	13.9 ± 1.3	0.648
25 mins	13.3 ± 0.5	14.3 ± 1.5	0.625
30 mins	13.0 ± 0.6	14.8 ± 1.5	1.154
40 mins	13.6 ± 1.0	11.8 ± 0.5	1.602
50 mins	12.7 ± 0.8	12.5 ± 0.6	0.238
60 mins	13.6 ± 0.7	12.0 ± 0.7	1.635
70 mins	15.1 ± 1.1	11.8 ± 0.7*	2.440
80 mins	13.1 ± 0.7	11.5 ± 0.6	1.745
90 mins	13.1 ± 0.3	12.4 ± 0.9	0.665

C Initial values taken 2 mins. prior to dosing.

a Mean ± S.E.M.

* Statistically significant difference ($p < 0.05$).

APPENDIX B

Table 2.

The Effect of Imipramine
on
Respiratory Rate

Time	Saline (n=7)	Imipramine (50 mg/kg) (n=7)	t values
C	90.0 $\pm$ 4.1 ^a	90.1 $\pm$ 6.3	0.189
10 mins	81.9 $\pm$ 8.1	131.4 $\pm$ 10.6*	3.706
20 mins	80.9 $\pm$ 5.5	126.0 $\pm$ 12.1*	3.200
25 mins	81.4 $\pm$ 5.9	107.7 $\pm$ 11.1	2.090
30 mins	82.4 $\pm$ 5.1	132.0 $\pm$ 5.8*	6.451
40 mins	89.2 $\pm$ 6.0	109.9 $\pm$ 7.0*	2.190
50 mins	90.9 $\pm$ 4.4	106.5 $\pm$ 7.9	1.804
60 mins	90.9 $\pm$ 5.2	101.1 $\pm$ 5.8	1.326
70 mins	95.3 $\pm$ 5.4	104.0 $\pm$ 4.3	1.229
80 mins	95.3 $\pm$ 5.2	94.8 $\pm$ 3.9	0.068
90 mins	89.4 $\pm$ 7.1	97.9 $\pm$ 4.7	0.988

C Initial values taken 2 mins. prior to dosing.

a Mean $\pm$ S.E.M.

* Statistically significant difference ($p < 0.05$).

Example of Slope Analysis

Rectal Temperature -
Imipramine + Vehicle versus Imipramine + Diazepam

Time	Control Group (Imipramine + Vehicle)			Treatment Group (Imipramine + Diazepam)		
	Slope	S.E.M.	t Value	Slope	S.E.M.	t Value
1 - 2	0.002	0.039		-0.039	0.031	
2 - 3	-0.027	0.027	-0.63	-0.049	0.031	-0.23
3 - 4	-0.023	0.029	-0.53	-0.050	0.035	-0.25
4 - 5	-0.032	0.018	-0.82	-0.044	0.018	-0.15
5 - 6	-0.028	0.022	-0.68	-0.048	0.020	-0.23
6 - 7	-0.034	0.022	-0.79	-0.037	0.023	0.04
7 - 8	-0.022	0.020	-0.56	-0.036	0.024	0.05
8 - 9	-0.026	0.019	-0.68	-0.036	0.024	0.07
9 - 10	-0.021	0.020	-0.54	-0.011	0.026	1.23

Comparison of Slopes of Control and Treatment Groups
from times 1 - 10 (i.e., 15 - 90 min)

Control Group		Treatment Group	
Slope	= -0.027 ± 0.002	Slope	= -0.038 ± 0.002
Y at time ₁	= 37.49	Y at time ₁	= 37.53
Y at time ₁₀	= 35.47	Y at time ₁₀	= 34.62
		t = -4.58	df = 270