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STUDIES ON EMETINE CARDIOTOXICITY
IN GUINEA PIGS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Richard Allen Davis, B.S.

* * * * *

The Ohio State University
1975

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ACKNOWLEDGMENTS

Many people have advised and helped me during my graduate training and are the object of my sincere gratitude.

My dear wife, Jane, has helped me in more ways than I can mention here. She has assumed more than her share of family responsibilities during my graduate training so that I could devote more time to my studies. Also, she has done an excellent job of typing this dissertation. For this and much more, I thank her.

Doctor Philip B. Hollander introduced me to pharmacology and provided me the financial support which enabled me to pursue my graduate education.

I am very grateful to Doctor John J. O'Neill, a true gentleman and the best teacher I have ever known. My association with him greatly enriched my graduate career.
I thank Doctor Robert W. Gardier and Doctor John O. Lindower for their careful reading and thoughtful critique of this dissertation.

Also, I thank Doctor Gerald P. Brierley and his staff for their expert help and use of their facilities in doing the mitochondrial studies.

I thank Richard Landis for his very able technical assistance in many of these studies.

To my parents who have always given me their best and have taught me to give my best, I dedicate this dissertation.
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Biochemical Pharmacology. Professor J.J. O'Neil.
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>DT</td>
<td>developed tension (isometric)</td>
</tr>
<tr>
<td>DPN⁺</td>
<td>diphosphopyridine nucleotide</td>
</tr>
<tr>
<td>dv/dt</td>
<td>action potential depolarization rate</td>
</tr>
<tr>
<td>FS</td>
<td>final supernate</td>
</tr>
<tr>
<td>GA</td>
<td>glutaraldehyde</td>
</tr>
<tr>
<td>G6P</td>
<td>glucose-6-phosphate</td>
</tr>
<tr>
<td>IP</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IS</td>
<td>initial supernate</td>
</tr>
<tr>
<td>K-H</td>
<td>Krebs-Henseleit</td>
</tr>
<tr>
<td>uc</td>
<td>microcurie</td>
</tr>
<tr>
<td>PEP</td>
<td>phosphoenolpyruvate</td>
</tr>
<tr>
<td>TPN⁺</td>
<td>triphosphopyridine nucleotide</td>
</tr>
</tbody>
</table>
INTRODUCTION

Therapeutic Use of Emetine

Treatment of Amebiasis. The principle use of emetine has been its effective use in the treatment of amebiasis. Amebiasis is the disease produced by infection with Entamoeba histolytica. This protozoan exists in two forms: the nonmotile cyst and the motile trophozoite. The cysts are highly resistant to environmental changes and as such are responsible for transmission of the disease. Upon ingestion of the cysts, trophozoites are produced which infect the wall and lumen of the colon resulting in ulceration and dysentery. Later the symptoms may subside with the disappearance of trophozoites from the intestine and the patient becomes an asymptomatic cyst passer. The organism may enter the portal circulation or lymphatic vessels and infest the liver. There the release of proteolytic enzymes from the amoeba causes tissue necrosis and formation of an abscess. If allowed to go untreated, this condition is fatal.

Emetine makes up over half of the active alkaloid
content of ipecac, an extract prepared from the root of *Cephaelis ipecacuanha*. During the 17th century this plant was used by Brazilian natives in treating dysentery. Ipecac was brought to Europe by the conquistadores and in 1817 Pelletier isolated a crystalline substance from it which he called emetine. Actually this substance was a mixture of three *ipecacuanha* alkaloids: emetine, cephaeline and psychotrine. In 1912 emetine hydrochloride was shown to be amebicidal *in vitro* (Vedder, 1912) and a short time later was effectively used in the treatment of amebiasis (Rogers, 1912). Since then emetine has been widely used in the treatment of extraintestinal amebiasis. However, the drug is not efficacious in the treatment of amebic dysentery. Nevertheless in combination with a luminal amebicide such as chloroquine, very high cure rates have been achieved (Powell, 1971; Powell, 1972; Datta et al., 1974).

Because of its lack of efficacy in intestinal amebiasis and its toxic side effects (see below), a long search for a less toxic and universally effective amebicide has been conducted. In 1959 dehydroemetine, a synthetic derivative of emetine, was introduced (Brossi et al., 1959) with claims of reduced toxicity due to its more rapid excretion. However, clinical studies have shown that dehydroemetine can produce the same toxic effects that emetine does (Dempsey and Salem, 1966; Lister, 1968;
Faure et al., 1972) and must be used with the same precautions (Powell, 1967). In 1966 metronidazole which had been used in the treatment of trichomoniasis was shown to be a universally effective, non-toxic amebicide producing relatively high cure rates (Powell, 1966; Powell, 1972). However, a greater tendency of relapse occurs with metronidazole. Also, a recent well controlled clinical trial showed that emetine in combination with chloroquine produced a higher cure rate than metronidazole (Datta et al., 1974). Therefore, emetine remains the most important drug used in the treatment of amebiasis.

The occurrence of amebiasis is worldwide (Elsdon-Dew, 1971) and has been estimated to afflict 10% of the world's population (Powell et al., 1961). There seems to be a higher incidence of amebiasis in the underdeveloped and tropical countries where there are lower standards of sanitation and hygiene. In the United States, incidences of 3-6% in the North and 7-10% in the South have been suggested (Burrows, 1961). However, incidences of up to 57% have been found in mental institutions and within groups of U.S. citizens traveling abroad such as tourists, war veterans, missionaries and Peace Corps volunteers. Also, there have been a number of acute outbreaks of amebiasis in the United States usually associated with some interruption of normal sanitary conditions (Juniper, 1971). These facts emphasize the importance of emetine as an effective
Cancer Chemotherapy. Shortly after its efficacy in the treatment of amebiasis was demonstrated, emetine was reported to cause tumor regression in two clinical studies (Lewisohn, 1918; Van Hoosen, 1919). However, interest in emetine as an antineoplastic agent declined probably because of reported toxic side effects. Recently this interest has been renewed with scattered reports over the last two decades of its efficacy in various types of human cancers (Isaka, 1950; Grollman, 1965; Abd-Rabbo, 1966; Wyburn-Mason, 1966). Animal studies have shown that emetine is quite effective in certain types of hematologic malignancies (Jondorf, 1970; Jondorf, 1971). The most recent phase I and phase II studies of emetine treatment for solid tumors have not been promising (Panettiere and Coltman, 1971; Siddiqui et al., 1973; Mastrangelo et al., 1973; Moertel et al., 1974). However, no hematologic toxicity has been observed with emetine treatment which may make it useful in combination chemotherapy. In fact, emetine in combination with cyclophosphamide has been shown to be quite effective in the treatment of lung cancer (Street, 1972).

The effective use of emetine in the treatment of amebiasis and in cancer chemotherapy requires daily administration of the drug for as long as two weeks. Under
these circumstances, emetine causes several toxic side effects which restrict successful therapy.

Emetine Toxicity

Toxic Effects in Man. Symptoms of toxicity always accompany prolonged clinical use of emetine. The most comprehensive study of emetine toxicity in man was done by Klatskin and Friedman while treating patients for amebiasis (Klatskin and Friedman, 1948). Table 1 taken from their report lists the various toxic symptoms, and the incidence and duration at which these symptoms were observed during chronic emetine administration. Many of the toxic symptoms subsided shortly after emetine administration had stopped or even during emetine administration. This was particularly true for nausea, diarrhea, tachycardia, hypotension and precordial pain. Generally, these symptoms were also the earliest to be observed. In contrast dyspnea, general muscular weakness, "neuritis" and electrocardiographic changes were slower in onset and usually did not subside until long after emetine administration had stopped. Headache, dizziness, nausea and precordial pain were transient in that they occurred shortly after administration of emetine and subsided within a few hours, although they recurred with subsequent doses.
<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Incidence</th>
<th>Subsided During Therapy</th>
<th>Subsided After Emetine Was Stopped</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Per Cent*</td>
<td>No.</td>
</tr>
<tr>
<td>Local pain</td>
<td>86</td>
<td>93.1</td>
<td>23</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>39</td>
<td>41.9</td>
<td>24</td>
</tr>
<tr>
<td>Nausea</td>
<td>29</td>
<td>31.2</td>
<td>20</td>
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<tr>
<td>E.K.G. changes</td>
<td>49</td>
<td>52.7</td>
<td>7</td>
</tr>
<tr>
<td>Fall in B.P.</td>
<td>33</td>
<td>35.5</td>
<td>22</td>
</tr>
<tr>
<td>Precordial pain</td>
<td>33</td>
<td>35.5</td>
<td>17</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>14</td>
<td>15.0</td>
<td>4</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>12</td>
<td>12.9</td>
<td>9</td>
</tr>
<tr>
<td>General Weakness</td>
<td>50</td>
<td>53.7</td>
<td>16</td>
</tr>
<tr>
<td>&quot;Neuritis&quot;</td>
<td>14</td>
<td>15.0</td>
<td>2</td>
</tr>
<tr>
<td>Dizziness</td>
<td>9</td>
<td>9.7</td>
<td>4</td>
</tr>
<tr>
<td>Headache</td>
<td>9</td>
<td>9.7</td>
<td>5</td>
</tr>
</tbody>
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x - Taken from Klatskin and Friedman, 1948
* - Refers to entire series of 93 subjects.
† - Refers to the number of subjects exhibiting that manifestation.
+ - Based on incomplete data; in many instances subjects were not followed until last signs of toxicity had subsided.
The cardiotoxic effects of emetine seem to be the most severe and life threatening in man. This is based on the observation of myocardial degeneration in several fatal cases without similar changes in other tissues (Kattwinkel, 1949; Gonzalez de Cossio, 1952; Brem and Konwaler, 1955; Wasserman, 1970; Faure et al., 1972). Electrocardiographic changes have been taken as an early sign of this condition. The most common changes in the ECG are flattening or inversion of the T wave and prolongation of the QT interval (Klatskin and Friedman, 1948; Gonzalez de Cossio, 1952; Powell, 1967; Pain and Wingfield, 1968). Also, nonspecific deviation of the S-T segment, changes in P waves and QRS waves, and prolongation of P-R interval have been occasionally observed. There have been several reports showing increased levels of serum glutamic-oxalacetic transaminase, serum glutamic-pyruvic transaminase, and serum creatine phosphokinase concurrent with onset of ECG changes (Dempsey and Salem, 1966; Kini et al., 1969; Mastrangelo et al., 1973; Siddiqui et al., 1973; Moertel et al., 1974). In two of these studies, values for these enzyme levels were obtained several times during and after the treatment period (Dempsey and Salem, 1966; Mastrangelo et al., 1973). In both cases the pattern of changes of serum enzyme levels was an increase during the treatment period followed by a decrease and then an even greater increase 1-2 weeks after the treatment.
period. The second increase of serum enzyme levels could not be correlated with ECG changes, muscle weakness or local pain at the injection site.

**Toxic Effects in Animals.** Emetine intoxication in animals is marked by general weakness, lethargy, anorexia, weight loss, and in cats and dogs, vomiting. The weight loss can be considerable and depends on duration of administration (Appelt and Heim, 1964). Dogs given 1.4 mg base/kg/day died after eight days and lost 30% of their initial body weight (Child et al., 1964). Cats and rabbits were reported to have lost 5% to 33% of their body weight after receiving a single lethal dose of emetine (Anderson and Leake, 1930). Rats lost an average 24% of initial body weight after receiving emetine hydrochloride 1.25 mg/kg/day for 15 days (Duane and Engel, 1970). Chatterjee et al. reported that rats given 2 mg/kg/day for 10 days lost 17% of their initial body weight whereas pair fed controls showed no significant change in body weight (Chatterjee et al., 1970). In another study rats given approximately 1 mg/kg/day for 28 days gained weight but pair-fed animals gained twice as much weight in the same period of time (Diamant, 1958). Thus, some action of the drug other than appetite suppression may be responsible for loss of body weight in animals.

Lesions and necrosis similar to those observed in
clinical fatalities have been reported to occur in rabbit cardiac muscle (Rinehart and Anderson, 1931) and rat skeletal muscle (Duane and Engel, 1970) after chronic emetine administration. A period of time was required for these changes to become apparent. Rinehart and Anderson (1931) saw changes in cardiac tissue from rabbits only if death occurred three days after a single lethal dose of emetine. Duane and Engel (1970) reported the earliest changes in rat skeletal muscle morphology did not occur until emetine had been administered daily for five days.

There have been several studies to determine the acute lethal dose of emetine. The best estimates of the LD\textsubscript{50} in rats are 12.1 ± 0.87 mg/kg (Radomski et al., 1952) and 17.2 ± 1.4 mg/kg (Gimble et al., 1948). In mice values of 62 ± 2.3 mg/kg (Gimble et al., 1948) and 45 mg/kg (Child et al., 1964) have been calculated for the LD\textsubscript{50}. There have been very few studies of emetine lethality due to chronic administration of sublethal doses. Johnson and Neal (1968) reported that a dose of 9.9 mg/kg given to mice for five consecutive days produced lethality in half of the animals. This cumulative dose of 49.5 mg/kg is very close to the acute doses mentioned above. However, Rosen et al. (1935) have shown that when a dose of emetine was divided up and administered to guinea pigs over a period of time, the mortality ratio was higher than it was when the entire dose was given at one time.
Pharmacology of Emetine

Absorption, Distribution, Metabolism and Excretion.

Emetine is readily absorbed after either oral or parenteral administration. The distribution of emetine does not seem to vary appreciably with route of administration, duration of administration or species (Gimble et al., 1948; Parmer and Cottrill, 1949; Child et al., 1964; Schwartz and Herrero, 1965). In general, the order of tissue concentrations is as follows: spleen > lung > kidney > liver > heart. After emetine administration has ended, this order of tissue concentrations does not change. However, the rates of decrease in tissue concentration differ, with liver having the highest rate and spleen and heart the lowest. The overall rate of elimination of emetine is slow so that chronic administration causes accumulation of the drug for several days before a steady state is reached (Child et al., 1964). Significant amounts of emetine have been found in animals 5-6 weeks after the drug was administered (Gimble et al., 1948; Child et al., 1964).

Emetine has not been shown to be biotransformed in any species. In fact Miller et al. (1970) demonstrated inhibition of drug metabolism in liver microsomal preparations obtained from rats pretreated with emetine. This inhibition of drug metabolizing activity was explained by two different actions of emetine; a direct competitive
inhibition and an indirect action lowering the cytochrome 
P-450 and cytochrome b5 content of liver. Under these 
circumstances it seems unlikely that emetine itself would 
be biotransformed (Johnson et al., 1971).

A recent study in humans reports that emetine is excreted 
in the urine (Synek, 1974). In this study nothing was said 
of fecal excretion except that no emetine was excreted 
into the bile and subsequently reabsorbed from the GI 
tract. Two animal studies have shown that emetine is excreted 
only in the feces (Gimble et al., 1948; Schwartz and 
Herrero, 1965).

Inhibition of Protein Synthesis by Emetine, Grollman 
(1966, 1968) was the first to show that emetine is a 
potent inhibitor of protein synthesis in eukaryotic cells 
only. He was able to completely inhibit protein synthesis 
with emetine in certain mammalian yeast and plant cells 
but not in E. coli. At an emetine concentration of 4 x 10^{-8} M 
a 50% inhibition of protein synthesis was observed in 
cultured Hela cells. The same degree of inhibition was 
observed in cell free preparations derived from Hela cells 
at a concentration of 8 x 10^{-5} M. This difference was 
partially explained by the fact that cells concentrate 
the drug 50-fold. The mechanism of this action was shown to 
be an inhibition of translocation of peptidyl-sRNA from 
the acceptor site to the donor site on the ribosomal
complex. Emetine did not affect activation of amino acids, synthesis of aminoacyl-sRNA, chain elongation or release of polypeptides from the ribosomal complex. In this respect the action of emetine was very similar to that of cycloheximide. However, the action of cycloheximide was shown to be reversible while that of emetine was irreversible.

Simultaneous with the inhibition of protein synthesis, emetine causes an inhibition of DNA synthesis (Grollman, 1968). Emetine inhibited protein synthesis in reticulocytes (which have no nucleus) at the same concentrations which were effective in nucleated cells. Therefore, it was thought that inhibition of DNA synthesis was the result of inhibition of protein synthesis and not vice versa. At concentrations which completely inhibit protein synthesis, emetine has no effect on RNA synthesis except after long incubation periods (Grollman, 1968). However at a much higher concentration (1.5 x 10^{-4} M), emetine immediately inhibits RNA synthesis by 80% (Gilead and Becker, 1971).

Perlman and Pinman (1970) reported that mitochondrial protein synthesis was not inhibited by concentrations of emetine which completely block cytoplasmic protein synthesis. However later reports have shown that inhibition of mitochondrial protein synthesis does occur at low concentrations of emetine although to a much smaller degree than that of cytoplasmic protein synthesis (Lietman, 1971; Chakrabarti et al., 1972). The specificity of
emetine in blocking only eukaryotic cytoplasmic protein synthesis at doses which minimally affect mitochondrial protein synthesis has made emetine an important research tool in various types of studies (Hogan and Gross, 1971; Schwartz et al., 1971; Mahler et al., 1971; Grollman and Huang, 1973).

Emetine has been shown to inhibit protein synthesis in heart and liver (Beller, 1968; Jondorf and Szapary, 1968), both in vitro and in vivo. In rat heart, $5 \times 10^{-7}$ M emetine inhibits protein synthesis by 50% in vitro (Beller, 1968). This effect was observed as a decreased incorporation of $^3$H-leucine into soluble and contractile proteins. When rats were given emetine 1.3 mg/kg/day for three days, a 68% inhibition of $^3$H-leucine incorporation into both types of protein was observed in vitro (Beller, 1968). However no change in protein content of heart was observed in rats given 2 mg/kg/day for 10 days (Chatterjee et al., 1970). It was suggested that this observation was explained by decreased protein degradation concurrent with decreased protein synthesis, yet emetine had no effect on transaminase activity in rat heart (Chatterjee and Roy, 1973).

**Effects of Emetine on Cardiac Metabolism.** Emetine completely blocks endogenous respiration in rat heart homogenates as well as respiration with butyrate and β-hydroxybutyrate as substrates (Appelt and Heim, 1964).
This effect was seen with in vitro concentrations of emetine greater than $10^{-4}$M and when the animals were injected with emetine at a dose of 2 mg/kg/day for 17 days. Quinine at similar in vitro concentrations produced the same degree of inhibition yet had no effect on cellular respiration when chronically administered to animals in very high doses. Therefore, the in vitro effect at high concentrations was considered nonspecific. Studies have shown that chronic emetine administration inhibits respiration of rat heart homogenates when citrate, malate, glutamate and α-ketoglutarate were used as substrates (Appelt and Heim, 1965). However, when succinate was used as the substrate for respiration in rat heart homogenates chronically treated with emetine, no inhibition was observed (Appelt and Heim, 1965). Emetine does not affect oxidative phosphorylation in that no change in the P:O ratio was observed in rat heart mitochondria after in vivo or in vitro administration of the drug (Appelt and Heim, 1964). Similarly, in vitro administration of emetine to respiring dog heart mitochondria did not affect the ADP:O ratio (Murphy et al., 1974).

The Mechanism of Emetine Cardiotoxicity. Considering the pathological changes observed in the heart with severe emetine toxicity, it seems most likely that a biochemical mechanism of action such as inhibition of protein synthesis
or inhibition of mitochondrial respiration would best explain the effects. Both of these actions occur \textit{in vitro} and \textit{in vivo} but neither has been correlated with the onset of cardiotoxicity. The basic question of which action is the primary mechanism or whether both in combination provide a mechanism for emetine cardiotoxicity has not been answered. However, there have been some interesting observations which allow one to speculate. The similarity between the concentrations of emetine \textit{in vitro} which inhibit mitochondrial respiration and mitochondrial protein synthesis is striking and suggests some correlation. Could it be that mitochondria \textit{in vivo} are exposed to such high concentrations due to intracellular pooling or nonuniform subcellular distribution?

Grollman noted the difference in emetine concentrations required to produce the same degree of protein synthesis inhibition in Hela cells and cell free extracts of Hela cells. About one fourth of this difference was made up by the fact that the cells concentrated emetine. The remaining difference he conjectured was explained by intracellular pooling and a greater availability of binding sites in the cell free extract. However another factor may be involved. In the cell free extract, energy for protein synthesis was provided in the form of ATP added to the reaction mixture, whereas the intact cells were required to produce their own ATP. Thus inhibition of
mitochondrial respiration and/or oxidative phosphorylation by emetine may play a role in its action on cellular protein synthesis. An observation made by Duane and Engel (1970) further supports this possibility. In their histochemical studies of skeletal muscle from emetine treated rats they saw myofibrillar lesions within the confines of regions with decreased mitochondrial activity. Whereas they never observed lesions without decreased mitochondrial activity, they often observed decreased mitochondrial activity without lesions.

Finally, the observations of Watkins and Guess (1968) on emetine toxicity in cultured embryonic chick heart cells are of interest. Emetine added to the culture medium caused a slow decrease in beating rate of individual cells. This effect could be reversed by the addition of TPNH, DPNH or ATP to the culture medium. No other pyridine or adenine nucleotides produced this effect.

The question of time is also important. What causes the delayed onset and long duration of emetine cardio-toxicity? Certainly cumulative uptake and distribution is involved but there is no information correlating uptake and distribution with the onset of toxicity. Some other time factor may be involved.
Objectives of the Present Study

The primary objective of this study is to gain information concerning the mechanism of emetine cardio­toxicity. Emetine toxicity in guinea pigs after chronic emetine administration was demonstrated and defined by electrocardiographic changes, loss of body weight and lethal dose studies. During chronic in vivo administration of emetine which produced a known degree of lethality in guinea pig populations, studies were done to correlate changes in myocardial uptake and subcellular distribution of emetine and in myocardial fine structure with onset of cardiotoxic effects. The results of these in vivo studies were compared with the results of similar or related in vitro studies. This was done in order to determine whether in vivo effects were due to the same drug actions which produced in vitro effects or whether in vivo effects were due to indirect drug actions not occurring in vitro. In other words, these studies were done in order to de­termine whether the mechanism of chronic emetine toxicity is the same as or different than the mechanism of acute emetine effects. Studies of myocardial adenine nucleotide and creatine-PO₄ concentrations and studies of heart mitochondrial function were done to determine if cardiac metabolism was inhibited at the time when cardiotoxic effects of emetine were observed.
MATERIALS AND METHODS

Whole Animal Experiments

**Injections.** Emetine was administered to animals by means of intraperitoneal (IP) injection at the same time each day for various periods of time. The injected volume was always 1 ml. Except for the radiolabelled drug studies, the sham injection solutions used for the various studies was sterile saline. Because the stock solution of $^3$H-emetine used to prepare drug injection solutions also contained 0.1 N HCl and 2% ethanol, the sham injection solution used in the radiolabelled drug studies contained an equivalent amount of HCl and ethanol in sterile saline.

**Lethal Cumulative Dose Studies.** Based on preliminary studies in which animals were given 0.5 to 16 mg/kg/day, 6 mg/kg/day was chosen as the dose which would produce significant toxic effects in ten days. This dose was given to a group of 16 male guinea pigs weighing 315 ± 3.8 grams. The animals were weighed and injected daily until death occurred. The lethal cumulative dose was
determined using log dose-probit analysis.

**Electrocardiogram Recording.** The three standard limb lead electrocardiograms were recorded daily from unanesthetized animals given emetine at doses of 2 to 6 mg/kg/day and from pair-fed sham injected controls. The animals were restrained on their backs and one half inch long 27 gauge syringe needles were inserted subcutaneously into each limb. To keep the electrically active area of the electrodes constant, all but one fourth inch at the tip of the needles was teflon coated. The potential difference between the electrodes was fed into a Grass model 5P5C EEG pre-amplifier and then into a Grass model 5C polygraph for recording.

Three guinea pigs were given emetine at a dose of 6 mg/kg/day for ten days. During this time daily electrocardiograms were recorded from the animals as described above. Twenty four hours after the last emetine injection electrocardiograms were recorded, then each animal was given 0.1 mg/kg atropine by intraperitoneal injection. Then electrocardiograms were recorded from each animal at one half hour intervals for two hours.

**Pair Feeding.** Animals given doses of emetine from 2 to 16 mg/kg/day lost body weight. Examination of gastric contents suggested that loss of body weight may have been
due to decreased consumption of food. Thus pair feeding experiments were necessary in order to determine whether some action of the drug other than anorexia was responsible for loss of body weight. Also pair fed sham injected animals served as the most proper controls for emetine treated animal studies.

Pair feeding was done by first selecting two animals with similar body weights. One was injected with emetine and given a known excess amount of food. Twenty four hours later, the food consumed by the emetine treated animal was determined by difference. The amount of food consumed was then given to the sham injected pair fed control animal. This was repeated every day for the entire administration period with the pair fed animal finishing its administration period one day after the emetine treated animal. Sham injected pair fed animals always consumed all food given them with this protocol.

Studies of Myocardial Fine Structure

Male guinea pigs weighing 309 ± 1.6 grams were given IP injections of emetine dihydrochloride in sterile saline at a dose of 6 mg/kg/day for periods of 3 (n=3), 6 (n=3), or 10 (n=5) days. The same number of sham injected animals received sterile saline without emetine and were pair fed with the emetine treated animals.
Twenty four hours after the last injection the animals were sacrificed by decapitation, and their hearts were prepared for Langendorff perfusion as explained below. Perfusion was begun with bicarbonate buffered Krebs-Henseleit (K-H) medium at room temperature containing 1 IU/ml heparin. Flow was maintained at 5 ml/min for a time just sufficient to wash blood out of the vascular space (about 5 minutes). Then fixation was begun by switching the perfusion medium to ice cold 1% glutaraldehyde (GA) fixative at a flow rate of 10 ml/min. The 1% GA fixative contained in per cent volume concentrations: 2% was 50% aqueous GA solution, 49% was 0.1 M Potassium phosphate buffer ph 7.4, 49% was 0.3 M sucrose solution. To this mixture was added 0.5 g/lit CaCl$_2$·2H$_2$O and 1.32 g/lit NaCl to make the complete 1% GA fixative. After 25 minutes the perfusion medium was switched to ice cold 6% fixative. The 6% GA fixative contained the same mixture of solutions as the 1% GA fixative except it contained more GA and no chloride salts. Ten minutes later perfusion was stopped and a section of left ventricle adjacent to the posterior descending branch of the right coronary artery was removed. This tissue was finely minced and incubated in cold 6% GA fixative for 1½ hours then in 1% OsO$_4$ fixative (according to Millonig in Pease, 1964).

After dehydration and embedding in Spurr Low-Viscosity
embedding media, the tissue blocks were thin sectioned using glass knives on a Porter-Blum Sorvall model MT-2 ultramicrotome. The sections were placed on copper grids and counterstained by immersing them in a saturated 50% ethanol solution of uranyl acetate for 20 minutes followed by lead citrate stain (Reynolds, 1963) for 20 minutes. The sections were then viewed on a Philips Model EM-300 electron microscope.

**Acquisition of Tritiated Emetine**

Fifty milligrams of emetine dihydrochloride was sent to New England Nuclear, Boston Massachusetts to be tritium labelled by the process of random catalytic exchange. The product of this reaction was returned with a total radioactivity of 580 millicuries.

**Purification of \( ^3\text{H} \)-Emetine.** A thin layer chromatographic method described by Stahl (1965) for the separation of ipecacuanha alkaloids uses Silica Gel G as the stationary phase and a solvent system of chloroform-methanol (85:15). The method of detection uses a 0.5% solution of iodine in chloroform as the spray reagent followed by heating at 60°C for 15 minutes. After this treatment emetine is identified by its yellow color in white light and yellow-blue fluorescence in long
wave ultraviolet light (365nm wavelength). The lower limit of emetine detection with this method is 0.002 microgram.

Using this TLC method, the product of the tritium labelling process was found to contain $^3$H-emetine and several other labelled compounds. Separation of $^3$H-emetine from the other compounds was accomplished by spotting the solution of labelled compounds on a preparative TLC plate (1mm thick Silica Gel G on a 20 cm x 20 cm glass plate) along with a separate standard emetine solution. The plate was developed and the emetine band as identified by the standard was scrapped off the plate. The silica gel making up the emetine band was placed in a small column and the $^3$H-emetine was eluted from it with methanol at a flow rate of about 0.3ml/min. After about 5 hours the radioactivity coming off the column was down to 12,000 counts/ml and elution was stopped. The collected effluent was acidified with 5% HCl-methanol and its volume reduced to about 10 ml. This solution was analysed for radiochemical purity as described below.

After radiochemical purity was established the remainder of the effluent was evaporated to dryness, the residue was weighed and then dissolved in 100 ml. of 0.1 N HCl with 2% ethanol added to act as a scavenger of reactive species produced by self-radiolysis (Bayly and Evans, 1966). Fifty milligrams of emetine dihydrochloride
was added to the solution to dilute the specific activity to 60.5 uc/mg. This stock solution was stored in an amber bottle at 4°C. Experiments utilizing $^3$H-emetine were completed within 14 months.

Criteria for Chemical and Radiochemical Purity. Two methods were used to establish chemical and radiochemical purity of $^3$H-emetine after purification. These methods were thin layer chromatography and spectrofluorometry. Radiochemical purity was determined immediately after purification and 18 months later.

Three solvent systems which produced different emetine RF's were chosen. These included the system used for purification and two others, cyclohexane-diethylamine (90:10) and chloroform-diethylamine (90:10). Three Silica Gel 60 pre-coated glass TLC plates of 0.25 mm layer thickness (EM Laboratories Inc., #5763) were spotted with 2 ug each of two standard emetine solutions, a solution of the labelled mixture from New England Nuclear and a solution of purified $^3$H-emetine. One of these plates was developed in each of the solvent systems, sprayed with iodine spray reagent and photographed in long wave ultraviolet light. The plate developed in chloroform-methanol was then used to analyze radiochemical purity. The pathways from origin to solvent front of the two radioactive solutions were divided into 1 cm segments, scrapped off and placed in
liquid scintillation vials. Fifteen milliliters of Bray's Solution was added to each vial. After allowing 24 hours for extraction of the labelled compounds into the scintillation cocktail, the radioactivity in the vials was counted in a Packard TriCarb Model 4312 Liquid Scintillation Spectrometer.

Using the Aminco-Bowman Spectrophotofluorometer (Cat. #4-8202), both the excitation and emission spectra of an emetine standard and \(^3\)H-emetine were determined in 0.1 N HCl with 2% ethanol. This method was compared with that of Davis et al. (1962).

Studies of Cardiac Uptake and Subcellular Distribution of \(^3\)H-Emetine

**In Vitro Langendorff Perfusion Studies.** Male guinea pigs weighing 255 ± 6.4 grams were sacrificed by decapitation. Their hearts and lungs were rapidly removed together and placed in Krebs-Henseleit (K-H) medium at room temperature. The K-H medium contained in m moles/liter: Na\(^+\), 145.3; K\(^+\), 5.8; Ca\(^{++}\), 2.5; Mg\(^{++}\), 1.2; Cl\(^-\), 127.9; SO\(_4^{--}\), 1.2; H\(_2\)PO\(_4^{--}\), 1.0; HCO\(_3^{--}\), 27.2; glucose, 11.1; with 1 IU/ml heparin and was well equilibrated with a gas mixture of 95% O\(_2\) - 5% CO\(_2\) so that the pH was 7.42.

The aorta was dissected free, cut in the region of
the aortic arch and perfused by means of a peristaltic pump with K-H medium minus heparin. The temperature of the perfusion medium was maintained at 25°C. After perfusion had started, a mass ligature was placed around all of the pulmonary veins just proximal to the left atrium and the lungs were removed.

Right intraventricular pressure was measured by attaching one end of a canula to a Statham model P23AC pressure transducer and placing the other end into the right ventricle via the pulmonary artery. After all tissue had been dissected away from the right atrium so that the opening to the superior vena cava was clear, the canula was tied in place. The output of the pressure transducer was fed into a Grass model 5P1H Low-Level DC Pre-amplifier and then into a Grass model 5C polygraph for recording. The signal from the pressure transducer was taken from the polygraph driver amplifier, differentiated by an R-C circuit (Baskin, 1971) and then recorded on another channel of the polygraph.

A surface electrogram was recorded from the perfused hearts by two electrodes, one attached to the metal perfusion post in the aorta and the other at the apex of the heart. The potential difference between the two electrodes was fed into a Grass model 5P5C EEG pre-amplifier and then into another channel of the polygraph.
Based on preliminary dose response studies, a $10^{-6} \text{M}$ concentration of emetine was chosen as the half maximally effective dose in this system. After an equilibration period of 70 minutes, $10^{-6} \text{M} \ ^3\text{H}-\text{emetine (7.8uc/mg)}$ in K-H medium was perfused for periods of either 4, 8, 16 or 64 minutes. This was followed by an eight minute washout period during which K-H medium was perfused without emetine. Samples of the perfusate coming out of the heart were collected during drug perfusion and washout.

After the washout period, both ventricles were dissected free of major vascular and connective tissue, weighed and then minced. The tissue was then homogenized in a Potter-Elvehjem homogenizer with a volume of sucrose-ethylenediamine tetraacetic acid (EDTA) solution ($0.33 \text{M sucrose}, 10^{-3} \text{M EDTA}$) sufficient to produce a 10% tissue homogenate.

The homogenate was fractionated by differential centrifugation according to the method of Dutta, Goswami, Lindower and Marks (Dutta et al., 1968) which is illustrated in figure 1.

A biuret protein assay (Gornall et al., 1949) as modified by Dutta et al. (1968) was used to measure protein contents of the five fractions and tissue homogenate. The assay mixture contained 0.1 ml. unknown protein solution, 1.2 ml. distilled water, 0.2 ml. 5% aqueous desoxycholate solution and 1.5 ml. of biuret reagent. The assay mixture
Figure 1

Procedure for fractionation of heart homogenates by differential centrifugation.
10% Tissue Homogenate

166,000 x G, 1 hour

Pellet

Initial Supernate

Resuspend

450 x G, 15 min.

Supernate

Nuclear Fraction

12,000 x G, 15 min.

Supernate

Mitochondrial Fraction

166,000 x G, 1 hour

Final Supernate

Microsomal Fraction
was incubated for 30 minutes at room temperature and then optical densities were read at 540nm on a Gilford model 240 spectrophotometer. Protein contents were calculated from a standard curve derived from assaying standardized amounts of bovine serum albumin.

Duplicate aliquots of the five fractions, the tissue homogenate, and the perfusate samples were placed in scintillation vials with 15 ml. of Bray's solution. The quantity of $^3$H-emetine in each sample was determined with the use of a Packard Tri-Carb Model 4312 Liquid Scintillation Spectrometer. Counting efficiency in each vial was determined with an automatic external standard.

The concentration of emetine in each fraction was calculated and expressed as picomoles/mg protein. The initial supernate/pellet activity ratio (IS/P) was calculated as follows:

Total homog. dpm = aliquot dpm x vol. of homog. fractionated
Total IS dpm = aliquot dpm x total volume of IS
Total pellet (P) dpm = total homog. dpm - total IS dpm

$$\text{IS/P} = \frac{\text{total IS dpm}}{\text{total pellet dpm}}$$

In a similar manner the final supernate/pellet activity ratio (FS/P) was calculated.
Controls were done to see if homogenization had any effect on the subcellular distribution of emetine. $^{3}H$-emetine was not perfused in these hearts but added directly to tissue before or after the 10% homogenate was made. The final concentration in the homogenate was close to that obtained in hearts perfused with $10^{-6} M$ $^{3}H$-emetine for 64 minutes. These homogenates were then treated as described for the perfused hearts.

Statistical analysis of the results of these studies was done using Student's t-test to compare group means.

In Vivo Administration Studies. Male guinea pigs weighing $314 \pm 2.0$ grams were given IP injections of $^{3}H$-emetine (10 uc/mg) at a dose of 6 mg/kg/day for periods of 1, 3, 6 or 10 days. In order to evaluate any change in uptake and subcellular distribution of individual doses over the 10 day administration period, two groups of animals were given 6 mg/kg/day injections of unlabelled emetine dihydrochloride for 5 or 9 days followed by a final 6 mg/kg injection of $^{3}H$-emetine on the 6th or 10th day. Two more groups of animals received sham injections and were pair fed with the $^{3}H$-emetine treated animals for the 5 or 9 day period prior to $^{3}H$-emetine administration on the final day.

Twenty four hours after the final injection, the animals were sacrificed by decapitation, blood samples
were collected and their hearts were quickly removed and prepared for Langendorff perfusion as described above. K-H medium containing 1 IU/ml heparin was perfused at a rate of 5 ml/min for four minutes. After this washout period both ventricles were homogenized and fractionated as described previously.

The same biuret protein assay was used to measure protein contents in the five fractions and homogenate except that more desoxycholate was needed to aid dissolution of tissue lipids which were found in greater amounts in these hearts. The assay mixture used here was the same except it contained only 0.1 ml distilled water and 1.3 ml of 2.5 aqueous desoxycholate solution.

In preparation for liquid scintillation counting, duplicate aliquots of the five fractions, homogenate and plasma were burned in a Packard Model 306 Sample Oxidizer. This instrument automatically burns each sample, condenses and collects the resulting tritiated water in scintillation vials and then pumps the desired amount of scintillation cocktail (15 ml of Packard Monophase-40 in this study) into the vial. It was necessary to do this because total extraction of emetine into Bray's Solution was not achieved.

Liquid scintillation counting and data processing were done as described above. Statistical analysis of these results was done using analysis of variance.
Electropharmacological Studies

**Dissection and Experimental Apparatus.** Male guinea pigs were sacrificed by decapitation, their hearts removed and placed in K-H medium (composition described above except without heparin). The right and left atria were removed together as a pair by cutting across the heart at the base of the ventricles. Paired atria were placed in a 50 ml bath containing K-H medium continuously aerated with 95% O₂ - 5% CO₂. The bath temperature was maintained at 30 ± 0.1°C. Isometric contractions were recorded with a Statham G-10-B force transducer. Zero tension during diastole was established, then the tissue was stretched until diastolic tension was increased to 900 mg (approximately 1.2 x rest length). The output of the force transducer was fed into a Statham Universal Transducer Readout model UR 5 and then into a Beckman type RS Dynograph for recording.

A papillary muscle from the right ventricle was dissected out and its base fixed to a punctate stimulating electrode assembly (Hollander, 1967). It was then placed in a 15 ml bath containing K-H medium continuously aerated with 95% O₂ - 5% CO₂ with the temperature maintained at 30°C ± 0.1°C and stimulated at a rate of 60/minute. The experimental set up of equipment for
recording intracellular potentials by standard micro-electrode techniques and isometric contractions of the papillary muscles has been reported (Ito et al., 1970). The length of the papillary muscles was measured by microscopy. Rest length was defined as the length of the muscle at which a developed tension of less than 20 mg was observed. After rest length was established the papillary muscles were stretched to 125% of rest length. At this length half maximal developed tension is obtained according to the length-tension relation for this preparation (Mark Strauch, personal communication). Since action potential characteristics have been shown to vary in different regions of guinea pig papillary muscles (Arlock, 1974), care was taken to record action potentials from only the center region of the papillary muscles.

**Experimental Protocol.** All tissues were allowed to equilibrate in the tissue bath for one hour before experimental protocol was begun. During this time the bath medium was changed every 20 minutes. Then there followed a control period of 30 minutes so that control values were established for each preparation. Three preparations from untreated animals were run for four hours after the equilibration period with bath medium changes every 60 minutes. This was done to establish the long term stability of the preparation. After equili-
bration and control periods, experimental protocol lasted no longer than 3½ hours and the bath medium was changed at least every 60 minutes.

A group of untreated animals weighing 384 ± 12.7 grams was used to establish long term preparation stability, paired atria dose-response relationships for acetylcholine and norepinephrine and the dose-response relationship for emetine dihydrochloride in both paired atria and papillary muscles.

The effect of emetine on the response to acetylcholine (Ach) and norepinephrine (NE) in both paired atria and papillary muscles was studied. This was done with preparations from a group of untreated animals, a group of animals weighing 314 ± 7.8 grams treated with emetine 6 mg/kg/day for 10 days, and a 10 day sham injected pair fed group weighing 306 ± 6.0 grams. The responses to two concentrations of both Ach and NE were obtained before and while the preparations were exposed to 3 x 10^-5 M emetine. The effect of 10^-5 M atropine on the response of paired atria to 10^-6 M Ach and 3 x 10^-5 M emetine was also studied.

Studies of Myocardial Adenine Nucleotide Concentrations

Preparation of Tissue Extracts, Three male guinea
pigs weighing 329 ± 7.0 grams were given emetine by IP injection at a dose of 6 mg/kg/day for 10 days. Three more animals weighing 301 ± 8.5 grams were given sham injections and pair fed for 10 days. Twenty four hours after the final injection, the animals were anesthetized with diethyl ether, the thorax was opened and a small piece of tissue (approximately 100 mg.) was cut from the apex of the beating heart. This tissue was immediately frozen with a Wollenburger clamp which had previously been cooled in liquid nitrogen. Tissues were stored in liquid nitrogen until extracts were made.

Frozen tissues were weighed then pulverized by a method first described by Stuart Simon (1968). A cleaned piece of tygon tubing was divided into two enclosed compartments with hemostat clamps. Approximately 0.5 ml ice cold 3M perchloric acid (HClO₄) was placed in one compartment and 50-100 mg of frozen tissue in the other. The tissue was pulverized by a blow with a mallet and immediately mixed with the HClO₄ by removing the hemostat separating the two compartments. The mixture was washed into a test tube with 0.2 ml ice cold HClO₄ and then incubated in an ice bath for 30 minutes. Approximately 2.8 ml of distilled water was added to the mixture diluting the HClO₄ to 0.6M. Then the mixture was homogenized with a high speed blade homogenizer (Tekmar Co., model SDT). The homogenate was centrifuged at 5000 x G for 15 minutes.
Three ml. of the clear supernate extract was removed and neutralized (pH 6.8 - 7.2) with a known volume of KOH-EDTA solution (contained 3M KOH, 0.015M EDTA). Then 0.1 ml of 1M imidazole (pH=7) was added as a buffer. The extract was stored at -90°C.

**Enzymatic Assay for Creatine-PO₄, ATP, ADP and AMP.** The assays used to determine creatine-PO₄ and adenine nucleotide contents of tissue extracts were based on methods developed by Lowry and Passonneau (1972). Specific enzyme reactions can be coupled to other reactions which are dependent on pyridine nucleotides as cofactors. When the coupled reactions are run to completion; the measured increase or decrease in fluorescence as the pyridine nucleotides are reduced or oxidized respectively is stoichiometrically equal to the assayed compound. In this study, kinetics for each enzyme reaction were routinely run before the assay. Ten half times were allowed for the reactions to go to completion.

ATP was assayed by running the coupled reactions below:

\[
\begin{align*}
\text{ATP} + \text{glucose} & \underset{\text{hexokinase}}{\rightarrow} \text{ADP} + \text{G6P} \\
\text{G6P} + \text{TPN}^+ & \underset{\text{dehydrogenase}}{\rightarrow} 6\text{Pgluconate} + \text{TPNH} + \text{H}^+
\end{align*}
\]
The increase in fluorescence due to the reduction of TPN$^+$ was measured with a Farrand Ratio Fluorometer.

Creatine-P$_4$ was assayed by coupling the creatine phosphokinase reaction (below) to the above reactions after they had gone to completion.

\[
\text{Creatine-P}_4 + \text{ADP} \xrightarrow{\text{CPK}} \text{ATP} + \text{creatine}
\]

ADP was assayed by the following coupled reactions:

\[
\text{ADP} + \text{PEP} \xrightarrow{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate}
\]

\[
\text{pyruvate} + \text{DPNH} + \text{H}^+ \xrightarrow{\text{lactic dehydrogenase}} \text{lactate} + \text{DPN}^+
\]

The result of these reactions is a decrease in fluorescence as DPNH is oxidized.

After the above reactions were completed, AMP was assayed by coupling the reaction below:

\[
\text{AMP} + \text{ATP} \xrightarrow{\text{myokinase}} 2\text{ADP}
\]

Statistical analysis of the data from these studies was done by comparison of group means using the Student's $t$-test.
Preparation of Guinea Pig Heart Mitochondria. The method of mitochondrial preparation described for rat heart by Pande and Blanchaer (1971) was used in this study. Hearts were quickly removed and placed in ice cold solution A containing 0.21M mannitol, 70mM sucrose and 0.1 mM EGTA. The ventricles were dissected free, washed with several portions of ice cold solution A and then minced. The minced tissue was mixed with 8 ml of solution A containing 10mM Tris base and 4 mg Nagarse at pH 7.4. After incubation in an ice bath for 8 minutes, 12 ml of solution A were added and the mixture was gently homogenized using a Potter-Elvehjem homogenizer with a pestle clearance of 0.66 mm until it was free of large tissue pieces. Following another 8 minute incubation period in an ice bath, 15 ml of solution A were added and the mixture was rehomogenized with a tighter pestle (0.20mm clearance) until no resistance to homogenization was felt (4-6 up and down passes at 300 rpm). The homogenate was centrifuged at 400 x G for five minutes at 0°C. The resulting supernate was centrifuged at 12,000 x G for 10 min. The pellet obtained was washed with solution A to remove the upper layer of white material and then suspended in 15 ml of solution A. The suspension was centrifuged at 12,000 x G for 8 minutes and the resulting
pellet was washed as before. The final pellet was carefully and uniformly suspended in 0.6 ml of solution A with 10 mM Tris (pH 7.4). The protein concentration of this suspension was measured with a biuret protein assay and then diluted to 25 mg/ml with solution A containing 10 mM Tris.

Mitochondria were prepared from a group of six animals which had been given emetine for ten days at a dose of 6 mg/kg/day and from six animals which were pair-fed and sham injected for 10 days. Hearts were pooled in groups of three for each preparation.

Assay of Mitochondrial Respiration and Oxidative Phosphorylation. ADP stimulated mitochondrial oxidations were measured polarographically. The reaction medium contained 100 mM KCl, 2 mM KH₂PO₄ buffer (pH 7.4) and 2 mM substrate. When succinate was used as the substrate 3.4 x 10⁻⁵ M rotenone was included. Oxygen content of the reaction medium was determined assuming air saturation at 25°C (room temperature). Five milligrams of mitochondrial protein (0.2 ml of mitochondrial suspension) were added to 5.8 ml of reaction medium to make the final reaction volume 6 ml. In vitro effects of 2 x 10⁻³ M emetine were observed by adding 60 µl of a 0.2 M aqueous emetine solution.
RESULTS

Whole Animal Studies: Chronic Emetine Administration

Estimation of the Median Lethal Cumulative Dose. The distribution of lethality due to chronic emetine administration in a group of sixteen guinea pigs was determined. These animals were given 6 mg/kg/day until death occurred and the number of deaths occurring at various total cumulative doses was recorded. Both the log dose-percent response curve and the regression line of the log dose-probit response curve for the observed frequency distribution are shown in figure 2. The median lethal cumulative dose (LCD$_{50}$) as computed from regression statistics is 98.9 mg/kg with a 95% confidence interval of 97.6 mg/kg - 100.2 mg/kg. According to this regression line, a total cumulative dose of 60 mg/kg when given at a rate of 6 mg/kg/day will produce lethality in 1% of a population of guinea pigs.

Effect on Body Weight. Chronic emetine administration at doses greater than 1 mg/kg/day caused a dose related decrease in the body weight of guinea pigs. This is shown in
Figure 2

The log dose-percent response curve and the computed regression line for log dose-probit response curve of lethality due to chronic emetine administration in guinea pigs.
PERCENT RESPONSE

LOG CUMULATIVE DOSE (mg/kg)

Y = 6.54X - 8.05

PROBIT RESPONSE
figure 3. Animals given 1 mg/kg/day for ten days gained weight like sham injected animals given food ad libitum. A dose of 2 mg/kg/day caused body weight to remain unchanged over the 10 day administration period, but this was a significant decrease when compared to sham injected animals. Doses of 4 mg/kg/day and 6 mg/kg/day caused actual decreases in body weight.

The changes in body weight of guinea pigs given 6 mg/kg/day and pair-fed sham injected controls are presented in figure 4. Food consumption of animals given 6 mg/kg/day decreased slowly over the 10 day administration period. The amount of food consumed on the first day was 13.9 ± 0.9 grams compared to the 4.8 ± 1.2 grams consumed on the tenth day. While the mean body weight of animals given 6 mg/kg/day for ten days decreased by 19.4%, the mean body weight of pair-fed sham injected animals did not change significantly over the same period. Also shown in figure 4 is the change in mean body weight of the group of animals used in the lethality studies. After the fifteenth day of treatment there was no significant change in the mean body weight of this group. At this time the mean body weight had decreased 23.8% from the initial mean body weight. However, the mean body weight of this group at death was 34.4% less than the initial mean body weight. Per cent body weight lost and time until death were not covarying factors because the correlation coefficient for
Figure 3

Per cent changes of mean body weight in groups of guinea pigs given different doses of emetine for ten days. The number of animals used in each experimental group are Sham = 3, 1 mg/kg/day = 2, 2 mg/kg/day = 4, 4 mg/kg/day = 2, 6 mg/kg/day = 11.
CHANGE BODY WEIGHT

- FED SHAM
- 1mg/kg
- 2mg/kg
- 4mg/kg
- 6mg/kg

% CHANGE BODY WEIGHT

DAY OF TREATMENT

0 2 4 6 8 10
Figure 4

The mean body weights of two groups of guinea pigs given emetine 6 mg/kg/day and a pair-fed sham injected group. The standard error for each data point is less than 10% of the mean. The number of animals used in each experimental group are pair fed sham = 21, 6 mg/kg/day, 10 days = 28, 6 mg/kg/day until death = 16.
BODY WEIGHT (GRAMS)

320
PAIR-FED SHAMS
300

280

260

6 mg/kg/day
10 DAYS

240

6 mg/kg/day
UNTIL DEATH

220

0 5 10 15 20 25 30
DAYS OF TREATMENT
these two variables is -0.089

**Effect on Myocardial Fine Structure.** The preliminary electron microscopic studies reported here did not show any changes in guinea pig myocardial fine structure due to chronic emetine administration. The myocardial fine structure of all animals given emetine at a dose of 6 mg/kg/day or pair-fed sham treatment for 3, 6 or 10 days appeared normal.

**Effect on Heart Rate.** The changes in heart rate observed during chronic emetine administration are shown in figure 5. The mean heart rate of animals given 2 mg/kg/day and sham injected animals increased early in the treatment period, then gradually decreased toward the day zero heart rate. The mean heart rates for these two groups at days 8, 9, and 10 did not significantly differ from their mean day zero heart rates. The pattern of mean heart rate changes for groups of animals given 4 mg/kg/day and 6 mg/kg/day was similar except the rate of decrease during the latter part of the treatment period was more rapid. When compared to the sham heart rate for the same day, significantly decreased mean heart rates were first observed at 6 and 9 days for the 6 mg/kg/day and 4 mg/kg/day groups respectively. It is interesting to note that these changes occur at the same cumulative dose, that is 36 mg/kg.
Figure 5

The mean heart rates of groups of guinea pigs given different doses of emetine for ten days. The standard error for each data point is less than 5% of the mean. The number of animals used in each experimental group are Sham = 7, 2 mg/kg = 4, 4 mg/kg = 2, 6 mg/kg = 22. The asterisks (*) indicate significant difference from the same day mean sham heart rate, P < 0.05.
HEART RATE (BEATS/MIN)

DAY OF TREATMENT

SHAM

2 mg/kg

4 mg/kg

6 mg/kg

0 2 4 6 8 10
Atropine injected IP at a dose of 0.1 mg/kg did not change the heart rate of three guinea pigs which had previously been given emetine 6 mg/kg/day for ten days.

**Effect on the Electrocardiogram.** The changes in lead II electrocardiogram configuration observed during the ten day treatment period are shown in table 2. There was no significant difference at day zero before drug injection between the measured parameters of sham injected animals and animals given 6 mg/kg/day. After ten days of treatment the QRS and T wave amplitude in both sham injected animals and animals given 6 mg/kg/day had increased. However, only the T wave amplitude of the treated group was significantly different from the sham injected group at day ten. While no changes in EKG durations were observed in the sham injected animals, significant changes were seen with chronic emetine administration. Both the P-Q and Q-T intervals of treated animals at day 10 were significantly greater than these values for sham injected animals at day 10 and treated animals at day zero. It is known that the duration of the P-Q and Q-T intervals vary with heart rate and the mean heart rate of treated animals at ten days was much lower than that of sham injected animals (figure 5). The mean heart rate of treated animals at day zero was very close to the mean heart rate at day six. Thus, a comparison of the P-Q and Q-T interval durations
### TABLE 2

Changes in Lead II Electrocardiogram Configuration During Chronic Emetine Administration

<table>
<thead>
<tr>
<th>TREATMENT (n)</th>
<th>P-Q INTERVAL msec</th>
<th>QRS AMPLITUDE µvolts</th>
<th>Q-T INTERVAL msec</th>
<th>T AMPLITUDE µvolts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham, Day 0 (7)</td>
<td>58 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>242 ± 21</td>
<td>106 ± 2</td>
<td>3 ± 4</td>
</tr>
<tr>
<td>Sham, Day 10 (7)</td>
<td>59 ± 1</td>
<td>300 ± 11</td>
<td>103 ± 1</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>6mg/kg, Day 0 (11)</td>
<td>60 ± 2</td>
<td>200 ± 30</td>
<td>109 ± 4</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>6mg/kg, Day 6 (11)</td>
<td>66 ± 2&lt;sup&gt;†&lt;/sup&gt;</td>
<td>308 ± 42&lt;sup&gt;†&lt;/sup&gt;</td>
<td>106 ± 2</td>
<td>52 ± 6&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>6mg/kg, Day 10 (4)</td>
<td>86 ± 6&lt;sup&gt;**†&lt;/sup&gt;</td>
<td>344 ± 77&lt;sup&gt;†&lt;/sup&gt;</td>
<td>138 ± 12&lt;sup&gt;**†&lt;/sup&gt;</td>
<td>88 ± 27&lt;sup&gt;**†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> - GROUP MEAN ± S.E.M.

<sup>†</sup> - Significant difference from Sham Day 10 mean, P < 0.05

<sup>**†</sup> - Significant difference from 6mg/kg Day 0 mean, P < 0.05
in treated animals at zero and six days should exclude differences due to changes in heart rate. Table 2 shows that the P-Q interval at day six was significantly greater than the P-Q interval at day zero but there was no difference between the Q-T intervals at these times.

A wide range of heart rates (240-427 beats/min) was observed in untreated animals at day zero. Using these data, correlation coefficients of -0.54 and -0.72 were computed for the variation of P-Q interval duration with heart rate and Q-T interval duration with heart rate respectively. These correlation coefficients indicate a highly significant degree (P<0.01) of covariation between heart rate and both interval durations. In order to estimate the functional dependence of the P-Q interval duration and the Q-T interval duration on heart rate, the data were subjected to Bartlett's three-group method of linear regression analysis. The regression equations obtained are shown below. Using these equations the P-Q and Q-T

\[
\begin{align*}
P-Q \text{ (msec)} & = (\text{Heart Rate in beats/min}) \times (-0.084) + 86 \\
Q-T \text{ (msec)} & = (\text{Heart Rate in beats/min}) \times (-0.187) + 171
\end{align*}
\]

interval durations were calculated for a heart rate of 265 beats/min which is the mean heart rate of treated animals at day ten. The predicted durations are 64 ± 6 msec for the P-Q interval and 121 ± 9 msec for the Q-T
interval. There was no significant difference between the predicted control Q-T interval and the mean Q-T interval in animals given emetine 6 mg/kg/day for ten days. There was however, a highly significant difference (P<0.01) between the predicted control P-Q interval and the ten day emetine treated P-Q interval.

No rhythm changes were ever observed in sham injected animals. Except for one case, rhythm changes were not observed in emetine treated animals until death. The one exception was the occurrence of two premature ventricular beats which quickly reverted to normal sinus rhythm in one animal given 6 mg/kg/day for 10 days. Electrocardiograms were recorded from three animals dying from chronic emetine administration. Two animals died shortly after a sudden change in atrioventricular conduction starting with 2nd degree and ending with 3rd degree heart block. The third animal died more slowly after suffering several attacks of multiple premature ventricular contractions which reverted back to sinus rhythm. Concurrent changes in QRS configuration indicated anomalies in intraventricular conduction. Finally, heart block occurred and the animal died.

Purity of $^3$H-Emetine

Chemical Purity. Plate I shows three thin-layer chromatograms comparing solutions of $^3$H-emetine before and after
Plate I

Thin-layer chromatograms comparing various emetine solutions. 1. Emetine standard in methanol. 2. Emetine standard in 0.1 N HCl with 2% ethanol. 3. $^3$H-emetine before purification in methanol. 4. $^3$H-emetine after purification in 0.1 N HCl with 2% ethanol. The following solvent systems were used: figure a, cyclohexane:diethylamine (90:10); figure b, chloroform:methanol (85:15); figure c, chloroform:diethylamine (90:10).
purification with two standard solutions of emetine dihydrochloride. Solvent systems of different polarities were used to develop each chromatographic plate so that it would be more likely that separation of contaminating compounds would be achieved. In all three chromatograms a small spot can be seen at the origin of each solution. This is probably due to the dihydrochloride salt of emetine. The two solutions of emetine standard and the solution of purified $^3$H-emetine produced only one other spot in all three systems. The solution of $^3$H-emetine before purification produced up to ten other spots one of which was emetine.

Figure 6 presents the fluorometric excitation and emission spectra of standard emetine dihydrochloride and purified $^3$H-emetine solutions. Both were dissolved in 0.1 N HCl with 2% ethanol. The spectrum labelled C is the emission spectrum for both solutions when an excitation wavelength of 290 nm was used. When an emission wavelength of 320 nm was used, slightly different excitation spectra were observed for the two solutions. The excitation spectrum labelled A in figure 6 is that of the standard emetine solution and the spectrum of $^3$H-emetine is labelled B.

The method of Davis et al. (1962) to determine the fluorometric spectra of emetine dihydrochloride uses a solvent of 0.01 N HCl with 0.05 N KCl. When this method was used, it was found that the solvent alone produced
Figure 6

Fluorometric spectra of standard emetine and purified $^3$H-emetine. A. Excitation spectrum of standard emetine dihydrochloride. B. Excitation spectrum of $^3$H-emetine. C. Emission spectrum of standard emetine dihydrochloride and $^3$H-emetine. Solvent used was 0.1 N HCl with 2% ethanol. Emission wavelength 320 nm, excitation wavelength 290 nm.
high levels of fluorescence even when previously extracted with diethyl ether as suggested by the authors. It was found that the blank fluorescence could be reduced to tolerable levels if the diethyl ether used to extract the HCl/KCl solvent was first extracted with distilled water. This reduced the amount of peroxides known to be present in the ether. Without prior extraction of the ether with distilled water, the peroxides were concentrated in the HCl/KCl solution.

Using the method of Davis et al. (1962) modified as described above, standard emetine dihydrochloride produced the same emission spectrum shown in figure 6. The excitation spectrum was similar to spectrum A in figure 6 the difference being a relative decrease in the intensity of the peaks at 275, 335 and 350 nm compared to the major peak at 320 nm.

Radiochemical Purity. The distribution of radioactivity along the chromatographic pathway of $^3$H-emetine before and after purification is shown in Plate II. The peak in radioactivity on the chromatographic pathway of $^3$H-emetine after purification has the same location as the emetine spot. Immediately after purification 97% of the radioactivity in the $^3$H-emetine solution was associated with the emetine spot. Eighteen months later 85% was associated with the emetine spot. Thus, 12% of the labelled compound had
Plate II

Distribution of radioactivity along the chromatographic pathway of $^3$H-emetine. Figure a (on the left) before purification. Figure b (on the right) after purification. The solvent system used in both cases was chloroform: methanol (85:15).
decomposed due to self-radiolysis over an 18 month period.

Effects of Emetine on Isolated Perfused Hearts

Functional Effects. Figure 7 presents the changes in heart rate, P-Q interval duration and right ventricular pressure observed during perfusion of Krebs-Henseleit medium with and without \(10^{-6}\)M emetine in isolated guinea pig hearts. The differences in the mean values of all three parameters at time zero between the emetine perfused and control groups were not statistically significant. There was very little change in all three parameters for the control group over the 60 minute perfusion period with no significant change from the zero time values. Emetine had no effect on heart rate until 40 minutes after emetine perfusion had begun. At this time 2nd degree heart block had occurred in 2 of the 5 hearts used in this group thus lowering the group mean heart rate. However, the sinus rate in these two hearts was not changed, in fact there was no change in the sinus rate of any of the emetine perfused hearts during the entire 60 minute perfusion period. The decreased mean heart rate of emetine perfused hearts at 60 minutes was not significantly different from the mean heart rate at zero time but was significantly different from the mean control heart rate at 60 minutes. The mean P-Q interval duration of emetine perfused hearts gradually
Figure 7

Changes in mean heart rate, mean P-Q interval duration and mean right ventricular systolic pressure during control and $10^{-6}$ M emetine perfusion of isolated guinea pig hearts. Control group $n = 3$, emetine group $n = 5$. 
increased for 40 minutes and then remained unchanged. This was a statistically significant change which occurred in all hearts. Because of the configuration of the electrogram recorded from isolated perfused hearts and the small n for this experimental group, other measurements could not be made with reliability. There was no significant change in the mean right ventricular pressure of emetine perfused hearts when compared to the zero time mean. However, there was a significant decrease in pressure when compared to the control group. This decrease may have been greater if there were no simultaneous decrease in mean heart rate. The changes in the first derivative of right intraventricular pressure were of the same magnitude and paralleled the pressure changes.

In Vitro Uptake of $^{3}H$-Emetine. Figure 8 shows the concentration of emetine in the perfusate after passing through the heart during drug perfusion and washout. The extraction of emetine by the heart reached a steady state after eight minutes. Under steady state conditions approximately 20% of the amount of drug perfused is taken up by the heart. Based on the initial rate of drug washout, 2 minutes was the estimated time required for washout of drug from the extracellular space. Thus, perfusion for 8 minutes can be assumed to completely washout the extracellular space so that no correction of uptake values for drug
Figure 8

The concentration of emetine in the perfusate after passing through isolated guinea pig hearts during $10^{-6}$ M emetine perfusion and washout. The number of hearts used to determine the perfusate concentrations during drug perfusion and washout are 1 min. = 8, 2 and 4 min. = 10, 8 min. = 8, 16 min. = 3, 64 min. = 4, 65 min. = 1, 66 min. = 2, 68 min. = 3, 72 min. = 4, 80, 96, and 128 min. = 1.
in the extracellular space is needed.

Figure 9 shows the concentration of emetine in the heart after various times of perfusion with $10^{-6}$M emetine in the perfusion medium. As would be expected from figure 8 there was a linear uptake of emetine after 8 minutes of perfusion.

In Vitro Subcellular Distribution of $^{3}$H-Emetine. The concentration of emetine in the various fractions of the heart during perfusion with $10^{-6}$M emetine are presented in figure 10. The microsomal fraction had the highest rate of uptake but the rate of mitochondrial uptake was also quite high. Uptake by the nuclear and supernate fractions proceeded at a much lower rate.

Table 3 compares the subcellular distribution of emetine when added to tissue before or after homogenization to the subcellular distribution observed after emetine was perfused. In all three cases the final concentration of emetine in the tissue homogenate was approximately 5 nm/ml. There was no significant difference between the subcellular distribution of emetine when added to tissue before or after homogenization. However, there were major differences between the subcellular distribution of emetine when added to tissue just before fractionation and when perfused for 64 minutes before fractionation. While there was no significant difference between the microsomal concentrations after these two
Figure 9

The concentration of emetine in isolated perfused guinea pig hearts after various times of perfusion with $10^{-6}$ M emetine. The number of hearts used for each perfusion time are 4, 8, and 16 min. = 2, 64 min. = 3.
EMETINE CONCENTRATION (nm/g TISSUE)

PERFUSION TIME (min)
Figure 10

The concentration of emetine in the subcellular fractions obtained from isolated perfused guinea pig hearts after various times of perfusion with $10^{-6}$ M emetine. The number of hearts used for each perfusion time are 4, 8 and 16 min. = 2, 64 min. = 3.
<table>
<thead>
<tr>
<th>FRACTION</th>
<th>ADDED BEFORE HOMOGENIZATION a (n=6)</th>
<th>ADDED AFTER HOMOGENIZATION a (n=3)</th>
<th>10⁻⁶ M PERFUSED FOR 64' (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMOGENATE</td>
<td>322 ± 17 t</td>
<td>376 ± 4</td>
<td>358 ± 21</td>
</tr>
<tr>
<td>INITIAL SUPERNATE</td>
<td>302 ± 27*</td>
<td>447 ± 90*</td>
<td>125 ± 34</td>
</tr>
<tr>
<td>NUCLEAR</td>
<td>182 ± 20</td>
<td>158 ± 7*</td>
<td>207 ± 8</td>
</tr>
<tr>
<td>MITOCHONDRIAL</td>
<td>356 ± 36*</td>
<td>379 ± 13*</td>
<td>580 ± 29</td>
</tr>
<tr>
<td>FINAL SUPERNATE</td>
<td>105 ± 17</td>
<td>146 ± 17*</td>
<td>92 ± 8</td>
</tr>
<tr>
<td>MICROSOMAL</td>
<td>672 ± 118</td>
<td>671 ± 10</td>
<td>787 ± 72</td>
</tr>
<tr>
<td>RATIO</td>
<td>IS/P 0.344 ± 0.016*</td>
<td>0.351 ± 0.015*</td>
<td>0.134 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>FS/P 0.081 ± 0.013*</td>
<td>0.071 ± 0.001*</td>
<td>0.044 ± 0.002</td>
</tr>
</tbody>
</table>

a - Emetine concentration ~ 5 nm/ml homogenate

† - Mean pm/mg protein ± S.E.M.

* - Significant difference from 64' perfused, P < 0.05
treatments, the mitochondrial concentration was significantly greater and the initial supernate concentration was significantly lower when emetine was perfused. This increase in mitochondrial uptake or binding was reflected in a decreased initial supernate/pellet activity ratio (IS/P). When emetine was added to tissue just before fractionation, the final supernate/pellet activity ratio (FS/P) was significantly larger than the FS/P ratio observed after perfusion.

Uptake and Subcellular Distribution of $^{3}\text{H}$-Emetine After Chronic In Vivo Administration

In Vivo Myocardial Uptake of $^{3}\text{H}$-Emetine. The myocardial concentration of $^{3}\text{H}$-emetine after 1, 3, 6 and 10 days of chronic administration and after single 6 mg/kg doses at 6 or 10 days is presented in figure 11. The bars labelled 1 day, 3 days, 6 days and 10 days indicate the accumulated concentration at the respective times after daily injection of 6 mg/kg doses of $^{3}\text{H}$-emetine. The bars labelled Sham, 6 days and Sham, 10 days indicate the concentration after single 6 mg/kg injections of $^{3}\text{H}$-emetine after 5 or 9 days of pair-feeding with sham injections. Likewise the bars labelled Emetine, 6 days and Emetine, 10 days indicate the concentration after single 6 mg/kg injections of $^{3}\text{H}$-emetine after 5 or 9 days of unlabelled emetine administration at a dose of 6 mg/kg/day. This
Figure 11

Myocardial concentration of emetine after chronic in vivo administration in guinea pigs. The bars labelled 1 day, 3 days, 6 days and 10 days indicate the accumulated concentration at the respective times after daily injection of 6 mg/kg doses of emetine. The bars labelled Sham, 6 days and Sham, 10 days indicate the concentration after single 6 mg/kg doses of $^3$H-emetine after 5 or 9 days of pair-feeding with sham injections. Likewise, the bars labelled Emetine, 6 days and Emetine, 10 days indicate the concentration after single 6 mg/kg doses of $^3$H-emetine after 5 or 9 days of unlabelled emetine administration at a dose of 6 mg/kg/day. The number of animals used in each treatment group are as follows: 1 day = 6, 3 days = 5, 6 days = 8, 10 days = 4, Sham 6 days = 4, Sham 10 days = 4, Emetine 6 days = 4, Emetine 10 days = 5.
EMETINE CONCENTRATION (nm/g heart)

CHRONIC EMETINE TREATMENT

EMETINE DAY 1 3 DAYS 6 DAYS 10 DAYS SHAM DAY 6 EMETINE DAY 6 SHAM DAY 10 EMETINE DAY 10

EMETINE CONCENTRATION (pm/mg prot.)
same format will be used for figure 12.

As shown in figure 11, the emetine concentration in the heart increased until day six and then decreased at day ten. These changes were evident whether emetine concentration was expressed on the basis of tissue weight or protein content. The decrease in concentration at day ten was not due to a change in water content of heart tissue. Tissue water content remained unchanged over the 10 day administration period at approximately 85%. The concentration of emetine found in the heart after individual injections at days 6 and 10 was less than the concentration observed after the first injection. This was true for both prior sham and emetine treatment but the decrease in concentration was greater with prior emetine treatment.

The plasma concentration of emetine increased over the ten day administration period from $1.33 \times 10^{-7} \text{M}$ at day one to $1.09 \times 10^{-6} \text{M}$ at day ten. Figure 12 presents the tissue/plasma concentration ratios for heart after various chronic in vivo administration procedures. This ratio was high at day one then decreased by 63% at day three, remained unchanged at day six then decreased again at day 10. While the distribution between heart and plasma of drug from individual injections at day 6 did not differ from that at day ten, the heart/plasma concentration ratios at these times were lower than the ratio at day one.
Figure 12

The heart/plasma emetine concentration ratio after chronic *in vivo* administration of emetine in guinea pigs. See figure 11 for label description.
In Vivo Myocardial Subcellular Distribution of 3H-Emetine. The distribution of 3H-emetine among the particulate fractions after chronic in vivo administration was similar to that observed in vitro. Generally, the changes in concentration of 3H-emetine in the particulate fractions followed the changes in tissue concentration.

During both chronic emetine administration and sham treatment there were changes in the protein content of the heart homogenate and most subcellular fractions. These changes as shown in table 4 were small and produced no apparent change in the subcellular distribution of emetine.

The distribution of emetine to the supernate fractions after in vivo administration was different from that observed in vitro even when the tissue concentration of emetine was similar. The concentration of emetine found in the final supernate was approximately the same after in vivo and in vitro administration. However after in vivo administration less emetine was found in the initial supernate than would be expected on the basis of in vitro results.

In Vitro Effects of Emetine on Paired Atria and Papillary Muscle

Emetine at concentrations of 10^{-5}M and greater, decreased contractility and slowed the intrinsic beating
TABLE 4

The Effect of Chronic In Vivo Administration of Emetine on the Protein Concentration of Heart Homogenate and Subcellular Fractions

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>EMETINE DAY 1 (n=7)</th>
<th>EMETINE DAY 3 (n=5)</th>
<th>EMETINE DAY 6 (n=16)</th>
<th>SHAM DAY 6 (n=4)x</th>
<th>EMETINE DAY 10 (n=13)</th>
<th>SHAM DAY 10 (n=4)x</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMOGENATE</td>
<td>1.3±0.01</td>
<td>1.3±0.01</td>
<td>1.4±0.01*</td>
<td>1.4±0.01*</td>
<td>1.4±0.01*</td>
<td>1.4±0.02*</td>
</tr>
<tr>
<td>INITIAL</td>
<td>0.3±0.00</td>
<td>0.3±0.01</td>
<td>0.4±0.00*</td>
<td>0.4±0.01*</td>
<td>0.3±0.00</td>
<td>0.3±0.01</td>
</tr>
<tr>
<td>SUPERNATE</td>
<td>1.0±0.01</td>
<td>1.1±0.03</td>
<td>1.0±0.01</td>
<td>1.0±0.03</td>
<td>1.0±0.01</td>
<td>1.0±0.02</td>
</tr>
<tr>
<td>NUCLEAR</td>
<td>0.8±0.01</td>
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<tr>
<td>MITOCHONDRIA</td>
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<td>1.0±0.01*</td>
<td>1.0±0.01*</td>
<td>0.9±0.01*</td>
<td>0.1±0.00</td>
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</tr>
<tr>
<td>FINAL</td>
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<td>0.2±0.00*</td>
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<td>0.1±0.00</td>
<td>0.1±0.00</td>
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<td>0.3±0.03*</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a - Mean mg protein/0.1 ml fraction ± S.E.M.
* - Significant difference from day 1 value, P < 0.05
† - Significant difference from same day Sham, P < 0.05
x - Received 6 mg/kg ³H-emetine on last day of treatment
rate of isolated paired atria. The dose response curve for the effect of emetine on atrial rate is shown in figure 13. Also shown in figure 13 are the dose-response curves for the effect of emetine on papillary muscle isometric developed tension (DT) and action potential depolarization rate (dv/dt) which are discussed below. The effect on atrial rate was slow in onset and reached a steady state after 30-45 minutes.

Figure 14 shows the negative chronotropic effect of acetylcholine (Ach) and the positive chronotropic effect of norepinephrine (NE) on atrial rate with and without emetine in the bath medium. The response to Ach and NE are presented in figure 14 as percent maximum response. Based on preliminary studies under the same experimental conditions the maximum response to Ach was a decreased atrial rate to zero beats/minute and the maximum response to NE was an increased atrial rate to 222 beats/minute. The control atrial rate was 122 beats/minute. The negative chronotropic effect of emetine was additive to the negative chronotropic effect of Ach and the positive chronotropic effect of NE. Emetine did not affect the magnitude of the chronotropic response to Ach or 3 x 10^{-8}M NE. However, in the presence of 3 x 10^{-5}M emetine, the response to 3 x 10^{-7}M NE was potentiated. Atropine at a concentration of 10^{-5}M completely reversed the effects of 10^{-6}M Ach on paired atria but did not alter the response to 3 x 10^{-5}M
Concentration-response curves for the \textit{in vitro} effects of emetine on intrinsic atrial rate, papillary muscle $dv/dt$, and papillary muscle developed tension. Each curve is based on the results from three preparations. In each papillary muscle preparation, electrical activity was recorded from at least two different cells during each emetine concentration.
The effect of emetine on the negative chronotropic response to acetylcholine and the positive chronotropic response to norepinephrine of isolated paired atria. Each data point is the mean response of five preparations.
A. 

% Maximum Response

<table>
<thead>
<tr>
<th>ACH Concentration</th>
<th>% Maximum Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3 \times 10^{-7}$ M</td>
<td>60</td>
</tr>
<tr>
<td>$3 \times 10^{-6}$ M</td>
<td>80</td>
</tr>
<tr>
<td>$3 \times 10^{-5}$ M</td>
<td>100</td>
</tr>
</tbody>
</table>

B. 

% Maximum Response

<table>
<thead>
<tr>
<th>NorEpi Concentration</th>
<th>% Maximum Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3 \times 10^{-8}$ M</td>
<td>60</td>
</tr>
<tr>
<td>$3 \times 10^{-7}$ M</td>
<td>80</td>
</tr>
</tbody>
</table>

Figure A: Effect of Emetine on ACH Concentration

Figure B: Effect of Emetine on NorEpi Concentration
The \textit{in vitro} effects of emetine on single cell action potentials recorded from isolated guinea pig papillary muscles is shown in figure 15. The only characteristic of electrical activity significantly changed by emetine at concentrations below $10^{-4}$M was the rate of depolarization (dv/dt). The onset of this effect was slow, gradually increasing for at least 45 minutes. When $10^{-4}$M emetine was added to the tissue bath for more than 30 minutes, more severe alterations of action potential configuration were observed. At this concentration there was a slight increase (\approx 7\%) in action potential duration at both the 20\% and 90\% repolarization level. Also there was a greater decrease of dv/dt and a decrease in the overshoot of the action potential past zero membrane potential.

At bath concentrations greater than $10^{-5}$M, emetine decreased the amplitude of isolated papillary muscle isometric contractions. The concentration-response curve for this effect is shown in figure 13. The rates of contraction and relaxation were decreased to the same degree as isometric developed tension. At a concentration of $10^{-4}$M there was a small decrease in the duration of isometric contractions.

Table 5 shows the effects of NE on papillary muscle dv/dt and developed tension before and after the addition of $3 \times 10^{-5}$M emetine to the bath medium. Without the
Figure 15

Reproduction of actual records showing the *in vitro* effects of emetine on guinea pig papillary muscle action potentials and dv/dt.
TABLE 5

Effects of Norepinephrine and Emetine on Isolated Guinea Pig Papillary Muscle $dv/dt$ and Developed Tension

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>ACTION POTENTIAL $dv/dt$ (v/sec)</th>
<th>DEVELOPED TENSION (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>$121 \pm 5.6^a$</td>
<td>$250 \pm 3.8^a$</td>
</tr>
<tr>
<td>$3 \times 10^{-8}$ M Norepi</td>
<td>$133 \pm 3.1^*$</td>
<td>$251 \pm 6.8$</td>
</tr>
<tr>
<td>$3 \times 10^{-7}$ M Norepi</td>
<td>$163 \pm 4.9^*$</td>
<td>$281 \pm 13.5^*$</td>
</tr>
<tr>
<td>$3 \times 10^{-5}$ M Emetine</td>
<td>$44 \pm 1.7^*$</td>
<td>$130 \pm 8.3^*$</td>
</tr>
<tr>
<td>Emetine + $3 \times 10^{-8}$ M Norepi</td>
<td>$72 \pm 4.5^+$</td>
<td>$134 \pm 8.3$</td>
</tr>
<tr>
<td>Emetine + $3 \times 10^{-7}$ M Norepi</td>
<td>$70 \pm 2.4^+$</td>
<td>$167 \pm 10.5^+$</td>
</tr>
</tbody>
</table>

- Each value is the mean response of six preparations ± S.E.M.
- * - Significant difference from CONTROL, $P < 0.05$
- † - Significant difference from $3 \times 10^{-5}$ M Emetine, $P < 0.05$
presence of emetine, \(3 \times 10^{-8} \text{M}\) and \(3 \times 10^{-7} \text{M}\) NE both increased the \(\text{dv/dt}\) but only \(3 \times 10^{-7} \text{M}\) NE increased DT. After the addition of \(3 \times 10^{-5} \text{M}\) emetine to the bath, both concentrations of NE produced a maximal effect on \(\text{dv/dt}\) whereas the DT response to NE was unchanged.

The control values for atrial rate and mechanical and electrical activity of papillary muscle taken from pair-fed sham injected animals were not different from normal animals. The responses to emetine, norepinephrine and acetylcholine described above were the same in pair-fed sham injected animals also. Control values for tissues taken from animals given emetine 6 mg/kg/day for ten days were not different from normal either. However, the response to acetylcholine was exaggerated and the responses to norepinephrine were less than normal in tissue taken from emetine pretreated animals.

Myocardial Creatine-\(\text{PO}_4\) and Adenine Nucleotide Concentrations

The effect of chronic emetine administration at a dose of 6 mg/kg/day for ten days on creatine-\(\text{PO}_4\) and adenine nucleotide concentrations in guinea pig heart is shown in table 6. The differences in ATP and AMP concentrations between ten day pair-fed sham controls and emetine treated animals are of marginal statistical
### TABLE 6

Effect of Chronic Emetine Administration on 
Creatine-PO$_4$ and Adenine Nucleotide Concentrations in Guinea Pig Heart

<table>
<thead>
<tr>
<th></th>
<th>Pair-fed Sham†</th>
<th>Chronic Emetine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine-PO$_4$</td>
<td>1.96 ± 0.36$^a$</td>
<td>1.73 ± 0.33</td>
</tr>
<tr>
<td>ATP</td>
<td>2.56 ± 0.14</td>
<td>2.27 ± 0.12</td>
</tr>
<tr>
<td>ADP</td>
<td>0.98 ± 0.08</td>
<td>1.10 ± 0.08</td>
</tr>
<tr>
<td>AMP</td>
<td>0.36 ± 0.05</td>
<td>0.51 ± 0.06</td>
</tr>
</tbody>
</table>

$^a$ - mean m moles/kg heart ± S.E.M.

* - 6 mg/kg/day for 10 days; n=3

† - pair-fed and given sham injections for 10 days; n=3
significance ($P<0.1$). The creatine-$\text{PO}_4$ and ADP concentrations are not significantly different. However, these results do indicate that chronic emetine treatment tends to decrease creatine-$\text{PO}_4$ and ATP concentrations and increase ADP and AMP concentrations.

**Heart Mitochondrial Respiration and Oxidative Phosphorylation**

The primary objective of these studies was to determine the effects of chronic emetine administration on guinea pig heart mitochondrial function. These results are presented in table 7. As shown by the decreased ADP:O ratio, oxidative phosphorylation by mitochondria prepared from guinea pigs given emetine 6 mg/kg/day for ten days was inhibited compared to pair-fed sham injected controls. The respiratory control ratio (state III rate of oxygen consumption/state IV rate of oxygen consumption) was not altered by chronic emetine administration. Table 7 shows the results for mitochondria respiring in the presence of 2mM glutamate plus 2mM malate. Similar results were obtained when 2mM succinate or 2mM $\beta$-hydroxybutyrate were used as the substrate.

Other limited studies of mitochondrial function indicated that $2 \times 10^{-3}$M emetine added *in vitro* had no significant effect on the ADP:O ratio or the respiratory
TABLE 7

Effect of Chronic Emetine Administration on Respiration by Heart Mitochondria in the Presence of Glutamate Plus Malate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ADP:O^x</th>
<th>Respiratory Control Ratio^y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control^a</td>
<td>2.5 ± 0.08^†</td>
<td>8.7 ± 0.8</td>
</tr>
<tr>
<td>Chronic Emetine^b</td>
<td>2.2 ± 0.03^*</td>
<td>9.0 ± 0.2</td>
</tr>
</tbody>
</table>

^a - guinea pigs pair-fed and sham injected for 10 days; values based on results from mitochondria prepared from two pooled groups of three hearts and three ADP stimulated oxidations

^b - guinea pigs given 6 mg/kg/day for 10 days; values based on results from mitochondria prepared from two pooled groups of three hearts and five ADP stimulated oxidations

^* - significant difference from control; P<0.005

^† - mean ± S.E.M.

^x - μ moles ADP phosphorylated/μ atoms O utilized

^y - state III rate of O₂ consumption/state IV rate of O₂ consumption
control ratio. This was observed with mitochondria prepared from pair-fed sham injected animals and animals given emetine 6 mg/kg/day for ten days.
DISCUSSION

Lethal Effects of Emetine

The lethality due to chronic emetine treatment has been attributed to the cardiotoxic effects of the drug. Cardiotoxic effects of emetine develop more prominently with chronic administration than with acute administration. Therefore, it was thought that a study relating the onset of cardiotoxic effects with lethality due to chronic emetine administration would generate useful information regarding possible mechanisms of emetine cardiotoxicity.

In the present study, lethality in guinea pigs was demonstrated with chronic emetine administration and a median lethal cumulative dose (LCD$_{50}$) of 98.9 mg/kg was calculated when given at a rate of 6 mg/kg/day. The validity of the method used to calculate the LCD$_{50}$ is substantiated by the fact that the prediction of 60 mg/kg as the LCD$_{1}$ was very close to actual experience. Of 77 animals given 6 mg/kg/day for ten days, only one death was recorded. Based on these results, the selection of 6 mg/kg/day for ten days as the dose and administration period for studying
emetine cardiotoxicity seemed ideal. Under these conditions, it was thought that significant toxicity would develop in all animals yet wasteful loss of animals due to death before completion of the experimental protocol would be avoided.

No other estimates of the lethal chronic dose of emetine have been made. In fact, there have been very few studies on emetine chronic toxicity. The assumption has been that because emetine is slowly excreted the lethality of emetine is the same whether administered in one large dose or divided into a number of smaller doses and given over a period of time. There is some evidence which has bearing on this premise. The mortality ratio (number of animals dead/number of animals used) was greater when a dose of emetine was divided into several smaller doses and given over a period of time, compared to the mortality ratio observed when the emetine dose was given at one time (Rosen et al. 1935). The acute $LD_{50}$ for emetine in mice has been estimated at $62 \pm 2.3 \text{ mg/kg}$ (Gimble et al. 1948) and $45 \text{ mg/kg}$ (Child et al. 1964). However, when smaller doses were administered to mice for five consecutive days the calculated $LD_{50}$ was $9.9 \text{ mg/kg/day}$, the total cumulative dose being $49.5 \text{ mg/kg}$ (Johnson and Neal, 1968). Therefore, it may be that the chronic $LCD_{50}$ is lower than the acute $LD_{50}$, yet there is no good evidence to support or refute this point. It is unfortunate that no acute toxicity studies have been done in guinea pigs so that an acute $LD_{50}$ could
be compared with the chronic $\text{LCD}_{50}$ found in this study. It would also be of interest to see how the $\text{LCD}_{50}$ varied if a different daily dose were used.

There is marked species variation in the sensitivity to both acute and chronic emetine toxicity. Estimates of the $\text{LD}_{50}$ in rat have been between 10 and 20 mg/kg whereas those for mice have been greater than 40 mg/kg. Chronic administration of 1.4 mg/kg/day or less to dogs caused death within ten days (Pearce et al. 1971, Child et al. 1964). In man, severe toxic symptoms develop within ten days when emetine is administered at a dose of about 1 mg/kg/day. Thus humans, dogs and rats seem more sensitive to emetine lethality than guinea pigs and mice.

Body Weight Loss With Emetine Treatment

Acute emetine administration in large doses and chronic emetine administration in small doses cause animals to lose body weight. Concurrent with the decrease in body weight is a decrease in food consumption (Diamant, 1958; Appelt and Heim, 1964). However decreased food consumption is responsible for only part of the weight loss since pair-fed shams do not lose as much body weight as emetine treated animals (Diamant, 1958; Chatterjee et al. 1970).

In this study loss of body weight in guinea pigs
due to chronic emetine treatment was shown to be dose and
time related (see figure 5). Animals given 6 mg/kg/day
for ten days slowly decreased their food intake by approxi-
mately 67% and lost about 20% of their body weight. Sham
injected animals which were pair-fed with the 6 mg/kg/day
animals showed no change in body weight over the ten day
treatment period. Sham injected controls given food ad
libitum increased their body weight by about 20% in the
same period. Thus about half of the difference in body
weight between animals given emetine 6 mg/kg/day and
ad libitum fed sham injected animals is due to decreased
food consumption.

The mechanism responsible for the decreased body weight
beyond that accounted for by decreased food consumption is
unknown. It is not due to an alteration of endocrine control
of carbohydrate metabolism since blood glucose levels
of rats chronically treated with emetine were not different
from pair-fed or ad libitum fed controls (Diamant, 1958;
Appelt and Heim, 1964). It seems most likely that the
effects of emetine on cellular respiration or protein
synthesis or a combination of both may be responsible for
this weight loss. However, emetine may act on central
nervous system mechanisms which control the release of
pituitary hormones involved in the regulation of metabolism
and growth. This possibility is suggested by the fact
that emetine has known actions on the central nervous
system which cause emesis (Wang, 1965).

The mean body weight of animals given emetine 6 mg/kg/day until death decreased until day 15 and then leveled off at approximately 75% of the mean initial body weight. However, the mean body weight at the time of death had decreased 34.4% from the initial body weight. Since the correlation coefficient between percent body weight lost and time until death is -0.089, animals surviving a longer period of emetine administration did not lose a greater portion of their body weight. Actually all animals had lost about the same portion of their body weight at the time of death. This suggests that the drug action which causes death also causes loss of body weight or that loss of body weight plays a role in the cause of death.

Myocardial Fine Structure Changes Due to Chronic Emetine Administration

Massive lesions visible with light microscopy have been observed in rabbit cardiac muscle after large acute doses of emetine (Rinehart and Anderson, 1931) and in rat skeletal muscle after emetine 1.25 mg/kg/day for ten days (Duane and Engel, 1970). Electron microscopic studies of the emetine treated rat skeletal muscle produced evidence of mitochondrial degeneration with and without myofibrillar degeneration (Duane and Engel, 1970). Electron microscopic
studies of rat cardiac muscle after emetine 1 mg/kg/day for ten days have shown extensive cellular lesions with myofibrillar lysis yet mitochondria were unaltered (Hatt et al. 1969). When dogs were given emetine at a dose of 0.8 mg/kg/day for a period of 5 to 32 days, electron microscopic examination of myocardial biopsies showed selective damage to mitochondria (Pearce et al., 1971). The mitochondrial damage consisted of partial or complete loss of cristae. The authors did not correlate the onset of this effect with duration of treatment.

The preliminary electron microscopic studies done here have shown that chronic emetine administration does not alter myocardial fine structure in guinea pigs as it does in rabbit, rat and dog. The lack of fine structure changes observed in guinea pig myocardium as compared to those reported for other species further emphasizes the difference in sensitivity to emetine cardiotoxicity between these species.

Purity of $^3$H-Emetine

The difference between the fluorometric spectra of emetine dihydrochloride standard and $^3$H-emetine dihydrochloride suggests the presence of a chemical impurity in the labelled emetine solution. The fact that the emission spectra of both solutions was the same suggests that the
impurity is an ipecacuanha alkaloid or an optical isomer of emetine. Both solutions produced a single spot when subjected to thin layer chromatography. Since the range of detection sensitivity of ipecacuanha alkaloids for the TLC method used is 0.01 ug to 0.001 ug, the impurity could make up no more than 0.5% of the $^3$H-emetine solution.

After 18 months of storage the stock $^3$H-emetine solution had undergone self-radiolysis. However experiments utilizing the labelled emetine were completed within 14 months so that it is likely that the degree of self-radiolysis was within tolerable limits: (<10%).

Myocardial Uptake of Emetine

Uptake of emetine by isolated perfused guinea pig hearts rapidly reached a steady state and remained constant for 64 minutes. The tissue concentration increased linearly for the entire 64 minutes. In vivo cumulative uptake of emetine during administration at a dose of 6 mg/kg/day produced tissue concentrations only one third as high as those observed after in vitro uptake. Tissue concentrations seen after in vivo administration increased until day six, then a decrease was observed at day ten. This was an actual decrease in tissue concentration since there was no change in the water content of the heart during the ten day treatment period and the decrease was also apparent
when tissue concentration was based on tissue protein content.

The amount of drug taken up by the heart from successive doses during the ten day treatment period decreased. This was apparent because the tissue concentrations produced by single doses of $^3$H-emetine at days six and ten after prior sham or unlabelled emetine treatment were lower than the tissue concentration observed at day one. Furthermore the heart/plasma concentration ratio observed after single doses of $^3$H-emetine at days six and ten with prior unlabelled emetine treatment were about half the ratio observed at day one. Both the tissue concentrations and heart/plasma concentration ratios observed after single doses of $^3$H-emetine at days six and ten with prior unlabelled emetine treatment were the same. Therefore, uptake was the same at days six and ten but less than uptake at day one.

The pattern of change in the heart/plasma concentration ratio during chronic $^3$H-emetine administration suggests that the heart has a greater affinity for emetine early in the treatment period which decreases rapidly. Further uptake simply reflects increases in plasma concentrations. The decreased heart/plasma concentration ratio at day ten after chronic $^3$H-emetine administration does not reflect a decrease in uptake or plasma concentration but a real decrease in tissue concentration.

The decreased tissue concentration observed at day
ten after chronic $^3$H-emetine administration is interesting. As discussed above, it does not reflect a change in water content of the heart, plasma concentration of emetine, or uptake by the heart. A possible explanation is an increased loss of drug from the heart which had been taken up during earlier injections.

Effect of Chronic Emetine Administration on Myocardial Protein Content

The biuret protein assay used in this study was not intended to be used as a sensitive measure of changes in myocardial protein concentrations. The intent of the assay was to provide a measure of the relative size of the various subcellular fractions so that the emetine concentration in each fraction could be expressed. Nevertheless, myocardial protein content in guinea pigs was shown to increase during chronic emetine administration and pair-fed sham treatment. Similar results have been observed in rats. Myocardial protein content in emetine treated rats did not change while differences in myocardial protein contents between emetine treated rats (2 mg/kg/day for ten days) and pair-fed controls were reported (Chatterjee et al., 1970).

The mechanism of apparent increase in myocardial protein content observed in pair-fed shams is unknown.
However, these animals were subjected to a considerable amount of stress including daily restraint during electrocardiogram recording which may account for this response. The biuret protein assay used did not detect differences in protein contents between emetine treated and sham controls. However, the tissue concentration of emetine was as high as $1.5 \times 10^{-5}$ moles/kg tissue. This concentration far exceeds concentrations known to inhibit protein synthesis in several other types of eukaryotic cells.

Myocardial Subcellular Distribution of Emetine

Control experiments showed that tissue homogenization did not cause an obvious change in subcellular distribution of emetine. In these studies emetine was added directly to tissue just before or after homogenization. When the distribution observed in these experiments was compared to that seen after emetine was perfused in isolated hearts, obvious differences were observed. With perfusion more drug was found in the mitochondrial fraction and less in the initial supernate. The concentrations of emetine in the other fractions did not differ when emetine was perfused or added directly to tissue.

The most important procedural difference between these two treatments seemed to be the time allowed for distribution to occur. Thus it was thought that with
in vivo administration, even greater concentrations of emetine in the mitochondrial fraction might have been observed. However, this did not occur. The pattern of emetine binding to all of the particulate fractions after in vivo administration was similar to that observed with in vitro perfusion. The changes in the concentrations of emetine in particulate fractions seem to reflect changes in tissue concentration after perfusion in vitro and chronic in vivo administration. However, the distribution of emetine to supernate fractions during chronic in vivo administration was different from that seen during in vitro perfusion. When emetine tissue concentrations were similar, the concentration of emetine in the final supernates were similar after chronic in vivo administration and in vitro perfusion. However, under the same conditions the concentration of emetine in the initial supernate during chronic in vivo administration was lower than that after in vitro perfusion. The decreased emetine concentration in the initial supernate after in vivo administration is consistent with the increased concentration after short duration distribution when the drug was added directly to tissue homogenates. This suggests that binding to the mitochondrial fraction is a slow process and a period of time is required to achieve a steady state.
Effects of Emetine on Electrical Activity of the Heart

Intravenous injection of emetine in dogs produces a widening of the QRS complex and increases the negative deflection of the S wave (Boyd and Sherf, 1941). Larger doses produce ventricular tachycardia which often change into ventricular fibrillation. No significant changes in P-R interval or T waves occur in dog. Similar changes occur with IV injection of dehydroemetine in rat except death results from atrio-ventricular block rather than ventricular tachycardia and fibrillation (Salako and Durotoye, 1972). Thus the response of dog heart to large acute doses of emetine is unique from that of other species. Rat, cat (Boyd and Sherf, 1941) and guinea pig (discussed below) succumb to complete heart block whereas in dog ventricular fibrillation is the terminal response.

In the present study, chronic administration of emetine in guinea pigs caused an increased P-Q interval duration and T wave amplitude. No obvious change in QRS configuration or duration was observed with the following exceptions; deepening of the S wave was observed shortly after IP injection of the drug during the 10 day treatment period and in the electrocardiograms recorded from animals shortly before death.

In Vitro effects of emetine on electrical activity
of guinea pig heart were observed in isolated perfused hearts and isolated papillary muscle. Perfusion of $10^{-6}$M emetine caused a 60% increase in the P-Q interval duration of electrograms recorded from isolated perfused hearts while sinus rate remained unchanged. This increase was sufficient to produce second degree heart block in two out of five hearts used in this experimental group. Bath concentrations of emetine greater than $10^{-7}$M caused a decrease in the rate of depolarization of action potentials recorded from isolated papillary muscle. This effect occurred without a change in stimulation rate or resting potential. The same effects on action potentials recorded from rabbit atria, cat papillary muscle and sheep heart purkinje fibers have been observed (DeHemptinne, 1965). Although single cell electrical activity was not observed in guinea pig atrial tissue, it seems likely that emetine would have the same effect on the atrial action potential $dv/dt$. It is generally agreed that conduction time is indirectly proportional to $dv/dt$ (Trautwein, 1963). Also the time course for the onset of the effects on P-Q interval and $dv/dt$ in this study were the same. This evidence suggests that the increased P-Q interval duration caused by emetine both in vivo and in vitro is due to an increased atrial conduction time which is the result of the decreased action potential depolarization rate.

Microelectrode studies of the cardiac action potential
have shown that the extracellular sodium ion (Na\(^+\)) concentration is directly proportional to the \(dv/dt\) and overshoot amplitude (Cranefield and Hoffman, 1958). Voltage clamp studies have shown that the early rapid inward current which is primarily responsible for depolarization of the cardiac cell is sensitive to the extracellular Na\(^+\) concentration and is blocked by tetrodotoxin (Johnson and Lieberman, 1971; Trautwein, 1973). Based on these results it is generally agreed that an early fast inward Na\(^+\) current is responsible for the depolarization of cardiac cells during conduction of an action potential. Also the effects of quinidine on the cardiac action potential have been reported to be very much like those described for emetine and quinidine decreases Na\(^+\) conductance (Trautwein, 1963). Thus, the \textit{in vitro} effects of emetine on the cardiac action potential suggest a specific blocking action on the inward Na\(^+\) current.

Changes in electrocardiogram amplitudes are usually explained by a change in size of the heart. Therefore, an increase in the size of the heart may explain the increased QRS and T wave amplitude observed in pair-fed sham injected guinea pigs. However, T wave amplitude changes have been classified as primary and secondary changes (Surawicz, 1966). Secondary T wave changes depend on changes in the QRS amplitude. Primary changes are due to direct effects on ventricular repolarization. Based on these definitions
one could say the T wave change observed with sham treatment is secondary whereas the T wave change seen with emetine treatment is due to both primary and secondary effects.

The mechanism of the primary T wave change observed after ten days of chronic emetine administration is unknown. As discussed below, changes in myocardial adenine nucleotide concentrations were observed at the same time. Decreased myocardial concentration of creatine-PO₄ and ATP have been correlated with increased potassium efflux (Scheuer and Stezoski, 1968; McDonald and Macleod, 1973). Thus the primary T wave change observed during chronic emetine administration may be due to an action of emetine on cardiac metabolism.

Effect of Emetine on Heart Rate

Intravenous injection of emetine into dogs produces a transient bradycardia (Boyd and Scherf, 1941; Bianchi et al., 1965). The same effect on heart rate is observed with the acute administration of dehydroemetine in cats (Salako and Durotoye, 1973) and rats (Salako and Durotoye, 1972). In the present study, chronic emetine administration at a dose of 6 mg/kg/day caused significant bradycardia in guinea pigs after six days.

Vagotomy and atropine do not alter the acute emetine effect on heart rate (Pellini and Wallace, 1916; Boyd
and Scherf, 1941; Salako and Durotoye, 1973). In this study, atropine had no effect on the bradycardia observed during chronic emetine administration. Therefore, the action of emetine causing bradycardia under both circumstances must not involve the parasympathetic innervation of the heart. Emetine has been shown to block sympathetic ganglionic transmission (Ng, 1966) and adrenergic nerve transmission (Ng, 1966; Abraham, 1968; Milelich et al., 1974). However, it seems unlikely that adrenergic blockade is involved in emetine bradycardia because prior administration of propanolol does not affect this response (Salako and Durotoye, 1973).

The fact that emetine decreases the intrinsic rate of isolated atria *in vitro* suggests a direct action on the sinus node. Certainly this direct action does not involve the muscarinic receptors of the sinus node because atropine does not alter the decreased atrial rate caused by emetine *in vitro*. The parallel noted above between the effects of emetine and quinidine on the ventricular action potential suggests that emetine may have effects similar to quinidine on membrane processes of pacemaker cells which decrease sinus rate.

The point of major interest here is whether or not the same drug action is responsible for bradycardia observed after both acute and chronic administration of emetine.
The plasma concentration of emetine was $8.4 \times 10^{-7}$M when significant bradycardia was first observed during chronic emetine administration. The minimally effective concentration of emetine which decreased the intrinsic rate of isolated atria in vitro was $3 \times 10^{-6}$M. The concentrations are quite close which suggests the same drug action is responsible for the decreased sinus rate both in vitro and in vivo. However, inhibition of cardiac metabolism is known to decrease heart rate (Trautwein, 1963; Cascarano et al., 1968; Dhalla et al., 1972) and as discussed below chronic emetine administration had inhibitory effects on cardiac metabolism. Therefore, the action of emetine on metabolism may contribute to the bradycardia observed after chronic administration.

**Effects of Chronic Emetine Administration on Myocardial Adenine Nucleotide Concentrations and Mitochondrial Function**

Chronic administration of emetine to guinea pigs at a dose of 6 mg/kg/day for ten days caused lower myocardial creatine-PO$_4$ and ATP concentrations. Simultaneously, ADP and AMP concentrations were increased. These differences from pair-fed sham injected controls were of marginal statistical significance probably because of the small number of animals used in each group.
Chronic emetine administration had effects on guinea pig heart mitochondria in accordance with the observed changes in myocardial adenine nucleotide concentrations. A decrease in the ADP:O ratio of mitochondria isolated from chronic emetine treated animals, indicates uncoupling of oxidative phosphorylation from respiration. Thus, less efficient production of ATP explains the observed changes in adenine nucleotide concentrations. The only other studies of chronic emetine effects on myocardial respiration were done by Appelt and Heim (1964, 1965). They found that chronic emetine administration (2 mg/kg/day for 17 days) decreased the rate of oxygen consumption of rat heart homogenates respiring in the presence of various substrates without affecting the P:O ratio. In this study, chronic emetine administration had no effect on the respiratory control ratio. It may be that changes in the rate of respiration similar to those observed in rat heart homogenates were not observed in this study because of the isolation procedure used in preparing the mitochondria. That is, one would expect that grossly abnormal mitochondria would be eliminated from the final preparation used in this study, whereas Appelt and Heim used a whole tissue homogenate possibly containing normal and abnormal mitochondria.

Emetine affects mitochondrial respiration in vitro also, but only at very high concentrations. In this study, \(2 \times 10^{-3}M\) emetine had no effect on respiring guinea pig
heart mitochondria. This concentration also had no effect on respiring dog heart mitochondria (Murphy et al., 1974). Appelt and Heim reported in vitro inhibition on the rate of oxygen consumption by rat heart homogenates at emetine concentrations greater than $10^{-4}$ M with complete inhibition at $3 \times 10^{-3}$ M. The fact that such high concentrations are required in vitro suggests that the mechanism of action causing inhibition of mitochondrial function during chronic emetine administration is different from the acute mechanism.
SUMMARY AND CONCLUSIONS

Emetine is the most important drug used in the treatment of amebiasis which is a disease that is a world wide problem. The effective use of emetine requires chronic administration but during chronic administration severe cardiotoxic symptoms usually occur which can indicate development of emetine myocarditis. The most prominent signs of this condition are electrocardiographic changes.

The objective of this study was to gain information concerning the mechanism of chronic emetine cardiotoxicity. To do this, the effects of chronic emetine administration on the guinea pig electrocardiogram were studied. Since emetine also has acute in vitro effects on the electrical activity of heart, it is important to determine whether acute and chronic effects differ with respect to mechanism of drug action. Thus, chronic in vivo cardiotoxic effects were compared with in vitro effects on isolated guinea pig heart.

The lethality of chronic emetine administration at a dose of 6 mg/kg/day in guinea pigs was quantitatively analyzed. When administered in this manner, the median lethal cumulative dose (LCD$_{50}$) was 98.9 mg/kg. All sub-
subsequent studies of chronic emetine toxicity used the predicted \( \text{LD}_{50} \), 60 mg/kg given at a rate of 6 mg/kg/day for ten days.

Guinea pigs treated in this manner presented the usual signs of emetine toxicity including, general muscular weakness, marked lethargy, anorexia and loss of body weight. Weight loss was shown to be dose dependent. The difference in body weight between \textit{ad libitum} fed and pair-fed sham injected animals was half the difference between \textit{ad libitum} fed shams and emetine treated animals. Thus about half of the weight lost by emetine treated animals was due to the anorexic effect. The remainder of the weight loss was due to an unexplained action of emetine.

Chronic emetine administration caused bradycardia at plasma concentrations close to concentrations effective in lowering the intrinsic rate of isolated atria \textit{in vitro}. This effect is due to a direct drug action on sinus rate and does not involve the parasympathetic innervation of the heart. However, the possibility that inhibitory effects of emetine on metabolism may also contribute to the decreased heart rate was not eliminated.

The electrocardiographic changes seen during chronic emetine administration were a prolonged P-Q interval, an increase in QRS amplitude and an increase in T wave amplitude. The increased QRS amplitude and part of the increase in T wave amplitude were not due to a direct drug action because
these increases were observed in pair-fed sham controls. A further increase in T wave amplitude observed in response to chronic emetine treatment could not be explained by in vitro effects on single cell electrical activity recorded from isolated guinea pig papillary muscle. It is known that alteration of cardiac metabolism such as that observed during chronic emetine administration can produce changes in potassium efflux from myocardial tissue which might explain this T wave change. However, the increased P-Q duration was due to increased atrial conduction time. This was a result of a decreased rate of depolarization which was an effect of emetine observed in vitro.

Results were obtained in this study which suggest subcellular alterations of myocardial tissue during chronic in vivo administration of emetine. An increase in protein content of myocardial tissue was observed in both pair-fed sham and chronic emetine treated animals. The earliest increases were observed in the microsomal and mitochondrial fractions. Studies of myocardial uptake and subcellular distribution of emetine after in vitro and in vivo administration did not produce information which would suggest a mechanism of action for emetine cardiotoxicity. However, observed changes in the myocardial concentration and binding of emetine during chronic in vivo administration seemed to provide further evidence that subcellular changes had occurred.
Because several *in vivo* effects were observed in controls, these studies point out the necessity of proper pair-fed sham injected control groups in experiments investigating the chronic *in vivo* effects of emetine. Much of the published work on *in vivo* emetine cardiotoxicity has not taken pair-fed sham effects into account.

It would seem that the *in vivo* effects of emetine seen in the present study are very early effects which occur prior to the development of severe cardiotoxicity. Yet it is possible that guinea pigs do not develop severe emetine cardiotoxicity since changes of myocardial fine structure were not observed. Indeed other species seem to be more sensitive to *in vivo* effects of emetine because myocardial lesions and weight loss have been shown to occur at lower doses than those used in this study. The chronic dose used in this study was equivalent to the LCD₁. It is possible that in guinea pigs the toxic dose response curve is not widely separated from the lethal dose response curve. As a result the LCD₁ may not produce severe toxic effects in guinea pigs.

Chronic emetine administration decreased the myocardial concentrations of creatine-PO₄ and ATP while the concentrations of ADP and AMP were increased. Studies of mitochondrial function after chronic emetine administration showed that the ADP:O ratio was decreased which suggested that the
observed changes in adenine nucleotide concentrations were due to uncoupled oxidative phosphorylation. These changes in cardiac metabolism occurred at the same time as the observed early effects of emetine cardiotoxicity in guinea pigs. This suggests that the action of emetine on cardiac metabolism during chronic administration may be the primary mechanism of chronic emetine cardiotoxicity.
BIBLIOGRAPHY


