ministered simultaneously with angiotensin or to rats with established renovascular hypertension. These findings were consonant with evidence indicating a preferential inhibitory or vasodilating effect of serotonin on constricted arterioles, the net effect of which is vasodepression (8, 11). The slight, insignificant increase in blood pressure noted in normotensive rats infused with serotonin appears to be the result of the vasoconstriction of large vessels induced by this agent in the absence of arteriolar constriction (11).

This information, as well as the failure to observe significant elevation of blood pressure in rats with a degree of unilateral renal artery constriction that was insufficient to produce hypertension indicate that serotonin does not augment renovascular hypertension in the rat as has been suggested to occur in man (3). Indeed, the converse was obtained in this species. Although renal blood flow and glomerular filtration rate may be decreased following administration of serotonin (12), its lack of effect on JGI suggests that any action of serotonin on blood pressure is not mediated by renal juxtaglomerular cells, the purported sites of renal renin formation. The failure to observe any significant effect of angiotensin infusions on JGI is unlike the effect of daily subcutaneous injections of pressor doses of this agent. Katz *et al.* (13) observed increased granularity during the first week of treatment after which time return to control values was noted.

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Keyphrases

Serotonin infusions—rats
Renovascular hypertension—experimental
Angiotensin effect—serotonin activity
Renal artery constriction—serotonin activity

Toxicity of Emetine to Isolated Embryonic Chick-Heart Cells

By W. DAVID WATKINS* and WALLACE L. GUESS

Isolated beating embryonic chick-heart cells were cultured and observed *in vitro* to investigate the toxic specificity of emetine for the myocardium. The results of this investigation indicated that emetine exerts its toxic effects by at least two modes of action: at high concentrations, a physical disruption of the plasma membrane of the cell; and, at lower concentrations, an intracellular inhibition of the process of forming high-energy phosphate compounds. The site of this inhibition is thought to occur at the enzymatic oxidation of substrates, other than succinate, mediated by nicotinamide-adenine dinucleotide (NAD).

EMETINE HYDROCHLORIDE is a compound which has classically been used in the treatment of amebic dysentery. Although its use for this purpose has been largely superceded by more effective and less toxic drugs, it is still used to treat certain types of amebiasis which are refractory to treatment by the therapeutic alternatives.

The predominance of toxicities resulting from emetine involve the cardiovascular system, and most investigators today agree that the typical cardiovascular symptoms of emetine toxicity are due to a direct myocardial depression. Attention has been called to the apparent hypersensitivity of the heart toward emetine (1).

Wenzel (2) has recently presented a relatively comprehensive review of the literature concerning emetine cardiotoxicity. Significant toxic mani-

Received May 27, 1968, from Drug-Plastic Research and Toxicology Laboratories, College of Pharmacy, University of Texas, Austin, TX 78712
Accepted for publication July 31, 1968.
Abstracted in part from a thesis presented to the Graduate School, University of Texas at Austin by W. David Watkins in partial fulfillment of the Master of Science degree requirements.
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festations of the drug include: electrocardiographic changes, ventricular fibrillation, diminished systolic force, tachycardia, and myocardial fatty degeneration and necrosis. Progressive cloudy swelling of the myocardium has also been reported (3) in conjunction with emetine administration.

A variety of investigations has been designed and undertaken to delineate the biochemical mechanism of emetine cardiotoxicity. Grollman (4) reported that emetine inhibits protein synthesis in a variety of cell types including mammalian L-cells and reticulocytes. It was demonstrated that emetine acts by inhibiting the aminoacyl-sRNA transfer reaction in protein biosynthesis. The suggested site of action of emetine is in the enzymatic formation of the peptide bond on the polyribosome.

Emetine has been shown (5) to inhibit the NAD—dependent oxidation of substrates by rat-heart homogenates, while the production of NAD has been shown to not be significantly altered.

Work done to date on emetine cardiotoxicity has generally been undertaken at three levels of biological organization: (*a*) the intact heart; (*b*) myocardial tissue; and (*c*) the heart homogenate. Certainly each of these approaches has offered valuable information necessary in the total toxicity evaluation of this compound.

There existed an apparent gap in the research efforts to date; none had reported the effects of emetine upon the heart cell *per se*. Since the cell is the functional "building-block" of organized life, it seemed reasonable that the explanation of toxicity should ultimately be found here also. Relatively recent advances in cell culture techniques have provided a unique means of further identifying and investigating such cellular toxicity.

MATERIALS AND METHODS

Culture Procedures—The routinely cultured contracting chick embryo heart cells were prepared from the hearts of 12–24 white leg-horn chick embryos 10–13 days *in ovo*. Aseptic techniques were used throughout all experimental procedures. The whole hearts were excised, placed in a phosphate-buffered saline solution (PBS 1×) (6), trimmed free of contiguous noncardiac tissue and membranes, and rinsed with PBS solution. The pooled hearts were placed in 10 ml. of freshly prepared 0.1% trypsin solution. Cell dispersion was initiated by gently forcing the cardiac tissue out of a 20-ml. syringe and into a flask. This flask and contents were incubated for 30 min. with frequent gentle agitation, then removed from the incubator. Gentle passage of the mince in and out of the syringe for about 5 min. was ordinarily sufficient time to complete the cell dispersion process. The resultant cell suspension was then centrifuged with a clinical centrifuge at three-fourths speed for 5 min., the trypsin solution decanted off, and the remaining packed cells washed twice by resuspension and centrifugation in PBS solution. Following the second washing, the cells were resuspended for the final time in Eagle's basal medium (BME 1×) (6) supplemented with 5% calf serum and 1% l-glutamine. The quantity of this basal medium used for the final suspension was 1.5 ml. of media per embryonic heart used. The approximate concentration of this suspension was 10^6 cells/ml. as determined by hemocytometer count.

A portion of the resulting heart-cell suspension was injected into sterile Sykes-Moore tissue culture chambers (Belco Glass, Inc., Vineland, N. J.) which, after cell settling and attachment to the glass coverslip, yielded a maximal working population of isolated cardiac cells. The remaining suspension was cultured in a sterile prescription bottle and incubated at 35° in a 5% CO_2 atmosphere. This culture provided the conditioned medium required for the cell-perfusion experiments described below.

Preparation of Isolated Atrial and Ventricular Cells—To develop the technique of visually distinguishing isolated beating atrial and ventricular cells, cultures were prepared from embryonic atrial and ventricular tissue, respectively, and characterized.

Cell Perfusion Procedure—A preliminary series of control cell-perfusion studies was performed to assure that any observed cellular effects following perfusion with emetine or other compounds could be related entirely to the particular compound, and not to certain environmental factors, such as changes in necessary nutrients, or tonicity effects. Preliminary experimentation and the work of others (7) indicated that various cellular alterations in response to fresh media could be prevented by perfusing with conditioned media. Such a conditioned medium was prepared by removing the cell-free fluid phase from the relatively large culture of heart cells. Multiple cell perfusions with this conditioned media produced no detectable variation in function or structure. As a result, conditioned medium was used to prepare all perfusion solutions used in this investigation.

To obtain useful and valid information from this study, it was necessary to prepare perfusion solutions which not only contained accurately determined quantities of perfusate compound, but which also closely approached the composition and tonicity of the original culture medium. Compounds to be perfused were accurately weighed and dissolved in PBS solution (1×). Serial dilutions of these solutions were prepared by taking a small, yet accurately measurable volume (0.1 ml.), and combining with this a relatively large volume (0.9 ml.) of conditioned media. The resulting perfusate fluid satisfied the above requisites.

It has been shown (8) that perfusion of 1 ml. through a 0.7-ml.-capacity Sykes-Moore chamber would result in 65% perfusate retention within the chamber. Perfusion of 2 ml. through the chamber results in essentially a complete medium change. In this report, all concentrations of perfused compound refer to the delivered concentration retained in the chamber following the perfusion process.

Perfusion of the cells contained within each cham-

ber could be initiated any time following cell attachment to the glass coverslip (15–20 hr.). The solutions and equipment to be used in the perfusion procedure were placed inside the microscope stage incubator for 15 to 20 min. so temperature equilibrium could be attained and thereby avoid physical perfusion alteration of the beating cells as much as possible. The inoculated chamber was firmly affixed to the microscope stage, and the cell(s) to be observed were located in the visual field. Perfusion of the cells was then performed using the procedures of Sykes and Moore (9).

Microscopy and Photography—All observations were made using a Nikon light-field inverted phase contrast microscope with stage encased in a thermostatically controlled incubator maintained at 35°. Photomicrographs were taken with a Nikormat FS 35-mm. camera using Kodak plus-X Panatomic film.

Drug Standardization—The quality and purity of emetine hydrochloride[1] used in this study were confirmed by IR and UV spectroscopy, melting point determinations, and differential thermal analysis.

RESULTS

The design of this experimental procedure was such that the following parameters associated with the functional status of the myocardial cells could be observed: (*a*) rate of beat (b.p.m.), (*b*) apparent strength of beat, (*c*) synchrony of beat, and (*d*) morphology.

Simply counting the b.p.m. of single cardiac cells represented a sensitive index of toxicity because, in essence, this analyzed the final effect of the drug on a delicate and highly organized enzyme system. Evidence indicating that the observed beat is an enzyme-regulated system stems from the observations (10) that in single cells, hydrogen ion concentration, temperature, salts, substrates, serum proteins, metabolic inhibitors, and a variety of chemical compounds affect the beating rate. Therefore, any alteration in beating rate was considered the initial indicator of toxicity, and cell lysis was taken to be the terminal manifestation of toxicity.

Rate of beating was recorded as b.p.m. and treated as strictly numerical data, and as such was relatively simple to collect. Recording cellular morphological changes also presented no significant problems in that photomicrographic techniques provided a means of recording at chosen intervals the condition of any cell in culture. Preliminary experience indicated that the apparent strength of beat and synchrony of beat, although purely qualitative parameters under these experimental conditions, were equally as vital to the measurement of cellular toxicity as rate of beat and morphology.

In most cultures, only a small proportion of the total population were observed to beat. This beating ranged from intermittent, irregular "twitches" to steady, deep rhythmic contractions at rates up to about 120 b.p.m. The majority of the beating cells, however, exhibited a beating range of about 30–80 b.p.m. It should be noted that each isolated beating cell maintained a rate independent of the other cells in the culture.

[1] Pfaltz and Bauer, Inc., Flushing, N. Y., and Ruger Chemcal Co., Inc., Long Island City, N. Y.

The characteristics of the untreated atrial and ventricular cell types used in the present study have been described by Mercer and Dower (11).

Observations of Emetine-Dosed Heart Cells—Emetine concentrations of 10^{-5} *M* or greater had severe and apparently equivalently toxic effects upon the beating atrial and ventricular cells. The inhibitory effects of emetine on beating were immediate and complete. In fact, the intended perfusate dosage could not be completely delivered before the cells terminated beating. After cessation of the converted contractions which formed the normal cellular beating pattern, weak irregular twitches were observed within the cytoplasmic regions. These occasionally continued up to the time of cell lysis. Within 10 min. after perfusion, the cells began retraction of the web-like pseudopodial projections and hyaloplasmic veil. Also, the entire cell, which under normal conditions was flattened and/or extended, slowly drew itself into a more compact, rounded form. Often the release of attachments of peripheral area during this rounding process were observable as rapid breaks, with subsequent withdrawal of the cellular component toward, and into the rounding cell. Cell lysis was almost always preceded by a rapid extrusion of the cytoplasmic contents into a portion of the plasma membrane. These large, spherical swellings are termed "superficial vacuoles." Normally, after the formation of these superficial vacuoles, only a short period of time elapsed prior to cell lysis, after which the superficial vacuoles were observed to remain intact and float freely in the media. In most cultures dosed with this level of emetine, a few cells of the population were observed to lyse within 30–45 min. Usually the first cells to undergo lysis were those which contained dense, heavy granules and were highly vacuolized. Progressive lysis of the cell population could be observed for 48 hr., after which no intact cells remained. Throughout this, and all other procedures, the pH was buffered between approximately 7.2 and 7.4. Most of the damage to the beating myoblasts occurred within the first hour after dosing. The cells which most effectively resisted the prolonged effects of emetine were those which were nonbeating and fibroblastic in nature. No further cell growth was observed in the period following drug perfusion.

Cells dosed with emetine concentrations less than 10^{-5} *M* responded differently than described above with the higher doses. Figure 1 illustrates the morphological effects 6 hr. after perfusion with 10^{-6} *M* emetine. Minutes after dosing, the portions of the plasma membrane normally engaged in pinocytotic activity, known as the "hyaloplasmic veil," were slowly retracted into the cell. The phenomenon known as "blebbing" was observed, but apparently this process could initiate at any time after cell perfusion. This process manifested itself as small swellings occurring on the plasma-membrane. These "blebs" would remain as such for intervals up to 2 or 3 min., after which they appeared to be resorbed back into the contour of the plasma membrane. While some blebs were being resorbed, others would spontaneously arise at apparently random sites of the plasma membrane which were exposed to the emetine. No superficial vacuolization or cell lysis was observed in the early portions of the observations. Figure 2 is a graphical

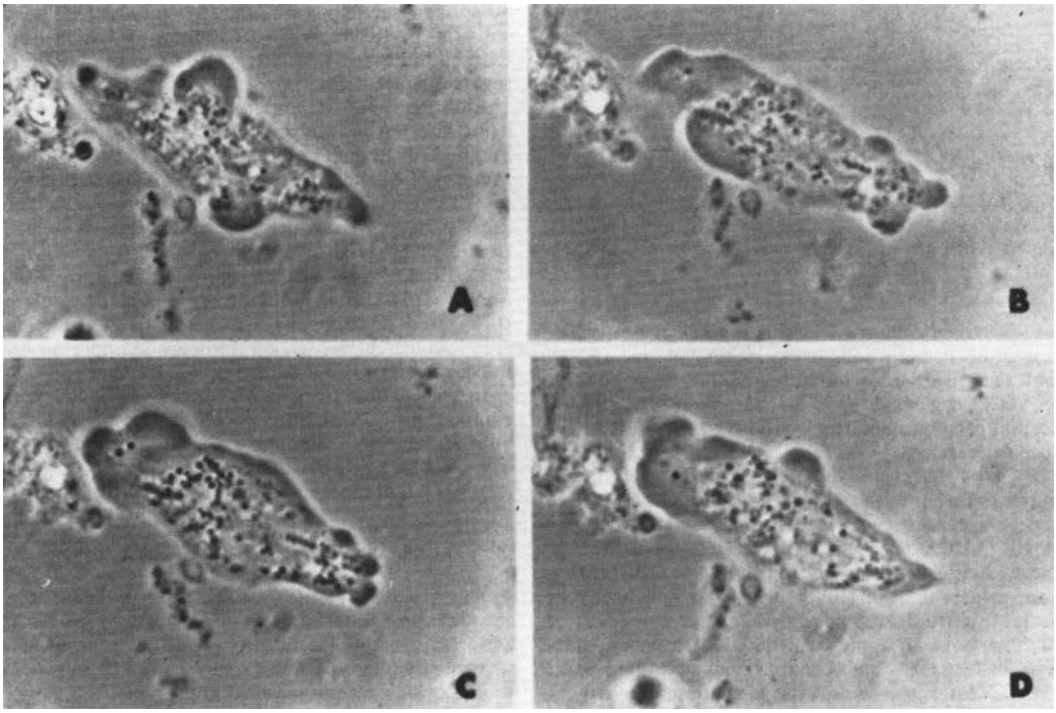

Fig. 1—Cardiac cell blebbing observed 6 hr. after perfusion with 10^{-6} M emetine. Photographs taken at 5-min. intervals (1,000×).

illustration of the typical immediate effects of 10^{-6} *M* emetine upon the beating rate of atrial and ventricular cells. It can be seen that the rate of beat decreased rapidly and progressively in both cell types. The strength of beat and synchrony of beat of the atrial cells was significantly more affected than the corresponding ventricular cells. The atrial cells appeared to maintain a regular rate, while superimposed on this rate were single or short bursts of weak, rapid contractions.

Observations up to 4 days after emetine dosing revealed progressive increases in the degree of vacuolization and signs of cell damage in doses ranging from 10^{-6} to 10^{-8} *M*. Cell growth and proliferation were observed to decrease at these dosage ranges also.

Ventricular cells dosed with 10^{-7} *M* and 10^{-8} *M* emetine exhibited short-term responses similar, yet not as pronounced, as those observed at the 10^{-6} *M* level. The atrial cells appeared to resist the effects of 10^{-7} *M* and 10^{-8} *M* emetine for periods of about 12 hr., after which were observed progressive decreases in rate, strength, and rhythmicity of beat similar to that found at the 10^{-6} *M* emetine level.

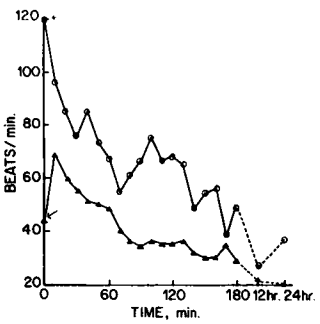

Fig. 2—Effect upon beating rate of 10^{-6} M emetine. Key: ○, atrial cell; ▲, ventricular cell; and ↑, perfusion.

Reversal Studies with Emetine-Dosed Cells—Having observed and characterized the usual effects of various concentrations of emetine upon both the atrial- and ventricular-type cells, it was decided to use 10^{-6} *M* emetine for subsequent studies. It was this concentration that elicited an observable toxic response from both atrial and ventricular beating cardiac cells. Subsequent experimentation consisted of perfusing the emetine-depressed cells with various compounds to determine their effects as possible toxicity-reversing agents. All initial perfusions with agents to counteract emetine toxicity were performed after the beating rate of the observed emetine-dosed cells had decreased to approximately one-half the preprocedural control rate.

Effect of Emetine Removal—The initial perfusion experiments consisted of removing the emetine from the culture chamber by washing with conditioned media containing no additional solutes. When compared to control cells dosed with emetine, multiple perfusion with conditioned media gave no observable protection against the toxicity, in terms of lengthened cell life, strengthening or retention of beating function, or reinitiation of beating after drug-induced cessation.

In order to evaluate and correlate the procedures reported by a variety of authors, using heart homogenates, isolated organs, or whole animals as test subjects, it was decided to appraise the activities of several biochemical compounds upon the eme-

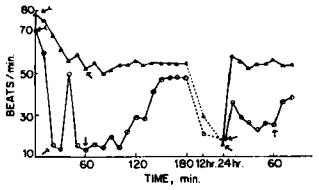

Fig. 3—Effect upon beating rate of 10^{-6} M emetine followed by 10^{-5} M NADH. Key: ▲, atrial cell; ○, ventricular cell; ⌇, emetine perfusion; ↑, NADH perfusion.

tine-poisoned cells used in this investigation. Control studies with adenosine triphosphate (ATP) and reduced nicotinamide-adenine dinucleotide (NADH) revealed that no significant effects on normal cells was elicited by these compounds in the dose ranges used.

Effect of NAD and NADH—Emetine-dosed cells were perfused with either NAD or NADH. No effects were observed with NAD at concentrations of 10^{-6}, 10^{-5}, or 10^{-4} *M*. NADH, on the other hand, induced significant stimulation of beating rate, apparent strength of beat, and synchrony of beat. Figure 3 graphically illustrates the effect of 10^{-5} *M* NADH upon both atrial- and ventricular-type cells which had been previously dosed with 10^{-6} *M* emetine. Following perfusion, the effects of the NADH were observable within 5–15 min. NADH appeared to possess more pronounced effects upon the ventricular cells than the atrial cells, although it should be noted that the atrial cells were not depressed as much as the ventricular cells. The total effects of NADH upon the observed emetine-dosed cells appeared to be a reversal of the symptoms chosen in this investigation to be considered toxicities. This reversal included three of the four measured parameters—rate of beating, synchrony of beat, and apparent strength of beat. No morphological differences were observable at the phase contrast level.

Following addition of the NADH to the cell environment, the cells would typically exhibit a brief time lag of usually from 5–10 min. before showing signs of response. After this brief period, the emetine reversal could be observed for periods lasting usually about 30–45 min. In a few instances, however, NADH reversed the observed toxic effects of emetine for periods up to several hours. Following the periods of emetine reversal the beating cells reverted back to a depressed status similar to that observed prior to the perfusion of NADH. Also noted was that the rate of beating following the effects of NADH was practically identical to the rate just prior to the NADH administration. After varying lengths of time, the rate of beating would diminish progressively until either cell death or additional NADH perfusion. After 24 hr., and in some experiments, 48 hr., further addition of NADH (also 10^{-5} *M*) to the depressed cells restored the rate, apparent strength, and rhythmicity of beat to a level at least equal to that obtained after the first administration of NADH. Often the observed reversal at this same time was in excess of reversals seen shortly after initiating the emetine toxicity. NADH (10^{-5} *M*) provided apparent protection against the effects of emetine (10^{-6} *M*) for periods up to 5 days after dosing.

Effects of Embryonic Chick-Heart Extract—The results of perfusion with an embryonic chick-heart extract indicated a slight, yet detectable, emetine-reversal effect.

Effects of ATP—Compared with the data obtained using the NADH as the perfusate compound, ATP at a dose of 10^{-6} *M* evoked a relatively brief, intense reversal of the emetine toxicity. The concentration of ATP eliciting maximum reversal was approximately 10-fold less than the most effective concentration of NADH.

It should be noted that the work of Harary and Farley (16) has demonstrated that cells such as those used in this study do respond to exogenous ATP and NADH, giving indirect support to the belief that these compounds do diffuse into the cells.

Effects of Other Compounds—None of the following compounds had significant effects upon atrial or ventricular cells: Adenine (10^{-6} *M*), flavin-adenine dinucleotide (FAD) (10^{-6} *M*), reduced FAD (10^{-6} *M*), and nicotinamide (10^{-5} and 10^{-6} *M*).

DISCUSSION AND CONCLUSIONS

The results of this investigation indicate that emetine possesses at least two possible modes of action upon the intact heart cell. The first, observable by phase-contrast microscopy in a relatively short period of time, occurs only at higher emetine concentrations, and is probably a nonspecific physical activity upon the plasma membrane. The second mode appears to be quite specific, and is believed to inhibit the production of high-energy phosphate compounds by the electron transport system.

Nonspecific Membrane Activity—Maintenance of an ionic membrane potential is known to be a requisite of the beating character of the cell types used in this investigation (12). Levinson and Green (13), also using cultured chick-heart cells, suggest that cell membrane injury from enzymatic trauma may result in swelling due to a loss of differential permeability to small electrolytes such as sodium and potassium. It seems reasonable, then, that the observed immediate cessation of beat is caused by an electrochemical imbalance resulting from the functional disruption of the cell membrane. It is also reasonable to assume that the irritation of the plasma membrane involves some mode of activity other than strictly a pH effect, or a phenolic-type protein denaturation since the media was buffered at pH 7.2–7.4, and since emetine is a nonphenolic alkaloid. One possible mode of activity upon the cell membrane in view of the abrupt change in activity observed between 10^{-5} and 10^{-6} *M* suggests a possible inhibition of enzymes of the plasma membrane. These enzymes, which catalyze and control the permeability of the membrane to most compounds, may be subject to inhibition by emetine only when the critical concentration of 10^{-5} *M* is attained.

Inhibition of Cellular High-Energy Phosphate—Whereas the physical irritation observed in the previous discussion appears to be totally based on membrane phenomenon, the second type of response to emetine is probably a result of the intracellular activities of the compound.

The importance of the TCA cycle and electron-transport system in providing sufficient energy to

support the periodic cellular contraction of cultured heart cells has been demonstrated by Harary and Farley (14). Succinate is oxidized directly by FAD, while the remaining TCA intermediates are oxidized first by NAD, which is in turn oxidized by FAD. The primary advantage of the NAD-mediated step resides in a considerable increase in ATP production. Succinate oxidation with concurrent inhibition of the oxidation of other normally occurring metabolites, results in an inhibition of heart muscle respiration (15). It is, therefore, apparent that a compound which inhibits the oxidation of NAD-mediated substrate, yet has no effect upon the succinoxidase system, would depress the energy-requiring processes of the myocardial cells.

Several facets of the experimental results from this investigation clearly indicate that cellular ATP production is seriously limited under the influence of emetine. The observed parameters of toxicity were reversed with NADH and NADPH. Perfusion of ATP had a similar, yet more rapid, intense, and short-lived reversal effect upon the beating activity of the emetine-dosed cells. It should be recalled that NADH must act throughout the electron transport system of the mitochondrion to produce the large amounts of ATP essential for myocardial activity. On the other hand, exogenously added ATP apparently by-passes the mitochondrial system and passes directly to the contractile myofibrils of the cell. This is then manifested as strengthening of beat and a rapid increase in the beating rate.

Applying this same concept, it is also logical that a longer time lag is observed before NADH reversal than ATP reversal. NADH represents potential ATP, while ATP, of course, is capable of utilization the moment it enters the cell. At this point, it should be noted that the investigations of Harary and Farley (16) offer indirect evidence that ATP does indeed permeate the ATP-deficient cell.

Of equal significance are the results in which NAD and NADP (oxidized forms) had no observable effect upon the emetine-dosed cells. This suggests that the activity of emetine is upon an enzyme, because uncatalyzed reduction of NAD requires relatively strong chemical measures which would not be available within the intact cell. In the absence of a functional enzyme system, exogenous NAD or NADP would, therefore, not be reduced and could provide no additional hydride to the electron transport system. In support of this view is the work of Marino *et al.* (17), who reported that the addition of NAD did not prevent electrocardiographic changes in the guinea pig heart after emetine administration. On the other hand, the results of this investigation and the work of Marino (18) indicate that embryonic heart extract has a reversal effect upon the observed emetine toxicity. Such an extract contains relatively high amounts of NADH and ATP, and would, therefore, be expected to elicit results similar to those observed when NADH is administered alone.

Additional indirect evidence that the emetine-dosed cell apparently lacks the ability to produce ATP was observed after performing two of the control experiments. Perfusing either ATP (10^{-6} M) or NADH (10^{-5} M) into previously untreated cultures of beating cells resulted in no observable response. The cells simply continued to maintain the beating function as though no extracellular changes had occurred. The cells apparently were producing sufficient amounts of high-energy phosphate compounds to efficiently maintain the functional status. Following emetine dosage (10^{-6} M), however, the cells appeared to rapidly utilize the exogenously provided NADH or ATP. This difference in response to exogenously provided NADH or ATP in normal cells and emetine-dosed cells, was interpreted as a facilitative mechanism of the cell to maintain chemical and functional homeostasis. This was indicated by the observavations that the normal cell, which produced sufficient endogenous ATP and NADH, had no apparent need for these compounds. However, when cellular energy production was impaired by emetine, the cell apparently turned to the ATP and NADH provided in the extracellular environment.

Two concepts have been proposed concerning the cause of cell blebbing, which was seen to occur at emetine doses of 10^{-6} M and less. The concept of Levinson and Green concerning cellular osmotic changes due to plasma membrane damage, has been discussed previously. Lettre (19), on the other hand, suggests that the plasma membrane, which requires ATP to maintain its integrity, receives an inadequate supply of the high-energy phosphates. The resultant weakening of the membrane is then manifest as the localized areas of spontaneous cell blebbing.

Lettre's concept appears to better explain the results observed at the lower emetine doses. More specifically, emetine enters the cell and effects an inhibition of the production of ATP by the electron transport system. The resultant decrease in ATP is then manifest first as relatively slow retraction of the hyaloplasmic veil. Soon following is progressive decrease in apparent strength, rhythmicity, and rate of beat, followed by cell blebbing, cessation of cell proliferation, and ultimately cell death. If the activity of emetine at this dosage level were directly upon the plasma membrane, as apparently was the case in the higher dosage levels, one would expect to see blebbing soon after emetine contacted the cells. However, such is not the case. Usually the blebbing phenomenon occurs at delayed periods after perfusion.

Based upon the reports of other authors (20), little biochemical significance can be placed upon the incidence of vacuolization in these cultured cells. However, the dose-related onset and degree of vacuolization of these emetine-treated cells lends itself to the concept of Yang *et al.* (21), who reported that the vacuole is an attempt by the cell to dilute the drug which is acting upon the cell.

Conclusions of Biochemical Effects of Emetine—The results of this study support, at the cellular level, the above-described findings of other investigators who used subcellular homogenates as the experimental system. The data suggest that emetine cardiotoxicity observed *in vitro* is primarily due to an affinity of the drug for the energy-producing portions of the cardiac tissue.

This study suggests that these isolated beating cells possess two levels of energy priorities, and budget the available ATP accordingly. Most fundamental to the survival of the cell is structural maintenance. However, only a relatively small portion of total cell energy is actually necessary for this function. The second level is maintenance of

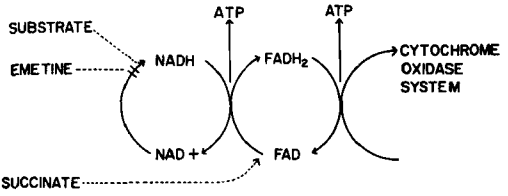

Fig. 4—Proposed site of cardiotoxic effects of emetine.

the contractile characteristics. These cells have differentiated to such an extent that the metabolism has been routed so as to provide continuously large quantities of energy for this latter function. From the observations of this study, it appears that rapid decreases in the available ATP supply, such as those induced by emetine, prevent the ability of the cell to "dedifferentiate." That is, enzymatic changes necessary to compensate for the drastic alteration in available energy cannot occur rapidly enough to maintain the cellular functions. Although much smaller quantities of ATP are produced, the same energy priorities exist. Inadequate energy is, therefore, provided to either of the abovementioned energy levels and the result is a loss of cellular function and integrity.

The proposed site of inhibition of cellular energy production is illustrated in Fig. 4. The results suggest that exogenous NADH by-passes the point of inhibition. It should be pointed out that despite the apparent functional ability of the electron transport system, the inhibition by emetine remains a major insult to the ability of the cell to efficiently produce high-energy phosphate compounds. This was evidence by the slow, yet progressive decreases in beating function observed when emetine and NADH were perfused simultaneously.

Pathological Implications of the Proposed Modes of Action—The apparent inhibition of mitochondrial activity by emetine points the way for a tentative explanation of pathological disturbances related to emetine toxicity. This may range from maintenance of electrolyte gradients, through fat and protein metabolism, to the maintenance of the cellular structure.

The reported gastrointestinal side-effects and prostration caused by emetine would be a direct result of the physical membrane effect associated with this drug. Goodman and Gilman (22) also believe the reported skeletal muscle effects are based solely upon the irritant effects upon the particular areas of drug administration.

The reported abnormalities in cardiac function indicate that emetine possesses a degree of preferential toxicity for the ventricular portions of the heart. The results of the present *in vitro* study have indicated also that isolated atrial cells can resist the toxic effects of emetine more effectively than ventricular cells.

Emetine has been reported to cause progressive cloudy swelling and fatty degeneration of the heart. It would be expected that emetine inhibition of NADH oxidation in the mitochondria would prevent the complete metabolism of the fatty acids being actively transported into the cell. The pathological result would be the observed fatty degeneration.

The dose-related inhibition of emetine upon cell growth, maintenance, and proliferation observed in this investigation indicates a likely inhibition of protein syntheses. Grollman (4) indicates that emetine may possess some structural similarity to the glutarimide antibiotics and acts similarly by physically binding at the ribosome and thereby inhibiting the aminoacyl-sRNA transfer reaction in protein biosynthesis. The results of this investigation neither confirm nor negate the possibility that emetine may act at the ribosomal level. However, it is suggested that the proposed concept of NADH inhibition by emetine is of more fundamental consequence to the cell. While protein synthesis may concomitantly be inhibited, it appears that the apparent inhibition of energy production more convincingly explains the cardiotoxic properties of this compound.

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Keyphrases

Emetine toxicity—embryonic chick-heart cells
Heart cells, embryonic chick—test cultures
Cell perfusion—emetine solution
Contractions, heart cells—emetine effect
NAD, NADH effect—emetine-dosed cells