The reaction was filtered and spin evaporated *in vacuo* to give white crystals. These crystals were collected, washed with ether, and recrystallized.

REFERENCES

(1) D. M. Shepherd and D. MacKay, *Prog. Med. Chem.*, **5**, 199 (1967).

(2) J. P. Green, in "Medicinal Chemistry," part II, A. Burger, Ed., Wiley–Interscience, New York, N.Y., 1970, p. 1633.

(3) J. L. Kelley, C. A. Miller, and H. L. White, *J. Med. Chem.*, **20**, 506 (1977).

(4) J. L. Kelley, C. A. Miller, and E. W. McLean, *ibid.*, **20**, 721 (1977).

(5) J. I. DeGraw, J. Engstrom, M. Ellis, and H. L. Johnson, *ibid.*, **20**, 1671 (1977).

(6) E. E. Smissman and J. A. Weis, *ibid.*, **14**, 945 (1971).

(7) L. R. Swett and T. O. Yellin, *ibid.*, **13**, 968 (1970).

(8) P. M. Ruoff and R. C. Scott, *J. Am. Chem. Soc.*, **72**, 4950 (1950).

(9) B. Robinson and D. M. Shepherd, *J. Chem. Soc.*, **1961**, 5037.

(10) K. Friedrich and H.-G. Henning, *Chem. Ber.*, **92**, 2756 (1959).

(11) R. A. Turner, *J. Am. Chem. Soc.*, **71**, 3476 (1949).

(12) C. F. Huebner, *ibid.*, **73**, 4667 (1951).

ACKNOWLEDGMENTS

The authors thank Dr. Helen L. White and her staff for the enzyme inhibition results and Dr. B. S. Hurlbert and his staff for the elemental analyses and NMR spectra.

Effects of Chronic Emetine Treatment on Mitochondrial Function

MICHAEL A. DUBICK * and WILLIAM C. T. YANG ˣ

Received March 10, 1980, from the *Department of Pharmacology and Nutrition, School of Medicine, University of Southern California, Los Angeles, CA 90033.* Accepted for publication September 8, 1980. *Present address: Department of Nutrition, University of California, Davis, CA 95616.

Abstract □ Emetine has long been recognized as a cardiotoxic drug, but its mechanism remains unknown. Since many studies reported an effect of emetine on cardiac metabolism, permanent damage to the heart could result from such treatment. To investigate this action of emetine in relation to the cardiotoxicity seen after a therapeutic regimen, New Zealand albino rabbits were given 2 mg/kg/day ip of emetine for 9 days. Heart and liver mitochondrial metabolism was assessed polarographically using pyruvate, pyruvate plus malate, or succinate as the substrate. Heart mitochondrial metabolism was reduced in emetine-treated rabbits in comparison to the normal controls. However, the metabolic activity of the pair-fed control group was equally reduced. Only the liver mitochondrial metabolism of the pair-fed control group was reduced in relation to the normal controls, while the liver mitochondrial metabolism of emetine-treated rabbits remained unchanged. These data indicated that the reduction in heart mitochondrial metabolism resulted from the inanition induced by the chronic emetine treatment and not from a primary effect of the drug.

Keyphrases □ Emetine—effects of chronic treatment on heart and liver mitochondrial metabolism □ Mitochondrial function—effects of chronic emetine treatment □ Cardiotoxicity—effects of chronic emetine treatment on mitochondrial function

Emetine remains important in the treatment of amebiasis. Its role, however, has been reduced to an alternative drug choice, primarily because of its numerous side effects, the most serious affecting the cardiovascular system. Many studies reported adverse effects of high concentrations of emetine on cardiac metabolism (1–4), although the mechanism of this cardiotoxicity remains unknown.

BACKGROUND

In a series of experiments, rats injected with ~2 mg/kg/day of emetine for 14–17 days showed a reduction in heart homogenate respiration with butyrate, β-hydroxybutyrate, citrate, α-ketoglutarate, malate, pyruvate, or lactate as the substrate (5–7). Since no effects were observed on the liver homogenate respiration, Appelt and Heim (5–7) concluded that emetine appeared to be selectively toxic to the heart. However, they reported that animals chronically treated with emetine reduced their food intake and lost weight. Since pair-fed controls were not included in their study, their conclusion must be reevaluated.

Few studies investigated the action of emetine on mitochondrial metabolism, but two reports indicated that therapeutic doses of emetine may affect heart mitochondria. Brink *et al.* (8) found a reduction in oxygen uptake and [^{14}C]carbon dioxide formation from glucose, pyruvate, and palmitate. If these effects are not secondary to the reduced contractility observed, a reduction in heart mitochondrial metabolism must be postulated. Pearce *et al.* (9) described the mitochondrion as the primary structure in the cardiac cell to be affected morphologically in response to therapeutic doses of emetine in dogs.

Since metabolic changes in the heart could indicate cardiac damage, further study of emetine is important in the overall evaluation of the cardiotoxicity seen clinically. Thus, heart mitochondrial metabolism was assessed in rabbits treated with therapeutic doses of emetine. Since emetine is toxic to the heart yet exerts a major therapeutic effect in the liver, liver mitochondrial metabolism was studied for comparison with the heart.

EXPERIMENTAL

Male New Zealand albino rabbits were given 2 mg/kg/day ip of emetine hydrochloride for 9 days. Since emetine-treated animals ate less, pair-fed controls were included to exclude effects due to inanition. Normal controls were fed *ad libitum*, and water was freely available to all groups.

Heart mitochondria were prepared as described previously (10). After removal of the heart, a piece of ventricle was removed and prepared for electron microscopy according to techniques outlined by Hayat (11). Rabbit liver mitochondria were prepared similarly. The homogenization and suspending media consisted of 0.25 *M* sucrose plus 1 m*M* ethylenediaminetetraacetic acid (pH 7.2).

Heart and liver mitochondrial metabolism was studied polarographically with an oxygraph. Pyruvate (5 m*M*), pyruvate (5 m*M*) plus malate (0.1 m*M*), and succinate (5 m*M*) were the substrates. The reaction medium for the heart mitochondrial studies contained 0.25 *M* mannitol, 10 m*M* KCl, 10 m*M* tris(hydroxymethyl)aminomethane hydrochloride, 0.1 m*M* ethylenediaminetetraacetic acid, and 5 m*M* phosphate buffer (pH 7.2). The reaction medium for the liver studies consisted of 0.25 *M* sucrose, 0.1 m*M* KCl, and 10 m*M* tris(hydroxymethyl)aminomethane phosphate buffer (pH. 7.2). Small volumes of concentrated adenine diphosphate and magnesium chloride were added to reach final concen-

0022-3549/81/0300-0343$01.00/0

Table I—Effect of Chronic Emetine Treatment on Pyruvate [a] Oxidation by Heart Mitochondria [b]

	Initial Rate	State 3	Magnesium Chloride	State 3	RC Ratio	ADP:O Ratio
Normal control (n = 10)	27.5 ± 3.4	433.3 ± 49.2	202.2 ± 15.0 135.6 ± 12.1	523.7 ± 47.8	12.8 ± 0.4 3.4 ± 0.3[c]	3.1 ± 0.1 3.6 ± 0.2[c]
Pair-fed control (n = 8)	18.7 ± 1.6[d]	306.0 ± 22.5[d]	147.6 ± 16.4[d] 106.8 ± 11.3	351.9 ± 24.8[e]	11.2 ± 0.6 3.0 ± 0.3[c]	3.2 ± 0.2 3.8 ± 0.1[c]
Emetine treated[f] (n = 8)	18.2 ± 1.5[d]	271.0 ± 9.3[e]	137.4 ± 8.7[e] 96.4 ± 3.7[e]	324.0 ± 14.4[e]	10.9 ± 0.5 3.3 ± 0.2[c]	3.3 ± 0.2 3.7 ± 0.2[c]

[a] Includes 0.1 mM malate. [b] Mean ± SE expressed as n atoms of oxygen consumed per minute per milligram of mitochondrial protein. [c] After the addition of 1.4 mM $MgCl_2$. [d] $p < 0.05$ from normal control. [e] $p \ll 0.05$ from normal control. [f] Given 2 mg/kg/day ip for 9 days.

Table II—Effect of Chronic Emetine Treatment on Succinate Oxidation by Heart Mitochondria [a]

	Initial Rate	State 3	Magnesium Chloride	State 3	RC Ratio	ADP:O Ratio
Normal control (n = 8)	119.7 ± 12.0	656.0 ± 43.9	359.7 ± 23.2	764.8 ± 74.4[b]	4.6 ± 0.2	2.3 ± 0.1
Pair-fed control (n = 3)	92.6 ± 8.7	508.3 ± 48.1	347.6 ± 33.7	523.5 ± 69.1	3.9 ± 0.5	2.5 ± 0.2
Emetine treated[c] (n = 3)	83.4 ± 5.3[d]	466.9 ± 28.9	310.7 ± 40.7	506.9 ± 15.2[d]	4.4 ± 0.3	2.6 ± 0.1

[a] Mean ± SE expressed as n atoms of oxygen consumed per minute per milligram of mitochondrial protein. [b] n = 4. [c] Given 2 mg/kg/day ip for 9 days. [d] $p < 0.05$ from normal control.

trations of 0.3 and 1.4 mM, respectively. All reactions were run at 28°. Protein determination was by the method of Lowry *et al.* (12).

RESULTS

With pyruvate plus malate or pyruvate alone as the substrate, the respiration rates of heart mitochondria isolated from the pair-fed control and emetine-treated rabbits were significantly lower than those recorded from normal control mitochondria (Table I). (Since both results were similar, only those with pyruvate plus malate are shown). This same trend was observed with succinate, but only the initial rate and postmagnesium State 3 respiration rates were significantly lower in the emetine-treated group than in the normal control group (Table II). In both cases, the respiration rates of mitochondria isolated from the pair-fed control group did not differ from the rates observed in the emetine-treated group. Also, as shown in Tables I and II, the respiratory control (RC) and adenosine 5′-phosphate–oxygen (ADP:O) ratios did not differ significantly in any of the three groups.

Liver mitochondria responded differently than heart mitchondria to chronic emetine treatment with all substrates used. Liver mitochondria from the pair-fed control rabbits, respiring in the presence of pyruvate plus malate or pyruvate alone, had significantly lower State 3 respiration rates than did both the normal control and emetine-treated mitochondria (Table III). In addition, the premagnesium RC ratio was significantly lower in the pair-fed control group than in the normal control group. With succinate as the substrate, only the premagnesium State 3 rate was significantly lower in the pair-fed control group in comparison to both the normal control and emetine-treated groups (Table IV). With any substrate used, the emetine-treated rabbits maintained mitochondrial respiration at normal control levels (Tables III and IV). No differences were detected in the ADP:O ratios in all liver mitochondrial preparations.

DISCUSSION

The results from the present experiments with heart mitochondria agreed with preliminary experiments using α-ketoglutarate as the substrate (13). In addition, they were similar to those obtained by Appelt and Heim (5–7) with rats and by Hanasono (14) with rabbits. The only discrepancy was that Appelt and Heim (6) observed no difference between control and emetine-treated heart homogenate respiration with succinate as the substrate. Our results on the reduced respiration of rabbit heart mitochondria with succinate as the substrate agreed with those of Hanasono using rabbit heart homogenates (14). The discrepancy may be due to species differences. Nevertheless, the major observation from this study was that although heart mitochondrial respiration in the emetine-treated group was reduced in comparison to the normal control group, respiration in the pair-fed control group was equally reduced. This finding indicated that the apparent depressive effects of emetine on heart mitochondrial respiration were not due to a direct action of the drug but were secondary to the nutritional state of the animal.

Other evidence to support this hypothesis comes from work by Gold and coworkers (15, 16). In rats semistarved or totally starved for 7 days, the activities of myocardial enzymes, including those found in the mitochondria, were reduced.

Table III—Effect of Chronic Emetine Treatment of Pyruvate [a] Oxidation by Liver Mitochondria [b]

	Initial Rate	State 3	Magnesium Chloride	State 3	RC Ratio	ADP:O Ratio
Normal control (n = 10)	8.3 ± 1.0	38.9 ± 2.4	22.1 ± 1.4	55.9 ± 3.3	3.8 ± 0.3 3.4 ± 0.3[c]	3.2 ± 0.2 3.5 ± 0.3[c]
Pair-fed control (n = 8)	6.2 ± 0.5	24.4 ± 1.3[d,e]	17.9 ± 0.8	34.3 ± 3.0[d,e]	2.8 ± 0.1[d] 2.6 ± 0.3[c]	3.7 ± 0.2 3.5 ± 0.0[c]
Emetine treated[f] (n = 7)	6.3 ± 0.5	35.8 ± 2.5	19.2 ± 1.1	51.8 ± 4.3	3.5 ± 0.2 2.9 ± 0.2[c]	3.4 ± 0.2 3.6 ± 0.2[c]

[a] Plus 0.1 mM malate. [b] Mean ± SE expressed as n atoms of oxygen consumed per minute per milligram of mitochondrial protein. [c] After the addition of 1.4 mM $MgCl_2$. [d] $p \ll 0.05$ from normal control. [e] $p \ll 0.05$ from emetine-treated rabbits. [f] Given 2 mg/kg/day ip for 9 days.

Table IV—Effect of Chronic Emetine Treatment on Succinate Oxidation by Liver Mitochondria [a]

	Initial Rate	State 3	Magnesium Chloride	State 3	RC Ratio	ADP:O Ratio
Normal control (n = 10)	17.3 ± 1.6	66.3 ± 4.6	31.3 ± 2.0	61.87 ± 4.04	3.6 ± 0.2	2.4 ± 0.1
Pair-fed control (n = 8)	11.1 ± 0.8[b]	45.3 ± 1.7[c]	26.5 ± 1.1	59.0 ± 3.9	3.3 ± 0.2	2.4 ± 0.1
Emetine treated[d] (n = 3)	17.2 ± 2.3	74.8 ± 5.3	33.3 ± 3.3	78.0 ± 5.4	3.8 ± 0.2	2.3 ± 0.1

[a] Mean ± SE expressed as n atoms of oxygen consumed per minute per milligram of mitochondrial protein. [b] $p \ll 0.05$ from normal control. [c] $p \ll 0.05$ from normal control and emetine-treated rabbits. [d] Given 2 mg/kg/day ip for 9 days.

Independent of the substrate used, the RC and ADP:O ratios were similar in all three groups. This finding indicated that neither emetine nor malnutrition affected the coupling of oxidation to phosphorylation or the oxidative phosphorylation process, respectively.

The results from the liver mitochondrial studies also agreed with the findings of Appelt and Heim (6, 7). Since the respiration rates were lower only in the pair-fed control group in comparison to the normal control group, liver mitochondrial metabolism apparently was reduced by malnutrition. This effect was overcome in the emetine-treated rabbits.

Basically, it seemed that liver mitochondria were less susceptible to the effects of inanition than heart mitochondria. This result was consistent with observations in semistarved rats (17) and probably reflects the higher metabolic rates and energy requirement of the heart.

As with heart mitochondria, emetine treatment did not affect oxidative phosphorylation in liver mitochondria. Since no consistent effects on the RC ratio were observed in the pair-fed control group, mitochondrial coupling probably was not significantly affected by malnutrition.

In the present experiments, the heart mitochondria from emetine-treated rabbits appeared unaltered, except for a slight swelling of the cristae, when examined under the electron microscope. However, these observations appeared to reflect metabolic effects. Similar morphological findings also were noted by Hatt *et al.* (18) in rats chronically treated with emetine, but they differed from the results of Pearce *et al.* (9) and may reflect species differences. As seen by the treatment schedule used by Pearce's group, the dog may be more sensitive to the actions of emetine than the rat and rabbit.

Thus, the results of these experiments indicate that chronic therapeutic doses of emetine produced no direct detrimental effect on heart mitochondrial metabolism. Any effects observed were secondary to the inanition induced by chronic treatment. The different response noted in liver mitochondria probably reflected the different metabolic states of the heart and liver, as well as the unique metabolic response of the liver during semistarvation or starvation (19). Thus, it appears unlikely that metabolic damage to the myocardium will result from chronic therapeutic doses of emetine. This view agrees with the clinical observation that after therapeutic doses of emetine, most patients recover from cardiovascular side effects without any compromise in cardiac function.

REFERENCES

(1) R. Deitrich and H. Heim, *J. Pharm. Sci.*, **45**, 512 (1956).

(2) A. Marino and S. Magliulo, *Arch. Int. Pharmacodyn. Ther.*, **132**, 331 (1961).

(3) H. Heim, H. Froede, and V. Erwin, *J. Pharmacol. Exp. Ther.*, **137**, 107 (1962).

(4) S. Imam, V. Rao, and K. Kar, *Biochem. Pharmacol.*, **21**, 3089 (1972).

(5) G. Appelt and H. Heim, *J. Pharm. Sci.*, **53**, 108 (1964).

(6) *Ibid.*, **54**, 1621 (1965).

(7) *Ibid.*, **57**, 1428 (1968).

(8) A. Brink, J. Kotze, S. Muller, and A. Lochner, *J. Pharmacol. Exp. Ther.*, **165**, 251 (1969).

(9) M. Pearce, R. Bulloch, and M. Murphy, *Arch. Pathol.*, **91**, 8 (1971).

(10) W. C. T. Yang and M. Dubick, *Life Sci.*, **21**, 1171 (1977).

(11) M. A. Hayat, "Basic Electron Microscopy," Van Nostrand-Reinhold, New York, N.Y., 1972, pp. 5, 6.

(12) O. Lowry, N. Rosenbrough, A. Farr, and R. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(13) M. A. Dubick and W. C. T. Yang, *Toxicol. Appl. Pharmacol.*, **54**, 311 (1980).

(14) G. Hanasono, Master's thesis, University of Southern California, Los Angeles, Calif., 1966.

(15) A. J. Gold and L. C. Costello, *Am. J. Physiol.*, **227**, 1336 (1974).

(16) A. J. Gold and S. R. Yaffe, *J. Nutr.*, **108**, 410 (1978).

(17) A. J. Gold and L. C. Costello, *ibid.*, **105**, 208 (1975).

(18) P. Y. Hatt, B. Swynghedauw, and J. C. Hayat, *Ann. N.Y. Acad. Sci.*, **160**, 799 (1969).

(19) S. Gaetani, A. M. Paolucci, M. A. Spadoni, and G. Tomasssi, *J. Nutr.*, **84**, 173 (1964).

COMMUNICATIONS

General Equation for Assessing Drug Removal by Extracorporeal Devices

Keyphrases □ Pharmacokinetics—general equation for measurement of drug removal by extracorporeal devices □ Extracorporeal devices—general equation for measurement of drug removal □ Drug removal—by extracorporeal devices, general equation

To the Editor:

The efficiency of extracorporeal drug removal commonly is measured as dialysance or dialysis clearance. Such terms, however, do not correlate directly with the amount of drug removed by the device. Previous investigators (1, 2) proposed an alternative parameter, f, defined as the fraction of drug in the body at the start of extracorporeal drug removal (EDR) that is removed by the device. This fraction is the product of the fraction of total elimination that EDR represents and the fraction of drug lost by all elimination routes during EDR and is given by:

$$f = \frac{Cl_D}{Cl + Cl_D}\left[1 - e^{[-(Cl+Cl_D)/V]t}\right] \qquad \text{(Eq. 1)}$$

where Cl_D is the EDR clearance, Cl is the total body clearance in the absence of EDR, V is the apparent volume of distribution of the drug, and t is the duration of EDR.

Equation 1 assumes one-compartment pharmacokinetics; thus, for most drugs, it may yield inaccurate estimates of the fraction of drug removed by an extracorporeal device. An alternative general equation that can be used for drugs following single or multicompartment kinetics is developed in this communication.

Consider the mass balance equation:

$$X_S = X_D + X_{el} + X_f \qquad \text{(Eq. 2)}$$

where X_S and X_f are the amounts of drug in the body at the beginning and the end of EDR, respectively, and X_D and X_{el} are the amounts of drug eliminated by EDR and by the body during EDR, respectively. Equation 2 may be expressed as:

$$X_S = Cl_D AUC_1 + ClAUC_1 + ClAUC_2 \qquad \text{(Eq. 3)}$$

where AUC_1 is the area under the plasma concentration–time curve during EDR and AUC_2 is the area under the

0022-3549/81/0300-0345$01.00/0