Effect of Chronic Poisoning by Emetine on Oxidative Process in Rat Heart II

Effects on Oxidation of Citric Acid Cycle Intermediates and Nicotinamide Adenine Dinucleotide Metabolism

By GLENN D. APPELT and HAROLD C. HEIM

An investigation of the effects of chronic emetine poisoning on certain oxidative processes in rat heart and liver was conducted. Conventional manometric techniques were employed to study the respiration of the tissue preparations in the presence of various citric acid cycle intermediates. In addition, the effect of chronic emetine poisoning on nicotinamide adenine dinucleotide levels in rat myocardium was studied utilizing a spectrophotometric assay. In animals chronically poisoned by emetine, the oxygen uptake by heart homogenates respiring in the presence of citrate, malate, or α-ketoglutarate was lower than that obtained with homogenates prepared from sham-injected control animals. With succinate as the substrate, however, this effect was not observed. Liver homogenates prepared from emetine-poisoned rats did not show an impaired ability to oxidize any of the substrates, including succinate, which were employed in the experiments with heart homogenates. Since succinate is not considered to be a prime substrate in the synthesis of high-energy phosphate intermediates by heart muscle, it is possible that the inhibition of the oxidation of certain metabolites, other than succinate, which have a greater potential for the synthesis of these energy rich compounds, may contribute to the apparent hypersensitivity of this organ to emetine. A considerable amount of experimental evidence indicates that in the oxidation of citrate, malate, or α-ketoglutarate electron transport involves the nicotinamide adenine nucleotides. With succinate, however, the electrons enter the transport system at the flavin nucleotide step. Under the conditions of this experiment, no changes were noted in nicotinamide adenine dinucleotide levels in heart homogenates prepared from emetine-poisoned rats when compared to sham-injected control animals.

MANY investigations have been made relative to the effect of emetine on cellular metabolism. A depression of glycogen synthesis by livers of emetine-poisoned rats has been demonstrated, together with a decrease in phosphorylase and aldolase activity (1). Furthermore, a depletion of liver glycogen in rats has been reported to occur after a single administration of emetine (2). It has been suggested that emetine may interfere with enzyme systems necessary for conversion of glycogen to contractile energy in the myocardium (3). Electrocardiographic changes have been induced in the guinea pig after the administration of sublethal doses of emetine, and it was observed that nicotinamide adenine dinucleotide evoked no protective effect in averting these electrocardiographic abnormalities (4). It has been shown that emetine has no effect on the synthesis of cocarboxylase by rat liver (5). Emetine increased the oxygen uptake of rat heart homogenates respiring in the presence of succinate (6), and a similar enhancement in mitochondrial preparations has been reported (7). It was also noted that emetine decreased the endogenous oxygen uptake of rat heart homogenates as well as the oxygen consumption in the presence of glucose, pyruvate, malate, and fumarate (6). It has been suggested that succinate is a poor source of energy for cardiac muscle and that inhibition of the oxidation of substrates other than succinate may lead to an impaired efficiency of the heart (8). Oxygen consumption of heart homogenates prepared from emetine-poisoned rats was found to be significantly lower than sham-injected controls when butyrate and β-hydroxybutyrate were utilized as substrates (9). It was also reported that emetine has no effect on oxidative phosphorylation by heart mitochondria respiring in the presence of α-ketoglutarate (9). Vitamin metabolism has been studied in emetine-poisoned rats and no apparent changes in the metabolism of vitamin A, riboflavin, nicotinic acid, or biotin were noted; however, it was observed that emetine-treated rats stored smaller amounts of thiamin and folic acid in the liver than did pair fed controls (10).

The distribution of emetine in rats has been studied, and the highest concentration of the alkaloid after intraperitoneal administration was found to be in the liver, whereas only small

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amounts were demonstrated to be present in the heart, brain, skeletal muscle, and blood (11). It seems of interest to note that, although the heart appears to contain small amounts of emetine after administration, profound cardiotoxic effects have been observed (12–14). The mechanism responsible for the toxic effects of emetine on the myocardium remains unknown and may involve a reduction in the efficiency of biochemical processes. This concept was explored in a previous communication (9), and it seemed of importance to expand investigations in this area further.

In this study, comparative experiments were conducted in which the effect of chronic emetine poisoning on the oxidation of certain citric acid cycle intermediates by rat heart and liver homogenates was investigated. In addition, the effect of emetine administration on nicotinamide adenine dinucleotide levels in rat myocardium was studied.

EXPERIMENTAL

To investigate the effects of chronic poisoning by emetine on the oxidation of substrates by heart and liver homogenates, young Sprague-Dawley rats of both sexes were employed as test animals. Animals weighing approximately 100 Gm. were separated into two groups of six animals each and given intraperitoneal injections daily for 14 days as follows: group *A*, 0.2 mg. emetine HCl in 0.1 ml. water; group *B*, 0.1 ml. water. All the animals were allowed food and water *ad libitum* during the test period. On the 14th day, the animals were stunned and exanguinated, the heart or liver tissue immediately removed, washed quickly in cold water, blotted dry, and weighed. The tissue was then transferred to a chilled Ten Broek tissue grinder containing sufficient cold 0.1 *M* potassium phosphate buffer, pH 7.4, so that the homogenates contained 80 mg. of fresh tissue per milliliter, unless otherwise noted. The homogenates were then pipeted into chilled Warburg vessels containing substrates and cofactors as follows. The main compartment of each flask contained 0.5 ml. of homogenate; 0.1 ml. of 5×10^{-4} *M* cytochrome c; 0.1 ml. of 1.5×10^{-2} *M* malate; 0.1 ml. of 2×10^{-2} *M* $MgCl_2$; 0.1 ml. of 2×10^{-2} *M* ATP; 0.1 ml. of 1.5×10^{-2} *M* nicotinamide; 0.3 ml. of 0.1 *M* substrate; 1.2 ml. of 0.1 *M* potassium phosphate buffer, pH 7.4; and 0.3 ml. of distilled water. When malate was employed as the substrate, 0.3 ml. of 1.5×10^{-2} *M* glutamate was substituted for the distilled water. In the experiments with succinate as substrate the flasks contained, in the main compartment, 0.5 ml. of homogenate; 0.3 ml. of 5×10^{-4} *M* cytochrome c; 1.0 ml. of potassium phosphate buffer containing 0.01 *M* EDTA, pH 7.4; and 0.6 ml. of distilled water. The sidearm contained 0.4 ml. of 0.1 *M* succinate which was tipped into the main compartment at zero time.

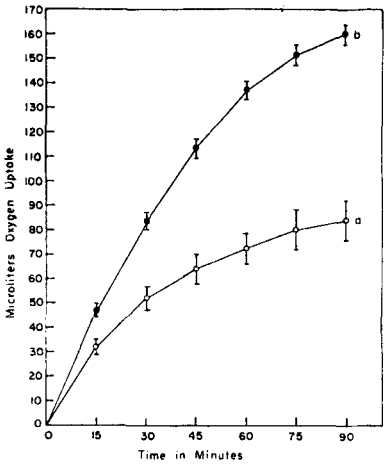

Fig. 2.—Oxidation of malate by heart homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

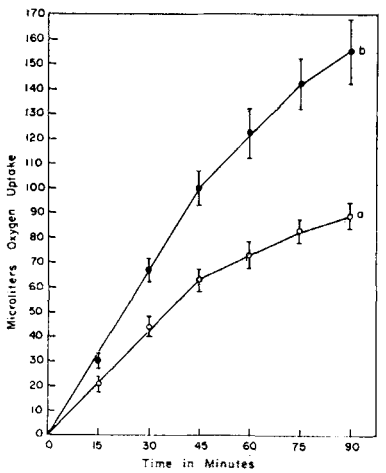

Fig. 1.—Oxidation of citrate by heart homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

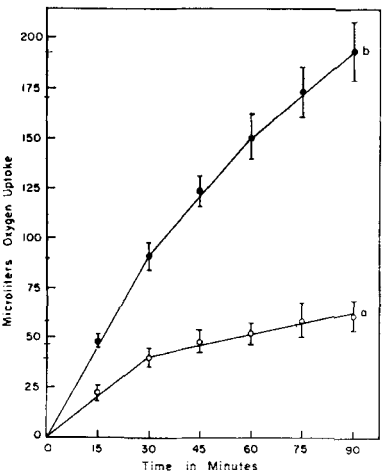

Fig. 3.—Oxidation of α-ketoglutarate by heart homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

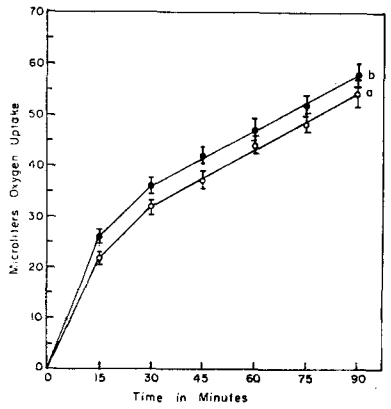

Fig. 4.—Oxidation of succinate by heart homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

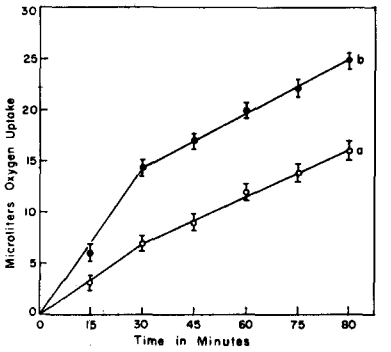

Fig. 5.—Endogenous oxygen uptake by heart homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

These procedures were used by Wolff and Ball (15) in their studies of the oxidation of succinate by heart homogenates. In all experiments the center well of each flask contained 0.2 ml. of 10% potassium hydroxide solution. The total volume of the flask contents was 3 ml. in all the oxidative studies. After a 10-min. equilibration period, oxygen consumption was measured for 90 min. according to conventional manometric techniques (16).

All oxygen uptake values represent the average of data obtained with at least six animals, and each determination was performed in duplicate. The pH of the contents of a representative number of flasks was determined at the end of the experimental period to insure that the observed effects were not due to a change in pH during the experiment. The standard error of the mean was calculated for each set of 15-min. oxygen uptake values and is designated by the vertical line through each point.

To determine the effect of emetine on nicotinamide adenine dinucleotide levels in ratmy ocardium, male albino rats weighing approximately 150 Gm. were separated into four groups of four animals each and given daily intrapertioneal injections as follows: group *A*, 65 mg. nicotinamide in 0.5 ml. water; group *B*, 65 mg. nicotinamide in 0.5 ml. water and 0.4 mg. emetine HCl in 0.2 ml. water; group *C*, 0.7 ml. water; group *D*, 0.4 mg. emetine HCl in 0.2 ml. water. The extraction of nicotinamide adenine dinucleotide from the myocardial tissue was accomplished by the method of Ciotti and Kaplan (17). The animals were sacrificed, and the ventricular tissue quickly removed, weighed, and transferred to a Ten Broek tissue grinder. Homogenization was then accomplished with 5 vol. of cold 5% TCA. After centrifugation, the TCA was removed from the supernatant by extraction with ether, and aliquots of the aqueous extract employed for nicotinamide adenine dinucleotide measurement. The nicotinamide adenine dinucleotide present was determined spectrophotometrically with a Beckman DB spectrophotometer at 340 mμ utilizing an ethanol–alcohol dehydrogenase system (17).

TABLE I.—TOTAL OXYGEN UPTAKE BY HEART HOMOGENATES FROM EMETINE-POISONED AND CONTROL RATS

Substrates	μl. Oxygen Uptake/90 min./40 mg. Wet Tissue Control	Emetine-Poisoned	% Change
Citrate	155	89	43[c]
Malate	160	83	48[c]
α-Ketoglutarate[a]	195	61	69[c]
Succinate[b]	58	54	7
None	25	16	36[c]

[a] Twenty milligrams per flask. [b] Ten milligrams per flask. [c] Significant $p < 0.001$.

RESULTS AND DISCUSSION

Figures 1 through 4 illustrate the effect of chronic emetine poisoning on the respiration of heart homogenates in the presence of the various substrates included in this study. The total oxygen uptake of heart homogenates from emetine-poisoned rats was significantly lower than that observed with the sham-injected controls when citrate, malate, or α-ketoglutarate were used as the substrates. The endogenous respiration was also decreased, as shown in Fig. 5. However, when succinate was employed

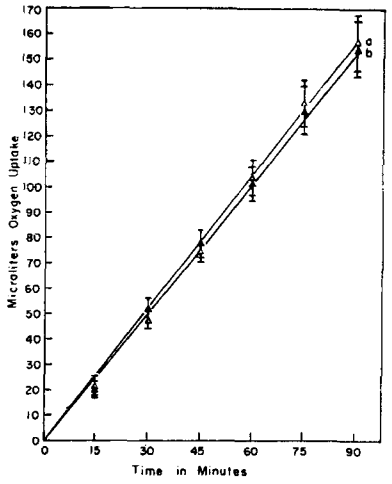

Fig. 6.—Oxidation of citrate by liver homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

as the substrate, chronic poisoning by emetine did not significantly alter the total oxygen uptake. A summary of these data is presented in Table I.

Figures 6 through 9 depict the effect of chronic emetine poisoning on the respiration of liver homogenates in the presence of the various substrates. The total oxygen uptake of homogenates prepared from the livers of emetine-poisoned rats was not significantly different from those obtained from the sham-injected control rats with citrate, malate, α-ketoglutarate, or succinate as substrates. The total endogenous oxygen uptake of liver homogenates prepared from emetine-poisoned rats was decreased as presented in Fig. 10, but this difference proved to be insignificant. A summary of these data appears in Table II.

The diminished oxygen consumption by heart homogenates prepared from emetine-poisoned rats and with citrate, malate, and α-ketoglutarate as substrates indicates an impairment of some oxidative process or processes involved in the degradation of these metabolites. Any interference with oxidative processes in cardiac muscle would be expected to impair the synthesis or utilization of high-energy compounds which are essential for contractility.

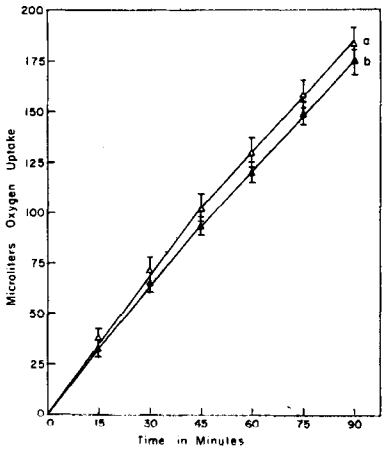

Fig. 7.—Oxidation of malate by liver homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

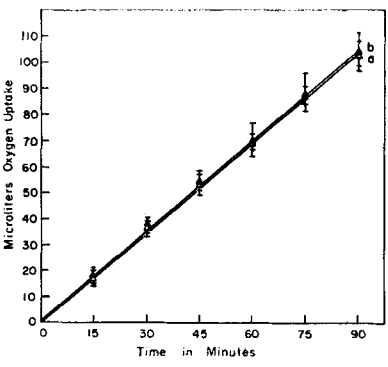

Fig. 8.—Oxidation of α-ketoglutarate by liver homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

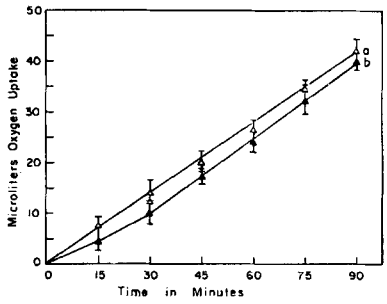

Fig. 9.—Oxidation of succinate by liver homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

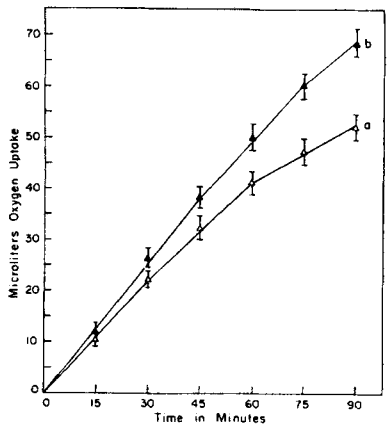

Fig. 10.—Endogenous oxygen uptake by liver homogenates from sham-injected and emetine-treated rats. Key: a, emetine-treated animals; b, sham-injected animals.

The oxidation of succinate by the heart was not altered by chronic poisoning with emetine. Such observation indicated that the succinoxidase system was not affected appreciably *in vivo* by the levels of emetine which are present in the rat heart after repeated administration of emetine. Deitrich and Heim (6) have noted that emetine *in vitro* augmented the activity of a partially purified succinoxidase system prepared from rat heart. This apparent discrepancy between the *in vitro* and *in vivo* effects of emetine on the metabolic process by which succinate is oxidized in rat heart may be partially attributed to the relative concentrations of the drug present. The amount of emetine required to enhance the activity of the succinoxidase system *in vitro* was considerably greater than the level of the drug found in the heart after administration of emetine. Furchgott and Shorr (18) have demonstrated that the oxidative process by which succinate is metabolized competes with processes involving the utilization of other normally occurring metabolites, resulting in the inhibition of respiration in heart muscle. Colowick and associates (19) have shown that succinate oxidation is coupled with phosphorylation, and Ochoa (20) has demonstrated that the efficiency of this coupled process is low as

TABLE II.—TOTAL OXYGEN UPTAKE BY LIVER HOMOGENATES FROM EMETINE-POISONED AND CONTROL RATS

Substrates	Control	μl. Oxygen Uptake/90 min./40 mg. Wet Tissue Emetine-Poisoned	% Change[c]
Citrate	154	159	3
Malate	184	178	3
α-Ketoglutarate[a]	103	103	0
Succinate[b]	42	48	12
None	68	52	24

[a] Twenty milligrams per flask. [b] Ten milligrams per flask. [c] None of the differences significant $p < 0.01$.

TABLE III.—NICOTINAMIDE ADENINE DINUCLEOTIDE LEVELS IN RAT HEART TISSUE

Group	Nicotinamide Adenine Dinucleotide Values in mcg./Gm. Tissue Mean	Range	S. E.
A			
Nicotinamide, 500 mg./Kg. i. p. daily	724	660–810	±32
B			
Nicotinamide, 500 mg./Kg. i. p. daily + Emetine HCl, 0.4 mg. i. p. daily	830	650–1135	±107
C			
Sham-injected distilled water i. p.	510	500–520	±4
D			
0.4 mg. Emetine HCl i. p. daily	488	470–500	±6

measured by the high-energy phosphate compounds formed during the oxidation of succinate. An inhibition of the oxidation of metabolites which have a greater potential for the formation of energy rich phosphate compounds and concomitant unaltered succinate oxidation could conceivably elicit a depressant effect upon the heart. This is in accord with the results reported by Forssman and Lindsten (21), who have demonstrated that succinate depresses the isolated rat heart. This concept may otherwise be explained by recalling that succinate oxidation yields fewer high-energy phosphate intermediates than does the oxidation of other citric acid cycle intermediates such as citrate, α-ketoglutarate, or malate.

The oxidation of citrate, malate, and α-ketoglutarate by liver homogenates prepared from emetine-poisoned rats was not significantly different from that of controls, even though a high concentration of emetine was present in this organ. In view of the fact that the concentration of emetine in the heart was considerably lower than that present in other organs, including the liver, kidney, spleen, and lung, it would appear plausible that the heart is more sensitive to this alkaloid than are other tissues. The results obtained in this investigation tend to support such a hypothesis because the oxidation of citrate, malate, or α-ketoglutarate was impaired in the heart of animals chronically poisoned by emetine. This effect could not be demonstrated with liver, however.

A considerable amount of experimental evidence indicates that electron transport in the oxidation of citrate, malate, or α-ketoglutarate involves the nicotinamide adenine nucleotides (22). In all substrates included in this study, with the exception of succinate, the electrons are transferred, for the most part, from the metabolite to the nicotinamide adenine nucleotides. With succinate as the substrate, however, nicotinamide adenine nucleotide is not required because the electrons enter the transport system at the flavin nucleotide step. Table III shows that chronic poisoning by emetine does not apparently affect the synthesis of nicotinamide adenine dinucleotide in the rat heart. Nicotinamide adenine dinucleotide levels were elevated in the rat myocardium after pretreatment with nicotinamide, and the concomitant administration of emetine did not alter this elevation significantly. Results also indicated that chronic emetine poisoning did not alter the levels of nicotinamide adenine dinucleotide in rat heart when compared to values for sham-injected control animals.

Although the chronic poisoning of experimental animals with emetine appears to impair the oxidation of certain citric acid cycle intermediates, it is not possible at this time to account for the toxicity of this alkaloid on the myocardium. It is conceivable, and even likely, that emetine may produce an aberration in many metabolic processes, and therefore, may elicit its effects at various levels in the oxidative process of myocardium.

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