combinations of active and inert principles, were evaluated for ulcerogenic effects in mice.

Phenylephrine hydrochloride was the initiating factor in the development of gastric ulcers.

Sucrose, glycerin, glucose, ethanol, lime, and vanillin potentiated phenylephrine-induced gastric ulcers in mice.

Phenylpropanolamine hydrochloride, similarly studied, produced little or no gastric toxicity.

These observations suggest that the composition of vehicle as well as the active principles may be important in reducing or eliminating the potential gastrointestinal side effects of sympathomimetic amine-containing liquid preparations.

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

Effects on Lipid Metabolism and Oxidative Phosphorylation

By GLENN D. APPELT* and HAROLD C. HEIM

Emetine at a concentration of $3 \times 10^{-3}M$ completely inhibited endogenous respiration of rat heart homogenates as well as the respiration in the presence of added butyrate or β-hydroxybutyrate. At a concentration of 10^{-4} *M*, the alkaloid elicited no effect. The inhibition observed was not specific for emetine, since quinine, at the same concentrations, was equally as effective in inhibiting respiration. Hearts from animals chronically poisoned by emetine, but not by quinine, showed impaired ability to oxidize these substrates. Chronic poisoning by emetine did not impair oxidative phosphorylation, nor did 10^{-3} *M* emetine added to mitochondria prepared from hearts of unpoisoned animals.

ABNORMALITIES in cardiac function following the use of emetine as an amebacide have been reported by numerous investigators. Ventricular fibrillation (1), diminished systolic force (2), and tachycardia (3) have been observed; other studies have shown that emetine may elicit a progressive cloudy swelling and fatty degeneration of the heart (4). Electrocardiographic changes have been induced by the drug in dogs and cats, evidenced by a widening of the initial P-R complex and by a frequently observed inversion of the T wave. These changes suggested a marked disturbance in energy utilization by the myocardium (5). Several studies have been conducted relative to the effects of emetine on cellular metabolism. It has been postulated that emetine may interfere with the enzyme systems which convert glycogen to contractile energy in the heart (6). Electrocardiographic alterations have been induced in the guinea pig by sublethal doses of the alkaloid, and these effects could not be prevented or abolished by the administration of diphosphopyridine nucleotide (7). It has also been shown that emetine evoked no effect on the synthesis of cocarboxylase by rat liver (8). The repeated administration of the alkaloid to young rats resulted in a reduction of food intake and marked inhibition of growth, findings which led to the suggestion that the drug caused nutritional and metabolic changes in certain tissues (10). A rapid depletion of liver glycogen has been reported to occur in young rats after a single administration of 0.1 to 0.2 mg. emetine/Kg. (11). Synthesis of glycogen by livers of emetine-poisoned rats was markedly depressed, and there also was observed a significant decrease in phosphorylase and aldolase activity in livers from the poisoned animals. Vitamin metabolism in emetine-poisoned rats has been studied; it was observed that the emetine-treated animals stored smaller amounts of thiamin and folic acid in the liver than did pair-fed controls. Poisoning by emetine did not alter metabolism of vitamin A, riboflavin, nicotinic acid, or biotin (12). The

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respiration of rat heart homogenates was inhibited by emetine in the presence of added glucose, pyruvate, malate, or fumarate (13). Oxygen uptake in the presence of added succinate, however, was augmented by emetine. Evidence was presented which suggested that the increase in oxygen consumption was due to a suppression of oxaloacetate accumulation, possibly through an inhibitory effect of emetine on malic dehydrogenase (14).

Although the toxic effects of emetine on the myocardium are prominent, it is not yet possible to explain the mechanism responsible for these phenomena. Some evidence exists, however, in support of the concept that the alkaloid may interfere with metabolism by the heart in such a manner as to reduce the efficiency of biochemical processes. To extend and explore this concept in more detail, a series of experiments was conducted in which the effects of emetine on fatty acid metabolism by heart homogenates and upon oxidative phosphorylation by mitochondria were investigated.

EXPERIMENTAL

Male rats of the Sprague-Dawley strain were used as the experimental animals and were young adults weighing approximately 200 Gm. unless otherwise noted. The animals were stunned and exsanguinated, the hearts were immediately removed; the ventricles were dissected away, washed quickly in cold water, blotted dry, and weighed. The ventricles were 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. fresh ventricle per milliliter. The homogenates were pipeted into chilled Warburg vessels containing substrates and cofactors listed in the tables and figures under *Results*. After a 10-minute equilibration period, oxygen consumption was measured for 90 minutes according to conventional manometric techniques (15).

To determine the effect of chronic poisoning by emetine on the oxidation of the substrates used in this study, young animals weighing approximately 100 Gm. were separated into three groups of eight animals each and given intraperitoneal injections daily for 17 days as follows: Group I, 0.2 mg. emetine hydrochloride in 0.7 ml. water; Group II, 25 mg. quinine hydrochloride in 0.7 ml. water; and Group III, 0.7 ml. water. The animals were provided with food and water *ad libitum;* on the seventeenth day, they were sacrificed, the hearts removed, the ventricles homogenized as outlined above, and the oxygen consumption of the homogenates determined. Blood samples were collected at the time the animals were sacrificed and glucose levels were determined according to the method of Somogyi (16).

All results represent the average of data obtained with at least eight animals, and each determination of oxygen consumption 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 verify that the observed effects were not caused by a change in pH during the experiment. Unless otherwise noted, the results are significant at the 99% probability level ($p < 0.01$) determined by the Fisher t test.

The effect of emetine on oxidative phosphorylation by heart mitochondria was determined by the method of Hunter (17). The mitochondria were isolated according to methods used by Plaut (18), and centrifugal separation was accomplished with a Servall RC-2 refrigerated centrifuge equipped with an SS-34 rotor and at 0°. Mitochondria representing 500 mg. fresh myocardium were added to each Warburg vessel, and the inorganic phosphate was determined by the method of Fiske and SubbaRow (19) with the Beckman DU spectrophotometer at 660 mμ.

RESULTS AND DISCUSSION

Figures 1–3 present data which show that the endogenous respiration of homogenates and the

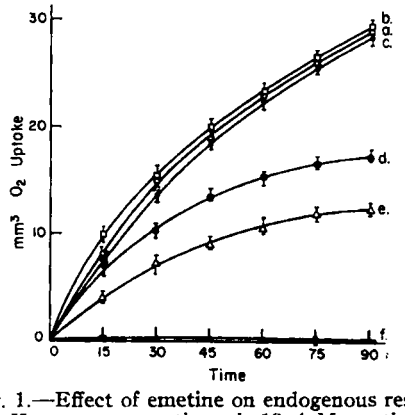

Fig. 1.—Effect of emetine on endogenous respiration. Key: *a*, no emetine; *b*, 10^{-4} *M* emetine; *c*, 2×10^{-4} *M* emetine; *d*, 5×10^{-4} *M* emetine; *e*, 10^{-3} *M* emetine; *f*, 3×10^{-3} *M* emetine. Flasks contained 0.5 ml. homogenate; 0.1×10^{-4} *M* cytochrome C; 0.1 ml. 2×10^{-2} *M* malate; 0.1 ml. 2×10^{-2} *M* ATP; 0.1 ml. 1.5×10^{-2} *M* nicotinamide; 0.1 ml. 10^{-3} *M* $MgCl_2$; 0.3 ml. H_2O; 1.0 ml. 0.1 *M* phosphate buffer, pH 7.4; 0.3 ml. emetine to yield concentrations listed; H_2O to make 3.0 ml. Center wells contained 0.2 ml. 10% KOH. Gas phase, air. Temperature, 30°C.

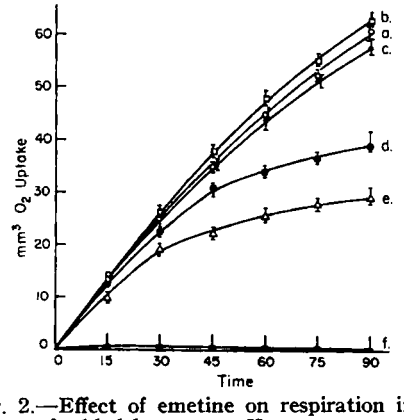

Fig. 2.—Effect of emetine on respiration in the presence of added butyrate. Key: *a*, no emetine; *b*, 10^{-4} *M* emetine; *c*, 2×10^{-4} *M* emetine; *d*, 5×10^{-4} *M* emetine; *e*, 10^{-3} *M* emetine; *f*, 3×10^{-3} *M* emetine. Experimental conditions and flask contents are identical to those in Fig. 1, except that 0.3 ml. 0.1 *M* butyrate was added instead of 0.3 ml. H_2O.

respiration in the presence of added butyrate or β-hydroxybutyrate were inhibited by emetine. The inhibition was complete at concentrations above 3×10^{-3} *M*, while at 1×10^{-4} *M* or 2×10^{-4} *M*, no significant inhibition was observed. At concentrations of 5×10^{-4} *M* and 1×10^{-3} *M* the inhibition was quite marked, being of the order of 40 to 60%. Concentrations of these magnitudes are high so as to be unrealistic when compared to the emetine found in the heart after administration of the drug to experimental animals (20), and the question arises as to whether the observed effects are specific for emetine. Figure 4 shows that quinine, at concentrations identical to those of emetine, inhibited the respiration of

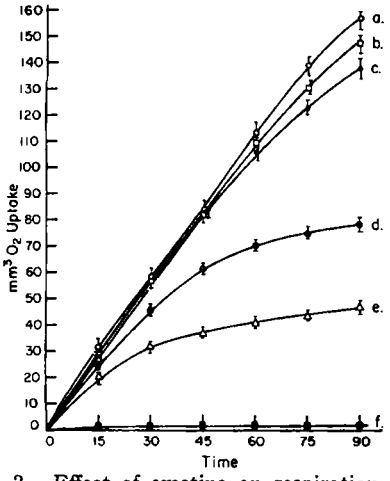

Fig. 3.—Effect of emetine on respiration in the presence of added β-hydroxybutyrate. Key: *a*, no emetine; *b*, 10^{-4} *M* emetine; *c*, 2×10^{-4} *M* emetine; *d*, 5×10^{-4} *M* emetine; *e*, 10^{-3} *M* emetine; *f*, 3×10^{-3} *M* emetine. Experimental conditions and flask contents are identical to those in Fig. 1, except that 0.3 ml. 0.1 *M* β-hydroxybutyrate was added instead of 0.3 ml. H_2O.

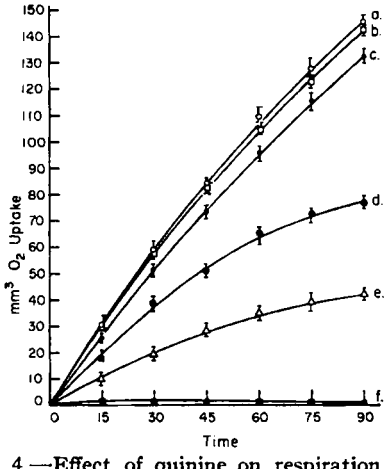

Fig. 4.—Effect of quinine on respiration in the presence of added β-hydroxybutyrate. Key: *a*, no quinine; *b*, 10^{-4} *M* quinine; *c*, 2×10^{-4} *M* quinine; *d*, 5×10^{-4} *M* quinine; *e*, 10^{-3} *M* quinine; *f*, 3×10^{-3} *M* quinine. Experimental conditions and flask contents are identical to those in Fig. 3, except that 0.3 ml. quinine hydrochloride solution, pH 7.4, was added instead of emetine to yield concentrations listed.

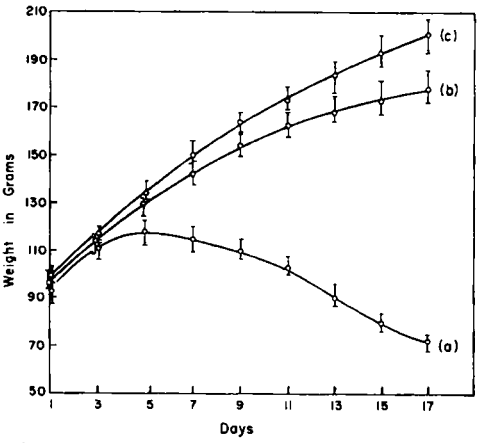

Fig. 5.—Effect of daily administration of emetine and quinine on weight gain of young rats. Key: *a*, emetine; *b*, quinine; *c*, sham-injected controls.

TABLE I.—EFFECT OF PROLONGED ADMINISTRATION OF EMETINE OR QUININE ON OXYGEN UPTAKE BY HEART HOMOGENATES[a]

Substrate	μl. O_2 Uptake/90 Min.	Variation from Controls, %
Sham-Injected Controls		
Butyrate	54 ± 4	...
β-Hydroxybutyrate	147 ± 9	...
None	29 ± 3	...
Emetine Treated		
Butyrate	35 ± 3	−35
β-Hydroxybutyrate	93 ± 6	−37
None	19 ± 3	−35
Quinine Treated		
Butyrate	61 ± 6	+11[b]
β-Hydroxybutyrate	138 ± 12	−10[b]
None	28 ± 3	+4[b]

[a] Water added to flasks in place of emetine or quinine, but other experimental conditions identical to those listed with Fig. 1. [b] Not significantly different from values obtained with sham-injected control animals.

homogenates in the presence of added β-hydroxybutyrate. Endogenous respiration, as well as that in the presence of added butyrate, was inhibited by quinine in the same concentrations; the magnitude of the inhibition was practically identical to that observed with emetine. Such observations suggest that the inhibition of respiration by high concentrations of emetine may be nonspecific and, therefore, of little value in explaining the cardiotoxic action of the drug. It would appear to be of greater importance to study the respiration of heart preparations made from animals chronically poisoned by emetine and quinine. Figure 5 shows that doses of 0.2 mg. of emetine daily caused a marked deterioration of the experimental animals evidenced by a pronounced weight loss. Animals given daily injections of 25 mg. of quinine gained weight almost as rapidly as did the sham-injected controls and, at the end of the experimental period, did not show the marked deterioration observed with the emetine group. Blood glucose levels of the emetine-treated animals were not significantly different from those of the control group or the quinine-treated group. Similar observations have been made by other in-

TABLE II.—EFFECT OF ADDED EMETINE AND OF CHRONIC EMETINE POISONING ON OXIDATIVE PHOSPHORYLATION BY HEART MITOCHONDRIA IN THE PRESENCE OF α-KETOGLUTARATE[a]

	Microatoms O Consumed	μm. P Consumed	P:O Ratio
Sham-injected animals			
No emetine added to flasks	5.8 ± 0.4	21.8 ± 3.1	3.8 ± 0.4
Sham-injected animals			
Flasks contained 10^{-3} *M* emetine	4.1 ± 0.5	15.7 ± 1.9	3.8 ± 0.3[b]
Emetine-poisoned animals	5.3 ± 0.4	19.0 ± 2.2	3.6 ± 0.3[b]

[a] Flasks contained in the main compartments 0.4 ml. 0.1 *M* potassium phosphate buffer, pH 7.4; 0.1 ml. 0.3 *M* $MgSO_4$; 0.1 ml. 4×10^{-4} *M* cytochrome c; 0.1 ml. 5×10^{-2} *M* adenosine triphosphate; 0.2 ml. 0.1 *M* α-ketoglutarate; 0.1 ml. 0.3 *M* potassium malonate; 0.8 ml. 0.25 *M* sucrose; 0.3 ml. H_2O (or emetine to yield molarity listed); and 0.4 ml. mitochondrial suspension, representing 500 mg. fresh ventricle. Sidearms contained 0.1 ml. 0.8 *M* glucose and 0.1 ml. purified hexokinase preparation (Sigma Chemical Co., St. Louis, Mo.) representing 600 units. Center wells contained 0.2 ml. 10% KOH together with a pleated strip of filter paper. Temperature, 30°; gas phase, air. Control flasks removed from bath at end of 5-minute equilibration period and reaction stopped by adding 0.5 ml. 30% trichloroacetic acid. Oxygen uptake measured in remaining flasks for 50 minutes, after which time the reaction was stopped with trichloroacetic acid and the inorganic phosphate determined. [b] Not significantly different from values obtained with sham-injected animals. Theoretical P:O ratio = 4.0 (8).

vestigators, who found that chronic poisoning by emetine did not evoke a change in blood glucose concentration (13). However, the intestinal tracts of the quinine-treated animals showed marked edema and the livers were discolored. Doses of quinine above 25 mg. per day caused death in an appreciable number of the animals and, for this reason, 25 mg. per day was chosen as the amount to be administered.

Table I shows that the oxygen consumption of heart homogenates prepared from emetine-poisoned animals is significantly lower than that observed either with the sham-injected controls or the quinine-treated animals. The heart has been reported to oxidize several substrates as energy sources among which are included pyruvate, glucose, lactate, and fatty acids (21). It has been demonstrated further that, under fasting conditions, the heart derives energy almost exclusively from the oxidation of fatty acids (22, 23). The rats poisoned by daily administration of emetine lost weight rapidly and resembled, in this respect, animals subjected to severe starvation. Furthermore, these animals consumed much less food than did the sham-injected or the quinine-treated animals. The hearts from the emetine-poisoned animals did not oxidize butyrate or β-hydroxybutyrate as efficiently as did the hearts from the unpoisoned animals or those given large daily doses of quinine. Such observations indicate that the effect of emetine on the heart is different from that which would be expected in animals subjected to severe starvation.

The efficiency of cellular oxidative processes is related not only to the actual utilization of oxygen but also to the concomitant production of high-energy phosphate, *i.e.*, oxidation is coupled to phosphorylation. Previous studies have shown that emetine enhances the oxidation of succinate by heart homogenates, an observation similar to that made by other investigators (24), who studied the effect of thyroxin on succinate oxidation. Thyroxin, under certain conditions, has been shown to uncouple oxidation from phosphorylation (25), but the results obtained in this study and set forth in Table II reveal that emetine evokes no significant effect on the P:O ratio when added to heart mitochondria respiring in the presence of α-ketoglutarate. Furthermore, oxidative phosphorylation by mitochondria prepared from the hearts of animals chronically poisoned by emetine was not significantly different from that observed with mitochondria from the sham-injected animals.

Although the chronic poisoning of experimental animals with emetine appears to impair the ability of hearts from these animals to oxidize fatty acids *in vitro*, such an effect does not necessarily account for the cardiotoxic action of this alkaloid. The oxidation of fatty acids ultimately involves the processes of the citric acid cycle, and it appears conceivable that emetine may elicit effects at various points on this "main line" of oxidation. Furthermore, the fact that the endogenous respiration of homogenates prepared from the chronically poisoned animals is lower than that observed with homogenates prepared from the control group indicates that the depression of oxidative processes is not limited to those involving butyrate or β-hydroxybutyrate. Indeed, preliminary experimentation indicates that the inhibitory effects of emetine *in vitro* can be reversed by the addition of nicotinamide adenine nucleotide coenzymes. Additional work in this area is now in progress and will be the subject of a subsequent publication.

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