Effect of Chronic Poisoning by Emetine on Oxidative Process in Rat Heart III. Effects on Oxidation of Pyruvate and Lactate

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The effect of chronic emetine poisoning on the oxidation of pyruvate and lactate by rat heart and liver homogenates was investigated. Oxygen consumption by heart homogenates was lower in the emetine-poisoned animals when compared to sham-injected controls. No significant difference was noted in liver homogenates. Heart slices from emetine-poisoned rats consumed less oxygen than slices from sham-injected controls. When diaphragm sections were employed, this difference was not noted.

MUCH EVIDENCE has been accumulated to show that the myocardium utilizes, as a source of energy, a variety of substrates including glucose, lactate, pyruvate, and fatty acids. In earlier communications, data were presented which described an inhibitory effect elicited by chronic emetine poisoning on the oxidation of fatty acids (1), and of certain citric acid intermediates (2) by rat heart homogenates. These inhibitory effects were not noted in the oxidation of these substrates by rat liver homogenates from emetine-poisoned animals. The heart contains a small amount of emetine after intraperitoneal injection, although a relatively high concentration of the drug is found in the liver (3). Cardiotoxicity, however, has been observed with emetine (4–6), and this effect may be related to a rather specific aberration of biochemical processes in the myocardium.

Since pyruvate and lactate may be utilized by the myocardium, and apparently represent substrates for the heart (7–9), it appeared of interest to study the effect of chronic emetine poisoning on the oxidation of these compounds by rat heart and liver homogenates. In addition, oxygen consumption by heart slices and diaphragm sections from emetine-poisoned rats in the presence of pyruvate was measured.

EXPERIMENTAL

To study the effects of chronic poisoning by emetine on the oxidation of pyruvate and lactate by heart and liver homogenates, young adult Sprague-Dawley rats of both sexes were employed as test animals. The animals weighed approximately 150 g. at the beginning of the experiment. The animals 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. Food and water were allowed *ad libitum*. After 14 days, the animals were decapitated, 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. The homogenates were pipeted into chilled Warburg vessels containing substrates and cofactors listed below.

When diaphragm sections were employed, the animals were treated for 14 days in the same manner as above, sacrificed, and the diaphragm removed rapidly and cut into segments of approximately 100 mg. each. Ventricle slices were prepared using the tissue slicer described by Stadie and Riggs (10). The individual slices or sections were quickly transferred to a torsion balance, the exact weight determined, and one slice or section placed in each iced Warburg vessel containing the reaction medium.

The main compartment of each flask contained 0.5 ml. of homogenate or a tissue slice, 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* pyruvate or lactate; 1.2 ml. of 0.1 *M* potassium phosphate buffer, pH 7.4, in flasks which contained homogenates, or 1.7 ml. in flasks which contained a diaphragm section or heart slice; and 0.3 ml. of distilled water. 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 according to conventional manometric techniques (11).

Oxygen uptake readings were taken at 15-min. intervals for 90 min. All values represent the average of data obtained with at least six animals, and each determination was performed in duplicate. The vertical line through each point designates the standard error of the mean for each set of values.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate the effect of chronic poisoning by emetine on the oxygen uptake by rat heart homogenates in the presence of pyruvate and lactate, respectively. The total oxygen consumption at the end of 90 min. was significantly lower ($p < 0.001$) in the preparations from emetine-poisoned rats when compared to values obtained from sham-

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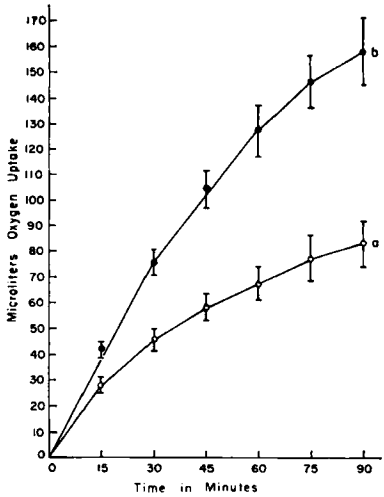

Fig 1—Oxidation of pyruvate by heart homogenates from sham-injected and emetine-treated rats. Key: Curve a, emetine-treated animals; Curve b, sham-injected animals.

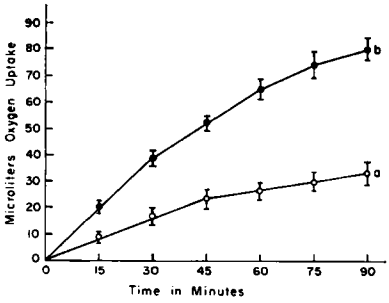

Fig. 2—Oxidation of lactate by heart homogenates from sham-injected and emetine-treated rats. Key: Curve a, emetine-treated animals, Curve b, sham-injected animals.

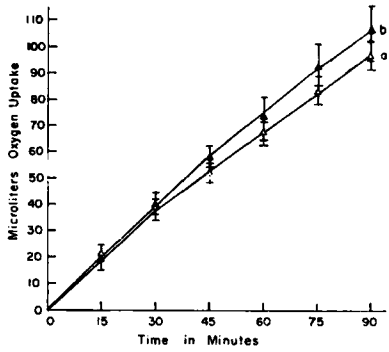

Fig. 3—Oxidation of pyruvate by liver homogenates from sham-injected and emetine-treated rats. Key: Curve a, emetine-injected animals; Curve b, sham-injected animals.

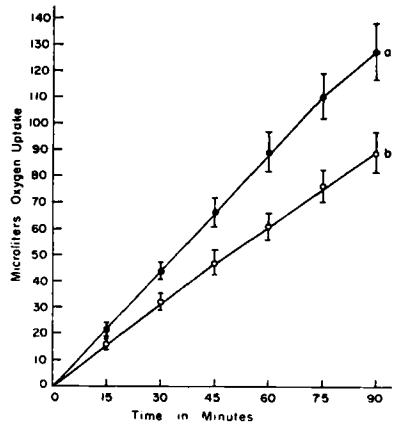

Fig. 4—Oxidation of lactate by liver homogenates from sham-injected and emetine-treated rats. Key: Curve a, sham-injected animals; Curve b, emetine-treated animals.

injected animals. This inhibitory effect represents a 48% decrease for pyruvate and a 59% reduction when lactate was the substrate.

Figures 3 and 4 depict oxygen consumption by rat liver homogenates when pyruvate and lactate were utilized as substrates. The 90-min. *r* readings from emetine-poisoned animals in this instance, however, were not significantly lower than the values from the sham-injected animals.

These data indicate that heart homogenates from emetine-poisoned rats were less efficient in the oxidation of these substrates than homogenates from control animals. Such a difference, however, was not noted in liver homogenates from emetine-poisoned rats.

Figure 5 shows a comparison of the respiration by diaphragm sections from emetine-poisoned rats with that of the sham-injected controls. Pyruvate was the substrate and, under the conditions of the experiment, no significant difference was observed between the test groups. Figure 6 illustrates that the total oxygen consumption of ventricle slices from emetine-poisoned rats was about 40% lower than that obtained with ventricle slices from the sham-injected controls. These results extend the idea that this inhibitory effect may be fairly specific for the myocardium, by including a muscle preparation for comparison.

It has been reported that pyruvate and lactate are utilized less efficiently in certain thiamine-deficient situations (12–14). Thiamine pyrophosphate is necessary in the biotransformation of pyruvate to acetyl-coenzyme A which represents a pathway whereby these substrates may be metabolized *via* the citric acid cycle. Diamant and associates (15) have reported that emetine-treated rats stored smaller amounts of thiamine in the liver than did control animals. It is conceivable that a similar situation exists in cardiac muscle resulting in a diminished utilization of pyruvate and lactate.

Nicotinamide adenine dinucleotide (NAD) functions as a coenzyme in the lactic dehydrogenase system, as well as a cofactor in the conversion of pyruvate to acetyl-coenzyme A. Deitrich and Heim (16) have noted that emetine decreased the oxygen uptake of rat heart homogenates in the presence of pyruvate and malate, however, an enhancement of the oxidation of succinate, an FAD-linked sub-

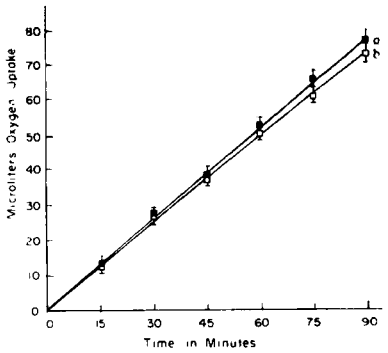

Fig. 5—Oxidation of pyruvate by diaphragm slices rom sham-injected and emetine-treated rats. Key: Curve a, sham-injected animals; Curve b, emetine-treated animals.

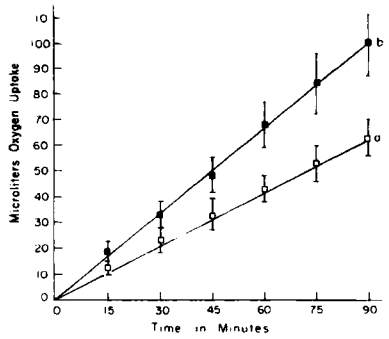

Fig. 6—Oxidation of pyruvate by heart slices from sham-injected and emetine-treated rats. Key: Curve a, emetine-treated animals; Curve b, sham-injected animals.

strate, was observed. A similar inhibitory activity of the oxidation of NAD-linked substrates in emetine-poisoned rats has previously been reported, although no alteration in NAD metabolism was noted (2).

At least one investigator (17) has suggested that emetine is most toxic to those tissues which involve contractility as the principal function. One might expect, therefore, that metabolic processes in tissue such as diaphragm would be altered by emetine. However, it was found that pyruvate was oxidized as rapidly by diaphragm sections from the emetine-poisoned animals as from the sham-injected animals. With heart slices it was found that chronic poisoning by emetine elicited an impaired ability to oxidize pyruvate. Such evidence tends to indicate that the effect of emetine, as far as oxidative processes are concerned, is not common only to those tissues in which contractility is the principal function, and further supports the view that the toxic effect of emetine is rather specific to the heart.

SUMMARY

1. The effects of chronic poisoning by emetine on the oxidation of pyruvate and lactate by various tissue preparations was studied.

2. Oxygen consumption by heart homogenates prepared from emetine-poisoned rats was lower than that observed with homogenates prepared from sham-injected controls.

3. The oxygen uptake by liver homogenates prepared from emetine-poisoned rats did not differ significantly from that observed with homogenates prepared from sham-injected controls with pyruvate and lactate as substrates.

4. Heart slices prepared from emetine-poisoned rats consumed less oxygen in the presence of pyruvate than did slices from the sham-injected controls. With diaphragm sections this difference was not observed.

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Keyphrases

Emetine poisoning, chronic—effects
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Pyruvate, lactate oxidation—emetine poisoning effect