On the metabolism of ascorbic acid in emetine-treated rats

Emetine reduces the rate of urinary ascorbic acid elimination in rats, the livers of which contained less L-ascorbic acid (Diamant, Halevy & Guggenheim, 1955) as a result of a reduction in L-ascorbic acid synthesis (Chatterjee, Datta & Ghosh, 1972). We have examined the effect of emetine on the oxidation by liver homogenates of L-gulonolactone to L-ascorbic acid, and the conversion of D-glucuronolactone to its free acid and of dehydro-L-ascorbic acid to 2,3-diketogulonic acid.

Male Wistar rats, 90–110 g, were divided into two groups of equal average body weight. One group was injected subcutaneously with emetine hydrochloride (0·2 mg day^{-1} for each 100 g wt) for 10 days. The other group served as pair-fed controls. The animals were maintained on a 18% casein diet, details of which were reported by Chatterjee & others (1972). The rats were killed under light ether anaesthesia 24 h after the tenth dose. Blood was collected from the hepatic vein and serum was separated by centrifugation. The livers were chilled in ice.

The L-gulonooxidase activity was assayed in a test system containing sodium phosphate buffer, pH 7·4 (20 mM), L-gulonolactone (5 mM), sodium pyrophosphate (50 mM), KCN (1 mM) and liver homogenate (in 0·25M sucrose) equivalent to 100 mg fresh tissue (for details see Chatterjee & others, 1972). The uronolactonase activity of liver homogenate (in 0·15M KCl) was determined by the method of Eisenberg & Field (1956), the dehydroascorbatase activity of liver tissues (homogenized in 0·15M KCl) was according to Kagawa, Takiguchi & Shimazono (1961) as described by Mukherjee, Kar & others (1968) and the protein content of liver homogenate according to Gornall, Bardawill & David (1949). Urine samples were collected for 24 h after the 2nd, 5th and last injections of emetine and their ascorbic acid contents were determined by the method of Roe & Kuether (1943).

The results show that emetine treatment for 10 days diminished both the *in vitro* L-ascorbic acid synthesizing ability and the dehydroascorbatase activity of the liver tissues of rats and these reductions were significant when the results were expressed on both a protein weight and a tissue weight basis (Table 1). Emetine treatment did

Table 1. *Effect of emetine 0·2 mg day^{-1} per 100 g weight for 10 days on the liver enzyme activities.* (The values are means ± s.e.)

Groups of animals	L-Gulonooxidase L-ascorbic acid synthesized		Uronolactonase D-glucuronolactone hydrolysed		Dehydroascorbatase 2,3-diketogulonic acid formed	
	μmol g^{-1} protein	μmol g^{-1} tissue	μmol g^{-1} protein	μmol g^{-1} tissue	μmol g^{-1} protein	μmol g^{-1} tissue
Pair-fed control	43·56±1·72 (6)	9·36±0·26 (6)	577·6±15·1 (8)	121·0±2·6 (8)	138·8±2·4 (8)	29·13±0·51 (8)
Emetine-treated	33·88±2·04 (5) P <0·01	6·17±0·34 (5) P <0·001	562·1±13·5 (8) P >0·05	99·51±2·74 (8) P <0·001	120·1±5·5 (8) P <0·01	21·71±0·90 (8) P <0·001

The figures in parentheses indicate the number of animals

not change the uronolactonase activity when this was expressed on a protein weight basis but caused a significant change when the results were expressed on a tissue weight basis. Urinary ascorbic acid elimination was significantly reduced only during the 24 h following the last emetine injection (emetine-treated group 0·94 ± 0·17 (s.e.); pair-fed controls 1·74 ± 0·19 μmol day^{-1} for every 100 g wt, P <0·02).

The reduction in the synthesis of L-ascorbic acid from D-glucuronolactone after emetine treatment was reported earlier (Chatterjee & others, 1972). The present studies also demonstrate a reduction in the synthesis of L-ascorbic acid from L-

gulonolactone. Impairment of liver protein synthesis was reported by a number of workers (Grollman, 1966; Beller, 1968; Pestka, 1971). The reduction in the activity of drug-metabolizing microsomal enzymes (Jondorf & Johnson, 1969; Miller, Johnson & others, 1970) and transaminases (Chatterjee & Ray, 1973) following emetine treatment was ascribed to the depressed synthesis of respective enzyme protein. The diminution in the activity of L-gulonooxidase after emetine treatment may be likewise attributed to the reduced synthesis of the enzyme protein. The significant change in liver uronolactonase activity on a tissue weight basis suggests that the synthesis of the apoprotein of uronolactonase is not affected as much as that of L-gulonooxidase. The depressed liver uronolactonase activity consequent upon emetine treatment excludes the possibility of the biosynthesis of liver ascorbic acid having been restricted by limiting the substrate concentration. The reduction in the dehydroascorbatase activity of liver on both a protein and a tissue weight basis indicates that the synthesis of the apoprotein of this enzyme is affected to a greater extent than is the synthesis of the apoprotein of uronolactonase. Thus all the enzymes involved in the metabolism of ascorbic acid are not affected to the same degree by emetine treatment. This favours the suggestion of a differential effect of emetine (Chatterjee & Ray, 1973).

Emetine treatment resulted in the reduction of urinary excretion of ascorbic acid only in the later part of the experimental period. Diamant & others (1955) and Kale, Joglekar & Balwani (1966) also noted similar diminished ascorbic acid excretion in the urine after emetine treatment. The reduced urinary elimination might have resulted from the lowered ascorbic acid level of serum in emetine-treated rats.

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