Short communications

Effects of emetine on the metabolism of ascorbic acid

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Treatment with emetine lowered the ascorbic acid concentrations of serum, liver and kidney, while the ascorbic acid concentration of adrenal tissue remained unaffected. The ability of the liver to synthesize (−)-ascorbic acid from (+)-glucuronolactone was also reduced after emetine treatment. The reduced concentration of ascorbic acid in liver and serum after emetine treatment may result from the diminished synthesis of ascorbic acid by the liver.

Increased awareness of the toxic properties of emetine has limited its wide application as a therapeutic agent against amoebic infections. Emetine is especially toxic to the heart, causing death by myocarditis (Brem & Konwaller, 1955). Cardiovascular disfunction (Klatskin & Friedman, 1948), abnormalities in electrocardiogram (Brem & Konwaller, 1955) and myocardial degeneration or necrosis (Turner, 1963) were observed. Emetine also inhibits protein synthesis in mammalian cells, plants and yeast (Grollman, 1966) and Beller (1968) found that emetine treatment for only 3 days inhibited the incorporation of tritiated leucine into both soluble proteins and actomyosin of the cardiac muscle. An inhibitory effect of emetine on the Krebs cycle and on glycolysis in the cardiac muscle was demonstrated by Brink, Kotze, Muller & Lochner (1969).

Diamant, Halevy & Guggenheim (1955) studied the metabolism of certain vitamins and the ascorbic acid content of different organs. As ascorbic acid plays a role in the development of the resistance of the body to drug toxicity, a study of ascorbic acid metabolism in rats after emetine treatment was carried out in emetine treated rats.

Materials and Methods.—Male albino rats (90-110 g) were divided into two groups, A and B. They were kept for 10 days on the following diet: protein (casein) 18%, carbohydrate (arrowroot starch) 71%, fat (groundnut oil) 7%, and salt mixture (Chatterjee, Roy, Dutta & Ghosh, 1970) 4%. Fat-soluble vitamins were supplied in the diet. Water-soluble vitamins were given daily to each rat by subcutaneous injection: thiamine hydrochloride, 12·5 μg; nicotinic acid, 20 μg; pyridoxine hydrochloride, 10 μg; riboflavine, 25 μg; calcium pantothenate, 100 μg; biotin, 2 μg; folic acid, 2 μg; and vitamin B_{12}, 0·01 μg. During this period one group of animals (B) was injected with emetine hydrochloride ((0·2 mg/100 g body weight)/day s.c.). The animals in group B were pair-fed with those of a control-group A. After 10 days the rats were killed, blood was collected and the serum separated. The liver, kidneys and adrenals were removed, chilled in ice and weighed.

Estimation of total ascorbic acid. Part of each tissue was weighed and ground in an all glass mortar with a small amount of sea-sand and cold 5% (w/v) metaphosphoric acid. The extract was made up to a known volume and filtered. The filtrate was treated with liquid bromine to convert ascorbic acid to dehydro-ascorbic acid. The excess bromine was removed by aeration. Serum was similarly treated with liquid bromine following deproteinization with 6% trichloroacetic acid. The total ascorbic acid contents of the protein-free filtrates of tissues and serum were determined by the dinitrophenylhydrazine method of Roe & Kuether (1943).

Biosynthesis of (−)-ascorbic acid. A portion of the liver was weighed and then homogenized with 4 volumes of cold 0·25 M sucrose solution. The total volume of the incubation mixture was 2·5 ml containing sodium phosphate buffer, pH 7·4 (20 mM), (+)-glucuronolactone (10 mM), liver homogenate (0·5 ml equivalent to 100 mg fresh liver) and potassium cyanide (50 mM). The mixture was incubated at 37° C for 60 min in a Dubnoff's shaker-bath. The reaction was stopped by the addition of 0·5 ml of 30% (w/v) cold metaphosphoric acid. The amount of (−)-ascorbic acid synthesized was determined by titrating against 2,6-dichlorophenolendophenol solution (Chatterjee, Ghosh, Ghosh & Guha, 1958).

TABLE 1. *Effect of emetine on the ascorbic acid concentration of serum and different tissues, and on the biosynthesis of ascorbic acid by the liver tissue*

	Ascorbic acid content								
	Serum	Liver		Kidney		Adrenal			μmol ascorbic acid synthesized/g liver protein
Groups of animals	mg/100 ml	μg/100 mg*	Total (mg/100 g) body weight	μg/100 mg*	Total (μg/100 g) body weight	μg/100 mg*	Total (μg/100 g) body weight	
Group A (Pair-fed control)	1·75 (5) ±0·14	28·80 (6) ±1·95	1·04 (6) ±0·148	20·50 (6) ±1·34	167·4 (6) ±9·5	293·2 (6) ±27·4	72·3 (6) ±8·2		23·44 (5) ±0·83
Group B (Emetine treated)	1·17 (4) ±0·17	17·13 (6) ±2·15	0·646 (6) ±0·107	15·72 (6) ±1·26	149·9 (6) ±11·3	280·5 (5) ±20·5	114·0 (5) ±6·4		16·85 (7) ±0·96
	t=2·634	t=4·021	t=2·136	t=2·606	t=1·179	t=0·371	t=4·01		t=5·193

The values are means±S.E.M. The figures in parentheses indicate the number of animals. *Wet weight.

Determination of tissue protein. The protein content of liver homogenate used in the study of the biosynthesis of (−)-ascorbic acid was determined by the biuret method (Gornall, Bardawill & David, 1949).

Results.—The average body weight of the emetine-treated rats was 94·8 g ± 1·7 (S.E.M.) compared to 96·8 g ± 1·6 of pair-fed control rats. But emetine treatment increased the weight of the different organs. For example, the weights (per 100 g body weight) of the liver, kidney and adrenal were 3·95 g ± 0·17 (S.E.M.), 0·961 g ± 0·047 and 40·92 mg ± 1·56, respectively, in emetine treated rats, whereas the corresponding organ weights in pair-fed control rats were 3·27 g ± 0·14, 0·829 g ± 0·029 and 25·25 mg ± 2·91. These observations are in agreement with the results reported earlier (Chatterjee *et al.*, 1970).

The total ascorbic acid contents of different tissues and of blood serum in control and emetine treated rats are shown in Table 1. The ascorbic acid content of liver and kidney was decreased whereas the total amount of the ascorbic acid in the respective tissues (mg or μg/100 g body weight) showed no change upon receiving emetine. The adrenal ascorbic acid concentrations (μg/100 mg) of the emetine treated rats were not changed, but the total amount of the ascorbic acid in the gland (μg/100 g body weight) showed a marked increase after emetine treatment.

Table 1 includes also the amount of (−)-ascorbic acid synthesized *in vitro* from (+)-glucuronolactone by the liver tissue of control and emetine treated rats. The capability of the liver of emetine treated rats to synthesize (−)-ascorbic acid was reduced considerably.

Discussion.—Emetine treatment of rats reduced the total ascorbic acid concentrations in liver, kidney and serum, but not in the adrenal glands. When expressed in μg/100 g body weight the adrenal ascorbic acid content was in fact increased. Diamant *et al.* (1955) observed a decrease in the content of free ascorbic acid in the liver of rats after emetine treatment. The reduced ascorbic acid concentration of the liver of emetine treated rats was accompanied by a diminished ability of the liver to synthesize (−)-ascorbic acid from (+)-glucuronolactone. The reduced serum ascorbic acid concentration in the emetine treated rats (Table 1) may be a result of the diminished synthesis of (−)-ascorbic acid by the liver tissue. Emetine treated rats also excrete less ascorbic acid in their urine (Diamant *et al.*, 1955). In emetine treated rats the liver protein concentration was decreased (Chatterjee *et al.*, 1970) as was the amount of labelled amino-acids incorporated into liver proteins (Jondorf & Szapary, 1968). This suggests impairment in the liver protein synthesis after emetine treatment. Thus the reduced ability of liver to synthesize ascorbic acid after emetine treatment may be ascribed to a decreased synthesis of enzymes involved in ascorbic acid biosynthesis.

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