Effect of Emetine on Certain Aspects of Protein Metabolism

Emetine has long been well appreciated as a therapeutic agent in the treatment of amoebic infections, but its recent use has been curtailed by increased awareness of its toxicity. The most serious manifestations of emetine-induced toxicity have been cardiac, and death due to myocarditis[1]. The toxic effects of this alkaloid are mainly concerned with the cardiovascular function[2], electrocardiographic abnormalities[1] and myocardial degeneration or necrosis[3-5]. Further, GROLLMAN[6] has shown that emetine is a potent inhibitor of protein synthesis in mammalian cells, plants and yeast. BELLER[7] demonstrated that incorporation of tritiated leucine into soluble proteins and actomyosin of myocardium is inhibited following treatment of animals with emetine for 3 days. Recent studies have indicated that emetine is an inhibitor of myocardial Krebs cycle activity and glycolysis, resulting in a significant depression in myocardial contractility[8]. The present studies are undertaken to find the effect on certain aspects of protein metabolism of emetine treatment for a greater period of time.

Male albino rats weighing 80–100 g were divided into groups A and B of equal average body weight. The carbohydrate used in the diet was a mixture of equal parts of arrowroot starch and sucrose. The percentage composition of the diet was: casein 18, carbohydrate 71, groundnut oil 7, and salt mixture 4[9]. Fat-soluble vitamins were supplied in the diet. Water-soluble vitamins were given daily to each rat by s.c. injection.

The rats of groups A and B were supplied with the 18% protein diet for 10 days. During these days animals of group B were injected s.c. with emetine hydrochloride at a dose of 0.2 mg/100 g body wt./day. This dose represents 11.8% of lethal dose (LD_{50}) of emetine hydrochloride which was reported to be 17 mg/kg body wt. for rat[10]. The animals group B were pair-fed with those of group A. After the experimental period was over, the animals were sacrificed. Their liver, heart and kidney tissues were excized, chilled in ice and weighed. A 10% homogenate of each of the tissues was made in ice-cold deionized water. RNA and DNA were isolated from the tissue homogenates by the modified Schmidt-Thannhauser method as recommended by MUNRO[11]. RNA and DNA contents of the fractions were determined by the orcinol[12] and diphenylamine reaction[13]. The protein contents of the tissue homogenates were determined by Biuret method[14].

Table I demonstrates the weight of the animals and different organs examined following emetine treatment. Emetine treatment for a period of 10 days caused liver and kidney enlargement. The results in Table II reveal that emetine treatment for the same period reduced the protein concentration of liver and kidney tissues, while RNA concentration of liver and kidney remained unaffected and consequently protein/RNA ratio was lowered. GROLLMAN[6] has shown that emetine is a potent inhibitor of protein synthesis in mammalian cells, plants and yeast. The incorporation of labelled amino acid into rat liver protein in vivo was also found to be inhibited by pretreatment of rats with emetine[15]. BELLER[7] studied the effect of emetine on protein synthesis in rat myocardium. Emetine was found to have both in vitro and in vivo inhibitory effect on the incorporation of tritiated leucine into soluble protein and actomyosin. But in the present study emetine treatment for 10 days does not seem to have an appreciable effect on protein and RNA concentrations of heart tissue and consequently protein/RNA ratio remained unaffected. The table further shows that emetine treatment causes unaltered protein/DNA ratio in each of the tissues studied. Assuming DNA per cell constant, the protein/DNA ratio is considered to be a measure of cellular protein concentration. So unaltered protein/DNA ratio in different tissues following emetine treatment suggests unimpairment in the cellular protein concentration of the tissues studied. The lowered protein content of the liver or kidney on wet weight basis upon emetine treatment may be ascribed to the enlargement of liver or kidney cell size resulting in reduced number of cells per unit weight of the tissues. The diminished DNA content per 100 g of liver or kidney tissue of emetine-treated rats is also suggestive of increased liver or kidney

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Table I. Effect of emetine on body and organ weight

Group	Body weight (g) At the beginning of treatment	At the end of treatment	Organ weight (g/100 g body weight) Liver	Heart	Kidney
Group A Pair-fed control (6)	106.5 ± 5.04	96.8 ± 4.17	3.36 ± 0.123	0.331 ± 0.015	0.753 ± 0.017
Group B Emetine treated (7)	108.1 ± 4.48	89.7 ± 2.62 $t = 1.442$	3.94 ± 0.197 $t = 2.457$	0.317 ± 0.011	0.931 ± 0.064 $t = 2.694$

The figures in the parentheses are the number of animals. The results are means ± S.E.M.

Table II. Effect of emetine on protein, RNA and DNA concentrations of different tissues

	Liver Group A Pair-fed control (6)	Group B Emetine-treated (7)	*t* value	Heart Group A Pair-fed control (6)	Group B Emetine-treated (7)	*t* value	Kidney Group A Pair-fed control (6)	Group B Emetine-treated (7)	*t* value
Protein (g/100 g)	15.30 ±0.60	11.68 ±0.61	4.230	11.73 ±0.32	11.06 ±0.15	1.895	12.02 ±0.12	10.39 ±0.21	6.739
RNA (g/100 g)	3.68 ±0.07	3.77 ±0.07	–	1.26 ±0.05	1.27 ±0.08	–	2.80 ±0.20	3.06 ±0.13	1.09
DNA (g/100 g)	0.450±0.045	0.345±0.006	2.313	0.174±0.006	0.181±0.003	–	0.578±0.017	0.528±0.012	2.403
Protein/RNA (ratio)	4.16 ±0.06	3.11 ±0.18	5.535	9.34 ±0.80	8.90 ±0.51	0.468	4.39 ±0.32	3.44 ±0.26	2.329
Protein/DNA (ratio)	34.15 ±0.98	34.33 ±1.80	–	67.97 ±3.74	61.27 ±1.15	1.71	20.86 ±0.55	19.72 ±0.50	1.532

The figures in the parentheses are the number of animals. The results are means ± S.E.M.

cell size. However, DNA content per 100 g of heart tissue does not change upon treatment suggesting thereby non-impairment in the cardiac cell size. The unaltered cellular protein concentration despite reduced protein synthesis in emetine-treated animals may be explained by the fact that probably diminished breakdown of protein occurs in addition to reduced protein synthesis.

It is further seen that RNA concentration of liver or kidney does not change following emetine treatment, but DNA concentration of liver or kidney is diminished under the same condition and accordingly RNA per unit amount of DNA is increased in liver or kidney after emetine treatment. This suggests, therefore, increased cellular concentration of RNA in liver or kidney of emetine-treated animals. Whether this increased cellular concentration of RNA in liver or kidney following emetine treatment is due to increased synthesis or reduced breakdown of RNA, or due to both, cannot be ascertained from the present studies. But heart tissue, unlike liver and kidney tissues, does not elicit depressed concentration of protein or increased cellular concentration of RNA upon emetine treatment. This differential response of the heart tissue to emetine appears more probable because of the differential ability of various organs to concentrate the drug[10]. GIMBLE et al.[10] have determined the relative concentrations of emetine of various organs and found that the heart muscle concentrates the drug less than other organs do.

Résumé. Le contenu de protéine, de RNA et de DNA dans les tissus de foie, de cœur et de rognon a été étudié chez des rats albinos traités à l'émétine. Le traitement a réduit la concentration de la protéine et du DNA dans le foie et dans le rognon. Dans le cœur, cette concentration ne fut pas altérée d'une manière significative. On suggère que l'émétine non seulement empêche la synthèse de la protéine, mais réduit aussi sa désagrégation.

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[16] Acknowledgement. The authors express their sincere gratitude to Prof. S. R. MAITRA, Head of the Department of Physiology, Calcutta University, for constant encouragement and his keen interest in the work.

Effect of an Inhibitor Isolated from Onion (*Allium cepa* Linn.) Bulbs on the Activity of Some of the Enzymes Involved in Starch Biosynthesis

Most fructosan-bearing plants do not contain starch, so it is possible that some type of inhibitor may be present in these plants which partially or completely inhibits the synthesis of starch. The presence of fructosan was previously noted in onion bulbs (*Allium cepa* Linn.)[1,2]. The observation[3] that juice of onion bulbs inhibits the formation of starch can be explained on the basis of inhibition of one of the enzymes involved in starch biosynthesis and degradation. In the present investigation, an inhibitor was isolated from the bulbs of *A. cepa* Linn. to study its effect on the activity of some of the enzymes of starch biosynthesis.

Material and methods. The inhibitor was isolated by the method employed by HART et al.[4] in the case of garlic bulbs, and its aqueous solution was used in the enzymic studies. Starch phosphorylase was prepared from potatoes by the method of GREEN and STUMF[5]. The reaction mixture consisted of 1. 0.4 ml starch phosphorylase preparation, 2. 0.3 ml of 0.1 *M* citrate buffer (pH 6.1), 3. 0.3 ml of 5% soluble starch solution, 4. 0.9 ml of either double distilled water or inhibitor solution, 5. 0.4 ml of 0.1 *M* glucose-1-phosphate solution. The reaction was carried out at 37 °C for different periods and stopped by the addition of 5 ml of 5% trichloroacetic acid and 2 ml of 2.5% ammonium molybdate in 5 *N* sulfuric acid. The mixtures were diluted to 25 ml with water and the suitable aliquot of the filtrate was analyzed for phosphorus by the method of FISKE and SUBBAROW[6].

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