Percentages of radiochemical protection in this experiment $\left[= \frac{^{3}\text{H-dibenamine}}{(^{3}\text{H}+^{14}\text{C})\text{-dibenamine}} \right.$ $\left. \frac{(\text{n moles})}{(\text{n moles})} \times 100 \right]$ were compared with the corresponding values calculated from the previous data (1). In this experiment (17 strips from 6 cats) tritium labeled dibenamine represents 'specific binding' and ^{14}C-labeled dibenamine 'non-specific binding'. At 10^{-7} M diphenhydramine, the radiochemical protection was 7.3 ± 1.4 (N=8) in the previous experiment, while 8.8 ± 0.6 (N=8) in this experiment. It was 19.5 ± 2.2 (N=9) in the former at 5×10^{-7} M diphenhydramine and 21.2 ± 1.0 at 3×10^{-7} M diphenhydramine in this experiment (N=9). Tritiated dibenamine bound in this experiment was approx. 30% of the corresponding value in the former.

The crucial point of this method is smaller deviations of the value of radiochemical protection, because the difference in sensitivity of a pair of muscle strips is irrelevant. The double labeling method may be a more useful and reliable method when more specific alkylating agents (10–12) or more selective photoaffinity labeling agents (13–15) are used.

REFERENCES

1) TAKAGI, K. AND UCHIDA, M.: *Japan. J. Pharmacol.* **20**, 448 (1970); 2) UCHIDA, M. AND TAKAGI, K.: *Japan. J. Pharmacol.* **23**, 337 (1973); 3) UCHIDA, M. AND TAKAGI, K.: *Japan. J. Pharmacol.* **27**, 1 (1977); 4) UCHIDA, M. AND TAKAGI, K.: *Japan. J. Pharmacol.* **27**, 9 (1977); 5) DIKSTEIN, S. AND SULMAN, F.G.: *Biochem. Pharmacol.* **14**, 881 (1965); 6) YONG, M.S. AND MARKS, G.S.: *Biochem. Pharmacol.* **18**, 1609 (1969); 7) MOTTRAM, D.R.: *Biochem. Pharmacol.* **23**, 1853 (1974); 8) TAKAGI, K., AKAO, M. AND TAKAHASHI, A.: *Life Sci.* **4**, 2165 (1965); 9) TAKAGI, K. AND TAKAHASHI, A.: *Biochem. Pharmacol.* **17**, 1609 (1968); 10) BURGEN, A.S.V., HILEY, C.R. AND YOUNG, J.M.: *Brit. J. Pharmacol.* **50**, 145 (1974); 11) BURGEN, A.S.V. AND HILEY, C.R.: *Brit. J. Pharmacol.* **51**, 279 (1974); 12) OHTA, K., TAKAYANAGI, I. AND TAKAGI, K.: *Pharmacometrics* **11**, 365 (1976); 13) WASER, P.G., HOFMANN, A. AND HOPPE, W.: *Experientia* **26**, 1342 (1970); 14) WASER, P.G.: *Brain Res.* **62**, 551 (1973); 15) TAKAYANAGI, I., YOSHIOKA, M., TAKAGI, K. AND TAMURA, Z.: *Europ. J. Pharmacol.* **35**, 121 (1976)

EFFECTS OF EMETINE ON SERUM PROTEINS OF RATS FED DIETS OF VARIOUS PROTEIN LEVELS

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The liver is considered to be the chief site of synthesis of plasma proteins (1–3) and emetine impairs the synthesis of proteins (4–7). It has also been demonstrated that the

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synthesis of liver proteins is depressed in emetine-treated rats (8, 9). The present investigation was an attempt to assess the effect of emetine on serum proteins and to determine whether the effects of emetine on serum proteins are modified by levels of dietary protein.

Male Wistar rats, 80 to 120 g, were separated into three groups, A, B and C and all animals had an equal average body weight. These animals were fed 18%, 5% and 40% protein (casein) diets, respectively. The percentages of carbohydrate (arrowroot starch) content of these diets were respectively 71, 84 and 49 and those of fat (groundnut oil) and salt mixture (10) were respectively 7 and 4. Water-soluble and fat-soluble vitamins were supplied in the manner described elsewhere (10).

Some of the rats in each group were given emetine hydrochloride (Parke-Davis, India) s.c. for 10 days in a dose of 0.2 mg/100 g body weight/day. The remaining rats in each group were pair-fed with the corresponding emetine-treated rats. After the experimental period for each dietary regimen was over, the rats were sacrificed and blood samples were withdrawn from the hepatic vein. Serum protein was estimated by the biuret method (11). Electrophoretic analysis of serum proteins was performed according to the method employed by Chatterjee (12). The paper strips containing the electrophoresed samples were dyed with bromophenol blue solution. With each paper strip a graph was plotted using densitometer (Photovolt Corporation, Model No. 510A, U.S.A.) and the area under each peak was measured using an Allbrit planimeter. The percentages of different fractions of serum proteins were calculated from the measured area under each peak. The absolute amount of each fraction was then calculated by comparing the percentage composition values to serum total protein levels.

Emetine treatment reduced the concentration of serum total proteins by 43.2% in rats on a 18% protein diet (Table 1). Emetine treatment also lowered the serum total protein

TABLE 1 Serum total proteins of emetine-treated rats fed diets of various protein levels

Dietary protein levels	Serum total proteins (g/100 ml)	
	Pair-fed control	Emetine-treated
5% (Low-protein diet)	8.97±0.24 (6)	4.84±0.17 (5) $P<0.001$
Percentage decrease		46.0
18% (Normal-protein diet)	7.99±0.37 (6)	4.54±0.30 (8) $P<0.001$
Percentage decrease		43.2
40% (High-protein diet)	8.21±0.11 (5)	5.03±0.13 (5) $P<0.001$
Percentage decrease		38.7

The figure in parentheses indicates the number of animals.
Values are means±S.E.M.

TABLE 2 Serum albumin and globulin fractions of emetine-treated rats on an 18% protein diet

Groups of animals	Serum protein fractions (g/100 ml)				
	Albumin	Globulins			
		α_1	α_2	β	γ
Pair-fed control (6)	2.02±0.19	1.40±0.10	0.78±0.12	1.83±0.11	1.95±0.15
Emetine-treated (8)	1.27±0.13 P<0.02	0.73±0.09 P<0.001	0.52±0.05 P>0.05	1.31±0.09 P<0.01	0.71±0.09 P<0.001
Percentage decrease	37.0	47.8	—	28.4	63.5

Values are means±S.E.M.

concentrations when the rats were fed a low-protein (5%) or a high-protein (40%) diet (Table 1). The percentage decrease in serum total protein concentration after emetine treatment was less (38.7%) in rats on a high-protein diet compared to 46% in rats on a low-protein diet. The ability of emetine to reduce the serum total protein level was, therefore, quite evident irrespective of dietary protein levels. However, this effect of emetine was slightly reduced in animals on the high-protein diet and slightly magnified in those on the low-protein diet. The fall in serum total protein level following emetine treatment in rats on the 18% protein diet was reflected in the decline of all the fractions with the exception of α_2-globulin (Table 2). It would appear that the percentage decrease was greatest for γ-globulin, least for β-globulin and intermediate for albumin and α_1-globulin. We previously suggested that emetine treatment reduces the synthesis of proteins in the liver (8). Other workers also found that emetine impairs the synthesis of proteins (4–7). Therefore it is possible that the reduced serum total protein level with fall in concentrations of its different fractions following emetine treatment may be the result of a diminished synthesis. The synthesis of the various fractions of serum proteins was probably affected to various extents and such may explain the various degrees of decrease in albumin, α_1-, β- and γ-globulin fractions after emetine treatment.

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REFERENCES

1) MILLER, L.L., BLY, C.G., WATSON, M.L. AND BALE, W.F.: *J. exp. Med.* **94,** 431 (1951); 2) MILLER, L.L. AND BALE, W.F.: *J. exp. Med.* **99,** 125 (1954); 3) MILLER, L.L., BLY, C.G. AND BALE, W.F.: *J. exp. Med.* **99,** 133 (1954); 4) GROLLMAN, A.P.: *J. biol. Chem.* **243,** 4089 (1968); 5) BELLER, B.M.: *Circulation Res.* **22,** 501 (1968); 6) JONDORI, W.R. AND SZAPARY, D.: *Archs Biochem. Biophys.* **126,** 892 (1968); 7) GROLLMAN, A.P.: *Ohio State Med. J.* **66,** 257 (1970); 8) CHATTERJEE, A.K., ROY, A.D., DATTA, S.C. AND CHOSH, B.B.: *Experientia* **26,** 1077 (1970); 9) CHATTERJEE, A.K., ROY, A.D., DATTA, S.C. AND GHOSH, B.B.: *Ind. J. Physiol. Allied Sci.* **25,** 43 (1971); 10) CHATTERJEE, A.K., DATTA, S.C. AND GHOSH, B.B.: *Brit. J. Pharmacol.* **44,** 810 (1972); 11) GORNALL, A.G., BARDAWILL, C.J. AND DAVID, M.M.: *J. biol. Chem.* **177,** 751 (1949); 12) CHATTERJEE, A.K.: *J. Nutr. Sci. Vitaminol.* **19,** 289 (1973)