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Actinomycin D-Sensitive Increase in the Biotinidase Activity in Mouse Liver and Serum after Ethionine Feeding

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Biotinidase (biotinamide amidohydrolase EC 3.5.1.12) is an enzyme which hydrolyzes biotin esters and amides with release of biotin. It is present in several but not in all microbial sources studied, and in animal tissues, notably liver and blood serum.[1-6] The total activity of serum biotinidase in some animal species (*e.g.* hog, rat, and mouse) is of the same magnitude as the total activity in liver. There is also evidence that in these animals biotinidase is mainly produced in the liver and secreted into serum.[5] The present studies with ethionine and actinomycin D were made in order to confirm these earlier findings. Administration of ethionine is known to inhibit protein and ribonucleic acid synthesis, possibly owing to the decreased concentration of adenosine triphosphate caused by the formation of *S*-adenosyl ethionine.[7-10] Actinomycin D is supposed to inhibit protein synthesis mainly by inhibition of RNA synthesis.

Experimental. Adult female mice of 22–27 g were used and kept on the routine laboratory diet (Hankkija, Helsinki, Finland) unless otherwise stated. The diets for the experimental groups are given in the tables. DL-Ethionine, DL-methionine, adenosine, and adenosine triphosphate were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Ethionine and methionine were given mixed in the diet. The dosage of adenosine and adenosine triphosphate was 40 mg intraperitoneally every 12th hour during the experiment. Actinomycin D was purchased from Merck, Sharp & Dohme, Rahway, N.J., U.S.A., and the single dosis used was 30 μg per animal given intraperitoneally. Biotinidase activities were determined as described earlier.[5]

Results and comments. Preliminary studies indicated that there is no change in the biotinidase activities in any group during the first day after the beginning of ethionine feeding. A marked and temporally sharp increase is seen in the liver biotinidase activity 36 h after the beginning of the experimental diet in groups III and IV, which had received ethionine (Table 1). The increase varied from 30 to 150 % in different experiments. After two days activity in the liver decreased almost to control level. Activity in the serum, on the other hand, continued to increase. After one week the activity of liver biotinidase in the ethionine group seems to have decreased below control level. On the other hand, an increase of about 100 % is still found in serum biotinidase activities in the ethionine groups. It is also seen that methionine does not inhibit this effect of ethionine, but rather potentiates it. Similar results were also obtained when ethionine was given intraperitoneally. Intraperitoneal administration of adenosine or adenosine triphosphate had no effect. Thus it seems improbable that this unexpected increase of biotinidase activity is due to a decrease in methylation or in the nucleotide pool. These results indicate that ethionine and methionine may not be antagonistic in this case. Damage of liver cells is not a likely cause as administration of carbon tetrachloride is known to decrease the biotinidase level in rat serum and liver.[5] A similar methionine-resistant increase in the re-

Table 1. Effect of ethionine on biotinidase activity in liver and serum of mouse. Each group consisted of eight animals. Four were killed after 1½ days and the rest 6½ days from the beginning of the diet. Ethionine and methionine were given mixed in the diet. The results are averages, with the range in brackets. The activities are expressed as nanomoles of *p*-aminobenzoate liberated per min per g wet weight of liver or per ml of serum.

	Biotinidase activity	
	1½ days on the diet	6½ days on the diet
Liver:		
I. Controls	26.0 (25.3–26.7)	24.3 (17.0–30.3)
II. 5 % Methionine	28.0 (26.3–30.0)	29.3 (26.7–31.3)
III. 0.5 % Ethionine	34.7 (31.7–37.3)	17.7 (12.0–23.3)
IV. 5 % Methionine + 0.5 % ethionine	32.3 (27.7–35.3)	26.0 (21.3–30.0)
Serum:		
I. Controls	6.7 (6.2–7.0)	5.7 (4.7–6.8)
II. 5 % Methionine	6.0 (4.8–7.7)	5.3 (4.5–6.3)
III. 0.5 % Ethionine	8.5 (7.7–9.2)	12.7 (11.3–14.5)
IV. 5 % Methionine + 0.5 % ethionine	9.5 (8.8–11.0)	14.5 (9.0–21.7)

Table 2. Effect of actinomycin D on the ethionine-induced increase in the biotinidase activity of liver and serum of mouse. Each group consisted of four animals. In experiment I actinomycin D (30 μg) was injected intraperitoneally at the beginning of the diet, and in experiment II 30 h after the beginning of the diet. The animals were killed 35 h after the beginning of the diet. In the ethionine group 0.5 % ethionine was mixed in the diet. The results are averages, with the range in brackets. The activities are expressed as nanomoles of *p*-aminobenzoate liberated per min per g wet weight of liver or per ml of serum.

	Biotinidase activity	
	Liver	Serum
Experiment I.		
Controls	25.0 (22.0–27.3)	5.6 (4.7–6.2)
0.5 % Ethionine	34.7 (31.7–36.7)	9.0 (8.0–10.3)
0.5 % Ethionine + 30 μg actinomycin D	24.0 (17.0–28.0)	6.8 (4.7–9.2)
Experiment II.		
Controls	20.7 (19.7–21.7)	4.7 (4.3–5.2)
0.5 % Ethionine	28.3 (26.0–29.7)	9.8 (9.5–10.3)
0.5 % Ethionine + 30 μg actinomycin D	28.3 (25.0–32.7)	8.7 (7.7–9.3)

duced glutathione concentration of liver after feeding ethionine has been reported by Hsu and Geller.[11] Increases in activities of some other enzymes after ethionine treatment have also been found [11–16] without final clarification of the effect.

In order to determine whether this increase was based on regulation at the transcriptional level several series of experiments were performed. Actinomycin D was injected into animals at different times during ethionine diet, and the biotinidase activity was followed as shown in Table 2. When actinomycin D was injected into animals at the beginning of the ethionine diet (exp. I) the increase in biotinidase activity was completely inhibited. However, when actinomycin D was injected only 5 h before killing the animals (exp. II) no inhibition was observed. These results suggest as one possibility that the increase in biotinidase activity after ethionine feeding is regulated directly or indirectly at the transcriptional level, and that it is probably due to an increase in the RNA directed protein

synthesis. We tried to use puromycin for confirmation of these results, but the synergistic effects of ethionine and puromycin did not allow the test animals to survive long enough.

We have also done similar experiments with guinea-pigs as test animals. This species has markedly low biotinidase activity both in liver and serum.[6] Ethionine feeding had no effect on the biotinidase activities in these animals.

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Solubility of Picric Acid in Light and Heavy Water and Isotopic Fractionation of Hydrogen between Water and Solid Picric Acid

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Standard free energies of transfer of sparingly soluble substances from H_2O to D_2O are usually calculated from the solubilities of the substances in the two waters.[1] However, if the solute in question contains hydrogen atoms that are exchangeable with those of the solvent, the "transfer free energy" obtained is actually the sum of several free energy changes. For example, when we compare the solubility of a solid compound HA in H_2O with that of DA in D_2O, the calculated free energy change includes the standard free energy changes of the isotope exchange reactions

$$HA(s) + \tfrac{1}{2}D_2O = DA(s) + \tfrac{1}{2}H_2O$$
$$HA(aq) + \tfrac{1}{2}D_2O = DA(aq) + \tfrac{1}{2}H_2O$$

as well as the genuine transfer free energy change when the solute is transferred from light to heavy water.

The above point is illustrated by the solubilities of proto- and deuteriopicric acids shown in Table 1. The solubility, 0.0569 M, of protopicric acid in light water containing no perchloric acid is in excellent agreement with the value, 0.05684 ± 0.00010 M, reported by Halban and Kortschak.[2]

The solubility products given in Table 1 were calculated from

$$K_s^\circ = y^2 c_{L^+} c_{Pi^-}, \quad (L^+ = H^+, \ D^+; \ Pi^- = \text{picrate anion}) \qquad (1)$$

in which the mean molar ionic activity coefficients y were estimated from a semiempirical Debye-Hückel approximation with parameter values given earlier.[3] A correction for the presence of undissociated picric acids was applied when calculating the ionic strengths and the molar concentrations of L^+ and Pi^-. If the activity coefficients of the undissociated acids remain unaltered in the solutions studied, their molar concentrations would be given by