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Intestinal and Hepatic Mixed Function Oxidase Activity in Rats Fed Methionine and Cysteine-Free Diets (40620)

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Lipotropic agents function in methyl group transfer or are methyl donors during the synthesis of phospholipids, which are essential for triglyceride transport from the liver. Rats fed diets marginally deficient in lipotropic agents show a greater incidence of tumors in response to several different carcinogens (1-4). In the above cited experiments the diets were low in the lipotropic agents choline, methionine, and folic acid, deficient in niacin, low in other indispensible amino acids, and high in fat. Supplementation of these diets with lipotropic agents or a mixture of indispensible amino acids decreased the number of diethylnitrosamine (DEN)-induced hepatic tumors (5). Methionine, which was contained in both the amino acid and lipotrope supplements may have contributed to reduced tumor incidence. In another study fewer aflatoxin B_1-induced hepatic tumors were found in rats fed a basal diet containing soybean protein supplemented with choline, methionine, and vitamin B_{12}. Soybean protein is first limiting in methionine (6).

Nutrition has major influence on hepatic or other mixed function oxidase (MFO) enzymes and may thereby alter cancer incidence. *In vitro* hepatic MFO activity is reduced in rats fed lipotrope-deficient diets (7, 8). *In vivo* studies show that the clearance of DEN from the blood is depressed in rats fed lipotrope-deficient diets (9). Pentabarbital-induced sleeping time is prolonged in rats fed lipotrope-deficient diets (10). The above studies suggest that the ability to metabolize and excrete the chemicals was decreased.

We report herein investigations with diets containing only L-amino acids as sources of dietary nitrogen to determine if withholding methionine and cysteine while keeping other lipotropic factors constant would depress MFO activity. Described are *in vitro* hepatic and small intestinal MFO activity of rats fed a sulfur amino acid-free diet for 4 days. Aryl hydrocarbon hydroxylase (AHH), a complex cytochrome *P*-450 (*P*-448)-dependent MFO system which metabolizes a variety of substrates including carcinogenic polycyclic aromatic hydrocarbons was chosen as the model for study. Cytochrome b_5, the only other cytochrome in the microsomal fraction, was also examined. Cytochrome b_5 has numerous metabolic functions (11) including desaturation of fatty acids and may also be involved in some P-450-linked drug oxidations (12). Pair-feeding techniques were employed to determine the influence of the sulfur amino acid deficiency independently of reduced food consumption which occurs when one or more essential amino acids are deficient.

Materials and methods. Male, Sprague-Dawley rats averaging 138 ± 2 g in initial body weight were fed the complete L-amino acid diet of Rogers and Harper (13) containing: dextrin 49.32%, sucrose 25.68%, corn oil 5%, Rogers and Harper salt mixture 5% (Teklad Test Diets, Madison, Wi.),[1] vitamin fortification mixture 1% (Teklad Test Diets, Madison, Wi.),[2] and L-amino acids 14%. The

[1] The Rogers and Harper salt mixture contained (in percent): $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 0.0025%; $CaCO_3$, 29.29%; $CaHOP_4\cdot 2H_2O$, 0.43%; $CuSO_4$, 0.156%; $Fe(C_6H_5O_7)\cdot 6H_2O$, 0.623%; $MgSO_4\cdot 7H_2O$, 9.98%; $MnSO_4\cdot H_2O$, 0.121%; KO, 0.0005%; KH_2PO_4, 34.31%; NaCl, 25.06%; $Na_2SeO_2\cdot 5H_2O$, 0.0015%; $ZnCl_2$, 0.020%. Catalog No. 170760. Teklad Test Diets, Madison, Wis. The salt mixture used is low in zinc and deficient in certain trace minerals such as chromium and fluoride. The use of this mixture in purified diets could confound results in studies of longer duration.

[2] The vitamin mix contained (in percent of vitamin mix): *P*-aminobenzoic acid, 1.10132; ascorbic acid, coated (97.5%), 10.16604; biotin, 0.00441; vitamin B_{12} (0.1% trituration in mannitol), 0.29736; calcium pantothenate, 0.66079; choline dihydrogen citrate, 34.96916; folic acid, 0.09982; inositol, 1.10132; menadione (vitamin K_3), 0.49559; niacin, 0.99119; pyridoxine HCl, 0.22026; riboflavin, 0.22026; thiamine HCl, 0.22026; retinyl pal-

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nitrogen lost by complete deletion of methionine plus cysteine was replaced with glycine. All of the diets were suspended in agar gel and refrigerated until fed. Thirty-six rats were fed the control diet for 3 days before their random assignment to dietary treatments (12 per diet): control diet fed *ad libitum,* methionine-free diet fed *ad libitum,* control diet pair-fed to the intake of rats fed the methionine-free diet. After consuming their experimental diets for 4 days the rats were killed by decapitation and microsomes were isolated by differential centrifugation from the liver and the mucosa from the first 25 cm of the small intestine. Microsomal protein was assayed according to Gornall (14), cytochrome *P*-450 and b_5 as described by Omura and Sato (15), and AHH according to Nebert and Gelboin (16). Statistical analysis was by the F-LSD method (17).

Enzyme data are expressed as specific activity and total tissue activity per body weight since exposure to environmental chemicals is usually related to some function of body weight.

Results. Body weights, weight gains, food intake, liver weight, and weight of the 25-cm intestinal segment are shown in Table I. The dry matter intake was reduced approximately 50% on Day 1 for the methionine + cysteine-free diet and pair-fed rats and remained so compared to controls. Body weights were significantly reduced by pair feeding, but the weight loss of rats fed the methionine + cysteine-free diet was even greater. There were no significant treatment effects on liver or intestinal wet weights.

The total hepatic microsomal protein was lower in rats restricted in food intake and there was a further reduction with sulfur amino acid deficiency (Table II). The hepatic content of cytochrome *P*-450 expressed as nmole/100 mg or as total liver content per unit body weight was significantly lowered by the deficiencies of methionine and cysteine. There were no significant differences in cytochrome b_5 content. The specific activity of AHH or total activity per unit body weight was significantly lower in the deficient rats compared to either the controls or pair-fed rats.

There were no differences in microsomal protein in the 25-cm intestinal segment (Table III). The specific activity per unit body weight of AHH was significantly lowered by sulfur amino acid deficiency but not by restricted food intake.

Discussion. A low intake of sulfur amino acids associated with a lipotrope deficiency may influence biological activity of foreign chemicals via several mechanisms. Methionine via its conversion to cysteine is a precursor for glutathione which is used by man and numerous other species to form glutathione conjugates of foreign substances (18). Depletion of hepatic glutathione has been correlated with enhanced toxicity and covalent binding of toxins to DNA and protein (19). However, Campbell *et al.* has shown that hepatic glutathione concentrations are not altered by lipotrope deficiency (8). Methionine also participates in the methylation of foreign compounds via the nucleotide, *S*-adenosyl methionine. Male rats, but not females, fed lipotrope-deficient diets show a decrease in hepatic *S*-adenosyl methionine (20).

Our present data show that dietary sulfur amino acids also participate in foreign compound metabolism by influencing the activity of MFO enzymes. AHH activity was decreased in the liver and intestine of rats fed a diet devoid of cysteine and methionine for 4 days. A similar reduction in hepatic cytochrome *P*-450 was observed with no effect on cytochrome b_5. The AHH complex converts polycyclic aromatic hydrocarbons into more hydrophilic products which are more readily excreted and usually less carcinogenic. However, during this process metabolites which are more carcinogenic than the parent compound may be transiently produced. Several studies have shown that depressed hepatic AHH activity at the time of exposure to carcinogenic polycyclic aromatic hydrocarbons is associated with enhanced tumor development (21, 22). Decreased activity of microsomal oxidases may contribute to the enhanced carcinogenesis by specific chemicals in lipotrope-deficient rats. Rogers *et al.* (7) observed a trend toward lower activity of hepatic AHH and other MFO enzymes in rats fed their lipotrope-deficient diet. Our

mitate (500,000 U/g), 0.39648; dry cholecalciferol (500,000 U/g), 0.04405; dry tocopheryl acetate (500 U/g), 2.42291; corn starch, 46.66878. Catalog No. 40060. Teklad Test Diets, Madison, Wis.

TABLE I. Body Weight, Weight Gains, Food Intake, Liver Weight, and Intestinal Weight of Growing Rats Fed an L-Amino Acid Diet With or Without Sulfur Amino Acids[a]

Treatment	Food intake (g dry matter / day)	Final body weight	Weight gain (g/day)	Liver wet wt (g)	Intestinal segment wet wt (g)
Control—*ad libitum* fed	11.6 ± 0.1^{x}	162 ± 1.7^{x}	5.8 ± 0.2^{x}	5.6 ± 0.5	1.93 ± 0.06
Control—pair fed	5.4 ± 0.1^{y}	133 ± 2.2^{y}	-1.5 ± 0.1^{y}	6.2 ± 0.6	1.85 ± 0.03
Met + Cys-free diet	5.5 ± 0.1^{y}	121 ± 1.5^{z}	-4.2 ± 0.3^{z}	5.8 ± 0.5	1.92 ± 0.04

[a] Data represents mean ± SEM. Data with significant differences ($P < 0.05$) show a different superscript.

TABLE II. Hepatic Mixed-Function Oxidase Activity in Growing Rats Fed an L-Amino Acid Diet With or Without Sulfur Amino Acids[a]

	Control—*ad libitum*	Control—pair fed	Met + Cys free
Total microsomal protein (mg)	295 ± 28^{x}	201 ± 13^{y}	167 ± 12^{z}
P-450			
nmole/100 mg protein	16.3 ± 4.0^{x}	14.3 ± 1.2^{x}	10.5 ± 0.7^{y}
nmole/100 g body wt[b]	29.7 ± 6.2^{x}	$21.6 \pm 4.0^{x,y}$	14.5 ± 3.4^{y}
b_5			
nmole/100 mg protein	2.0 ± 0.5	2.7 ± 0.4	2.1 ± 0.2
nmole/100 g body wt[b]	3.6 ± 0.7	4.1 ± 0.6	2.9 ± 0.4
Aryl hydrocarbon hydroxylase			
Specific activity[c]	15.9 ± 2.1^{x}	16.3 ± 2.4^{x}	4.6 ± 1.3^{y}
Activity/g body wt[d]	28.9 ± 3.9^{x}	24.6 ± 3.1^{x}	6.3 ± 1.8^{y}

[a] Data represets mean ± SEM. Data with significant differences ($P < 0.05$) show a different superscript.
[b] nmole/100 mg protein × total microsomal protein ÷ body wt × 100.
[c] nmole 3-OH benzo(a)pyrene/30 min/mg protein.
[d] Specific activity × total microsomal protein ÷ body wt.

TABLE III. Intestinal Mixed-Function Oxidase Activity in Growing Rats Fed an L-Amino Acid Diet With or Without Sulfur Amino Acids[a]

Treatment	Control—*ad libitum*	Control—pair fed	Met–Cys deficient
Total microsomal protein, 25-cm segment (mg)	15.7 ± 0.4	14.8 ± 0.5	15.0 ± 0.4
Aryl hydrocarbon hydroxylase			
Specific activity[b]	2.3 ± 0.3^{x}	1.7 ± 0.3^{x}	0.7 ± 0.1^{y}
Activity/intestine[c]	36.1 ± 5.1^{x}	25.6 ± 4.0^{y}	9.8 ± 0.6^{z}

[a] Data represents means ± SEM. Data with significant differences ($P < 0.05$) show a different superscript.
[b] Nanomoles of 3-hydroxybenzo(a)pyrene produced per 30-min assay/mg microsomal protein.
[c] Specific activity × total microsomal protein per intestinal segment.

studies show that a deficiency of sulfur amino acids alone can reduce MFO activity. It is impossible to extrapolate the results obtained from methionine and cysteine deficiencies to other indispensible amino acids. Prior studies have shown that specific amino acid deficiencies cause alterations in hepatic MFO activity unique to the amino acid. Although a 7-day tryptophan deficiency decreased hepatic cytochrome *P*-450 by 40% a similar deficiency of isoleucine or valine produced no significant changes (23).

Summary. Intestinal and hepatic mixed function oxidase (MFO) activity was studied in rats fed sulfur amino acid free diets. Male, Sprague–Dawley rats (~140 g) were randomly assigned to three diets containing L-amino acids as the only source of nitrogen and fed for 4 days. The treatments were the control diet fed *ad libitum* (C), the control diet minus methionine and cysteine (M), and the control diet pair fed (P) with the M-deficient partners. There were no significant differences in total microsomal protein in the first 25 cm of the small intestine but total liver microsomal protein was depressed by decreased food intake and by the sulfur amino acid deficiency ($P < 0.05$). Intestinal aryl hydrocarbon hydroxylase (AHH) specific activity (nmol 3-OH BP/30 min/mg) was (C) 2.30 ± 0.27, (P) 1.75 ± 0.28, (M) 0.65 ± 0.06, and hepatic AHH specific activity

was (C) 15.9 ± 2.1, (P) 16.3 ± 2.4, (M) 4.6 ± 1.3. The data show that sulfur amino acid deficiency causes a similar reduction in intestinal and hepatic MFO activity.

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