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CHANGES IN THE MIXED FUNCTION OXIDASE ENZYMES AS A RESULT OF INDIVIDUAL AMINO ACID DEFICIENCIES*

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The activity of the hepatic mixed function oxidase (MFO) enzyme system in animals is changed by the quality and quantity of protein consumed. Protein deficient diets reduce microsomal activity and decrease the metabolism rate of drugs in vivo (1,2). Different sources of dietary protein also cause changes in the metabolism of drugs (3). The experiments herein reported describe some effects of specific amino acid deficiencies on MFO activity.

Male Sprague Dawley rats with initial body weights of 130–160 g were maintained in pairs in cages with mesh floors and no bedding. The synthetic control L amino acid diet was that of Rogers and Harper (4). For feeding, the agar gel suspensions of the diet were formed by mixing the dry diet with an equal quantity of boiling 3% agar suspension (4). All diets were isonitrogenous and refrigerated until fed. Glycine was used to replace the nitrogen for the particular amino acid which was removed to create a deficiency. All rats were allowed 3 days to adjust to the control gel diet. Then fifteen pairs were separated into three dietary treatment groups (5 pairs/group) for each deficiency experiment. One group was fed the control diet ad libitum, a second, the amino acid deficient diet fed ad libitum; and a third was given the control diet pair-fed to the second group. Liver microsomes were prepared by differential centrifugation. Cytochrome P-450 and cytochrome b_5 were determined as described by Omura and Sato (5). Aldrin epoxidase was assayed as described by Krieger and Wilkinson (6). K_m and V_{max} were obtained by regression analysis according to Lineweaver and Burk (7).

The quantity of food eaten, weight gain, and liver weight as % of body weight were altered uniquely for each specific amino acid deficiency (Table 1). A tryptophan deficiency per se reduced the content of cytochrome P-450 per g body weight whereas deficiencies of

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Table 1 EFFECT OF AMINO ACID DEFICIENCIES ON FOOD CONSUMPTION AND GROWTH OF MALE RATS

Experiment	Dietary Treatment	Parameter: Food Intake g/7 days	Parameter: Weight Gain g/7 days	Parameter: Liver Weight as % of body wt
Tryptophan Deficiency	Control-- ad libitum fed	224±1[a]	+35±2[a]	4.0±0.2[a]
	Control-- pair-fed	108±1[b]	-12±1[b]	2.9±0.1[b]
	Deficient-- ad libitum fed	108±1[b]	-21±6[c]	3.4±0.1[c]
Isoleucine Deficiency	Control-- ad libitum fed	224±2[a]	+28±3[a]	4.5±0.2[a]
	Control-- pair-fed	82±2[b]	-15±2[b]	2.9±0.1[b]
	Deficient-- ad libitum fed	82±2[b]	-38±2[c]	3.7±0.1[c]
Valine Deficiency	Control-- ad libitum fed	231±3[a]	+26±2[a]	3.7±0.2[a]
	Control-- pair-fed	99±1[b]	- 9±1[b]	2.9±0.1[b]
	Deficient-- ad libitum fed	99±1[b]	-27±2[c]	3.2±0.1[c]

Data represent means ± SE for 5 samples, each pooled for 2 animals. Data with statistically significant differences ($P<0.05$) show a different superscript for particular amino acids.

isoleucine or valine caused no reduction (Table 2). Restricted food intake due to isoleucine deficiency caused a reduction in P-450 as evidenced by data for pair-fed animals. Cytochrome b_5 content per g body weight was not changed for the tryptophan pair-fed controls. However isoleucine and valine deficient rats pair-fed the control diet had significantly less b_5 due to depressed food intake alone. Tryptophan deficiency per se reduced the aldrin epoxidase V_{max} per 100 g body weight, and the restricted food intake accounted for no difference in V_{max} for this enzyme. The K_m was elevated in response to the restricted food intake caused by the tryptophan deficiency. Isoleucine deficiency did not alter the V_{max} per 100 g body weight but restricting intake of the control diet to that of the isoleucine deficient animals caused a 74% reduction in the V_{max}. The K_m was not altered by the isoleucine deficiency per se or by the restricted food intake. Valine deficiency did not alter the V_{max} or K_m for aldrin epoxidase.

Table 2 EFFECT OF AMINO ACID DEFICIENCIES ON CYTOCHROME P-450 AND b_5 AND KINETIC PARAMETERS FOR THE EPOXIDATION OF ALDRIN BY RAT LIVER MICROSOMES

Experiment	Dietary Treatment	Parameter**			
		Cytochrome P-450	Cytochrome b_5	Aldrin Epoxidase	
				V_{max}*	K_m
		(n moles/g body wt)	(n moles/g body wt)	(100 g body wt)	(μM)
Tryptophan Deficiency	Control--ad libitum fed	0.235±0.030[a]	0.232±0.018[a]	68±9[a]	7.86±1.22[a]
	Control--pair-fed	0.256±0.015[a]	0.229±0.020[a]	65±4[a]	16.44±0.83[b]
	Deficient--ad libitum fed	0.148±0.012[b]	0.212±0.022[a]	34±3[b]	7.44±0.95[a]
Isoleucine Deficiency	Control--ad libitum fed	0.361±0.019[a]	0.228±0.009[a]	62±5[a]	7.93±1.05[a]
	Control--pair-fed	0.159±0.015[b]	0.162±0.020[b]	16±3[b]	9.00±1.06[a]
	Deficient--ad libitum fed	0.323±0.011[a]	0.349±0.058[a]	27±4[c]	7.18±0.36[a]
Valine Deficiency	Control--ad libitum fed	0.201±0.016[a]	0.224±0.017[a]	54±2[a]	7.00±0.33[a]
	Control--pair-fed	0.195±0.021[a]	0.171±0.016[b]	32±4[b]	9.55±2.10[a]
	Deficient--ad libitum fed	0.158±0.012[a]	0.258±0.026[a]	34±5[b]	7.74±0.44[a]

*Micromoles of dieldrin produced per minute per 100 g body weight.

**Data represent mean ± SE for 5 pooled samples, each from 2 animals assayed in duplicate at five substrate levels (5-50 mM). Data having statistically significant differences ($P<0.05$) show different symbols for a particular amino acid.

These experiments show: (1) that specific amino acid deficiencies caused unique alterations in hepatic microsomal levels of cytochrome P-450, cytochrome b_5, and the apparent V_{max} and K_m of aldrin epoxidase and (2) that the effects of specific amino acid deficiencies were caused by the deficiency per se and/or an associated depressed feed intake.

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