Some Biochemical Changes in Hamsters Fed Excess Phenylalanine Diets.* (31243)

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Previous studies in this laboratory have revealed that feeding excess quantities of phenylalanine to rats influences the activity of various liver enzymes(1,2,3), the quantity of brain serotonin(2,4), plasma amino acid levels, and affects these parameters in the developing fetus(5,6). Behavioral responses of rats fed such diets have also been evaluated (7,8). Other studies by Waisman and Harlow(9,10) have provided convincing data that the Rhesus monkey is a better animal than the rat for both biochemical and behavioral experiments designed to study the effect of hyperphenylalaninemia.

In attempts to develop still other animal models of hyperaminoacidemias, hepatic phenylalanine hydroxylase activities of animals of several species were determined and it was surprising to find that the Syrian hamster *(Cricetus auratus)* had but 18% of the hydroxylase activity of the rat(11). The purpose of this study was to determine the effect of different phenylalanine-supplemented diets on growth, plasma phenylalanine and tyrosine levels, phenylalanine hydroxylase, phenylalanine-pyruvate transaminase, and tyrosine-α-ketoglutarate transaminase activity in the liver of the growing hamster.

Materials and methods. Weanling hamsters, 25 days of age, were obtained from a local vendor (Con Olson Co., Madison, Wis.) and placed in individual cages, in a room with 3-hour alternate dark-light cycles as previously described(12). The animals were randomized in groups of six, 3 ♂ and 3 ♀ to a group, and fed different diets. The first 5 groups received commercial diet,‡ supplemented with varying amounts of L-phenylalanine, or L-phenylalanine + DL-phenylalanine; a group which received only commercial diet served as controls. Eight other groups received synthetic diets consisting of varying quantities of casein and dextrose plus corn oil, minerals,§ and vitamins. This basic diet was supplemented with 5% L-phenylalanine or a mixture of L-phenylalanine and DL-phenylalanine, as listed in Table I.

Determinations of plasma phenylalanine and tyrosine were made weekly on samples obtained by bleeding the animals from the ophthalmic venous plexus under light ether anesthesia(13). The samples were always drawn at the same time of day, immediately at the end of a dark cycle, a period during which the animals had consumed food. Phenylalanine was determined by the fluorimetric method of McCaman and Robins(14) as modified by Wong *et al*(15) and tyrosine by the method described by Udenfriend and coworkers(16). The animals were weighed twice weekly. After 4 weeks on diet, when the hamsters were 53 days old, the last blood sample was drawn and the animals sacrificed by decapitation. The livers were excised immediately, weighed, and placed in beakers in an ice bath. Phenylalanine hydroxylase was determined by the method of Freedland *et al* (17) as modified by Voss *et al*(11). Liver transaminase activity was determined according to the method of Lin *et al*(18).

Results. Growth. Fig. 1 shows the mean weights and standard deviations for each of the 13 different dietary groups after they had been on the diet for either 2 or 4 weeks.

Plasma amino acid levels. Plasma levels of phenylalanine and tyrosine are presented in Table I. Values and standard deviations are given for animals which received the specific diet for 4 weeks. As expected, the addition of phenylalanine, in L or DL form, to the

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‡ Ground Wayne Lab Blox, Allied Mills, Inc.

§ USP XIV Salt Mixture.

TABLE I. Plasma Amino Acids and Liver Enzymes After 4 Weeks on the Diets.

Diet		Plasma Ø-alanine (mg/100 ml)	Plasma tyrosine (mg/100 ml)	Liver Ø-alanine hydroxylase activity/100 g b.w.	Liver Ø-alanine pyruvate transaminase (μm/mg protein/hr)	Liver tyrosine-α-ketoglutarate transaminase (μm/mg protein/hr)
Chow	(24)†	2.5 ± .1‡	2.8 ± .4	42.8 ± 2.5	.85 ± .12	2.39 ± .37
+5% L-Øal.	(18)	11.2 ± 2.7*	9.3 ± 1.0*	31.6 ± 8.2*	.88 ± .14	2.76 ± .53
+2.5% L +2.5% DL-Øal.	(12)	10.4 ± 2.0*	7.0 ± .8*	31.7 ± 1.7*	.79 ± .14	2.36 ± .44
+4% L +4% DL-Øal.	(6)	13.8 ± 2.6*	11.2 ± 2.5*	48.7 ±10.3		
+7% L-Øal.	(6)	42.4 ±27.0*	11.8 ± 5.2*	40.6 ±13.7		
9% Casein	(6)	2.3 ± .3	3.5 ± .5*	16.7 ± 4.9*	.61 ± .19*	1.56 ± .57*
+5% L-Øal.	(6)	21.1 ±11.0*	49.7 ±13.3*	18.4 ± 6.4	.64 ± .10	3.73 ± .64*
18% Casein	(12)	2.6 ± .6	4.2 ± .2*	38.5 ± .7	.69 ± .10	1.87 ± .56
+5% L-Øal.	(6)	10.4 ± 4.0*	15.7 ± 6.3*	28.5 ±11.4	.67 ± .08	2.20 ± .36
+2.5% L +2.5% DL-Øal.	(6)	15.0 ± 4.6*	15.8 ± 5.0*	31.5 ± 9.9	.70 ± .10	1.79 ± .40
25% Casein	(6)	2.7 ± .4	3.4 ± .6*	53.1 ±18.8	.82 ± .16	2.38 ± .53
+5% L-Øal.	(6)	17.7 ± 8.4*	27.4 ±18.9*	35.3 ±16.5	.81 ± .16	2.72 ± .85
60% Casein	(6)	2.9 ± .7	4.6 ± 1.4*	42.6 ± 6.0		

* $P < .01$. Each supplemented group is compared to its own control. Unsupplemented groups are compared to the chow control.

† No. of animals.

‡ Mean ± standard deviation.

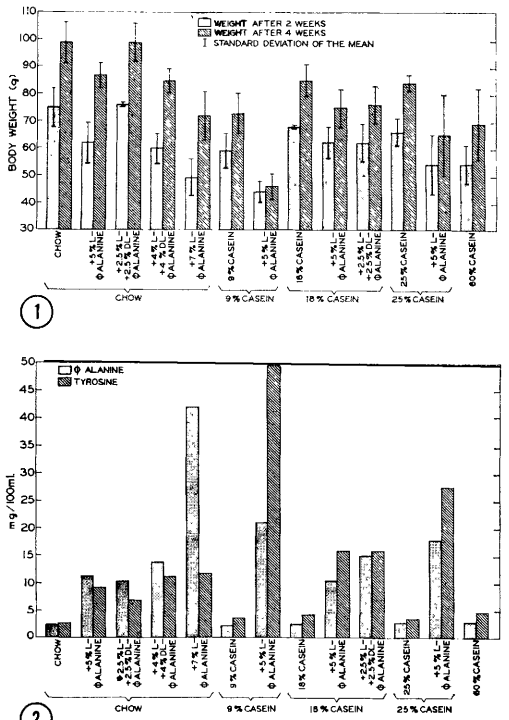

FIG. 1. Mean weights and standard deviations of hamsters fed various diets.

commercial diet, produced an elevation in the plasma phenylalanine and tyrosine concentrations, the phenylalanine level always being higher than that of tyrosine. The elevated phenylalanine and tyrosine plasma concentrations were approximately the same when either 5% L, 2.5% L + 2.5% DL or 4% L + 4% DL-phenylalanine were added to the control diet. When 7% L-phenylalanine was added, very high phenylalanine blood levels were reached. Fig. 2 presents data that indicate that addition of phenylalanine to casein diets produced significantly higher plasma levels of tyrosine than were obtained with the commercial diets. This effect was especially remarkable in the animals fed a diet containing 9% casein + 5% phenylalanine.

Maximum plasma phenylalanine levels were obtained in the first week but these decreased during the next 3 weeks, more strikingly on the commercial diets than on the casein diets (Table II).

FIG. 2. Plasma phenylalanine and tyrosine levels of hamsters fed various diets.

Enzymes. It can also be seen from Table I that the addition of 5% L-phenylalanine and of 2.5% L + 2.5% DL-phenylalanine to the commercial diet produced a significant decrease of the liver phenylalanine hydroxylase activity. Strangely this effect was not observed in the animals fed 4% L + 4% DL-phenylalanine or 7% L-phenylalanine. Similar liver phenylalanine hydroxylase activities were found in hamsters fed either 18% casein or chow. The enzyme activity in animals fed a 9% casein diet was extremely low, but no

TABLE II. Changes in Plasma Phenylalanine and Tyrosine Levels.

Diet	Plasma phenylalanine (mg/100 ml) at:				Plasma tyrosine (mg/100 ml) at:			
	1 wk	2 wk	3 wk	4 wk	1 wk	2 wk	3 wk	4 wk
Chow	2.7 ± .2*	2.4 ± .2	2.6 ± .3	2.5 ± .1	2.8 ± .2	2.5 ± .2	2.6 ± .2	2.8 ± .4
+5% L-Øal.	47.6 ± 6.5	22.9 ± 1.2	14.0 ± 1.3	11.2 ± 2.7	11.1 ± 1.0	11.8 ± .2	9.3 ± .7	9.3 ± 1.0
+2.5% L +2.5% DL-Øal.	15.2 ± 1.8	13.7 ± 1.8	11.9 ± 3.6	10.4 ± 2.0	9.5 ± .3	9.2 ± .5	7.2 ± .8	7.0 ± .8
+4% L +4% DL-Øal.	57.9 ±10.7	30.0 ±14.5	24.5 ± 4.6	13.8 ± 2.6	10.0 ± 1.4	13.4 ± 2.9	17.1 ±12.4	11.2 ± 2.5
+7% L-Øal.	59.6 ± 6.2	72.0 ±17.0	60.3 ±21.2	42.4 ±27.0	10.7 ± 2.0	12.3 ± 2.4	17.8 ±13.4	11.8 ± 5.2
9% Casein	2.1 ± .4	2.6 ± .5	2.3 ± .3	2.3 ± .3	2.9 ± .7	4.6 ± 1.2	4.0 ± .7	3.5 ± .5
+5% L-Øal.	21.0 ±10.7	13.8 ± 7.6	20.4 ± 9.2	21.1 ±11.0	45.0 ±25.0	45.0 ±23.2	42.3 ±24.7	49.7 ±13.3
18% Casein	3.3 ± .5	2.5 ± .1	3.2 ± .6	2.6 ± .6	6.0 ± 1.6	4.9 ± 1.3	4.5 ± 1.0	4.2 ± .2
+5% L-Øal. +2.5% L	21.5 ± 8.6	17.7 ± 3.6	15.8 ± 4.2	10.4 ± 4.0	48.0 ±25.8	30.4 ±14.3	26.3 ±11.6	15.7 ± 6.3
+2.5% DL-Øal.	26.9 ±17.0	11.7 ± 4.0	14.0 ± 5.7	15.0 ± 4.6	29.5 ±19.2	19.7 ±13.7	15.3 ± .5	15.8 ± 5.0
25% Casein	3.1 ± .4	2.8 ± .5	3.3 ± .6	2.7 ± .4	4.9 ± 1.1	3.3 ± 1.0	4.4 ± 1.3	3.4 ± .6
+5% L-Øal.	17.9 ±16.1	14.4 ±11.4	20.0 ± 8.0	17.7 ± 8.4	14.9 ± 4.9	27.1 ±16.6	41.7 ±21.0	27.4 ±18.9
60% Casein	—	3.2 ± 1.4	—	2.9 ± .7	—	—	—	4.6 ± 1.4

* Mean ± standard deviation.

further lowering was produced by the addition of 5% L-phenylalanine to this diet. Wide variation was observed in animals fed 25% casein, and the addition of phenylalanine to this diet did not influence this enzyme activity.

The low protein diet (9% casein) had a marked effect in lowering the activity of phenylalanine-pyruvate transaminase and tyrosine-α-ketoglutarate transaminase. No further lowering of the phenylalanine-pyruvate transaminase activity was observed by addition of 5% L-phenylalanine to this diet, but a significant increase in the activity of tyrosine-α-ketoglutarate transaminase was noted.

Discussion. It is apparent from the data that the hamster is a very satisfactory animal for studying the effect of several diets containing excess phenylalanine on various biochemical parameters. The plasma phenylalanine levels were comparable to those found in phenylketonuric children. Similar high levels have also been obtained in some rats fed phenylalanine-supplemented diets(19), but the levels have varied markedly in different experiments on rats, and plasma phenylalanine levels were sometimes much lower(4,6) although the same procedures were used.

The higher plasma tyrosine levels observed in the hamsters fed casein diets than in animals fed commercial diets have no ready interpretation at present. The hamsters were evidently able to convert large amounts of phenylalanine to tyrosine despite their relatively low activity of phenylalanine hydroxylase. The markedly elevated tyrosine concentrations in those animals fed the 9% casein diet + 5% L-phenylalanine cannot be explained by the presence of unused substrate due to a decrease in the transamination reaction, since the liver tyrosine-α-ketoglutarate transaminase activity was actually high. This elevated enzyme activity may represent an adaptive response to the high plasma levels of tyrosine. The marked increase in tyrosine is probably attributable to a net decrease in protein synthesis and a greater "imbalance" of amino acids on the low protein regimen.

The low activity of all 3 enzymes studied in hamsters fed a 9% casein diet is most probably due to inadequate synthesis of the enzyme proteins when the diet contains insufficient protein. No significant effect on either transaminase was produced when phenylalanine was added to the commercial diet or to the 18% and 25% casein diets; this observation was also previously made in rats (1,2). Other work from this laboratory has shown that the growth rate of rats on a commercial diet + 5% L-phenylalanine is impaired(12); this effect was even greater when higher amounts of phenylalanine were fed. In hamsters addition of 5% L-phenylalanine or 2.5% L + 2.5% DL to the diet did not influence growth (Fig. 1). The depression in growth in hamsters fed 7% phenylalanine was not as great as in the rat. A 10% mortality after 3 weeks was observed in rats fed 7% L-phenylalanine(12) but no deaths occurred in hamsters fed this diet for the same period.

The markedly low weight gain of the animals fed casein diets as compared to the weight gain of the hamsters fed commercial diet to which the same amount of phenylalanine was added may be due to the fact that casein is slightly deficient in its sulphur amino acid content and this imbalance is probably accentuated by the addition of excess phenylalanine. The poor growth observed in rats fed 60% casein is well known(12) and was confirmed in hamsters.

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Response of Mammalian Cardiac Muscle to Certain Sympathomimetics in Presence of Morphine. (31244)

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It has long been accepted that exposure of the myocardium to therapeutic concentrations of morphine does not result in significant alterations in cardiac performance. The heart rate is either unaffected or slightly decreased. There appear to be no consistent effects on cardiac output or blood pressure, nor is the ECG altered. However, large doses apparently produce bradycardia (Goodman and Gilman(1); Drill(2)). Although therapeutic amounts of morphine thus appear to have little effect upon the myocardium of the intact animal, its beneficial action in patients with acute left ventricular failure, pulmonary edema, and paroxysmal hyperpnea associated with cyanotic congenital heart disease is well recognized. However, the mechanism(s) whereby morphine exerts these beneficial effects is not well understood. Wood(3) suggested that paroxysmal hyperpnea was due to obstructive spasm of the right ventricular infundibulum which others speculated might be the result of an increase in endogenous myocardial catecholamine release (Johnson(4) and Honey *et al*(5)); the implication being that morphine acts by inhibiting the positive inotropic action of catecholamines upon the myocardium.

Since the cardiovascular effects of morphine are complicated by its central as well as peripheral actions, we decided to test the hypothesis that morphine may antagonize the action of catecholamines upon the myocardium by employing *in vitro* techniques.

Methods. The cat papillary muscle preparation has previously been described in detail (Tanz(6)). Briefly, cats were sacrificed by cardiectomy under light ether anesthesia. Two papillary muscles from the right ventricle were quickly isolated with minimal trauma, mounted on muscle holders and placed in 2 chambers containing a modified Krebs-Ringer bicarbonate solution enriched with glucose. The perfusate was maintained at a constant temperature of 37.5°C and a mixture of 95% O_2 and 5% CO_2 bubbled through it. Tension for each muscle was adjusted so that stimulation would yield a maximal force of contraction. Stimulation was at suprathreshold voltages, with a frequency of 1/sec and a duration of one millisecond using separate Grass (Model S-4) stimulators. Isometric contractile force was recorded using the amplified output of Sanborn (fta 30) transducers which were monitored on a direct-writing Sanborn 2-channel recorder.

The following experimental groups were studied: a) untreated controls; b) morphine HCl (1 to 100 μg/ml); c) isoproterenol (IPNE) and epinephrine controls (0.2 μg/