Enhanced Hepatic Protein Synthesis in Rats Force-Fed a Tryptophan-Devoid Diet[1] (35107)

HERSCHEL SIDRANSKY AND ETHEL VERNEY

Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

A number of investigators (1–6) have reported that the livers of fasted animals respond rapidly to one feeding of protein or of a complete amino acid mixture with a shift in polyribosomes from lighter to heavier aggregates and with enhanced protein synthesis. A similar response has been observed within 1 or 2 hr when fasted animals were fed an amino acid mixture devoid of single amino acids, arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine, or valine (7, 8). However, this effect was not observed when animals were fed an amino acid mixture devoid only of tryptophan (1, 3, 7). Tryptophan administration alone to fasted animals resulted in a shift of hepatic polyribosomes to heavier aggregates and in enhanced protein synthesis (7, 9, 10). This suggested that tryptophan has a special role in influencing hepatic protein synthesis (7, 11).

Because of our earlier interests in hepatic protein synthesis in a kwashiorkor-like model in which young rats were force-fed for 3 to 7 days purified diets devoid of single essential amino acids (12–16), we decided now to investigate the influence of force-feeding a tryptophan-devoid diet. Our earlier results (12–16) demonstrated that rats force-fed a purified diet devoid of single essential amino acids, other than of tryptophan, developed enhanced hepatic protein synthesis when studied *in vivo* or *in vitro*. This change developed even after only 1 day of force-feeding (3 feedings) a threonine-devoid or a phenylalanine-devoid diet (17). Therefore, because of the reported special role of tryptophan, it became of interest to determine whether young rats force-fed a purified diet devoid of this amino acid for 1 day (3 feedings) would respond with unchanged hepatic protein synthesis as observed in fasted animals within 1 or 2 hr after one tube-feeding or with increased hepatic protein synthesis as observed after 1 or more days in rats force-fed other single essential amino acid devoid diets. The results of the present experiments indicate that young rats force-fed a tryptophan-devoid diet for 1 day show enhanced hepatic protein synthesis *in vivo* similar to that observed earlier with threonine- or phenylalanine-devoid diets (17).

Materials and Methods. Female rats of the Sprague-Dawley strain were maintained on a commercial diet for several days before the experiments were begun. In all experiments, groups of animals, each of the same age and weight, were used. In Expts. 329 and 340 the rats weighed 61 g while in Expt. 334 they weighed 114 g.

The basal diet was the same as that used in earlier experiments (14–18). The percentage of dietary components was as follows: essential amino acids, 9.2; nonessential amino acids, 8.1; vitamin–sucrose mixture, 5; salt mixture, 4; corn oil, 5; cod liver oil, 1.5; and dextrin, 67.2. Dextrin was substituted for the tryptophan in the tryptophan-devoid diet. The diets were blended with distilled water so that each milliliter of diet mixture contained 0.5 g of diet. Rats were force-fed using plastic tubes at 8 a.m., 1 p.m., and 6 p.m. and received daily, 1.1 g of diet/10 g of initial body weight. The complete basal diet was force-fed to the control rats throughout and to the experimental animals for 3 days prior to beginning the deficient diet. Rats had free access to water. They were housed

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TABLE I. Liver Weight, Protein, and Incorporation of ^{14}C-Leucine into Hepatic Protein and Plasma Protein of Rats Force-Fed a Complete or Tryptophan-Devoid Diet for 1 Day.

			Liver				Plasma
					^{14}C-leucine incorporation[b]		
Expt.	Group[a]	No. of rats	Wt (g)	Protein (mg)	(cpm/mg of protein)	(cpm/total protein $\times 10^{-3}$)	^{14}C-leucine incorporation[b] (cpm/mg protein)
329	C	3	2.63	543	857	465	1186
	Tryp D	4	2.99	685	1404	963	1760
340	C	4	2.67	630	773	484	1126
	Tryp D	6	3.17	683	1045	709	1216
334[c]	C	5	4.61	930	477	445	702
	Tryp D	5	5.31	1012	580	588	780
Summary[d]			%	%	%	%	%
	C	12	100 ± 0.3	100 ± 3.3	100 ± 5.7	100 ± 5.9	100 ± 4.8
	Tryp D	15	116 ± 1.8[e]	114 ± 2.2[e]	140 ±10.9[e]	161 ±15.1[e]	121 ± 8.9

[a] C indicates complete diet; Tryp D indicates tryptophan-devoid diet.

[b] All rats received 2.5 μCi of ^{14}C-L-leucine (UL, sp act, 10 μCi/μmole) intraperitoneally 1 hr before killing.

[c] Rats weighed 114 g in this experiment while in the other two experiments they weighed 61 g.

[d] In each experiment the experimental group was compared with its control group (100%). Values are mean ± SEM.

[e] $p < 0.01$.

in individual wire cages with raised bottoms and kept in an air-conditioned room.

Rats were weighed at the beginning and at the end of each experiment. The animals were anesthetized with ether and exsanguinated the following morning, 16 hr after the last feeding. All animals received 2.5 μCi (10 μCi/μmole) L-leucine, ^{14}C uniformly labeled, intraperitoneally 1 hr before killing. The liver, left gastrocnemius muscle, spleen, and left kidney were rapidly removed and weighed.

The methods used for chemical analyses of lipid, protein, and radioactivity have been described in detail in earlier studies (12–16). Radioactivity in protein was measured using a Packard liquid scintillation spectrometer.

Results. Rats force-fed the complete diet gained 3.3 g while rats force-fed the tryptophan-devoid diet gained 2.7 g during the 1-day experiments.

Liver weight, protein, and *in vivo* incorporation of ^{14}C-leucine into liver protein and plasma protein are summarized in Table I. The liver weight and protein showed small, but significant increases. The incorporation of ^{14}C-leucine into hepatic but not into plasma proteins was significantly increased in the animals force-fed the tryptophan-devoid diet in comparison with those force-fed the complete diet. In all experiments, radioactivity in the acid-soluble fractions of homogenates of livers was measured to obtain some insight into the precursor pool size. The acid-soluble radioactivity per total liver showed an average 24% increase in the experimental compared with control groups. In two experiments (Expts. 329 and 334), the levels of hepatic free leucine were obtained by amino acid analysis (16). The livers of the experimental animals had an average 19% increase in free leucine per liver over that found in livers of the control animals. Also, in Expt. 334, the specific activity of leucine in the livers at sacrifice was determined and revealed a 3% increase in level in the experimental animals over that in the control animals. Thus, the changes, in regard to pool size of precursor, cold or isotopically labeled, could not account for the average 61% increase in

TABLE II. Analyses of Gastrocnemius Muscle, Spleen, and Kidney of Rats Force-Fed a Complete or Tryptophan-Devoid Diet for 1 Day.

			Gastrocnemius muscle[b]			Spleen[b]			Kidney[b]		
Expt. no.	Group[a]	No. of rats	Wt (g)	Protein (mg)	^{14}C-Leucine incorporation[c] (cpm/mg of protein)	Wt (g)	Protein (mg)	^{14}C-Leucine incorporation[c] (cpm/mg of protein)	Wt (g)	Protein (mg)	^{14}C-Leucine incorporation[c] (cpm/mg of protein)
329	C	3	0.38	50.2	103				0.33	62.4	401
	Tryp D	4	0.30	33.0	82				0.30	54.6	522
340	C	4	0.34	64.9	90	0.20	43.4	665	0.35	69.8	504
	Tryp D	6	0.34	69.0	65	0.17	33.1	512	0.36	69.1	474
334[d]	C	5	0.73	104.0	48	0.42	72.7	367	0.54	105.3	316
	Tryp D	5	0.71	103.7	35	0.34	66.7	239	0.53	98.0	254
Summary[e]			%	%	%	%	%	%	%	%	%
	C		100	100	100	100	100	100	100	100	100
	Tryp D		92 ± 6.6	90 ± 12.2	75 ± 2.1[f]	83 ± 4.0	84 ± 8.0	71 ± 6.0	97 ± 3.3	93 ± 3.5	99 ± 14.9

[a] C indicates complete diet; Tryp D indicates tryptophan-devoid diet.

[b] Organs from each group in each experiment were pooled.

[c] All rats received 2.5 μCi of ^{14}C-L-leucine (UL, sp act, 10 μCi/μmole) intraperitoneally 1 hr before killing.

[d] Rats weighed 114 g in this experiment while in the other two experiments they weighed 61 g.

[e] In each experiment, the experimental group was compared with its control group (100%). Values are mean ± SEM.

[f] $p < 0.01$.

^{14}C-leucine incorporation into total hepatic protein of the experimental animals.

In one experiment, Expt. 334, sucrose density gradient patterns of hepatic polyribosomes (14) were studied using deoxycholate-treated postmitochondrial supernatants of pooled livers of control and experimental animals. The livers of the experimental group contained relatively more heavier aggregates and fewer monomers and dimers than in those of the control group. These changes in patterns were similar to those described earlier with rats force-fed a threonine-devoid diet for 1 day (17) and with rats force-fed a tryptotphan-devoid diet for 3 days (9).

Liver total lipid was assayed in three experiments. In two experiments, Expts. 329 and 334, there was a 16 and 8% increase, respectively, in the experimental groups while in Expt. 340 there was a 3% decrease in the experimental group when compared with the control group.

The weights, protein content, and *in vivo* incorporation of ^{14}C-leucine into protein of gastrocnemius muscle, spleen, and kidney are summarized in Table II. There were decreases in ^{14}C-leucine incorporation into proteins of gastrocnemius muscle (significant decrease) and of the spleen of the experimental animals, but no change in incorporation of ^{14}C-leucine into kidney protein.

Discussion. The results of this study demonstrate that rats force-fed a tryptophan-devoid diet for 3 feedings during 1 day and killed the next morning, 16 hr later, have enhanced *in vivo* hepatic protein synthesis when compared to control animals force-fed a complete diet. These results are similar to those obtained after force-feeding a threonine-devoid diet for 1 (17), 3 (14–16), or 7 (12) days. Similar results were obtained after force-feeding a purified diet devoid of phenylalanine, leucine, or isoleucine (13). Thus, enhanced hepatic protein synthesis appears to be a common reaction pattern in young animals following the force-feeding of purified diets devoid of one of many single essential amino acids for 1 to 7 days.

It is important to stress that the results obtained with multiple (3) feedings of a purified diet devoid of tryptophan for 1 day are different from those obtained in fasted animals (rats or mice) force-fed a single feeding of a tryptophan-devoid amino acid mixture. In the latter studies (7, 11) it has been demonstrated that there is a decreased rate of incorporation of labeled amino acids into hepatic proteins in animals receiving the tryptophan-devoid amino acid mixture in comparison with those receiving the complete amino acid mixture. Thus, the imbalance or the response to it induced in young animals after 1 or more days of force-feeding an amino acid-devoid diet (regardless whether the diet is devoid of tryptophan, threonine, phenylalanine, isoleucine, or leucine) must be quite different from the response in the liver induced within minutes or hours by a single tube-feeding of an amino acid mixture deficient in tryptophan (7, 11).

In earlier reports (7, 11) it was speculated that since tryptophan is the least abundant amino acid both in the free amino acid pool and in the proteins formed from the pool, it may be of special importance in the regulation of hepatic proteins synthesis, especially in the fasted animal. This would take into consideration the results observed earlier when fasted rats or mice received a single feeding of a tryptophan-devoid amino acid mixture (1, 3, 7) or of tryptophan alone (7, 9). In the latter case, tryptophan administered alone, but not a single administration of threonine, methionine, or isoleucine (7) elicited a stimulating hepatic response which was similar to that obtained with a complete amino acid mixture. On the other hand, it has been speculated that the enhanced hepatic protein synthesis reported in young rats force-fed a purified diet devoid of an essential amino acid, such as a threonine-devoid diet, for 1 or more days is related to alterations in skeletal muscle protein metabolism (synthesis is decreased and catabolism is increased) (19), whereby skeletal muscle contributes amino acids, including the one missing in the diet, into the circulation and to the liver. Thus, when rats are force-fed a tryptophan-devoid diet for 1 day, tryptophan is most probably supplied to the liver from skeletal muscle catabolism and is therefore not limit-

ing. The exact mechanism for the enhanced hepatic protein synthesis under these experimental conditions is still unexplained.

Summary. Young Sprague-Dawley rats force-fed a tryptophan-devoid diet for 1 day show enhanced hepatic protein synthesis and decreased gastrocnemius muscle protein synthesis in comparison with animals force-fed a complete diet. These changes are similar to those observed previously in rats force-fed diets devoid of other single essential amino acids for 1 to 7 days.

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