Hyperphenylalaninemia: Disaggregation of Brain Polyribosomes in Young Rats

Abstract. *A single injection of* L-*phenylalanine in 7-day-old rats produced disaggregation of brain polyribosomes and inhibition of in vitro protein synthesis in cell-free systems prepared from brain. Liver polyribosomes and in vitro protein synthesis in hepatic systems were not affected. In 4-week-old rats these effects on brain protein synthesis did not occur.*

The mechanism responsible for brain damage in phenylketonuria (PKU) is still unknown, despite intensive investigation of this problem. Several theories have been proposed to explain the damage that takes place during brain development in affected individuals. The fact that several abnormal metabolites of phenylalanine are excreted by patients with PKU (*1*) has led to the concept of a "toxic metabolite" of phenylalanine, which could, in some unspecified manner, alter the normal course of brain development or neurological function. Phenylethylamine has been proposed as a candidate for the toxic metabolite role in PKU (*2*). The observation that plasma levels of 5-hydroxytryptophan are lowered in PKU has led Pare *et al.* (*3*) to propose that a cerebral serotonin deficiency is the cause of impaired mental ability in PKU. More recently, Appel (*4*) has suggested that the mental retardation associated with PKU may result from a disturbance in brain protein synthesis during development.

The availability of animal models of hyperphenylalaninemia, which is characteristic of PKU, as developed by Waisman and co-workers (*5*) has made a neurochemical approach to the problem possible. Two recent reports of neurochemical disturbances in hyperphenylalaninemia are of considerable interest. Swaiman *et al.* (*6*) have reported that young rabbits made hyperphenylalaninemic by the injection of L-phenylalanine have impaired in vitro brain protein synthesis, and McKean *et al.* (*7*) have found that the injection of L-phenylalanine into young rats results in a significant reduction in the brain levels of tryptophan and several other amino acids. The report of reduced tryptophan levels in brain assumes special interest in view of the recently reported ability of tryptophan to regulate polyribosome aggregation (*8*).

We studied in vitro protein synthesis in tissues from hyperphenylalaninemic rats and our data suggest that the injection of phenylalanine into young rats produces a marked disaggregation of polyribosomes in brain but not in liver. The polyribosome disaggregation is paralleled by a depletion of brain tryptophan levels, and it is suggested that the depletion of tryptophan is the mechanism by which protein synthesis is inhibited in the brains of rats with experimental PKU. Brain polyribosome aggregation in older rats is not affected by injection of phenylalanine.

Long-Evans rats (7 days old or 4 weeks old) were used in all experiments. Hyperphenylalaninemia was produced by the intraperitoneal injection of a 2.5 percent solution of L-phenylalanine in 0.42 percent NaCl (1 g per kilogram of body weight). Control rats received injections of 0.85 percent NaCl. Animals were killed 1 hour after injection, and liver and cerebral cortex were rapidly removed and rinsed with ice-cold saline. Tissues were homogenized in 9 volumes of buffer A (sucrose, 0.25*M*; KCl, 0.025*M*; MgOAc, 0.004*M*; tris-HCl, 0.05*M*, *p*H 7.4) and microsomes or ribosomes and *p*H 5 fractions were prepared according to Zomzeley *et al.* (*9*). Polyribosomes were isolated according to Campagnoni and Mahler (*10*), and polysome profiles were generated by sucrose density centrifugation. Cell-free protein synthesis was assayed in an incubation medium (*9*) containing: microsomal or ribosomal protein, 0.10 mg; *p*H 5 protein, 0.15 mg; Na_2ATP, 2 m*M*; Na_2GTP, 0.25 m*M*; creatine phosphate, 20 m*M*; creatine phosphokinase, 0.01 mg; sucrose, 0.25*M*; KCl, 100 m*M*; $MgCl_2$, 12 m*M*; tris-HCl, 0.05*M*, *p*H 7.4, and L-[^{3}H]lysine, 2.5 μc, in a total volume of 150 μl. After incubation for 30 minutes at 37°C the tubes were transferred to an ice bath and incorporation of [^{3}H]-lysine into protein was determined by the paper disc method of Mans and Novelli (*11*).

Mix-and-match systems of protein synthesis were used to assess the ability of all possible combinations of microsomes and *p*H 5 enzymes to support in vitro protein synthesis. When tissues from 7-day-old rats were used in such experiments, cell-free systems that included brain microsomes from rats injected with phenylalanine evidenced an impaired capacity for in vitro protein synthesis (Table 1). The effect was reproducible in many replicate experiments, and a similar degree of inhibition was found when ribosomes rather than microsomes were used. When 4-week-old rats were used in similar experiments, a slight stimulation of cell-free protein synthesis in brain systems was found. Hepatic systems showed no inhibition of protein synthesis after injection of phenylalanine (Table 1).

Polyribosome profiles from cerebral cortex of 7-day-old control rats con-

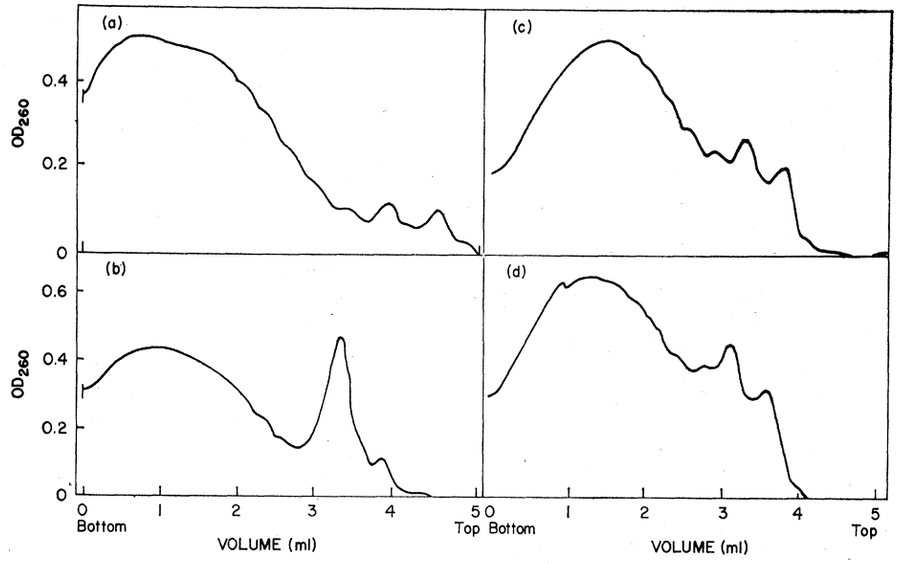

Fig. 1. Sucrose density gradient profiles of cerebral cortex polyribosomes. One hour after receiving an intraperitoneal injection of L-phenylalanine solution or 0.85 percent saline, animals were killed and polyribosomes were prepared. Polyribosome profiles were obtained by centifugation through 4.6 ml sucrose density gradients (15 to 40 percent) for 2 hours at 25,000 rev/min in a Spinco SW 39L rotor. Saline-injected 7-day-old (a); phenylalanine-injected 7-day-old (b); saline-injected 4-week-old (c); and phenylalanine-injected 4-week-old (d). OD, optical density.

Table 1. The effects of L-phenylalanine injection on in vitro protein synthesis. Animals were killed 1 hour after an intraperitoneal injection of either 0.85 percent NaCl or L-phenylalanine solution. The incorporation of L-[^{3}H]lysine into protein was determined. Tissues from ten animals of each group were pooled to provide the cellular fractions; dpm, disintegrations per minute.

Source of microsomes	Source of *p*H 5 fraction	7-Day-old rats		4-Week-old rats	
		Protein specific activity (dpm/mg of protein)	Percent of control	Protein specific activity (dpm/mg of protein)	Percent of control
		Cerebral cortex			
Control	Control	31501		14734	
Control	Phenylalanine	33387	106	18226	124
Phenylalanine	Control	19703	62	15649	106
Phenylalanine	Phenylalanine	19738	63	16732	114
		Liver			
Control	Control	25253		18013	
Control	Phenylalanine	26600	105	16557	92
Phenylalanine	Control	26382	104	17723	98
Phenylalanine	Phenylalanine	27891	110	18783	104

Table 2. The effects of a single injection of L-phenylalanine on tissue levels of phenylalanine and tryptophan in the rat. Injections were given according to the procedure described in the legend to Table 1. Each value represents the mean value from five rats. Values are expressed in milligrams per 100 grams of tissue.

Tissue	Group	Phenylalanine		Tryptophan	
		7-Day-old rats	4-Week-old rats	7-Day-old rats	4-Week-old rats
Brain	Control	0.90	1.38	0.55	0.32
Brain	Phenylalanine	14.96*	7.50*	.31†	.16*
Liver	Control	2.00	2.24	.81	.66
Liver	Phenylalanine	33.08*	18.77*	.90	.70

* Difference from control significant, $P < .001$. † Difference from control significant, $P < .01$.

sisted almost entirely of large aggregates, with small oligosome peaks. Injection of L-phenylalanine caused pronounced disaggregation of polyribosomes (Fig. 1). Liver polyribosomes were not affected by injection of phenylalanine. In 4-week-old rats, however, phenylalanine injection did not cause disaggregation of brain polyribosomes (Fig. 1). Large polyribosomes are more active in protein synthesis than are monosomes (*12*); thus the finding of polyribosome disaggregation is consistent with impaired protein synthesis.

Phenylalanine and tryptophan levels were determined 1 hour after injection of phenylalanine. A decreased level of tryptophan was found in brain, whereas tryptophan levels in liver were not significantly altered by phenylalanine injection (Table 2). Hepatic levels of phenylalanine were found to be greater than those of brain, after the injection of phenylalanine, but no impairment of in vitro protein synthesis nor disaggregation of liver polysomes was found. This indicates that high levels of phenylalanine per se are not responsible for the inhibition of brain protein synthesis in phenylalanine-injected rats. Brain tryptophan levels are lowered by phenylalanine injection, while hepatic levels of tryptophan are unchanged, which suggests a mechanism of polysome disaggregation involving tryptophan depletion, such as has been reported to take place in the livers of fasting mice (*8*). Tryptophan depletion also takes place in the brains of 4-week-old rats following phenylalanine injection, but no polysome disaggregation follows (Fig. 1). This may indicate that free polysomes, which predominate in 7-day-old rat brain (*13*), are more easily disaggregated than are membrane-bound polysomes, which predominate in 4-week-old rat brains. A recent report of Sarma *et al.* (*14*) indicates that free, but not bound, polysomes of liver are disaggregated by actinomycin D, providing a basis for this hypothesis.

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15. Supported by PHS grant No. HD-0231. We wish to thank Dr. Harry A. Waisman for his continuing interest and advice.
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13 November 1969

Hydroxyurea: Suppression of Two-Stage Carcinogenesis in Mouse Skin

Abstract. *Hydroxyurea, a selective cytotoxic agent for cells in DNA synthesis, injected intraperitoneally at 24 and 48 hours after the first painting with 1 percent croton oil, significantly reduced the tumor yield in the two-stage chemical carcinogenesis in mouse skin. A comparable group of mice receiving hydroxyurea only once at 24 hours had a tumor induction similar to that in controls.*

Chemical carcinogenesis in mouse skin can be a two-stage process: initiation and promotion. A subthreshold dose of a carcinogenic hydrocarbon can initiate the tumor which then develops upon repeated application of a promoter (*1, 2*). Croton oil has been used extensively as a promoting agent for the study of the mechanism of skin carcinogenesis (*3*). After one application of croton oil to mouse skin, the rate of uptake of tritiated thymidine in the epidermal basal cells is elevated with a maximum at 22 to 24