Effect of Tryptophan on Hepatic Polyribosomal Disaggregation Due to Ethionine[1] (36519)

HERSCHEL SIDRANSKY, ETHEL VERNEY, AND D. S. R. SARMA[2]
Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

In this laboratory, we have been concerned with the effect of diet and dietary components, particulaly of tryptophan, on hepatic polyribosomes and protein synthesis in fasted animals (1–8). Fleck, Sheperd and Munro (9), Wunner, Bell and Munro (10), Munro (11) and we (2, 3) have reported that when fasted rats or mice are tube-fed a complete amino acid mixture there occurs a rapid shift in hepatic polyribosomes from lighter to heavier aggregates. This does not occur when the fasted animals are tube-fed a complete amino acid mixture devoid of tryptophan. Subsequently we (3, 4) and also others (12–14) have reported that fasted mice or rats respond rapidly to a single feeding of tryptophan but not to one feeding of other single amino acids with a shift of hepatic polyribosomes from lighter to heavier aggregates and with enhanced hepatic protein synthesis. Recently, we have also reported that when tryptophan was tube-fed to nonfasted mice, similar changes as found with fasted animals were observed (5).

In other studies from our laboratory, we have been concerned with the effect of certain toxic agents, such as actinomycin D (15, 16), ethionine (17), puromycin (18) and sparsomycin (19) on hepatic polyribosomes and protein synthesis. Recently, we have investigated whether the prior nutritional status (fasted or nonfasted) would influence the degree of hepatic polyribosomal disaggregation and protein synthesis due to actinomycin D (20) or ethionine (17) and observed that fed animals became resistant to the induction of polyribosomal disaggregation due to these agents. Also, we observed that tryptophan administration following that of actinomycin D to fasted rats acts to correct the disaggregation of hepatic polyribosomes due to actinomycin D (20).

In the present study we investigated whether the administration of tryptophan to previously ethionine-treated mice or rats would influence the hepatic polyribosomal disaggregation and inhibition of hepatic protein synthesis due to ethionine. The results indicate that the administration of tryptophan after ethionine treatment causes a corrective effect on hepatic polyribosomes and protein synthesis.

Material and Methods. White female mice (20–25 g) of the CF 1 strain (Carworth, New City, NY) and female rats (150–200 g) of the Sprague-Dawley strain (Sprague-Dawley, Inc., Madison, WI) were used in these experiments. Animals were fed a commercial ration (Wayne Lab-Blox). All animals were fasted overnight before the experiments were begun. The following morning the experimental animals received intraperitoneally a solution (25 mg/ml water) of DL-ethionine, 1 mg/g body weight. At selected intervals thereafter, 1–2 hrs, groups of animals were tube-fed a solution (10 mg/ml) of L-tryptophan (16 mg/mouse and 30 mg/100 g body wt of rat) or water alone and 1 hr later all animals were killed by decapitation (Table I).

The livers were removed and freed of gallbladders in the case of mice. Postmitochondrial supernatants (PMS) of livers pooled from 4 to 5 mice or from 1 to 3 rats in each

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[2] Present address: Fels Research Institute, Temple University School of Medicine, Philadelphia, PA.

group were prepared as described earlier (3). Size distribution of polyribosomes of deoxycholate-treated PMS was determined by layering samples on linear sucrose gradients (0.3 to 1.1 *M* sucrose containing TKM (0.05 *M* Tris (pH 7.5); 0.025 *M* KCl and 0.005 *M* $MgCl_2$) and centrifuging in a swinging bucket rotor in a model L2-65 Spinco ultracentrifuge at 2° (3, 4). In the experiments using livers of mice, the linear sucrose gradients were prepared in 17 ml tubes and centrifuged in a Spinco SW 27 rotor at 25, 000 rpm for 2.5 hr. In the experiments using livers of rats, the linear sucrose gradients were prepared in 12 ml tubes and centrifuged in a Spinco SW 41 rotor at 38,000 rpm for 1 hr. The degree of hepatic polyribosomal disaggregation under the different experimental conditions was evaluated from the patterns obtained from sucrose density gradients by calculating the relative distribution of monomer-dimers in relation to total ribosomes. *In vitro* incorporation of ^{14}C-leucine, uniformly labeled, (10 mCi/mmole) into proteins using PMS and the preparation of samples for radioactive determination were carried out as described earlier (3).

Results. Studies with Mice. The effect of tryptophan administration on hepatic polyribosomes of mice that had been treated previously with ethionine is presented in Fig. 1. Sucrose density gradient patterns of hepatic polyribosomes 2 hr after ethionine treatment revealed disaggregation of polyribosomes with an increase in monomers. However, administration of tryptophan 1 hr after the ethionine treatment caused a partial corrective effect on the polyribosomal pattern, shifting toward that of the control, nontreated animals. Table I summarizes the results of the experiments in which mice were treated with ethionine for 1 or 2 hr and then received tryptophan 1 hr before killing. The results express the degree of hepatic polyribosomal disaggregation by using the ratio of monomer-dimers to total ribosomes for each group. Tryptophan administration appeared to cause a corrective effect on the polyribosomal disaggregation due to ethionine. The administration of tryptophan alone caused a shift of hepatic polyribosomes toward heavier aggregation similar to that described earlier (2, 3).

Table II summarizes the results of *in vitro* hepatic protein synthesis in the experiments previously described (Table I). It is apparent that ethionine treatment inhibited protein synthesis by 16% after 2 hr and by 33% after 3 hr. Tryptophan administration improved the hepatic protein synthesis after ethionine treatment. Control mice receiving tryptophan revealed a 75% increase in hepatic protein synthesis which was similar to the increase reported in earlier studies (2, 3).

Studies with Rats. The effect of tryptophan administration on hepatic polyribosomes of rats that had been treated previously with ethionine (Fig. 2 and Table I) is similar to that described for mice treated similarly. Also the results of *in vitro* hepatic protein synthesis of rats treated with ethionine followed by tryptophan (Table II)

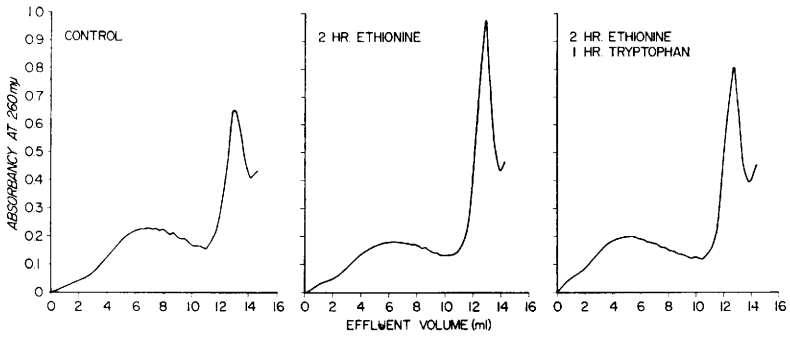

FIG. 1. Sucrose gradient patterns of hepatic polyribosomes of fasted control mice, of mice treated with ethionine 2 hr before being killed, and of mice treated with ethionine 2 hr and with tryptophan 1 hr before being killed.

TABLE I. Effect of Tryptophan on Hepatic Polyribosomes of Mice and Rats Treated with Ethionine 2–3 hr Before Killing.

	Experimental conditions[a]				No. of expts.[b]	Disaggregation of hepatic polyribosomes: Monomer-dimers / Total ribosomes × 100
	9 a.m.	10 a.m.	11 a.m.	12 a.m.		
Mice						
1	0	0	Kill		5	48.6 ± 1.2[c]
2	Eth	0	Kill		5	58.7 ± 0.9[d] (21)
3	Eth	Tryp	Kill		3	50.4 ± 3.0[e] (14)
4	0	Tryp	Kill		2	28.8 ± 0.6[f] (41)
5	0		0	Kill	3	47.8 ± 1.8
6	Eth		0	Kill	3	64.4 ± 2.7[g] (35)
7	Eth		Tryp	Kill	3	54.7 ± 2.0[h] (14)
Rats						
8	0	0	Kill		6	47.1 ± 0.4
9	Eth	0	Kill		10	59.8 ± 2.0[i] (27)
10	Eth	Tryp	Kill		10	52.1 ± 1.9[j] (13)
11	0	Tryp	Kill		3	37.3 ± 1.0[k] (21)

[a] Mice were treated with 25 mg ethionine (Eth) (25 mg/ml) intraperitoneally and 16 mg tryptophan (Tryp) (10 mg/ml) or water (0) by stomach tube. Rats were treated with ethionine (25 mg/ml), 1 mg/g body weight intraperitoneally and tryptophan (10 mg/ml), 0.3 mg/g body weight or water by stomach tube.

[b] Livers from 4 to 5 mice or from 1 to 2 rats were pooled for each group in each experiment.

[c] Mean ± SEM. Percentage change in parentheses.

[d] $p < .01$; 2 compared to 1.

[e] $.05 > p > .01$; 3 compared to 2.

[f] $p < .01$; 4 compared to 1.

[g] $p < .01$; 6 compared to 5.

[h] $.05 > p > .01$; 7 compared to 6.

[i] $p < .01$; 9 compared to 8.

[j] $.05 > p > .01$; 10 compared to 9.

[k] $p < .01$; 11 compared to 8.

were similar to those found with mice after similar treatments.

In order to determine the effect of simultaneous administrations of ethionine and of tryptophan upon hepatic polyribosomes and protein synthesis, in two experiments rats were given ethionine intraperitoneally and/or tryptophan by stomach tube and killed 1 hr later. The results indicated that rats receiving tryptophan alone or tryptophan with ethionine showed a similar degree of shift toward heavier polyribosomal aggregation and *in vitro* hepatic protein synthesis was also similar (only 6–11% greater in the tryptophan group than in the ethionine plus tryptophan group). Thus, it appears that under these experimental conditions ethionine is not or is only slightly inhibitory to the effect of tryptophan.

Discussion. The present study demonstrates that the administration of tryptophan after ethionine treatment leads to a corrective effect on hepatic polyribosomes and protein synthesis. Earlier studies have reported that ethionine administration to fasted animals leads to polyribosomal disaggregation and inhibition of protein synthesis in the liver (17, 21). The mechanism implicated for this effect of ethionine has been attributed to the lowering in ATP concentration as the result of the formation of *S*-adenosylethionine in the liver (21). Also, it has been shown that the administration of adenine or other adenine nucleotide precursors prevents or reverses the

TABLE II. Effect of Tryptophan on *in Vitro* Incorporation of ^{14}C-Leucine into Proteins by Post-mitochondrial Supernatants of Livers of Mice and Rats Treated with Ethionine 2–3 hr Before Killing.

	Experimental conditions[a]				No. of expts.[b]	Incorporation of ^{14}C-leucine into hepatic proteins[c] (cpm/sample; %)
	9 a.m.	10 a.m.	11 a.m.	12 a.m.		
Mice						
1	0	0	Kill		5	100
2	Eth	0	Kill		5	84 ± 7.1[d]
3	Eth	Tryp	Kill		3	122 ± 9.0 (45)[e]
4	0	Tryp	Kill		2	175 ± 9.5[f]
5	0		0	Kill	3	100
6	Eth		0	Kill	3	67 ± 8.6[g]
7	Eth		Tryp	Kill	3	107 ± 15.2 (60)
Rats						
8	0	0	Kill		3	100
9	Eth	0	Kill		3	83 ± 6.0[h]
10	Eth	Tryp	Kill		5	105 ± 9.7 (27)[i]
11	0	Tryp	Kill		2	140 ± 9.0[j]

[a] Mice were treated with 25 mg ethionine (Eth) (25 mg/ml) intraperitoneally and 16 mg tryptophan (Tryp) (10 mg/ml) or water (0) by stomach tube. Rats were treated with ethionine (25 mg/ml), 1 mg/g body weight intraperitoneally and tryptophan (10 mg/ml), 0.3 mg/g body weight or water by stomach tube.

[b] Livers from 4 to 5 mice or from 1 to 2 rats were pooled for each group in each experiment.

[c] Values for control groups 1, 5 and 8 were arbitrarily set at 100%. Values are mean ± SEM.

[d] $p = .05$; 2 compared to 1.

[e] $.05 > p > .01$; value in parentheses is percentage increase; 3 compared to 2.

[f] $p < .01$; 4 compared to 1.

[g] $.05 > p > .01$; 6 compared to 5.

[h] $.05 > p > .01$; 9 compared to 8.

[i] $p = .05$; value in parentheses is percent increase; 10 compared to 9.

[j] $.05 > p > .01$; 11 compared to 8.

effect of ethionine (21). Whether tryptophan which likewise induces a corrective effect may act in a similar or in a different manner as does adenine is at present unknown.

It is important to point out that tryptophan has been demonstrated to correct or improve the hepatic polyribosomal profiles in a number of other conditions or states in which the polyribosomes become disaggregated. Tryptophan has a beneficial effect on hepatic polyribosomes of fasted animals (3–5), of animals treated with actinomycin D (20) or puromycin (22), and of livers following alcohol perfusion (23). Thus, a number of conditions in which hepatic polyribosomes become disaggregated due to different mechanisms can be improved by tryptophan.

Recently we have reported that the administration of tryptophan to fasted mice caused an increase in the levels of cytoplasmic messenger RNA (mRNA) in the liver (8). Also, studies with fasted mice in which RNA was prelabeled with orotic acid-6-^{14}C and then treated with actinomycin D to inhibit RNA synthesis before tube-feeding tryptophan revealed elevated levels of mRNA and a shift in polyribosomes toward heavier aggregates in the livers of the experimental animals compared to controls (8). This latter finding suggests that tryptophan may increase the availability of relatively stable mRNA in the liver. This speculation is somewhat similar to that thought to be responsible for the effect of adenine after its administration to previously ethionine-treated animals (24).

Summary. Mice or rats fasted overnight

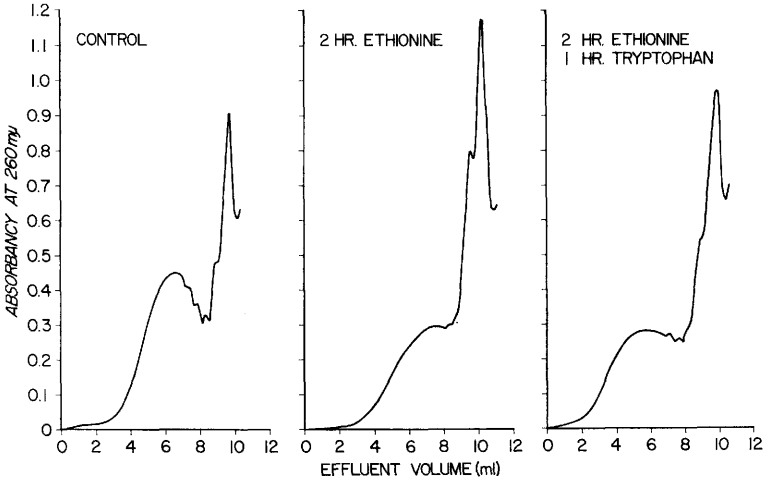

FIG. 2. Sucrose gradient patterns of hepatic polyribosomes of fasted control rats, of rats treated with ethionine 2 hr before being killed, and of rats treated with ethionine 2 hr and with tryptophan 1 hr before being killed.

were treated intraperitoneally with ethionine and were killed 2 or 3 hr later. Hepatic polyribosomal disaggregation and decreased *in vitro* hepatic protein synthesis were observed. The administration of L-tryptophan by stomach tube after ethionine treatment and 1 hr before killing caused a corrective effect on hepatic polyribosomes and protein synthesis. Simultaneous administration of ethionine and tryptophan to fasted rats 1 hr before killing induced a shift of hepatic polyribosomes toward heavier aggregation similar to that observed after administering tryptophan alone.

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