PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 309-314 (1988)

Effect of Tryptophan on Livers of Pregnant and Lactating Rats and Their Fetuses and Pups[1] (42669)

HERSCHEL SIDRANSKY AND ETHEL VERNEY

Department of Pathology, The George Washington University Medical Center, Washington, D.C. 20037

Abstract. The effect of the administration of L-tryptophan on hepatic polyribosomes and protein synthesis in pregnant rats and their fetuses and in lactating rats and their pups was investigated. Pregnant rats tube-fed tryptophan 1 hr before killing revealed increased hepatic protein synthesis but essentially unmodified polyribosomal aggregation of maternal livers while no changes were observed in fetal livers in comparison to controls (water-treated). Lactating rats tube-fed tryptophan 1 hr before killing revealed increased polyribosomal aggregation and protein synthesis of the livers in comparison to controls. Pups of these mothers that received tryptophan intraperitoneally 1 hr before killing did not reveal a significant change in the hepatic polyribosomes or protein synthesis.

Investigators have studied the effects of elevated dietary tryptophan on animals during pregnancy (1, 2). The adverse effects of long-term ingestion of high levels of tryptophan, consisting of increased neonate mortality and decreased neonate weight, have been attributed to increased serotonin (3, 4). In the present study, we were concerned with the short-term (within 1–2 h) effect of administering tryptophan on protein synthesis in the livers of pregnant rats and their fetuses and of lactating rats and their pups. This would supplement our earlier data dealing mainly with mature animals (5, 6).

Studies from other laboratories and from our laboratory have reported that the ingestion of L-tryptophan by mice or rats rapidly stimulated hepatic protein synthesis (5, 6). This effect appeared to be unique for tryptophan since other single essential amino acids did not elicit such a response (5, 6). The ability of tryptophan to stimulate hepatic protein synthesis has also been demonstrated in rats which were treated with a variety of hepatotoxic agents, such as actinomycin D, α-amanitin, CCl_4, cordycepin, ethanol, ethionine, hypertonic NaCl, puromycin, and NaF (5, 6) and also in rats which were treated in order to increase hepatic protein synthesis, as after administering cortisone acetate (7) and phenobarbital (7) and after force-feeding a threonine-devoid diet (8).

In the present study we investigated whether or not tryptophan would cause increased aggregation of polyribosomes and an increase in protein synthesis in the livers of (1) rats that were pregnant as well as their fetuses and (2) lactating rats and their pups. The results revealed that the ingestion of tryptophan acted on the livers of pregnant and lactating rats in a stimulatory manner but not in the livers of fetuses or in young pups.

Materials and Methods. *Animals.* Female rats of Sprague–Dawley strain (Harlan Sprague–Dawley, Bethesda, MD) weighing on the average 184 g were used. Pregnant rats at 15 days of gestation were used, and lactating rats at 8 to 21 days postpartum were used. The rats, maintained in an air-conditioned room, were fed a commercial diet (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL). Pregnant rats were removed from diet overnight and lactating rats were kept on diet up until killing.

In the experiments L-tryptophan (30 mg in 3 ml distilled water per 100 g body wt) was administered to the pregnant or lactating animals by stomach tube 1–2 hr before killing. Control groups were tube-fed equivalent amounts of distilled water. In experiments where pups were used, they received tryptophan (3.8 mg) or water (0.38 ml) intraperitoneally 1 hr before killing. In a previous report

[1] This work was supported by U.S. Public Health Service Grants AM-27339 from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases and CA-41832 from the National Cancer Institute.

0037-9727/88 $1.50

(9), it has been demonstrated that the effects of tryptophan on mature rats were similar whether administered by stomach tube or intraperitoneally.

Preparation of polyribosomes. Postmitochondrial supernatants were prepared from homogenates of livers of each group and were used for size distribution analysis of polyribosomes after addition of deoxycholate as described earlier (10). The degree of polyribosomal aggregation of livers under the experimental conditions was evaluated from the patterns obtained by centrifugation in sucrose density gradients. The relative distribution of monomer–dimers in relation to total ribosomes was calculated by measuring the area under the monomer and dimer peaks and the area under the entire pattern (monomer–dimer plus the other polyribosome fractions) of each gradient pattern.

In vitro protein synthesis. Postmitochondrial supernatants of livers were used for studies involving amino acid incorporation into protein *in vitro* as described earlier (10). L-[U-^{14}C]Leucine (0.5 μCi; sp act 330 mCi/mmol) (Amersham/Searle, Arlington Heights, IL) was added to each incubation tube. Radioactivity in protein was measured using a liquid scintillation spectrometer (Beckman Instruments, Palo Alto, CA).

In vivo protein synthesis. In one group of experiments L-(U-^{14}C)leucine (sp act 276 mCi/mmole), 5 μCi/100 g body wt, was injected intraperitoneally into lactating rats 30 min before killing. Its incorporation into total proteins was measured using total liver homogenates.

Results. *Tryptophan effect on pregnant rats and their fetuses.* The short-term effects of tube-feeding tryptophan on protein synthesis in the livers of pregnant rats and their fetuses are summarized in Table I. When pregnant rats (15 days) were fasted overnight and then tube-fed tryptophan (30 mg/100 g body wt) 1 hr before killing, the hepatic polyribosomes of the mothers and of the fetuses were essentially unchanged in comparison to their controls (Fig. 1). The hepatic polyribosomes of the fetuses appeared to be relatively more aggregated than the hepatic polyribosomes of the mothers and the polyribosomes peaked at the levels of sextomers rather than at higher levels in the case of the mothers. The *in vitro* [^{14}C]leucine incorporation into protein using postmitochondrial supernatants of livers was significantly increased due to tryptophan in the maternal but not in the fetal livers.

In order to determine whether or not changes in pool sizes of leucine in the livers may have influenced the [^{14}C]leucine incorporation into proteins (Table I), we measured in one experiment the free leucine levels in the livers of the pregnant mothers and their fetuses. The free leucine levels were changed in the livers of the pregnant mothers (+18.1%) and of the fetuses (−13.1%) of the tryptophan-treated animals compared to the

TABLE I. EFFECT OF TRYPTOPHAN ON THE HEPATIC POLYRIBOSOMES AND PROTEIN SYNTHESIS IN PREGNANT RATS AND THEIR FETUSES[a]

Group[b]	Tryptophan administered	Status of polyribosomes (Monomer–dimers/ total ribosomes ×100)	*In vitro* [^{14}C]leucine incorporation into protein using postmitochondrial supernatants (cpm/mg RNA[c] (%))
Mothers, pregnant	−	(6) 35.8 ± 1.57	(5) 100
Mothers, pregnant	+	(6) 33.9 ± 1.74	(5) 144.3 ± 6.0[d]
Fetuses (15 days)	−	(4) 28.6 ± 0.88[d]	(4) 100
Fetuses (15 days)	+	(3) 28.1 ± 1.17[d,e]	(4) 116.3 ± 36.8

[a] Number of experiments in parentheses. In each experiment livers from two mothers and 7–14 fetuses of each group were pooled. Tryptophan (30 mg per 100 g body wt) was tube-fed to mothers 1 hr before killing. Values presented as means ± SEM.

[b] Pregnant mothers weighed on the average 321 g and fetuses weighed on the average 186 mg.

[c] The mean value for the control mothers was 2612 and for the control fetuses was 2079.

[d] $P < 0.01$, compared to mothers + water (control) group.

[e] $0.05 > P > 0.01$, compared to mothers + tryptophan group.

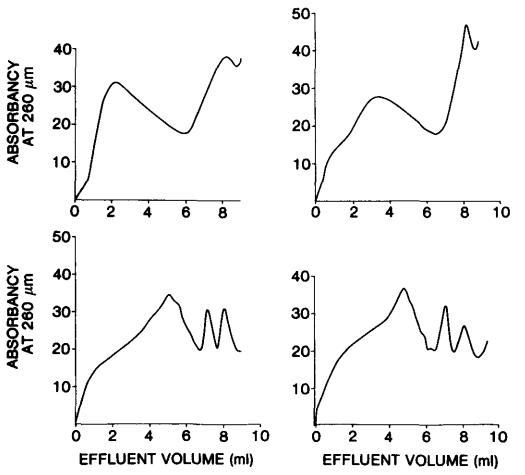

FIG. 1. Sucrose density gradient patterns of hepatic polyribosomes of deoxycholate-treated postmitochondrial supernatants of pregnant (15 days) rats (upper figures) and of their fetuses (lower figures) that were tube-fed water (left) or tryptophan (right) 1 hr before being killed.

control animals. Therefore, the increase of [^{14}C]leucine incorporation into protein using hepatic postmitochondrial supernatants of pregnant mothers treated with tryptophan (+44.3%) should not be attributed to the free leucine pool size. Likewise, the free leucine pool size of the livers of tryptophan-treated fetuses does not influence the insignificant change in [^{14}C]leucine incorporation into protein.

In view of the discrepancy between the findings with the polyribosomes (Fig. 1) and with *in vitro* protein synthesis (Table I) using postmitochondrial supernatants of livers of pregnant rats treated with tryptophan in comparison with controls, we investigated in three experiments the influence of microsomes and of cytosol (cell sap fraction) of the livers of control and experimental rats on *in vitro* protein synthesis. The microsomes of the livers of the experimental rats showed essentially little change (+9.9%) in comparison to those of control rats when using a constant cytosol in studying [^{14}C]leucine incorporation into protein. On the other hand, the cytosol of the livers of the experimental rats showed a 20.7% increase ($P < 0.05$) in comparison to that of control rats when using constant (control or experimental) microsomes in measuring [^{14}C]leucine incorporation into protein. As indicated in the preceding paragraph, the changes in free leucine levels would not account for the differences observed with cytosol. Thus, it appears that the cytosol and not the microsomes (polyribosomes) is chiefly responsible for the enhanced hepatic protein synthesis in the pregnant rats treated with tryptophan.

Tryptophan effect on lactating rats and their pups. In one preliminary experiment lactating rats (21 days postpartum) were fasted overnight and then tube-fed tryptophan (30 mg/100 g body wt) or water 1 hr before killing. The liver revealed an increase in polyribosomal aggregation (38.4 vs 51.6; monomer–dimers/total ribosomes × 100; of experimental compared to control) and a 41.3% increase in *in vitro* [^{14}C]leucine incorporation into protein (using postmitochondrial supernatants) in experimental compared to controls (Table II). In this experiment hepatic levels of free leucine were assayed and the levels were decreased (17.7%) in the experimental compared to the control group. This change could have been responsible for some of the 41.3% increase in [^{14}C]leucine incorporation into protein.

In subsequent experiments we used lactating rats which were 8–13 days postpartum and fed until killing and pups aged 8–13 days. In three experiments lactating rats were tube-fed tryptophan (30 mg/100 g body wt) or water 2 hr before killing and these rats also received [^{14}C]leucine intraperitoneally 30 min before killing. The hepatic polyribosomal profiles revealed greater aggregation in the experimental than in the control group (Fig. 2 and Table II). The *in vivo* [^{14}C]leucine incorporation into protein (sp act, cpm/mg protein) of the livers of the tryptophan-treated group was 29% higher than that in the control (water-treated) group (Table II). In these *in vivo* experiments, a rough estimate of the possible changes in pool size of leucine was determined by measuring the acid-soluble radioactivity of the livers of tryptophan-treated and control lactating rats. The results revealed essentially no difference (3.1% more in the experimental than in the control group).

In several experiments nursing pups (8–13 days old) were used. They received tryptophan (30 mg/100 g body wt) or water intra-

TABLE II. EFFECT OF TRYPTOPHAN ON THE HEPATIC POLYRIBOSOMES AND PROTEIN SYNTHESIS IN LACTATING RATS AND THEIR PUPS[a]

Group[b]	Tryptophan administered	Status of polyribosomes (monomer–dimers/ total ribosomes ×100)	[^{14}C]Leucine incorporation into protein[c]	
			In vivo (cpm/mg protein (%))	*In vitro* using postmitochondrial supernatants (cpm/mg RNA (%))
Mothers (8–13 days postpartum)	–	(3) 40.5 ± 1.42	(2) 100	(1) 100[d]
Mothers (8–13 days postpartum)	+	(3) 32.7 ± 1.71[e]	(2) 129.1 ± 11.8	(1) 141.3[d]
Pups (8–13 days)	–	(7) 37.4 ± 2.15		(7) 100
Pups (8–13 days)	+	(7) 39.4 ± 1.42		(7) 89.8 ± 3.8[e]

[a] Number of experiments in parentheses. In each experiment livers from two mothers and three to five pups of each group were pooled. Tryptophan (30 mg per 100 g body wt) was tube-fed to mothers 1 hr before killing. Values presented as means ± SEM.

[b] Lactating mothers weighed on the average 306 g and pups weighed on the average 12.6 g.

[c] The mean value for the control mothers was 641 and for the control pups was 3310.

[d] In this experiment, livers of mothers (21 days postpartum) were used.

[e] $0.05 > P > 0.01$.

peritoneally 1 hr before killing. It is apparent from the data in Table II and Fig. 2 that tryptophan did not stimulate the hepatic polyribosomes and protein synthesis (as measured *in vitro*) of the animals in comparison with controls. Hepatic protein synthesis actually was decreased in the experimental animals.

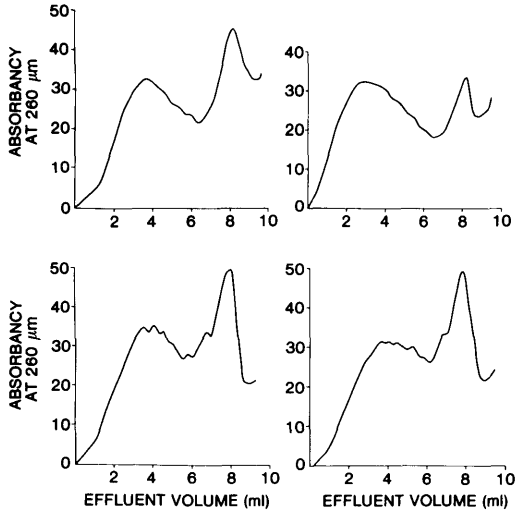

FIG. 2. Sucrose density gradient patterns of hepatic polyribosomes of deoxycholate-treated postmitochondrial supernatants of lactating (8 days postpartum) rats (upper figures) and of their pups (8 days old) (lower figures) that were treated with water (left) or tryptophan (right) 1 hr before being killed.

Discussion. In previous studies it has been reported that tryptophan could act to stimulate hepatic protein synthesis in animals in which certain endocrine alterations had been induced. Using mice which had been adrenalectomized 2 days before and then fasted overnight, we observed that the stimulation of hepatic protein synthesis due to tryptophan was independent of adrenal cortical hormones (10). Also, we reported earlier that tryptophan administered to rats that had hepatic protein synthesis stimulated by the administration of cortisone acetate showed further stimulation of hepatic protein synthesis (*in vitro*) and a shift of hepatic polyribosomes toward heavier aggregation (7). Jorgensen and Majumdar (11, 12) have reported that tube-feeding of tryptophan to well-fed adrenalectomized and adrenalectomized–diabetic rats stimulated amino acid incorporation into plasma albumin, transferrin, and fibrinogen and into liver ferritin *in vivo*. However, Cammarano *et al.* (13) reported that there was involvement of adrenal steroids in the changes of polyribosome organization during feeding of high levels of L-tryptophan. In the present study we have observed that pregnant and lactating rats responded to the administration of trypto-

phan with stimulated hepatic protein synthesis. Thus, it appears that tryptophan is able to have a stimulatory effect on hepatic protein synthesis in animals undergoing a variety of alterations in endocrine status.

It is of interest that the hepatic polyribosomes of pregnant rats did not reveal a significant shift in polyribosomal aggregation due to tryptophan ingestion yet there was an enhancement of hepatic protein synthesis (Table I and Fig. 1). In previous studies (5, 6, 10), a shift of hepatic polyribosomes toward heavier aggregation has paralleled the increase in hepatic protein synthesis due to tryptophan. In our earlier studies the livers of animals receiving tryptophan have always demonstrated that both the polyribosomes as well as the cytosolic components (cell sap fraction), when used to measure *in vitro* protein synthesis, showed a stimulatory effect (10). However, in the present study the main effect of tryptophan on the liver in the pregnant rat is on the cytosol in relation to the enhancement of protein synthesis.

In an attempt to explain why the tube-feeding of tryptophan to pregnant rat stimulated hepatic protein synthesis in the mother but not in the fetal liver, the presence of different levels of tryptophan in the blood in the two (maternal and fetal) circulations was considered. It has been reported that the level of most amino acids is considerably higher in fetal than in maternal blood (14–16). Tryptophan, as well as total amino acid concentration, in plasma decreases significantly with pregnancy (16) while in the fetus (21 days) the plasma tryptophan level is higher (65–91%) than in the mother (16, 17). This suggests that the plasma tryptophan level in the fetus may already be high so that an additional elevation via the maternal ingestion of tryptophan may not have a further stimulatory effect. In general, it has been reported that there is a faster turnover of tissue proteins in the fetus than in the adult (18). Also, it is known that the fetal liver is involved in active hematopoiesis and the numerous hematopoietic cells alter the cellular population in the fetal liver. These cells may not be responsive to tryptophan.

The reason why the livers of young (8–13 days old) pups do not respond to the administration of tryptophan with enhanced hepatic protein synthesis is not known. It is possible that such young pups still react like fetuses and may have altered circulating tryptophan levels in the livers. Indeed, it has been reported that the intracellular concentrations of free amino acids in the rat liver are low at time of birth, but by the 10th day they increase and peak at 19 days (19). Measurement of the concentration of free tryptophan shows a similar trend (19). Thus, an increase of hepatic level of tryptophan by intraperitoneal injection may be unable to further stimulate the already elevated protein synthesis which has been induced in response to the high liver free amino acid pools, including that of tryptophan. However, the administration of tryptophan to mature animals in which hepatic protein synthesis has already been stimulated by dietary (8, 20) or hormonal (7) means, as well as following partial hepatectomy (21), has been demonstrated to further enhance hepatic protein synthesis. Thus, one must search for other reasons, possibly such as immaturity or endocrine alterations, to account for the resistance of pups or fetuses to the stimulatory effect of tryptophan on the livers.

In earlier studies using normal mature animals, we have described that tryptophan has a stimulatory effect on (1) nucleocytoplasmic translocation of RNA (22, 23), (2) nuclear envelope nucleoside triphosphatase activity (24), (3) nuclear poly(A)polymerase activity (25), (4) hepatic glycoprotein synthesis (26), and also (5) the degree of binding of tryptophan to hepatic proteins, particularly in nuclear envelopes (27, 28). Whether or not such changes occur due to the administration of tryptophan in the livers of the fetus or pup needs to be investigated. Such studies may reveal differences in the responsiveness related to immaturity.

1. Matsueda S, Niiyama Y. The effects of excess amino acids on maintenance of pregnancy and fetal growth in rats. J Nutr Sci Vitaminol **28**:557–573, 1982.
2. Meier AH, Wilson JM. Tryptophan feeding adversely influences pregnancy. Life Sci **32**:1193–1196, 1983.
3. Paulson E, Robson JM, Sullivan FM. Teratogenic effect of 5-hydroxytryptamine in mice. Science **141**:717–718, 1963.
4. Hammer R. Embryotoxic effects of serotonin dur-

ing early pregnancy in the rat. Anat Rec **196:** 71A, 1980.
5. Sidransky H. Tryptophan: Unique action by an essential amino acid. In: Sidransky H, Ed. Nutritional Pathology. Pathobiochemistry of Dietary Imbalances. New York, Dekker, pp1–62, 1985.
6. Sidransky H. Effects of tryptophan on protein synthesis by liver. In: Scarpelli DG, Migaki G, Eds. Nutritional Disease: Research Directions in Comparative Pathobiology. New York, A. R. Liss, pp71–90, 1986.
7. Verney E, Sidransky H. Further enhancement by tryptophan of hepatic protein synthesis stimulated by phenobarbital or cortisone acetate. Proc Soc Exp Biol Med **158:**245–249, 1978.
8. Sidransky H, Verney E. Effect of tryptophan or phenobarbital administration on hepatic polyribosomes and protein synthesis in rats force-fed a complete or threonine-devoid diet. J Nutr **107:**730–738, 1977.
9. Sidransky H, Verney E, Murty CN. Effect of tryptophan on hepatic polyribosomal disaggregation due to hypertonic sodium chloride. Lab Invest **34:**291–297, 1976.
10. Sidransky H, Sarma DSR, Bongiorno M, Verney E. Effect of dietary tryptophan on hepatic polyribosomes and protein synthesis in fasted mice. J Biol Chem **243:**1123–1131, 1968.
11. Jorgensen AJF, Majumdar APN. Bilateral adrenalectomy: Effect of a single tube-feeding of tryptophan on amino acid incorporation into plasma albumin and fibrinogen in vivo. Biochem Med **13:**231–240, 1975.
12. Jorgensen AJF, Majumdar APN. Bilateral adrenalectomy: Effect of tryptophan force-feeding on amino acid incorporation into ferritin, transferrin, and mixed proteins of liver, brain and kidneys in vivo. Biochem Med **16:**37–46, 1976.
13. Cammarano P, Chinali G, Gaeteni S, Spandoni MA. Involvement of adrenal steroids in the changes of polysome organization during feeding of imbalanced amino acid diets. Biochim Biophys Acta **155:**302–304, 1968.
14. Snyderman SE. Protein and amino acid metabolism. In: Stave U, Ed. Physiology of the Perinatal Period. New York, Appleton-Century-Crofts, pp441–456, 1970.
15. Dancis J, Jansen V, Levitz M. Transfer across perfused human placenta. IV. Effect of protein binding on free fatty acid. Pediatr Res **10:**5–10, 1976.
16. Palou A, Arola L, Alemany M. Plasma amino acid concentrations in pregnant rats and in 21-day foetuses. Biochem J **166:**49–55, 1977.
17. Marquis SM, Leichter J, Lee M. Plasma amino acids and glucose levels in the rat fetus and dam after chronic maternal alcohol consumption. Biol Neonate **46:**36–43, 1984.
18. Mestyan J, Soltesz G. Maternal, fetal and neonatal amino acids and protein metabolism. In: Beard RW, Nathanielsz PW, Eds. Fetal Physiology and Medicine, Second Revised Edition. New York, Dekker, pp177–210, 1984.
19. Miller SA. Protein metabolism during growth and development. In: Munro HN, Ed. Mammalian Protein Metabolism. New York, Academic Press, Vol. **3:**pp183–233, 1969.
20. Sarma DSR, Verney E, Bongiorno M, Sidransky H. Influence of tryptophan on hepatic polyribosome and protein synthesis in non-fasted and fasted mice. Nutr Rep Int **4:**1–7, 1971.
21. Sidransky H, Verney E. Effect of nutritional alterations on protein synthesis in transplantable hepatomas and host livers of rats. Cancer Res **39:**1995–2000, 1979.
22. Murty CN, Verney E, Sidransky H. The effect of tryptophan on nucleocytoplasmic translocation of RNA in rat liver. Biochim Biophys Acta **474:**117–128, 1976.
23. Murty CN, Verney E, Sidransky H. In vivo and in vitro studies on the effect of tryptophan on translocation of RNA from nuclei of rat liver. Biochem Med **22:**98–109, 1979.
24. Murty CN, Verney E, Sidransky H. Effect of tryptophan on nuclear envelope nucleoside triphosphatase activity in rat liver. Proc Soc Exp Biol Med **163:**155–161, 1980.
25. Kurl RN, Verney E, Sidransky H. Effect of tryptophan on rat hepatic nuclear poly(A)polymerase activity. Fed Proc **46:**1000, 1987.
26. Sidransky H, Murty CN, Verney E. Evidence for the role of glycosylation of proteins in the tryptophan-induced stimulation of mRNA in rat liver. Lab Invest **54:**93–99, 1986.
27. Sidransky H, Murty CN, Verney E. Nutritional control of protein synthesis. Studies relating to tryptophan-induced stimulation of nucleocytoplasmic translocation of mRNA in rat liver. Amer J Pathol **117:**298–309, 1984.
28. Kurl R, Verney E, Sidransky H. Tryptophan-binding sites on nuclear envelopes of rat liver. Nutr Rep Int **36:**669–677, 1987.

Received June 22, 1987. P.S.E.B.M. 1988, Vol. 187.
Accepted November 5, 1987.