THE INFLUENCE OF TRYPTOPHAN ON HEPATIC POLYRIBOSOMES AND PROTEIN SYNTHESIS IN FASTED MICE*

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It has been reported that the livers of fasted rats respond rapidly to a single feeding of proteins or of a complete amino acid mixture with a shift in polyribosomes from lighter to heavier aggregates and with enhanced in vitro protein synthesis (Fleck, Shepherd and Munro, 1965; Webb, Blobel and Potter, 1966). In addition, Fleck, Shepherd and Munro, 1965 and Wunner, Bell and Munro, 1966 reported such an effect with fasted rats tube-fed a complete amino acid mixture but not when fed a complete amino acid mixture devoid of tryptophan. We observed previously that the livers of mice fasted for short intervals rapidly responded to tube-feeding of a casein hydrolysate with a similar change in polyribosome pattern and in protein synthesis (Staehelin, Verney and Sidransky, 1967). In a further extension of this study, we have now found that tryptophan alone, but not certain other single essential amino acids such as threonine, isoleucine, or methionine, is as effective as a complete amino acid mixture in shifting the hepatic polyribosome pattern from lighter to heavier aggregates and in enhancing in vitro hepatic protein synthesis in fasting mice. Our findings indicate that dietary tryptophan plays a special role in the regulation of polysome patterns and protein synthesis in the liver.

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Materials and Methods

Female mice, of CF1 strain from Carworth Farms weighing 15-20 g, were fasted overnight. The following morning the animals were tube-fed a complete amino acid mixture (Fleck et al, 1965), the same mixture but devoid of tryptophan, threonine, isoleucine or methionine, or one of the following single amino acids; tryptophan, threonine, isoleucine or methionine. Each animal received 100 mg of the complete or incomplete amino acid mixture in 1 ml distilled water by stomach tube. $NaHCO_3$ was substituted for the missing amino acid. In experiments where animals received single amino acids the amount administered in 1 ml of distilled water is stated in Table 2 and was equivalent to that contained in 100 mg of the complete amino acid mixture. The animals were killed by decapitation one hour after the feeding. In all experiments four animals were used in each group and their livers were pooled.

Postmitochondrial supernatant (PMS) of liver homogenate was prepared in Medium B (0.03 M Tris pH 7.5, 0.15 M NH_4Cl and 0.0035 M $MgCl_2$) after removal of unbroken cells, debris, nuclei and mitochondria and used for protein synthesis in vitro, or after addition of desoxycholate (0.7% final concentration), for direct sucrose density gradient analysis. Size distribution analysis of polyribosomes was performed with sucrose density gradient centrifugation using 15 ml of 0.3-1.1 M linear sucrose gradients in Medium B. An amount corresponding to approximately 68 mg liver was layered on top of 15 ml of a sucrose gradient. The gradient tubes were centrifuged in a Spinco SW 25.3 swinging bucket rotor in a Model L 2-65 Spinco ultracentrifuge at 25,000 rev/min for 150 minutes at 4° C. The bottoms of the tubes were punctured with a hypodermic needle, and the fluid was passed by continuous flow through a ultraviolet spectrophotometer (cell of 4 mm diameter) attached to a stripchart recorder which scanned absorbency at 260 mμ. A constant flow rate was maintained by a precision pump. In vitro C^{14}-amino acid incorporation into protein was assayed with PMS in Medium B. The incubation mixture

contained PMS (corresponding to approximately 44 mg liver), ATP 0.5 μmole, GTP 0.2 μmole, 5 μmoles phosphoenolpyruvate (tricyclohexylammonium salt), 3.25 μg crystalline pyruvate kinase, Tris buffer, NH_4Cl and $MgCl_2$ in the same concentration as specified earlier for Medium B and 0.25 μc C^{14}-leucine. C^{14}-leucine (Chicago Nuclear) with a specific activity of 30 mc/m mole or 165 mc/m mole was used. Incubation was carried out for one hour at 25° C. Hot trichloroacetic acid insoluble radioactivity was measured in a Packard liquid scintillation spectrometer.

Results and Discussion

The polyribosome patterns of the livers of all the various groups fell generally into one of two basic patterns (A or B) which are portrayed in Figure 1. All groups fed

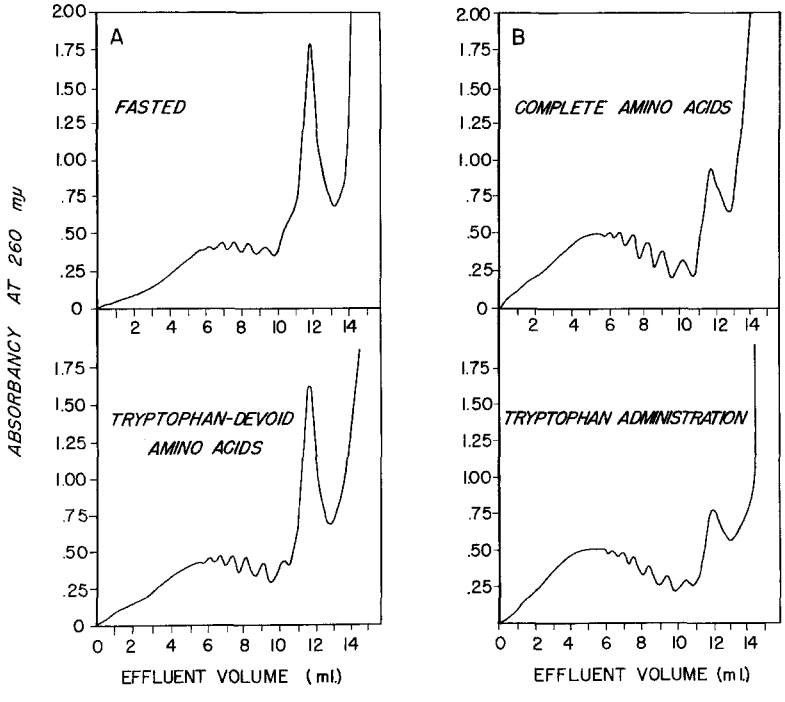

Figure 1: Hepatic polyribosomes of fasted mice (A, upper left) one hour after receiving by stomach tube a complete amino acid mixture (B, upper right), a tryptophan-devoid amino acid mixture (A, lower left) or tryptophan alone (B, lower left). Other conditions corresponding to A and B are described in Tables 1 and 2.

diets containing tryptophan, whether complete or incomplete with respect to other amino acids, showed the B polysome pattern, while the tryptophan-devoid group had the A pattern (Table 1). As seen in Table 1, the A pattern of polysomes was associated with depressed protein synthesis while all other groups had control or above control levels of C^{14}-leucine incorporation into protein.

In the second group of experiments (Table 2) in which single amino acids were tube-fed, tryptophan was unique in that only with this amino acid was a B polysome profile observed together with a considerably enhanced level of C^{14}-leucine incorporation into protein. The groups fed other amino acids all showed group A profiles and essentially little or no change of leucine incorporation into protein.

In one experiment of the first group of experiments (Table 1) the levels of free amino acids in the mouse livers of the five groups as well as of the fasted group were analyzed on a Spinco Amino Acid Analyzer, model 120B. These values enabled us to determine the specific activity of the free leucine in each incubation mixture. These values were very similar in all groups fed the different amino acid mixtures and therefore introduced essentially no corrections for the hepatic protein incorporation values of the different groups. After correction for the leucine pools in the fasted and complete amino acid mixture groups, the incorporation of C^{14}-leucine into proteins of the latter group was found to be 97% greater than that in the fasted group.

The results of this study as well as of other studies (Webb, Blobel and Potter, 1966; Sox and Hoagland, 1966; Staehelin, Verney and Sidransky, 1967) have indicated that the hepatic polyribosomes of fasted animals (rats or mice) are able to respond rapidly (shifting from lighter to heavier polyribosomes) following the ingestion of protein or complete amino acids. Our present study indicates the importance of tryptophan in eliciting the rapid hepatic polyribosome response as well as in enhancing hepatic protein synthesis when measured in vitro using C^{14}-leucine incorporation into proteins of cell-free

TABLE 1

IN VITRO INCORPORATION OF C^{14}-LEUCINE INTO HEPATIC PROTEINS OF FASTED MICE FED AN AMINO ACID MIXTURE WHICH WAS COMPLETE OR DEVOID OF SINGLE ESSENTIAL AMINO ACIDS AND KILLED ONE HOUR LATER

Amino Acid Mixture	No. of Experiments#	C^{14}-Leucine Incorporation: CPM Into Protein per Sample	C^{14}-Leucine Incorporation: Percentage of Control*	Polyribosome Profile (Figure 1)
Complete	7	2365±231x	100	B
Tryptophan-devoid	7	1548±169+	66	A
Threonine-devoid	6	1962±189	89	B
Isoleucine-devoid	4	2563±221	128	B
Methionine-devoid	4	2967±152	149	B

\# In each experiment determinations were performed in triplicate on pooled livers of four mice.

* The activity of the control flask containing components of the livers of mice fed the complete amino acid mixture was arbitrarily set at 100 in each experiment.

x Mean ± standard error of the mean.

\+ $P<0.02$

preparations. Such a response was not encountered after administration of other single essential amino acids, threonine, isoleucine or methionine. Likewise, an amino acid mixture lacking only tryptophan failed to elicit the response produced by a complete amino acid mixture or one devoid of each of the other three essential amino acids studied.

The action of amino acids, particularly of tryptophan, on the regulation of polysomes appears to be at the translational level rather than at the transcriptional level. Experiments with actinomycin support this concept. The rapid response of hepatic polysomes and in vitro protein synthesis of fasted mice to a single feeding of casein hydrolysate occurred even after inhibition of RNA synthesis due to actinomycin D (Staehelin, Verney

TABLE 2

IN VITRO INCORPORATION OF C^{14}-LEUCINE INTO HEPATIC PROTEINS OF FASTED MICE TUBE FED SINGLE ESSENTIAL AMINO ACIDS AND KILLED ONE HOUR LATER

Amino Acid Administration	Amount Used mg	No. of Experiments#	C^{14}-Leucine Incorporation CPM into Protein per Sample	Percentage of Control*	Polyribosome Profile (Figure 1)
None	0	9	3765±499×	100	A
L-tryptophan	4	9	7079±1059+	186	B
None	0	3	2687±643	100	A
L-threonine	5.6	3	2384±339	93	A
L-isoleucine	6.4	3	2573±576	99	A
L-methionine	6.4	3	3222±562	124	A

\# In each experiment determinations were performed in triplicate on pooled livers of four mice.
* The activity of the control flask containing components of the livers of fasted mice was arbitrarily set at 100 in each experiment.
× Mean ± standard error of the mean.
\+ $P<0.02$

and Sidransky, 1967). We have likewise found that pretreatment with actinomycin D did not alter the induced response in fasted mice given tryptophan alone. In fasted rats, Fleck, Shepherd and Munro, 1965 and Wunner, Bell and Munro, 1966 found that actinomycin did not influence the difference in response of animals fed the complete amino acid mixture and of those fed the amino acid mixture devoid of tryptophan.

The importance of tryptophan deficiency in rabbit reticulocytes has recently been described by Hori, Fisher and Rabinovitz 1967. Of eight amino acids tested, only the omission of tryptophan resulted in polyribosome disaggregation. The authors attached

special significance to tryptophan since it is located exclusively near the amino-terminal ends of both chains of rabbit globulins. Whether tryptophan has a similar role in many of the proteins of the mouse liver remains to be established. Since tryptophan is present only in small amounts (much lower than most of the other amino acids) in mammalian hepatic proteins (Block and Weiss, 1956), its presence might be of special importance in the regulation of hepatic protein synthesis on the level of translation especially following deprivation produced by fasting.

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