DAILY RHYTHMS IN HEPATIC POLYSOME PROFILES AND TYROSINE TRANSAMINASE ACTIVITY: ROLE OF DIETARY PROTEIN

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Abstract.—Hepatic polysome profiles vary in untreated rats as a function of time of day. The ratio of polysomes to total ribosomes increases from 50 to 73 per cent in darkness. There is also a daily rhythm in tyrosine transaminase activity which resembles but does not coincide with the polysome rhythm. Both rhythms are dependent on the cyclic ingestion of dietary protein, and disappear in rats given a protein-free diet.

The activities of several hepatic enzymes have been shown to vary diurnally in untreated rats.[1] If rats are kept under alternating 12-hour light and dark periods each day, tyrosine transaminase activity is almost four times as great early in the dark period as it is during the light period.[2–4] The rhythm in hepatic tyrosine transaminase is largely the consequence of the tendency of the rat to consume dietary protein cyclically, and is extinguished if protein (or tryptophan) is omitted from the diet,[3, 4] or if the feeding cycle is perturbed.[2] The synthesis of proteins, such as tyrosine transaminase, requires that the messenger RNA coded for these proteins be bound with large aggregates of ribosomes to form polysomes.[5, 6] Dietary amino acids are necessary for polysome aggregation, and availability of tryptophan seems to be the limiting essential amino acid in this regard.[7–9] Hence, it has been suggested that the tyrosine transaminase rhythm might reflect cyclic synthesis of the enzyme, resulting from an underlying rhythm in polysome aggregation.[3] Data have been obtained which demonstrate the existence of such a rhythm in polysome aggregation.

Materials and Methods.—Sprague-Dawley male rats weighing 100–150 gm and caged individually were kept under controlled lighting (lights on and off at 12-hr intervals). Approximately 50–100 foot-candles of light were provided by cool white fluorescent bulbs. The rats were given access *ad libitum* to Purina Chow or to special synthetic diets containing various levels of protein from 24 to 0%. In some cases, the diets were made up in agar gel.[10] In the experiments with these synthetic diets, the amount of food eaten during an interval of several hours was estimated by weighing the food container and its contents periodically. In the case of the agar gel diets, corrections were made for the decrease in the weight of the food which resulted from loss of water to the atmosphere. Groups of three to six animals were decapitated by a guillotine at various times of the 24-hr cycle. A portion of each liver was used immediately for the preparation of polysome profiles; another portion was frozen at −20°C and later assayed for tyrosine transaminase activity.

To obtain polysome profiles,[11] samples of liver were chilled in ice-cold Medium A, 0.375 *M* sucrose in TKM buffer (0.05 *M* Tris, 0.025 *M* potassium chloride, 0.005 *M* magnesium chloride, pH 7.6). All subsequent operations were performed at temperatures near 0°C. After being minced with scissors, the livers were gently homogenized in two

volumes of Medium A in a Kontes homogenizer with Teflon pestle (clearance 0.010 in.), and antiferritin serum was added in the proportion of one to ten. The post-mitochondrial supernatant fraction was prepared by centrifugation at 13,000 × *g* for 20 min in a SS34 rotor using the Sorvall centrifuge. Sodium deoxycholate was added to the supernatant, after it was filtered through glass wool, to give a concentration of 1%. Samples of 0.2 ml post-mitochondrial supernatant were applied to linear 10–50% sucrose (in TKM) gradients to the top of which a layer of 0.2 ml 5% sucrose in TKM had been added to trap soluble proteins. The gradients were spun in a SW 50 rotor at 38,000 rpm for 90 min using the L2 Spinco ultracentrifuge. The extinction profile at 260 mμ was recorded automatically with a Gilford Model 2000 spectrophotometer, as the gradient was displaced upwards through a flow cell. Polysome profiles were quantitated by using a compensating planimeter to measure the area under the monosome, disome, and polysome peaks and then calculating the percentage of the total area due to the polysomes and that portion due to the monosomes and disomes. The variation in the ratio of polysomes to total ribosomes with time of day was used to demonstrate a diurnal rhythm in polysome aggregation.

In an experiment designed to test the effect of ribonuclease on the breakdown of polysomes to monosomes and disomes during the above procedure, ribonuclease inhibitor was prepared according to Blobel and Potter.[12] A post-mitochondrial supernatant (S_2) was prepared by homogenizing rat liver ten strokes in Medium A and then centrifuging the homogenate in the SS34 rotor for 10 min at 17,000 × *g*. The post-mitochondrial supernatant fraction was centrifuged in a Spinco Ti 50 rotor for 4 hr at 40,000 rpm, and the resulting supernatant fraction (S_3) was frozen at −20°C. When testing the ribonuclease activity of S_3, a sample of liver was homogenized in Medium A as usual to obtain polysome profiles, while a sample of the same liver was homogenized in a sucrose-S_3 medium (9 parts Medium A to 1 part S_3).

Hepatic tyrosine transaminase activity was assayed by a modification[2] of the method of Diamondstone.[13] Tyrosine transaminase activity was shown to be linear under the conditions of the assay.

The data were analyzed statistically by analysis of variance.

Results.—A diurnal rhythm in polysome aggregation would be expected to manifest itself as a tendency for the ratio of polysomes to total ribosomes to vary predictably as a function of time of day. On the basis of previous studies on the rhythm in tyrosine transaminase activity, it was anticipated that polysomal aggregation in rat liver might increase around the time of the onset of the dark period. Hence, livers from groups of six rats fed *ad libitum* on laboratory chow diet were taken for examination at the fifth and eleventh hours of the light period, and the fourth and eighth hours of the dark period.

A definite rhythm was observed in polysome profiles. The ratio of polysomes to total ribosomes was significantly greater during the dark period than during the daylight hours (Fig. 1 and Table 1). This peak coincided with maximal activity in tyrosine transaminase. Although diurnal changes in the polysome and enzyme rhythms were generally similar at other points in the cycle, the temporal patterns were not coincident. The polysome ratio was greater at L5 than at L11, while tyrosine transaminase activity did not change significantly during this interval. Further, the polysome ratio remained elevated between D4 and D8, whereas transaminase activity fell significantly.

To examine the possibility that hepatic ribonuclease, acting during preparation to dissociate the polysomes, was responsible for the observed polysome rhythm, polysomes prepared from livers taken at L4 and D4 were examined following addition of extra ribonuclease inhibitor at the homogenization step, as suggested

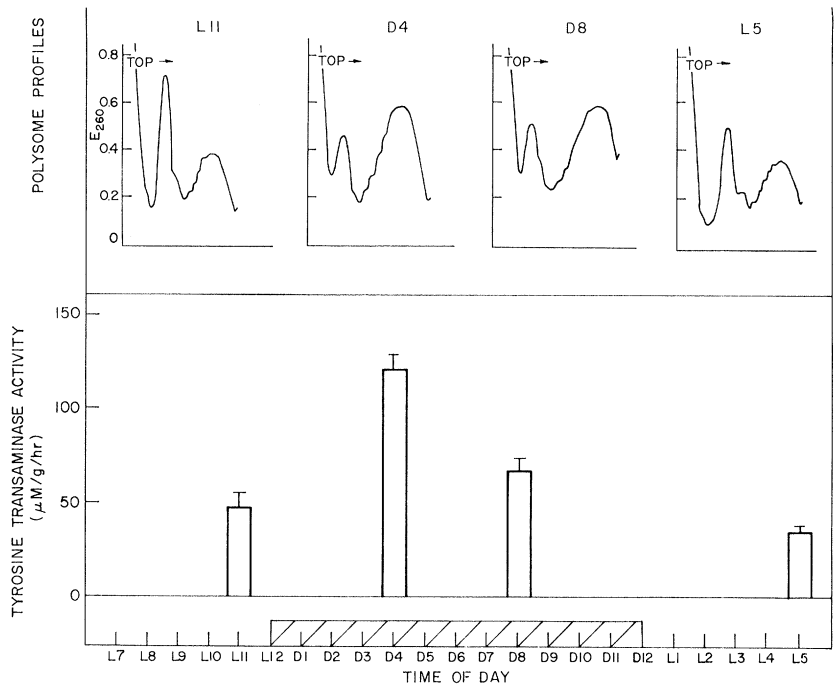

FIG. 1.—Hepatic polysome profiles and tyrosine transaminase activity among groups of six rats fed Purina Chow and killed at each time indicated. Vertical bars indicate standard errors of the mean, and hatching along the abscissa indicates the daily dark period.

by Blobel and Potter.[12] Profiles prepared from rats killed at L4 contained 48 per cent polysomes when prepared with ribonuclease inhibitor, and 39 per cent without it; profiles prepared from rats killed at D4 contained 65 per cent polysomes with the inhibitor, and 58 per cent without it. These changes are minor compared with the amplitude of the diurnal rhythm. Hence, although there may have been breakdown of polysomes during preparation, this factor does not obscure or affect the polysome rhythm.

To examine the dependence of the polysome rhythm on the cyclic ingestion of dietary protein, rats were fed various synthetic diets of known protein composition, and their polysome patterns and tyrosine transaminase activities were then examined. Animals previously maintained on Purina Chow were given a synthetic dry diet containing 6 per cent protein for one day. On the next day, half of the animals received a 0 per cent protein dry diet, and half a 24 per cent protein dry diet, starting early in the light period (L3). Six rats eating the 24 per cent protein diet and three fed the protein-free diet were killed at each time interval reported, and the polysome profiles and transaminase activities were evaluated for individual animals.

Rats on 24 per cent protein diets showed rhythms in polysome profiles and tyrosine transaminase activity similar to those observed in the animals given Purina Chow (Table 2). In the absence of dietary protein, rat liver showed no

TABLE 1. *Polysome profiles and tyrosine transaminase activity of rats given access to Purina Chow.*

	Polysome profiles (% polysomes)	Tyrosine transaminase activity (μM/g/hr)
L5	60 ± 1.7	35 ± 2.2
L11	50 ± 3.0	49 ± 9.6
D4	73 ± 2.9	122 ± 9.7
D8	67 ± 3.1	66 ± 8.4

Rats were kept under controlled illumination and given access to Purina Chow *ad libitum* for one week prior to the experiment. Six animals were killed at each time. Results are given as mean ± standard error in all tables. Both diurnal rhythms were significant at $P < 0.001$.

TABLE 2. *Polysome profiles and tyrosine transaminase activity of rats given access to 0% or 24% protein dry diets.*

	Polysome Profiles (% Polysomes)		Tyrosine Transaminase Activity (μM/g/hr)	
	0% protein*	24% protein†	0% protein‡	24% protein§
L8	51 ± 1.4	44 ± 4.9	28 ± 6.1	37 ± 6.2
L12	29 ± 5.0	...	70 ± 29.2	...
D4	32 ± 2.5	62 ± 3.3	63 ± 9.4	117 ± 6.5
D8	34 ± 2.7	...	50 ± 1.4	...

Rats kept under controlled illumination for one week received a 6% protein dry diet for 24 hr starting early in the light period (L3). On the following day half of the animals were given access to a 0% protein dry diet, and the rest received a 24% protein dry diet. Groups of 6 (24% protein diet) or 3 (0% protein diet) animals were killed at each time tested.

* Significant decrease from L8 ($P < 0.001$); no significant diurnal rhythm.

† Significant diurnal rhythm ($P < 0.025$).

‡ No significant diurnal rhythm.

§ Significant diurnal rhythm ($P < 0.001$).

increase in heavy polysomes during the dark period; instead, the relative abundance of polysomes decreased significantly and remained repressed. The tyrosine transaminase rhythm was eliminated among these animals.

Besides the dry synthetic diets used in the above experiments, agar-gel based diets were used to correlate protein intake with polysome profiles and tyrosine transaminase activity. Groups of three or six rats prepared as above (i.e., animals given a diet containing 6 per cent protein for one day prior to the experiment) received synthetic gel diets containing 0, 18, or 24 per cent protein starting early in the light period (L5). Polysome profiles and tyrosine transaminase activity were examined at the eleventh hour of the light period and the fourth and eighth hours of the dark period.

A small but significant rhythm in polysome pattern was observed among the rats eating the 24 per cent protein gel diet;[14] however, the characteristics of this rhythm differed from those seen in livers of animals fed dry 24 per cent protein diets, in that gel diets produced a polysome:total ribosome ratio that was greater at L11 (64 ± 4.5%) than at D4 (56 ± 2.0%). The ratio at D8 was greater (73 ± 0.8%) than at the other two times studied. The tyrosine transaminase rhythm was also abnormal in animals on the 24 per cent protein gel diet in that enzyme activity did not fall significantly between D4 and D8.[14] No polysome rhythm was observed among rats eating gel diets containing no protein or 18 per cent protein.

All groups of animals studied displayed diurnal rhythms in food consumption similar to those reported previously.[3, 4] Rats generally ate about twice as much or more per hour early in the dark period as they did in the middle of the light period (Table 3). Animals given a protein-free diet consumed only 74 per cent as much food per day as rats given the dry diet containing 24 per cent protein.

Discussion.—The rhythm in polysome profiles reported in this study appears to be related to the protein content of the food and the tendency of the rat to eat cyclically. The influence of nutritional factors on polysome profiles has been examined in depth by Munro and colleagues.[5] Postprandial influx of amino acids through the portal vein to the liver enhances protein synthesis, diminishes breakdown of proteins, and entrains more free ribosomes into polysomes.[15, 16] The effects of protein intake on polysome patterns appear to be mediated by the availability of dietary tryptophan.[8, 9] On the other hand, our data show that feeding rats nonprotein diets mainly composed of carbohydrates fails to sustain the polysome rhythm, which is replaced by a reduction in polysome aggregates throughout the period of observation. This correlates with earlier studies by Munro and others showing that the carbohydrate in diets low in protein causes the liver to lose significant amounts of protein, presumably through a depression of protein synthesis.[17]

It is also of significance that the rhythm in liver tyrosine transaminase appears to be generated by tryptophan intake, inasmuch as rats consuming an amino acid mixture without tryptophan fail to display the usual rhythm, and, furthermore, hepatic free tryptophan content increases significantly several hours before the daily rise in enzyme activity.[18] Thus our data indicate that dietary tryptophan may be unique in the regulation of tyrosine transaminase activity in rat liver because of its special role in the aggregation of hepatic polysomes, an index of intensity of protein synthesis.

The changes in the two rhythms among rats fed the agar-based gel diets may result from the rates at which amino acids are released and absorbed from the nondigestible gels; these must be mechanically disintegrated in order to release

TABLE 3. *Rates of food intake (in grams per hour) in rats that were d protein and nonprotein diets.*

(*a*) *Dry diets*

Protein (%)	L3-L8	L8-L11	L11-D2
0	0.45 ± 0.04(6)*	0.81 ± 0.13(3)	...
24	0.52 ± 0.22(6)	0.62 ± 0.22(3)	0.92 ± 0.07(3)
	D2-D4	D4-D12	
0	...	...	
24	1.40 ± 0.60(3)	1.38 ± 0.40(3)	

(*b*) *Gel diets*

	L5-L11	L11-D4	L11-D8
0	0.62 ± 0.23(18)	1.03 ± 0.21(3)	1.20 ± 0.19(3)
18	0.52 ± 0.09(9)	1.97 ± 0.21(3)	2.22 ± 0.13(3)
24	0.53 ± 0.15(9)	1.70 ± 0.44(3)	1.84 ± 0.44(3)

* The number of rats averaged to determine the rate of food intake in each interval is given in parentheses.

nutrients, and thus may retard absorption. This retarded and more uniform flow of amino acids to the liver could explain unusually large amounts of polysomes found at L11, and the retention of large aggregates at D8. This hypothesis would also explain the slow rate at which tyrosine transaminase activity declined after attaining its peak at D4. It is evident from these results that the consistency of the diet may be an important factor in demonstrating the hepatic rhythms.

Polysome profiles and tyrosine transaminase activity are similar in certain respects: Both show diurnal rhythms among rats given access *ad libitum* to Purina Chow or to a synthetic dry diet containing 24 per cent protein; both rhythms are absent in rats maintained on a protein-free diet. The aggregation of polysomes and the rhythmic increase in tyrosine transaminase activity are also both influenced by tryptophan in the diet. Nevertheless, these rhythms are not perfectly isomorphic, possibly because polysomes represent all of the heterogeneous messenger-ribosomal RNA complexes in the cell, while tyrosine transaminase is a homogeneous protein with a known short half-life ($t^1/_2$ = 2–3 hours).[19] Thus, it would not be expected that the polysome and tyrosine transaminase rhythms should be perfectly coincident.

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[1] Wurtman, R. J., in: *Mammalian Protein Metabolism*, ed. H. N. Munro, in press.
[2] Wurtman, R. J., and J. Axelrod, these PROCEEDINGS, **57,** 1594 (1967).
[3] Wurtman, R. J., W. J. Shoemaker, and F. Larin, these PROCEEDINGS, **59,** 800 (1968).
[4] Zigmond, M. J., W. J. Shoemaker, F. Larin, and R. J. Wurtman, *J. Nut.*, **98,** 71 (1969).
[5] Munro, H. N., *Federation Proceedings*, **27,** 1231 (1968).
[6] Wettstein, F. O., T. Staehelin, and H. Noll, *Nature*, **197,** 430 (1963).
[7] Baliga, B. S., A. W. Pronczuk, and H. N. Munro, *J. Mol. Biol.*, **34,** 199 (1968).
[8] Pronczuk, A. W., B. S. Baliga, J. W. Triant, and H. N. Munro, *Biochim. Biophys. Acta*, **157,** 204 (1968).
[9] Sidransky, H., M. Bongiorno, D. R. S. Sarma, and E. Verney, *Biochim. Biophys. Acta*, **87,** 525 (1967).
[10] (*a*) Each kilogram of protein-free dry diet contained the following: dextrose, 272 gm; sucrose, 217 gm; dextrin, 272 gm; corn oil, 150 gm; Harper's salt mix, 40 gm; choline (50%), 4 ml; alphacel, 35 gm; vitamin mix (3), 10 gm. (*b*) 6 and 24% protein dry diets contained per kilogram the following differing from (*a*): casein, 60 or 240 gm; dextrose, 247 or 187 gm; sucrose, 207 or 147 gm; dextrin, 247 or 187 gm. (*c*) Gel diets contained per kilogram the following differing from dry diets: casein, 0, 60, 180, or 240 gm; dextrose, 272, 247, 207 or 187 gm; sucrose, 217, 107, 167, or 147; dextrin, 272, 247, 207, or 187 gm; water, 1000 ml; agar, 35 gm; and no alphacel.
[11] Drysdale, J. W., and H. N. Munro, *Biochim. Biophys. Acta*, **138,** 616 (1967).
[12] Blobel, G., and V. R. Potter, *J. Mol. Biol.*, **28,** 39 (1967).
[13] Diamondstone, T. I., *Anal. Biochem.*, **16,** 395 (1966).
[14] The 24% protein gel diet showed significant rhythms in polysome profiles ($P < 0.01$) and tyrosine transaminase activity ($P < 0.005$). No significant rythhms were observed with the 18% protein gel in polysome profiles and tyrosine transaminase activity or with the 0% protein gel diet in tyrosine transaminase activity. The 0% protein gel diet showed a significant decrease in percentage of polysomes after L11 ($P < 0.025$).
[15] Fleck, A., J. Shepherd, and H. N. Munro, *Science*, **150,** 628 (1965).
[16] Wunner, W. H., J. Bell, and H. N. Munro, *Biochem. J.*, **101,** 417 (1966).
[17] Munro, H. N., in *Mammalian Protein Metabolism*, ed. H. N. Munro, and J. B. Allison (New York: Academic Press, 1964), vol. 1, p. 440.
[18] Civen, M., C. Wilson, C. B. Brown, and D. Granner, Abstract No. 69, Fifty-first Meeting of the Endocrine Society, June 27–29, 1969, New York, p. 65.
[19] Shimke, R. T., in *Mammalian Protein Metabolism*, ed. H. N. Munro, in press.