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Hepatic Tyrosine Transaminase Rhythm: Interaction of environmental lighting, food consumption and dietary protein content [1]

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ABSTRACT Hepatic tyrosine transaminase activity was measured over a 24-hour period in animals maintained under three environmental situations: normal lighting (lights on from 9 AM to 9 PM) and ad libitum dietary protein; reversed lighting (lights on from 9 PM to 9 AM) and ad libitum dietary protein; and normal lighting and a nonprotein diet, with protein added at various times during the day. In each case, the increase in tyrosine transaminase activity was not observed until after the initial ingestion of protein. These results support the hypothesis that the daily rhythm in the activity of this enzyme in rat liver is generated by the cyclical ingestion of protein.

The activity of the hepatic enzyme tyrosine transaminase varies fourfold as a function of the time of day (1–3). When rats are kept under normal environmental lighting (e.g., lights on from 6 AM to 6 PM) and given access to food and water ad libitum, enzyme activity is at a minimum early in the morning, begins to rise late in the afternoon and reaches a maximum several hours after the onset of darkness.

A similar rhythm exists in food intake (4). Rats eat relatively little until late in the light period, and consume most of their food in the hours following the onset of darkness. Rats acutely deprived of food show no rhythm in tyrosine transaminase activity (5). Moreover, rats fed a nonprotein diet also show no enzyme rhythm, although the feeding rhythm persists (5). It appeared, therefore, that the tyrosine transaminase rhythm is generated by the cyclical ingestion of dietary protein. To examine this hypothesis further, we altered the rhythm in protein intake and observed the consequent effects on the enzyme rhythm.

In the rat, the feeding rhythm is synchronized by light. An alteration in the time of the daily light exposure is followed within a few days by a similar change in the pattern of food intake. If an increase in the rate of protein consumption provides the necessary signal for the daily rise in tyrosine transaminase, such a change in the feeding rhythm should be accompanied by a parallel change in the enzyme rhythm. Similarly, if protein consumption is altered independently of the feeding rhythm (by giving rats maintained under a standard lighting schedule protein-free food ad libitum and then replacing this food with a protein diet at different times during the day), the rise in tyrosine transaminase activity should follow protein ingestion and not simply the feeding rhythm. Experiments are described which test these hypotheses.

MATERIALS AND METHODS

Female rats of the Sprague-Dawley strain, weighing 180 to 200 g at the onset of the experiment, were obtained commercially.[2] Animals were housed individually and exposed to 50 to 70 ft-c of illumination from cool-white fluorescent bulbs. Measurements of food and water consumption were made by weighing the containers and correcting for the loss due to evaporation. At intervals during a 24-hour period,

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[2] Charles River Laboratories, Inc., Wilmington, Mass.

groups of three or four animals subjected to each experimental treatment were decapitated; the livers were removed and stored on dry ice until assayed. Tyrosine transaminase was assayed by a modification (1) of the method of Diamondstone (6). Diets containing zero percent or 18% protein were prepared as previously described (5).

RESULTS

In experiment 1, Purina Laboratory Chow [3] and water were available ad libitum, and food and water intake were measured at the beginning and end of the daily light period (9 AM to 9 PM). Food consumption showed a marked 24-hour rhythm; about 80% of the total daily intake was consumed during the dark period. The lighting schedule was then reversed; lights were now on from 9 PM to 9 AM. Animals were killed at intervals during day 3 or 6 of exposure to the new lighting regimen.

The rhythms in feeding behavior and tyrosine transaminase activity appeared to shift phase at the same rate: after 3 days under reversed lighting conditions, the rats ate 40% of their food during the dark period, and showed a 4- to 6-hour shift in the time of peak tyrosine transaminase activity. After 5 days of reversed lighting, rats ate 77% of their daily food intake during the new dark period, and on day 6 the peak in enzyme activity had shifted by a full 12 hours (fig. 1). Other animals killed after 11 days of exposure to reversed lighting showed a rhythm similar to that seen after 6 days, but with less variance.

In experiment 2, animals under a normal lighting schedule (lights on from 7 AM to 7 PM) previously given access to Purina Laboratory Chow were given a nonprotein diet beginning at 10 AM. This was replaced by an isocaloric diet containing 18% casein at 1 PM, 6 PM, 1 AM or not at all. The expected rhythm in food intake was observed in each group, although animals eating the protein-free diet ate somewhat less than animals given the diet containing protein (table 1).

The livers of rats which ate the nonprotein diet showed a low level of tyrosine transaminase activity throughout the 24-hour period; no marked rise in tyrosine transaminase occurred until after this diet was replaced by 18% casein. Thus, animals receiving protein at 1 PM showed a significant increase in enzyme activity by 6 PM, animals given protein at 6 PM had elevated tyrosine transaminase activity by 10 PM, and animals receiving protein at 1 AM showed a similar increase by 5 AM (fig. 2).

DISCUSSION

It has previously been suggested (5) that the daily rhythm in tyrosine transaminase activity resulted from the cyclical intake of dietary protein, and was not a direct response to the time of day, the light cycle, or an endogenous "biological clock." The experiments reported here support this hypothesis.

If the rhythm in food intake generates the rhythm in transaminase activity, experimental alterations of the former should be accompanied by simultaneous changes in the latter. This was observed in experiment 1: reversal of the light cycle was followed by coupled changes in the timing of the food and enzyme rhythms. Moreover, if the generating signal within food is protein, dissociating the time of day that the animal consumes protein and the time that it elects to eat should produce a parallel dissociation between the transaminase rhythm and the rhythm in food intake. In experiment 2, animals initially fed a nonprotein diet showed a feeding rhythm but no enzyme rhythm. The rise in enzyme activity was not observed until after protein was added to the diet.

Under normal laboratory conditions, the liver of the rat receives a large influx of dietary protein via the portal circulation for part of every 24-hour period. A parallel rhythm in tyrosine transaminase activity appears to be one of the adaptive responses of the liver to this influx (5, 7). Tyrosine transaminase activity is normally high during the time of day that the animal ingests tyrosine (i.e., in dietary protein) at the greatest rate. The enzyme rhythm may thus have the effect of dampening the large fluctuations which might otherwise occur in the tyrosine concentrations of blood or liver.

[3] Ralston Purina Company, St. Louis, Mo.

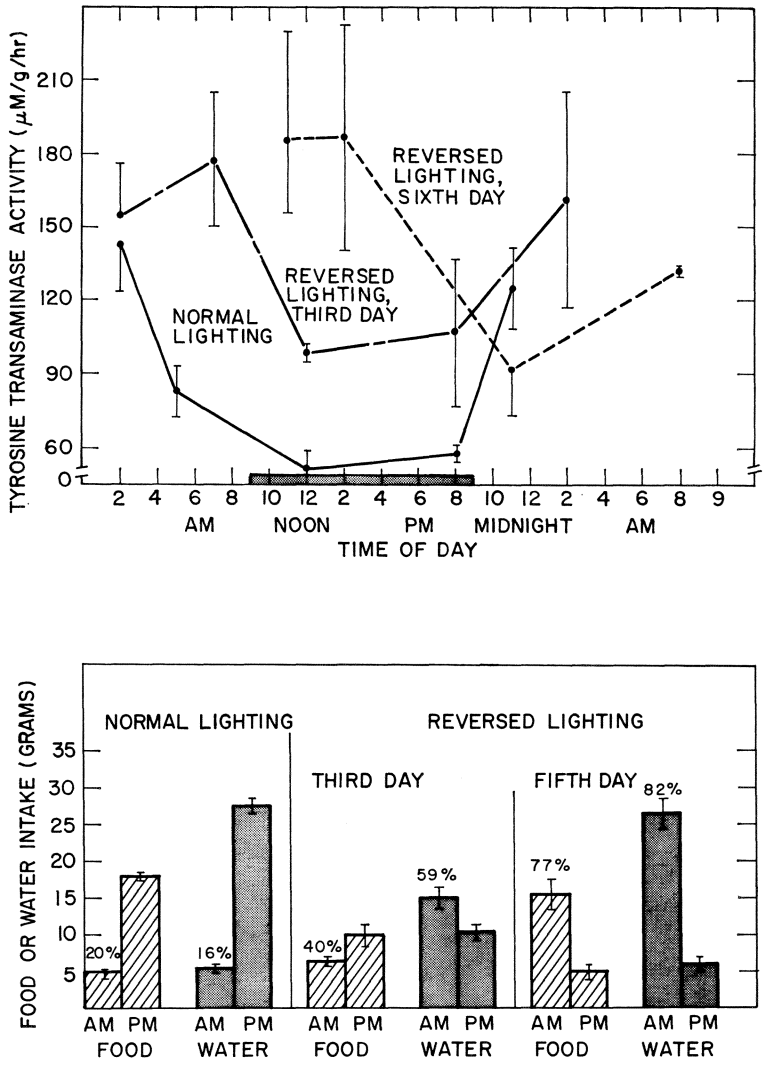

Fig. 1 Tyrosine transaminase activity and food and water intake in animals under normal (9 AM to 9 PM) or reversed (9 PM to 9 AM) lighting conditions. a) Tyrosine transaminase activity after 3 or 6 days of reversed lighting is compared with enzyme activity in rats kept under normal lighting. Each point represents the average of three animals. Shaded horizontal bar along abscissa indicates hours of darkness under new (i.e., reversed) lighting conditions. b) Food and water intake during the light and dark hours are compared for the last day of normal lighting, and days 3 and 5 of reversed lighting. Each bar represents the average of 12 (reversed lighting) or 30 (normal lighting) animals. Vertical lines represent the estimated standard error of the mean.

The tyrosine transaminase rhythm is a reflection of the laboratory feeding habits of the rat. An animal with a different feeding pattern (such as the human) might be expected to have a different enzyme rhythm. Although maximal hepatic enzyme activity is normally observed at night in the adult rat, it has been shown that rats fed during the hours of 8 AM and 12 noon have an early morning peak in enzyme activity (7) and that before weaning, the suckling rat (whose feeding activity is maximal during the early morning hours) shows maximal tyrosine transaminase ac-

TABLE 1

Rate of food intake in rats fed protein and nonprotein diets [1]

	10 AM to 1 PM	1 PM to 6 PM	6 PM to 10 PM	10 PM to 1 AM	1 AM to 5 AM	5 AM to 10 AM
	g/hr	*g/hr*	*g/hr*	*g/hr*	*g/hr*	*g/hr*
Nonprotein diet [2]	0.61±0.21 [3]	0.53±0.07	1.05±0.17	1.02±0.23	0.69±0.13 [4]	
Protein from 1 PM	—	0.66±0.11	1.10±0.13	1.67±0.22	0.92±0.72	0.97±0.15
Protein from 6 PM	—	—	1.22±0.18	1.43±0.26	1.92±0.42	0.57±0.23
Protein from 1 AM	—	—	—	—	2.38±0.19	0.50±0.40

[1] All animals received a nonprotein diet at 10 AM. This was replaced by a similar diet supplemented with 18% casein at 1 PM, 6 PM, 1 AM or not at all.
[2] Data represent average food intake of all animals receiving nonprotein diet during each time interval.
[3] Mean ± estimated SE of the mean.
[4] Food intake was measured only once between 1 AM and 10 AM in this group.

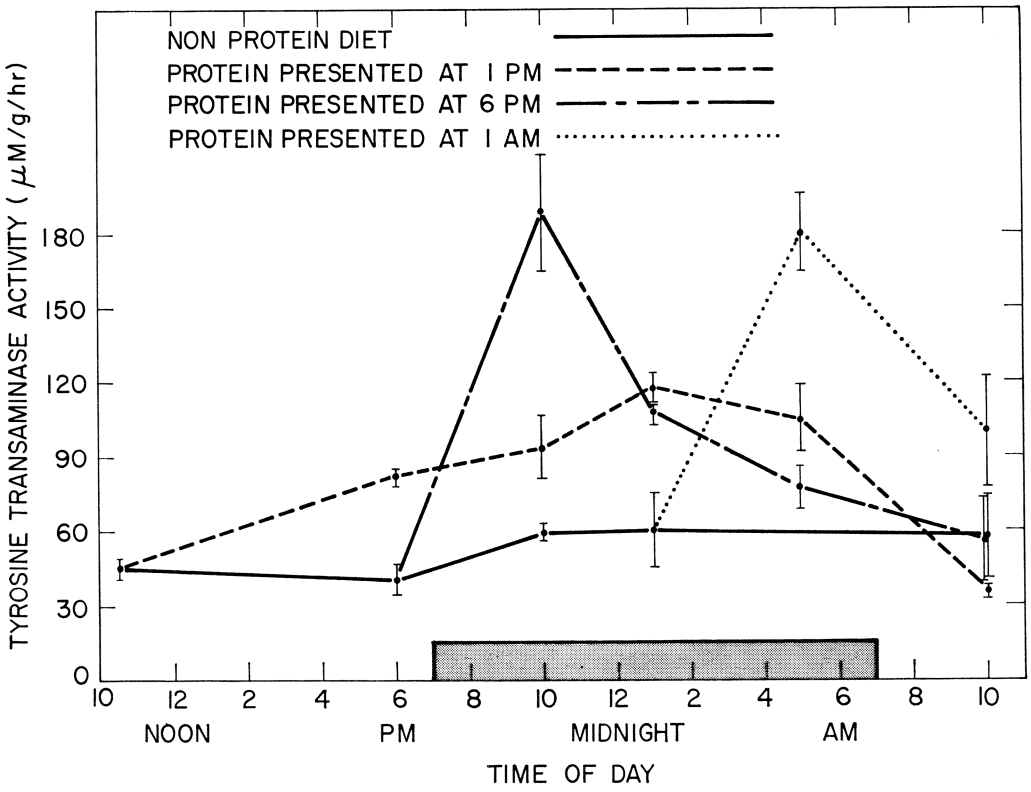

Fig. 2 Tyrosine transaminase activity in animals given a nonprotein diet at 10 AM, and then an 18% protein diet at 1 PM, 6 PM, 1 AM or not at all. Lights were on from 7 PM to 7 AM. Each point represents the average of three or four animals. Lines connect enzyme activity determined the first time after protein was made available with activity observed the previous time that it was sampled. Vertical lines represent the estimated standard error of the mean.

tivity during the light period (8). Preliminary data suggested that rats trained to eat at a constant rate throughout a 24-hour period would show no rhythm in tyrosine transaminase activity.[4]

Tyrosine transaminase activity increased only slowly among rats receiving protein at 1 PM. In contrast, rats first given pro-

[4] Cohn C., and R. J. Wurtman, unpublished observations.

tein 5 hours later showed a much faster rise (fig. 2). This difference may be related to the relative rates at which protein was initially ingested: the rate of food consumption is much slower in the afternoon than in the early evening (table 1). It might also result from an underlying rhythm in the extent to which the enzyme can be induced.

The small but significant variation in transaminase activity seen among animals receiving no protein (fig. 2) has been noted in previous experiments (4). Because the concentration of corticosterone in the blood varies rhythmically (1, 9) and because the administration of pharmacological doses of this compound is known to elevate tyrosine transaminase activity (10), it is possible that the steroid rhythm may make some contribution to the enzyme rhythm. Such a contribution, however, would normally be of minor significance compared with the effects of dietary protein: the amplitude of the transaminase rhythm is unaffected following hypophysectomy or adrenalectomy, so long as rats continue to have access to dietary protein (1).

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