*MECHANISM OF THE DAILY RHYTHM IN HEPATIC TYROSINE TRANSAMINASE ACTIVITY: ROLE OF DIETARY TRYPTOPHAN**

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That there are daily fluctuations in the activity of hepatic tyrosine transaminase when rats are kept under normal laboratory conditions (i.e., lights on 12–14 hr per day; access *ad libitum* to Purina Chow) has been demonstrated[1] and confirmed.[2,3] Enzyme activity is almost four times as great several hours after nightfall as it is in the morning. Although tyrosine transaminase activity is increased by treatment with certain adrenocortical steroids whose concentration in the blood also varies rhythmically,[4,5] the enzyme rhythm is not produced by the hormone rhythm, inasmuch as it persists in adrenalectomized or hypophysectomized animals.[1]

This report presents evidence that the tyrosine transaminase rhythm is generated both by the tendency of the rat to consume most of its food during certain hours of the day and by the presence of amino acids, especially tryptophan, in the diet.

Methods.—Sprague-Dawley female rats weighing 140–180 gm were kept under controlled lighting (lights on from 6 A.M. to 6 P.M.) and given access *ad libitum* to Purina Chow or to special diets prepared as described below. In some experiments the amount of food eaten during an interval of several hours was estimated by weighing the food container and its contents periodically. Corrections were made for the decrease in the weight of the food which resulted from loss of water to the atmosphere. Light was provided by cool-white fluorescent bulbs; animals were exposed to 50–70 ft-c of illumination. Groups of four to six animals were killed by neck fracture at various times during the day; their livers and, in some experiments, a cardiac blood sample were removed and chilled on dry ice. The livers were assayed for tyrosine transaminase activity by a modification[1] of the method of Diamondstone.[6] In some experiments the data obtained using this spectrophotometric method were compared with those from an isotopic assay;[7] there were no significant differences between these results. The tyrosine concentrations of liver and blood were determined using the method of Waalkes and Udenfriend;[8] hepatic tryptophan was measured by the method of Denckla and Dewey.[9] Hypophysectomized animals were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Results.—Relation between hepatic tyrosine transaminase rhythm and concentrations of tyrosine in blood and liver: Tyrosine injections can produce a rise in tyrosine transaminase activity in the intact animal.[5] Hence, the afternoon rise in enzyme activity may result from a previous increase in the availability of the substrate, tyrosine. This increase might, in turn, be reflected by a rise in blood or liver tyrosine concentration several hours before the daily increase in transaminase activity. To examine this possibility, we sacrificed groups of intact or hypophysectomized rats at intervals and determined the tyrosine concentrations of plasma and liver. Hepatic tyrosine transaminase activity was also measured.

Both intact and hypophysectomized animals demonstrated the expected daily rise in tyrosine transaminase activity (Figs. 1 and 2). In neither type of

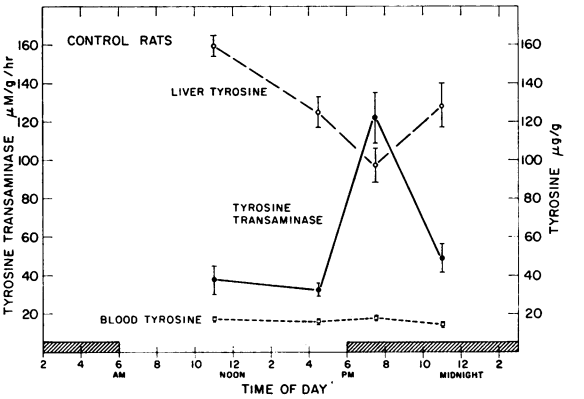

FIG. 1.—Relation between blood and liver tyrosine concentrations and hepatic tyrosine transaminase activity in unoperated rats fed Purina Chow. In all figures, vertical bars indicate standard error of the mean, and hatching along abscissa indicates daily dark period.

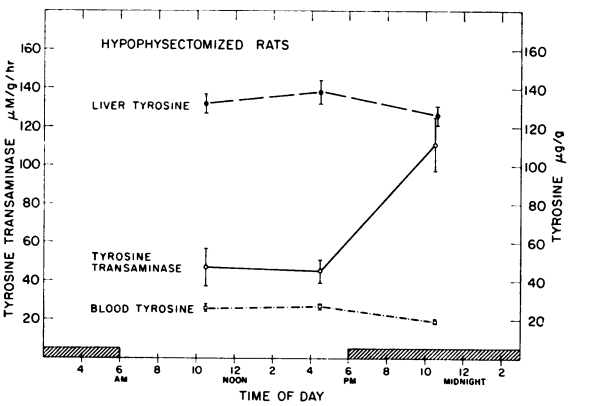

FIG. 2.—Relation between blood and liver tyrosine concentration and hepatic tyrosine transaminase activity in hypophysectomized rats fed Purina Chow.

experimental animal, however, was this elevation preceded by a rise in plasma or liver tyrosine concentration. Plasma and liver tyrosine levels tended to fall *after* the transaminase activity had risen (Figs. 1 and 2), suggesting that more of the amino acid was being metabolized. Statistically significant decreases were observed in the hepatic tyrosine content of intact rats and in the plasma tyrosine concentration of hypophysectomized animals.

Relation between transaminase rhythm and concentration of tryptophan in liver: Administration of tryptophan to intact rats has also been reported to increase tyrosine transaminase activity.[5] We therefore performed experiments in which hepatic tryptophan content and tyrosine transaminase activity were measured in animals killed at different times of day. A typical study is illustrated in Figure 3. Hepatic tryptophan concentration was lowest at 9 A.M., and by 2 P.M. it had risen to levels of 14.5 μg/gm. Thereafter it did not change significantly for 12 hours, whereupon it fell to 9 A.M. levels. Tyrosine transaminase activity was lowest in this experiment at 2 P.M.; it rose fivefold during the next ten hours. Hence, an increase in hepatic tryptophan levels preceded by several hours the daily elevation in tyrosine transaminase activity.

Dependence of transaminase rhythm upon availability of dietary protein: To determine whether the availability of dietary tryptophan or of other amino acids

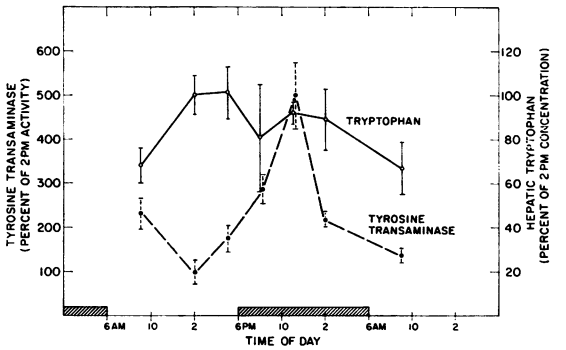

FIG. 3.—Relation between hepatic tryptophan concentration and tyrosine transminase activity in animals fed Purina Chow.

was a prerequisite in the genesis of the tyrosine transaminase rhythm, we prepared three sets of animals. All were given access to Purina Chow for several days, and a group of six control rats was killed at 9 A.M. At 11 A.M. one set was deprived of all food; the second set was given access *ad libitum* to a protein-free diet;[10a] and the third set was maintained on Purina Chow. Groups of animals from each experimental set were killed at intervals over the next 36 hours, and their livers examined for tyrosine transaminase activity. Starved animals showed no daily rhythm in the activity of the transaminase (Fig. 4). Enzyme activity remained at low levels for about 24 hours, and then rose sharply. Animals given the low-protein diet had low hepatic transaminase activity at all times studied. This activity tended to vary over a narrow range, but the variation was considerably less than that observed in the animals kept on Purina Chow (i.e., a fivefold rise between 2 P.M. and 11 P.M.) and did not demonstrate clear 24-hour periodicity. Access to dietary protein is, then, an absolute requirement for the production of the tyrosine transaminase rhythm.

Persistence of transaminase rhythm in rats given a synthetic mixture of amino acids, and its disappearance with the absence of tryptophan: Dietary proteins are normally degraded to their constituent amino acids in the intestines. These subunits are then absorbed and delivered to the liver via the portal circulation. In order to determine whether amino acids arising from dietary protein provide the signal which generates the daily rise in hepatic tyrosine transaminase activity,

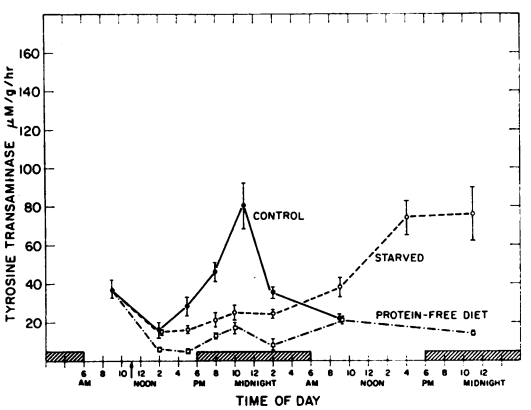

FIG. 4.—Hepatic tyrosine transaminase activity at different times of day in rats given access to special diets starting at 11 A.M.

animals previously maintained on Purina Chow were fed the protein-free diet fortified with 18 amino acids, including all of those previously determined to be necessary for the rat.[10b,c] In three separate experiments, control animals were killed at 9 A.M., and others were presented the experimental diet starting at 10:30 A.M. A typical study is illustrated by Figure 5. Animals given access to

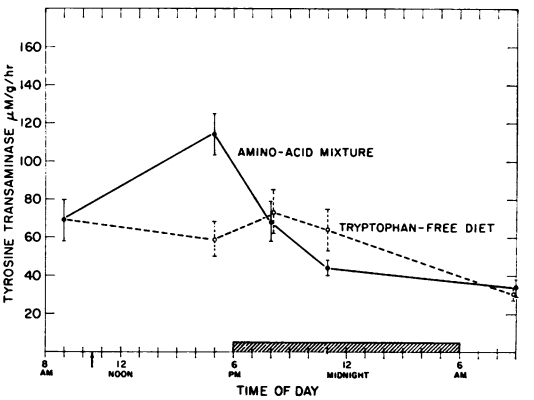

FIG. 5.—Hepatic tyrosine transaminase activity at different times of day in rats given access to special diets starting at 10:30 A.M.

amino acids instead of to protein showed a definite rhythm in hepatic tyrosine transaminase activity; however, the time of peak enzyme activity came several hours before that seen in animals fed protein. Enzyme activity at 5 P.M. was more than threefold greater than at 9 A.M. the following morning. When rats were maintained on the amino acid diet for two days, a similar afternoon rise in enzyme activity was observed on the second day of treatment.

When tryptophan was omitted from the amino acid mixture, the daily rise in tyrosine transaminase activity did not occur, and no rhythm was demonstrated (Fig. 5).

Failure of diets containing normal levels of tyrosine or tryptophan alone to generate transaminase rhythm: Rats previously maintained on Purina Chow were divided into four sets and given access to different diets starting at 10:30 A.M. The first set received a diet which contained no protein or amino acids ("0% protein"); the diets of the second and third sets were fortified with 1/2 per cent tryptophan and 1 per cent tyrosine, respectively. The last set received 18 per cent casein. Groups of animals from each set were killed at intervals and their livers assayed for tyrosine transaminase. Only those from the last set showed the characteristic rhythm in transaminase activity (Fig. 6). Among all the other animals, enzyme activity was low and unrelated to time of day.

Production of rhythmic changes in transaminase activity by a 6 per cent tryptophan diet: Diets which contained normal concentrations of tyrosine and tryptophan may have failed to produce oscillations in tyrosine transaminase activity because, in the absence of other amino acids, large fractions of the tyrosine or tryptophan were utilized for transamination or gluconeogenesis. Therefore, rats were given access to a diet very rich in tryptophan so that even if most of the amino acid were transformed in the body, large amounts would still be left to act on the liver. Starting at 10:30 A.M., animals were presented with a

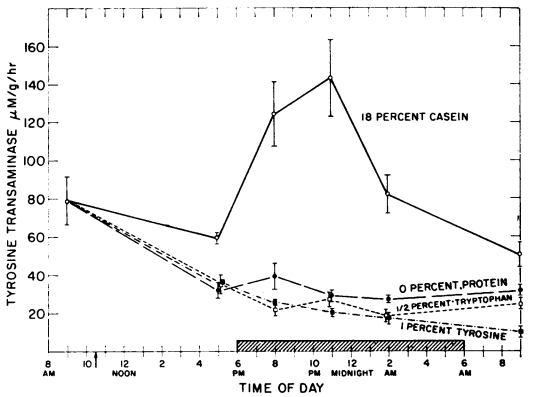

FIG. 6.—Hepatic tyrosine transaminase activity at different times of day in rats given access to special diets starting at 10:30 A.M.

protein-free diet fortified with 6 per cent tryptophan. Groups of rats were killed at intervals, and hepatic tyrosine transaminase activity and tryptophan content were measured. The concentration of tryptophan in the liver rose between 10:30 A.M. and 5 P.M. (Fig. 7). Subsequently, tyrosine transaminase activity increased almost twofold. Between 8 P.M. and 11 P.M. the concentration of tryptophan in the liver was low; during this interval tyrosine transaminase activity also fell. Between 11 P.M. and 9 A.M. hepatic tryptophan levels rose considerably; this elevation was also accompanied by an increase in tyrosine transaminase activity.

We could not perform a similar experiment involving a diet rich in tyrosine because of the toxicity of this amino acid.[11]

Pattern of food intake of rats fed various experimental diets: The rat is a nocturnally active animal that consumes most of its food during the hours of darkness.[12] To prove that dietary amino acids provide the signal responsible for the hepatic tyrosine transaminase rhythm, it is necessary first to show that the animals do, in fact, eat during the hours preceding the enzyme rise. We therefore measured food consumption at several times of the day, using several of the experimental diets.

No significant differences existed among the different diets, either in the total amount of food eaten per day or in the temporal pattern of its consumption.

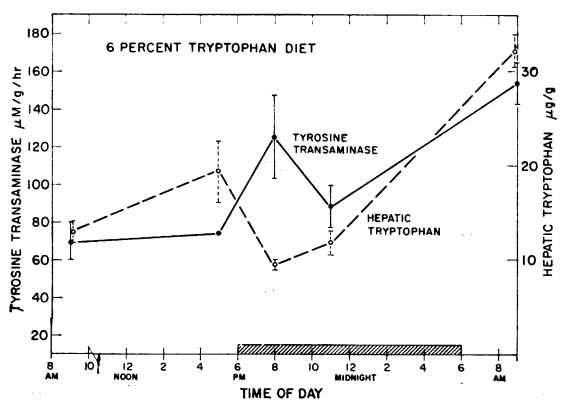

FIG. 7.—Relation between hepatic tryptophan concentration and tyrosine transaminase activity in animals given access to a diet containing 6% tryptophan starting at 10:30 A.M.

During the end of the daily light period, animals ate about 0.58–0.70 gm of food per hour; this increased about twofold soon after the onset of darkness, and then declined toward the end of the dark period. Significant amounts of food are ingested during the hours preceding the daily rise in tyrosine transaminase activity.

Discussion.—These observations demonstrate that the rat must have access to dietary protein or amino acids in order to undergo an afternoon rise in hepatic tyrosine transaminase activity. The transaminase rhythm appears to be generated by the interaction of both an endogeneous and an exogeneous factor: e.g., by the tendency of the rat to consume most of its food during certain hours of the day, and by the presence of protein or amino acids in its foodstuffs. The participation of both endogenous and exogenous factors in the genesis of the tyrosine transaminase rhythm appears to distinguish this cycle from other daily rhythms in mammals whose mechanisms have been elucidated. Most daily rhythms persist when all known cyclic inputs from the environment (e.g., light, temperature, access to food) have been removed.[13–16] The environmental cycles function only as *Zeitgebern*, or "time-givers"; they influence the phasing of the rhythm and keep its period from drifting too far from exactly 24 hours. A second group of daily rhythms appears to be generated entirely by a particular environmental cycle: that of daylight and darkness. Thus, when rats are blinded or kept in continuous darkness, 24-hour rhythms in pineal norepinephrine content[17] and melatonin synthesis[18] are rapidly extinguished. The absolute requirement of the tyrosine transaminase rhythm for exogenous protein suggests that this rhythm should not be termed *circadian*.[19]

The endogenous component of the tyrosine transaminase rhythm (the tendency of the rat to eat cyclically) probably responds to the lighting cycle as to a *Zeitgeber*, in a manner similar to other endogenous rhythms. Preliminary experiments indicate that the daily rise in tyrosine transaminase activity can be shifted 12 hours by reversing the lighting schedule, provided sufficient time is allowed for the daily eating cycle to adjust to this new schedule.[20] Moreover, Potter and his colleagues[21] have shown that when rats are maintained on a 60 per cent protein diet presented for only eight hours each day (9 A.M. to 5 P.M.; animals kept in darkness from 9 A.M. to 9 P.M.), the daily rise in tyrosine transaminase activity also occurs six to seven hours after the onset of feeding.

The afternoon rise in tyrosine transaminase activity is preceded by an increase in hepatic tryptophan concentration (Fig. 3). This enzyme rise is not observed when tryptophan is omitted from the diet (Fig. 5), and large amounts of ingested tryptophan can generate oscillations in tyrosine transaminase activity in the absence of other amino acids. These observations all suggest a special relationship between tryptophan and the transaminase rhythm. This is perhaps surprising, considering that tryptophan is a poor substrate for the enzyme.[22]

The rat liver contains relatively small quantities of tryptophan; its concentration of this amino acid is usually no greater than that found in the blood.[9] Hence, the postprandial increase in the delivery of tryptophan to the liver via the portal vein is probably of greater physiologic significance than that of other amino acids whose stores in liver are larger. Munro and his colleagues[23] have demon-

strated that dietary tryptophan enhances protein synthesis in the rat liver by a specific mechanism which involves the aggregation of the synthesizing unit, the polysome. Tryptophan appears to be unique among the amino acids in this action.[24,25] The tyrosine transaminase rhythm may thus result from cyclic changes in hepatic tryptophan content, changes which result from the ingestion of protein and which allow preformed messenger RNA to direct protein synthesis more efficiently at certain times of day.

Dietary tryptophan alone may initiate the processes responsible for the afternoon rise in tyrosine transaminase activity. However, the lack of other amino acids might quickly limit the formation of the enzyme protein, unless a full amino acid diet were presented or other amino acids were generated *in vivo* by protein breakdown and transamination. If the transaminase rhythm is generated by dietary tryptophan, it seems reasonable to anticipate that other hepatic enzymes will be found whose activities vary with a daily rhythm in response to protein ingestion.

The physiological significance of the tyrosine transaminase rhythm is not yet clear. It has been demonstrated, however, that the concentration of tyrosine (the chief substrate for this enzyme) in human blood also varies as a function of time of day.[26] It is possible that the metabolic fate of dietary tyrosine depends upon the hour of its ingestion.

Summary.—Studies are described on the mechanism of the daily rhythm in hepatic tyrosine transaminase activity. The daily rhythm is rapidly extinguished when the rat is deprived of dietary protein. It can be restored when the animal is given access to a mixture of amino acids, but only if this mixture includes tryptophan. The afternoon rise in enzyme activity is preceded by an increase in hepatic tryptophan concentration and followed by a decline in the concentration of tyrosine in blood and liver. In the absence of other amino acids, dietary tryptophan alone can produce gross oscillations in tyrosine transaminase activity, provided this amino acid is presented in high concentrations (6%). The rat normally consumes significant quantities of food during the hours which precede the daily rise in tyrosine transaminase activity.

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[10] (*a*) Each kilogram of protein-free 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; water, 1000 ml; agar, 35 gm; vitamin mix, 10 gm. Each kilogram of vitamin mix contained the following: vitamin A acetate, 1 gm; vitamin E acetate, 40 gm; vitamin K, 0.5 gm; thia-

mine HCl, 1 gm; riboflavin, 2 gm; niacin, 5 gm; vitamin C, 20 gm; pyridoxine, 1 gm; PABA, 10 gm; biotin, 0.05 gm; calcium pentothenate, 5 gm; folic acid, 0.2 gm; inositol, 20 gm; vitamin B_{12}, 5 gm; sucrose, 890.27 gm. (*b*) The full amino acid diet contained the following, per kilogram: dextrose, 240 gm; sucrose, 185 gm; dextrin, 240 gm; Harper's salt mix, 40 gm; corn oil, 150 gm; vitamin mix (as in 10*a*), 10 gm; choline (50%), 4 ml; water, 1000 ml; agar, 35 gm; amino acid mixture (as in ref. 10*c*), 95 gm. (*c*) Khairallah, E. A., and G. Wolf, *J. Nutr.*, **87**, 469 (1965).

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