

## Influence of Feeding Habits and Adrenal Cortex on Diurnal Rhythm of Hepatic Tyrosine Transaminase Activity<sup>1</sup> (34497)

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The hepatic enzyme of the rat, tyrosine transaminase, L-tyrosine-2-oxoglutarate aminotransferase, TT (EC 2.6.1.5), exhibits a 4 fold variation in activity over a 24-hr period when the animal has free access to food and water and is kept in an environment of 12 hr of light and 12 of darkness (1-3). The enzymatic activity is at a nadir early in the light phase, commences to rise at the end of this period, and reaches a maximum some 4-6 hr after the start of darkness. It has been demonstrated that dietary protein is the primary input responsible for this enzyme rhythm (4, 5, 8) and that the diurnal light cycle (7, 8) and adrenocortical hormones (1, 2, 6, 7) also influence the rhythm.

Light (or darkness) is considered to have only an indirect effect on the enzyme, in that the rat normally commences to eat toward the end of the light phase and consumes most of its food early in the dark period. This type of response is observed regardless of when in a 24-hr period light and darkness are imposed on the animals (8). If the rat is starved, or the diet is protein-free (4, 8), the cyclic activity of the transaminase is largely extinguished; when protein or amino acids are included in the diet, a rise in activity occurs soon after this nitrogen source is ingested, to reach a peak some 6 hr later.

That adrenal cortical hormones can influence TT activity has been shown in several ways—(a) enzyme activity increases after animals are injected with adrenocortical steroids (6) and (b) the amplitude of the

24-hr TT rhythm is diminished in the adrenalectomized animal (1, 2). The rate at which the rat adrenal secretes glucocorticoids also varies diurnally; peak secretion occurs several hours before the daily maximum in TT activity among undisturbed animals given access to protein *ad libitum*. Further information on the significance of cyclic adrenocortical secretion and the cyclic pattern of food ingestion in generating the TT rhythm could be obtained if aliquots of the day's ration of nutrients were "continuously" fed and eaten in the presence and absence of the adrenals. We have performed such experiments and have observed that by abolishing the periodicity of food intake, the amplitude of the TT rhythm is decreased in intact animals and no significant rhythm is seen in adrenalectomized ones.

*Methods.* Male Holtzman rats, received when 120-140 g in body weight, were used. They were individually caged in an air-conditioned room and offered a semisynthetic diet (9) containing 18.5% protein either *ad libitum* or in 24 feedings/day at hourly intervals (10). The total daily food consumption of the animals fed hourly approximated the average intake of the control rats given free access to food; the 24 feedings were equal in amount, and essentially all of the food presented was consumed. The normal animals had free access to distilled water and the adrenalectomized rats had access to a 1% saline solution.

Three experiments were performed. In experiments I and III, animals were exposed to light from 0600 to 1800 hours; in experiment II, light was provided between 1800 and 0600 hours. Experiments I and II util-

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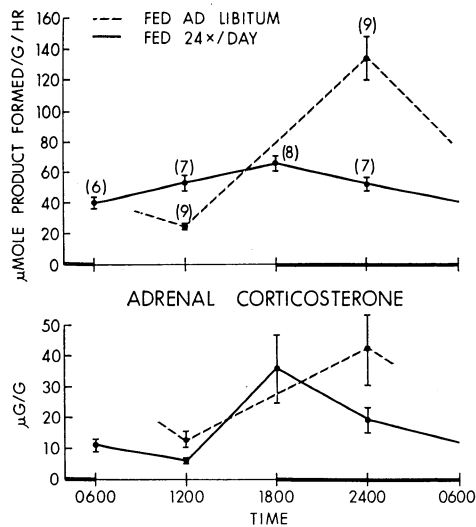


FIG. 1. Hepatic tyrosine transaminase activities and adrenocortical corticosterone contents at different times of the day in rats eating *ad libitum* (A. L.) and in those fed hourly (H). In this and subsequent figures, the numbers above the points indicate the number of animals killed at a given time period, the vertical bars indicate the standard error of the means, and the black areas along the abscissa denote the daily dark period. In this experiment, the  $p$  values for the differences between nadir and peak for each feeding schedule were: (a) for tyrosine transaminase activities: (A. L.),  $p = <.001$ , (H),  $p = <.025$ ; (b) for corticosterone contents: (A. L.),  $p = <.05$ , (H),  $p = <.01$ .

ized intact rats; experiment III was accomplished with adrenalectomized ones. In all experiments, animals eating *ad libitum* were compared with those fed 24 times a day. After feeding under the above conditions for 3–4 weeks, the rats were killed by decapitation at 0600, 1200, 1800, and 2400 hr; a portion of the liver and both adrenals (when present) were removed, frozen in dry ice, and subsequently analyzed for tyrosine transaminase (11) or corticosterone content (12).

**Results.** In confirmation of previous findings (1), intact rats with free access to food exhibited cyclicity in both hepatic tyrosine transaminase activity and adrenal corticosterone content. Rats fed 24 times a day continued to display a significant rhythm in adrenal corticosterone content that did not differ from the rhythm observed in animals eating *ad libitum*. The amplitude of the daily

rhythm in hepatic TT activity was markedly decreased, however, from 400 to 50% by the hourly feedings (Fig. 1). Reversing the lighting schedule caused a parallel change in the time of peak enzyme activity among both *ad libitum* and wheel-fed rats (experiment II, Fig. 2). In the absence of the adrenal (experiment III, Fig. 3), the amplitude of the tyrosine transaminase rhythm was reduced in animals eating *ad libitum*, and in animals fed hourly no significant rhythm was seen.

**Discussion.** The results presented above are in accord with previous data indicating that the rhythm in tyrosine transaminase activity is generated primarily by the periodicity of food intake (4, 5, 8). Other factors which might theoretically generate the rhythm—time of day, environmental lighting, (except as it influences food intake), and “biological clocks” appear to have only a secondary influence upon it.

Normal animals which had eaten *ad libitum* displayed a tyrosine transaminase rhythm whose amplitude and temporal characteristics were similar to those previously described (1–3). Peak TT values were seen near the midpoint of the dark period, a relationship that is probably attributable to a high rate of protein consumption in the preceding few hours (4, 5, 8). Normal ani-

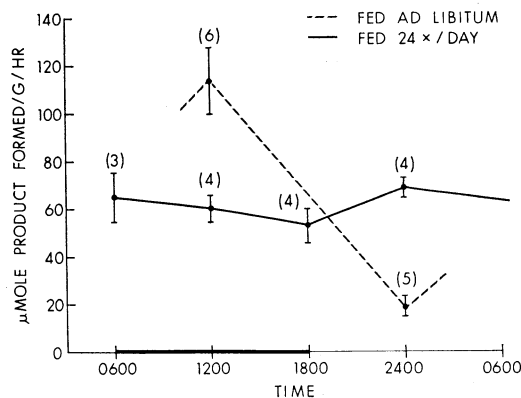


FIG. 2. Hepatic tyrosine transaminase activities at different times of the day in rats eating *ad libitum* and in those fed hourly, with a reversed lighting schedule. The  $p$  values for the differences between nadir and peak for each feeding schedule were: (A.L.)  $p = <.001$  and (H),  $p = <.05$ .

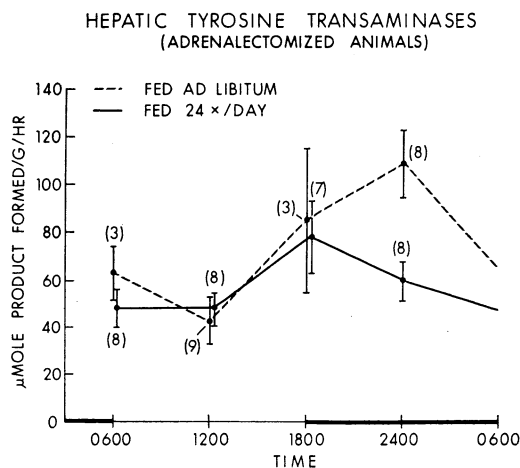


FIG. 3. Hepatic tyrosine transaminase activities at different times of the day in adrenalectomized rats eating *ad libitum* and in those fed hourly. The *p* values for the differences between nadir and peak for each feeding schedule were: (A. L.),  $p < .05$  and (H), no significant difference.

mals fed hourly exhibited a tyrosine transaminase rhythm of markedly reduced amplitude. In this sense, these animals resembled rats which had been starved or fed acutely a protein-free diet (4). These data are compatible with the hypothesis that the cyclic delivery of amino acids to the rat liver via the portal vein is the major, but not the only, factor which causes hepatic TT activity to display diurnal rhythmicity.

In the absence of the adrenal cortex, the rats eating *ad libitum* demonstrated a TT rhythm that differed from normal only in having a smaller amplitude. This difference could be attributed to a lack of rhythmic stimulation of the liver by adrenal steroids or by the decreased food intake that characterizes the adrenalectomized preparation (13). The role of the adrenocortical secretory rhythm in producing the daily changes in activity could be examined conveniently in animals fed at hourly intervals; in these rats the contribution of the rhythm in protein consumption to TT rhythmicity was removed, and the separation of hormonal from other endogenous factors which might produce the rhythm was exposed. Removal of the adrenals caused the remaining TT rhythm to be extinguished, suggesting that the adrenal secretory cycle was a major fac-

tor in producing the rhythm in wheel-fed intact rats.

In contrast to the activity of the hepatic enzyme, rhythm in the corticosterone content of the adrenal persisted in the animals fed hourly. This finding indicates that the adrenal cycle is not generated by rhythms in dietary intake.

**Summary.** The amplitude of the 24-hr rhythm in hepatic tyrosine transaminase activities of rats fed hourly was markedly reduced when compared to the activities of the enzyme in rats eating *ad libitum*. Reversing the lighting schedule reversed the rhythms but did not change their amplitudes. The TT rhythm of adrenalectomized rats eating *ad libitum* was reduced in amplitude and the rhythm extinguished in animals fed hourly. Hourly feedings did not influence the cyclicity of the corticosterone content of the adrenal. It is concluded that rhythms in food ingestion and adrenal cortical secretions play roles in the generation of the rhythmicity of hepatic tyrosine transaminase activities but that the diurnal variation in adrenal corticosterone content is not related to the cyclicity of food intake.

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