# *DAILY RHYTHMIC CHANGES IN TYROSINE TRANSAMINASE ACTIVITY OF THE RAT LIVER**

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*Communicated by Seymour S, Kety, April 13, 1967*

Several mammalian enzymes have been shown to exhibit regular variations in activity during each 24-hour period. When rats are kept in a lighting schedule of 12 hours of light alternated with 12 hours of darkness, adrenal succinic dehydrogenase activity rises 50 per cent above basal levels at the end of the daily light period.[1] Under similar conditions, renal transaminase activity in mice is lowest toward the end of the dark period, and increases by 30 per cent during the first 6 hours of light.[2] The tryptophan pyrrolase activity of the rat liver rises toward the end of the light period, doubling within 8 hours; it then falls to basal levels, where it remains for the better part of the day.[3] The rhythmic changes in this last enzyme are abolished when the animals are adrenalectomized.[3]

We have observed that the activity of tyrosine transaminase in the rat liver varies each day over a fourfold range. This rhythm appears to be remarkably synchronous among animals maintained on the same lighting schedule, and persists with altered phase relationships following the removal of the pituitary or the adrenals.

*Methods.*—Sprague-Dawley female rats weighing 160–200 gm were subjected to hypophysectomy, adrenalectomy, or no operation, and kept under controlled lighting (lights on for 12 hours daily, starting at 6, 7, or 8 A.M.) in clear plastic cages for 7–10 days. Food and water were provided *ad libitum*. Adrenalectomized rats were given a 0.3 per cent sodium chloride solution to drink. Light was provided by cool-white fluorescent bulbs; animals were exposed to 50–70 ft-c of illumination. Groups of five or six rats were killed by neck fracture at each time of day, and their livers and adrenals were rapidly removed and frozen on dry ice. Hepatic tyrosine transaminase was assayed by a modification of the method of Diamondstone.[4] The liver was homogenized in 10 volumes of isotonic potassium chloride. The homogenate was centrifuged for 20 minutes at 50,000 *g*, and 10 microliters of the supernatant fraction were incubated for 20 minutes with 6 $\mu$M of l-tyrosine, 9 $\mu$M of potassium alpha-ketoglutarate, 0.1 $\mu$M of pyridoxal phosphate, and 100 $\mu$M of pH 7.6 phosphate buffer in a final volume of 1 ml. The reaction was stopped by adding 100 microliters of 10 *N* potassium hydroxide, and the para-hydroxybenzaldehyde formed was measured at 331 m$\mu$ in a Guilford spectrophotometer. Adrenal corticosterone was measured by the method of Glick *et al.*[5]

*Results.*—Unoperated rats kept in light from 8 A.M. to 8 P.M. daily were killed at 8 A.M., 1:30 P.M., 6 P.M., or 10:30 P.M. Hepatic tyrosine transaminase activity was examined in the liver and found to be lowest at the end of the dark period (Fig. 1). Enzyme activity did not change significantly during the first half of the light period; it then rose slowly, attaining levels about twice those observed at 8 A.M. during the next four hours. With the onset of darkness, enzyme activity rose more

rapidly; by 10:30 P.M., it was more than four times the 8 A.M. level. Subsequently, enzyme activity fell to the lowest observed levels at the end of the dark period. The content of corticosterone in the adrenal gland varied with a similar rhythm. Steroid levels were lowest at 8 A.M. and did not change significantly by 1:30 P.M. They rose to a peak of three times the 8 A.M. values by 6 P.M., where they remained for the first few hours after the onset of darkness, subsequently falling to lowest levels at the end of the dark period (Fig. 1).

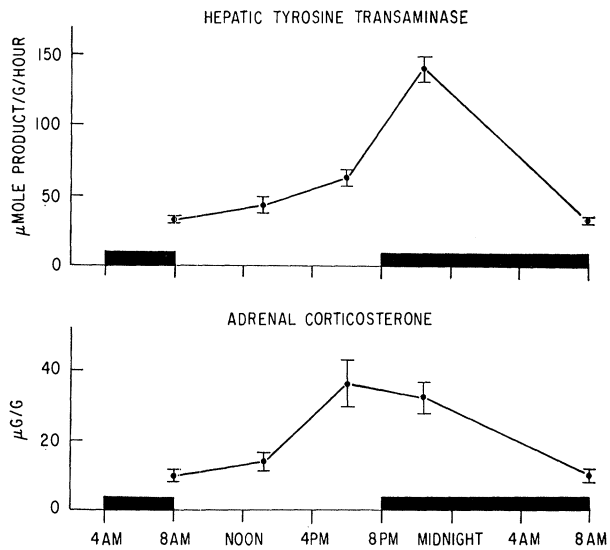


FIG. 1.—Daily rhythms in hepatic tyrosine transaminase and adrenal corticosterone content. Rats were kept under controlled illumination (lights on: 8 A.M. to 8 P.M.) for 1 week prior to assay. Vertical lines in both figures represent standard errors of the mean.

Tyrosine transaminase activity is known to be increased when rats are treated with carbohydrate-active steroids.[6, 7] Thus the phase relationships of the afternoon rise in adrenal corticosterone level, and the subsequent increase in transaminase activity suggested that the hepatic rhythm might be produced by the changes in adrenal secretion. To explore this possibility, the rhythmic changes in enzyme activity were examined in animals that had been hypophysectomized ten days earlier. In this study, rats were kept under lighting from 6 A.M. to 6 P.M., and killed at various times during the day. Unoperated rats showed diurnal changes in enzyme activity similar to those seen in the first experiment (Fig. 2). Among hypophysectomized rats, a distinct rhythm could still be discerned in enzyme activity. However, the phase of this rhythm had shifted somewhat, so that maximum and minimum enzyme activities were observed four to eight hours earlier than in unoperated rats. The amplitude of the rhythm was about three times the lowest values.

The persistence of the transaminase rhythm following hypophysectomy suggested that although this rhythm might normally be synchronized by the adrenocortical cycle, its generation did not depend upon variations in adrenal function. How-

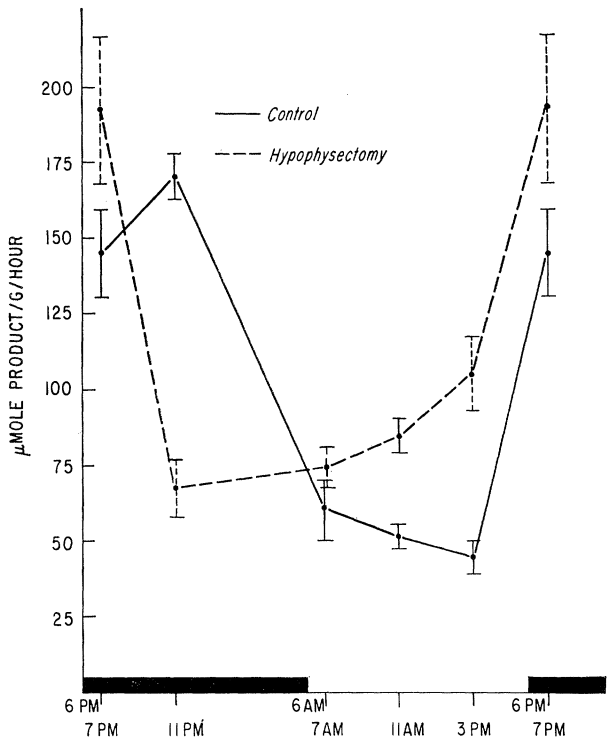


FIG. 2.—Effect of hypophysectomy on the daily rhythm in hepatic tyrosine transaminase activity. Rats were hypophysectomized and kept under controlled illumination (lights on: 6 A.M. to 6 P.M.) for 10 days prior to assay.

ever, it was still possible that sufficient intrinsic rhythmicity might have persisted in adrenocortical secretion after hypophysectomy to have produced the enzyme rhythm. Alternatively, aldosterone (whose cyclic secretion is not dependent upon the pituitary gland) might have generated the tyrosine transaminase rhythm. To examine these possibilities, the enzyme was assayed in liver from adrenalectomized rats. Animals were kept under light from 7 A.M. to 7 P.M., and killed at various times during the day. Following adrenalectomy, the characteristics of the transaminase rhythm were similar to those observed after hypophysectomy (Table 1). The rhythm persisted, with an amplitude of about three times the lowest values; however, the time of maximum enzyme activity occurred several hours earlier than in unoperated rats.

*Discussion.*—These observations demonstrate that the activity of tyrosine transaminase in the rat liver varies over a fourfold range during each 24-hour day.

TABLE 1

EFFECT OF ADRENALECTOMY ON THE DAILY RHYTHM IN HEPATIC TYROSINE TRANSAMINASE ACTIVITY

| Time of day | μMole Product/Gm/Hr | |
|---|---|---|
| | Control | Adrenalectomy |
| 9 A.M. | 36.2 ± 3.2 | 29.1 ± 4.7 |
| 1 P.M. | 36.5 ± 6.2 | 31.3 ± 7.7 |
| 6 P.M. | 93.0 ± 9.1 | 69.6 ± 6.8 |
| 8 P.M. | 134.0 ± 7.2 | 99.7 ± 2.9 |
| 10 P.M. | 145.5 ± 6.9 | 73.6 ± 7.4 |

Rats were subjected to bilateral adrenalectomy and kept under controlled lighting for 10 days (lights on: 7 A.M. to 7 P.M.). Results are given as mean ± standard error.

Enzyme activity is lowest at the start of the light period, and rises sharply for 6 hours beginning several hours before the onset of darkness, after which it returns to the lowest levels. The rhythm exhibits a striking degree of synchrony among animals maintained under the same environmental conditions: e.g., in none of the experiments reported was there any overlap among observations made early in the morning, in the late afternoon, or in the evening.

In intact rats, the phase relationships of the tyrosine transaminase rhythm appear to be entrained by the adrenocortical rhythm. However, the enzyme rhythm clearly persists in animals deprived of adrenal rhythmicity (by hypophysectomy), or of the adrenal gland itself. Thus another mechanism must exist which can generate the rhythm in the absence of adrenocortical steroids.

It is possible that the 24-hour cycle in the ingestion of food could produce the enzyme rhythm. Evidence has been presented that several dietary amino acids can increase tyrosine transaminase activity, when administered to the intact rat.[6, 7] The rat consumes most of its food after the onset of darkness; thus the rise in enzyme activity during the first few hours of darkness could result from the increased availability of these amino acids to the liver. This hypothesis is not easily reconciled with the observations that enzyme activity is already increased before the onset of darkness, and that transaminase activity falls during much of the dark period. Furthermore, in rats deprived of a pituitary or adrenal the peak enzyme activity is achieved even earlier in the day.

It is possible that the rhythm could be generated by neural information, or by circulating compounds whose levels are not controlled by the pituitary gland. Evidence has been presented that the contents of norepinephrine in the pineal and salivary glands[8] and the excretion of this sympathetic neurotransmitter into the urine[9] vary with a daily rhythm. Thus the transaminase could be related to cyclic variations in sympathetic nervous function. The release of serotonin from the rat pineal gland[10] and the synthesis of melatonin within this organ[11] have been shown to vary diurnally. Both of these pineal rhythms depend upon information transmitted to the gland by its sympathetic nerves. Serotonin and other indoles have also been shown to increase tyrosine transaminase activity when administered to intact rats.[6, 12] These observations suggest that the enzyme rhythm might be related to cyclic alterations in pineal function.

Finally, it is possible that the tyrosine transaminase rhythm is intrinsic to the hepatic cells themselves, and that it can be entrained by a variety of cyclic functions that provide information to the liver. These might include such factors as adrenocortical secretion, dietary amino acid intake, and environmental lighting (presumably acting by controlling the timing of the adrenal cycle). Tyrosine transaminase activity is an important variable in determining the metabolic fate of ingested aromatic amino acids. It is possible that the enzymatic transformation of tyrosine, tryptophan, and phenylalanine[13] proceeds at a more rapid rate during the hours that tyrosine transaminase activity is elevated than at other times of day. The studies described in this report suggest that significant economies could be effected in the use of limited protein resources by encouraging the ingestion of protein at times of day when its constituent amino acids are less likely to be metabolized by the liver.

Many reports have presented evidence that various drugs or procedures can

elevate tyrosine transaminase activity.[6] Most of these reports included no information about the times of day that the animals were killed, or information about the lighting conditions under which they had been maintained. The experiments described here indicate that a rise in enzyme activity as great as that produced by certain inducing agents can occur spontaneously in the untreated animal. Similarly, previous studies which have found that hypophysectomy and adrenalectomy have no effect on tyrosine transaminase activity[6] should now be interpreted as proving only that at the particular time of day that the animals were assayed, these procedures appeared to have no effect. Information about the lighting schedule to which animals were exposed, and the times of day that they were killed, should probably be provided in all investigations dealing with tyrosine transaminase.

*Summary.*—The activity of tyrosine transaminase in the rat liver varies markedly during each 24-hour period. Soon after the onset of darkness, enzyme activity is four times greater than it is during the early part of the light period. The rhythm persists following hypophysectomy or bilateral adrenalectomy, but appears to be entrained by the adrenocortical secretory cycle in the intact animal.

*Note:* Dr. W. R. Beisel and his colleagues at Fort Detrick, Maryland, have independently observed a 24-hour rhythm in tyrosine transaminase activity.

We thank Dr. Beverly Peterkofsky, National Institute of Arthritis and Metabolic Diseases, for advice in setting up the tyrosine transaminase assay.

* This research was supported in part by USPHS grants AM-11709 and AM-11237.

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