Circadian Rhythms of Liver Enzymes and Their Relationship to Enzyme Induction

Abstract. *Tyrosine alpha ketoglutarate transaminase, which is rapidly induced by various agents, shows circadian rhythmicity in the intact rat. This rhythmicity is only slightly altered after adrenalectomy, indicating that adrenal hormones do not play a major role in the metabolic control of the activity of tyrosine alpha ketoglutarate transaminase. On the other hand, phenylalaninepyruvate transaminase, which is not inducible over the same time period, does not show circadian variation. The results suggest that the sensitivity of an enzyme's regulating system to inducing agents may be related to the inherent circadian rhythm of the enzyme.*

The detection of great circadian changes in the activities of several liver enzymes that metabolize amino acids (*1*) has shown the necessity for controlling the time factor in the study of the physiology of enzyme regulation. These enzymes have also been shown to be inducible within a few hours when treated with glucocorticoids, glucagon, and casein hydrolyzate (*2*). However, there is still the question of whether these compounds, at physiological concentrations, are active as inducers of enzyme. In the case of the inducible enzyme mouse liver tryptophan pyrrolase (TP), Rapoport *et al.* (*3*) found a relation between the circadian changes in plasma corticosterone and liver TP activity. Furthermore, they reported that adrenalectomy abolishes the very pronounced circadian rhythm of this enzyme, indicating that changes in the endogenous glucocorticoids might play a major role in the physiological induction of TP. Thus, circadian changes in enzyme activity have proved useful in the identification of endogenous inducers of an enzyme.

Another question of interest is the actual physiological significance of enzyme induction and its relation to circadian changes in enzyme activity. Are these rapid changes which occur after administration of various inducing agents simply a reflection of an enzyme's tendency to undergo circadian changes? Thus, the sensitivity of an enzyme's regulatory system to inducing agents would be a reflection of the basal circadian rhythm of the enzyme. If so, an enzyme with a marked circadian rhythm should be inducible in a short period of time (a few hours), whereas one which has no circadian rhythm would not be inducible in this time period.

We looked for circadian changes in two liver enzymes, tyrosine α-ketoglutarate transaminase (TKT) and phenylalanine-pyruvate transaminase (PPT). The former is inducible by glucocorticoids and glucagon, and it reaches maximum activity 4 to 6 hours after glucocorticoid treatment (*2*) and 2 to 4 hours after glucagon (*4*). On the other hand, PPT activity changes only slightly after repeated injections of glucocorticoids. Although its activity shows a small increase (30 percent) 6 hours after glucagon treatment, it only reaches a maximum of a threefold increase 24 hours after glucagon induction (*4*).

Male Sprague-Dawley rats (Berkeley-Pacific Laboratories), weighing 250 to 300 g, were used. The animals were housed in individual cages in an isolated room with controlled temperature (25° ± 1°C) and illumination (5 a.m. to 5 p.m.) and were freely given Purina Lab Chow. To establish a regular rhythmic change in the concentrations of plasma corticosterone, they were placed in their cages for a period of 2 weeks before being killed. Adrenalectomized animals were given 0.9 percent saline and were used 2 weeks after surgery. The rats were killed by guillotine, and the blood was collected in heparinized beakers. The blood was chilled and centrifuged in the cold, and the plasma was removed and frozen for later determination of corticosterone (*5*). These results are expressed as micrograms of corticosterone per 100 ml of plasma. The livers were rapidly removed and chilled on ice. The finely minced liver tissue was homogenized in Krebs-Ringer bicarbonate medium (Na:K, 1 : 1, *p*H 7.4) at 2°C in eight strokes in a glass homogenizer with a Teflon pestle driven by a constant torque motor (400 rev/min). The final homogenate concentration was 10 percent (weight per volume). The homogenates were centrifuged at 13,000*g* for 30 minutes; the lipid layer was removed, and the clear supernatant was used for the assay of TKT and PPT by the continuous spectrophotometric method of Lin *et al.* (*6*). The results are expressed as micromoles of product formed per milligram of protein per hour at 37° ± 0.1°C and represent the averages of 8 to 12 animals for each time period.

The TKT activity at various times of the 24-hour period in intact rats is shown in Fig. 1A. In the intact animal, TKT undergoes a 387 percent change, with minimum TKT activity at 11 a.m. and maximum activity at 9 p.m. The plasma corticosterone rises from its minimum level at 6 a.m. to its maximum at 6 p.m., with an increase of 1870 percent. The peak TKT activity occurs 3 hours after the maximum corticosterone level. Rapoport *et al.* (*3*) found that the peak activity in liver TP in the mouse occurred about 7 hours after maximum corticosterone levels. The circadian variation of TP in the rat should be compared with that in the mouse to see whether the

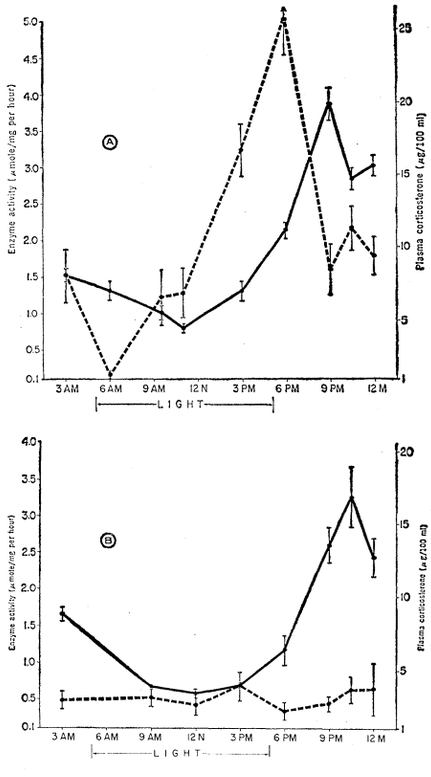

Fig. 1. Relation in normal male rats (A) and in adrenalectomized rats (B) of TKT activity and plasma corticosterone concentrations over a 24-hour period. Each point represents mean ± standard error. Solid lines represent TKT activity; dashed lines represent plasma corticosterone concentrations.

time relation in fluctuations of corticosterone and TP would coincide.

Figure 1B shows that the circadian rhythm of TKT is not appreciably altered after adrenalectomy. There appears to be a lowering of both the maximum and minimum points in the curve; however, this is not statistically significant. Our experiments indicate that adrenal hormones do not play a major role in determining the circadian rhythmic pattern of TKT. Our results are in agreement with the findings of Wurtman and Axelrod (*7*) who also studied the circadian variation of rat liver TKT. Our findings imply that caution is necessary before assuming that a hormone, capable of inducing an enzyme when administered in pharmacological amounts, is the primary physiological inducer of the enzyme. The natural inducer or inducers of TKT remain to be determined.

It has been shown that TKT activity is increased after starvation, whereas TP activity is decreased (*8*). In man, starvation produces significant increases in glucagon (*9*). Studies in this laboratory have shown that glucagon can act as an inducer of TKT activity, but not as an inducer of TP activity, independently of the pituitary adrenal axis (*4*). Thus, it is possible that glucagon may be one of the hormones involved in the control of the circadian rhythm of TKT.

The other liver transaminase studied, PPT, does not show any circadian variation. This enzyme is not significantly changed in 4 to 6 hours after glucocorticoids and glucagon (*4*), the period at which the maximum increase in both TP and TKT activities is obtained. Thus, there appears to be a relation between an enzyme's short-term inducibility by exogenous inducing agents and its natural circadian variation.

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Gynogenesis and Triploidy in the Viviparous Fish Poeciliopsis

Abstract. *Three all-female strains of the viviparous fish* Poeciliopsis *occur in the Río Fuerte of Sinaloa, Mexico.* Poeciliopsis lucida, *a bisexual species, provides sperm for these monosexual forms which I designate as Cx, Cy, and Cz. Form Cy is a triploid that when test-mated to males of various species produces all-female, triploid offspring devoid of paternal characters. Both Cx and Cz are diploid and express characteristics of both parents.*

Poeciliopsis lucida (order Cyprinodontiformes, family Poeciliidae) is a viviparous fish of northwestern Mexico. Associated with it is an all-female form designated as Cx. Males of *P. lucida* provide sperm for both the *P. lucida* females and the monosexual Cx form. This mating triangle occurs in all three rivers inhabited by *P. lucida*, namely, the Ríos Mocorito, Sinaloa, and Fuerte (*1*). On the basis of external morphology there is little difference in the Cx-*lucida* complex from one river system to the next. Hybridization experiments with distantly related species of *Poeciliopsis* now reveal that the monosexual form in the Río Fuerte actually consists of at least three distinct all-female types, Cx, Cy, and Cz. All of these produce exclusively female progeny when mated to males of *P. lucida* in the laboratory and apparently rely mainly on *P. lucida* sperm for fertilization in nature. Other species of *Poeciliopsis* that occur in the Fuerte are *latidens, monacha,* and *prolifica* (*1*).

Form Cx has been studied most extensively. After 16 laboratory generations from matings of Cx with *P. lucida* giving rise to over 800 offspring, the first male has yet to be produced. Form Cx mates successfully with the males of nine other species of *Poeciliopsis.* As long as the paternal species is one that is closely related to *P. lucida*, the offspring are all females; but when males are from other species groups, progeny of both sexes are produced. Sex ratios vary, however, from one mate combination to another. Since all offspring from Cx females exhibit characteristics of both parents, regardless of the father, they must be considered true hybrids and not the product of parthenogenesis or gynogenesis. Paternal chromosomes of the F_1 generation, however, are not transmitted through the ova; only those characteristics of the female germ line pass to the next generation (*2, 3*).

One species particularly useful for tracing the flow of genetic material in crosses with monosexual forms is *P. latidens*, which is boldly marked with black bars and spots and has dense black pigment about the genitalia. These characters are in stark contrast to the uniform olive coloration and sparse genital pigment of the monosexual forms and *P. lucida.* Other equally contrasting traits are found in the shapes of the jaws, lips, and teeth. When Cx and *P. latidens* are crossed (Fig. 1), the F_1 generation has fewer spots than *P. latidens* and no bars; in other characters it is more or less intermediate (*3*).

Stocks of Cz and Cy perpetuated in the laboratory by matings with *P. lucida* males were thought to be Cx until individuals were mated to *P. latidens.* Form Cz, instead of giving birth to progeny of both sexes, as Cx does, produced only males. In matings to Cz, Cy, *P. lucida*, and *P. latidens* these males failed to reproduce; they probably are sterile. Although males from matings of Cz with *P. latidens* have a few weakly expressed *P. latidens* traits, they also have characteristics of *P. monacha*, another species that lives in the Fuerte. This suggests that Cz has had a hybrid origin that involved *P. monacha.* The reproductive mechanism that results in these all-male progeny has not been determined. The third all-female form, Cy, when mated to *P. latidens*, produces only female offspring morphologically indistinguishable from the mother. It is with these that this investigation is primarily concerned.

The original laboratory stocks of Cy and Cz are each descendants of