Feeding Schedule Alteration of Daily Rhythm in Tyrosine Alpha-Ketoglutarate Transaminase of Rat Liver

Abstract. *Liver tyrosine alpha-ketoglutarate transaminase has a daily rhythm such that in rats fed on an unrestricted basis the activity is highest at approximately 11:00 p.m. In contrast, rats fed only from 8:00 a.m. to noon show a markedly different rhythm in the enzyme, with maximum activity at 11:00 a.m. Controlling the time of food intake seems to be a useful means of studying the mechanism of the daily changes in this enzyme.*

The enzyme L-tyrosine : 2-oxoglutarate aminotransferase (E.C. 2.6.1.5) has been reported to undergo a daily rhythm in rats (*1*), with highest activity at night. Normally, rats are nocturnal feeders. We now describe alteration in the rhythm of this enzyme, commonly called tyrosine α-ketoglutarate transaminase (TKT), by a change in the feeding pattern of rats.

Male Sprague-Dawley rats (from Southern Animal Farms) were kept in individual, hanging, wire cages with lights on from 7:30 a.m. to 4:30 p.m. and were fed Purina Lab Chow. One group of rats had food and water available continuously. The second group had water available at all times but had food only from 8:00 a.m. to noon. These rats adapted within a few days and began to gain weight; such animals are used routinely in studies of appetite suppressants. After 2 weeks, rats from both groups were decapitated in groups of five at 3-hour intervals during a 24-hour period. The average body weight was 200 g for rats fed on an unrestricted basis and 162 g for rats fed only in the morning. The livers were rapidly removed and frozen on dry ice. They were later homogenized in four volumes of 0.025*M* phosphate buffer (*p*H 7.4), and the homogenates were centrifuged at 30,000*g* for 30 minutes. The clear supernatant was diluted 1 to 10 in buffer and used for TKT assay (*2*) which was carried out at 37°C in a Gilford multiple-sample absorbance recorder.

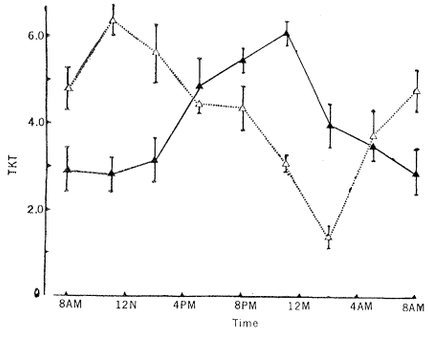

Fig. 1. Liver TKT in rats. Solid line (black triangles), rats fed on an unrestricted basis; dotted line (open triangles), rats fed only from 8:00 a.m. to noon. Enzyme activity is expressed as micromoles of tyrosine transaminated per minute per gram of liver (wet weight). Means and standard errors of the data obtained with five rats for the determination at each point are shown.

For rats with food available continuously (Fig. 1), our findings confirm those of others (*1*), the highest activity of TKT being at 11:00 p.m. When rats were fed during the day, the rhythm was entirely different, TKT being highest at 11:00 a.m. (a shift of 12 hours).

In interpreting these results, we must take into account many factors. The enzyme can be induced by its substrate (*3*); however, absorption of tyrosine from digested food cannot explain the rapid increase in TKT before 8:00 a.m. in those rats that received food only at that time. Adrenal, thyroid, pituitary, and pancreatic hormones influence the rhythmicity or activity of liver TKT in varying degrees (see *1, 4*).

The increases in TKT shown in Fig. 1 occur several hours after the end of feeding (if we assume that rats fed on an unrestricted basis ate little during the day) and may represent one of the responses to a demand for increased gluconeogenesis. Greengard and Baker (*5*) suggested that factors including dietary changes may induce or enhance TKT induction by stimulating the secretion of glucagon.

It may be preferable to consider the variation in TKT as a regulatory phenomenon rather than as a biological rhythm. The term *circadian* (*6*) should probably be avoided because this is not a 24-hour rhythm that is free-running, that is, independent of exogenous controlling influences. Further study of the variation in liver TKT must take into account the profound influence of the feeding schedule. The control of food intake may provide a useful tool for investigating the mechanism of the changes in liver TKT.

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Structure of Membranes: Reaction of Red Blood Cell Membranes with Phospholipase C

Abstract. *Treatment of human red blood cell membranes with phospholipase C releases 68 to 74 percent of the total membrane phosphorus into solution, through hydrolysis of membrane phospholipids to diglycerides and water-soluble phosphorylated amines. In spite of this drastic change, the membrane remains intact in phase microscopy, and the average protein conformation in the membranes, as determined by circular dichroism measurements in the ultraviolet, is unaffected. These results are readily explained by a model of membrane structure that is stabilized by hydrophobic interactions and in which the polar and ionic heads of lipids are on the outer surfaces of the membrane, in contact with the bulk aqueous phase and accessible to the action of phospholipase C.*

We have recently reported (*1*) on the optical rotatory dispersion and circular dichroism spectra, in the wavelength region of the peptide bond absorption bands, of intact red blood cell membranes and of fragments of *Bacillus subtilis* membranes. The spectra for the two very different membrane preparations are remarkably similar; they are also similar to the optical rotatory dispersion spectra obtained by others with chloroplast-lamellae fragments (*2*) and to membrane preparations from tumor cells (*3*) and from mitochondria (*4*). The spectra are characteristic of the protein portion of the membrane, with only negligible contributions from the lipid constituents (*1, 5*); and they indicate that the protein is in a partially helical conformation. The closely similar spectra suggested that a major fraction of the protein of these different membranes is closely similar in conformation and therefore in chemical struc-