## 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 $\alpha$-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.

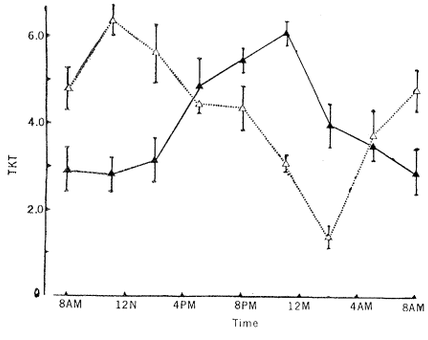


Fig. 1. Liver TKT in rats. Solid line (black triangles), rats fed on an unrestricted basis; dotted line (open triangles), rats fed only from 8:00 a.m. to noon. Enzyme activity is expressed as micromoles of tyrosine transaminated per minute per gram of liver (wet weight). Means and standard errors of 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-

ture; the protein was inferred to be the membrane "structural protein" of Green and his colleagues (*6*, *7*). This inference has found support in the recent work of Steim and Fleischer (*5*) on the structural protein of mitochondria.

In connection with these data, we proposed a new model for the general structural organization of the components of membranes (*1*). Wallach and Zahler (*3*) independently proposed a model that is similar in many respects. In this model, the membrane components are held together predominantly by noncovalent hydrophobic interactions, as had been suggested earlier (*6*, *8*). The ionic and polar heads of the phospholipids, together with the charged groups of the proteins, are all situated at the exterior surfaces of the membranes in contact with the bulk aqueous phase. The interior of the membrane contains the hydrophobic tails of the phospholipids, the rest of the protein, and other hydrophobic components, such as cholesterol. By contrast, membrane models of the Davson-Danielli-Robertson type (*9*) have the ionic and polar heads of the phospholipids submerged under a monolayer of protein on both surfaces of the membrane, the entire structure held together predominantly by electrostatic interactions between the ionic heads of the phospholipids and the charged groups of the protein monolayers.

We now report some results of the modification of red blood cell membranes with preparations of phospholipase C. This enzyme specifically catalyzes the hydrolysis of phospholipids to diglycerides and water-soluble phosphorylated amines (*10*). Human red blood cell membranes were prepared by the method of Dodge *et al.* (*11*), as in our previous studies (*1*). The intact membranes were then thoroughly dialyzed into a buffer containing 5 m*M* imidazole, 0.1*M* KCl, and 2 m*M* $CaCl_2$ at *p*H 7.3. A commercial preparation of phospholipase C from *Clostridium welchii* (*12*) was added to membranes at a concentration corresponding to about 1 unit per milligram of membrane protein. The mixture was incubated at 37°C and the extent of reaction was followed by measuring the phosphorus released into the supernatant after dilution and centrifugation of the membranes for 60 minutes at 30,000*g*. Phosphorus was assayed by the procedure of Bartlett (*13*).

Under these conditions 68 to 74 percent of the total membrane phosphorus was released into the supernatant within the first 10 minutes of the reaction. Since by far the largest part of the membrane phosphorus is in the form of phospholipids (see *14*), this figure represents the amount of phospholipid that was hydrolyzed. Neither longer incubation times nor a second addition of enzyme raised this value. The supernatant did not contain detectable amounts of cholesterol (*15*) or of carboxylic acid esters (*16*), which indicates that lipid components and diglycerides were not released from the membrane by treatment with phospholipase C. The absorbance at 280 m$\mu$ of the supernatant after treatment was identical with that of an untreated control, showing that protein was not released from the membrane. The membranes remained intact when viewed in the phase microscope; no electron microscopic observations were made.

The circular dichroism spectrum of the membranes treated with prosopholipase C was indistinguishable from that of an untreated control (*1*); the minimum at 224 m$\mu$ and the smaller minimum at 210 m$\mu$ were unchanged in either magnitude or position by action of the enzyme (*17*).

These results indicate that rapid cleavage and release of a major fraction of the ionic heads of the phospholipids occur upon the action of phospholipase C on intact cell membranes, without disruption of the membrane or alteration of the overall conformation of the protein in the membrane. This suggests that: (i) the phosphoester bonds that are hydrolyzed are readily accessible to the phospholipase C molecule in the intact membrane; and (ii) electrostatic interactions between phospholipids and membrane proteins play only a secondary role, presumably to hydrophobic interactions, in maintaining the integrity of the membranes and in determining the conformation of the membrane proteins. These data are, therefore, more consistent with the model of membrane structure we have proposed (*1*) than with the Davson-Danielli-Robertson (*9*) model.

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## Genetic Selection for Voluntary Alcohol Consumption in the Albino Rat

Abstract. *By outbreeding Wistar rats and selecting for breeding animals that differ in their alcohol consumption, we have raised two genetically different lines. Marked differences between the sexes and the strains were evident by the eighth generation. Selection is reflected in the regression coefficient .754, which accounts for 65.9 percent of the variance. The heritabilities differ significantly in the two sexes,* $h^2$ *for the males being .263, and for the females .371; this difference seems mainly ascribable to sex-linkage of some of the genetic factors controlling voluntary consumption of alcohol.*

The underlying assumption in experiments on voluntary selection of alcohol is that heritable biochemical reactions which control physiological mechanisms also to some extent regulate alcohol consumption. This hypothesis has been the basis of numerous animal experiments used to clarify the heritability of voluntary consumption of alcohol. Reed (*1*) investigated the selection of alcohol by six different inbred lines of rats, and, although the investigation was not designed for genetical research, the results reveal differences between the strains. Mardones (*2*) set out to raise two inbred rat strains that would differ in their alcohol habits; he found the differences to be genetically determined, although