Feeding Habits and Daily Rhythms in Tissue Glycogens in the Rat[1] (35777)

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The glycogen contents of liver and muscle of fed and fasted rats, rabbits and mice are one of a number of body constituents that have been shown to vary in a daily rhythmic cycle (1–3). The peaks in glycogen concentrations usually are seen during the dark hours and the nadirs during the light hours. Changing the feeding habits of rats from one of free access to food (consumed primarily during the dark hours of a 24-hr day) to one in which the food is available (and eaten) for only a limited time period during the day or night alters the phase of these rhythms of glycogen levels in liver, fat, and diaphragm (4–6). Hence it is believed that dietary habits, with their consequent effect on the time of influx of nutrients, play a role in generating the daily rhythms that are observed in tissue carbohydrate concentrations.

In the present experiments, the timing and periodicity of food intake by rats were either permitted to continue in the characteristic cyclic pattern or were maintained constant throughout the 24-hr day; concentrations of glycogen present in fat, liver, and diaphragm were determined at 6-hr intervals. The results obtained are supporting evidence that the cyclic changes in tissue glycogen levels are secondary to the manner of food ingestion.

Methods. Male Holtzman rats, received at a body weight of 100–120 g, were used in the experiments. The animals were divided into four groups and kept in individual metabolism cages, all of a similar size, in a temperature controlled room. The rats were allowed to eat *ad libitum* or were fed hourly 1/24th of the same daily total amount of food consumed by those eating *ad libitum* (7). Animals were adapted to a 24% protein, moderate carbohydrate, semisynthetic diet (9), to the feeding habits, and to 12 hr of alternating light and darkness (lights on at 0600 and off at 1800 hr). Experiments were performed with normal rats (35 eating *ad libitum*; and 36, hourly) and with bilaterally adrenalectomized ones (20 eating *ad libitum*; and 32, hourly); the adrenalectomies were done at least 3 weeks prior to the sacrifice of the animals. The normal rats had free access to distilled water and the adrenalectomized ones to 1% saline.

After 3–4-weeks adaptation to the above conditions, approximately equal numbers of animals of a group were decapitated at 0600, 1200, 1800, and 2400 hr; a portion of the right lobe of liver, diaphragm, and epididymal fat, and in some instances heart and kidney, were removed, frozen between slabs of Dry Ice and subsequently analyzed for glycogen. The glycogen was isolated, hydrolyzed, and determined as glucose with glucose oxidase (9, 10).

Results. a. Tissue glycogen levels in rats eating ad libitum. Rats with free access to food, regardless of whether they were intact or adrenalectomized, exhibited a daily cyclicity in the concentrations of glycogen in liver, fat and diaphragm (Figs. 1 and 2). The peak levels were observed usually at 0600 hr and the nadirs at 1800 hr. The kidneys and hearts of the normal rats likewise demonstrated a daily rhythm in glycogen levels; the phase of the former tissue followed those of the above three organs but the cardiac gly-

[1] This research was aided by a Grant from the Erna Heller Fund and a Grant from General Research Support Grant 5 FR 05476-08 from the National Institutes of Health.

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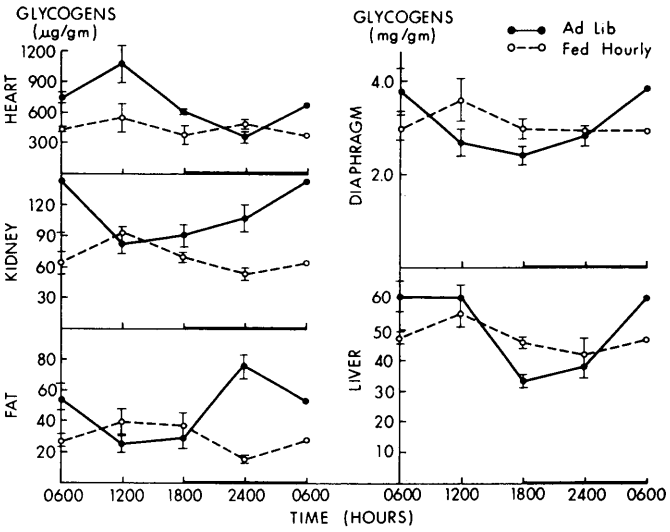

FIG. 1. Effect of feeding habits: tissue glycogen levels of normal rats eating *ad libitum* or hourly 1/24th of the same daily food allotment.

cogen rhythm tended to be 12 hr out of phase. Since liver weight exhibits a daily rhythm that is synchronized with that of liver glycogen concentrations (2, 5) (unpublished observations), the absolute quantities of glycogen present in this tissue at any one time were greater (or less) than indicated by the concentrations alone. The differences between nadir and peak values for every tissue were all highly significant, irrespective of whether the animals were intact or adrenalectomized; the p values varied between <0.05 and <0.001.

b. Tissue glycogen levels in rats fed hourly. Rats offered (and eating) hourly 1/24th of their daily food allotment demonstrated either a marked decrease in the amplitude of the glycogen rhythm in a given tissue, a quenching of the rhythm or a shift in phase of the rhythm (Figs. 1 and 2). The concentrations of tissue glycogen tended to be smaller in amount for these rats, when compared to the rats eating *ad libitum*, in that the levels of the hourly-fed rats approximated either the nadir or mean 24-hr values exhibited by the cyclic eaters. However, statistical analyses of the differences between nadir and peak levels showed $p < 0.05$ for the fat and liver of the normal and $p < 0.01$ for the diaphragm and liver of the adrenalectomized animals. It should be mentioned that the amplitudes of the daily rhythms of liver weights of the animals fed hourly were smaller than in the animals eating *ad libitum*.

Discussion. The cyclicity of food intake by the rat has been considered to play a role in the generation of the daily rhythm that characterizes tissue glycogen levels. Evidence for this belief is based on observations of the temporal relationship of food intake and glycogen deposition. Thus, rats with free access to food usually consume some 60–80% of their nutrients during the dark hours; this peak in food intake is followed by many metabolic adjustments, including glycogen deposition in fat, liver, and diaphragm. However, when the food intake is restricted in time to a limited portion of the light or dark hours, the deposition of glycogen occurs shortly thereafter (4–6). This cause and effect relationship satisfies one of the criteria proposed to demonstrate that an exogenous oscillator can act as an input for generating a daily rhythm, *i.e.*, an exogenous stimulus can induce its effect at any time of the day or night (11).

Additional data suggesting that eating habits are linked to tissue glycogen rhythms are available from the results obtained from the rats fed hourly 1/24th of their daily food. With the rate of nutrient intake maintained constant over the 24-hr day, the amplitudes

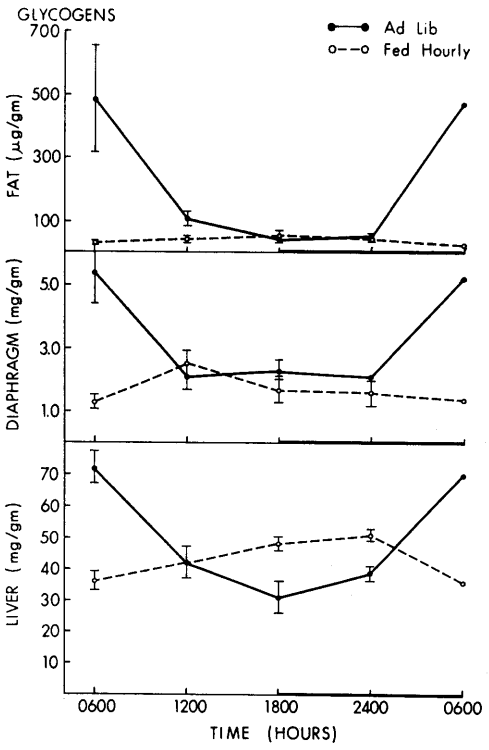

FIG. 2. Effect of feeding habits: tissue glycogen levels of adrenalectomized rats eating *ad libitum* or hourly 1/24th of the same daily food allotment.

of the daily cycles of all tissues were markedly reduced. This type of response might be anticipated if an exogenous input is maintained constant in magnitude throughout the 24 hr (11). However, as is evident from Figs. 1 and 2, not all rhythms were extinguished, suggesting that some additional factor(s) may act as inputs.

Daily rhythms in the rates of secretion of hormones, which might influence glycogen cycles, are known to exist (12–14). Studies were done, therefore, in the adrenalectomized rat because of the report of Agren (15) that adrenalectomy abolishes the daily rhythm in hepatic glycogen concentrations. The results of the persent study are not only at variance with the previous data but, in addition, reveal that daily rhythms in glycogen content are present for epididymal fat and diaphragm. It is clear also that a combination of adrenalectomy and hourly feedings did not completely quench the rhythms in diaphragm and liver.

Nutritional status and endocrine secretions alter cardiac glycogen levels. Fasting, a decrease in insulin, and an increase in growth hormone release rates have been reported to increase the content of glycogen in this organ (16, 17). We observed the peak in cardiac glycogen concentrations to occur in the middle or at the end of the light hours, at a time when the animals are at their nadir of food intake; temporally, this corresponds to a minimum insulin secretion but peaks in growth hormone release and in serum free fatty acid levels (6). Since there are known daily rhythms in nutrient ingestion and in hormonal secretions, it appears likely that one or a combination of these hormonal-nutritional influences could act as input(s) for generating the daily rhythm in the glycogen content of the heart.

Summary. Normal rats, eating *ad libtium*, exhibit synchronized daily rhythms in the glycogen contents of fat, diaphragm, liver, and kidney while cardiac glycogen demonstrates a cycle which is 12 hr out of phase with the other rhythms. Feeding animals hourly 1/24th of their daily food allotment either quenched the rhythms or markedly decreased their amplitudes. The fat, liver, and diaphragm of adrenalectomized rats fed hourly or allowed free access to food reacted as did the tissues of the normal rats. Feeding habits, combined with their effects on the rates of hormonal release, appear to play a major role in the generation of tissue glycogen cycles.

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Received March 26, 1971. P.S.E.B.M., 1971, Vol. 137.