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Diurnal Variations of Liver Folate Metabolism in Rats Maintained Under Controlled Feeding Schedules (37867)

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There is now considerable evidence to suggest that a daily rhythm occurs in various metabolic activities in the laboratory rat in association with cycles of light and darkness, food intake, and activity (1–6).

Since the demonstration of a daily oscillation in hepatic tyrosine aminotransferase by Potter (7), many other enzyme activities have been shown to fluctuate daily (9–13).

The incorporation of labeled precursors into RNA (5), the endoplasmic reticulum (14), and DNA synthesis itself (15) have also shown diurnal variation.

In this light, in the course of studies on the regulation of folate metabolism in mammals by hormones (16–18) and vitamins (19, 20), we have undertaken the study of this metabolism in the liver of rats maintained under controlled feeding schedules. The liver was studied since it is the most active organ in terms of C1 unit metabolism.

The possible diurnal variations found during the 24-hr cycle could help in establishing a precise background to evaluate more exactly the quantitative phenomena found in the different experimental conditions studied.

Materials and Methods. Seven-week-old male Wistar albino rats weighing 200–220 g were housed since weaning in an air-conditioned windowless room with an inverted and displaced lighting schedule. The lights were on from 9:00 PM (21:00) to 9:00 AM (9:00) in a 24-hr cycle. The food, a standard lab diet, was supplied just before the lights were switched off and was removed 8 hr later, according to the "8 + 16" feeding schedule developed by Potter *et al.* (21). Water was supplied *ad lib.*

Rats were killed by decapitation at the time of day indicated in the single experiments and the livers quickly removed and frozen at −80°.

To determine the folate coenzymes[1], a part of the single livers was homogenized with 1% potassium ascorbate, pH 6.0. The homogenate was heated at 95° for 5 min, cooled, and centrifuged. The supernatant obtained was incubated for 4 hr at 37° with pig kidney conjugase. The folate derivatives were determined by the "aseptic" and "autoclaved" microbiological assay described by Bird *et al.* (22).

The enzyme activities were measured on the supernatant obtained by homogenizing part of the single livers with 0.05 *M* Tris–HCl, pH 7.5, then centrifuging at 15,000*g* for 20 min. Tetrahydrofolate dehydrogenase (EC 1.5.1.3) activity was determined by measuring the decrease in absorbance at 340 nm caused by the conversion of NADPH into $NADP^+$ (23). Serine hydroxymethyltransferase (EC 2.1.2.1) activity was determined by measuring photometrically both the free and the bound formaldehyde in 5,10-CH_2–H_4folate with the acetylacetone reagent (24). N^5,N^{10}-Methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5) (25) and 10-HCO–H_4folate synthetase (EC 6.3.4.3) (26) activities were assayed by determining spectrophotometrically the 5,10-CH=H_4folate formed.

[1] The following abbreviations are used: H_4folate, tetrahydrofolate; 5,10-CH_2–H_4folate, N^5,N^{10}-methylenetetrahydrofolate; 10-HCO–H_4folate, 10-formyltetrahydrofolate; 5,10-CH=H_4folate, N^5,N^{10}-methylidynetetrahydrofolate.

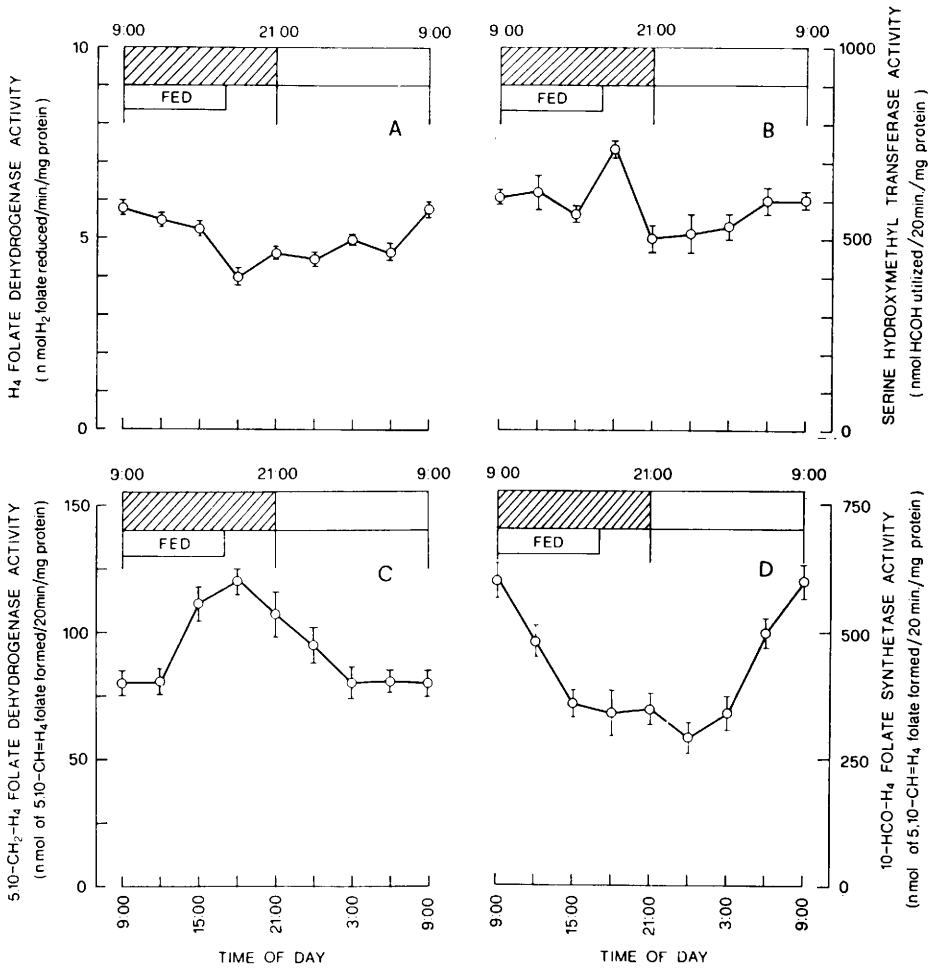

FIG. 1. Hepatic enzyme activities of folate metabolism as a function of time of day. (A) H_4Folate dehydrogenase; B, serine hydroxymethyl transferase; (C) 5,10-HCO–H_4folate dehydrogenase; (D) 10-HCO–H_4folate synthetase. Each point represent the mean ± SEM of 6–8 rats.

Results and Discussion. Figure 1 reports the daily pattern of the four enzyme activities studied. Graph A refers to the H_4folate dehydrogenase activity and shows that there was little variation in the range of the 24-hr cycle. Only a slight decrease occurred immediately after the removal of food. This decrease was followed by a progressive recovery during the next 12 hr to again reach the same level. This behavior seems to indicate an almost constant availability of the substrate and its consequent constant conversion to the active form.

Serine hydroxymethyl transferase activity (Graph B) shows on the contrary a significant increase of about 25% over a rather constant background level of about 550 nmoles HCOH utilized/20 min/mg protein maintained through the whole day ($P < .001$). This is consistent with the possibility that the food intake increases the availability of the serine substrate.

More evident diurnal variations are found in both of the other enzyme activities studied. The 5,10-CH_2–H_4folate dehydrogenase activity (Graph C) begins to increase 6 hr after the onset of the dark period, which coincides also with the food availability. This enzyme activity peaks immediately after the food has been removed and decreases thereafter to reach again the background level during the light period. An opposite be-

havior has been found for the 10-CHO–H_4-folate synthetase activity, which shows a progressive decrease from 9:00 AM to 12:00 PM (24:00) to increase again thereafter. The fastest decrease of this enzyme activity coincides with the availability of food, and this leads us to think that the CHO–H_4folate forms are more actively synthesized by other alternate pathways induced by the food intake.

Experiments performed on the liver content of the folate coenzymes during the day failed to show any significant diurnal variation in the concentration of these compounds.

Our results indicate that during the 24-hr cycle in the rat liver, there is a different need of the folate coenzymes in association with the daily rhythms of the other liver metabolic activities and that the constant level of the folate coenzymes is maintained by the diurnal variations of the enzyme activities involved in their formation. Besides this, the present results stress the need for controlled feeding schedules in order to obtain more valuable indications from the experiments and establish a precise daily background pattern for further experiments on the regulation of folate metabolism in the rat liver.

Summary. Rats maintained under controlled feeding schedules exhibit daily rhythms of the enzyme activities involved in folate metabolism. Tetrahydrofolate dehydrogenase and serine hydroxymethyl transferase show only small variations in their activity in the range of the 24-hr cycle. The 5,10-CH_2–H_4folate dehydrogenase shows a peak of activity at 6 PM (18:00), while the 5,10-CH_2–H_4folate synthetase activity reaches its lowest level between 3:00 PM (15:00) and 3:00 AM (3:00). The liver content of folate coenzymes does not change significantly during the whole day.

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