Effect of riboflavin deficiency on in vivo incorporation of C^{14} from alanine-1-C^{14} into liver glycogen (averages ± S.E.)

Nutritional state	No. of animals	Liver			
		Weight g/100 g body wt.	Glycogen in mg		Glycogen specific activity[a]
			per g	per 100 g body wt.	
Group A (control)	6	3.21 ± 0.10	0.158 ± 0.004	0.509 ± 0.029	3.96 ± 0.58
Group B (riboflavin deficient)	7	3.84 ± 0.13	0.190 ± 0.001	0.730 ± 0.028	7.2 ± 0.54

[a] Glycogen specific activity = counts per min per mg of glycogen.

correction for back ground and self absorption. They were expressed as counts/min/mg of glycogen. The Table shows that the increased glycogen content in liver in riboflavin deficiency is associated with increased incorporation of C^{14} into it from labelled alanine.

It has been reported earlier[4] that increased glycogen deposition in liver is associated with enhanced alanine transaminase activity in the liver of riboflavin deficient rats. There are evidences[5,6] suggesting direct correlation between alanine transaminase activity and glycogen deposition in liver through gluconeogenesis. Studies of LONG et al.[7] and WELT et al.[12] established that adrenal steroid hormones, when administered to the whole animal, increases the rate of gluconeogenesis. Further in vivo administration of cortisol to rats has been found to increase the incorporation of alanine carbon into liver glycogen[13]. VON HOLT et al.[14] demonstrated, using C^{14}-labelled substrates, that the synthesis of glucose from amino acids and the incorporation of this glucose into liver glycogen are enhanced following treatments of rats with cortisol. In the present investigation also, riboflavin deficiency causes increased incorporation of C^{14} from alanine-1-C^{14} into liver glycogen. In vitro effect of adrenal steroids on hepatic gluconeogenesis has been demonstrated[8,9,15-17]. These hormones stimulate synthesis of carbohydrate from L-alanine by liver slices of normal[15,17], adrenalectomized[8] or pyridoxine-deficient rats[16]. This was reflected in an increased incorporation of labelled carbon from alanine into glucose[8] or into glucose and glycogen[15]. So these observations, along with the results reported earlier[4] and in the present investigation, suggest that riboflavin deficiency causes increased conversion of the amino acid into pyruvate by transamination. This is possibly effected by increased adrenal cortical secretion as riboflavin deficiency produces increased adrenal cortical activity[4,10]. This increased conversion of alanine to pyruvate might ultimately lead to increased incorporation of labelled carbon from alanine into liver glycogen[18].

Zusammenfassung. Männliche Albino-Ratten, die während 45 Tagen mit Riboflavin-armer Kost ernährt wurden, zeigten erhöhten Glykogengehalt in der Leber, verbunden mit einer gesteigerten Aufnahme von C^{14} aus Alanin-1-C^{14} im Leberglykogen. Die Befunde lassen auf erhöhte Glykoneogenese in der Leber Riboflavin-armer Ratten schliessen.

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Circadian Rhythmycity of some Key Metabolites in the Fasted and Fed Weanling Female Rat[1]

Circadian rhythmycity[2] has been established for a great number of biological parameters[3]. The present report deals with the circadian fluctuations observed in several key metabolites in weanling female Holtzman rats.

8 groups of 8 rats each were accommodated in single cages in a room kept at a constant temperature of 22.5 °C and with 12 h light (06.00–18.00) and 12 h dark (18.00 to 06.00). They were given tap water and lab chow ad libitum. After 4 days on the above regimen the rats of group 1 were fasted from 08.00–08.00 the following day; similarly, the rats of groups 3, 5 and 7 were fasted from 14.00, 20.00 and 02.00, respectively, for the next 24 h. During this time the corresponding controls (groups 2, 4, 6 and 8) were fed ad libitum. This was done because food intake and fasting are known to affect several parameters measured in this study. The rats were sacrificed by decapitation, trunk blood was received and serum obtained for the determination of inorganic phosphorus, glucose, urea nitrogen and total protein (Beckman Ultramicroanalytical System, Model 150, Technical Bulletins No. 6079D, 6073D, 6075D and TB-6074D, respectively),

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and serum sodium and potassium by flame photometry.

Fed rats. Significant peaks in serum glucose, serum sodium (Na^+) and serum potassium (K^+) were found in the animals sacrificed at 20.00. Low values were observed in serum Na^+ and K^+ in the animals killed at 08.00, again of serum Na^+ and K^+ at 14.00 and at the same time also of glucose and total protein. At 20.00 and 02.00 total protein values were significantly lower than those at 08.00. No significant diurnal fluctuations were observed in serum inorganic phosphorus and urea nitrogen (Figures 1 and 2).

Fasted rats. Significant peaks were seen in total serum protein at 08.00, in urea nitrogen at 14.00 and in serum Na^+ at 20.00. Significant troughs and low values, respectively, were observed in inorganic phosphorus at 08.00, of total protein, urea nitrogen and inorganic phosphorus at 02.00. No significant fluctuations were found in serum glucose and K^+.

The present data show significant diurnal fluctuations in some metabolites but not in others. As expected, some parameters that fluctuate in fed rats do not do so in fasted animals. Thus, high serum glucose values at 20.00 in fed animals are likely related to increased activity and/or food intake around that time. RICHTER[4] has reported that rats show the period of their greatest activity after the onset of darkness, which in RICHTER's and the present studies was 18.00. In keeping with this assumption is the lack of diurnal fluctuations in serum glucose in fasted rats. It is noteworthy that in the fasted rats a number of parameters, i.e. total serum protein, showed a peak value in the morning hours (08.00) that was significantly higher than the levels found during the subsequent 18 h. Similarly, urea nitrogen was high at 08.00 and 14.00 while subsequent determinations (20.00 and 02.00) showed significantly lower values.

Serum and plasma inorganic phosphorous levels have been related to growth hormone activity[5-7]. The significant peak in this parameter in the fasted rats at 14.00 suggests a possible peak of plasma growth hormone levels at this time. Preliminary results on pituitary and plasma growth hormone levels by radioimmunoassay obtained in collaboration with Dr. L. A. FROHMAN of the Buffalo General Hospital, Department of Medicine, suggest that this might indeed be the case.

Whatever the mechanism(s) are for these phenomena – be it (they) fluctuations of 1 or several endocrine secretions – the fact that they occurred in fasting animals, i.e. without the exogenous stimulus of food intake, points to a dependence on nervous and neuroendocrine agents that in turn seem to be subject to fluctuations in the activity of the 'Biological Clock'[8,9].

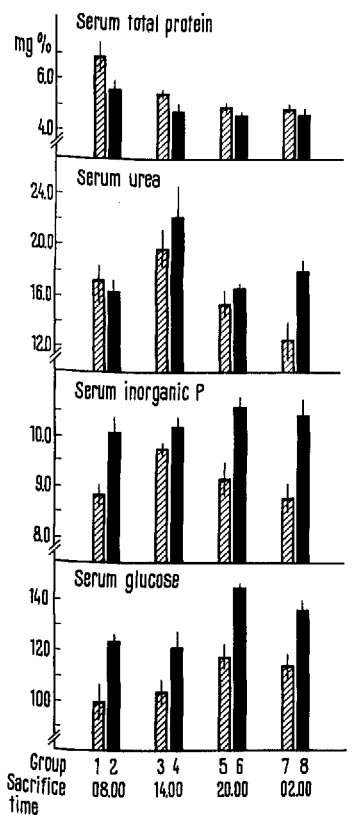

Fig. 1. Levels of several metabolites in fasted (horizontally-hatched) and fed (solid black) rats that were sacrificed at 08.00, 14.00, 20.00 and 02.00 h; group number and sacrifice time are shown at bottom of graph.

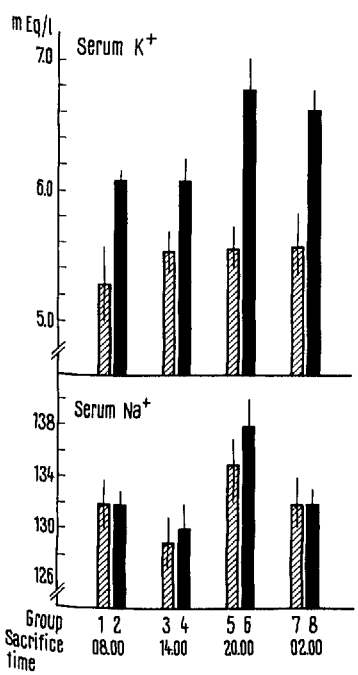

Fig. 2. Levels of serum sodium and potassium. For legend see Figure 1.

Zusammenfassung. Bei je 32 Ratten im Hungerzustand und mit normaler Fütterung wurden Tagesschwankungen von Na, K, Protein, Harnstoff, Phosphor und Glukose im Serum gefunden. Die Schwankungen spiegeln zweifellos die Aktivität mehrerer endokriner Sekretionen wider, welche wiederum von der Biologischen Uhr[8] beeinflusst werden.

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