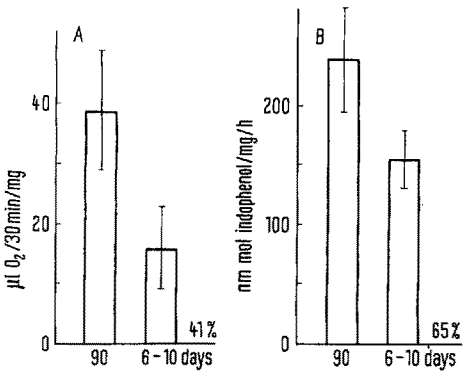
The presence of phenazine-methasulphate, however, did not decrease the difference in sarcosine dehydrogenase activity between adult and infant rats.

In 7-day-old rats mitochondrial sarcosine dehydrogenase activity represents 83 ± 17% of the total activity in liver homogenates. Hence it is unlikely that the lower activity in mitochondria from 7-day-old rats is due to the release of intramitochondrial proteins during isolation of mitochondria.



Sarcosine dehydrogenase activity of rat liver mitochondria from young and adult rats determined manometrically (A) and colourimetrically (B). Enzyme activity is expressed as μl O_2/30 min/mg mitochondrial protein (A) and mμmoles of indophenol reduced/h/mg protein (B).

The lower activity of sarcosine dehydrogenase in liver mitochondria from infant rats might signify a change in the ratio of membrane and soluble protein fraction in them. This, however, was found not to be the case, since this ratio was found to be the same in the mitochondrial fraction (600–5000 *g*) of 7-day-old and adult rats, i.e. 64% of membrane proteins and 36% of soluble proteins.

Thus the change with age in sarcosine dehydrogenase activity described here seems to be due to a change in enzyme activity that is not related to an overall increase in soluble proteins in mitochondria.

Zusammenfassung. Die Aktivität der Sarcosinedehydrogenase in Lebermitochondrien 7tägiger Ratten ist um 50% kleiner als in adulten Tieren. Dieser Unterschied ist nicht durch das Elektrontransportflavoprotein limitiert und kann auch nicht durch eine Änderung in dem relativen Wert der löslichen Proteine in den Mitochondrien erklärt werden.

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Effect of Riboflavin Deficiency on in vivo Incorporation of C^{14} from Labelled Alanine into Liver Glycogen

MORGAN et al.[1,2] observed an increased gluconeogenesis in the liver at the beginning of riboflavin deficiency and a depressed gluconeogenesis in the later part of riboflavin deficiency. MOOKHERJEA et al.[3] demonstrated increased transaminase activity in the liver of riboflavin deficient rats. Recently, increase in alanine transaminase activity of the liver of riboflavin deficient rats was found to be associated with a greater deposition of glycogen in the liver[4]. NICHOL et al.[5] and EISENSTEIN[6] observed a direct correlation between alanine transaminase activity and glycogen deposition in liver through gluconeogenesis. They have studied this association in various ways, including the use of the pyridoxine deficient rats. Further studies of LONG[7] demonstrated that adrenocortical hormone administration increased body carbohydrate stores. The rise in liver glycogen and blood sugar were attributed to gluconeogenesis. HAYNES[8] and OKUNO[9] demonstrated that adrenal steroids stimulate carbohydrate synthesis from alanine when incubated in vitro with rat liver, findings which support the idea that a primary effect of these hormones is to enhance gluconeogenesis. The present experiments were therefore carried out with alanine-1-C^{14} to study whether increased glycogen content in riboflavin deficient liver as reported earlier[4] resulted from increased gluconeogenesis or not.

Young male albino rats of 80–100 g were divided into 2 groups of equal average body weights. Group A consisted of control animals, and group B of riboflavin deficient rats. The animals were pair-fed on 16% protein for 45 days. Particulars regarding the diet have been reported elsewhere[10]. Water-soluble vitamins were supplied daily by s.c. injection.

After the experimental period was over, the rats were kept fasting overnight and then injected i.p. with 5 μc of alanine-1-C^{14} (specific activity 1.38 mc/m*M*) and after 6h they were sacrificed. The livers were removed, cleaned of adherent blood, weighed, and chilled in ice. A weighed quantity of liver was digested in 30% KOH. The digested tissue was then subjected to processings to extract glycogen[11]. The glycogen extracted was then suspended in 2 ml of water. 1 ml of aliquot was used on planchet for radioactive counts in a windowless gas flow counter. The remaining part was used for determination of glycogen after hydrolysis[11]. Radioactive counts were subjected to

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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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