Effect of riboflavine deficiency on incorporation in vivo of [^{14}C]amino acid into liver proteins of rats

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(*Received* 15 *September* 1969—*Accepted* 20 *January* 1970)

1. The effect of riboflavine deficiency on in vivo incorporation of [2-^{14}C]glycine into proteins of liver homogenates and its subcellular fractions has been studied on rats maintained on a 16% protein diet.

2. Riboflavine deficiency did not seem to affect the in vivo incorporation of [2-^{14}C]glycine into proteins of liver homogenates. But riboflavine deficiency caused increased and reduced in vivo incorporation of [^{14}C]amino acid into mitochondrial and microsomal proteins, respectively. There was no significant change in the in vivo incorporation of [^{14}C]amino acid into proteins of nuclear, ribosomal and soluble fractions in riboflavine deficiency.

3. Riboflavine deficiency caused enhanced and reduced proportions of liver mitochondrial and microsomal proteins, respectively.

4. The results are discussed as suggestive of enhanced and reduced protein synthesis in the mitochondrial and microsomal fractions, respectively, of riboflavine-deficient rats.

A number of investigators demonstrated a direct relationship between liver storage and utilization of riboflavine and the level of dietary protein intake (Sarett, Klein & Perlzweig, 1942; Sarett & Perlzweig, 1943; Unna, Singher, Kensler, Taylor & Rhoads, 1944; McQuarrie & Venosa, 1945; Czaczkes & Guggenheim, 1946). Further, riboflavine is known to have an important role in protein assimilation and tissue protein synthesis (Sure, 1941, 1944; Sure & Dichek, 1941; Sure & Ford, 1942). Studies on the relationship between the intake of riboflavine and the degree of nitrogen storage have also been made on many occasions (Borgström & Hammersten, 1944; Seifter, Harkness, Rubin & Muntwyler, 1948; Mayfield & Hedrick, 1949; Doisy & Westerfeld, 1952). All these studies emphasize the importance of riboflavine in protein metabolism.

Guggenheim & Diamant (1959) noted liver enlargement in riboflavine deficiency, but the concentration of nitrogen in liver was found to have remained unaffected. There was, however, a decrease in the nitrogen content of the carcass, principally that of muscle. Mookerjea & Hawkins (1960) observed that deprivation of riboflavine does not seem to impair the ability of the rat to synthesize important constituents of liver and blood. Unimpairment of the capacity of the liver to regenerate proteins has also been observed by Mookerjea & Jamdar (1962). These observations suggest that riboflavine deficiency may not affect the synthesis of liver proteins, but the subject requires further study. The present investigation was therefore undertaken to study the in vivo incorporation of ^{14}C-labelled amino acid into liver proteins in riboflavine-deficient rats.

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EXPERIMENTAL

Animals and diets. Male albino rats weighing 80–100 g were allocated to control and riboflavine-deficient groups, A and B respectively. Control rats were pair-fed with riboflavine-deficient rats. The diet contained 16% protein (vitamin-free casein) along with other ingredients, which were the same as those described by Jamdar & Mookerjea (1962) and Chatterjee, Roy & Ghosh (1969). Water-soluble vitamins were supplied daily by subcutaneous injections. The animals were maintained on the experimental diet for 45 d.

After the experimental period was over, the rats were fasted overnight and then 1 μCi radioactivity as [2-^{14}C]glycine (specific activity, 1 mCi/m-mole) per 100 g body-weight was injected into the tail vein. The rats were killed by a blow on the head 1 h after the injection.

Removal and analysis of liver. After the rats had been killed the livers were exposed and blood was drawn from the liver through the hepatic vein. Each liver was excised and washed with 0·9% (w/v) NaCl. It was then blotted dry and weighed. A weighed portion of liver was homogenized in ice-cold 0·25 M-sucrose solution, using an all-glass homogenizer. Some liver homogenate was also spun in an ordinary centrifuge in the cold to remove cell debris and was then subjected to differential centrifugation according to Schneider & Hogeboom (1950) in an ultracentrifuge, successively at 3000 *g* for 10 min, 10000 *g* for 15 min, 30000 *g* for 30 min and 105000 *g* for 1 h, to separate nuclear, mitochondrial, microsomal and ribosomal fractions respectively. The supernatant liquid obtained after centrifugation at 105000 *g* formed the soluble fraction. Proteins were precipitated from portions of tissue homogenate and subcellular fractions (resuspended in 0·25 M-sucrose solution) of liver tissue by the addition of an equal volume of 10% trichloroacetic acid and treated according to the procedure of Rabinovitz, Olson & Greenberg (1954). The dried protein was dissolved in a known volume of 1 N-NH_4OH solution. Portions of this solution were used for protein determination (Gornall, Bardawill & David, 1949) and for taking radioactive counts in a windowless gas-flow counter. Sufficient counts were recorded and corrections were applied for the background and self-absorption. The results have been expressed as specific activities (counts/min per mg protein).

Statistical analysis. The significance of changes in protein specific activity in riboflavine deficiency was determined by the *t* test (Fisher, 1936).

RESULTS

Table 1 shows reduced body-weight ($t = 4{\cdot}81$) and enlarged liver ($t = 2{\cdot}31$) in riboflavine-deficient rats. Table 2 demonstrates that riboflavine-deficient rats did not show any significant change ($t = 1{\cdot}21$) in the in vivo incorporation of [^{14}C]amino acid into proteins of liver homogenates. But riboflavine deficiency caused increased ($t = 2{\cdot}16$) and reduced ($t = 2{\cdot}15$) in vivo incorporation of [^{14}C]amino acid into mitochondrial and microsomal proteins respectively. There was no significant change in the in vivo incorporation of [^{14}C]amino acid into proteins of nuclear, ribosomal and

soluble fractions in riboflavine deficiency. Table 3 reveals that the riboflavine-deficient rats had increased and decreased proportions of mitochondrial and microsomal protein fractions respectively.

Table 1. *Effect of riboflavine deficiency on body-weight and liver weight*

(Mean values with their standard errors for eight rats)

Group	Body-wt (g)	*t* Value	Liver wt (g/100 g body-wt)	*t* Value
Pair-fed control	112·7 ± 3·50	4·81	3·18 ± 0·21	2·31
Riboflavine-deficient	90·5 ± 3·01		3·97 ± 0·27	

Table 2. *Effect of riboflavine deficiency on the in vivo incorporation of [2-^{14}C]glycine into proteins of whole liver and its subcellular fractions*

(Mean values with their standard errors for eight rats)

Tissue	Protein specific activity (counts/min mg protein): Pair-fed control	Riboflavine-deficient	*t* Value
Whole liver	108·0 ± 13·5	90·0 ± 6·1	1·21
Fractions of liver homogenates:			
Nuclear	69·5 ± 10·2	63·8 ± 7·5	
Mitochondrial	145·9 ± 11·0	180·6 ± 11·7	2·16
Microsomal	171·4 ± 9·1	141·5 ± 10·5	2·15
Ribosomal	101·7 ± 13·1	98·8 ± 15·2	—
Soluble	83·7 ± 13·0	72·7 ± 12·5	—

Table 3. *Effect of riboflavine deficiency on proportions of subcellular protein fractions in liver*

(Mean values with their standard errors for eight rats)

Subcellular fraction	Protein content of subcellular fraction*: Pair-fed control	Riboflavine-deficient	*t* Value
Nuclear	35·3 ± 2·15	35·6 ± 1·45	—
Mitochondrial	18·9 ± 0·85	24·9 ± 1·03	4·498
Microsomal	20·7 ± 0·98	14·4 ± 0·97	4·572
Ribosomal	11·6 ± 1·09	12·4 ± 1·20	—
Soluble	13·4 ± 1·03	12·6 ± 1·4	—

* As % of liver total protein.

DISCUSSION

The induction of riboflavine deficiency caused liver enlargement (Table 1). This confirmed observations which had been made before (Guggenheim & Diamant, 1959; Mookerjea & Jamdar, 1962; Chatterjee, Jamdar & Ghosh, 1966; Kim & Lambooy, 1969; Fass & Rivlin, 1969). The incorporation of [^{14}C]amino acid into proteins of liver was found to be unaffected in riboflavine deficiency (Table 2). The cellular

protein concentration is a function of the rates of anabolism and catabolism. In the liver, the rate of protein synthesis has been correlated with the RNA:DNA ratio and the free amino acid levels (Allison, Wannemacher & Banks, 1963). Riboflavine deficiency for 45 d did not affect the RNA and DNA levels of liver (Chatterjee *et al.* 1969) and consequently liver RNA:DNA ratio remained unaffected. However, the total free amino acid nitrogen concentration in liver was found to be increased in riboflavine deficiency (Chatterjee & Ghosh, 1968). The protein concentration in liver was also found to be unimpaired in riboflavine deficiency (Guggenheim & Diamant, 1959; Fass & Rivlin, 1969). These studies show unaltered protein:RNA and protein:DNA ratios in liver of riboflavine-deficient rats, suggesting that the synthesis and catabolism of liver proteins is unimpaired in riboflavine deficiency. The unaltered liver protein synthesis in riboflavine deficiency is further confirmed by the unimpaired incorporation of [^{14}C]amino acid into liver protein as noted in the present investigation (Table 2). That liver protein synthesis is unimpaired in riboflavine deficiency, as found in the present experiment, agrees with the results of Mookerjea & Hawkins (1960) and Mookerjea & Jamdar (1962), who noted that the capacity of the riboflavine-deficient liver to regenerate proteins was not affected by partial hepatectomy or protein depletion.

It is noteworthy that the mitochondrial fraction of riboflavine-deficient liver showed enhanced incorporation of [^{14}C]amino acid into protein, while the microsomal protein showed reduced uptake of [^{14}C]amino acid (Table 2). The incorporation of [^{14}C]amino acid into proteins of nuclear, ribosomal and soluble fractions remained uninfluenced by riboflavine deficiency (Table 2). Burch, Hunter, Combs & Schutz (1960) noted an increase in the mitochondrial population in riboflavine deficiency with an increase in the fraction of liver protein associated with mitochondria. Kim & Lambooy (1969) also found increased numbers of liver mitochondria in riboflavine deficiency. The present study also showed an increased proportion of liver mitochondrial protein (Table 3). Thus, this increase in liver protein in the mitochondrial fraction was found to be associated with the enhanced incorporation of [^{14}C]amino acid into mitochondrial protein as noted in the present investigation. This suggests, therefore, increased protein synthesis in mitochondria. Reis, Coote & Work (1959) and Rendi (1959) demonstrated the presence of amino acid activating enzymes and ribonucleoprotein particles in mitochondria. Whether the enhanced mitochondrial protein synthesis in riboflavine deficiency is related to the amino acid activating enzymes and ribonucleoprotein particles of mitochondria cannot be ascertained from the present studies. Investigation on the subcellular fractions of rat liver showed that transaminase enzymes are mostly located in the mitochondrial fraction (Eichel & Bukovsky, 1961). In riboflavine deficiency both aspartate aminotransferase and alanine aminotransferase activities are enhanced in liver (Mookerjea & Jamdar, 1962; Chatterjee *et al.* 1966). These increased transaminase activities may be related to the enhanced mitochondrial protein synthesis resulting in increased synthesis of apoprotein of transaminases in riboflavine deficiency. Tables 2 and 3 demonstrate that the reduced incorporation of [^{14}C]amino acid into microsomal protein was associated with a diminished proportion of microsomal protein in the liver of the riboflavine-deficient rats. This might suggest reduced

microsomal protein synthesis in the liver in riboflavine deficiency. It is possible that the distribution of certain enzymes in various subcellular fractions may differ in riboflavine deficiency from those that are found under normal condition (Burch *et al.* 1960). The possible influence of adrenal cortex on the incorporation of radioactive amino acid into microsomal protein has been emphasized by Korner (1960). He demonstrated that microsomes isolated from rat liver after adrenalectomy incorporate more radioactive amino acid into their protein in vitro than microsomes from normal rat liver. In the present investigation microsomes showed reduced incorporation of [^{14}C]amino acid into their proteins (Table 2). Studies reported earlier (Chatterjee *et al.* 1966; Chatterjee & Ghosh, 1967, 1968, 1969; Jamdar & Udupa, 1967) suggested stimulated activity of the adrenal cortex in riboflavine deficiency. Whether the diminished incorporation of [^{14}C]amino acid into microsomal proteins in riboflavine deficiency is an effect of stimulated adrenal cortical activity cannot be ascertained from the present findings. It is a basic assumption of the hypothesis that the ability of the microsomes to incorporate amino acids into protein is enhanced or diminished when there is, at the site of incorporation, a high or low concentration of amino acids available for incorporation. Korner (1960) suggested that corticosteroid-induced gluconeogenesis can check the amino acid incorporation by the liver microsomes into protein and this check is removed by adrenalectomy. In riboflavine deficiency there is also an increased gluconeogenesis in liver (Chatterjee & Ghosh, 1967). This increased gluconeogenesis may divert the amino acids from the microsomes, resulting in decreased concentration of amino acids at the site of incorporation in the microsomes. This reduced concentration of amino acids in the microsomes may lead to a diminution in the ability of the microsomes to incorporate amino acids into protein in riboflavine deficiency.

The authors express their sincere gratitude to Professor S. R. Maitra, Head of the Department of Physiology, Calcutta University, for constant encouragement and for his keen interest in the work. They are also grateful to Mr K. B. Udupa of Saha Institute of Nuclear Physics, Calcutta, for assistance in making some of the radioactive counts.

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Printed in Great Britain