Effect of riboflavine deficiency on nucleic acid metabolism of liver in the rat

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1. The effect of riboflavine deficiency on liver ribonuclease activity, RNA and DNA content, and ^{32}P incorporation into RNA and DNA has been studied in rats maintained on a 16% protein diet, a protein-free diet and on a protein-free diet subsequently replaced with a 40% protein diet.

2. Rats maintained on a riboflavine-deficient diet for 45 days showed decreased incorporation of ^{32}P into liver RNA but no effect on the RNA content of liver. The concentration of DNA in liver and ^{32}P incorporation into it remained unaffected. After a deficiency period of 70 days, both the RNA and DNA contents of liver were found to be decreased. When the riboflavine-deficient or control rats were given the protein diet for 30 days and then a protein-free diet for 15 days, the RNA content of their livers decreased, while the liver DNA content was increased. Repletion with a 40% protein diet restored the RNA and DNA content in both control and riboflavine-deficient rats.

3. Liver ribonuclease activity was decreased after a deficiency period of 45 days, whereas it was increased after a deficiency period of 70 days.

4. A correlation between liver RNA level and liver ribonuclease activity in riboflavine deficiency is suggested.

With regard to the role of riboflavine in the metabolism of protein in liver, it is of interest to note that there is 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, a number of investigators (Sure 1941; 1944; Sure & Dichek, 1941; Sure & Ford, 1942) demonstrated the importance of riboflavine in protein assimilation and tissue protein synthesis. The relationship between the intake of riboflavine and the degree of nitrogen storage has also been studied on many occasions (Borgström & Hammersten, 1944; Seifter, Harkness, Rubin & Muntwyler, 1948; Mayfield & Hedrick, 1949; Doisy & Westerfeld, 1952). All these observations indicate the importance of riboflavine in protein metabolism.

Guggenheim & Diamant (1959) observed liver enlargement in riboflavine deficiency, but the concentration of nitrogen in liver 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). The important role of nucleic acids in protein

metabolism is well established. The present investigation was therefore undertaken to study whether riboflavine deficiency causes any change in the nucleic acid metabolism of liver.

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. Three diets, varying in protein content, were used. Their percentage compositions were: vitamin-free casein 16, 40 or 0 with corresponding carbohydrate at 73, 49 or 89 respectively, groundnut oil 7 and salt mixture (Hawk & Oser, 1931) 4. Water-soluble vitamins were supplied daily by subcutaneous injections.

The group of control rats was subdivided into four subgroups. One subgroup (A_1) was fed 16% protein for 45 days only; subgroup (A_2) was fed 16% protein for 30 days followed by 0% protein for 15 days; subgroup (A_3) was fed 16% protein for 30 days followed by 0% protein for 15 days and then fed 40% protein for another 7 days; subgroup (A_4) was fed 16% protein for 70 days. The riboflavine-deficient rats (group B) were similarly subdivided.

After the experimental period for each dietary regimen was over, the rats were fasted overnight and then 5 μc [^{32}P]phosphate (specific activity, 5 mc/mM) per 100 g body-weight were injected intraperitoneally. The rats were killed 2 h after the injection.

Removal and analysis of liver. Under ether anaesthesia, blood was drawn from the liver through the hepatic vein. Then the rats were killed by exsanguination. The liver was excised and washed with 0·9% (w/v) NaCl. A weighed portion of liver was homogenized in ice-cold distilled water, using an all-glass homogenizer. RNA and DNA were isolated from the tissue homogenates by the modified Schmidt-Thannhauser method, as recommended by Munro (1966). The RNA and DNA contents of the fractions were determined by the orcinol reaction (Brown, 1946) and diphenylamine reaction (Dische & Schwarz, 1937). Portions of RNA- and DNA-fractions were used for determination of organic phosphorus (Umbreit, Burris & Stauffer, 1957) and radioactivity in an end-window beta counter. The radioactivity of the inorganic phosphate of the liver was measured by the procedure described by Davidson, Frazer & Hutchison (1951). The results have been expressed as specific activities (counts/min per 100 μg P) and also as relative specific activities (specific activity of the organic P as a percentage of the specific activity of the inorganic phosphate fraction of the same liver). For determination of ribonuclease activity a weighed portion of liver was homogenized in 0·1 M-acetate buffer, pH 5·0, and the ribonuclease activity was determined according to the method based on ultraviolet absorption of acid-soluble degradation products from DNA (Josefsson & Lagerstedt, 1962).

Statistical analysis. The significance of the changes in RNA and DNA content, ^{32}P incorporation into RNA and DNA, and ribonuclease activity of liver in riboflavine deficiency was determined by the *t* test (Fisher, 1936).

RESULTS

Tables 1 and 2 show that, on the 16% protein diet riboflavine-deficient rats did not show any change in the RNA and DNA content of liver, but showed decreased incorporation of ^{32}P into RNA ($t = 7{\cdot}73$). Incorporation of ^{32}P into DNA, however, remained unaltered. The protein-free diet decreased the liver RNA content of both

Table 1. *Effect of riboflavine deficiency on nucleic acid content and ribonuclease activity of liver of rats under conditions of varied dietary protein*

(Mean values with their standard errors)

Dietary regimen (total period = 52 days)	Group of animals	Nucleic acid content (mg/10 g liver) RNA	Nucleic acid content (mg/10 g liver) DNA	Ribonuclease activity (μg/mg liver)
16% protein for 45 days	Control (A_1)	136·6(5) ±3·10	13·72(5) ±0·71	12·34(5) ±0·70
	Riboflavine-deficient (B_1)	130·7(5) ±4·91	13·00(5) ±0·98	7·45(5) ±0·21
16% protein for 30 days followed by 0% protein for 15 days	Control (A_2)	98·8(5) ±2·87	19·71(5) ±0·77	18·73(5) ±0·66
	Riboflavine-deficient (B_2)	102·5(5) ±2·64	20·46(5) ±0·91	10·91(5) ±0·71
16% protein for 30 days followed by 0% protein for 15 days plus 40% protein for 7 days	Control (A_3)	148·8(5) ±4·87	12·75(5) ±0·91	13·61(5) ±1·83
	Riboflavine-deficient (B_3)	135·0(5) ±10·59	11·81(5) ±0·74	6·30(5) ±0·62

Figures in parentheses are the number of experiments. Each experiment involved investigation on pooled samples from three or four rats.

control ($t = 8{\cdot}93$) and riboflavine-deficient rats ($t = 5{\cdot}24$), but the decrease was less marked in riboflavine-deficient rats. On the protein-free diet, liver DNA was increased in both control ($t = 5{\cdot}71$) and riboflavine-deficient rats ($t = 5{\cdot}56$). ^{32}P uptake by liver RNA of both control ($t = 6{\cdot}47$) and riboflavine-deficient rats ($t = 4{\cdot}07$) was increased on the protein-free diet, but the protein-free diet did not seem to have an effect on ^{32}P uptake by liver DNA in either control or riboflavine-deficient rats. Repletion with the 40% protein diet restored the RNA and DNA content of both control and riboflavine-deficient rats. ^{32}P uptake by liver RNA was restored in both control and riboflavine-deficient rats on repletion with the 40% protein diet.

Table 1 shows decreased liver ribonuclease activity in riboflavine-deficiency ($t = 6{\cdot}64$), and the protein-free diet increased the ribonuclease activity in both control ($t = 6{\cdot}63$) and riboflavine-deficient rats ($t = 4{\cdot}64$). The increase was less intense in the riboflavine-deficient rats. Repletion with the 40% protein diet restored the liver ribonuclease activity in both control and riboflavine-deficient rats.

Table 3 demonstrates that, after a deficiency period of 70 days, both the RNA

(t = 4·23) and DNA (t = 2·63) contents of liver were decreased in riboflavine deficiency. Liver ribonuclease activity was increased after a deficiency period of 70 days (t = 3·00).

Table 2. *Effect of riboflavine deficiency on ^{32}P incorporation into RNA and DNA of liver of rats under conditions of varied dietary protein*

(Mean values with their standard errors)

Dietary regimen (total period = 52 days)	Group of animals	Specific activity* RNA	Specific activity* DNA	Relative specific activity† RNA	Relative specific activity† DNA
16% protein for 45 days	Control (A_1)	132·5(6) ±5·76	56·2(6) ±2·91	39·6(6) ±0·55	16·8(6) ±0·48
	Riboflavine-deficient (B_1)	110·8(6) ±4·69	57·2(6) ±5·28	31·2(6) ±0·94	16·1(6) ±0·97
16% protein for 30 days followed by 0% protein for 15 days	Control (A_2)	157·9(6) ±7·20	56·8(6) ±4·14	46·8(6) ±0·97	17·2(6) ±0·73
	Riboflavine-deficient (B_2)	126·8(6) ±4·81	58·2(6) ±6·03	36·2(6) ±0·79	16·6(6) ±0·78
16% protein for 30 days followed by 0% protein for 15 days plus 40% protein for 7 days	Control (A_3)	122·3(6) ±5·69	51·4(6) ±2·93	38·8(6) ±0·44	16·3(6) ±1·09
	Riboflavine-deficient (B_3)	101·4(6) ±7·61	52·1(6) ±1·96	30·4(6) ±1·07	15·5(6) ±0·58

Figures in parentheses are the number of animals.

* Counts/min per 100 μg P.

† Specific activity of the organic P as a percentage of the specific activity of the inorganic phosphate fraction of the same liver.

Table 3. (*Total period = 70 days*). *Effect of riboflavine deficiency on the nucleic acid content and ribonuclease activity of liver of rats receiving a diet containing* 16% *protein for* 70 *days.*

(Mean values with their standard errors)

Group of animals	Liver: Nucleic acid content (mg/10 g liver) RNA	Liver: Nucleic acid content (mg/10 g liver) DNA	Liver: Ribonuclease activity (μg/mg liver)
Control (A_4)	126·9(5) ±8·49	12·06(5) ±0·57	13·27(5) ±0·72
Riboflavine-deficient (B_4)	85·5(5) ±4·86	8·82(5) ±1·09	19·34(5) ±1·88

Figures in parentheses are the number of experiments. Each experiment involved investigation on pooled samples from three or four rats.

DISCUSSION

Although the RNA and DNA contents of liver on a tissue weight basis remained unaffected after the deficiency period of 45 days (Table 1), they were decreased after a deficiency period of 70 days (Table 3). Jamdar, Boral & Bhattacharya (1965) also

reported a diminished DNA content in the liver of riboflavine-deprived rats after a deficiency period of 60 days.

A protein-free diet caused, in both control and riboflavine-deficient rats, a slight increase in liver DNA, expressed on a tissue weight basis (Table 1). A number of investigators (Thomson, Heagy, Hutchison & Davidson, 1953; McIndoe & Davidson, 1952; Fukuda & Sibatani, 1953; Campbell & Kosterlitz, 1947, 1948, 1952) have reported that no significant increase in the average DNA content of liver cell nuclei can be demonstrated when these organelles are isolated from the livers of rats fed on protein-deficient diets. On the other hand, Ely & Ross (1951) and Lecomte & DeSmul (1952), using histochemical methods, have shown that a significant increase in the DNA content of the liver cell nucleus occurs when rats are fed on nitrogen-free or protein-deficient diets. Moreover, studies carried out on total liver homogenates (Muntwyler, Seifter & Harkness, 1950; Cooper, 1953; Villela, 1952) showed also that there is a slight increase in the DNA concentration of the liver in protein-malnourished rats. The increase in liver DNA content on a protein-free diet may be explained by protein deprivation. Williams (1961) also found an increased DNA concentration per mg of liver, on giving a protein-free diet.

Tables 1 and 3 show that liver ribonuclease activity was decreased after 45 days on the riboflavine-deficient diet, whereas it was increased after 70 days on the riboflavine-deficient diet. The importance of ribonuclease activity in the maintenance of RNA level has been emphasized (Allison, Wannemacher, Banks, Wunner & Gomez-Brenes, 1962). There is other evidence to suggest an interrelationship between the ribonuclease activity and RNA. With increasing nitrogen intake, increased RNA content of the liver was found to be associated with decreased ribonuclease activity (Zigman & Allison, 1959; Allison, Wannemacher, Parmer & Gomez-Brenes, 1961). Further, in riboflavine deficiency, both vitamin B_{12} and folic acid are significantly reduced in liver (Bhagwat & Sohonie, 1954, 1955; Donaldson & Keresztesy, 1959). Vitamin B_{12} deficiency is known to lower the nucleic acid content of liver (Donaldson & Keresztesy, 1959). Folic acid is also known to play a part in the formation of purines and nucleic acids (Greenberg, 1945; O'Brien, 1962). Reduction in the amount of vitamin B_{12} and folic acid in the liver may ultimately lead to decreased synthesis of RNA in the liver of riboflavine-deficient rats. Thus although there may be reduced synthesis of RNA, the normal RNA level, observed after 45 days of riboflavine deficiency, is probably maintained by the decreased ribonuclease activity. As the ribonuclease activity of the liver is increased after 70 days of riboflavine deficiency, the diminution in the amount of liver RNA observed after 70 days of receiving a riboflavine-deficient diet is likely to have resulted from the reduced synthesis as well as from the increased breakdown of ribonucleic acids.

Studies carried out by other investigators (Kosterlitz, 1947; Campbell & Kosterlitz, 1948, 1952; Munro & Clark, 1960) have demonstrated that liver cells of rats given a protein-deficient diet lose some of their ribonucleic acid. Munro & Clark (1960) studied extensively the interrelationship between dietary protein intake and RNA content of liver cells. They have emphasized the association of certain types of RNA with the endoplasmic reticulum, which increases and decreases in concentration with

the increase and decrease in nitrogen intake respectively. Table 1 shows that a protein-free diet decreases the RNA content of liver in both control and riboflavine-deficient rats, but the effect is less pronounced in riboflavine-deficient rats. This relatively less-diminished liver RNA found in riboflavine deficiency on a protein-free diet may be correlated with the smaller increase in ribonuclease activity (Table 1), which may result in relatively less breakdown of RNA.

The relatively smaller uptake of ^{32}P into liver RNA by the riboflavine-deficient rats (Table 2) and the maintenance of normal RNA level at the 16% dietary protein level (Table 1) are also suggestive of a decreased breakdown of RNA after a deficiency period of 45 days. Giving a protein-free diet for several days has been found to increase considerably the uptake of ^{32}P into liver RNA (Munro, Naismith & Wikramanayake, 1953). Since protein deficiency does not reduce the rate of RNA synthesis (Campbell & Kosterlitz, 1948, 1952; Munro *et al.* 1953), the increased ^{32}P activity of RNA in rats on protein-free diet may be explained by the increased breakdown of RNA; feeding of protein terminates this breakdown (Munro & Allison, 1964). Table 2 shows that a protein-free diet increases the ^{32}P uptake into liver RNA of both control and riboflavine-deficient rats, and repletion with 40% protein reduces the ^{32}P uptake into liver RNA to normal level. Riboflavine deficiency does not seem to affect the ^{32}P uptake into liver DNA under varied conditions of protein intakes.

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