EFFECTS OF RIBOFLAVIN DEFICIENCY ON OXIDATIVE PHOSPHORYLATION, FLAVIN ENZYMES AND COENZYMES IN RAT LIVER*

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SUMMARY

Riboflavin deficiency in rats caused a decrease in the activities of hepatic succinate dehydrogenase (50 %), L-α-glycerophosphate dehydrogenase (50 %) and xanthine oxidase (70 %). It also reduced to 50 % the rate of mitochondrial oxidation of succinate, β-hydroxybutyrate, α-ketoglutarate, glutamate, pyruvate and malate without changing ADP : O ratios, thus showing that riboflavin deficiency interferes with electron transport along the respiratory chain without noticably affecting phosphorylation.

Several biochemical studies have been carried out on livers of riboflavin deficient rats. Measurements of oxidative phosphorylation in hepatic mitochondria, from riboflavin deficient rats, have been reported to be normal with such substrates as succinate and glutamate (1, 2, 3). Burch et al (4) on the other hand found that with succinate and β-hydroxybutyrate as substrates there was reduction in the rates of oxidation and P:O ratios whereas with α-ketoglutarate only the rate of oxidation was reduced while P:O ratio remained normal.

In view of these conflicting results we investigated the effect of riboflavin deficiency on (i) oxidative phos-

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phorylation (ii), succinate dehydrogenase, mitochondrial L-α-glycerophosphate dehydrogenase and xanthine oxidase, and (iii) levels of riboflavin and flavin nucleotides in rat liver. To accelerate the onset of riboflavin deficiency, young rats were fed a riboflavin-free diet supplemented with galactoflavin*. Although it has been shown that the latter cannot be incorporated into tissues (5), the exact mechanism whereby galactoflavin enhances riboflavin deficiency is still unknown. It has been suggested that it might interfere with uptake of riboflavin by tissues or increase the rate of excretion of riboflavin (5).

EXPERIMENTAL

Animals : 21 days old litter-mate male Wistar rats were placed on a normal diet or a riboflavin-free diet supplemented with galactoflavin (see Table 1). The rats were sacrificed after 10 weeks.

Methods : Mitochondria were prepared by the method of Myers and Slater (6) except that 1 mM tris HCl buffer (pH 7.4) was used. Oxidative phosphorylation measurements were performed at 31°C in 3 ml of incubation medium adjusted to pH 7.4 and consisting of 0.05 M tris HCl buffer, 0.05 M KCl, 0.05 M sucrose, 0.01 M KH_2PO_4, 0.005 M $MgSO_4$ and 0.001 M EDTA. To 2.9 ml of this medium were added 50 ul of 0.004 M substrate and 50 ul of mitochondrial suspension (7). Respiratory control ratios and ADP:O ratios were determined by the method of Estabrook (8). Succinate dehydrogenase was measured by

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the 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride-indicator method (9). L-α-glycerophosphate dehydrogenase was assayed in homogenised liver mitochondria according to Lee et al (10), except that an oxygen electrode was used to measure oxygen consumption. Xanthine oxidase was estimated, in the supernatant of a liver homogenate which had been centrifuged at 30.000 xg for 30 min., by the slightly modified method of Roussos (11). Riboflavin and flavin nucleotides were determined according to Cerletti et al (12). Protein was estimated by the biuret method (13).

RESULTS AND DISCUSSIONS

The decrease in the levels of riboflavin (85 %), FMN (77 %) and FAD (74 %) compared with the normal values (Table 1) emphasised the degree of riboflavin deficiency. This deficiency was further reflected in the activities of FAD-dependent enzymes. Activities of succinate dehydrogenase and L-α-glycerophosphate dehydrogenase, which are both membrane-bound mitochondrial enzymes, were reduced to 50 %. On the other hand a decrease of 70 % in the activity of xanthine oxidase, a soluble cytoplasmic enzyme, paralleled that of FAD. This difference of 20 % in the sensitivities of the mitochondrial enzymes and the cytoplasmic enzyme to flavin deficiency could be explained in at least three ways : (a) it might be due to the fact that a native molecule of xanthine oxidase contains two molecules of FAD (14) whereas mammalian succinate dehydrogenase (15) requires only one molecule of FAD and indications are that L-α-glycerophosphate dehydrogenase (16) may also contain one molecule of FAD per molecule of

Table 1

Effect of riboflavin deficiency* on flavin enzymes and coenzymes

	Activity (units/mg)					
Animals	Succinate dehydrogenase[a]	L-α-Glycerophosphate dehydrogenase[b]	Xanthine oxidase[c]	Riboflavin[d]	FMN	FAD
Control (5)	33.96 ± 0.88	12.78 ± 0.05	60.63 ± 5.44	0.77 ± 0.05	8.93 ± 0.07	26.00 ± 0.17
Riboflavin deficient (5)	17.12 ± 0.55	6.40 ± 0.04	18.37 ± 2.36	0.11 ± 0.03	2.05 ± 0.38	6.75 ± 0.88

Figures in parenthesis indicate number of animals used.

A unit was defined as a) mg of formazan formed/min; b) n moles of O_2 consumed/min, and c) change of 0.001 in optical density/30 min. d) Riboflavin and flavin nucleotides were expressed as ug/gm wet weight of liver.

* Normal diet

Vitamin-free casein (18 %), vegetable oil (10 %), U.S.P. Salt mixture No 2 (4 %), Sucrose and "Vitamins" (68 %).

Following "Vitamins" per 100 lb of diet :

Vit. A (900.000 units), Vit. D (100.000 units), -Tocopherol (5 gm), Ascorbic acid (45 gm), Inositol (5 gm), Choline chloride (75 gm), Folic acid (90 mg), Vitamin B_{12} (1.35 mg), Menadione (2.25 gm), p-aminobenzoic acid (5 gm), Thiamine hydrochloride (1 gm), Biotin (20 mg) Niacin (4.5 gm), Pyridoxine hydrochloride (1 gm), Tryptophan supplement (0.05 %), Riboflavin (40 mg), Calcium Pentothenate (3.00 gm).

In riboflavin-free diet riboflavin was replaced by galactoflavin (60 mg/100 lb diet). Diets were provided by Hope Farms b.v., Woerden, Netherlands.

enzyme; (b) there might be difference in half-lives of the mitochondrial enzymes and the cytoplasmic enzyme; or (c) it could be that compartmentalization and localization on the membrane affords mitochondrial enzymes some protection against flavin deficiency.

Furthermore our results are consistent with those of other workers (17, 4) who found that there was no direct relationship between the level of flavin nucleotides and flavin enzymes. Each enzyme showed different sensitivity to riboflavin deficiency. It must be pointed out that these

Table 2

Effect of flavin deficiency on oxidative phosphorylation[a]

		n moles Oxygen consumed/min/mg			
Substrates	Animals	State 3	State 4	Respiratory control Ratio	ADP:O ratio
Succinate	control	136.99 ± 7.93	43.32 ± 2.03	3.16 ± 0.16	1.76 ± 0.08
	riboflavin deficient	70.63 ± 3.50	41.34 ± 3.23	1.52 ± 0.04	1.67 ± 0.06
β - hydroxy-butyrate	control	62.66 ± 2.44	16.31 ± 1.49	3.89 ± 0.30	3.05 ± 0.05
	riboflavin deficient	30.05 ± 2.50	14.09 ± 1.57	1.93 ± 0.23	3.03 ± 0.06
α - ketogluta-rate	control	76.78 ± 2.53	16.20 ± 2.70	4.85 ± 0.65	2.92 ± 0.13
	riboflavin deficient	33.62 ± 0.12	16.45 ± 0.42	2.05 ± 0.05	2.70 ± 0.20
Glutamate	control	72.56 ± 5.06	12.23 ± 0.42	6.02 ± 0.67	3.02 ± 0.07
	riboflavin deficient	40.50 ± 0.50	12.75 ± 0.80	2.96 ± 0.03	2.90 ± 0.04
Pyruvate	control	45.28 ± 0.74	11.74 ± 0.98	3.91 ± 0.27	2.73 ± 0.13
	riboflavin deficient	25.33 ± 1.68	12.33 ± 1.17	1.90 ± 0.15	2.71 ± 0.13
Malate	control	50.57 ± 3.32	14.60 ± 0.60	3.48 ± 0.37	2.77 ± 0.07
	riboflavin deficient	25.31 ± 0.50	13.50 ± 0.06	1.87 ± 0.37	2.67 ± 0.12

a : each value was obtained from duplicates of 5 animals.

workers did not assay L-α-glycerophosphate dehydrogenase.

As referred to earlier, Beyer et al (1) working with succinate and glutamate and Kielley (2) using succinate found that feeding adult rats a riboflavin deficient diet for 10-14 weeks produced no difference in mitochondrial oxidation of these substrates. Similarly Frei et al (3) reported normal oxidative phosphorylation with succinate in fla-

vin deficient mitochondria. In contrast to these results, while working with weanling rats which are much more susceptible to dietary manipulations, we found that with all the substrates tested, riboflavin deficiency caused a decrease in oxygen consumption of the order of 50 % by hepatic mitochondria (Table 2). This reduction was produced by a decrease in the rate of state 3 oxidation. Rate of state 4 oxidation like ADP:O ratios remained normal. These results agree with those of Burch et al (4) in sofaras oxidation of succinate, β-hydroxybutyrate and α-ketoglutarate were reduced. But unlike these workers we observed no alteration in P:O ratios with any of the substrates (Table 2).

Therefore our results show that, in addition to causing a fall in the activities of certain flavin enzymes, riboflavin deficiency reduced the efficiency of electron transport along the respiratory chain without noticably interfering with the phosphorylation steps.

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