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CORRELATION OF H_2O_2 PRODUCTION AND LIVER CATALASE DURING RIBOFLAVIN DEFICIENCY AND REPLETION IN MAMMALS

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A substantial decrease in liver peroxisomal catalase was found during riboflavin deficiency in rats. This decrease is greater than that found among other hemoproteins and seems to follow decrease in flavin-dependent peroxisomal oxidases. This is not due to a general depression of peroxisomal enzymes, since Cu-dependent urate oxidase activity was not changed. Furthermore, the level of catalase activity as well as flavin-dependent oxidases was restored by riboflavin repletion. These results suggest that hydrogen peroxide, the substrate for catalase produced by several flavoprotein oxidases, induces catalase in mammals as has been indicated for certain bacteria.

Catalase, (EC 1.11.1.6) a heme-dependent enzyme, had been reported to decrease in riboflavin-deficient mice (1) and rats (2); however, a mechanism for the dependence of catalase on riboflavin status had not been suggested. Since flavocoenzyme-dependent oxidases among other flavoproteins decrease in riboflavin deficiency, there is less H_2O_2 produced. It seems plausible that H_2O_2 may have some regulatory effect on catalase biosynthesis in mammals, as specific induction of catalase by H_2O_2 has been reported in certain microorganisms (3,4).

To study this hypothesis, two experiments were performed and are reported in this communication. In the first, several hemoproteins in hepatocytes isolated from riboflavin deficient rats were quantitated to see whether or not the decrease in catalase in riboflavin deficiency is relatively specific or general among hemoproteins. Functionally, the hemoproteins, viz. cytochromes

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$c + c_1$, $b_{561} + b_{566}$ and $a + a_3$ in mitochondria, cytochromes P-450 and b_5 in endoplasmic reticulum and catalase in peroxisomes, are closely coupled to flavoprotein oxidation-reduction reactions. In the second experiment, the effects of riboflavin depletion and repletion on peroxisomal flavoproteins, viz. FMN-dependent glycolate oxidase (EC 1.1.3.1) and FAD-dependent D-amino acid oxidase (EC 1.4.3.3), were compared with those on urate oxidase (EC 1.7.3.3) and catalase to determine if the change in catalase observed during change in riboflavin status is due to a general effect on all peroxisomal redox enzymes or is the reflection of change in H_2O_2-producing flavoprotein oxidases with consequent change in H_2O_2-induced catalase.

MATERIALS AND METHODS

Materials. Bovine serum albumin, collagenase, D-alanine, uric acid and hydrogen peroxide were purchased from Sigma, St. Louis, MO. Riboflavin was purchased from Aldrich, Milwaukee, WI. Other chemicals and reagents used were of analytical grade. Powdered riboflavin-deficient synthetic diet was purchased from ICN Nutritional Biochemicals, Cleveland, OH.

Animals. Weanling, male Sprague-Dawley rats (King Animal Lab., Oregon, WI) weighing 50 to 60 g were divided into two groups and housed individually in wire-bottom cages. The deficient group was fed the riboflavin-deficient diet; in the sufficient group this diet was supplemented with 2 µg of riboflavin/g. After 5 weeks, the deficient group was then fed the riboflavin-supplemented diet. In the first experiment, four animals from the deficient group at 3 weeks were anesthetized with ether and hepatocytes isolated by a collagenase recirculating perfusion method (5). In the second experiment, three to six rats from each group at 0,1,3,5,6,8, and 10 weeks were killed by decapitation. Livers were removed and minced in a ninefold volume of cold isotonic buffer (0.01 M sodium phosphate-saline buffer, pH 7.4) and homogenized using a motor-driven Potter-Elvehjem glass homogenizer. The homogenates were centrifuged at 4°C for 5 minutes at 800 x g and the supernatants were used for enzyme assay.

Assay of hemoproteins. Concentration of catalase as well as the other hemoproteins (cytochromes $c + c_1$, $b_{561} + b_{566}$, $a + a_3$, P-450, b_5) and total heme-pyridine hemochromogen were measured spectrophotometrically as previously described (6).

Assay of peroxisomal enzymes. Samples for catalase assay were prepared as described by Cohen et al. (7). Catalase activities were determined by the disappearance of hydrogen peroxide at 240 nm (8). Urate oxidase activity was determined by measuring the decrease in absorbance at 290 nm resulting from the oxidation f

uric acid to allantoin (9). Activities of D-amino acid oxidase with D-alanine (10) and glycolate oxidase with sodium glycolate (1) were determined by the rate of oxygen uptake using an oxygen-sensitive electrode assembly. Protein was determined by the method of Lowry et al. (12) with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

As shown in Table 1, catalase concentration in riboflavin-deficient rats was reduced to only 50% of that in normal rats. This result is consistent with previous observations (1,2). Less change was seen with cytochromes P-450 and b_5 which decreased only 30 and 10%, respectively, as a result of deficiency. It had been reported that rat liver cytochrome P-450 content was essentially independent of liver flavin status (13). The small decrease observed in the present study may be due to the decreased size of hepatocytes and concomitant decreased amount of endoplasmic reticulum in aribroflavinosis. The concentrations of cytochromes $a + a_3$, $c + c_1$ and $b_{561} + b_{566}$ remained the same in the two groups, in agreement with an earlier report (14). Presumably, the insensitivity of such activities as NADH dehydrogenase

TABLE 1. HEMOPROTEIN CONCENTRATIONS IN ISOLATED HEPATOCYTES OF RIBOFLAVIN-DEFICIENT RATS

Component	Deficient [a]	Normal [b]
	nmol/10^6 cells	nmol/10^6 cells
Catalase	0.10 ± 0.01	0.22 ± 0.01
Cyt. P-450	0.18 ± 0.03	0.26 ± 0.05
Cyt. b_5	0.08 ± 0.01	0.09 ± 0.02
Cyt. $a + a_3$	0.17 ± 0.01	0.18 ± 0.01
Cyt. $c + c_1$	0.17 ± 0.03	0.17 ± 0.02
Cyt. $b_{561} + b_{566}$	0.14 ± 0.01	0.14 ± 0.02
Pyridine hemochromogen	0.80 ± 0.08	1.04 ± 0.04

[a] Values given are means from 4 animals ± SE.
[b] Data taken from Ref. 6.

to riboflavin status may relate to the insensitivity of cytochromes a + a_3, c+ c1 and b_{561} + b_{566}. In summary results from the first experiment show that, among the hepatocyte hemoproteins, only catalase level is strongly decreased in riboflavin deficiency.

Peroxisomal enzyme activities expressed as the % of control level are shown in Fig. 1. Depression of glycolate oxidase and D-amino acid oxidase found in the present study is compatible with a previous finding (14). The FMN-dependent glycolate oxidase decreased somewhat more rapidly and to a greater extent than FAD-dependent D-amino acid oxidase probably because, as observed previously, FMN is lost from liver more rapidly (14) and to a greater extent (15) than FAD during riboflavin depletion.

Activity of urate oxidase was relatively constant regardless of riboflavin status. The finding that this flavin-independent activity is unaltered in riboflavin deficiency suggests that

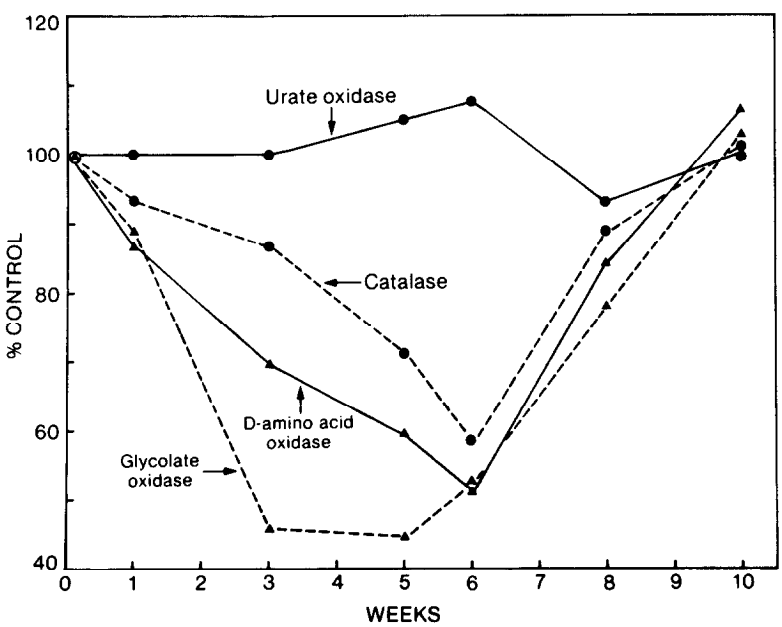

Figure 1. Changes of liver peroxisomal enzymes in rats fed a riboflavin-deficient diet which was supplemented after 5 weeks. Each point represents the mean from 3 to 6 animals; relative standard errors of the means were from 10 to 20% of values shown.

general damage of peroxisomes which would influence all redox enzymes present does not occur.

Catalase activity in the deficient group decreased fairly slowly during the depletion period, but became significantly lower than the controls at 5 weeks. After riboflavin supplementation, catalase activities exhibited a lag in recovery, decreasing to 60% of the control level at 6 weeks prior to reaching 90% of control by 8 weeks. It has been observed that certain anaerobic bacteria contain no detectable catalase, whereas all the aerobes examined exhibited significant catalase (16); moreover, catalase activity has been specifically induced with hydrogen peroxide in Rhodopseudomonas spheroides (3,4). The lack of substrate H_2O_2 due to the depression of flavin-dependent oxidases during riboflavin deficiency in mammals also appears to be a contributing factor to the decrease in catalase in liver peroxisomes which are important sites of hydrogen peroxide metabolism.

The association of catalase with peroxisomal oxidases is biologically meaningful. Since glutathione peroxidase is also decreased in riboflavin deficiency (17), both enzymes which protect against toxic peroxides (18) are decreased and the increase of lipid peroxides observed in ariboflavinosis is more comprehensible.

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