Tissue Distribution and Turnover of [^{3}H]Riboflavin During Respiratory Infection in Mice

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Studies in children and mice suggest that respiratory infections cause a mobilization of riboflavin from the tissues to the blood, resulting in increased urinary loss of this vitamin. To verify this observation, the tissue distribution and turnover of [^{3}H]riboflavin were investigated in control and low-riboflavin–fed mice infected with *Klebsiella pneumoniae*. Infection significantly reduced [^{3}H]riboflavin levels in the liver and kidney of low-riboflavin–fed mice and in the liver of control mice. Such changes were not observed in tissues such as muscle, small intestine, and brain. Urinary excretion of [^{3}H]riboflavin increased significantly during the acute phase of infection and the biological half-life of [^{3}H]riboflavin was shorter in the low-riboflavin–fed group. The results confirm that the mobilization of riboflavin from tissues to blood during infection results in a deterioration of riboflavin status. Thus, the study supports the hypothesis that respiratory infection is a nondietary factor contributing to the high prevalence of subclinical riboflavin deficiency in children of developing countries like India.

STUDIES IN DEVELOPING countries like India have revealed a high incidence of riboflavin deficiency in women and children in low-income groups as judged by the biochemical test, erythrocyte glutathione reductase activation coefficient (EGR-AC).[1-7] Prior investigations in children showed that respiratory infections alter riboflavin metabolism, resulting in an increased urinary loss of this vitamin.[8] This could be due to the mobilization of riboflavin from the tissues to the blood, since liver flavin adenine dinucleotide (FAD) levels were decreased in mice infected with *Klebsiella pneumoniae*.[9] To confirm these observations, the tissue distribution and turnover of [^{3}H]riboflavin were investigated in mice infected with *K. pneumoniae*.

MATERIALS AND METHODS

Male weanling mice of the Swiss/NIN strain were divided equally into control and low-riboflavin–fed groups. The animals were housed individually in screen-bottomed cages at optimal temperature (22° to 25°C) and humidity (55% ± 10%) with a 12-hour light-dark cycle. They were fed ad libitum on a purified diet containing 70% sucrose, 20% vitamin-free casein (Sigma Chemical, St Louis, MO), 5% peanut oil, 4% salt mixture, and 1% vitamin mixture. The composition of the salt and vitamin mixture was described previously.[10] The riboflavin content of the low-riboflavin diet was 0.5 mg/kg. This level of riboflavin is expected to simulate the human situation in developing countries like India, where riboflavin is one of the limiting nutrients. The control diet contained 13.3 mg riboflavin/kg diet.

After feeding the mice for 18 days with the respective diets, all animals were injected intraperitoneally with [^{3}H]riboflavin (200 nCi/20 g body weight, specific activity 44 mCi/mmol; Radio Chemical Center, Amersham, UK). Twenty-four hours later, a sublethal dose of *K pneumoniae* was injected intraperitoneally to half of the animals in each group. At this dose, no deaths were observed in preliminary experiments to determine the 50% lethal dose. This organism was isolated from the lungs of the rat (Wistar/NIN strain) and purified. The remaining animals received the vehicle intraperitoneally and served as uninfected controls.

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Urine and feces were collected at 24-hour intervals for 12 consecutive days after injecting the label.

Seventy-two hours after injection of the organism, the animals showed signs of acute infection such as loss of appetite and reddening of the snout. At this stage, six animals from each group were killed, and the remaining animals were killed 11 days after injection of the organism. A sample of blood was collected from the ocular plexus. The liver, kidney, muscle, small intestine, and brain were collected for analysis.

The riboflavin status of the mice was assessed by measuring EGR-AC.[11] Total flavins in the tissues were estimated by the fluorimetric method of Bessey et al[12] as modified by Bamji et al.[13]

The liver, kidney, small intestine, brain, and feces were homogenized in 0.1N HCl to obtain a 20% homogenate. This was autoclaved at 15 lbs for 15 minutes. Urine samples acidified with HCl to a final concentration of 0.1N were also autoclaved in a similar manner.[14] Aliquots of the samples were counted in an LKB Rack Beta Liquid Scintillation Counter (model 1219; Wallac, Sweden).

The biological half-life of [^{3}H]riboflavin was calculated according to the standard method using the formula $t_{½} = 0.693/Ke$, where Ke is the elimination constant.

RESULTS

There was a nonsignificant reduction in feed intake on days 1 and 2 after infection, but it did not significantly affect the body weight of the low-riboflavin and control groups. However, riboflavin restriction per se reduced the weight gain (Table 1).

EGR-AC values increased significantly in the low-riboflavin–fed group, confirming riboflavin deficiency. In the low-riboflavin–fed infected group, EGR-AC values were lower compared with the respective uninfected group ($P < .05$). After recovery from infection, the values were similar to those observed in the corresponding uninfected group. Infection had no effect on EGR-AC values in the control group (Table 1).

Riboflavin restriction per se produced a significant reduction in the total flavin content of both the liver and the kidney. Infection contributed to a further reduction in the total flavin content in both tissues (Table 2). In the control group also, infection caused a reduction in the total flavin content in these two tissues, but of lesser magnitude. On recovery from infection, the total tissue flavin levels tended to normalize (Table 2). While riboflavin deficiency reduced the total flavin content of the small intestine and the muscle, infection had no effect on the flavin content of these tissues (Table 3). Neither riboflavin restriction nor infection had any effect on brain flavin levels

Table 1. Effect of Infection on Body Weight and EGR-AC

Parameter	Low-Riboflavin Group		Control Group	
	Uninfected	Infected	Uninfected	Infected
Body weight (g)				
Period I	17.4 ± 0.60[a]	16.8 ± 0.71[a]	24.0 ± 0.80[b]	23.2 ± 0.73[b]
Period II	18.2 ± 0.72[a]	18.5 ± 0.70[a]	26.2 ± 0.91[b]	25.4 ± 0.82[b]
EGR-AC				
Period I	1.27 ± 0.05[b]	1.06 ± 0.06[ax]	1.09 ± 0.07[a]	1.06 ± 0.05[a]
Period II	1.29 ± 0.04[b]	1.26 ± 0.08[by]	1.08 ± 0.06[a]	1.08 ± 0.08[a]

NOTE. Values are the mean ± SEM of 8 observations. Values not sharing a common superscript are significantly different ($P < .05$) by ANOVA and LSD multiple-range test in a period (a and b) or between periods in a group (x and y). Period I, peak period of infection (72 hours); period II, after recovery (11 days).

(data not shown). In the body, the liver and kidney have better stores of flavins than the intestine, muscle, and brain.

Riboflavin deficiency increased the specific activity of [3H]riboflavin in the liver and kidney, but not in the other tissues. Infection produced a reduction in the specific activity of [3H]riboflavin in the liver, as well as the kidney, the reduction being more marked and significant in the restricted riboflavin-fed mice.

Data on the urinary excretion of [3H]flavins showed a significantly higher level of excretion during the peak period of infection (72 hours) in the low-riboflavin group (Table 4). The peak urinary level of [3H]flavin was observed on day 4 after injection of the label, and the levels were similar to values in the uninfected group from day 5. Although a similar trend was observed in the control infected group, the difference was not statistically significant (Fig 1). Fecal excretion of the label was not sensitive to riboflavin restriction or infection. While riboflavin deficiency increased the biological half-life of [3H]riboflavin, infection reduced it in the deficient group but not in the control group (Table 4).

DISCUSSION

A reduction in EGR-AC and an increase in urinary and erythrocyte flavin levels were reported in children with upper-

Table 2. Distribution of [3H]Flavin in Liver and Kidney During Infection

Parameter	Low-Riboflavin Group		Control Group	
	Uninfected	Infected	Uninfected	Infected
Liver				
Total flavin nmol/g tissue				
Period I	52.20 ± 1.76[b]	41.89 ± 1.66[ax]	84.75 ± 2.75[d]	75.52 ± 2.71[c]
Period II	56.23 ± 2.84[a]	52.04 ± 2.56[ay]	82.21 ± 3.63[b]	82.92 ± 3.39[b]
nCi [3H]riboflavin/nmol total flavin				
Period I	0.72 ± 0.04[dy]	0.52 ± 0.02[cy]	0.34 ± 0.02[by]	0.26 ± 0.01[ay]
Period II	0.53 ± 0.03[dx]	0.35 ± 0.02[cx]	0.21 ± 0.02[bx]	0.15 ± 0.01[ax]
Kidney				
Total flavin nmol/g tissue				
Period I	53.81 ± 4.36[b]	40.24 ± 2.37[a]	70.82 ± 5.06[c]	66.85 ± 2.42[c]
Period II	49.62 ± 3.02[a]	48.60 ± 2.99[a]	70.67 ± 2.43[b]	70.49 ± 2.70[b]
nCi [3H]riboflavin/nmol total flavin				
Period I	0.61 ± 0.03[cy]	0.47 ± 0.03[by]	0.33 ± 0.02[ay]	0.33 ± 0.02[ay]
Period II	0.50 ± 0.02[cx]	0.32 ± 0.03[bx]	0.21 ± 0.01[ax]	0.20 ± 0.02[ax]

NOTE. Values are the mean ± SEM of 3–4 observations in period I and 6 observations in period II. Values not sharing a common superscript are significantly different ($P < .05$) by ANOVA and LSD multiple-range test in a period (a, b, and c) or between periods in a group (x and y). Period I, peak period of infection (72 hours); period II, after recovery (11 days).

Table 3. Distribution of [3H]Flavin in Small Intestine and Muscle During Infection

Parameter	Low-Riboflavin Group		Control Group	
	Uninfected	Infected	Uninfected	Infected
Small intestine				
Total flavin nmol/g tissue				
Period I	11.82 ± 1.08[a]	12.39 ± 1.23[a]	16.29 ± 1.84[b]	17.63 ± 2.19[b]
Period II	11.60 ± 0.67[a]	12.36 ± 1.35[a]	18.58 ± 1.24[b]	17.54 ± 1.43[b]
nCi [3H]riboflavin/nmol total flavin				
Period I	0.38 ± 0.04[y]	0.36 ± 0.04[y]	0.35 ± 0.02[y]	0.32 ± 0.02[y]
Period II	0.27 ± 0.02[x]	0.24 ± 0.02 [x]	0.24 ± 0.08[x]	0.20 ± 0.02[x]
Muscle				
Total flavin nmol/g tissue				
Period I	6.39 ± 0.77[a]	6.23 ± 1.29[a]	9.66 ± 0.53[b]	9.23 ± 0.61[b]
Period II	6.81 ± 0.95[a]	6.39 ± 0.71[a]	9.74 ± 1.17[b]	10.43 ± 1.63[b]
nCi [3H]riboflavin/nmol total flavin				
Period I	0.57 ± 0.07[by]	0.58 ± 0.05[by]	0.33 ± 0.03[ay]	0.33 ± 0.03[ay]
Period II	0.38 ± 0.03[bx]	0.45 ± 0.05[bx]	0.25 ± 0.01[ax]	0.22 ± 0.02[ax]

NOTE. Values are the mean ± SEM of 3–4 observations in period I and 6 observations in period II. Values not sharing a common superscript are significantly different ($P < .05$) by ANOVA and LSD multiple-range test in a period (a and b) or between periods in a group (x and y). Period I, peak period of infection (72 hours); period II, after recovery (11 days).

Table 4. Urinary and Fecal [^{3}H]Flavin Levels During Acute Phase of Infection and Biological Half-Life of [^{3}H]Riboflavin

Parameter	Low-Riboflavin Group		Control Group	
	Uninfected	Infected	Uninfected	Infected
Urinary [^{3}H]flavin (nCi/72 h)	11.17 ± 0.857^a	22.64 ± 1.512^b	14.22 ± 1.426^a	19.09 ± 1.72^{ab}
Fecal [^{3}H]flavin (nCi/72 h)	3.21 ± 0.17	3.54 ± 0.15	3.34 ± 0.13	3.66 ± 0.18
Half-life (d)	24.3 ± 1.66^b	13.5 ± 0.48^a	14.96 ± 0.48^a	11.96 ± 1.12^a

NOTE. Values are the mean ± SEM of 6 observations. Values not sharing a common superscript are significantly different ($P < .05$) by ANOVA and LSD multiple-range test in a period.

respiratory infections and measles.[8] Similar changes were also observed in riboflavin-deficient mice infected with *K pneumoniae*.[9] In addition, the prior study showed that an increased erythrocyte flavin level was associated with the major metabolite, FAD. This would explain the reduction in EGR-AC reported previously,[8,9] as well as that found in the present study. The transient increase in erythrocyte flavin levels during the peak period of infection might have resulted from the mobilization of riboflavin from the liver and kidney to the blood, since total flavin levels were reduced in these two tissues during infection (Table 2).

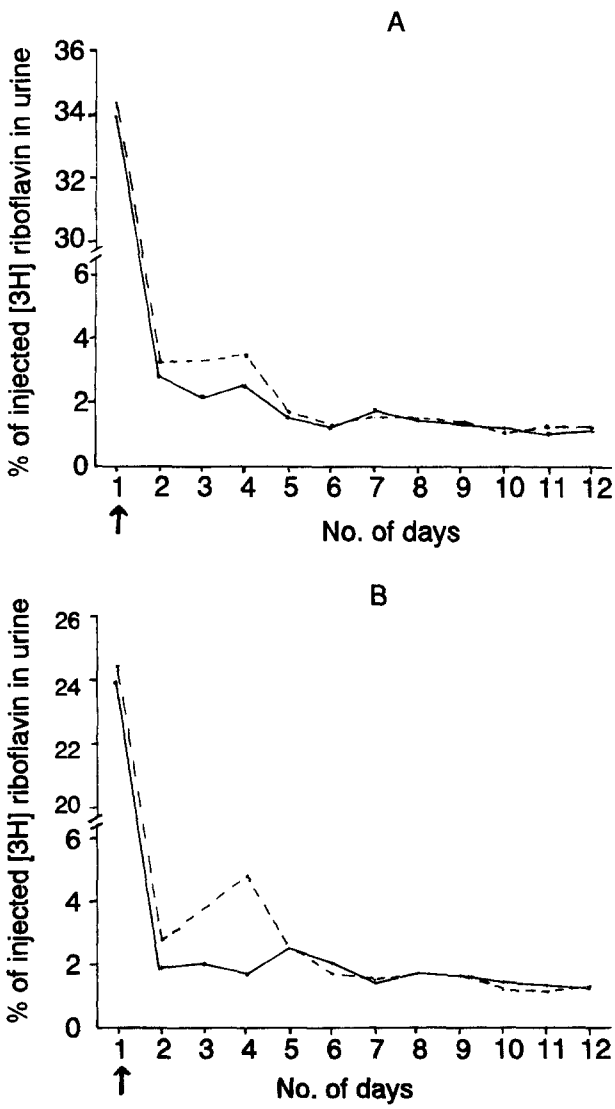

Fig 1. Urinary excretion of [^{3}H]flavins (mean ± SE) in uninfected (-) and infected (---) control (A) and low-riboflavin-fed (B) mice. Arrows indicate day on which organism was injected.

The riboflavin content of the control diet was about twice the recommended dietary allowance. A greater amount of the vitamin was added to maintain the riboflavin status of the control animals during the peak period of infection, since there was a slight reduction in food intake for 1 or 2 days during that period.

The amount of [^{3}H]riboflavin in the tissues calculated as nanocuries per gram of tissue at 72 hours was in the order, liver > kidney > small intestine, in both the control and low-riboflavin–fed groups. The muscle and brain (data not shown) had the lowest amount of [^{3}H]riboflavin compared with the other tissues. A similar order was reported by Yang and McCormick[14] at 24 hours after administration of [2-^{14}C]riboflavin.

As observed by Burch et al,[15] total flavin levels were lower in the liver, kidney, small intestine, and muscle of the riboflavin-restricted group compared with the controls, whereas brain total flavin levels were not sensitive to riboflavin status. During infection, riboflavin was lost from the liver and kidney of low-riboflavin–fed mice, but only from the liver of control mice.

Prior studies have indicated that there are two pools of riboflavin, one with a rapid turnover rate (10 to 12 hours) and the other with a slow turnover rate.[14] A biexponential-type riboflavin elimination was described, indicating the existence of a peripheral compartment of distribution.[16,17] A two-compartment open model has been confirmed for humans by pharmacokinetic study.[18] The present study also indicates the presence of two pools of riboflavin, although [^{3}H]riboflavin levels in the urine were not measured earlier than 24 hours. The longer biological half-life of the label in the low-riboflavin–fed group is understandable since the retention of administered riboflavin was greater in this group. As there was an increased mobilization of riboflavin from the liver and kidney during the acute phase of infection in the low-riboflavin group, the biological half-life of [^{3}H]riboflavin was reduced in this group compared with the corresponding uninfected group.

Yang and McCormick[14] reported that body retention data do not fit exactly into a straight line and tend to curve upward, especially for data on the latter days. Similar results were observed in the present study as well. As suggested earlier, it may be due to the growth of the animals during the experimental period. The biological half-life of 15 days for riboflavin observed in the control mice compares well with the half-life of 16 days and 14 days reported for rats by Yang and McCormick[14] and Amos et al,[19] respectively.

The reduction of [^{3}H]flavin levels in the liver and kidney, the increase in urinary [^{3}H]flavin, and the shorter half-life of [^{3}H]riboflavin during infection in the low-riboflavin–fed group confirm the hypothesis that there is a mobilization of riboflavin from the tissues during respiratory infection. Similar changes were also observed in the control infected animals, but the magnitude of change was smaller. These results support the hypothesis that repeated respiratory infection is a nondietary factor contributing to the high prevalence of riboflavin deficiency in poor communities where the diet is deficient in riboflavin.[8]

Control studies will be performed to examine the effects of different types of infections on riboflavin metabolism during mild and moderate riboflavin deficiency.

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