Riboflavin deficiency in the rat: effects on iron utilization and loss

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Iron absorption and daily loss of Fe were measured in riboflavin-deficient (B_2^-) Norwegian hooded rats and controls (B_2^+). Animals were fed on a test meal extrinsically labelled with ^{59}Fe and whole-body radioactivity measured for 15 d. Riboflavin deficiency led to a reduction in the percentage of the ^{59}Fe dose absorbed and an increased rate of ^{59}Fe loss. All post-absorption ^{59}Fe loss could be accounted for by faecal ^{59}Fe, confirming that the loss was gastrointestinal. Fe concentrations and ^{59}Fe as a percentage of retained whole-body ^{59}Fe were higher in the small intestine of riboflavin-deficient animals than their controls, 14 d after the test meal. A separate experiment demonstrated that riboflavin deficiency was associated with a significant proliferative response of the duodenal crypts of the small intestine. These observations may explain the enhanced Fe loss in riboflavin deficiency.

Riboflavin: Iron: Crypt cell proliferation: Rat

There is considerable evidence from studies in both animals and humans that poor riboflavin status can disturb iron economy (Buzina *et al.* 1979; Powers *et al.* 1983*a*, *b*; Adelekan & Thurnham, 1986). It seems as though riboflavin may be important at several stages of Fe utilization including mobilization of Fe from stores, and absorption (Sirivech *et al.* 1977; Powers, 1986; Powers *et al.* 1988). Attempts to elucidate the mechanisms of this involvement have concentrated on the effects of riboflavin deficiency on the activity of a flavin-dependent oxidoreductase (ferriductase) system which can, at least in vitro, release Fe from ferritin (Zaman & Verwilghen, 1977; Ulvik & Romslo, 1981; Powers *et al.* 1983*a*). A previous study investigated the effects of riboflavin deficiency on Fe absorption in vivo in rats and demonstrated very clearly that not only was Fe absorption impaired, but also that daily Fe loss was increased (Powers *et al.* 1988). We have repeated and extended this earlier study to try and provide some further insight into the mechanism of the effect of riboflavin deficiency on Fe metabolism.

MATERIALS AND METHODS

Fe utilization study

Seventy female Norwegian hooded 5-week-old weanling rats (90–130 g) were fed on a basal diet containing (g/kg) arachis oil 30, sucrose 700, casein 200 (supplying 0·52 mg riboflavin (vitamin B_2)/kg diet) and a vitamin and salt mixture (Powers, 1987). Rats were allocated

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to one of two dietary groups, one group to receive the basal diet (treatment B_2^-) and the other group to receive the basal diet with an additional 15 mg riboflavin/kg diet (treatment B_2^+). For 2 weeks, treatment B_2^- animals were fed *ad lib.* Treatment B_2^+ animals were paired by weight to treatment B_2^- animals. The food intake of each control (treatment B_2^+) animal was adjusted daily in order to maintain a similar growth rate to that of its treatment B_2^- counterpart. For the following 3 weeks animals were trained to meal feed so that a 1 d food allowance was consumed within a 2 h period during the morning; treatment B_2^+ animals again had their intakes restricted to maintain the weight of their treatment B_2^- partner. All animals were then given a fixed amount (12 g) of their diet each morning for 3 d in order to standardize Fe intakes, since dietary Fe intake can influence subsequent Fe absorption from a test meal (Fairweather-Tait & Wright, 1984); the result of a short-term mucosal effect which can last for up to 3 d after any dietary change (Fairweather-Tait *et al.* 1985). Following a 22 h fast, each animal was given a test meal of 3 g of a cooked starch–sucrose (1:1, w/w) paste containing 120 μg Fe (as ferrous sulphate in 0·1 M-hydrochloric acid) extrinsically labelled with 37 kBq ^{59}Fe (ferric chloride in 0·1 M-HCl, 110–740 MBq/mg Fe; Amersham International Plc, Amersham, Bucks.). At 5 h after finishing the test meal all animals were given 12 g of their respective diets and were maintained at 12 g food/d for the duration of the experiment.

Each rat was placed in a small-animal, whole-body gamma-counter (NE 8112; NE Technology, Beenham, Berks.) immediately after consuming the test meal (designated day 0) as previously described (Powers *et al.* 1988), to establish the exact initial ^{59}Fe dose. All rats consumed at least three-quarters of the test meal within 1 h and none was excluded from the experiment. Fifteen rats from each group killed after 7 d had an initial and day 7 count only, whereas twenty rats from each group killed after 14 d were counted daily. These latter forty rats also had their faecal output collected daily, starting immediately after the day 5 whole-body count, and this was monitored for ^{59}Fe in a Phillips PW 4580 automatic gamma-counter. True Fe absorption and the daily rate of Fe loss were calculated for the rats counted daily, as follows: for each rat, the $\log_{10}$ ^{59}Fe retention (as % dose) (Y axis) for days 8–14 inclusive (X axis) were subjected to regression analysis to predict (a) the true ^{59}Fe absorption (antilog$_{10}$ Y-axis intercept) and (b) the percentage rate of ^{59}Fe loss per d (100(1 − antilog$_{10}$ slope)).

On day 7 after receiving the test meal fifteen animals from each dietary group were weighed and killed by exsanguination under anaesthesia. Liver and small intestine (SI) were removed and washed in isotonic saline (9 g sodium chloride/l). Samples of blood (1 ml), liver and duodenum (proximal 10% SI), ileum (distal 50% SI) and jejunum (remainder of SI) were monitored for ^{59}Fe in the Phillips automatic gamma-counter. The liver and sections of SI were then kept frozen until an estimation of total Fe content could be performed. Riboflavin status of the rats on the day before receiving the test meal and at the time of death was determined by measuring blood glutathione reductase (NAD(P)H) (*EC* 1.6.4.2) activation coefficient (BGRAC) (Powers *et al.* 1983*a*). Packed cell volume was measured in blood samples at the time of death of the animals.

On day 14 after receiving the test meal the remaining twenty rats from each group were killed in a similar manner to those killed on day 7. All livers were freeze-dried, weighed and crushed to a homogeneous powder. Sections of SI were oven-dried for 16 h at 85° for determination of dry weight. Each dried section of SI and a small sub-sample from each powdered liver were then ashed in a muffle furnace for 48 h at 480°, dissolved in a minimum volume of 11·7 M-HCl and further diluted with distilled water. The Fe content was determined by atomic absorption spectroscopy using a PU9000 (Pye Unicam, Cambridge).

Crypt cell study

Six female Norwegian hooded rats were fed on a riboflavin-deficient diet (initially *ad lib.*) as described, from weaning (21 d) for 5 weeks. A further six animals receiving the complete diet were weight-matched to their riboflavin-deficient partners. Meal feeding was introduced after 2 weeks on the diets. After a 22 h fast, crypt cell proliferation was measured in the upper part of the SI of each animal using a stathmokinetic method.

The metaphase arrest agent, vincristine, was administered intraperitioneally (1 mg/kg) at time zero. Animals were killed by diethyl ether anaesthesia and cervical dislocation 150 min later. The abdomen was opened and the entire SI from pylorus to caecum removed. Its length was measured and a duodenal segment excised, at 50 mm distal to the pylorus.

Duodenal specimens were opened longitudinally and placed mucosal side uppermost on cards, fixed in Clarke's solution (ethanol–acetic acid (75:25, v/v)) for 24 h and stored in ethanol (750 ml/l) until staining. Specimens were bulk-stained by the Feulgen reaction: each was hydrated through descending concentrations of ethanol and hydrolysed in 1 M-HCl at 60° for 5·5 min before staining with Schiff reagent for 35 min. Under a stereomicroscope the serosal and muscle layers were removed and individual villus-crypt units microdissected. The depths and widths of ten well-orientated intact crypts were measured with an eyepiece micrometer. The tissue was then gently squashed under a cover slip in acetic acid (450 ml/l) and the number of metaphase arrest figures per crypt counted. A mitotic index was calculated as the mean number of metaphase arrest figures per ten crypts per animal. This method of measuring crypt cell proliferation has been well validated (Wright & Appleton, 1980; Wright & Irwin, 1982).

The riboflavin status of the animals was determined by measuring BGRAC.

Statistical methods

Fe utilization study. BGRAC and body-weights, SI length, true ^{59}Fe retention, percentage rate of loss of absorbed ^{59}Fe per d and loss of initially-retained ^{59}Fe over 5–14 d accounted for in faecal output were compared by Student's unpaired *t* test. Tissue Fe, specific activity of ^{59}Fe, ^{59}Fe either as percentage dose or percentage total ^{59}Fe retained at day of kill, and tissue dry weights, were subjected to two-way analysis of variance with the variables diet and time.

Crypt cell study. SI length, crypt depth and width, and mitotic index were compared using the Student's unpaired *t* test.

RESULTS

Fe utilization study

Table 1 shows the mean body-weights of the rats at the time of dosing and the time of killing. Deficient (B_2^-) animals killed 14 d after the dose were lighter than their controls (B_2^+).

BGRAC values $\geqslant$ 1·30 are considered to indicate biochemical ariboflavinosis. Rats fed on the riboflavin-deficient diet (treatment B_2^-) were evidently deficient at the time of dosing and remained so for the duration of the experiment (Table 2). Rats fed on the control diet (treatment B_2^+) had BGRAC values significantly lower than those of the depleted (B_2^-) group although some rats had values > 1·30 (Table 2). In our experience, rats that are weight-matched or paired-fed with treatment B_2^- rats do sometimes show a BGRAC greater than 1·30 despite having tissue flavin concentrations indicative of adequate riboflavin status (Powers *et al.* 1983*a*; Duerden & Bates, 1985; Powers, 1986).

The percentage of the ^{59}Fe dose absorbed was 43·0% for the B_2^- treatment group and 52·2% for the B_2^+ treatment group. The difference was significant ($P < 0{\cdot}01$; Table 3). The

Table 1. *Effect of riboflavin deficiency on body-weights of rats at the time of dosing (test meal extrinsically labelled with ^{59}Fe) and at the time of killing*†

(Mean values with their standard errors)

	Body-wt (g)								
	Time of dosing (day 0)			Time of killing					
				Day 7			Day 14		
Treatment group	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
B_2^-	35	160·8	1·6	15	170·1	1·6	20	168·6***	2·1
B_2^+	35	162·0	1·3	15	178·1	1·7	20	181·6	1·6

B_2^-, rats fed on a riboflavin-deficient diet; B_2^+, rats fed on a complete diet (control).
Mean value was significantly different from that of control animals: ***$P < 0.001$.
† For details of diets and procedures, see p. 488.

Table 2. *Riboflavin status of rats, measured at the time of dosing (test meal extrinsically labelled with ^{59}Fe) and at the time of killing*†

(Mean values with their standard errors)

	BGRAC								
	Time of dosing (day 0)			Time of killing					
				Day 7			Day 14		
Treatment group	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
B_2^-	33	2·10***	0·09	14	2·27***	0·16	20	1·89**	0·14
B_2^+	35	1·32	0·06	15	1·19	0·05	20	1·37	0·10

B_2^-, rats fed on a riboflavin-deficient diet; B_2^+, rats fed on a complete diet (control); BGRAC, blood glutathione reductase (*EC* 1.6.4.2) activation coefficient.
Mean values were significantly different from those of control animals (Student's *t* test): **$P < 0.01$, ***$P < 0.001$.
† For details of diets and procedures, see p. 488.

daily rate of loss of absorbed ^{59}Fe, expressed as percentage daily loss, was significantly higher ($P < 0.01$) in the B_2^- treatment group (0·72) than the B_2^+ treatment group (0·41; Table 3).

A very rapid fall in whole-body ^{59}Fe up to day 5 reflected the excretion of unabsorbed Fe. Faecal ^{59}Fe loss of absorbed Fe was calculated as a daily percentage loss of whole-body ^{59}Fe from day 5 to day 14. Recovery of faecal ^{59}Fe represented 104 (SE 3) % of that lost from the body in the B_2^- treatment group and 102 (SE 3) % in the B_2^+ treatment group. All of the ^{59}Fe lost from the body over this period could, therefore, be accounted for by faecal ^{59}Fe loss.

The distribution of retained ^{59}Fe between the liver, blood and SI, is shown in Table 4. The B_2^- treatment group had a significantly higher percentage of ^{59}Fe in blood than the B_2^+ treatment group ($P < 0.001$) and, although the blood values remained constant between days 7 and 14 in the B_2^+ treatment group, the ^{59}Fe (as a percentage of total body count) increased in the B_2^- treatment group ($P < 0.05$).

Table 3. *Effect of riboflavin-deficiency on ^{59}Fe absorption and daily rate of loss in groups of twenty rats killed 14 d after administration of a 3 g cooked starch–sucrose test meal containing 120 μg Fe and 37 kBq ^{59}Fe*†

(Mean values with their standard errors)

Treatment group...	B_2^-		B_2^+	
	Mean	SE	Mean	SE
^{59}Fe absorption‡ (%)	43·0**	2·1	52·2	1·8
Rate of ^{59}Fe loss/d‡ (%)	0·72**	0·08	0·41	0·06

B_2^-, rats fed on a riboflavin-deficient diet; B_2^+, rats fed on a complete diet (control).
Mean value was significantly different from that of control animals: $^{**}P < 0.01$.
† For details of diets and procedures, see p. 488.
‡ Calculated from regression analysis, see p. 488.

Table 4. *Effect of riboflavin deficiency on tissue distribution of absorbed ^{59}Fe (percentage of whole-body count) from a test meal extrinsically labelled with ^{59}Fe, at time of killing**

(Mean values with their standard errors)

Treatment group...	Day 7				Day 14				Statistical significance of effect (*P*)† of:	
No. of rats...	B_2^- 15		B_2^+ 15		B_2^- 20		B_2^+ 20			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Diet	Time
Liver	31·53	1·90	35·90	2·11	27·11	1·40	29·41	1·00		< 0·01
Blood (/ml)	4·91	0·17	4·64	0·23	5·91	0·16	4·60	0·24	< 0·001	< 0·05
Duodenum	0·280	0·014	0·256	0·009	0·277	0·010	0·241	0·008	< 0·01	
Jejunum	0·729	0·034	0·657	0·033	0·647	0·021	0·494	0·023	< 0·001	< 0·001
Ileum	1·103	0·072	0·839	0·043	0·973	0·032	0·749	0·059	< 0·001	< 0·05
Total SI	2·089	0·112	1·752	0·081	1·897	0·052	1·484	0·079	< 0·001	< 0·01

SI, small intestine.
* For details of diets and procedures, see p. 488.
† Values were analysed by a 2-way analysis of variance.

The B_2^- treatment group showed a greater proportion of retained ^{59}Fe in each section of the SI than in the B_2^+ control animals. With increasing time interval after ^{59}Fe dose there was a decrease in the proportion of body ^{59}Fe in the jejunum and ileum which was not seen in the duodenum. Similarly, when values for each section of the SI were summed for individual rats it was evident that riboflavin deficiency was associated with a greater retention of ^{59}Fe. The proportion of ^{59}Fe found in the SI decreased with the time interval after dosing. In order to investigate the effects of isotope dilution on the distribution of absorbed ^{59}Fe the specific activity of ^{59}Fe was calculated for the liver and SI. Table 5 shows the specific activity of ^{59}Fe to be the same in the liver and ileum of the two experimental groups and lower in the duodenum, jejunum and total SI of the B_2^- treatment group than the B_2^+ control group. Though the proportion of ^{59}Fe retained at day 14 was higher per ml of circulating blood in the B_2^- treatment group (Table 4), this was offset by a lower whole-body ^{59}Fe retention (Table 3), such that the absolute amount of ^{59}Fe/ml of circulating blood was not significantly different between groups.

Preferential retention of body Fe in the SI in riboflavin deficiency is also evident from

Table 5. *Effect of riboflavin deficiency on specific activity (Bq/μg) absorbed ^{59}Fe in small intestine and liver at time of killing**

(Mean values with their standard errors)

	Day 7				Day 14					
Treatment group...	B_2^-		B_2^+		B_2^-		B_2^+		Statistical significance of effect (*P*)† of:	
No. of rats...	15		15		20		20			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Diet	Time
Liver	3·90	0·53	4·43	0·37	3·04	0·30	3·71	0·25		< 0·05
Duodenum	3·88	0·24	4·14	0·14	3·24	0·20	3·91	0·13	< 0·01	< 0·05
Jejunum	3·27	0·29	4·08	0·20	3·44	0·19	3·89	0·13	< 0·01	
Ileum	2·04	0·13	2·13	0·11	2·08	0·21	2·56	0·33		
Total SI	2·45	0·16	2·82	0·13	2·50	0·19	3·01	0·19	< 0·05	

SI, small intestine

* For details of diets and procedures, see p. 488.

† Values were analysed by a 2-way analysis of variance.

Table 6. *Effect of riboflavin deficiency on tissue distribution of body iron (μg/g dry weight) and total liver iron stores (μg)**

(Mean values with their standard errors)

	Day 7				Day 14					
Treatment group...	B_2^-		B_2^+		B_2^-		B_2^+		Statistical significance of effect (*P*)† of:	
No. of rats...	15		15		20		20			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Diet	Time
Liver: μg total	1258	48·3	1399	59·4	1373	44·5	1489	27·3	< 0·01	< 0·05
μg/g	752·1	21·2	797·0	34·8	803·0	34·9	809·7	15·9		
Duodenum (μg/g)	54·3	1·5	54·0	1·8	57·5	2·7	54·4	1·4		
Jejunum (μg/g)	75·3	8·5	55·1	5·0	52·7	2·1	43·4	1·3	< 0·01	< 0·001
Ileum (μg/g)	116·0	6·5	103·2	6·2	109·2	5·2	82·9	5·5	< 0·001	< 0·05
Total SI (μg/g)	92·5	4·4	77·7	3·9	80·2	2·9	64·0	3·0	< 0·001	< 0·001

SI, small intestine

* For details of diets and procedures, see p. 488.

† Values were analysed by 2-way analysis of variance.

Table 6, which shows the concentration of Fe in different parts of the SI at time of kill. B_2^--treated rats clearly have significantly lower total liver Fe stores than B_2^+-treated control rats.

Crypt cell study

Riboflavin-deficiency was evident in the B_2^- treatment group, which had a mean BGRAC of 1·91 (SE 0·09) when killed. This value was significantly higher than that of the control group (1·16 (SE 0·07)).

The effects of riboflavin deficiency on crypt morphometry and mitotic activity are shown in Table 7. There was no effect on body-weights at the time of kill, or SI lengths. However, the B_2^--treated animals showed a significant proliferative response of the duodenal crypts; this was characterized by an increase in crypt depth and width and an increase in the mitotic index (metaphase arrest/crypt). The magnitude of the effect is clearly seen in Fig. 1.

Table 7. *Effect of riboflavin deficiency on body-weights, and characteristics of the upper small intestine of rats*†

(Mean values with their standard errors for six rats/group)

Treatment group...	B_2^-		B_2^+	
	Mean	SE	Mean	SE
Body-wt (g)	123	4·6	125	3·7
Small intestine length (mm)	765	27	767	20
Crypt depth (μm)	267***	3·2	193	4·0
Crypt width (μm)	62**	2·6	54	1·2
Mitotic index‡	48***	1·5	26	1·0

B_2^-, rats fed on a riboflavin-deficient diet; B_2^+, rats fed on a complete diet (control).
Mean values were significantly different from those of control animals: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.
† For details of diets and procedures, see pp. 488–489.
‡ No. of cells in metaphase arrest/crypt.

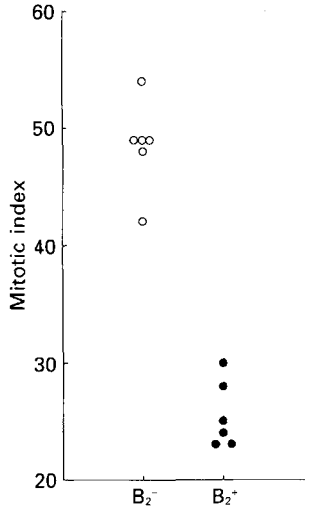

Fig. 1. Mitotic index in upper small intestinal epithelium of six riboflavin-depleted (B_2^--treated) animals and six control (B_2^+-treated) animals. For details of treatments, see p. 488. Mitotic index is the mean number of metaphase arrests/ten crypts per animal 2·5 h after an intraperitoneal injection of 1·5 mg vincristine.

DISCUSSION

The purpose of the study described here was threefold: (1) to confirm the effect of riboflavin-deficiency on Fe absorption and loss which we observed in a previous experiment, (2) to clarify the route of the Fe loss, (3) to investigate the effect of riboflavin deficiency on crypt cell proliferation in the small intestine.

Fe utilization

Riboflavin deficiency was clearly associated with an impaired Fe absorption, an increased rate of loss of absorbed Fe and lower liver Fe stores. Having absorbed a smaller percentage of the ^{59}Fe dose the riboflavin-deficient (B_2^--treated) rats distributed this Fe differently from the B_2^+-treated controls. Thus, the blood contained a greater proportion of the

absorbed Fe, presumably in response to the high requirements of the erythroid marrow. A refinement of the experimental technique, compared with the earlier study, was to give both groups of animals the same quantity of diet after the test meal. This was in order to ensure that dietary Fe intakes were the same in the two groups, and thereby to control for any influence that dietary Fe intake might have on whole-body Fe turnover. The effect of this was that B_2^--treated animals, with a lower efficiency of food utilization, were slightly lighter than B_2^+-treated controls at day 14. This should not have influenced the distribution of the absorbed Fe.

The magnitude of the difference in rate of Fe loss was not as great as previously observed (Powers *et al.* 1988) but was nevertheless highly significant. The fact that the daily loss of absorbed ^{59}Fe could all be accounted for in faecal output confirms our previous suggestion that the loss occurred entirely through the gastrointestinal tract. However, the precise route of the post-absorption Fe loss, and the mechanism for the enhanced loss in riboflavin deficiency, requires careful consideration of the findings. Riboflavin deficiency was associated with an increased uptake of absorbed ^{59}Fe by the SI as was evident from the distribution values 14 d after the ^{59}Fe dose. There was no evidence that differences in specific activity of ^{59}Fe between the two experimental groups could have accounted for this observation. In fact the specific activity of ^{59}Fe in the SI was lower in the B_2^- treatment group than the control group. This is to be expected in the light of the observed reduction in ^{59}Fe absorption and increase in the concentration of Fe in the SI of the B_2^- treatment group.

The increased concentration of Fe in the SI of the B_2^--treated animals might contribute to an elevated daily loss of Fe during the normal course of mucosal cell turnover.

Post-absorption Fe excretion has been observed by other groups of workers in humans (Green *et al.* 1968; Björn-Rasmussen *et al.* 1980; Johnson, 1984). A number of hypotheses have been put forward to explain the observation but none has been thoroughly investigated and the observation has generally received little recognition. The post-absorption loss of Fe observed in our study cannot be due simply to the exfoliation of mucosal cells carrying ^{59}Fe that never passed into the blood as it continued long after the normal lifespan of the mucosal cell. On the other hand, ^{59}Fe which was completely absorbed could have been made available in the blood for the synthesis of Fe-containing compounds within newly synthesized mucosal cells and subsequently lost by exfoliation (Linder & Munro, 1977). There is some evidence in the literature that Fe may be lost by a serosal-to-mucosal transfer of Fe, independent of mucosal cell turnover, possibly by excretion through goblet cells (Guy & Schachter, 1975; Refsum & Schreiner, 1980; Schreiner & Refsum, 1983). Finally, it cannot be ruled out that there is some capacity for enteroenteric recirculation, with the Fe being released from mucosal cells as they slough off being reabsorbed lower in the gut. If this were a significant component of the Fe movement in our experiment, it might be expected that the duodenum would actually be depleted of Fe more rapidly than the jejunum and ileum, which was not the case.

Crypt cell proliferation

Riboflavin deficiency, of a severity similar to that associated with an increased daily rate of loss of Fe, was associated with an enhanced proliferative response of the mucosal epithelia of the upper SI. It remains to be determined whether this is associated with an accelerated villus enterocyte loss and an influx of immature enterocytes. An accelerated enterocyte turnover would be expected to increase the daily rate of loss of Fe from the body. To our knowledge, there is only one other report in the literature of an effect of riboflavin deficiency on mucosal cell turnover. Miyaji & Hala (1965) reported that riboflavin deficiency in the mouse was associated with an increased turnover rate of the mucosal epithelial cells of the SI.

The deleterious effect of riboflavin deficiency on Fe absorption may be a direct result of the mechanism leading to enhanced Fe loss but as there is no published information describing a relationship between mucosal cell turnover rate, hypertrophy, and Fe absorption, this is merely speculation. There are as yet no grounds on which to reject the hypothesis that the impaired Fe absorption seen in riboflavin deficiency is due to a reduced activity of a flavin-dependent oxidoreductase in the mucosal cells of the SI, as described in an earlier study (Powers, 1986).

In conclusion, riboflavin deficiency leads to impaired Fe absorption and an increased Fe loss from the gastrointestinal tract. An increased turnover of the mucosal epithelia of the SI and preferential accumulation of body Fe by the SI could both be factors contributing to the disturbed Fe metabolism. The precise mechanisms remain to be elucidated.

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