Role of Magnesium in Glutathione Metabolism of Rat Erythrocytes[1]

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ABSTRACT The effects of dietary magnesium (Mg) on glutathione (GSH) metabolism were studied in rat erythrocytes. Young male and young adult female rats were fed a powdered diet containing either 12 ppm Mg (deficient rats) or 662 ppm Mg (control rats) for 14 to 28 days. Results showed that Mg deficiency in male rats had a decreased body weight gain, lower values in plasma hematocrit and Mg ion, but increased organ weights. The concentrations of erythrocyte GSH as measured by alloxan were reduced in male and female rats receiving a Mg-deficient diet. This decrease was confirmed by determining GSH with DTNB reagent [5,5'-dithiobis(2-nitrobenzoic acid)]. Mg-deficient rats, however, had higher levels of liver and kidney GSH, but no effect in other soft tissues examined. The decrease of erythrocyte GSH was not due to feed intake, the availability of its precursors, or the activities of GSH-related enzymes and can be reversed by Mg supplementation. Mg-deficient rats had a reduced blood ATP and a 6-fold increase in the activity of plasma gamma-glutamyl transpeptidase. These findings suggest that Mg is essential in the maintenance of GSH concentration to protect against oxidative damage in the erythrocyte membrane. J. Nutr. *112:* 488–496, 1982.

INDEXING KEY WORDS Mg · glutathione · erythrocyte

Erythrocytes contain large amounts of reduced glutathione (GSH). Although not all of the functions of this sulfhydryl compound are fully understood, it is believed that one role of GSH is to protect hemoglobin and other thio-group containing proteins from denaturation under oxidative stress (1, 2). When the red cell GSH decreases, as in patients deficient in glucose-6-phosphate dehydrogenase who have been treated with the drug 8-aminoquinolin (3), or in sheep (4) and humans (5) with copper toxicity, there is a hemolytic crisis characterized by hemoglobinemia and hemoglobinuria.

Rats placed on a Mg-low diet become anemic (6), which is associated with an altered membrane structure (7), a shortened erythrocyte survival (8, 9), reticulocytosis (7, 10), spherocytosis (11), microcytosis (10, 12) and erythroid hyperplasia of the bone marrow (8, 12). The numerous morphological and biochemical abnormalities in rat erythrocytes during Mg deficiency have been reviewed comprehensively by Elin (13). The cause of this anemia is unclear; however, it has been demonstrated that the adenosine triphosphate (ATP) content in the erythrocytes of the Mg-deficient rats is decreased and that the cell assumes a spherocytic shape (8, 11). Since rat erythrocytes are able to synthesize and destroy GSH (14) and the synthesis of GSH is completely dependent on ATP (15) and Mg (16), the depression of ATP and Mg could reduce GSH concentration which may possibly be the mechanism in producing the hemolytic anemia in Mg-deficient rats.

In the present work, we investigated the concentrations of GSH in the erythrocytes and other target organs of Mg-deficient, Mg-repleted rats and their respective pair-fed

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TABLE 1

Composition of Mg-deficient diet

Ingredient	g/kg
Casein vitamin free	200.0
DL-methionine	3.0
Dextrose monohydrate	667.8
Corn oil	70.0
Vitamin mix[1]	10.0
Mineral mix[2]	49.2

[1] Vitamin mix obtained from Teklad Test Diets, Madison, WI, provided the following compounds per kilogram diet: vitamin A palmitate, 39.64 mg; vitamin D-2, 4.405 mg; vitamin E acetate, 242 mg; P-aminobenzoic acid, 110 mg; ascorbic acid (coated 97.5%), 0.9912 g; biotin, 441 μg; vitamin B-12 (0.1% trituration in mannitol), 29.7 μg; calcium pantothenate, 66.079 mg; choline dihydrogen citrate, 3.496 g; folic acid, 1.98 mg; inositol, 110 mg; menadione (vitamin K-3), 49.56 mg; niacin, 99 mg; pyridoxine HCl, 22.0 mg; riboflavin, 22.0 mg; thiamine HCl, 22.0 mg; corn starch, 4.666 g. [2] Mineral mix obtained from Teklad Test Diets, Madison, WI, provided the following compounds per kilogram diet: $NaHCO_3$, 12.6 g; $KHCO_3$, 15.1 g; Na, 0.03 g; $CaHPO_4$, 14.3 g; $CaCl_2$, 5.7 g; $Fe(C_6H_5O_7) \cdot 5H_2O$ (10.7% Fe), 1.24 g; $CuSO_4 \cdot 5H_2O$, 0.013 g; $ZnSO_4 \cdot 7H_2O$, 0.024 g; $MnSO_4 \cdot H_2O$, 0.174 g.

control animals. The activities of glutathione peroxidase (GSH-px) and glutathione reductase (GSH-red) in the erythrocytes were also determined in order to evaluate whether Mg status of animals affected sulfhydryl-related enzymes.

MATERIALS AND METHODS

Chemicals. All organic solvents were reagent grade. The co-enzymes, glutathione reductase (GSH-red), catalase, phosphoglycerate phosphokinase, glyceraldehyde phosphate dehydrogenase, GSH, and GSSG (oxidized glutathione) used in enzyme assays were of highest available purity (Sigma Chemical Co., St. Louis, MO).

Animals and diets. Five-week-old male Sprague-Dawley rats were randomly divided into two groups by matched age and body weight. The first group (deficient rats) received a powdered diet (table 1) containing 12 ppm Mg. The second group (control rats) was pair fed the identical diet except Mg levels were increased to 662 ppm. In one experiment, eight-week-old female rats were similarly used in this investigation. All the rats were housed individually in suspended stainless steel cages at an ambient temperature of 28°. Deionized water was allowed ad libitum.

Preparation of the specimens. At appropriate intervals the rats after overnight fasting were anesthetized with ether and decapitated. For determination of ATP and GSH-red, acid-citrate-dextrose (ACD) solution (17) in a ratio of 1.25 ml to 10 ml blood was used as an anticoagulant. The remaining blood was collected into heparinized tubes. Various tissues were excised within 5 minutes after death. These specimens were immediately chilled with ice-cold saline and used for enzyme assays as described below.

GSH assay. The concentration of GSH in blood and other tissues was primarily determined by the method of Alloxan 305 (18). For comparison, GSH concentrations in various tissues were also measured using DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) reagent of Beutler et al. (19).

Enzyme assays

Glutathione peroxidase (GSH-px). GSH-px was measured using a modification of the method of Paglia and Valentine (20). Briefly, heparinized blood was washed with saline-phosphate buffer, pH 7.4 (21). After centrifugation, the buffy coat was removed and the washed red cells were hemolyzed in 7.5 volumes of deionized water. The clear hemolysate was subsequently used for the GSH-px assay. One unit of glutathione-px activity was defined as the oxidation of 1 nanomole NADPH per minute.

Glutathione reductase (GSH-red). GSH-red was measured using the procedure of Tillotson and Sauberlich (22). Fresh blood was collected in a tube containing ACD solution (16) and washed twice with 5 volumes of ice-cold saline. The washed red cells were hemolysed in 5 volumes of deionized water followed by 10 minutes of freezing then thawing. After centrifugation the clear hemolysate was assayed for GSH-red.

One unit of glutathione-red activity was defined as oxidation of 1 nanomole NADPH per minute. Erythrocyte GSH-red assays were also expressed in terms of "activity coef-

ficients" (AC), representing the degree of stimulation resulting from the in vitro addition of FAD. AC was expressed as the reduction of absorbance with added FAD divided by the reduction of absorbance without added FAD.

Catalase. Catalase activity was measured by following the rate of disappearance of sodium perborate as described by Goldstein (23). The catalase activity was determined in an oxygen monitor (Model 53, Yellow-Springs Instrument Co., Yellow Springs, OH).

γ-glutamyl-transpeptidase (GGTP). Plasma GGTP was measured according to the method of Naftalin et al. (24). The assay was based on the transfer of the glutamyl group from the substrate γ-L-glutamyl-p-nitroanilide to the acceptor glycylglycine catalyzed by GGTP. The liberated p-nitroaniline was diazotized and the absorbance of the pink azo-dye measured at 540 nm was proportional to GGTP activity. One unit of GGTP was defined as that amount of activity that will liberate 1 mumole (1 nanomole) of p-nitroaniline per minute at 25° under the specified conditions.

ATP assay. Fresh blood was treated with ACD solution and used for the determination of ATP which was based on enzymatic reactions described by Bucher (25) as modified by Adams (26). Reagents and enzymes used in this assay were obtained commercially (Sigma Chemical Co., St. Louis, MO). By determining the decrease in absorbance at 340 nm, a measurement of the amount of ATP present is obtained when NADH was oxidized to NAD.

Determination of serum Mg and Ca. Serum Mg was measured by the colorimetric method of Gindler and Heth (27). Serum Ca determinations were done by the method of Connerty and Briggs (28) utilizing O-cresolphthalein complexone which forms a color complex with Ca.

Amino acid analysis. Plasma was deproteinized by addition of sulfosalicylic acid (50 mg/ml), denatured protein was removed by centrifugation and 0.1 ml aliquot of supernatant was taken for analysis of free amino acids (Model 119CL, Amino Acid Analyzer, Beckman Instruments, Palo Alto, CA).

The statistical significance of experimental observations were determined by the Student's *t* test.

RESULTS

General response to Mg depletion. A marked hyperemia was apparent in all Mg-deficient rats between 5–7 days of depletion and subsided in 12–16 days. This was followed by a coarsening of the hair and the

TABLE 2

Growth, hemoglobin and hematocrit values and plasma Mg and Ca concentrations[1]

Type of diet	Initital body weight	Final body weight in 20 days	Hematocrit	Hemoglobin	Mg	Ca
	g	*g*	*%*	*g/dl*	*meq/L*	
Male						
Mg-supplemented pair fed	97 ± 8[2] (5)[2]	213 ± 25	43.6 ± 1.8	14.36 ± 0.58	1.99 ± 0.26	5.82 ± 0.27
Mg-deficient	91 ± 8 (5)	172 ± 20[a]	40.8 ± 1.3[b]	13.22 ± 0.90	0.72 ± 0.12[a]	5.66 ± 0.37
Female						
Mg-supplemented pair fed	187 ± 12 (8)	232 ± 22	45.1 ± 1.8	—	1.64 ± 0.12	5.18 ± 0.11
Mg-deficient	176 ± 27 (8)	216 ± 32	43.9 ± 1.8	—	0.69 ± 0.21[a]	5.26 ± 0.31

[1] Data presented as means ± SD. [2] Figures in parenthesis indicate number of rats used. [a] Significantly different from respective Mg-supplemented pair-fed rats ($P < 0.01$). [b] Significantly different from respective Mg-supplemented pair-fed rats ($P < 0.05$).

TABLE 3

Organ weights of Mg-deficient and Mg-supplemented rats[1]

Type of diet	Number of rats	Liver	Kidney	Spleen	Testis	Adrenal
		g/100 g body wt				*mg/100 g body wt*
Male						
Mg-supplemented pair-fed	5	4.06 ± 0.18	0.79 ± 0.06	0.315 ± 0.041	0.981 ± 0.181	17.38 ± 1.98
Mg-deficient	5	4.79 ± 0.49[b]	0.95 ± 0.10[b]	0.471 ± 0.121[b]	1.289 ± 0.201[c]	20.20 ± 3.90
Female						
Mg-supplemented pair-fed	8	2.68 ± 0.08	0.74 ± 0.03	0.213 ± 0.041	—	26.91 ± 3.71
Mg-deficient	8	3.36 ± 0.21[a]	0.99 ± 0.09[a]	0.421 ± 0.100[a]	—	28.51 ± 5.01

[1] Results are expressed as means ± SD. [a] Significantly different from Mg-supplemented pair-fed female rats ($P < 0.01$). [b] Significantly different from Mg-supplemented pair-fed male rats ($P < 0.02$). [c] Significantly different from Mg-supplemented pair-fed male rats ($P < 0.05$).

development of ulcerated lesions around the nose, face, neck and paws. A growth retardation was seen in young male rats after 20 days of being fed Mg-deficient diet. The weight gains were similar, however, between Mg-deficient young adult female rats and their respective controls. Plasma Mg levels shown in table 2 decreased significantly in Mg-deficient rats of both sexes. No significant differences were seen in the plasma Ca levels between the two groups. The Mg-deficient male rats exhibited a mild anemia as judged by the decrease in hematocrit percentage. Mg deficiency did not alter hemoglobin values.

Organ weights. At autopsy, a number of organs were weighed. The data are presented in table 3. The most consistent change observed in these experiments was splenomegaly which occurred in both Mg-deficient male and female rats. The weight increases in the kidney, liver and testis of Mg-deficient rats were also registered in this study. Although the dietary regimen seemed stressful, changes in adrenal weight were not significant.

GSH levels. The concentrations of GSH in the erythrocytes were significantly decreased after 14–23 days of feeding a Mg-deficient diet in both male and female rats (table 4).

TABLE 4

Effect of Mg-deficiency on glutathione contents in erythrocyte and liver[1]

Days on diet	Erythrocyte μmole/100 ml packed cells		Liver μmole/100 g wet weight	
	Mg-supplemented pair-fed	*Mg-deficient*	*Mg-supplemented pair-fed*	*Mg-deficient*
Male				
14	150 ± 9	111 ± 11[a]	396 ± 11	419 ± 3[a]
18–20	224 ± 28	145 ± 18[b]	467 ± 50	559 ± 71[a]
Female				
14–16	216 ± 20[1]	134 ± 15[b]	360 ± 11	400 ± 40[a]
20–23	229 ± 42	129 ± 22[b]	405 ± 31	476 ± 42[a]

[1] Results are the average of six male or six female rats ± SD. [a] Significantly different from the rats receiving Mg-supplemented diet ($P < 0.05$). [b] Significantly different from the rats receiving Mg-supplemented diet ($P < 0.01$).

In contrast, liver GSH levels were significantly increased in deficient animals (table 4). The extent of GSH change is greater in erythrocytes than in liver. Mg deficiency also increased GSH concentration in the kidney (table 5), but had no effect on other soft tissues.

To verify our results on GSH values, this tripeptide was determined by both alloxan 305 and DTNB methods in the erythrocytes, liver, and kidney. Results shown in table 6 again indicate that the erythrocyte GSH concentrations of Mg-deficient rats were reduced approximately to 67% of normal values. The magnitude of the differences shown by two independent methods was identical. In the liver and kidney, GSH concentrations measured by DTNB were higher than the values obtained by alloxan 305 test. Regardless of the method used, GSH concentrations in the liver and kidney were consistently increased by Mg-deficient rats. These diametrically opposed changes resulting from Mg depletion require further elucidation of its mode of action in mammalian tissues.

The data summarized in table 7 demonstrated that the refeeding for one week with Mg-supplemented diet to Mg-deficient rats restored the values of GSH to normal in erythrocytes. Thus, the defects in impairment of GSH metabolism appeared to be readily reversible.

Plasma amino acids. The concentration of plasma free amino acid was generally higher in Mg-deficient rats than in Mg-supplemented controls (table 8). Significant increases of plasma phenylalanine, glutamic acid and taurine were observed in Mg-deficient rats. Also, glutamine values were significantly decreased by Mg deficiency.

ATP and enzyme assays. Mg depletion reduced the activity of glutathione peroxidase but the decrease did not reach statistical significance. It would be of importance to determine whether the enzyme activity will be affected in prolonged Mg-deficient rats since this enzyme catalyzes the destruction of peroxides by the oxidation of GSH (29). Glutathione reductase activities were determined in the absence and in the presence of FAD. Results (table 9) indicate Mg defi-

TABLE 5

Effect of Mg-deficiency on glutathione contents in tissues[1]

	Mg-supplemented pair-fed	Mg-deficient
Kidney	172 ± 17	231 ± 8[a]
Pancreas	219 ± 26	218 ± 39
Heart	185 ± 13	177 ± 10
Spleen	261 ± 14	300 ± 54
Testes	281 ± 24	274 ± 41
Brain	179 ± 19	177 ± 24
Muscle	131 ± 28	123 ± 18

[1] Results expressed as μmole/100 g wet weight are average of seven male rats ± SD. [a] Significantly different from the rats receiving Mg-supplemented diet ($P < 0.05$).

TABLE 6

Comparative measurement of glutathione content by alloxan 305 and DTNB methods[1] *in erythrocyte, liver and kidney*[2]

	Mg-supplemented	Mg-deficient
Erythrocyte		
μmole/100 ml packed cells		
Alloxan 305	247 ± 21	168 ± 2[a]
DTNB	247 ± 8	169 ± 21[a]
Liver		
μmole/100 g wet tissue		
Alloxan 305	416 ± 13	555 ± 23[b]
DTNB	507 ± 7	732 ± 6[b]
Kidney		
μmole/100 g wet tissue		
Alloxan 305	198 ± 3	236 ± 7[b]
DTNB	240 ± 11	329 ± 23[a]

[1] Alloxan 305 method (18): The reaction of GSH with alloxan was used to produce a specific substance with a maximum absorbance at 305 nm. DTNB method (19): This method is based upon the development of a relatively stable yellow color when 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. Like the color-developing reagent used in the nitroprusside and bis(-nitro-phenyl)disulfide(PNPD) method, the DTNB reagent reacts with many different types of sulfhydryl groups. The higher values of GSH in the liver and kidney obtained by DTNB method are related to the non-specificity of analytical procedure. [2] Results are average of six male rats ± SD on experimental diet for 20 days. [a] Significantly different from rats receiving Mg-supplemented diet ($P < 0.05$). [b] Significantly different from rats receiving Mg-supplemented diet ($P < 0.01$).

TABLE 7

Effect of Mg repletion on erythrocyte glutathione content[1]

Type of diet	Number of rats	Final body weight	Glutathione
		g	*μmole/100 ml packed cells*
Mg-supplemented pair-fed	6	153 ± 11	223 ± 7
Mg-deficient	6	110 ± 8	150 ± 25[a]
Mg-repleted[2]	5	150 ± 7	245 ± 3

[1] Means ± SD. [2] Male rats receiving Mg-supplemented diet ad libitum for one week after they had Mg-depletion of three weeks. [a] Significantly different from rats receiving Mg-supplemented diet and from Mg-repleted rats ($P < 0.01$).

ciency did not affect enzyme activity in either condition. Likewise, catalase activity was not changed by Mg deficiency.

In agreement with the report of Elin et al. (11) and Oken et al. (8), blood ATP values in Mg-deficient rats were significantly decreased. Table 9 also shows that the dietary depletion of Mg resulted in a 6-fold increase in plasma GGTP activity.

DISCUSSION

The purpose of this investigation was to determine whether the depression of ATP and Mg ions in the blood of Mg-deficient rats is associated with the changes of erythrocyte GSH concentration. Data presented here clearly show that male and female rats fed a Mg-deficient diet had a decreased Mg level in the plasma and a reduction of GSH concentration in the erythrocytes. Since GSH content in other organs was not reduced, the erythrocytes appear to be the primary target in the metabolic disturbance during Mg deficiency. The fact that GSH concentration decreased only in Mg-deficient erythrocytes and not in the soft tissue with normal Mg contents (30) suggests that this ion is directly responsible for the depletion of erythrocyte GSH. Decrease in glutathione could be due to either a decrease in synthesis or an increase in degradation of glutathione or as a consequence of both.

Erythrocytes are known to contain high amounts of GSH (1) which have been implicated in a wide variety of biological reactions (31). For instance, it acts as a protector of SH-enzymes (31) and the integrity of erythrocyte membranes (32). The reported defects in erythrocyte membrane structure, the shortening of erythrocyte survival (13) in connection with the reduction of erythrocyte GSH concentration observed from this study support the view that Mg is essential in the maintenance of GSH to protect against oxidative damage in the erythrocyte membrane. Thus, a lack of GSH appears to be one of the mechanisms leading to hemolysis of the erythrocyte during a state of Mg deficiency.

The question arises whether the reduction of GSH is associated with the changes of the enzyme synthesizing GSH. According to Ta-

TABLE 8

Concentration of plasma-free amino acid levels[1]

Amino acid	Mg-supplemented pair-fed (8)[2]	Mg-deficient (8)
	μmole/L	
Essential		
Arginine	151 ± 41	174 ± 18
Histidine	59 ± 10	57 ± 11
Isoleucine	100 ± 19	114 ± 15
Leucine	161 ± 31	180 ± 25
Lysine	533 ± 167	643 ± 69
Methionine	49 ± 7	57 ± 13
Phenylalanine	54 ± 5	66 ± 8[a]
Threonine	291 ± 73	356 ± 69
Tyrosine	63 ± 12	78 ± 16
Valine	197 ± 47	218 ± 37
Non-Essential		
Alanine	488 ± 77	515 ± 187
Asparigine	98 ± 20	101 ± 19
Cystine half	14 ± 3	14 ± 4
Glutamic acid	114 ± 25	165 ± 28[a]
Glutamine	629 ± 45	531 ± 62[a]
Glycine	310 ± 121	331 ± 56
Ornithine	50 ± 11	56 ± 17
Proline	161 ± 49	138 ± 18
Serine	306 ± 52	264 ± 53
Taurine	257 ± 85	356 ± 83[b]

[1] Results expressed as means ± SD. [2] Number of male rats. [a] Significantly different from rats receiving Mg-supplemented diet ($P < 0.01$). [b] Significantly different from rats receiving Mg-supplemented diet ($P < 0.05$).

TABLE 9

Effect of Mg deficiency on ATP and glutathione related enzymes in rat blood[1]

	Mg-supplemented pair-fed (6)[2]	Mg-deficient (7)	P
Mg mEq/l	1.89 ± 0.15	0.63 ± 0.21	<0.001
GSH μmole/100 ml packed red cells	248 ± 11	130 ± 25	<0.01
GSH peroxidase u/mg Hb	52.9 ± 10.4	38.4 ± 10.0	NS
GSH reductase − FAD u/mg Hb	2.57 ± 1.18	2.24 ± 1.39	NS
+ FAD u/mg Hb	3.88 ± 1.92	2.74 ± 1.63	NS
A/C[3]	1.58 ± 0.62	1.25 ± 0.16	NS
Catalase u/mg Hb	657 ± 97	532 ± 50	NS
ATP μmole/100 ml blood	51.61 ± 3.56	37.89 ± 2.78	<0.01
μmole/g Hb	4.08 ± 0.15	3.38 ± 0.21	<0.02
GGTP u/100 ml plasma	30 ± 10	194 ± 119	<0.001

[1] Data presented as means ± SD. [2] The numbers in parentheses represent number of male rats used in each group. They were on experimental diets for 21 days. [3] A/C: Reduction of absorbance with FAD divided by reduction of absorbance without FAD. NS: Not significant.

teishi et al. (33), the increase in liver GSH on feeding starved rats does not appear to involve de novo production of the enzyme synthesizing GSH. This conclusion was based on these two findings: *a*) lack of inhibition by actinomycin D and cycloheximide on the increase of liver GSH and *b*) no significant difference between the GSH-synthesizing activities in fasted and refed rats. Therefore, it appears that the concentrations of the available substrates and cofactors determine the synthetic rate in liver cells. Whether this is equally true in erythrocytes remains to be experimented.

Another factor determining the erythrocyte GSH concentration is its degradation which is thought to be catalyzed mainly by GGTP (34). The relationship between the marked increase of plasma GGTP and the decrease of erythrocyte GSH in Mg-deficient rats is unknown. It has not been shown that this soluble enzyme originating from the kidney, pancreas and liver (35, 36) is able to enter red cells and to alter metabolism. Elevated serum GGTP activity has been reported in diseases of the liver, bile and pancreas (37), certain neurologic diseases (38) and frequently following myocardial infarction (39). Whether the striking elevation of plasma GGTP is the result of the reported functional impairment in the liver (40), pancreas (41), kidney (42) and heart (43) of Mg-deficient rats is not clear. Recent studies have shown that glutathione is translocated from lymphoid cells into culture medium (44) and from certain cells in vivo into the blood plasma (45), which is utilized by GGTP. In the present study, we have documented significant increase in plasma GGTP in Mg-deficient rats compared to Mg-supplemented pair-fed rats. GSH, leaking out of red blood cells, probably would be degraded faster in plasma of Mg-deficient rats. This would cause a downward gradient and faster efflux of GSH from erythrocytes to plasma, resulting in lower levels of erythrocyte GSH. Further studies are needed to investigate the rate of synthesis and degradation of GSH in erythrocytes of Mg-deficient rats.

Because of the activities of catalase, GSH reductase and GSH peroxidase were not significantly altered by dietary Mg depletion, they do not seem to contribute substantially to the reduction of erythrocyte GSH.

Another factor which may regulate the synthesis is the concentration of adenine nucleotide, because low ATP levels have been shown to depress GSH production (46) and because ATP is one of the substrates for the synthetic reactions (15). In addition, ADP

and AMP are also reported to inhibit one or both reactions (47). Therefore, the decrease in the blood ATP of Mg-deficient rats (table 9) which is comparable to the findings of Oken et al. (8) and Elin et al. (11), should contribute to the lowering of GSH level.

Elin et al. (48) reported that rats on a Mg-deficient diet for 2 weeks had a significant increase in the percentage of reticulocytes. The increase of reticulocytes percentages resulted in the change in the activities of RBC glycolytic enzymes and glucose-6-phosphate dehydrogenase (13). Since GSH synthetase levels are not effected by reticulocyte counts (16), it is unlikely that the increased reticulocyte population is the cause of erythrocyte GSH reduction.

The disturbances of protein metabolism resulting from dietary deprivation of Mg has been reported (49) are also illustrated by the changes of several amino acids in the plasma of Mg-deficient rats (table 8). There were no significant differences in the concentration of glycine, cystine and combined glutamic acid and glutamine between Mg-deficient and Mg-supplemented rats. Therefore, the observed GSH decrease cannot be attributed to the availability of its precursors. The increase of free phenylalanine and possibly tyrosine might be a reflection of the impairment in thyroid function. The increase of glutamic acid with the simultaneous decrease of glutamine suggests a disruption of the interconversion between these two amino acids and perhaps is associated with the depression of ATP.

Although plasma taurine was significantly increased in Mg-deficient rats, the knowledge concerning taurine content in various tissues is needed to determine the effect of Mg deficiency on taurine synthesis. Since cysteine is the principal precursor for GSH and taurine, the decrease in GSH concentration might enhance the utilization of cysteine for taurine synthesis.

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