Erythrocyte Survival in Magnesium-Deficient Rats (37198)

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Rats placed on a magnesium-deficient diet become anemic (1–5). The cause of the anemia is unclear; however, it has been shown that the ATP content of the magnesium-deficient erythrocyte is decreased and that the cell assumes a spherocytic shape (4, 5). Oken *et al.* reported a shortened erythrocyte survival in three magnesium-deficient rats (5). The present study evaluates the survival of normal and magnesium-deficient erythrocytes in control and magnesium-deficient inbred rats after 6 and 12 wk on the respective diets.

Materials and Methods. Seventy Fisher 344 inbred rats (10 rats in each erythrocyte survival group) weighing about 150 g were individually caged and provided a magnesium-deficient test diet (4.5 mg magnesium/100 g, General Biochemicals, Chagrin Falls, OH) and deionized water *ad libitum* (6). Fifty Fisher rats (6 rats in each erythrocyte survival group) were handled in a like manner but received the same diet with added magnesium (65 mg magnesium/100 g). Six animals in each group were sacrificed for a blood sample (intracardiac puncture) using heparin as an anticoagulant after 6 and 12 wk on the respective diets to determine plasma and erythrocyte magnesium concentrations with an atomic absorption spectrometer (4). The erythrocytes from each group for the survival studies were washed three times with phosphate-buffered saline (PBS) and suspended in 10 ml of PBS. The washed erythrocytes were labeled with ^{51}Cr (5). The labeled erythrocytes were washed three times with PBS and then injected into magnesium-deficient and control rats via the tail vein using a 23 gauge needle (Minicath-23, Desert Pharmaceutical Co., Sandy, UT). Ten blood samples were obtained from the retrobulbar sinus during the next 24 days using a disposable heparinized 100 μl pipet (Microcaps, Drummond Scientific Co., Broomwall, PA). The filled pipet was incorporated into a gamma counting vial containing 2 ml of distilled water to lyse the erythrocytes. The counting vials were stored and counted together for each animal at the conclusion of the study using a crystal scintillation counter (Nuclear-Chicago Corp., Des Plaines, IL). Only those rats who survived 24 days after erythrocyte injection were included in the study. The half-life of the labeled erythrocytes was calculated for each rat by the method of least squares using the logarithm of the percentage of surviving erythrocytes at each sample time (7). The mean of the erythrocyte half-life and plasma and erythrocyte magnesium concentrations was calculated for each group with the standard error of the mean. Groups were compared using Student's t test.

Results. After 6 wk, significant differences ($p < 0.001$) were present between the plasma and erythrocyte magnesium concentrations in the two animal groups (Table I). The survival of normal and magnesium-deficient erythrocytes was significantly shortened ($p < 0.001$) in magnesium-deficient rats at 6 wk (Table II). Also, the survival of magnesium-deficient erythrocytes was shortened ($p < 0.025$) in control rats. The magnesium concentration of ^{51}Cr-labeled magnesium-deficient erythrocytes was 4.46 mg% and in normal erythrocytes, 6.20 mg%.

After 12 wk, a further decrease occurred in the plasma and erythrocyte magnesium concentrations in animals on the magnesium-deficient diet (Table I). A progressive shortening of erythrocyte survivals was found in magnesium-deficient rats (Table II). Also,

TABLE I. Plasma and Erythrocyte Magnesium Concentrations.

Time on diet (wk)	Magnesium (mg/100 ml)			
	Control rats		Magnesium-deficient rats	
	Plasma	RBC	Plasma	RBC
6	1.95 (6)[a] ±0.02	5.97 (6) ±0.12	0.72 (6) ±0.06	4.36 (6) ±0.16
12	1.94 (6) ±0.03	5.83 (6) ±0.08	0.48 (4) ±0.07	3.50 (4) ±0.21

[a] The results are expressed as the mean ± the standard error of the mean. The number in parentheses represents the number of animals.

the survival of magnesium-deficient erythrocytes in control rats was now significantly decreased ($p < 0.005$). The magnesium concentration of the labeled magnesium-deficient erythrocytes fell to 3.66 mg% with normal erythrocytes 5.94 mg%.

Discussion. The changes in the magnesium-deficient erythrocyte cannot be entirely corrected by placing the cell in an environment with normal electrolyte concentrations. Erythrocyte magnesium falls slowly in magnesium deficiency compared with the rapid drop in plasma magnesium (4). Studies by Tufts and Greenberg (8) suggested the magnesium content of erythrocytes reflected the plasma magnesium level at the time of erythropoiesis. Ginsburg *et al.* (9), using ^{28}Mg *in vitro,* could find no appreciable exchange between intracellular erythrocyte magnesium and the ^{28}Mg in the medium. They concluded that the erythrocyte magnesium content after erythrocyte formation did not change appreciably with changes in plasma electrolytes. This stability of the intracellular erythrocyte magnesium content after synthesis may explain the shortened survival of magnesium-deficient erythrocytes injected into control animals in this study since these erythrocytes would be unable to correct their intracellular magnesium deficiency in the presence of a normal plasma magnesium concentration. However, the survival of magnesium-deficient erythrocytes was decreased further ($p < 0.001$ at 6 wk and $p < 0.005$ at 12 wk) in magnesium-deficient animals compared with controls (Table II). Therefore, the combination of a magnesium-deficient erythrocyte in a magnesium-deficient host acts synergistically to reduce erythrocyte survival.

A magnesium-deficient host can significantly shorten ($p < 0.001$) the survival of normal erythrocytes. Studies by Welt (10) have shown that a magnesium-deficient environment significantly reduces erythrocyte membrane ATPase activity. The energy for the membrane-localized cation pump of erythrocytes is derived from membrane ATPase activity (11). Therefore, a magnesium-deficient environment may impair the activity of the cation pump of erythrocytes which would induce an accumulation of sodium in excess of potassium loss resulting in an obligatory

TABLE II. Erythrocyte Half-Lives in Magnesium Deficiency (Days).

Time on diet (wk)	RBC half-life (days)			
	Control rats		Magnesium-deficient rats	
	Normal RBC	Mg-deficient RBC	Normal RBC	Mg-deficient RBC
6	15.63 (6)[a] ±0.55	14.02 (6) ±0.49	9.16 (7) ±0.71	8.30 (9) ±0.37
12	15.36 (6) ±0.68	11.26 (6) ±0.94	8.57 (5) ±0.63	5.20 (3) ±0.46

[a] The results are expressed as the mean half-life ± the standard error of the mean. The number in parentheses represents the number of animals in the group.

gain of cell water and, hence, the observed spherocytic shape of the magnesium-deficient erythrocyte (12).

The biochemical and physical changes in the magnesium-deficient erythrocyte probably result in their shortened survival due to an increased rate of sequestration in the spleen. The ATP content of the magnesium-deficient erythrocyte is significantly diminished (4, 5). Nakao, Nakao and Yamazoe (13) showed the shape and rigidity of the erythrocyte are dependent on the ATP content. Namely, as the ATP content decreases the erythrocyte becomes spherocytic and progressively more rigid. These observations coincide with the observed spherocytic shape of the magnesium-deficient erythrocyte. A study of organ and body weights in magnesium-deficient rats showed an increase in the splenic weight in magnesium-deficient animals compared with control animals but the opposite trend was found for body weights (14). This indicates splenomegaly in magnesium-deficient rats. Humans with the disease congenital spherocytosis have splenomegaly and a shortened erythrocyte survival that is frequently corrected by splenectomy. The shortened erythrocyte survival in this disease is related to an increased splenic sequestration of spherocytes and a reduction of the resistance of the spherocytes to extrasplenic hemolytic activity after release from the spleen into the general circulation (15). The shortened survival of spherocytic magnesium-deficient erythrocytes may be due to similar mechanisms. Further studies will be needed to clarify the role of the spleen.

The decrease in erythrocyte survival in magnesium deficiency is directly related to the erythrocyte and plasma magnesium concentrations. With progressive magnesium deficiency, most likely a greater percentage of erythrocytes exhibit the "critical defect" responsible for sequestration in the spleen or hemolysis. Therefore, erythrocyte survival in magnesium deficiency can be separated into two components: the environment of the erythrocyte deficient in magnesium and an intracellular erythrocyte magnesium deficiency. This study showed that these two components act synergistically to shorten erythrocyte survival.

Summary. The shortened erythrocyte survival in magnesium deficiency is directly related to the duration of magnesium deprivation. The etiology of the diminished erythrocyte survival can be divided into a decreased magnesium concentration in the environment (plasma) of the erythrocyte and a decreased intracellular magnesium content of the erythrocyte. These two components act synergistically in effecting a shortened erythrocyte survival.

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