Magnesium Metabolism of Human and Rabbit Erythrocytes

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THE CURRENT PAUCITY of information concerning magnesium metabolism in erythrocytes is due in part to the lack of a reliable method for determining magnesium in red cells, and in part to the fact that a radioactive isotope of magnesium suitable for tracer studies has only recently become available. The purposes of this study were fourfold: 1) to devise a reliable method for determining magnesium in erythrocytes; 2) to relate erythrocyte magnesium concentration to reticulocyte count; 3) to study the in vitro uptake of Mg^{28} by erythrocytes; and 4) to study the Mg^{28} uptake of various tissues in experimental animals with reticulocytosis induced by phenylhydrazine.

MATERIAL

Mg^{28} was received as $MgCl_2$ in concentrated HCl, 200 μc being contained in 25 to 30 mEq. of stable magnesium.* The material was neutralized with 1N NaOH and then diluted in physiologic saline solution to a concentration of 0.2 mEq. of mg./ml.

METHODS AND RESULTS

Radioactivity Assay

Samples of plasma, tissues, and precipitate were assayed for gamma ray activity with a well-type scintillation counter. A total of 10,000 counts were made on each sample. All determinations were corrected for physical decay of the isotope.

Magnesium Content of Red Cells

The method devised by Simonsen, Westover, and Wertman[1] for determining serum magnesium was modified as follows: Within two hours after blood was drawn, erythrocytes were separated from the heparinized blood by centrifugation. They were then washed twice in physiologic saline solution, and resuspended in saline. (Such washing does not alter the magnesium content of erythrocytes.[2]) After a hematocrit reading on this suspension, aliquots of the suspension were used in the magnesium determination.

The erythrocytes were hemolyzed by the addition of two volumes of distilled water and one drop of concentrated ammonium hydroxide. As in the method for serum magnesium, the calcium was then removed by precipitation as oxalate, and the magnesium in the supernate was precipitated as magnesium ammonium phosphate. The phosphate content was determined colorimetrically with molybdivanadate. Although most previous methods prescribe precipitation of protein after hemolysis, recovery of magnesium was better when protein was not removed. Mg^{28} added to the hemolysate was found trapped in the

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Table 1.—*Correlation of Reticulocyte Count and Erythrocyte Magnesium Concentration in Patients*

Case	Reticulocyte Count (%)	RBC Magnesium Content (mEq./L.)
1	0.6	4.0
2	0.7	4.7
3	1.8	4.0
4	2.8	5.3
5	3.0	5.5
6	5.5	6.0
7	39.7	10.6

precipitate. Complete recovery of magnesium was accomplished when the calcium oxalate precipitate was washed twice with distilled water and the wash solution added to the original supernate.

By this method, triplicate determinations of erythrocyte magnesium concentration in blood from healthy hospital personnel showed no more than a 5 per cent variation. The range of concentration was 3.47 to 6.52 mEq./L., with a mean of 4.67 ± 0.92 mEq./L.

Reticulocytosis and Erythrocyte Magnesium Concentration

There are scattered reports in the literature of an increased erythrocyte magnesium concentration associated with reticulocytosis.[3,4] In order to determine whether there is a true correlation between the reticulocyte count and the magnesium content of erythrocytes, blood was obtained from seven patients (five with reticulocytosis) and the reticulocyte counts were determined by counting 1,000 cells.

Table 1 reveals a direct correlation between the reticulocyte count and the magnesium content of erythrocytes. This clinical observation was then subjected to controlled experimentation.

Preliminary studies in rabbits revealed that the repeated subcutaneous injection of phenylhydrazine very readily reduced the hematocrit and red cell count and produced a marked reticulocytosis. This method was more effective in changing the blood picture than the withdrawal of 200 ml. of blood by repeated cardiac punctures.

The mean magnesium concentration in the erythrocytes of six normal rabbits was found to be 7.8 ± 0.63 mEq./L. In five of these animals, hemolysis of erythrocytes and resultant reticulocytosis were induced by phenylhydrazine administered subcutaneously in doses of 12 to 25 mg. at intervals of one to six days, according to the schedule shown in figure 1. The frequency of administration was determined by the hematocrit and erythrocyte levels. Figure 1 is typical of the results obtained in all five rabbits. The maximum reticulocyte count (85 per cent) was accompanied by a red cell magnesium concentration of 28.4 mEq./L. In all cases the changes in reticulocyte count paralleled the changes in red cell magnesium concentration. The increase in reticulocytes and in magnesium values usually occurred 24 hours after the injection of phenylhydrazine, and was accompanied by a proportionate *decrease* in the hematocrit and red cell count. These results suggest that immature erythrocytes have a higher magnesium content than mature red cells.

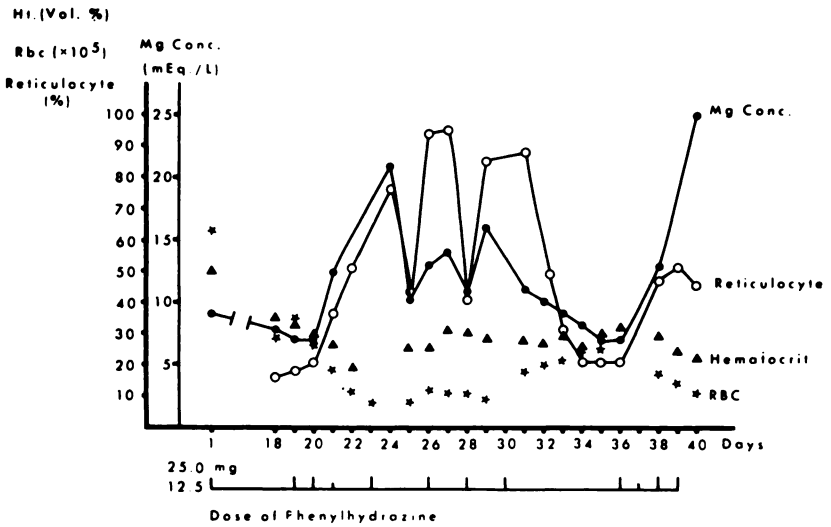

Fig. 1.—Effect of phenylhydrazine on hematologic values in rabbit.

In Vitro Uptake of Mg^{28} by Erythrocytes

The next experiment was designed to determine whether there is in the mature circulating erythrocytes of human beings an exchange with extracellular magnesium. Such an exchange has previously been reported[5,6] in the sheep and the rat.

Mg^{28} was used as a tracer in these studies, which were performed under aseptic conditions. Blood was obtained from nine human subjects and treated with heparin: In five instances the erythrocytes were separated by centrifugation and washed twice in physiologic saline solution; in the remaining four, erythrocytes remained suspended in their own plasma. Aliquots of *saline suspensions of washed cells* were added to isotonic solutions of saline, glucose, or both; each solution contained a tracer amount of Mg^{28} and stable magnesium in a concentration of 8 mEq./L. The *whole blood samples* were mixed with equal amounts of isotonic saline solution containing Mg^{28} and Mg^{24}. All the preparations were incubated for 24 hours in a water bath at 37 C. The radioactivity content of the erythrocytes was determined at 12 and 24 hours. No significant uptake of Mg^{28} by human red cells occurred in any of these preparations. Mature human red cells, therefore, do not exchange magnesium in significant amounts in the peripheral circulation.

To investigate the possibility that immature cells might take up Mg^{28}, similar in vitro studies were performed with red cells from normal rabbits and from those with reticulocytosis induced by phenylhydrazine. Washed red cells were incubated at 40 C. for 18 hours in saline containing Mg^{28} and stable magnesium at a concentration of 4 mEq./L., and the uptake of Mg^{28} was then determined. In cells from five normal rabbits with a reticulocyte count of 1 per cent, the Mg^{28} concentration was 1.7 to 3.3 per cent of that in the suspending medium. In red cells from five rabbits with a reticulocyte count as high as 94 per cent, the maximum Mg^{28} concentration was 3.7 per

cent of that in the suspending medium. Thus, there was no significant difference in the in vitro uptake of Mg^{28} by mature and immature rabbit erythrocytes.

These studies suggest that the greatly increased content of magnesium in immature erythrocytes cannot be due to uptake of magnesium in the peripheral circulation. It appears likely that the reticulocytes attain this high concentration of magnesium prior to their release into the peripheral circulation. This being the case, the Mg^{28} uptake of the bone marrow should be higher in rabbits with reticulocytosis than in normal animals.

Effect of Phenylhydrazine on Tissue Uptake of Mg^{28}

The following experiment was performed to test the above hypothesis, and also to determine the effect of phenylhydrazine on the magnesium uptake of other tissues.

Eighteen rabbits with reticulocyte counts ranging from 44.6 to 64.6 per cent were used in the test group. Four hours after receiving intravenous injections of 1.77 mEq. of magnesium containing a tracer of Mg^{28}, they were killed by air embolism. Samples of skeletal muscle, skin, liver, appendix, heart, kidney, bone cortex, bone marrow, spleen, and lung (approximately 1 Gm. of each tissue) were assayed for Mg^{28} content. The results are expressed as relative radioactivity:

$$\frac{\text{cpm/Gm. wet weight of tissue}}{\text{cpm/ml. of serum}}$$

The mean values were compared with those obtained previously in normal untreated rabbits,[7] and the significance of the difference between the means was tested with the "t" test.[8] The mean relative radioactivity in the bone marrow was significantly higher than that of the control mean, while those in the heart, spleen, and kidney were significantly lower (table 2).

The heart and kidney appeared normal in size and appearance. The mean weight of the spleen in the rabbits given phenylhydrazine was 3.6468 Gm.; it was 1.1086 Gm. in the control animals.

Comments

A slight modification of the method devised by Simonsen, Westover and Wertman[1] for determining serum magnesium resulted in a procedure suitable for the determination of red cell magnesium concentration. Previous reports[2,9-14] of magnesium concentration in human erythrocytes range from 1.6 to 6.2 mEq./L. Results of the present study agree best with those reported by Hald and Eisenman.[12] The magnesium concentration in normal rabbit erythrocytes was considerably higher than that in normal human red cells. In both species, reticulocytosis was accompanied by an increase in erythrocyte magnesium concentration. An increased magnesium content appears to be a property of newly formed red cells.[15]

Although the exact mechanism responsible for the effect of phenylhydrazine on erythrocytes is not understood, it is thought to be primarily a hemolytic

Table 2.—*Effect of Phenylhydrazine on Relative Mg^{28} Activity* in the Rabbit*

Tissue	Control Group†	Phenylhydrazine Group‡
Bone marrow	1.96 ± 0.23§	4.89 ± 0.42‖
Heart	8.44 ± 0.36	6.67 ± 0.40‖
Kidney	10.46 ± 0.61	7.74 ± 0.41‖
Spleen	4.97 ± 0.23	3.15 ± 0.15‖
Skin	1.36 ± 0.13	1.34 ± 0.12
Liver	5.10 ± 0.42	5.67 ± 0.21
Lung	3.77 ± 0.33	3.29 ± 0.16
Muscle	0.76 ± 0.13	0.45 ± 0.06
Appendix	7.66 ± 0.28	6.08 ± 0.36
Bone cortex	12.90 ± 1.13	9.73 ± 0.93

*Relative Mg^{28} Activity $= \frac{\text{cpm/Gm. wet tissue}}{\text{cpm/Gm. serum}}$.

†Eight normal rabbits.

‡Eighteen rabbits with reticulocyte counts ranging from 44.6 to 64.6 per cent.

§Mean ± standard deviation.

‖Statistically significant difference between the means of the test and the control groups.

agent acting on non-nucleated red cells, either directly on hemoglobin or on the cell membrane.[16,17] That its administration regularly produces reticulocytosis is well known.[18,19] It has been suggested that phenylhydrazine not only has a direct hemolytic effect but also affects the erythropoietic factor in the plasma.[19]

The fact that the reticulocytosis and increased erythrocyte magnesium concentration occurred within 24 hours after the injection of phenylhydrazine in rabbits suggests that the increased magnesium concentration is due to the release of immature red cells into the peripheral circulation. This interpretation is strengthened by the negligible *in vitro* uptake of Mg^{28} by normal human and rabbit erythrocytes and immature red cells from rabbits; immature red cells released into the circulation have a higher concentration of magnesium than mature cells, but once in the peripheral circulation they do not exchange magnesium very readily with that in the extracellular environment. The finding that the relative activity of Mg^{28} in the bone marrow of animals given phenylhydrazine is considerably higher than that in normal controls suggests that the uptake of magnesium is increased in tissue which is stressed to activity and presumably has an accelerated turnover of relatively immature cells.

Phenylhydrazine has previously been shown to be toxic to the kidney and the liver.[18] In the present study, the relative radioactivity in the heart, kidney, and spleen was decreased after administration of this drug. If magnesium uptake by cells is related to cell anabolism or multiplication, then functional tissue damage, not necessarily evident as anatomic abnormality or change in gross appearance, would be expected to suppress the uptake of Mg^{28}—and such a suppression was observed in the kidney and heart. Since the spleen is involved in the removal of damaged erythrocytes, and since phenylhydrazine rapidly produces intravascular hemolysis, the splenic mass would be increased (as observed) by the damaged erythrocytes and a decrease in the splenic uptake of Mg^{28} would result.

The results in this series of experiments strengthen the hypothesis that one of the primary factors affecting the rate of Mg^{28} uptake by various tissues is their metabolic rate or rate of growth. In a previous study, thyroxine was found to increase the uptake of Mg^{28} in certain tissues stimulated by this drug. In another study[21] of the placental transfer and fetal tissue uptake of Mg^{28}, it was shown that the uptake of Mg^{28} was greater in the fetal tissue than in the mother. Conversely, the cells of organs damaged by pharmacologic agents (as the kidney and heart are damaged by phenylhydrazine) have a decreased uptake of Mg^{28}. In certain other tissues,[22] the administration of a metabolic antagonist such as desoxypyridoxine also suppressed the uptake of Mg.[28]

Summary

A modification of the magnesium ammonium phosphate precipitation method for the determination of serum magnesium was devised to determine the magnesium content of erythrocytes.

The concentration of magnesium in the red cells of healthy hospital personnel was 4.67 ± 0.92 mEq./L.

An increase in erythrocyte magnesium concentration was observed in patients with reticulocytosis. Experimental production of reticulocytosis by the administration of phenylhydrazine to rabbits confirmed these clinical observations.

No significant in vitro uptake of Mg^{28} from the suspending medium occurred in mature human erythrocytes or in mature or immature erythrocytes from rabbits.

The relative tissue uptake of Mg^{28} in the bone marrow was significantly increased in animals in whom anemia and marked reticulocytosis were produced by phenylhydrazine. Relative activity was decreased in the hearts, spleens, and kidneys of these animals.

Since there is no evidence for significant exchange of magnesium in immature or mature erythrocytes in the peripheral circulation, it is concluded that the magnesium content of erythrocytes is increased in the bone marrow prior to their release into the peripheral circulation.

Summario in Interlingua

Un modification del methodo a precipitation de phosphato ammoniaco-magnesian pro le determination del magnesium del sero esseva elaborate pro determinar le contento de magnesium de erythrocytos.

Le concentration de magnesium in le erythrocytos de normal empleatos de hospital esseva 4,67 ± 0,92 mEq./L.

Un augmentate concentration de magnesium esseva observate in le erythrocytos de patientes con reticulocytosis. Iste observationes clinic esseva confirmate per le production experimental de reticulocytosis in conilios per le administration de phenylhydrazina.

Nulle significative acceptation de Mg^{28} ab le medio de suspension occurreva in vitro in matur erythrocytos human o in matur o immatur erythrocytos ab conilios.

Le relative acceptation de Mg^{28} per le tissus esseva significativemente augmentate in le medulla ossee de animales in le quales anemia e un marcate reticulocytosis habeva essite producite per phenylhydrazina. Le relative activitate esseva reducite in le cordes, splenes, e renes de iste animales.

Proque nulle evidentia esseva trovate pro un significative excambio de magnesium in erythrocytos immatur o matur in le circulation peripheric, il es concludite que le contento de magnesium del erythrocytos es augmentate in le medulla ossee ante lor liberation ad in le circulation peripheric.

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