BRIEF REPORT

Microcytic Anemia with Erythroblastosis in Offspring of Magnesium-deprived Rats

By Sidney Q. Cohlan, Valerie Jansen, Joseph Dancis and Sergio Piomelli

Magnesium deprivation of pregnant rats produces in their offspring depression of intrauterine growth and severe anemia. The anemia is characterized by microcytosis, red cell fragmentation, increase in number of normoblasts and markedly decreased osmotic fragility.

Magnesium deficiency has been extensively studied in growing and mature animals, but there is no information concerning the effect on the fetus of Mg deprivation during pregnancy.

Anemia has never been reported in Mg-deficient adult animals, but in acute deprivation experiments, decreased Mg levels, rates of glycolysis and ATP content were observed and ascribed to impairment of hexokinase. In chronic deficiency these effects gradually disappeared and when dietary Mg was restored there was an increase to levels above normal of Mg, hexokinase and ATP.

In the course of a systematic study of specific nutritional deficiencies in the pregnant rat, it was observed that maternal Mg deprivation produces a depression of intrauterine growth and severe anemia in the offspring.

Material and Methods

Animals: Rats 7 days pregnant were obtained from the Charles River Laboratories.

Diet: The experimental and control diets (General Biochemicals, Chagrin Falls, No. 170490) differed only in their Mg content (1.04 and 120 mEq./Kg.).

Plasma and red cell Mg were determined by atomic absorption spectrophotometry. Mg was measured directly in the plasma. In the red cells it was measured after digestion with concentrated nitric acid.

Hematological indices (MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration) were determined according to Cartwright. Hemoglobin was determined by a modified cyanmethemoglobin method. Hematocrits were determined by the capillary method. Red cell counts were obtained with a Celloscope Model 201 electronic counter with dual channels pulse analysis. The solution

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used for counting has been previously described. Size distribution curves were obtained by counting at 20 equally spaced discriminator levels on one channel simultaneously with repeated counts at threshold level on the other channel.

Osmotic fragility was estimated from the percentage of hemoglobin released in the supernatant after exposure of whole blood to buffered NaCl solution of varying concentrations for 30 minutes at 20°C. One-hundred per cent hemolysis was obtained in an isotonic buffered NaCl solution containing 200 parts per million of saponin. The mean corpuscular fragility (MCF) and its standard deviation were estimated by the probit plot of the cumulative percentage hemolysis versus the salt concentration.

Interference microscopic studies of red cells were performed on cells fixed for 20 minutes in 200 volumes of buffered 0.5 per cent glutaraldehyde, pH 7.4, followed by three washes in distilled water, with a Zeiss photomicroscope equipped for interference, according to Nomarsky, using a mercury burner as light source.

Statistical analysis: t test on paired and nonpaired samples were performed with an Olivetti Programma 101 table computer according to C.A.B. Smith.

Experimental design: On the tenth day of gestation, pairs of pregnant rats were fed, respectively, Mg-deficient (experimental group) and control diets ad libitum. In five experiments, the animals were pair fed by weighing the daily diet intake of the experimental animal and then feeding the control animal the same amount. On the 21st day of gestation, the animals were anesthetized and the fetuses were removed by caesarean section. Blood samples were collected with heparin from the mother by cardiac puncture, and from the fetuses by severing axillary vessels.

RESULTS

The experimental dams exhibited the characteristic hyperemic ears and feet previously described in Mg-deficient animals but appeared otherwise healthy. The average litter sizes in the control and experimental groups were 9.6 and 9.4, respectively. The offspring of the Mg-deficient dams were on the average small (2.9 Gm. versus 3.5 Gm. in the controls), pale and inactive but viable and free of congenital malformations. In the most severely affected, polyhydramnios and edema were occasionally observed. In the pair-feeding experiment, fetal growth retardation was identical; thus it was not a result of maternal anorexia.

Biochemical and hematological findings are summarized in Table 1.

The mean plasma and red cell Mg were both decreased in the experimental mothers. The mean fetal plasma Mg was also decreased in the experimental newborns but there was no difference in the red cell Mg.

Hemoglobin concentration was initially measured in 22 control and 28 experimental individual newborns. The mean values were, respectively, 11.9 Gm./100 ml. (S.E.M. 0.45) in the controls and 8.1 Gm./100 ml. (S.E.M. 0.36) in the experimental animals (p < .001).

The characteristics of the anemia were then further investigated in pooled blood samples from 14 paired litters and 10 paired dams. Marked anemia was observed in all but one of the deficient litters. Since plasma Mg had not been simultaneously measured, it is impossible to exclude, in this litter, a failure to obtain a significant Mg deficiency. The mean hemoglobin concentration and hematocrit were significantly reduced in comparison to the controls but there was no difference in the mean number of RBC/cu. mm. Thus the experimental fetuses exhibited an anemia characterized by reduced MCV and MCH but insignificantly reduced MCHC.
Table 1.—Mg Levels and Hematological Data

<table>
<thead>
<tr>
<th></th>
<th>Fetal Litters (14)</th>
<th>Mothers (10)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>x</td>
<td>S.E.M.</td>
</tr>
<tr>
<td>Plasma Mg* (mEq/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contr.</td>
<td>3.51</td>
<td>0.13</td>
</tr>
<tr>
<td>Mg def.</td>
<td>1.05</td>
<td>0.28</td>
</tr>
<tr>
<td>R.B.C. Mg† (mEq/L)</td>
<td></td>
<td></td>
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<tr>
<td>Contr.</td>
<td>7.88</td>
<td>0.50</td>
</tr>
<tr>
<td>Mg def.</td>
<td>8.08</td>
<td>0.67</td>
</tr>
<tr>
<td>Hemoglobin (Gm. %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contr.</td>
<td>10.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Mg def.</td>
<td>7.68</td>
<td>0.51</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td></td>
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<tr>
<td>Contr.</td>
<td>39.92</td>
<td>0.73</td>
</tr>
<tr>
<td>Mg def.</td>
<td>32.74</td>
<td>1.39</td>
</tr>
<tr>
<td>RBC (× 10⁶/cu. mm.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contr.</td>
<td>2.17</td>
<td>0.13</td>
</tr>
<tr>
<td>Mg def.</td>
<td>2.13</td>
<td>0.05</td>
</tr>
<tr>
<td>MCV (µl)</td>
<td></td>
<td></td>
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<tr>
<td>Contr.</td>
<td>187.44</td>
<td>9.86</td>
</tr>
<tr>
<td>Mg def.</td>
<td>154.54</td>
<td>5.76</td>
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<tr>
<td>MCH (γγ)</td>
<td></td>
<td></td>
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<tr>
<td>Contr.</td>
<td>42.24</td>
<td>2.59</td>
</tr>
<tr>
<td>Mg def.</td>
<td>36.53</td>
<td>2.19</td>
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<tr>
<td>MCHC (%)</td>
<td></td>
<td></td>
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<tr>
<td>Contr.</td>
<td>25.91</td>
<td>2.53</td>
</tr>
<tr>
<td>Mg def.</td>
<td>24.52</td>
<td>3.83</td>
</tr>
<tr>
<td>MCF (=NaCl Gm. %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contr.</td>
<td>0.50</td>
<td>0.60</td>
</tr>
<tr>
<td>Mg def.</td>
<td>0.39</td>
<td>1.30</td>
</tr>
<tr>
<td>Normoblasts (× 10⁶/mm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contr.</td>
<td>15.58</td>
<td>1.32</td>
</tr>
<tr>
<td>Mg def.</td>
<td>41.50</td>
<td>6.31</td>
</tr>
</tbody>
</table>

* Measured in 12 pairs only.
† Measured in eight pairs only.

The peripheral smears (Fig. 1) showed intense polychromasia in both control and experimental fetuses, reflecting the extreme reticulocytosis in both groups (94 per cent and 95 per cent, respectively). In the experimental fetuses there was in increased number of normoblasts, intense aniso- and poikilocytosis, a large percentage of fragmented cells and of very small schistocytes. In many red cells, there were membrane creases and hemoglobin content appeared decreased. Under the interference microscope (Fig. 1), the aniso- and poikilocytosis was even more obvious, most cells showed evidence of membrane alteration, and the discoid shape was replaced by a flatter, crater-like shape, suggesting a disproportion between normal size of the outer envelope and reduced cell content.

The red cell size distribution curves showed a second minor population of smaller cells, together with a major population of cells of normal volume (Fig. 2).

In the experimental animals, the mean osmotic fragility was grossly decreased with a larger standard deviation (Fig. 3). These findings correlated well with the decreased mean size of the red cells as well as with their wider size distribution. In the one litter that showed no evidence of anemia, the osmotic fragility was practically identical to that of the red cells from the control newborn group.

Anemia, in a much lesser degree, was also observed in the Mg-deficient mothers. Because of the small group size and the lesser magnitude of the
Fig. 1.—A and B: control rat fetuses; C and D: Mg-deficient rat fetuses. A and C, Wright's stain. × 550; B and D, interference microscope. × 1350. These preparations were obtained from nonpooled, individual blood samples.

effect, the only significant difference was in the hemoglobin level. The hematocrit, MCV, and MCH also showed a trend toward lower values concordant with that observed in the fetuses.

**DISCUSSION**

Plasma Mg in the deficient fetuses was decreased, but it was still higher than in their mothers' plasma. Thus, in these experimental conditions, a fetal-maternal gradient of Mg was maintained, presumably through an active
transplacental transport mechanism. In other experiments in this laboratory, with a different type of Mg-deficient diet, it has been observed that fetal plasma Mg can decrease to the same level as maternal plasma Mg if a more severe Mg deprivation is obtained.8

Despite the decreased plasma level, the red cell Mg concentrations in the deficient fetuses was similar to that of the controls. The maturing red cells apparently succeed in concentrating Mg, even from a grossly depleted pool, thus suggesting a critical need of Mg for formation and release of new cells. In the Mg-deficient mothers, the Mg concentration of the red cells was, instead, markedly reduced. This discrepancy may be explained by a progressive depletion of red cell Mg during circulation in Mg-poor environments; this cannot occur in the fetal red cells, as these, being newly formed, have been in the circulation for only a short period of time.

The anemia observed in the Mg-deficient fetuses was characterized by microcytosis, presence of a large number of very small and fragmented red cells, marked increase in the number of normoblasts and grossly decreased osmotic fragility. Since in the newborn rat practically all circulating red cells are reticulocytes and indirect bilirubin is cleared through the placenta, neither reticulocyte nor bilirubin concentrations can be used to assess whether the anemia is due to accelerated destruction of red cells or to defective production. Iron deficiency as a cause of the anemia can be ruled out since total body Fe content is not reduced in the Mg-deficient fetuses.15 Moreover, the fact that the reticulocyte count was not decreased and the percentage of normoblasts was increased indicates at least the presence of active erythropoiesis.

The deficiency in the body Mg pool results in profoundly altered red cells. The Mg concentration per unit weight is unchanged, but, as the red cells are much smaller, the Mg content of the individual cells is decreased; the volume (but not the number) of red cells is decreased; the hemoglobin content of individual cells is reduced; there are gross structural abnormalities, indicated by abnormal osmotic fragility, laxity of the membrane and tendency of the red cells to fragmentation. These alterations can be interpreted as the result
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Fig. 3.—Size distribution curves of red cells. Points for maternal cells represent the average of three individual animals. Points for fetal cells represent the average of four individual animals from four different litters.

of a deficient synthesis of hemoglobin, leading to formation of cells whose membranes cannot be distended to normal shape, tend to form creases (clearly visible with the interference microscope but apparent also on stained smears) and are easily fragmented, producing small schistocytes.

The morphological appearance of the red cells of the Mg-deficient fetal rats bears a striking similarity to those of patients with Cooley’s anemia (another situation where hemoglobin synthesis is impaired). It is of interest, at least as coincidence, that in these patients a decreased level of plasma Mg has been reported.16

Further studies are in progress to clarify whether defective hemoglobin synthesis and/or severe hemolysis is the predominant factor in the anemia of Mg deficiency in the fetal rat.

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