Magnesium Deficiency Anemia in the Rat Fetus

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Summary

Magnesium-deficient fetuses exhibited malformations (44%), anemia, and edema. Maternal plasma magnesium levels at day 21 of pregnancy reflected the level of dietary magnesium (2.43 ± 0.09 mg Mg/100 ml, control; 0.74 ± 0.02 mg Mg/100 ml, deficient). Plasma magnesium levels of deficient fetuses showed similar decreases although all fetal magnesium values at term were higher than maternal values from the same group (3.29 ± 0.22 mg Mg/100 ml, control; 1.78 ± 0.07 mg Mg/100 ml, deficient). Magnesium deficiency did not appear to affect the maternal blood parameters. However, when fetal blood was examined, all of the parameters measured were altered in magnesium-deficient fetuses (Table 2). No abnormalities in hemoglobin bands or plasma proteins were seen between any groups by electrophoresis. Measurement of total protein showed no differences between maternal blood protein contents, but total plasma protein from magnesium-deficient fetuses was significantly lower than controls (2.00 ± 0.14 versus 2.62 ± 0.13 g/100 ml), thus establishing a factor in fetal edema production.

Morphologic data showed that in magnesium-deficient fetuses, fetal erythropoiesis was significantly greater in liver, adrenal glands, and spleen than in controls and that maturation was normoblastic. Stained and unstained peripheral blood smears of magnesium-deficient fetuses showed an obvious macrocytosis and at least 50% of the red cells stained abnormally, exhibiting pale areas. Erythrocytic morphology seen in fetal magnesium deficiency is consistent with inadequate filling of the cell by hemoglobin as suggested by Cohan *et al.* (5), a probable cause of membrane collapse. The inadequate filling of magnesium-deficient red blood cells (RBC) with hemoglobin might be explained by a reduction in hemoglobin synthesis which is consistent with the reduced mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC) of the deficient fetal red cells. The role of magnesium in protein synthesis is also compatible with a reduction in hemoglobin synthesis, yet may not completely explain the abnormalities and resultant shortened lifespan of the red cells.

Speculation

The present studies show the effects of magnesium deficiency during pregnancy in the rat to be severe, producing congenital malformations and fetal anemia. Very little is understood about magnesium deficiency in humans, although it is generally agreed that there is depression in plasma magnesium levels toward the end of pregnancy and a possible relationship between magnesium and pregnancy toxemia. The question of whether or not magnesium deficiency is a contributing factor to fetal anemia in humans remains. Magnesium deficiency in rats, however, provides an experimental model to study fetal anemia and its subsequent effects during the perinatal period.

The effects of magnesium deficiency in the pregnant rat were recently reported (2, 8, 12, 19). A diet severely deficient in

magnesium given from the 6th to the 14th day of pregnancy produced fetal death and resorption or gross congenital malformations (11). A diet less severely deficient in magnesium fed throughout pregnancy also produced congenital malformations and some resorptions. In addition, these fetuses were edematous and anemic (9, 10). Cohan *et al.* (5) have also observed anemia resulting from maternal magnesium deficiency but not congenital anomalies. The present report describes the morphologic aspects of fetal anemia resulting from magnesium deficiency together with the hematologic parameters and plasma protein concentration in fetuses shown to have a high percentage of congenital malformations.

MATERIALS AND METHODS

Female Sprague-Dawley rats were purchased from a commercial source (24) and were maintained as previously described (11). At mating, as determined by observation of sperm in the vaginal smear (day 0 of pregnancy) females were fed purified diets made with either casein or soybean protein (25), and containing varying levels of magnesium. In addition, one group was fed a commercial rat stock diet (26) containing 125 mg Mg/100 g diet (11). The females were fed these diets from day 0 to day 21 of gestation. In order to test the effects of a shorter deficiency period additional pregnant rats were fed the magnesium-deficient, soybean protein diet only from days 0-19, 0-17, 0-15, 0-13, 13-21, and 15-21 of gestation.

On day 21 of gestation the females were anesthetized with ether and maternal blood was taken by cardiac puncture in heparinized syringes. The fetuses were removed by cesarian section and a sample of fetal blood was also taken from the heart.

HEMATOLOGY

Red blood cell counts were made on maternal and fetal blood with standard diluting pipettes and counting chamber. Packed cell volume was determined using heparinized microhematocrit tubes centrifuged for 15 min (27). Hemoglobin concentration was measured by the standard acid hematin method (28).

ELECTROPHORESIS

Heparinized fetal blood was examined electrophoretically for hemoglobin species by the method of Graham and Grunbaum (7). Plasma protein distribution of maternal and fetal blood was examined by the electrophoretic method of Narayan (16, 17) and total plasma protein of the same samples was determined by the method of Lowry *et al.* (13).

LIGHT MICROSCOPY

Blood smears were made of fetal blood; one smear from each sample was stained with Wright's stain, the other was left unstained and examined by phase and interference microscopy. Whole fetuses were fixed in Bouin's fixative, transferred to 70%

alcohol, embedded in paraffin, and sectioned either in transverse or sagittal fashion. The 4 μ m sections were stained with hematoxylin and eosin. Special attention was given to the fetal liver, spleen, bone marrow, and adrenal glands.

ELECTRON MICROSCOPY

Samples of fetal liver were fixed in 1% osmium buffered in 2 M sodium cacodylate, pH 7.4, for 3 hr dehydrated in alcohol, and Epon embedded. Sections were stained for 30 min in 1% uranyl acetate, 10 min in a modified Reynold's lead citrate stain, and viewed with RCA EMU-3G and AEI-6B transmission electron microscopes. (29). Fetal blood samples were treated in the same manner except that they were prefixed in 4% glutaraldehyde (buffered in 2 M Na-cacodylate, pH 7.4). Fetal blood samples fixed in glutaraldehyde were dehydrated in acetone, air dried, coated with silver and gold, and examined with a scanning electron microscope (14, 30).

RESULTS

The effects of magnesium deficiency on reproductive performance are presented in Table 1. The magnesium-deficient casein diet containing 2.5 mg Mg/100 g had the most severe effect on maternal weight gain during pregnancy. Both the deficient casein diet containing 2.5 mg Mg/100 g and the deficient soybean diet containing 5 mg Mg/100 g increased intrauterine death. In these two groups the weights of the fetuses surviving to term were also reduced significantly. Even when given for short periods during gestation the magnesium-deficient soybean diet decreased fetal survival and weight.

Plasma magnesium levels at day 21 of pregnancy reflected the level of magnesium in the maternal diet. Maternal plasma magnesium was reduced significantly in all groups given deficient diets for 21 days, and in most groups given deficient diets for

shorter periods. Plasma magnesium levels of deficient fetuses showed similar decreases, although all fetal magnesium values at term were higher than maternal values from the same group. When the magnesium-deficient dams were fed the magnesium control diet from 2–8 days before term, the plasma magnesium at day 21 increased to normal after 2 days of repletion in the fetuses and only approached normal after 8 days in the dams. If the magnesium-deficient diet was fed through days 15–21 and 13–21 only, the 21-day plasma magnesium levels were reduced in both maternal and fetal plasma. In the group fed a high level of dietary magnesium (200 mg Mg/100 g) the maternal plasma magnesium was increased to values similar to normal fetal values, whereas the fetal plasma magnesium remained unchanged.

HEMATOLOGY

Magnesium deficiency appeared to affect only the magnesium concentration of maternal red blood cells whereas packed cell volume, hemoglobin concentration, and the RBC count remained normal (Table 2). This decrease in magnesium concentration was also seen in the mean corpuscular Mg concentration index. Females fed the higher than normal magnesium diet (200 mg Mg/100 g) had higher RBC magnesium and mean corpuscular Mg concentration values than the controls.

When fetal blood was examined, all of the parameters measured were altered in magnesium-deficient fetuses (Table 2). The RBC count, packed cell volume, and hemoglobin concentration were reduced in the 0–21-day, deficient groups. Even in groups fed the deficient diets for shorter periods during gestation these changes were evident. These decreases were reflected in the mean corpuscular hemoglobin and corpuscular hemoglobin concentration indices. Fetal RBC magnesium was higher than in maternal blood and did not appear to be affected by lowered dietary magnesium. When mean corpuscular volume was calculated, both deficient groups (2.5 mg Mg/100 g casein diet and 5

Table 1. Effect of maternal magnesium deficiency on reproduction and fetal development

Experimental group	Days of gestation	No. fetuses ¹	Maternal weight gain, g	Resorption, %	No. fetuses	Fetal wt at term, g	Plasma Mg at term, mg/100 ml	
							Maternal	Fetal
Stock diet	0–21	10	108.0 $\pm$ 6.0	5.5	128	5.1 $\pm$ 0.04	2.87 $\pm$ 0.15	3.52 $\pm$ 0.27
Purified control								
Casein (40 mg Mg/100 g)	0–21	13	67.5 $\pm$ 6.7	7.2	120	5.3 $\pm$ 0.03	2.43 $\pm$ 0.09 ²	3.29 $\pm$ 0.22
Soybean (40 mg Mg/100 g)	0–21	13	74.5 $\pm$ 5.5	5.0	133	5.3 $\pm$ 0.03	2.34 $\pm$ 0.06	3.30 $\pm$ 0.08
Magnesium-deficient								
Casein (2.5 mg Mg/100 g)	0–21 ³	61	48.6 $\pm$ 2.1	94.0	52	3.2 $\pm$ 0.07	0.74 $\pm$ 0.02 ^{4,5}	1.78 $\pm$ 0.07 ^{4,5}
Casein (5.0 mg Mg/100 g)	0–21	5	86.8 $\pm$ 6.1	9.8	54	4.8 $\pm$ 0.03	0.79 $\pm$ 0.12 ^{4,5}	1.68 $\pm$ 0.35 ^{6,7}
Soybean (5.0 mg Mg/100 g)	0–21	6	92.3 $\pm$ 3.7	60.0	42	3.2 $\pm$ 0.05 ⁸	0.82 $\pm$ 0.07 ⁵	1.75 $\pm$ 0.12 ⁷
	0–19	6	81.8 $\pm$ 12.6	30.0	40	3.8 $\pm$ 0.09 ⁵	1.58 $\pm$ 0.15 ⁵	3.68 $\pm$ 0.18 ⁸
	0–17	5	84.8 $\pm$ 13.4	49.0	42	4.2 $\pm$ 0.12 ⁵	1.71 $\pm$ 0.19 ⁹	3.61 $\pm$ 0.35
	0–15	5	97.5 $\pm$ 4.4	44.0	29	4.7 $\pm$ 0.16 ⁷	1.79 $\pm$ 0.12 ¹⁰	3.20 $\pm$ 0.57
	0–13	5	107.0 $\pm$ 6.5	4.0	64	4.5 $\pm$ 0.08 ⁵	2.13 $\pm$ 0.07 ⁷	2.84 $\pm$ 0.22
	15–21	8	91.8 $\pm$ 9.1	10.0	93	5.0 $\pm$ 0.04 ¹⁰	0.60 $\pm$ 0.08 ⁵	1.19 $\pm$ 0.18 ⁵
	13–21	5	89.4 $\pm$ 7.1	3.0	65	4.9 $\pm$ 0.05 ¹¹	0.58 $\pm$ 0.08 ⁵	2.11 $\pm$ 0.29 ⁷
Mg-supplemented casein (200 mg Mg/100 g)	0–21	4	94.8 $\pm$ 3.4	8.0	46	5.1 $\pm$ 0.05	3.20 $\pm$ 0.22 ⁸	3.28 $\pm$ 0.13

¹ With at least one implantation site.

² Significantly lower than stock control, $P < 0.05$.

³ Malformations included cleft palate, micrognathia, clubbed feet, syndactyly, short and curly tail, and herniation (44% of 25 fetuses dissected were malformed).

⁴ Significantly lower than stock control, $P < 0.001$.

⁵ Significantly lower than purified diet control, $P < 0.001$.

⁶ Significantly lower than stock control, $P < 0.01$.

⁷ Significantly lower than purified diet control, $P < 0.01$.

⁸ Significantly higher than purified diet control, $P < 0.01$.

⁹ Significantly lower than purified diet control, $P < 0.05$.

¹⁰ Significantly lower than purified diet control, $P < 0.02$.

¹¹ Significantly higher than days 0–21, deficient, $P < 0.001$.

Table 2. Effect of maternal magnesium deficiency on hematologic parameters in maternal and fetal blood¹

Experimental group	Days of gestation	Sample	RBC, $\times 10^6$	PCV, %	Hgb, g/dl	RBC Mg, mg/dl	MCV, μ^3	MCH, pg corpuscle	MCHC, g/dl	MCMgC, mg/100 ml	MCMg, mg corpuscle
Maternal											
Stock diet	0-21	5-10		37.9	11.3 $\pm$ 0.2	5.9 $\pm$ 0.6			30	15	
Purified control casein (40 mg Mg/100 g)	0-21	13	5.2 $\pm$ 0.2	36.8	10.4 $\pm$ 0.3	4.3 $\pm$ 0.2 ²	71	20	28 ³	12 ²	8
Mg-deficient											
Casein (2.5 mg Mg/100 g)	0-21	13	4.9 $\pm$ 0.2	36.6	10.7 $\pm$ 0.2	4.2 $\pm$ 0.2 ²	75	21	29	11 ²	8
Casein (5.0 mg Mg/100 g)	0-21	5		37.5	11.7 $\pm$ 0.3	4.5 $\pm$ 0.2			31 ⁴	12	
Mg-supplemented casein (200 mg Mg/100 g)	0-21	4		37.7	11.2 $\pm$ 0.8	5.8 $\pm$ 0.4 ⁴			30	15 ⁴	
Fetal											
Stock diet	0-21	4-14 ⁵	2.2 $\pm$ 0.2	35.8	10.8 $\pm$ 0.4	12.6 $\pm$ 0.4	247	71	28	32	79
Purified control											
Casein (40 mg Mg/100 g)	0-21	5	1.6 $\pm$ 0.1	30.8	8.0 $\pm$ 0.4 ²	12.4 $\pm$ 2.2	193	50	26	42	79
Soybean (40 mg Mg/100 g)	0-21	5	2.2 $\pm$ 0.2	37.0	11.4 $\pm$ 0.6	12.5 $\pm$ 1.3	173	53	31	34	57
Mg-deficient											
Casein (2.5 mg Mg/100 g)	0-21	5	1.0 $\pm$ 0.1 ^{2, 4}	25.4 ³	3.6 $\pm$ 0.4 ^{2, 4}	14.9 $\pm$ 1.4	268 ⁶	38 ⁶	14 ^{2, 4}	63	168
Casein (5.0 mg Mg/100 g)	0-21	4-12	1.6 $\pm$ 0.1 ³	32.8	9.2 $\pm$ 0.5 ³						
Soybean (5.0 mg Mg/100 g)	0-21	6	1.0 $\pm$ 0.1 ⁴	25.0 ⁴	5.0 $\pm$ 0.4 ⁴	15.7 $\pm$ 1.5	262 ⁶	48	19 ⁴	65	176 ⁴
	0-19	6	1.2 $\pm$ 0.1 ⁴	30.0 ⁶	6.0 $\pm$ 0.7 ⁴	15.1 $\pm$ 0.5	261 ⁶	48	19 ⁴	53 ⁶	141 ⁴
	0-17	5	1.4 $\pm$ 0.1 ⁶	34.0	8.0 $\pm$ 0.9 ⁶	10.1 $\pm$ 2.2	246 ⁶	60	26	30	74
	0-15	5	1.7 $\pm$ 0.3	37.0	10.0 $\pm$ 1.1	10.5 $\pm$ 1.0	199	52	27	28	55
	0-13	5	2.1 $\pm$ 0.1	42.0 ⁶	12.0 $\pm$ 0.4	11.6 $\pm$ 0.9	205	56	28	28	56
	15-21	8	2.0 $\pm$ 0.1	37.0	10.0 $\pm$ 0.3	13.2 $\pm$ 2.1	181	49	28	41	70
	13-21	5	1.4 $\pm$ 0.2 ⁶	33.0	6.3 $\pm$ 0.9 ⁴	13.0 $\pm$ 0.9	244 ⁶	46	19 ⁴	41 ⁶	102
Mg-supplemented											
Casein (200 mg Mg/100 g)	0-21	4-8	1.6 $\pm$ 0.1	32.9	10.6 $\pm$ 0.4						

¹ RBC: red blood cells; PCV: packed cell volume; Hgb: hemoglobin concentration; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCMgC: mean corpuscular magnesium concentration:

$$\left(\frac{\text{RBC Mg (mg/100 ml)} \times 100}{\text{PCV}} \right); \text{ MCMg: mean corpuscular magnesium: } \left(\frac{\text{RBC Mg (mg/100 ml)} \times 10}{\text{RBC count (millions)}} \right).$$

² Different from stock control, $P < 0.01$.

³ Different from stock control, $P < 0.05$.

⁴ Different from purified diet control, $P < 0.01$.

⁵ Each sample of fetal blood represented pooled blood from one litter.

⁶ Different from purified diet control, $P < 0.05$.

mg Mg/100 g soybean diet) had larger red cell volumes than did the control groups. When the magnesium-deficient diets were given from day 0 to day 19 or 21 of gestation the mean corpuscular magnesium was increased above normal.

ELECTROPHORESIS

Fetal blood studied electrophoretically on cellulose acetate showed no significant differences between the magnesium-deficient (5 mg Mg/100 g soybean diet) and control (40 mg Mg/100 g soybean diet) litters. All hemolysates, both maternal and fetal, from normal and from magnesium-deficient fetuses exhibited five distinct hemoglobins. In each instance, the electrophoretic pattern of the fetus was identical with that of its mother. There was one notable exception consisting of a normal fetus and its mother who exhibited identical patterns of seven species of hemoglobin (Fig. 1).

Electrophoresis of plasma proteins showed no differences in protein motility between groups although there were some

changes, highly significant, in some of the peak areas from the 10 bands measured. Measurement of total protein in the same samples showed that there was no difference between control and magnesium-deficient maternal blood protein content; however, total plasma protein from magnesium-deficient fetuses was significantly lower in the control (Table 3).

MORPHOLOGIC DATA

In magnesium-deficient fetuses, fetal erythropoiesis was significantly greater in liver, adrenals, and spleen than in the controls (Fig. 2). Maturation was normoblastic in all areas (similar in all groups), and megaloblastic erythropoiesis was conspicuously absent. Although light microscopy showed no difference in maturation, electron microscopy of magnesium-deficient fetal liver did show differences in the stage of maturation present. Red blood cells appeared to stop development at the reticulocyte stage, after nuclear extrusion, with many organelles and membranes remaining in the cells. Many RBC near maturation or

Fig. 1. Tracing of a hemoglobin electrophoretogram (densitometric curve) from control fetal blood. There are seven distinct bands; one band shows two hemoglobin peaks.

Table 3. Plasma protein concentration in maternal and fetal blood

	No.	Total plasma proteins, g/100 ml	
		Maternal	Fetal ¹
Purified control diet, soybean (40 mg Mg/100 g)	8	7.98 ± 0.29	2.62 ± 0.13
Mg-deficient, soybean (5 mg Mg/100 g)	8	7.27 ± 0.27	2.00 ± 0.14 ²

¹ Pooled samples.

² Lower than controls, $P < 0.01$.

matured were bizarre in shape and contained vacuoles or holes. Few of these cell types were found in control fetal liver (Fig. 3).

Peripheral blood smears of all control fetuses showed erythrocytes to be round and spherocytic, varying from 8–10 μ m in diameter with a minimum diameter of 7 μ m. The staining was fairly uniform with only a rare cell exhibiting slight central pallor (Fig. 4). Normoblasts were seen occasionally and the most immature cell present was the late erythroblast.

In the magnesium-deficient fetuses, stained and unstained peripheral blood smears revealed extreme alteration in red cell morphology characterized by abnormalities in size, shape, amount of hemoglobin, membrane conformation, and the number and type of nucleated red cells. There was an obvious macrocytosis associated with numerous microcytes and red cell fragments (Fig. 4b). In any given microscopic field, there were many red cells at least 1.5 times the size of the largest cells seen in the controls. At least 50% of the red cells stained abnormally. Most were severely hypochromic with large, irregular, and sometimes eccentric areas of pallor. A few cells were hyperchromic. Nucleated cells were frequent and consisted mainly of early or midstage erythroblasts. Normoblasts were relatively infrequent. Distortions of red cell shape and the presence of red cell fragments, although seen in stained smears, were more evident by phase microscopy (Fig. 5). Many cells showed bud-like excrescences and there were numerous irregular, small, pointed fragments (schistocytes). Interference microscopy showed that areas of pallor were associated with folding or "sinking in" of the cell membrane.

Scanning electron microscopy of peripheral red blood cells from magnesium-deficient fetuses showed these abnormalities in shape, excessive discocytic forms, blebs or protrusions, and holes (Fig. 6, a and b). Electron microscopy of the same peripheral sample showed the flattened discocytic forms were not due to vacuoles but to absence of hemoglobin in the center of the enlarged, flattened RBC or torocytes (Figs. 3c and 6c).

DISCUSSION

The plasma magnesium levels on day 21 were indicative of the dietary level of magnesium fed before sampling. These results

substantiate earlier work in rats showing rapid and extreme drops in plasma magnesium after maternal magnesium deficiency (11). The fetal plasma magnesium appeared to be most easily affected by changes in dietary magnesium levels. Although both fetal and maternal plasma decreased markedly in the magnesium-deficient, fed groups, when the diet was repleted with magnesium the fetal plasma responded after only 2 days. These results plus the higher fetal plasma magnesium might be explained by studies in the pregnant rabbit and fetus using ²⁸Mg. Initially the concentration of radioactive Mg in the placenta rapidly increased over maternal plasma levels and the turnover of ²⁸Mg was found to be more rapid in the fetal tissues than maternal; the highest uptake was in fetal bone. ²⁸Mg uptake in most tissues was the same in nonpregnant as well as pregnant females, although uptake in bone and muscle was slower in pregnant animals (1). Therefore, a magnesium deficiency state during pregnancy appears to produce reduction of plasma magnesium in both fetus and dam, but the preferentially high turnover in the placenta can conceivably bring the fetal levels back to normal first, presumably at the expense of maternal bone and muscle.

The magnesium-deficient fetuses exhibited malformations, anemia, and edema. The presence of edema, anemia, and an increase in circulating nucleated red cells is suggestive of hydrops fetalis-erythroblastosis in the human. With rare exceptions, this condition is due to an isoagglutinin-induced hemolytic anemia (23). In these rat fetuses, the hemolytic anemia has no immunologic basis and derives from a defect in erythropoiesis producing abnormal erythrocytes with a shortened lifespan. As in the human, the edema may be attributed to hypoxic heart failure, but is abetted in the rats by hypoproteinemia. The significantly increased erythropoiesis in liver, spleen, and marrow bespeak an inadequate attempt to keep pace with increased red cell destruction.

Erythrocytic morphology is consistent with inadequate filling of the cell by hemoglobin as suggested in Cohlan *et al.* (5). The collapse of membrane to produce folds and grooves and "holes" is quite apparent by interference and scanning electron microscopy. Transmission electron microscopy establishes that there are no vacuoles underlying the "holes," merely a circular collapse of membrane. The presence of red cell fragments, red cell holes, and other distortions of red cell shape might suggest microangiopathy with a "fibrin-knife" mechanism (3, 4). The electron microscopic studies and the absence of intravascular fibrin clots in the histologic sections render this explanation untenable.

Reduction in hemoglobin synthesis by magnesium deficiency is consistent with a reduced MCH and MCHC of the deficient fetal red cells. Interestingly, the fetal MCH and MCHC values were higher in all fetal groups compared to maternal values. However, this can be explained by the increased corpuscular size found in all fetal groups. This increase in size may enhance the altered oxygen dissociation curve in fetal cells found to be physiologically more advantageous to the fetus (21). The role of magnesium in protein synthesis (20) is also compatible with a reduction in hemoglobin synthesis. However, inadequate hemoglobin filling may not completely explain the abnormalities and resultant shortened lifespan of the red cells. The importance of magnesium in ATP generation is well known and magnesium is a constituent of the red cell membrane. Weed and Lacille (22) have suggested that ATP depletion results in a sol-gel transformation at the membrane interior interface and that this change is dependent on the levels of ATP, Mg⁺⁺, and Ca⁺⁺. ATP depletion has also been shown to transform erythrocytes from discs to spheres (15). In this connection, it is pertinent that magnesium deficiency in adult rats produced spherocytic anemia (6, 18). It is possible, therefore, that in these magnesium-deficient fetal cells part of the defect is due to membrane abnormality. This may have some relationship to the high RBC magnesium and low plasma magnesium observed in these fetuses. Dissociation of magnesium from its position on the RBC mem-

Fig. 2. *a*: control fetal rat spleen, exhibiting diffuse moderate erythropoiesis. Magnification $\times 40$. *b*: magnesium-deficient fetal rat spleen. Note the increased erythropoiesis. Magnification $\times 40$. *c*: higher magnification of magnesium-deficient fetal rat spleen shows erythropoiesis with normoblastic maturation. Magnification $\times 128$.

Fig. 3. *a*: control fetal rat liver with erythrocytes in varying stages of maturation. Magnification $\times 16,000$. *b*, *c*: erythrocytes from magnesium-deficient fetal liver which show bizarre shapes, abundance of intracellular organelles, and holes or vacuoles. Magnification $\times 16,000$.

Fig. 4. *a*: control fetal rat erythrocytes. Wright stain, magnification $\times 304$. *b*: anemic erythrocytes from magnesium-deficient fetuses showing hyperchromic macrocytes. Hypochromic macrocytes, normocytes, and microcytes are also present. Magnification $\times 304$.

Fig. 5. *a*: Unstained phase photograph of control fetal erythrocytes. Magnification $\times 304$. *b*: magnesium-deficient fetal erythrocytes exhibit schistocytes, microcytes, streaks, and areas of pallor. Magnification $\times 304$.

Fig. 6. *a*: scanning electron microscopic study of control fetal rat erythrocytes. Magnification $\times 2,450$. *b*: magnesium-deficient fetal rat erythrocytes showing an abundance of enlarged flattened cells or torocytes. Magnification $\times 2,450$. *c*: transmission electron microscopic study of a magnesium-deficient erythrocyte from the same fetal sample. Note severe vacuolization and disruption of the outer membrane. Magnification $\times 12,500$.

brane, possibly due to this gradient, could cause structural and metabolic changes.

Observations presented in this paper also suggest that a level of 40 mg magnesium/100 mg diet is not optimal for pregnancy in rats. Females given this level of dietary magnesium showed lower plasma levels of the element and lower weight gain during pregnancy than stock-fed controls.

CONCLUSION

Maternal magnesium deprivation in the rat has been shown to produce fetal death, congenital malformation, and fetal anemia. The anemia is characterized by edema, increased extramedullary hematopoiesis and macrocytosis, poikilocytosis, and erythroblastosis in the circulating blood. There is no evidence of alteration in the chemical structure of the hemoglobin molecule. The anemia is the direct result of red cell malformation. The morphologic features are consistent with the hypothesis that reduction of hemoglobin synthesis and abnormalities in the red cell membrane are the most important factors in the production of the anemia.

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- Ralston Purina Company, St. Louis, Mo.
- Wayne Lab Blox, Ft. Wayne, Ind.
- International microcapillary centrifuge, model MB.
- As outlined in the Klett-Summerson Clinical Manual and read in a Klett colorimeter.
- RCA EMU-3G and AEI-6B electron microscopes.
- Cambridge Mark II Stereoscan.
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