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Evidence for Active Maternofetal Transfer of Magnesium across the *in Situ* Perfused Rat Placenta

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ABSTRACT. Mechanisms of maternofetal Mg transfer have been investigated across the *in situ* perfused rat placenta at 21 d gestation (term = 23 d). The fetal placental circulation was perfused with Mg-free Krebs-Ringer solution and clearance of Mg from maternal plasma across the placenta [unidirectional maternofetal clearance (K_{mf}) Mg] compared with that for ^{45}Ca and ^{51}Cr-EDTA, the latter being used as a diffusional marker. Because diffusion coefficients determined for these solutes were similar (6.8–7.6 × 10^{-6} $cm^2 \cdot sec^{-1}$), greater K_{mf} values determined for Mg and ^{45}Ca (mean ± SD: 26.7 ± 9.2 and 93.1 ± 29.8 $\mu L \cdot min^{-1} \cdot g^{-1}$ placenta, respectively) compared to ^{51}Cr-EDTA (3.2 ± 0.9 $\mu L \cdot min^{-1} \cdot g^{-1}$) suggest that maternofetal transfer of these cations occurs by mechanisms in addition to diffusion. K_{mf} Mg was also greater than K_{mf} ^{51}Cr-EDTA when measured across the dually perfused rat placenta, in which the maternal uterine artery was additionally perfused with Mg-containing (0.5 $mmol \cdot L^{-1}$) Krebs-Ringer solution. Decreasing the Mg concentration in the maternal perfusate by 90% reduced Mg appearance in the fetal perfusate by 87% within 8 min; this suggests that K_{mf} Mg across the *in situ* perfused placenta largely reflects Mg transfer from maternal plasma and not simply elution of a placental Mg pool. Addition of KCN (1 $mmol \cdot L^{-1}$) to the fetal perfusate or lowering perfusate temperature from 37 to 26°C significantly reduced K_{mf} Mg and K_{mf} ^{45}Ca across the *in situ* perfused placenta. In contrast, K_{mf} ^{51}Cr-EDTA was increased by KCN and unaffected by temperature. Both total and ultrafiltrable Mg concentrations were higher in fetal compared with maternal plasma, indicating that maternofetal Mg transfer occurs against a chemical gradient. These data collectively suggest that maternofetal transfer of Mg, as well as that of Ca, is dependent on placental metabolism. (*Pediatr Res* 27: 622–625, 1990)

Abbreviations

D, diffusion coefficient
K_{mf}, unidirectional maternofetal clearance
Pd, potential difference

Mg concentrations are reported to be higher in fetal compared with maternal plasma in rats (1, 2), rabbits (3), sheep (4) and humans (5, 6). Furthermore, studies in rats indicate that this gradient does not reflect greater protein binding in fetal plasma, ultrafiltrable Mg concentrations also being higher in fetal compared with maternal plasma (1). These observations indicate that placental transfer of Mg from mother to fetus must occur against a chemical gradient. Using ^{28}Mg, Care *et al.* (4) determined maternofetal and fetomaternal Mg fluxes of 0.042 and 0.012 $mg \cdot h^{-1} \cdot kg^{-1}$ fetus, respectively, in sheep at 13 d preterm. These fluxes suggest the presence of an active mechanism for the maternofetal transfer of Mg.

In our study, maternal and fetal plasma Mg concentrations (total and ultrafiltrable) were measured in the near term rat and maternofetal Mg transfer was investigated using the *in situ* (umbilically) perfused rat placenta preparation (7). K_{mf} of ^{24}Mg was compared with that of ^{51}Cr-EDTA, used as a diffusional marker (7), and also with that of ^{45}Ca, a divalent cation with which Mg may share transcellular transport systems (8). Previous studies (9) suggest that Ca is actively transferred across the *in situ* perfused rat placenta. The effects of adding KCN to the perfusion fluid or decreasing perfusate temperature were therefore examined for effects on possible active placental transfer of Mg as well as of ^{45}Ca. Experiments were also performed using a dually perfused rat placenta in which the maternal uterine artery was additionally perfused with Krebs Ringer solution with or without Mg (10). These were performed to confirm that Mg transfer (as measured using the *in situ* perfused placenta) reflects maternofetal transfer and not simply elution of a placental Mg pool. A preliminary report of this work has appeared elsewhere (11).

MATERIALS AND METHODS

Plasma Mg determinations. Pregnant Sprague Dawley rats (300–400 g body wt) were anesthetized by intraperitoneal injection of 110 $mg \cdot kg^{-1}$ sodium thiobutabarbital (Inactin, BYK Gulden, Hamburg, FRG) on d 21 of gestation (term = 23 d). After laparotomy, a maternal blood sample (2–3 mL) was withdrawn from the inferior vena cava using a 23-gauge needle. Hysterotomy was then performed and a fetus delivered. Fetal blood (0.3–0.5 mL) was withdrawn by gentle suction into a plastic tube connected to a 24-gauge needle inserted into the umbilical artery (12). This method causes minimal cell damage since maternal blood obtained from the inferior vena cava using this device had a similar plasma Mg concentration to that obtained as above. Heparinized blood samples were centrifuged (2000 rpm for 2 min) and plasma from four to six fetuses pooled. To remove protein bound Mg, samples (300 μL) of plasma were further centrifuged (1000 rpm for 15 min) in ultrafiltrate tubes (Amicon, Gloucester, UK). After appropriate dilution in 5% $LaCl_2$ solution, Mg concentrations were determined by atomic absorption spectrophotometry (Perkin Elmer 2380, Perkin Elmer Corp., Norwalk, CT; linear working range up to 80 $\mu mol \cdot L^{-1}$ Mg; sensitivity 0.3 $\mu mol \cdot L^{-1}$ Mg for 1% absorption). Plasma

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total and ultrafiltrable Mg concentrations were also measured in nonpregnant female Sprague Dawley rats.

Placental perfusion procedure. Pregnant Sprague Dawley rats were anesthetized as above on d 21 of gestation and the trachea, a jugular vein (for tracer administration), and a carotid artery (for monitoring maternal blood pressure and blood sampling) cannulated. The rat was then immobilized on its back in a thermostated (37°C) bath of isotonic saline. After laparotomy and hysterotomy, a fetus was delivered and its umbilical artery and vein cannulated as previously described (7). The placenta was perfused (500 $\mu L \cdot min^{-1}$) with Mg-free Krebs-Ringer solution containing: ($mmol \cdot L^{-1}$) NaCl 118, KCl 4.7, $NaHCO_3$ 24.9, $CaCl_2 \cdot 2\ H_2O$ 1.40, KH_2PO_4 1.18, D-glucose 0.2%, Dextran (40 000 mol wt) 3.5%, pH 7.4 adjusted by continuous gassing with 95% O_2, 5% CO_2. The solution was warmed to 37°C using a heat exchanger prior to perfusion. Perfusion pressure was monitored via a side arm in the arterial catheter. For K_{mf} determination (see below), tracers (10 μCi $^{45}CaCl_2$, 0.071 mg $Ca \cdot mL^{-1}$; 50 μCi ^{51}Cr-EDTA, Amersham International, Buckinghamshire, UK) were injected at time zero and maternal blood samples (0.5 mL) taken at 2, 12, 24, 36, and 44 min. Consecutive 4-min collections of fetal venous effluent were made between 3 and 43 min. Aliquots of plasma (50 μL) and perfusate collections (0.4 mL) were analyzed for ^{45}Ca (Packard Tricarb 2000CA counter, Packard Instrument Co., Inc., Downers Grove, IL) and ^{51}Cr-EDTA (Packard Autogamma 800 counter). Since the Mg isotope (Mg^{28}) was not available, K_{mf} Mg was determined by measuring ^{24}Mg in plasma samples and that transferred to the Mg free perfusate by atomic absorption spectrophotometry. Plasma samples were diluted 100-fold and perfusate samples 5-fold with 0.5% $LaCl_2$ solution to obtain Mg in the assay range. Background concentrations of Mg in the perfusion fluid was always <10% that in perfusate effluent. In some experiments, the effect of changing to a KCN-containing (1 $mmol \cdot L^{-1}$) perfusate or rapidly cooling perfusate temperature from 37°C to 26°C (by addition of cold water to the heat exchanger) on K_{mf} values was investigated. The criteria for a successfully perfused placenta were as previously described (7).

K_{mf} for Mg and ^{51}Cr-EDTA were also determined across rat placentas in which the maternal uterine artery as well as the umbilical circulation was perfused (dual perfusion). The uterine artery was catheterized (24-gauge) and perfused at 1.8 $mL \cdot min^{-1}$ with Mg-containing (0.5 $mmol \cdot L^{-1}$) Krebs-Ringer solution (other constituents as above). Maternal blood vessels to placentas other than that being perfused were ligated; maternal effluent was allowed to drain from an incision in the uterine vein (10).

Diffusion coefficients. These were determined for Mg, ^{45}Ca, and ^{51}Cr-EDTA using the method of Berhe *et al.* (13), in which 2-mL syringes with injection ends cut off were filled with a 1% agar solution containing 150 $mmol \cdot L^{-1}$ NaCl, 40 $mmol \cdot L^{-1}$ $CaCl_2$ and placed in a stirred medium of 150 $mmol \cdot L^{-1}$ NaCl, 40 $mmol \cdot L^{-1}$ $MgCl_2$ with 0.2 $\mu Ci\ mL^{-1}$ $^{45}CaCl_2$ (0.071 mg $Ca \cdot mL^{-1}$), 0.4 $\mu Ci \cdot mL^{-1}$ ^{51}Cr-EDTA and maintained at 37°C for 4 hr. The cylinder of gel was then expelled and cut with a razor blade into 1-mm sections that were each allowed to equilibrate in 2.5 mL of a 0.5% $LaCl_2$ solution. The concentration of ^{45}Ca and ^{51}Cr-EDTA ($cpm \cdot mL^{-1}$), and of Mg ($mmol \cdot L^{-1}$) in each slice was then determined (as above) and diffusion coefficients estimated by nonlinear regression as previously described (13).

Calculations and statistics. K_{mf} for Mg, ^{45}Ca, and ^{51}Cr-EDTA were calculated using the equation

$$K_{mf} = \frac{[v]Q}{[A]wt}\ \mu L \cdot min^{-1} \cdot g^{-1}$$

where [v] is the isotope or Mg concentration in the umbilical vein effluent (background perfusate Mg subtracted), [A] is the corresponding concentration in maternal plasma (estimated for the midpoint of each collection period) or uterine arterial perfusate (in the dually perfused preparation), Q is the fetal perfusate flow rate, and wt is the placental wet wt.

Data are shown as mean ± SEM or ± SD where appropriate and statistical comparisons have been made using *t* tests, paired or unpaired as appropriate.

RESULTS

As shown in Table 1, total and ultrafiltrable Mg concentrations were both lower in maternal compared with fetal rat plasma at 21 d gestation. Maternal plasma Mg concentrations were also lower than those in the nonpregnant female. However, in each case, 64–65% of plasma total Mg was ultrafiltrable.

K_{mf} (across the *in situ* perfused placenta in rats maintained at 37°C) and D values for Mg, ^{45}Ca, and ^{51}Cr-EDTA are shown in Table 2. K_{mf} for the first three effluent periods were averaged; there was little change in K_{mf} values for any solute with time up to 15 min (Figs. 1 and 2). Mean ± SD placental and fetal wt were 0.50 ± 0.05 g and 4.12 ± 0.55 g, respectively ($n = 21$). D for these solutes were of a similar order of magnitude, whereas K_{mf} for Mg and ^{45}Ca were markedly greater than for the diffusional marker ^{51}Cr-EDTA. This is reflected by higher K_{mf}/D ratios for Mg and ^{45}Ca compared with ^{51}Cr-EDTA.

The effects of adding KCN to the fetal perfusate (1 $mmol \cdot L^{-1}$ final concentration) or lowering perfusate temperature from 37 ± 1°C to 26 ± 1°C on K_{mf} for Mg, ^{45}Ca, and ^{51}Cr-EDTA are shown in Figures 1 and 2, respectively. Data are expressed as a percentage of the first collection period. In all cases, K_{mf} for Mg, ^{45}Ca, and ^{51}Cr-EDTA during this period were similar to those in Table 2 and K_{mf} values did not significantly differ between control and treatment groups at this time. K_{mf} for Mg and ^{45}Ca were each significantly reduced within 15 min of KCN addition compared with controls. In contrast, K_{mf} ^{51}Cr-EDTA was increased by this treatment (Fig. 1). Decreasing perfusate temperature also reduced K_{mf} for Mg and ^{45}Ca compared with controls, whereas K_{mf} ^{51}Cr-EDTA was not significantly affected (Fig. 2).

K_{mf} Mg and K_{mf} ^{51}Cr-EDTA measured using the dually perfused rat placenta ($n = 4$) were 52.7 ± 18.4 (mean ± SD) and 9.7 ± 10.0 $\mu L \cdot min^{-1} \cdot g^{-1}$ placenta, respectively; these values are greater than those determined using the *in situ* perfused preparation (Table 2). Reducing Mg concentration in the uterine arterial perfusate by 90% was paralleled by an 86% decrease in Mg concentration in the fetal perfusate effluent within 8 min

Table 1. *Plasma Mg concentrations ($mmol \cdot L^{-1}$) in rats**

	Total	Ultrafiltrable
Nonpregnant female	0.79 ± 0.04†	0.51 ± 0.03† (65%)
Maternal	0.55 ± 0.02	0.36 ± 0.02 (65%)
Fetal	0.84 ± 0.01‡	0.54 ± 0.04† (64%)

* Data are mean ± SEM. Percent ultrafiltrable Mg are in parentheses ($n = 8$/group).

† Significance compared to maternal concentrations, $p < 0.01$ (*t* test, two-tailed).

‡ Significance compared to maternal concentrations, $p < 0.001$ (*t* test, two-tailed).

Table 2. *K_{mf} (across in situ perfused rat placenta), D, and K_{mf}/D values for Mg, ^{45}Ca, and ^{51}Cr-EDTA**

	K_{vf} ($n = 21$) ($\mu L \cdot min^{-1} \cdot g^{-1}$)	D ($n = 6$) ($cm^2 \cdot s^{-1} \times 10^6$)	K_{mf}/D ($cm \cdot g^{-1}$)
Mg	26.7 ± 9.2	6.9 ± 0.7	64.8
^{45}Ca	93.1 ± 29.8	7.6 ± 0.5	204.2
^{51}Cr-EDTA	3.2 ± 0.9	6.8 ± 0.7	7.8

* Data are shown as mean ± SD.

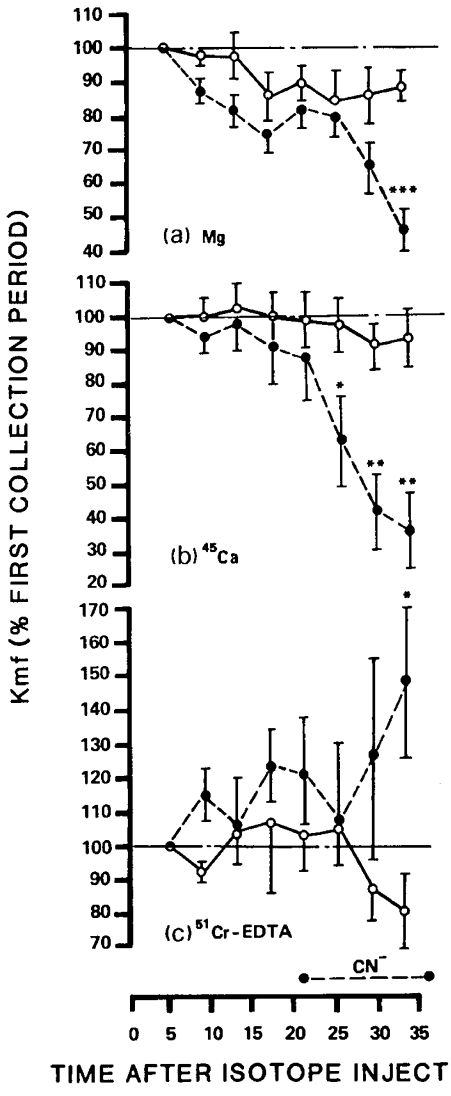

Fig. 1. Effects of adding KCN to fetal perfusate (1 mmol/L final concentration) on K_{mf} for (*a*) Mg, (*b*) ^{45}Ca, and (*c*) ^{51}Cr-EDTA. Mean K_{mf} values ± SEM are normalized to that for the first collection period (n = 6/group). Statistical significance between treated (●) and control (○) groups: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (t test, two-tailed).

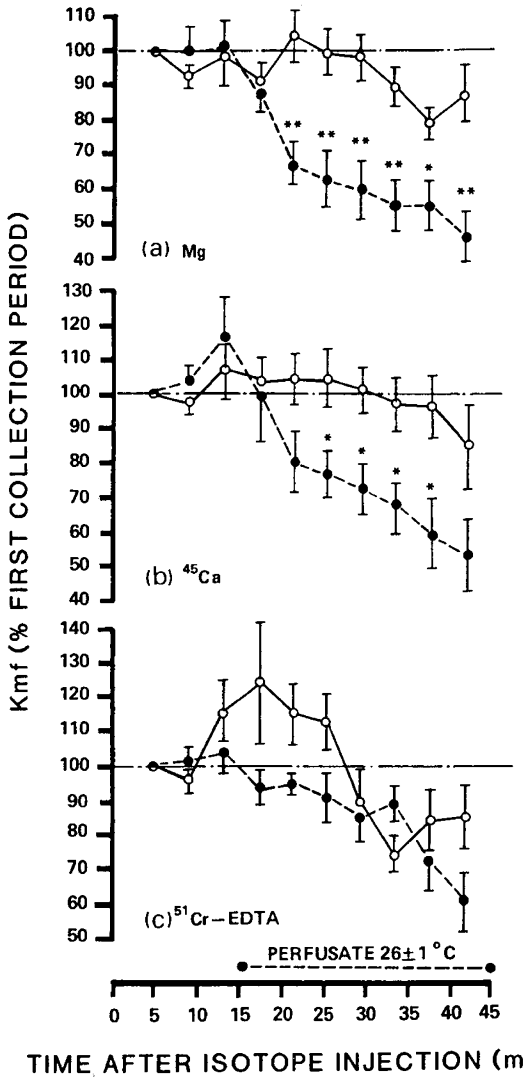

Fig. 2. Effects of lowering perfusate temperature from 37 ± 1°C to 26 ± 1°C on K_{mf} for (*a*) Mg, (*b*) ^{45}Ca, and (*c*) ^{51}Cr-EDTA. Mean K_{mf} values ± SEM are normalized to that for the first collection period (n = 6/group). Statistical significance between treated (●) and control (○) groups: *, $p < 0.05$; **, $p < 0.01$ (t test, two-tailed).

(Fig. 3). In contrast, K_{mf} ^{51}Cr-EDTA did not significantly change during the time course of this experiment (data not shown).

DISCUSSION

In our study, both total and ultrafiltrable plasma Mg concentrations were lower in pregnant compared with nonpregnant rats (Table 1). Lowered plasma Mg concentrations have been reported previously in pregnant rats (1) and rabbits (3), the latter study suggesting that this may partly reflect maternofetal Mg transfer. Studies in humans, however, suggest that a decrease in maternal plasma Mg concentration during pregnancy is related to a dilutional process inasmuch as plasma protein and Mg concentrations were correlated (14). In agreement with previous studies (1, 2), both total and ultrafiltrable Mg concentrations in maternal plasma were also lower than in fetal plasma (Table 1). This transplacental gradient, in the absence of a transplacental Pd, favors an active mechanism for the maternofetal transfer of Mg.

K_{mf} values for ^{45}Ca and ^{51}Cr-EDTA measured across the *in situ* rat placenta perfused using Mg-free fetal Ringer solution (Table 2) are comparable to those previously described in which Mg was present (7, 9, 15). This suggests that removal of Mg from the fetal perfusate does not increase paracellular permeability or affect placental Ca transfer, which is known to have an active component (9). ^{51}Cr-EDTA was used as a paracellular diffusional marker (7) and, in the absence of flow limitation, K_{mf} for such solutes across the rat placenta is a direct function of D (16). The higher K_{mf}/D ratios found for Mg and ^{45}Ca compared with that for ^{51}Cr-EDTA (Table 2) therefore suggest that transfer mechanisms are available for these cations in addition to paracellular diffusion. It should be noted that K_{mf} values (and consequently K_{mf}/D ratios) for Mg and ^{45}Ca would have been even higher had they been calculated on the basis of the free maternal plasma electrolyte. Thus, 35% of maternal plasma Mg is nonfiltrable (Table 1) and this fraction, which is probably protein bound, may not be available for placental transfer.

K_{mf} Mg across the dually perfused rat placenta was twice that obtained across the *in situ* perfused preparation. This could partly reflect the absence of protein-bound Mg in the Krebs-Ringer solution used to perfuse the uterine artery in the dually perfused preparation, whereas in the *in situ* perfused preparation, 35% of the maternal plasma Mg concentration (used in the calculation of K_{mf}) is not filtrable and consequently may not be exchangable. However, in agreement with Mohammed *et al.* (10), K_{mf} Cr-EDTA was also greater in the dually perfused compared with the

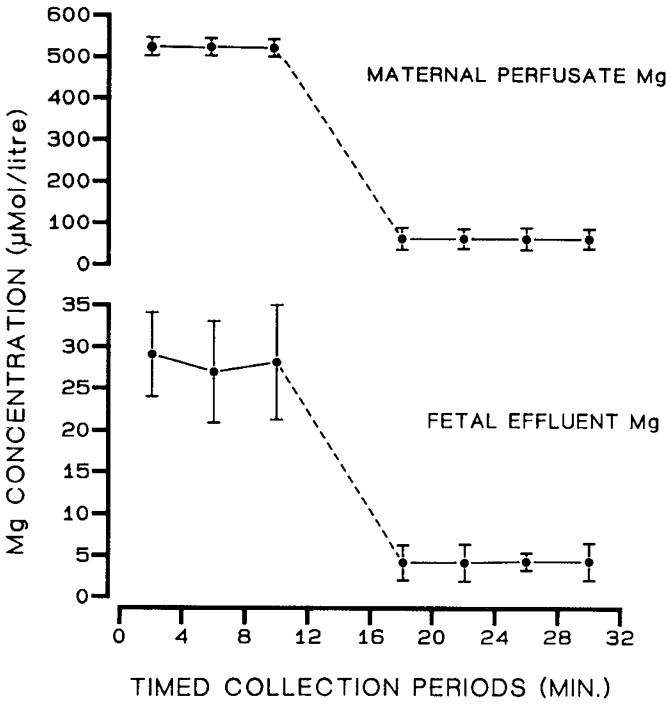

Fig. 3. Effects of reducing maternal perfusate Mg concentration on that in the fetal perfusate effluent in the dually perfused rat placental preparation. Data are shown as mean ± SEM ($n = 4$).

in situ perfused placenta, probably reflecting a greater permeability in the former preparation. In our study, a 90% decrease in the maternal perfusate Mg concentration was rapidly followed by a similar decrease in Mg concentration in the fetal perfusate effluent (Fig. 3). This suggests that K_{mf} Mg determined across the *in situ* perfused preparation largely reflects Mg transfer from maternal plasma and not simply elution of a placental Mg pool.

The finding that K_{mf} Mg and K_{mf} ^{45}Ca are both reduced after adding KCN to the fetal perfusate (Fig. 1) or lowering perfusate temperature (Fig. 2) suggests that the maternofetal transfer of these cations is at least partly dependent on placental metabolism. It is unlikely that these data reflect a decrease in placental permeability since K_{mf} ^{51}Cr-EDTA was significantly increased by KCN and unaffected by perfusate temperature (Figs. 1 and 2). The effect of KCN on K_{mf} ^{45}Ca is consistent with that of NaCN and the metabolic inhibitor dinitrophenol previously described (9). These data could reflect direct effects on transcellular transport mechanisms for Mg and Ca or, alternatively, an indirect effect on a placental Pd. The magnitude and polarity of any transplacental Pd is not known although the maternofetal Pd between catheters placed in maternal and fetal extracellular space at sites removed from the placental exchange area has been reported to be 17 mV fetus positive (17). The relationship between this and any transplacental Pd is not certain (16, 18), but a similar Pd with this polarity across the placenta could not drive maternofetal cation transfer. This suggests that the effects of the metabolic inhibitors resulted from direct actions on transport systems for Mg and Ca.

Much evidence now exists for ATP-dependent maternofetal Ca transport (16, 19), and it is possible that Mg may compete for this system (8). However, studies by Mimouni *et al.* (20) suggest that maternofetal Ca transfer in the rat is unaffected by increasing maternal plasma Mg concentrations. Gunther *et al.* (21) reported that injection of amiloride or furosemide into pregnant rats at 19 d gestation reduced maternofetal transfer of an acute ^{28}Mg label within five h. From these data, the authors suggested the presence of a placental Na/Mg antiport and Mg/HCO_3 symport, respectively. This clearly needs further investigation.

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