The Cellular Transport of Magnesium in Rat Liver

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ABSTRACT   The bidirectional transport of Mg in rat liver was studied using slices labeled with ^{28}Mg in a closed two-compartment system under steady-state conditions. The influx (K_{bs}) and efflux (K_{sb}) transfer coefficients governing transport between the extracellular phase and a rapidly exchanging cell fraction were 0.074 and 0.019 per min, respectively. An increased extracellular concentration of Mg^{++} caused a 30% decrease in K_{bs} and a 31% increase in K_{sb}. A decreased extracellular Mg^{+++} had an opposite effect. At 0°C, both transfer coefficients were reduced by 65%. Increased pH and NaCN increased transport, whereas Ca^{++} reduced transport. Reduced pH, altered Na^{+}:K^{+} ratio, Sr^{++}, glucose deletion, iodoacetate, ethanol, and lactate had no significant influence. Dinitrophenol reduced K_{sb} but had no effect on K_{bs}. These data support the thesis that the intracellular concentration of Mg is in part regulated by a reciprocal change in the influx transfer coefficient and a parallel change in the efflux transfer coefficient in response to altered extracellular concentrations of Mg^{++}. The qualitative and quantitative similarities of Mg and Ca transport in this system suggest that Mg and Ca share a common transport mechanism which is primarily dependent upon the binding of these divalent cations to macromolecular ligands within the cell membrane or within the cell.

The importance of Mg in the regulation of a variety of cellular processes including membrane permeability, intracellular enzyme activity, mitochondrial function, and protein synthesis has become increasingly evident in recent years. However, little information is available regarding the factors which control the permeability of cell membranes to Mg, the concentration and physical state of intracellular Mg, and the quantitative relationships between intracellular Mg and regulatory activity. Compartmental analyses of radiomagnesium (^{28}Mg) turnover studies in human beings have suggested that three or more kinetically distinct types of Mg exist in tissues (24). In vivo studies in experimental animals and human beings have provided evidence that the cellular transport of Mg may be influenced by the extracellular concentration of Mg, parathyroid hormone, thyroid hormone, and vitamin D (8, 9, 14, 21, 20, 24). The present work was undertaken to devise a cellular

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system for the study of the bidirectional transport of Mg which would be suitable for in vitro investigations of the effects of physical, chemical, and metabolic factors. The rat liver slice was chosen for this work because of its content of homogeneous, metabolically active cells whose Mg readily exchanges with administered ^{28}Mg in vivo (20). This report is concerned with the characteristics of this system and the influence of the physical and chemical milieu on Mg transport.

METHODS

Mature male Sprague-Dawley rats (C-D strain, Charles River Laboratories, North Wilmington, Mass.), 4–8 months old and weighing 350–500 g were used for all studies. The rats were decapitated and their livers were sliced freehand to a thickness of 0.3 mm with a Stadie-Riggs blade. In preliminary studies designed to define the characteristics of uptake of ^{28}Mg by liver slices, 1.0 or 2.5 g samples of slices were incubated in a 10-fold greater volume of a buffered pH 7.4 medium containing 148 mM Na$^+$, 5.0 mM K$^+$, 2.5 mM Mg^{++}, 128 mM Cl$^-$, 25 mM HCO$_3^-$ 11.0 mM glucose, and 2–5 μCi of ^{28}Mg (Brookhaven National Laboratories). Incubation was carried out for 2.5–180 min at 37°C in a Dubnoff metabolic shaker at 105 oscillations per min with a gas phase of 95% O$_2$, 5% CO$_2$. At the end of incubation, the slices were decanted and blotted. The ^{28}Mg content and the Mg concentrations of the postincubation slices and media were determined.

In order to determine the bidirectional transport of Mg, the following standard procedure was employed. 7.5 g of fresh liver slices was placed in 50 ml of a buffered pH 7.4 medium identical with that described above, except that 10–15 μCi of ^{28}Mg was added. The slices were incubated at 37°C in a Dubnoff metabolic shaker at 105 oscillations per min with a gas phase of 95% O$_2$, 5% CO$_2$. After 30 min, the labeled slices were removed and washed twice for 5 min periods in an identical nonradioactive medium. Samples of 2.5 g of washed, blotted slices were then reincubated in 25 ml of nonradioactive medium for 180 min. For studies at pH 6.8, the gas phase was 80% O$_2$, 20% CO$_2$ and for studies at pH 8.6, the gas phase was 100% O$_2$. Periodically, 0.25 ml samples of medium were removed for ^{28}Mg analyses. At the end of incubation, the slices were removed and blotted. The water, Mg, and ^{28}Mg concentrations of the pre- and postincubation slices were determined. Mg analyses and pH measurements were performed on pre- and postincubation samples of the medium.

Radioactivity measurements were made at constant geometry in a scintillation spectrometer calibrated to detect gamma rays between 0.1 and 1.6 Mev, thus eliminating emissions from the ^{28}Al daughter of ^{28}Mg. After counting, the liver samples were dried at 105°C for 48 hr and their water content determined by differential weight. The dried slices and postincubation media were prepared for Mg analysis by homogenization in 10% trichloroacetic acid. Traces of Ca present in the trichloroacetic acid extracts were first precipitated as the oxalate salt, and the Mg content of the supernates was determined by complexometric titration with EDTA (20, 22).

Calculations for Bidirectional Transport The slices were assumed to contain interstitial and cellular Mg fractions. After equilibration of the slice interstitium with the

medium, Mg exchange between the cellular and extracellular phases could be expressed by a differential equation describing a closed two-compartment system,

$$\frac{d\,(\mathrm{Mg})_s}{dt} = -K_{sb}\,(\mathrm{Mg})_s + K_{bs}\,(\mathrm{Mg})_b \qquad (1)$$

where $(\mathrm{Mg})_s$ = radiomagnesium concentration of slices (cpm/g)

$(\mathrm{Mg})_b$ = radiomagnesium concentration of medium (cpm/ml)

K_{sb} = efflux transfer coefficient

K_{bs} = influx transfer coefficient

The solution of this differential equation has been outlined in a previous publication describing studies of Ca transport using a similar experimental technique (23). The efflux and influx transfer coefficients are given by the following equations:

$$K_{sb} = \frac{S\,(\mathrm{Mg})_{b\infty}\left(\dfrac{v}{m}\right)}{(\mathrm{Mg})_{so}} \qquad (2)$$

$$K_{bs} = [S - K_{sb}]\left(\frac{v}{m}\right) \qquad (3)$$

where $(\mathrm{Mg})_{b\infty}$ = the radiomagnesium concentration of the medium at isotopic equilibrium

$(\mathrm{Mg})_{so}$ = the radiomagnesium concentration of the slices at the beginning of incubation

S = the slope of the straight line obtained when experimental values of

$1 - \dfrac{(\mathrm{Mg})_b}{(\mathrm{Mg})_{b\infty}}$ are plotted semilogarithmically against time

v = the volume of the medium (25 ml)

m = the mass of the liver slices (2.5 g)

In each experiment, a semilogarithmic plot of $1 - \dfrac{(\mathrm{Mg})_b}{(\mathrm{Mg})_{b\infty}}$ against time yielded a linear relation between approximately 10 min and 90% of equilibrium, validating the assumption of a closed two-compartment system. Between 0 and 10 min, a curve of greater slope was obtained which was assumed to represent equilibration of $^{28}\mathrm{Mg}$ initially in the slice interstitium with the medium. In order to eliminate interstitium-medium equilibration from consideration, the following equation was substituted for equation 2

$$K_{sb} = \frac{S[(\mathrm{Mg})_{b\infty} - (\mathrm{Mg})_{b_o}]\left(\dfrac{v}{m}\right)}{(\mathrm{Mg})_{so} - (\mathrm{Mg})_{b_o}\left(\dfrac{v}{m}\right)} \qquad (4)$$

where $(\mathrm{Mg})_{b_o}$ = the apparent extracellular concentration of $^{28}\mathrm{Mg}$ at time zero. This value was determined by extrapolation of the linear portion of the curve to zero time

and the use of the intercept value of $1-\dfrac{(\text{Mg})_b}{(\text{Mg})_{b\infty}}$ to calculate $(\text{Mg})_{b_o}$. A digital computer

program was devised to yield a least squares fit of values of $1-\dfrac{(\text{Mg})_b}{(\text{Mg})_{b\infty}}$ against time

between 10 min and 90% of equilibrium. The slope and intercept of this line were then utilized to calculate K_{sb} and K_{bs}, employing equations 3 and 4. For the purpose

of illustration, a semilogarithmic plot of $1-\dfrac{(\text{Mg})_b}{(\text{Mg})_{b\infty}}$ against time for a representative

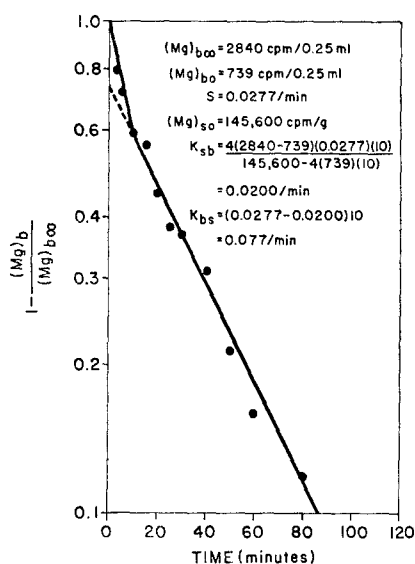study and the method of calculation are shown in Fig. 1.

FIGURE 1.   Semilogarithmic
plot of $1 - \dfrac{(\text{Mg})_b}{(\text{Mg})_{b\infty}}$ vs. time for a
representative study at 37°C.
The method of calculation of
K_{sb} and K_{bs} is shown.

RESULTS

In order to define the initial rate of accumulation of [28]Mg by liver slices, several 1 g samples of slices from the same liver were incubated for 2.5-25 min. Fig. 2 shows the mean data for four such experiments. The [28]Mg contents of the slices were corrected for interstitial [28]Mg, assuming an extracellular space of 22% of slice weight (25). The cellular uptake of [28]Mg was linear for 10 min and then proceeded at a slower rate between 10 and 25 min. The rate of accumulation of [28]Mg between 0 and 2.5 min was slower than between 2.5 and 10 min, presumably because of a short lag period required for complete equilibration between the medium and the interstitium of the slices. The exchangeability of cellular Mg during a longer period of incubation is shown in Fig. 3. In four separate experiments, 2.5 g samples of slices from the same liver were incubated for 30, 60, and 180 min. At 30 min, 20% of the cellular Mg had exchanged with [28]Mg and at 180 min, exchange had increased to 55%.

In the studies of bidirectional transport, the concentration of Mg of individual samples of washed, labeled slices ranged from 5.35 to 11.0 mmoles/kg wet weight. Mean Mg concentrations of the various experimental groups ranged from 6.40 ± 0.34 (SEM) to 9.70 ± 0.40 mmoles/kg wet weight. The 2.5 g samples of washed slices initially consisted, on the average, of 0.52–0.58 g solids and 1.92–1.98 ml water. During subsequent incubation, mean losses of 0.10–0.17 g solids occurred. The mean loss of water by the slice samples ranged
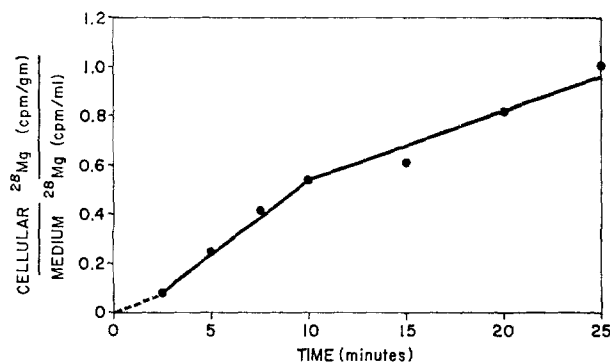

FIGURE 2.   Uptake of ^{28}Mg by rat liver slices during a 25 min period. Accumulation of ^{28}Mg occurred by a biphasic process.
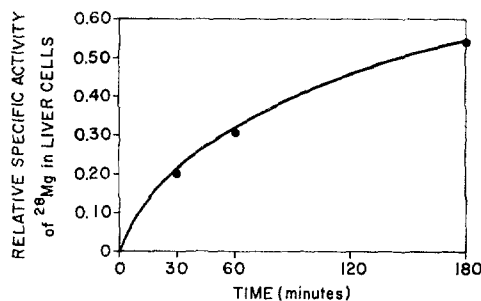

FIGURE 3.   Uptake of ^{28}Mg by rat liver slices during a 180 min period. 20% of cellular Mg exchanged with ^{28}Mg in 30 min, and 55% in 180 min.

from 0.09–0.38 ml except when the medium contained K^+ as the only univalent cation, or the metabolic inhibitors, iodoacetate, cyanide, and dinitrophenol, were used. Under these four conditions, the change in the water content of the slice samples ranged from −0.04 to +0.10 ml. At the end of incubation in nonradioactive media, and when the concentration of ^{28}Mg in the media had reached a constant value, the mean specific activity of the slices exceeded that of the media by 10–48% in the control and in all but three of the experimental conditions studied. When the concentration of Mg^{++} in the medium was reduced to 0.5 mM, the mean specific activity of the slices at the end of incubation was 96% of the specific activity of the medium. When the concentration of Mg^{++} in the medium was increased to 25 mM, the mean specific activity of

the slices at the end of incubation exceeded that of the medium by 71%. The addition of 22.5 mM Ca^{++} to the medium resulted in a mean specific activity of the slices which exceeded that of the medium by 110%. The effects of the various experimental conditions studied on the influx and efflux transfer coefficients and on the concentrations of Mg of the slices are shown in Tables I, II, and IV.

Effect of Mg Concentration (Table I)   At 37°C, pH 7.4, and a concentration of Mg^{++} in the medium of 2.5 mM, the mean influx and efflux transfer

TABLE I

EFFECT OF Mg CONCENTRATION, pH AND
TEMPERATURE ON BIDIRECTIONAL MAGNESIUM
TRANSPORT IN RAT LIVER SLICES

	No. of experiments	K_{bs}	K_{sb}	Initial slice magnesium	Change in slice magnesium
		per min	*per min*	*mmoles/kg*	*mmoles/kg*
2.5 mM Mg^{++}, pH 7.4, 37°C	22	0.074 ± 0.003	0.019 ± 0.001	7.75 ± 0.34	−0.33 ± 0.20
25 mM Mg^{++}, pH 7.4, 37°C	6	0.052 ± 0.002 ($p < 0.001$)	0.025 ± 0.001 ($p < 0.001$)	6.50 ± 0.22	+19.8 ± 0.85 ($p < 0.001$)
0.5 mM Mg^{++}, pH 7.4, 37°C	7	0.098 ± 0.004 ($p < 0.001$)	0.016 ± 0.001 ($p < 0.05$)	6.55 ± 0.27	−3.76 ± 0.29 ($p < 0.001$)
2.5 mM Mg^{++}, pH 8.6, 37°C	6	0.111 ± 0.004 ($p < 0.001$)	0.030 ± 0.003 ($p < 0.001$)	7.35 ± 0.38	−0.78 ± 0.38
2.5 mM Mg^{++}, pH 6.8, 37°C	7	0.073 ± 0.005	0.019 ± 0.001	7.35 ± 0.38	−0.97 ± 0.32
2.5 mM Mg^{++}, pH 7.4, 0°C	8	0.029 ± 0.003 ($p < 0.001$)	0.0067 ± 0.0006 ($p < 0.001$)	7.50 ± 0.45	−0.55 ± 0.47*

* After 60 min at 0°C and 160 min at 37°C

coefficients (K_{bs} and K_{sb}) were 0.074 and 0.019 per min, respectively, and the Mg concentration of the liver slices was maintained at approximately 7.7 mmoles/kg, a level within the normal range for the Mg concentration of mammalian liver (20). A 10-fold increase in the concentration of Mg^{++} in the medium to 25 mM caused a 30% decrease in the influx transfer coefficient ($p < 0.001$) and a 31% increase in the efflux transfer coefficient ($p < 0.001$). A fivefold decrease in the concentration of Mg^{++} in the medium to 0.5 mM resulted in a 33% increase in the influx coefficient ($p < 0.001$) and a 16% decrease in the efflux transfer coefficient ($p = 0.05$). The alterations in the concentration of Mg^{++} in the medium were of a greater magnitude than the resultant changes in the transfer coefficients, and the mean Mg concentration

of the slices increased by 19.8 mmoles/kg in 25 mM Mg^{++}, and decreased by 3.8 mmoles/kg in 0.5 mM Mg^{++}.

Effect of pH (*Table I*)   An increase in the pH of the medium to 8.6 resulted in increases in the influx and efflux transfer coefficients of 50 and 58%, respectively ($p < 0.001$), but had no effect on the concentration of Mg in the liver slices. A decrease in pH to 6.8 had no influence on either transfer coefficient or the concentration of Mg in the slices.
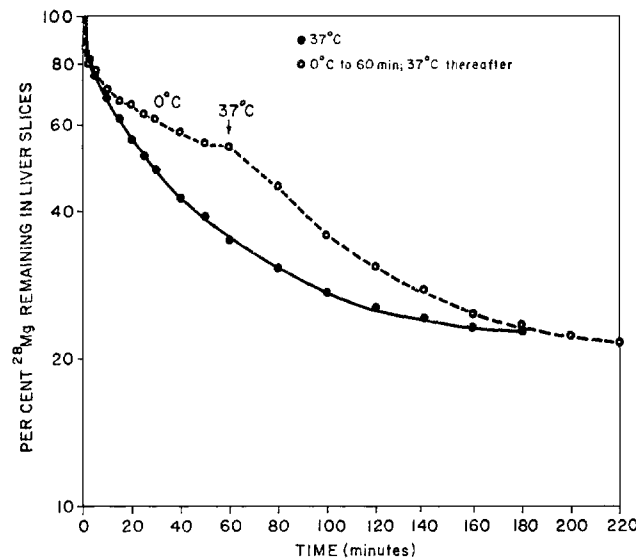

FIGURE 4.   Comparison of the rates of release of ^{28}Mg from rat liver slices at 37 and 0°C.

Effect of Reduced Temperature (*Table I*)   The rate of release of ^{28}Mg from the liver slices was slow at 0°C compared to 37°C (Fig. 4). Since an inordinately long period of time would therefore be required to achieve isotopic equilibrium at 0°C, a modification in procedure was introduced for studies of Mg transport at 0°C. After 60 min of incubation in the nonradioactive medium at 0°C, the temperature was increased to 37°C and the total period of incubation extended to 220 min. The transfer coefficients were determined from the slope of log $[1-(Mg)_b/(Mg)_{b\infty}]$ vs. time between 10 and 60 min, and the value of b_∞ at 37°C (Fig. 5). Because of the possibility that Mg transport might be qualitatively as well as quantitatively different at reduced temperature, it was necessary to validate this modification in procedure. Validation was accomplished by comparing values of K_{sb} obtained by the method shown in Fig. 5 with those obtained by determining the rate of decrease of ^{28}Mg in the slices between 10 and 40 min. It was assumed that the latter would approximate the efflux transfer coefficient since the accumulation of ^{28}Mg in the medium and

the uptake of released [28]Mg by the slices at 0°C would both be small during a short time period. The mean fractional rate of decrease of [28]Mg in the slices of 0.0066 ± 0.0005 (sem) per min agreed with the mean value for K_{sb} at 0°C of 0.0067 ± 0.0006 and indicated that despite the radically different conditions which existed at 0°C, Mg transport was quantitatively but not qualitatively altered. As shown in Table I, a reduction in temperature to 0°C caused mean decreases in the influx and efflux transfer coefficients of 61 and 65%, respectively ($p < 0.001$).
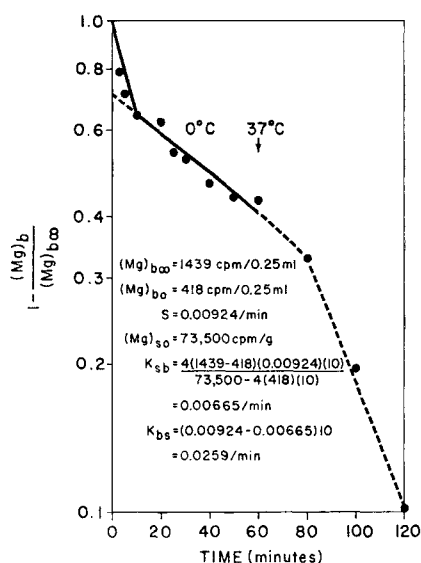

$(Mg)_{b\infty} = 1439 \text{ cpm}/0.25 \text{ ml}$

$(Mg)_{bo} = 418 \text{ cpm}/0.25 \text{ ml}$

$S = 0.00924/\text{min}$

$(Mg)_{so} = 73,500 \text{ cpm/g}$

$K_{sb} = \dfrac{4(1439-418)(0.00924)(10)}{73,500 - 4(418)(10)}$

$= 0.00665/\text{min}$

$K_{bs} = (0.00924 - 0.00665)10$

$= 0.0259/\text{min}$

FIGURE 5.   Semilogarithmic plot of $1 - \dfrac{(Mg)_b}{(Mg)_{b\infty}}$ vs. time for a representative study at 0°C. The incubation temperature was increased to 37°C after 60 min. The method of calculation of K_{sb} and K_{bs} is shown.

Effect of Altered Cation Concentrations (*Table II*)   The addition of 22.5 mM $CaCl_2$ to the medium significantly decreased the influx and efflux transfer coefficients for Mg by 18 and 16%, respectively ($p < 0.02$, $p < 0.05$), and decreased the concentration of Mg in the slices by 28% ($p < 0.01$). In contrast, the addition of a similar amount of $SrCl_2$ had no effect on Mg transport. It was not possible to make accurate measurements of the Mg concentration of the media or slices during $SrCl_2$ addition because of difficulties in precipitating all the added Sr^{++} as the oxalate salt prior to complexometric titration.

Confirmation of the inhibitory effect of Ca^{++} on the transport of Mg was obtained in the collateral studies shown in Table III in which the influence of added Ca^{++} on the concentrations of slice Mg was studied in media containing Mg^{++} at concentrations both below and above that used for the [28]Mg transport studies. Regardless of the concentration of Mg^{++} in the medium or the slice concentration of Mg, the addition of Ca^{++} resulted in a 15–20% decrease in the concentration of Mg in the slices ($p < 0.02$, $p < 0.001$).

Graded replacement of the Na^+ of the medium with K^+ caused a small but

progressive decrease in the influx transfer coefficient for Mg which just missed significance at the 5% level when total replacement with K^+ was produced (Table II). The addition of K^+ had no consistent effect on the efflux transfer coefficient or the concentration of Mg in the slices.

TABLE II

EFFECT OF ALTERED CATION CONCENTRATIONS
ON BIDIRECTIONAL Mg TRANSPORT IN RAT LIVER
SLICES (2.5 mM Mg^{++}, pH 7.4, 37°C)

	No. of experiments	K_{bs}	K_{sb}	Initial slice magnesium	Change in slice magnesium
		per min	per min	mmoles/kg	mmoles/kg
Control	22	0.074 ± 0.003	0.019 ± 0.001	7.75 ± 0.34	−0.33 ± 0.20
CaCl₂, 22.5 mM	7	0.061 ± 0.004 ($p < 0.02$)	0.016 ± 0.001 ($p < 0.05$)	6.40 ± 0.34	−1.80 ± 0.36 ($p < 0.01$)
SrCl₂, 22.5 mM	8	0.078 ± 0.004	0.019 ± 0.002	8.00 ± 0.75	—
Na^+, 74 mM; K^+, 74 mM	6	0.070 ± 0.006	0.020 ± 0.002	8.50 ± 0.60	−1.00 ± 0.35
K^+, 148 mM	6	0.064 ± 0.004	0.017 ± 0.001	8.25 ± 0.60	−1.05 ± 0.46

TABLE III

EFFECT OF Ca^{++} ON Mg CONCENTRATIONS
OF RAT LIVER SLICES (pH 7.4, 37°C)

Medium calcium concentration	Medium magnesium concentration	No. of experiments	Initial slice magnesium	Final slice magnesium
mM	mM		mmoles/kg	mmoles/kg
1.5	0.75			5.06 ± 0.17
		13	7.90 ± 0.19	
14.0	0.75			4.39 ± 0.18 ($p < 0.02$)
1.5	10.0			13.9 ± 0.43
		7	7.81 ± 0.22	
25.0	10.0			10.9 ± 0.31 ($p < 0.001$)

In each experiment, liver slices from the same animal were divided into three samples of 2.0 g each. One sample was analyzed to obtain the initial concentration of Mg^{++}. The two remaining samples were incubated for 3 hr in 25 ml of a buffered medium of the following composition (mM): 80 Na^+, 5.0 K^+, 88 Cl^-, 50 Tris, 11.1 glucose, and the CaCl₂ and MgCl₂ concentrations shown above. Incubation was conducted in a Dubnoff metabolic shaker at 105 oscillations/min with 100% O_2. Values are means ±SEM.

Effect of Metabolic Inhibition (Table IV) Neither the deletion of glucose from the medium nor the addition of 10 mM Na iodoacetate had a significant influence on the bidirectional transport of Mg or the concentration of Mg in the liver slices. The addition of 10 mM NaCN increased the influx and efflux

transfer coefficients by 37% (p < 0.001) and 16% (p < 0.02), respectively. As a consequence of the stimulation of transport in both directions, NaCN did not alter the concentration of Mg in the liver slices. Dinitrophenol, in 10 mM concentration, had no influence on the influx transfer coefficient but decreased the efflux transfer coefficient by 21% (p < 0.001). Despite this, the concentration of Mg in the liver slices was not significantly altered.

Effect of Ethanol and Lactate (*Table IV*)   The addition of 0.10 M ethanol to the medium caused increases in both the influx and efflux transfer coefficients of 15–20%. However, because of the high standard error of the mean, these

TABLE IV

EFFECT OF METABOLIC INHIBITION, ETHANOL,
AND LACTATE ON BIDIRECTIONAL Mg TRANSPORT IN
RAT LIVER SLICES (2.5 mM Mg^{++}, pH 7.4, 37°C)

	No. of experiments	K_{bs}	K_{sb}	Initial slice magnesium	Change in slice magnesium
		per min	*per min*	*mmoles/kg*	*mmoles/kg*
Control	22	0.074 ± 0.003	0.019 ± 0.001	7.75 ± 0.34	−0.33 ± 0.40
Glucose deletion	5	0.082 ± 0.002	0.019 ± 0.001	9.70 ± 0.40	−0.75 ± 0.30
Na iodoacetate, 10 mM	6	0.083 ± 0.006	0.020 ± 0.001	6.95 ± 0.22	−0.80 ± 0.40
NaCN, 10 mM	6	0.101 ± 0.005 (p < 0.001)	0.022 ± 0.001 (p < 0.02)	7.40 ± 0.37	0 ± 0.27
Dinitrophenol, 10 mM	6	0.073 ± 0.003	0.015 ± 0.001 (p < 0.001)	7.75 ± 0.85	−1.15 ± 0.50
Ethanol, 0.10 M	6	0.089 ± 0.011	0.022 ± 0.002	7.25 ± 0.40	−0.55 ± 0.21
Na lactate, 10 mM	6	0.079 ± 0.008	0.019 ± 0.001	7.00 ± 0.41	−0.65 ± 0.25

changes were not significant. Na lactate, in 10 mM concentration, had no influence on Mg transport. Neither agent caused a significant change in the Mg concentration of the liver slices.

DISCUSSION

These studies indicate that a quantitation of bidirectional transport of Mg in an in vitro system is feasible and that in the rat liver slice, Mg transport is susceptible to certain alterations in the physical and chemical environment. Although the slices are several cells thick, it is reasonable to assume that the interior cells are as labeled with exchangeable [28]Mg as cells closer to the surface of the slices, and an appropriate correction in the calculations has been made for the passage time of the deeply located [28]Mg from the interstitial space between the cells to the bulk medium. No suitable radioisotope of Mg exists with which to prepare radioautographs supporting this assumption. However, in studying calcium transport in an identical system, [45]Ca has been utilized to prepare radioautographs using a cryostatic technique to prevent

translocation of the label. These radioautographs show that although there is some variation from lobule to lobule in the concentration of the ^{45}Ca, possibly reflecting random variation in the metabolic activity of the hepatocytes, deeply located cells are as well-labeled as surface cells (A. B. Chausmer, B. S. Sherman, and S. Wallach, unpublished observations). Slight heterogeneity of radioisotope distribution and exchange might nevertheless occur, but the use of a uniform procedure of slicing and a large mass of slices should correct for this.

Under the control condition of an external concentration of Mg^{++} of 2.5 mM, a steady state of flux is present with influx and efflux transfer coefficients of 0.074 and 0.019 per min, respectively. The applicability of these values to the hepatic transport of Mg in vivo is suggested by studies in dogs with renal pedicle ligations (20). Injected ^{28}Mg disappears from the plasma of such dogs at an initial rate of 5% per min. Since liver has a high in vivo exchangeability with ^{28}Mg, and some recycling of ^{28}Mg accumulated by the tissues back to the extracellular space is also occurring, an influx transfer coefficient for liver in excess of 0.05 per min can be assumed for the in vivo condition. Therefore, the influx transfer coefficients for Mg in liver appear to be similar in vivo and in vitro, further supporting the validity of the in vitro studies.

Certain characteristics of the system suggest that Mg distribution in the liver cell may actually be more complex than the two-compartment model used for the interpretation of the transport data. The linearity of slice uptake of ^{28}Mg during the first 10 min of incubation in radioactive media indicates exchange in a kinetically homogeneous cellular fraction of Mg. However, when incubation is continued for a longer period, a progressively slower rate of additional accumulation occurs which is not quantitatively accounted for by concurrent efflux of the ^{28}Mg. This finding, as well as the persistence of a greater specific activity of Mg in the liver slices than in the medium at isotopic equilibrium after incubation in nonradioactive media, suggests that in addition to a cellular fraction of Mg which exchanges rapidly with the extracellular phase, other intracellular Mg fractions are present which have significantly slower rates of exchange. The additional possibility that a cellular pool of Mg also exists which exchanges more rapidly than the fraction whose exchange is quantitated in this system cannot be discounted since there was a 10 min period of washing and the first 10 min of release of ^{28} Mg was deleted from the calculations. On the other hand, the intercept value of the linear portion of the curve fell between 0.7 and 0.8 (Figs. 1 and 5), a range compatible with the reported cellular fraction of liver (25). Therefore, if such rapid exchange were present, it probably accounts for only a small fraction of total exchange. The presence of multiple cellular pools is in accord with the results of ^{28}Mg turnover studies in human beings which indicate that at least three kinetically different fractions

of magnesium exist within the body (24). The present data suggest that these kinetically different fractions may exist within the same cell.

A consideration of the quantitative relationships between the transport coefficients of the exchanging cellular fraction and the extracellular and slice concentrations of Mg suggests that the concentration, as well as the kinetics, of Mg within the cell is discontinuous. Since the in vitro system is essentially in a steady state under control conditions, the concentration of the intracellular fraction of Mg which is exchanging with the extracellular phase can be estimated as $(0.074:0.019)$ (2.5) = 9.7 mmoles/kg. Assuming that the slices have an extracellular space of at least 22% (25), the average intracellular concentration of Mg is approximately 7.8 − (0.22) (2.5) = 7.2 mmoles/kg. Thus, the exchanging intracellular fraction has a Mg concentration at least 35% higher than the average cell concentration of Mg. It is unlikely that this exchanging intracellular pool represents diffusible ionic Mg since the existence of an ionic pool of such a magnitude, unbound to cell constituents, is unlikely. It is more probable that most if not all of the exchanging intracellular pool is bound to macromolecular species within the cell, but the affinity of binding is low and the pool therefore participates in exchange across the cell membrane. The finding of a kinetically distinct intracellular Mg pool with a concentration in excess of the average cell Mg provides evidence for significant gradients for Mg within the cell and is consonant with studies showing variable concentrations of Mg in separated cell organelles (6).

The development of a slightly greater specific activity of Mg in the liver slices than in the medium during incubation also does not invalidate the assumption that exchange between the rapidly exchanging cell fraction and other intracellular fractions is slow compared to exchange between the rapidly exchanging fraction and the extracellular phase. Therefore, despite the complexity of the system, measurements of the rate of accumulation of ^{28}Mg in the extracellular phase and the utilization of a closed two-compartment model are valid in quantitating cellular transport of Mg. Perhaps the most significant finding of this study is the ability of the cell membrane to respond to changes in the extracellular concentration of Mg^{++}. The influx coefficient changes reciprocally and the efflux coefficient changes in parallel with the extracellular gradient. It is tempting to speculate that the reciprocal change in the influx coefficient is triggered by the extracellular concentration and the parallel change in the efflux coefficient by the intracellular concentration of Mg at a sensitive cell locus. The data do not distinguish whether the concentration dependence of influx is a specific or a general effect of extracellular Mg^{++} to alter membrane permeability reciprocally to the Mg^{++} concentration. In any case, the control of Mg efflux cannot be explained by a nonspecific alteration in membrane permeability and regardless of the exact mechanism, liver cells

appear to exert a significant degree of autocontrol over the intracellular content of Mg without the need for an external negative feedback system. The influx transport system is probably a primary control, with the efflux transport system coming into play if the former is overwhelmed by an extreme change in gradient. This hypothesis is supported by studies of ^{28}Mg turnover in uremic human beings with hypermagnesemia (24) in whom the extracellular gradient is not as greatly altered as in the present study. In the hypermagnesemic human being, the fractional rate of Mg influx into tissues is decreased, the exchangeable tissue pools of Mg are normal, and the fractional rate of Mg efflux from tissues is unaltered.

The transport process for Mg is both temperature- and pH-dependent, as is Ca transport studied by an identical technique (23), but the effects of reduced temperature and altered pH are less extreme for Mg than for Ca. Other features which the in vitro transport of Mg and Ca in rat liver have in common are the lack of inhibition of transport by iodoacetate and cyanide, and the minor influence of alterations in the concentrations of univalent cations. It is unlikely that the graded inhibition of Mg transport observed with substitution of K^+ for Na^+ in the medium is physiologically important since the data fail to achieve significance at the minimum criterion of $p < 0.05$. The ability of univalent cations to interact with divalent cations has been demonstrated in other systems (7, 16) but does not appear to be a significant characteristic of liver (23). Interestingly, cyanide stimulates both Mg and Ca transport. The stimulatory effect on Ca transport has been ascribed to the induction of cellular damage (23). Although certain types of experimental liver damage have been reported to increase Mg uptake (18), this is not invariably so (10). The imposition of the in vitro condition, itself an injurious influence, leads to a small loss of liver Mg (unpublished observations) and an increase in the extracellular concentration of Mg^{++} to the range of 2.5 mM is required to maintain a steady-state concentration of tissue Mg in vitro. Therefore, it is possible that the stimulation of divalent cation transport by cyanide may have a metabolic basis other than cellular injury. The lack of effect of glucose deletion and the small effect of dinitrophenol in inhibiting efflux also suggest that cellular injury, per se, does not stimulate Mg transport. Regarding the effect of dinitrophenol, Aikawa and Reardon (1) have noted alterations in Mg transport in several tissues in intact rabbits, and Averill and Heaton (4) have presented similar data for renal tubular transport of Mg in rats given dinitrophenol. Neither study, however, permits a clear definition of the specific event in Mg transport affected by this inhibitor. It has been claimed that both ethanol and lactate alter Mg transport in studies of renal tubular handling of Mg in human beings (11, 19). However, continuous alcohol administration to rats does not alter skeletal muscle concentrations of Mg (17), and the present study does not suggest an influence of either agent in the rat liver slice.

The ability of Ca to inhibit Mg transport is reminiscent of the inhibition of Ca transport by Mg previously observed in the rat liver slice (23). Quantitatively, the effect of Mg on Ca transport is considerably greater than the opposite effect. Nevertheless, this reciprocal inhibition suggests a common transport mechanism for the two divalent cations which shows some characteristics of saturation kinetics and which is not shared by Sr. A common transport system for Ca and Mg has been incriminated in gastrointestinal mucosa (2, 12) and the renal tubules (15), but fragmentary evidence has been heretofore available as to a similar situation in other cellular systems (3). In contrast, several examples of competition between Ca and Mg for binding to subcellular fractions are available (7, 16).

As for Ca transport, the energy requirements for Mg transport in the rat liver slice are undefined and the arguments previously advanced to support the theory that Ca transport is dependent upon the binding of Ca to cellular ligands (23) can be advanced for Mg with equal force. The fact, in contrast to Ca, that the average intracellular concentration of Mg is considerably higher than the extracellular concentration does not alter the thesis since it is likely that the bulk of intracellular Mg is bound and not in ionic form. The degree of temperature and pH dependence, the inhibition by Ca, and the lack of a major inhibitory effect by the other conditions studied, especially those utilizing metabolic inhibitors, are compatible with a passive diffusion process for magnesium influx. The diffusion process, however, might be a facilitated one despite the absence of evidence for a membrane-bound carrier, on the basis of the observed rates of transport, the magnitude of the temperature and the pH dependence, and the evidence that Mg binds competitively to cellular ligands.

The ability of such a transport process to be influenced by physiologic perturbations is not necessarily impaired by the lack of a requirement for a direct source of metabolic energy. Indeed, in studies to be published, 1-thyroxine treatment of the rat has been found to stimulate both Ca and Mg transport in liver slices, confirming and extending earlier studies of radiocalcium (5, 13) and radiomagnesium turnover (8) in human beings with thyroid disease. Parathyroid hormone stimulates Ca but not Mg transport in rat liver slices and causes a parallel increase in Ca binding by liver homogenates (unpublished observations). Thus, the Mg transport system appears to be capable of exerting homeostatic control on the intracellular concentration of Mg, and at the same time to permit selective variation of transport with appropriate physiologic stimuli.

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