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MAGNESIUM EXCHANGE IN RAT VENTRICLE

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SUMMARY

1. The exchange of cellular Mg with external ^{28}Mg in the rat left ventricle was measured *in vivo* and, under conditions approximating a steady state, in an isolated, working rat heart perfused and contracting at 36–38° C.

2. About 98 % of cellular Mg exchanged at a single rate.

3. The rate of exchange *in vivo* was the same as that observed in independent *in vitro* measurements of the influx and efflux at the physiological external Mg^{2+} concentration of 0·56 mM. The rate was 0·15 ± 0·02 m-mole/(kg dry ventricle.min) or 0·21 ± 0·02 p-mole/(cm².sec).

4. In the perfused heart the dependence of the influx on the external Mg concentration was hyperbolic with an apparent V_{max} of 0·31 ± 0·04 m-moles/(kg dry weight.min) and an apparent K_m of 0·57 ± 0·08 mM.

5. The Mg efflux into a solution containing 2·8 mM-Mg was markedly faster than that into a Mg-free solution.

6. These results are interpreted as consistent with a carrier-mediated transport of Mg across the plasma membrane.

INTRODUCTION

Although the adenine nucleotides utilized by heart muscle in contraction and relaxation are believed to exist in heart muscle cells as Mg compounds, surprisingly little is known about the transport of Mg across cardiac cell membranes or about the state and distribution of this ion within the cells. It has been reported that, as in other types of muscle (Gilbert, 1960), Mg enters cardiac cells (Rogers & Mahan, 1959; Glaser & Brandt, 1959) and that Mg is transported across the inner membrane of cardiac mitochondria (Brierley, Bachmann & Green, 1962; Brierley, 1967). However, there is no information about the rate of the processes by which Mg enters cardiac cells.

In this paper we report a study of the exchange of ventricular cellular Mg with ^{28}Mg both *in vivo* and in the isolated, perfused rat ventricle. The

results suggest that in this tissue nearly all of cellular Mg exchanges at a single rate. The influx exhibits a hyperbolic dependence on the external Mg concentration, and the efflux demonstrates the phenomenon of counter transport (Wilbrandt & Rosenberg, 1961).

Portions of this work have previously appeared in abstract (Polimeni & Page, 1971).

METHODS

Experimental design

Experiments in intact rats

Twelve rats from the same batch (Sprague–Dawley females weighing 190–250 g) were injected I.P. with 0·5 ml. ^{28}Mg-labelled solution. The solution had the same chemical composition as the medium used to perfuse isolated hearts (see below), but contained no Mg except that serving as carrier for the ^{28}Mg-labelled $MgCl_2$ (44 μc/kg body weight, total Mg injected 0·019 m-mole/kg body wt.). At intervals of from 0·5 to 30 hr after injection of ^{28}Mg the animals were injected I.P. with heparin; they were then stunned, a sample of mixed venous blood was obtained from the inferior vena cava, and the left ventricles were excised and analysed for Mg content, wet weight, and dry weight. The radioactivities of the muscle and plasma were assayed in a well type crystal scintillation counter and the total Mg was measured by atomic absorption spectrophotometry (Page, Mobley, Johnson & Upshaw, 1971*b*).

Experiments in isolated perfused hearts

Female Sprague–Dawley rats weighing 190–250 g were injected with heparin, then stunned by a blow on the head. For flux measurements under a particular experimental condition the hearts from a single group of litter mates were used to reduce biological variation. The hearts were rapidly excised for perfusion of the whole heart with ^{28}Mg. The perfusion apparatus was that previously reported (Page, Power, Borer & Klegerman, 1968; Neely, Liebermeister, Battersby & Morgan, 1967). This arrangement yields a totally isolated rat heart which is contracting against an elastic aortic pressure at a constant inflow pressure of 10 cm water. Its contractile performance has been characterized by haemodynamic measurements by Neely *et al.* (1967). It has been found suitable in our laboratory for measurements of ion fluxes with excellent time resolution by virtue of an experimental arrangement which minimizes the transit time of radioactivity in the various components of the dead space (Page *et al.* 1968). To measure the ^{28}Mg influx with this apparatus, hearts were pre-perfused for not less than 10 min with non-radioactive control solution; this solution was then changed to one of identical chemical composition labelled with ^{28}Mg and the perfusion was continued for 1–91 min. All perfusions were carried out at 36–38° C, a temperature at which the hearts contracted spontaneously at a rate of 180–220 beats/min. At the end of the perfusion the heart was removed from the cannulae and the left ventricular wall was cut free from the interventricular septum. The wet weight, dry weight, radioactivity and magnesium content of the left ventricular wall were measured and compared with the radioactivity and chemical Mg content of the perfusate.

Analyses

The analytical methods have previously been described in detail (E. Page & E. G. Page, 1968; Krames & Page, 1968; Page *et al.* 1971*b*).

Determination of surface/volume ratio. The ratio (area of external sarcolemmal membrane + area of T-tubular membrane)/(cell volume) was determined from photographic prints of electron micrographs of osmium-perfused ventricles (Krames & Page, 1968), and a combination of point counting and lineal integration (Page, McCallister & Power, 1971*a*; Mobley & Page, 1972).

Solutions

Intact hearts were perfused with the modified Krebs-Henseleit solution previously used (Page *et al.* 1968). The solution contained: NaCl, 118 mM; $NaHCO_3$, 25·0 mM; KCl, 4·7 mM; KH_2PO_4, 1·2 mM; $CaCl_2$, 2·5 mM; $MgCl_2$, 0·56 mM; EDTA calcium disodium salt, 0·5 mM, and dextrose, 5·5 mM. The pH was maintained at 7·3 by continuously bubbling 5 % CO_2–95 % O_2 through this solution.

Calculation of the fraction of exchangeable cellular Mg in isolated, perfused hearts

During influx and efflux experiments hearts were perfused from an effectively infinite reservoir of ^{28}Mg-labelled solution which was not recirculated. The specific activities of the solutions used for loading the heart with radioactivity were therefore constant and the specific activity of the solution used for washing out radioactivity was zero. Under these conditions the radioactivity of the total Mg in an initially non-radioactive left ventricular wall at any time after the beginning of a perfusion with ^{28}Mg-labelled solution is given by

$$P^*_{tot}Mg_{tot} = P^*_iMg_i + P^*_oMg_o$$

in which P^*_{tot}, P^*_i, and P^*_o are the specific activities (in counts per minute/m-mole) of the magnesium contents, Mg_{tot}, Mg_i and Mg_o (in m-mole), respectively; the subscripts *tot*, *i*, and *o* refer to the total ventricular muscle, the intracellular compartment and the extracellular compartment, respectively. If the specific activity of Mg in a perfusion solution (P^*_{ps}) is constant, then the fractions of total ventricular Mg, cellular Mg, and extracellular Mg that have exchanged (f_{tot}, f_i, and f_o, respectively) are P^*_{tot}/P^*_{ps}, P^*_i/P^*_{ps}, and P^*_o/P^*_{ps}. Then $f_i = (f_{tot}\,Mg_{tot} - f_oMg_o)/Mg_i$, in which Mg_o is given by $S_o \cdot H_2O_{tot} \cdot [Mg]_{ps}$; S_o is the (dimensionless) extracellular fraction of total ventricular water content, H_2O_{tot} (g), and $[Mg]_{ps}$ is the concentration of Mg in the perfusion solution (mM). Left ventricular S_o in the *in vivo* and *in vitro* preparations were, respectively, 0·33 (Scharff & Wool, 1965) and 0·44 (Klegerman & E. Page, unpublished observations).

If the extracellular space is equilibrated with the perfusing solution sufficiently long so that $P^*_{ps} = P^*_o$, then f_i can be calculated from the experimentally measurable quantities P^*_{tot}, P^*_{ps}, and H_2O_{tot}. Since the extracellular space equilibrates very rapidly in the preparation used for these experiments (Page *et al.* 1968), P^*_o was assumed to be effectively the same as P^*_{ps} within 5 min after beginning perfusion with ^{28}Mg.

In preliminary experiments on the isolated perfused heart, we found that the left ventricle loses about 0·1 % of its Mg content/min. Because of this slow net loss of Mg we have kept the *in vitro* experiments as brief as possible. Experiments designed to measure the influx of ^{28}Mg were limited to 50 min, including a 10-min period of pre-equilibration with non-radioactive solution; in other experiments, the duration of perfusion never exceeded 100 min.

RESULTS

Unless otherwise indicated results in the text and Tables are expressed as mean ± S.E. of the mean. Lines were fitted by the method of least squares. A weighted least squares method was used when the variances of points on a slope were appreciably different.

Experiments on intact rats

Rogers & Mahan (1959) injected ^{28}Mg-labelled $MgCl_2$ I.P. into rats and compared the specific activities of the hearts and plasma as a function of time, using values for the chemical Mg content of rat hearts tabulated in the literature. They observed that the value of the cardiac specific activity divided by the plasma specific activity reached and then significantly exceeded unity within 150 min, after which the ratio did not measurably change for about 4 hr. They concluded that under the conditions which prevail *in vivo* cardiac Mg exchanges completely within 3 hr.

This conclusion would suggest that ^{28}Mg-exchange *in vivo* is substantially more rapid than that observed under the *in vitro* conditions of experiments reported later in this paper. Accordingly we undertook some experiments to investigate this apparent discrepancy in exchange rates, as well as to obtain some information about the exchangeability of cardiac Mg *in vivo*.

Fig. 1*A* shows the time course of the specific activities of cellular Mg in the left ventricle (P_i^*) and plasma (P_{pl}^*) after injection of ^{28}Mg-labelled $MgCl_2$; Fig. 1*B* shows the time course of the ratio P_i^*/P_{pl}^*. It is apparent from Fig. 1*A* that the temporal relation between the specific activities of Mg in the heart and in the plasma is complex. Fig. 1*B* shows that P_i^*/P_{pl}^* significantly exceeds unity about 3 hr after the injection, reaches a maximum of 1·5 from 6–9 hr after injection, and then falls slowly over a period of hours with a half-time of approximately 10 hr.

The interpretation of these curves is not simple, but the data clearly do not support Rogers & Mahan's conclusion that cardiac Mg equilibrates completely within 3 hr. The observation that P_i^*/P_{pl}^* reaches and exceeds unity reflects the differences in the time courses of the specific activities of cardiac and plasma Mg. During the period when the two time courses are so different, the time course of the ratio is not equal to the time course of the exchange of cardiac Mg with ^{28}Mg, as it would be if the specific activity of plasma were constant.

After a sufficiently long time, the exchange of cellular Mg with plasma Mg should approach completion in all tissues. Assuming that any additional uptake by the extracardial tissues is slow relative to the ventricular transmembrane flux, P_{pl}^* should decline very slowly while P_i^*/P_{pl}^* should approach a constant value and give a good approximation of the fraction of cardiac

Mg exchanged with ^{28}Mg. The last six points in Fig. 1*B* are the results of a second experiment in which the animals from a second litter were sacrificed from 20–30 hr after injection of ^{28}Mg-labelled $MgCl_2$. After 20 hr the

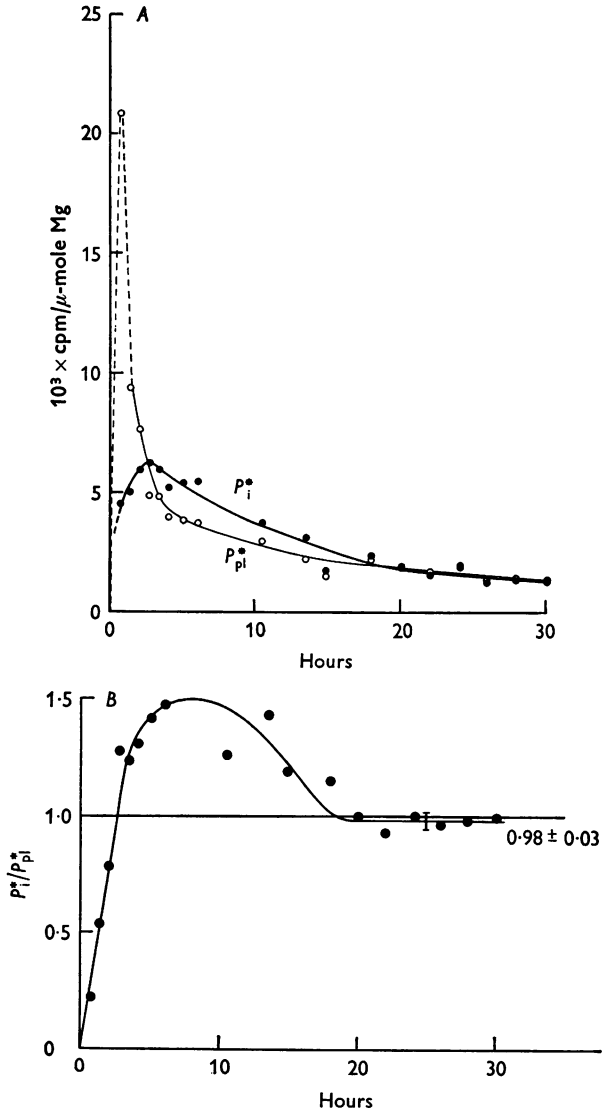

Fig. 1. *A*, Specific activities of Mg in ventricular muscle (P_i^*, filled circles and heavy line) and plasma Mg (P_{pl}^*, open circles and thin line) in the intact rat as a function of time after injection of ^{28}Mg. *B*, the ratio P_i^*/P_{pl}^* as a function of time after ^{28}Mg injection calculated from the data in Fig. 1*A*. The last six points were used to derive the fraction of exchangeable Mg, as discussed in the text, yielding a value of 0·98 ± 0·03 after 20 hr.

half-time for the loss of ^{28}Mg from the plasma is only about one-eighth of that for the cellular Mg exchange described in the next section. Since the loss of radioactivity from the plasma into compartments other than the heart is so much slower than the exchange of ^{28}Mg between the heart and the plasma, the ratio P_i^*/P_{pl}^* should approach constancy at a value given by the maximal fraction exchanged. A least-squares plot through the last six points yields an horizontal line, indicating that the fraction of myocardial Mg exchanged is $0{\cdot}98 \pm 0{\cdot}03$.

Cellular Mg exchange in the isolated perfused heart

The steady state approximation

Preliminary experiments indicated a slow net loss of Mg from the isolated, perfused heart. Before measuring the exchange of cellular Mg with ^{28}Mg, we determined the rate and magnitude of this loss to evaluate

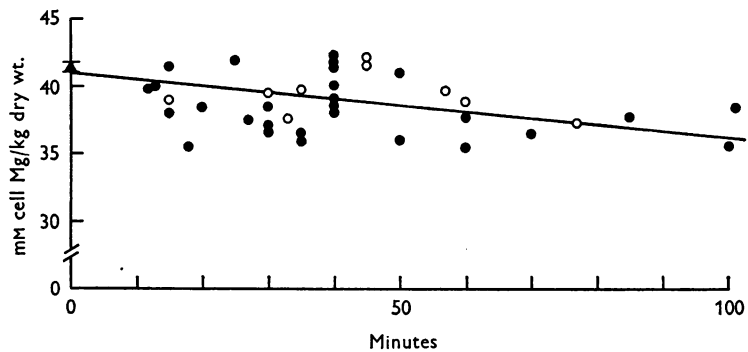

Fig. 2. Cellular Mg content of the left ventricle during perfusion with modified Krebs-Henseleit solution containing 0 mM-Mg (open circles) and 0·56 mM-Mg (filled circles). The cellular Mg content of twenty-eight unperfused left ventricles (triangle) was found to be $41{\cdot}4 \pm 0{\cdot}3$ m-mole Mg/kg dry wt. The slope of the line indicates a net loss of $0{\cdot}05 \pm 0{\cdot}01$ m-mole Mg/(kg dry wt. . min), or about 0·1 % of the cellular Mg per min. The net Mg losses in Mg-free and Mg-containing solutions were not statistically different. The line extrapolates to an intercept of $40{\cdot}9 \pm 0{\cdot}3$ m-mole Mg/kg dry wt.

the duration during which a steady-state approximation might be applicable. Since the isotopic exchange measurements were to be carried out over a range of external Mg concentrations, we also examined the effect of $[Mg]_o$ on the net loss of Mg. These measurements were carried out over the entire range of $[Mg]_o$ (0–0·56 mM) used during experiments on ^{28}Mg exchange. As expected from the literature (Hoffman & Cranefield, 1960), changes of $[Mg]_o$ over this range produced no changes in the frequency of spontaneous contraction. The results of these experiments are summarized in Table 1 and Fig. 2.

Fig. 2 illustrates the decline in cellular Mg content which occurs during 101 min of perfusion in left ventricles perfused, respectively, with Mg-free solution and with a solution containing the highest $[Mg]_o$ used for quantitative measurements of the exchange rate (0·56 mM). There is no discernible difference between the time courses of cellular Mg content at the two Mg concentrations. The point at time zero is the cellular Mg content in the

TABLE 1. Mg and water contents of rat left ventricles

Expt	Cellular Mg content (m-mole Mg/kg dry wt.)	Total water/wet wt. (kg/kg)
Unperfused ventricles (28)	41·4 ± 0·3	0·763 ± 0·001
Perfused ventricles (28) $[Mg]_o$ = 0·56 mM	40·9 ± 0·3	0·825 ± 0·003
Perfused ventricles (14) $[Mg]_o$ = 0 mM	40·2 ± 0·5	0·830 ± 0·004

Figures in parentheses give the number of experiments. Extracellular Mg content of unperfused ventricles was taken as 1·5 m-mole/kg dry wt., calculated from a value of 0·249 ml./kg wet wt. for the *in vivo* inulin space of ventricles from nephrectomized rats (Scharff & Wool, 1965), and the residual blood in ventricular myocardium from bled rats (Sharpe, Culbreth & Klein, 1950), the tissue haematocrit of the rat ventricle (Everett, Simmons & Lasher, 1956), and a plasma Mg concentration of 1·04 ± 0·02 mM measured on venous plasma obtained from eighteen rats. The Mg contents of the perfused ventricles fell linearly as a function of perfusion duration up to at least 100 min. (Fig. 2). The cellular Mg contents of these ventricles were calculated by extrapolating the regression equations for $[Mg]_o$ = 0 mM and $[Mg]_o$ = 0·56 mM to the respective intercepts at t = 0. The Mg content of the right ventricle (41·5 ± 0·5 m-mole Mg/kg dry wt., ten rats) did not differ from that of the left ventricle.

unperfused left ventricle (41·4 ± 0·3 m-mole Mg/kg dry wt. (Table 1)). The slope indicates a net loss of 0·05 ± 0·01 m-mole Mg/(kg dry wt. . min.) This result indicates that about 6 % of cellular Mg would be lost during a 50-min perfusion. The steady-state approximation is therefore rather good for experiments of this duration.

^{28}*Mg-influx at physiological* $[Mg^{2+}]_o$

In Fig. 3 the exchange of cellular Mg with ^{28}Mg at the physiological $[Mg^{2+}]_o$ of 0·56 mM has been plotted semilogarithmically against time. It is apparent that about 15 % of cellular Mg exchanged during the 40-min influx, and that the exchange occurred at a single rate. The extrapolated line intersects the ordinate at $1-f_i$ = 0·99 ± 0·01. The closeness of this value to unity suggests that any cell fraction which exchanges faster than the rate indicated by the slope, would have to be very small. The slope of the least squares line yields an exchange of 0·0038 ± 0·0004 of cellular Mg per minute.

The rate of perfusion of the spontaneously beating preparations used to obtain the data in Fig. 3 varied. The perfusion rate could not be accurately determined in this preparation because the effluent was composed of fluid perfusing the coronary vessels and fluid leaking from the open pulmonary veins. We have therefore tested whether there is a relationship between the flow rate of perfusion solution and ^{28}Mg flux, using the Langendorff preparation in which leakage from the pulmonary veins is not a problem. The flows in this preparation ranged from 8–12 ml./min. Since there was

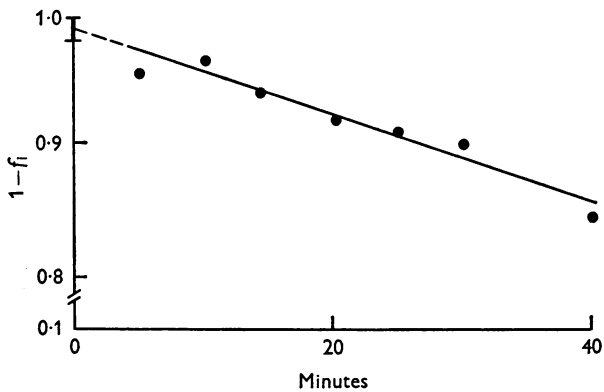

Fig. 3. Determination of rate of cellular Mg exchange from ^{28}Mg uptake in 0·56 mM-Mg. The fraction of cellular Mg remaining unexchanged, $1-f_i$, is plotted semilogarithmically against perfusion duration, t. The graph yields an exchange rate of $0{\cdot}0038 \pm 0{\cdot}0004$ min^{-1}.

no correlation between the observed exchange rate and the perfusion rate ($r = -0{\cdot}067$), the perfusion rate was evidently not rate-limiting for the ^{28}Mg influx.

Mg efflux

Five rats were injected intraperitoneally with ^{28}Mg at least 20 hr before the heart was to be removed for perfusion *in vitro*. The 20 hr interval was chosen because the *in vivo* experiments already described indicated that all of the cellular Mg which is capable of exchange with ^{28}Mg (about 98 % of total cellular Mg) would have equilibrated after this time (Fig. 1*B*). At the end of the 20 hr the hearts were excised, mounted in the usual manner, and perfused for 30–90 min with non-radioactive solution. The specific activity of cellular Mg at the beginning of the perfusion was determined from the specific activity of a blood plasma sample obtained at the time the heart was removed.

Fig. 4 is a plot of the fraction of cellular Mg which has exchanged with external Mg against the duration of perfusion. The points can be fitted to

a line corresponding to an exchange of 0·0040 ± 0·0007 of cellular Mg per minute, a value which agrees well with that of 0·0038 ± 0·0004 derived from the influx data. The intercept of 0·98 ± 0·04 of cellular Mg is also consistent with the conclusion, previously reached on the basis of the *in vitro* uptake data, that little if any cellular Mg exchanges at a rate faster than that indicated by the efflux slope.

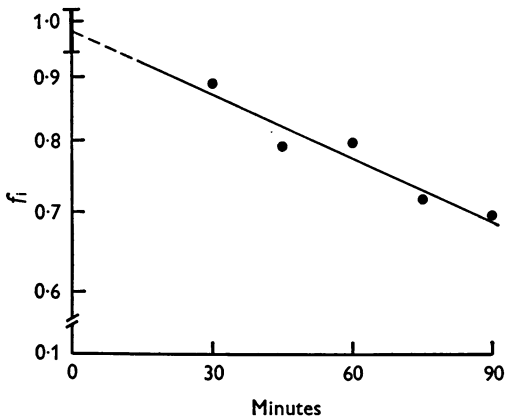

Fig. 4. Determination of rate of cellular Mg exchange from ^{28}Mg efflux in 0·56 mM-Mg. The graph yields a rate of 0·0040 ± 0·0007 min^{-1}. The intercept at $t = 0$ is 0·98 ± 0·04 and agrees with the *in vivo* measurement of the fraction of exchangeable cellular Mg (Fig. 1*B*).

Dependence of ^{28}Mg influx on external Mg concentration

To examine the relationship between Mg exchange and $[Mg]_o$, hearts were perfused with ^{28}Mg-labelled Krebs–Henseleit solution containing between 0·011 and 0·56 mM-Mg. After a 10-min equilibration period with inactive solution, the ^{28}Mg influx was determined as a function of time, separate hearts being used for each point at a given concentration. In order to minimize scatter due to biological variation, the influx at each Mg concentration was measured on hearts obtained from batch-mates matched with respect to body weight. Although we determined the Mg uptake over a period of 90 min, we have considered only the first 30 min of uptake in quantifying the rate of uptake (Fig. 5*A*). This experimental design was chosen to obtain the initial linear segment of the uptake, to make measurements at an approximately constant cellular Mg concentration, and to avoid any errors that might be caused by the deleterious effects of a prolonged perfusion. A plot of the initial uptake rate as a function of $[Mg]_o$ appeared hyperbolic. When the initial uptake rates are replotted as a double reciprocal plot according to the method of Lineweaver & Burk (Fig. 5*B*), the points fall on a straight line; the exception is the value for

the lowest $[Mg]_o$ (0·011 mM), which clearly lies above the line (Fig. 5*B*, insert). Extrapolation of the weighted least squares line through the other four points (Fig. 5*B*) yields intercepts on the ordinate and abscissa corresponding to an apparent V_{max} of $0{\cdot}0075 \pm 0{\cdot}0011$ min^{-1}, or $0{\cdot}31 \pm 0{\cdot}04$ m-mole Mg/(kg dry wt..min), and an apparent K_m of $0{\cdot}57 \pm 0{\cdot}08$ mM.

The most probable explanation for the deviation of the points at $[Mg]_o$ = 0·011 mM is that at this extremely low concentration a significant fraction of Mg is bound to extracardiac sites of low binding capacity, for example, the surfaces of the perfusing apparatus.

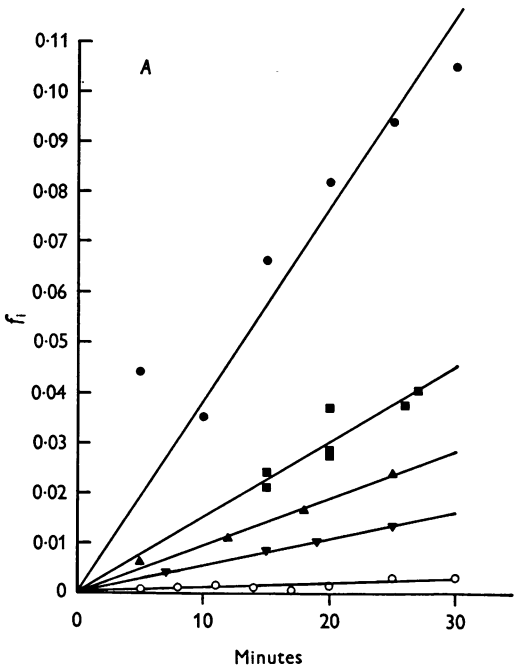

Fig. 5*A*. For legend see opposite page.

Dependence of Mg efflux on external Mg concentration

The finding that the ^{28}Mg influx shows a hyperbolic dependence on $[Mg]_o$ is compatible with a carrier-mediated transport of Mg. In order to investigate this point further, we carried out experiments designed to demonstrate the presence of isotopic counter-transport (Wilbrandt & Rosenberg, 1961), that is, a dependence of the ^{28}Mg efflux on the external Mg concentration. For this purpose we compared the ^{28}Mg efflux into Mg-free solution with an efflux into a solution containing a Mg concentration sufficient to nearly saturate the transport system.

In a preliminary experiment (Fig. 6) the heart was perfused for 30 min with a ^{28}Mg-labelled solution containing 0·56 mM-Mg. The accumulated

^{28}Mg was then washed out for 3 min with a non-radioactive solution containing no Mg and the effluent was sampled with a fraction collector. The non-radioactive washout solution was then abruptly changed from one containing no Mg to an otherwise identical solution containing 5·6 mM-Mg.

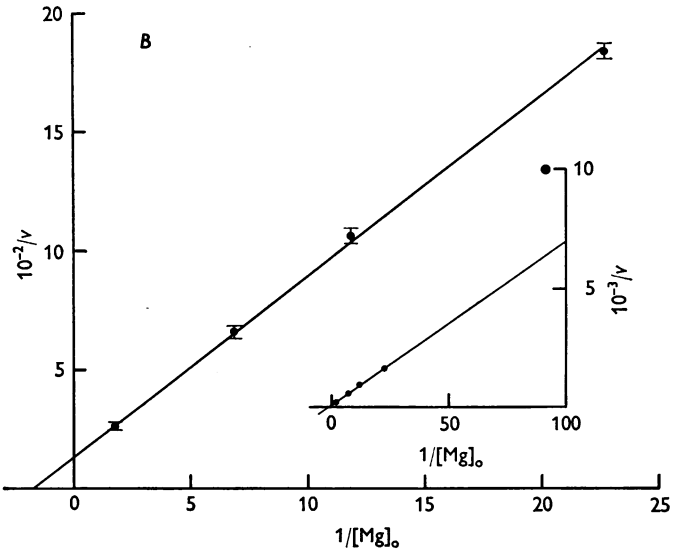

Fig. 5. Determination of apparent V_{max}, apparent K_m, and the exchange constant from the initial uptake velocity as a function of $[Mg]_o$. *A*, uptake of ^{28}Mg is plotted against the duration of perfusion with solutions containing different concentrations of external Mg. All curves were computed from the initial, linear segment of the uptake curve by the least-squares method with the lines constrained to pass through the origin. The slopes of the curves represent the uptake velocities (v) at different $[Mg]_o$: 0·011 mM (○), 0·044 mM (▼), 0·084 mM (▲), 0·145 mM (■), and 0·56 mM (●). *B*, a double-reciprocal plot of $1/v$ as a function of $1/[Mg]_o$ for values of $[Mg]_o$ between 0·044 mM and 0·56 mM yields a weighted least-squares line which corresponds to an apparent V_{max} of $0·0075 \pm 0·0011$ min^{-1}, or $0·311 \pm 0·044$ m-mole Mg/(kg dry wt..min), and an apparent K_m of $0·567 \pm 0·082$ mM, respectively. The uptake velocity at the lowest concentration of external Mg ($[Mg]_o$ = 0·011 mM, see insert of Fig. 5*B*, symbol ⊙) deviated from saturation kinetics, presumably due to an ion exchange with the surface of the perfusing apparatus; it was therefore excluded from the Lineweaver–Burk analysis.

This produced a transient interruption in the initially rapid decline of effluent radioactivity, indicated by the hump in Fig. 6. These results suggested that the ^{28}Mg efflux from myocardial cells may depend on $[Mg]_o$. However, as this type of experiment is difficult to quantify and is particularly subject to artifactual interferences, we turned to a different experimental design, the results of which are summarized in Table 2.

The experimental procedure was to equilibrate cardiac Mg with ^{28}Mg *in vivo* for 23–33 hr, that is, long enough to achieve specific activity equilibrium between cardiac and plasma Mg. A blood sample was then drawn from the inferior vena cava, and the heart was excised and mounted on the perfusion apparatus. Thereafter the preparation was perfused for

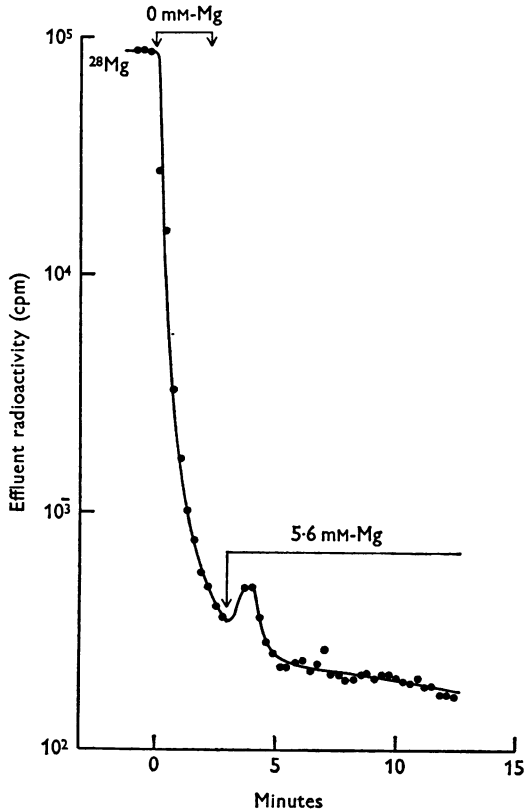

Fig. 6. Effect of external Mg concentration on ^{28}Mg efflux. Effluent radioactivity from the ^{28}Mg-perfused (30 min, $[Mg]_o$ = 0·56 mM) rat heart is plotted versus perfusion time. Effluent radioactivity from a rat heart preperfused with ^{28}Mg-labelled solution containing 0·56 mM-Mg has been potted *vs.* time of perfusion. At the first arrow the perfusion was switched from the radioactive solution to a non-radioactive, Mg-free solution. After 3 min (second arrow), the external Mg concentration was abruptly raised to 5·6 mM, with a consequent increase in the effluent radioactivity.

40 min with non-radioactive solution containing either 0 or 2·8 mM-Mg, as follows: all hearts were pre-perfused with Mg-free solution during the initial 15 min. This period of pre-perfusion was then followed by a 25-min period of perfusion with the test solution. In alternate experiments the test solution contained 2·8 mM-Mg or 0 mM-Mg. Ten minutes after the start of

the test period the effluent dripping out of the heart was collected for 10 min and its radioactivity was later measured. At the end of the perfusion the heart was removed and analysed for residual radioactivity and for total Mg content.

Table 2 presents a comparison of the residual cellular radioactivities (expressed as f_1) in hearts washed out during the test period with 0 and 2·8 mM-Mg, respectively. The Table also shows the radioactivity of the effluent. The results are clear-cut: significantly less ^{28}Mg remains in the heart and significantly more ^{28}Mg appears in the effluent when the perfusing solution contains 2·8 mM-Mg than when it is Mg-free.

TABLE 2. Efflux of ^{28}Mg from ^{28}Mg-equilibrated left ventricle into Mg-free and high $[Mg]_o$ perfusing solutions

Expt	$[Mg]_o$ (mM)	f_1 Measured	f_1 Predicted	^{28}Mg in effluent $\left(\frac{\text{cpm/min}}{\text{g wet wt.}} \times 10^{-2}\right)$
In vivo (6)	1·03 ± 0·04	0·98 ± 0·03	—	—
In vitro (5)	0	0·99 ± 0·01	0·98 ± 0·03	8 ± 1
In vitro (5)	2·8	0·80 ± 0·03	0·84 ± 0·03	24 ± 2

The *in vivo* data were obtained from the hearts represented by the six points of the plateau in Fig. 1*B*. The two groups of *in vitro* hearts were equilibrated *in vivo* with ^{28}Mg for 23–33 hr and then perfused *in vitro* for 40 min with non-radioactive solutions. One group was perfused with Mg-free solution throughout, while the other group was perfused sequentially with two solutions containing, respectively, 0 mM-Mg (15 min) and 2·8 mM-Mg (25 min). Assuming that the exchangeable Mg at the beginning of the perfusion was initially equilibrated with ^{28}Mg, the fraction of ^{28}Mg remaining in the cell after perfusion with non-radioactive solutions for duration t is predicted by $f_1 = 0{\cdot}98 \exp\{V_{max}.t/(1+K_m/[Mg]_o)\}$ where V_{max} and K_m were derived independently from the influx measurements (Fig. 5*B*) and have values of 0·0075 ± 0·0011 min^{-1} and 0·57 ± 0·08 mM, respectively.

The residual radioactivities after perfusion with solutions containing 0 and 2·8 mM-Mg agree quantitatively with values predicted from the concentration dependence of the influx (Fig. 5*B*). At the end of a 40-min perfusion the cell should have lost 2 m-mole Mg/kg dry wt. However, since there can be no exchange of cellular ^{28}Mg against extracellular ^{24}Mg in Mg-free solution, the specific activity of cellular Mg should remain the same during the washout. The data in Table 2 are in accord with this prediction. When $[Mg]_o$ is raised to 2·8 mM, the rate of exchange is given by $V_{max}/(1+K_m/[Mg]_o)$, or 0·0063 ± 0·0009 min^{-1}. It can readily be shown that after 25 min f_1 should be 0·84 ± 0·03, a figure which agrees well with the observed value of 0·80 ± 0·03.

The values for the amount of ^{28}Mg in the effluent are only useful as qualitative checks on the residual cardiac ^{28}Mg contents, which can be determined with much greater precision. This is true because the effluents

are unavoidably contaminated with radioactivity from the surfaces of the heart and apparatus.

Effect of external cardiac work on Mg uptake

To investigate whether the exchange of cellular Mg depends on the external work of contraction, ^{28}Mg-uptake was measured at two different aortic pressures. The cardiac output from the heart contracting against the lower aortic pressure was kept relatively low by adjusting the left atrial inflow pressure. Four pairs of hearts were studied; for one of each pair the aortic pressure was 50 and 100 cm H_2O, respectively. The external work performed by the two pairs of hearts was calculated to be 0·07 and 0·18 kg.m/(min.g dry ventricle), respectively. The coronary vascular flow could not be measured precisely in this preparation because of the possibility of leakage from the aortic valves and left atrium; the calculation of external work is therefore only an approximation. For the four consecutive pairs of hearts, the ratio of the Mg influx at high external work performance to that at low external work performance was found to be $1{\cdot}10 \pm 0{\cdot}04$. The experiments are sufficiently complex so that this small difference, although apparently significant, must be accepted with caution. The results do, however, indicate clearly that if there is any dependence of the Mg flux on external work, it must be very small. Indeed, the observed flux increment was in no way commensurate with the nearly threefold increase in external work.

DISCUSSION

Exchangeability of cellular Mg

The experimental results suggest that nearly all cellular Mg in rat left ventricles exchanges at a single rate. The rate *in vivo* was apparently the same as that in the isolated ventricle perfused and beating at 37° C. The evidence for these conclusions is summarized in Fig. 7.

The observation that nearly all cellular Mg in the rat's ventricle exchanges at a single rate differs from the results obtained by Gilbert (1960) on the only other striated muscle for which detailed kinetic measurements on Mg exchange are available. Gilbert found that about 75–80 % of cellular Mg in isolated frog sartorius was inexchangeable with ^{28}Mg. The findings of Brandt, Glaser & Jones (1958) suggest that the differences between our observations on heart muscle and Gilbert's observations on skeletal muscle represent real differences between heart and skeletal muscle; thus Brandt *et al.* found that 24 hr after injection of ^{28}Mg into dogs, heart muscle contained about 10 times more isotope (per dry weight of tissue) than skeletal muscle.

We have previously reported that 2–3 % of the cellular Mg in rat left ventricles is inexchangeable with ^{28}Mg even after prolonged glycerination

of the tissue (Page, Polimeni, Zak, Earley & Johnson, 1972). This fraction of cellular Mg is sequestered within the thin filament during the polymerization of actin (Oosawa, Asakura, Asai, Kasai, Kobayashi, Mibashi, Oii, Taniguchi & Nakano, 1964; Drabikowski & Strzelecka-Golszewska, 1963; Weber, Herz & Reiss, 1969). The present observation that $98 \pm 3\%$ of cellular Mg exchanges *in vivo* is consistent with the existence of a small sequestered fraction, although the dispersion of the data do not permit an exact estimation of it.

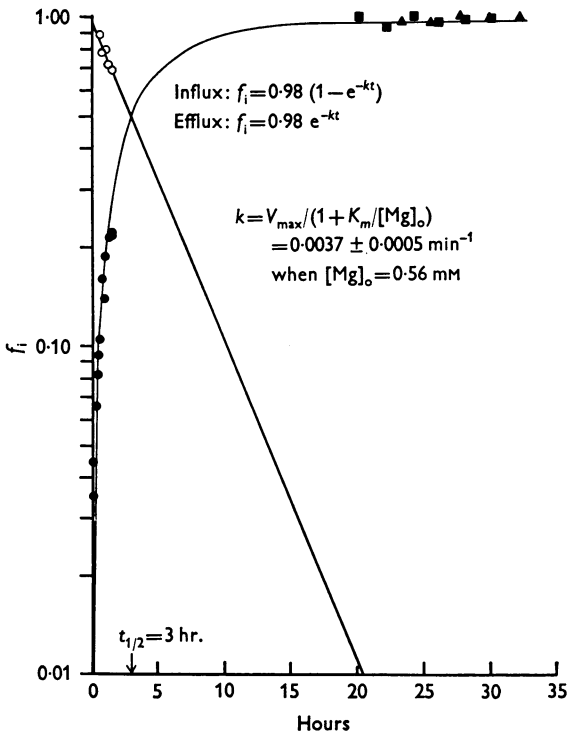

Fig. 7. Comparison of data obtained from *in vitro* and *in vivo* influx measurements and *in vitro* efflux measurements with curves calculated on the assumption that 98 % of cellular Mg exchanges at a single rate derived from the Lineweaver–Burk analysis. For $[Mg]_o$ = 0·56 mM, the exchange rate given by Fig. 5*B* is $0{\cdot}0037 \pm 0{\cdot}0005\ min^{-1}$. The *in vivo* data is compared with the calculated uptake curve on the assumption that the concentration of plasma Mg in the ionic state is approximately half that of total Mg. Experimental points were obtained as follows: (●) *in vitro* uptake of ^{28}Mg; (○) *in vitro* ^{28}Mg efflux into 0·56 mM solution following equilibration with ^{28}Mg *in vivo*; (■) *in vivo* uptake of ^{28}Mg; and (▲) *in vitro* ^{28}Mg efflux into Mg-free solution following equilibration with ^{28}Mg *in vivo*. The fact that the experimental points for the initial and terminal parts of the uptake, as well as the initial part of the efflux, fall on the predicted curves, supports the assumption of a single rate for the entire *in vivo* and *in vitro* exchange.

Calculation of Mg exchange per unit of sarcolemmal area

The ratios (area of external sarcolemma)/(cell volume) and (area of external sarcolemma + T-system)/(cell volume), as determined for five hearts by the application of point counting and lineal integration to electron micrographs (Page *et al.* 1971*a*; Mobley & Page, 1972) were $0{\cdot}30 \pm 0{\cdot}02\ \mu^2/\mu^3$ and $0{\cdot}38 \pm 0{\cdot}02\ \mu^2/\mu^3$, respectively. These two values correspond to absolute Mg fluxes of $0{\cdot}27 \pm 0{\cdot}02$ and $0{\cdot}21 \pm 0{\cdot}02$ p-mole Mg/(cm^2.sec), respectively. The magnitude of the Mg flux in rat left ventricles is thus of the same order as or slightly smaller than that reported for Ca fluxes in vertebrate heart muscle. For example, Niedergerke (1963) reported a total Ca-influx (resting + tension-related) of 0·034 p-mole/(cm^2.sec) at the much lower temperature of 7° C for frog hearts contracting at a rate of 10 beats/min; and Winegrad & Shanes (1962) found a Ca influx of 0·295 p-mole/(cm^2.sec) for guinea-pig atria contracting at 30 beats/min at 24–26° C.

The nature of the transport mechanisms for Mg

The simplest assumption is that the rate limiting process for the Mg exchange measured by us is localized to the sarcolemma and T-system. According to this assumption the exchange of Mg in the sarcoplasm, in mitochondria and in other cellular subcompartments would be fast relative to that across the plasma membrane. It is then necessary to consider the nature of the transport mechanism by which Mg crosses the plasma membrane.

This paper presents three observations which suggest that Mg transport may be carrier-mediated: a hyperbolic relation between the rate of exchange and $[Mg]_o$; a dependence of Mg efflux on $[Mg]_o$; and a low passive permeability to Mg. A low passive permeability seems probable from the fact that the rate of *in vitro* loss of cellular Mg to Mg-free solution is similar to the rate at which Mg is lost into a solution containing an approximately physiological concentration of Mg ion. (The slow net loss of Mg which occurs *in vitro* is in any case an unphysiological process which is absent *in vivo*.) A low passive Mg permeability in cardiac muscle is also implied by the observation that neither the resting nor the action potential is noticeably affected by changing $[Mg]_o$ by more than an order of magnitude (Brooks, Hoffman, Suckling & Orias, 1955). A low passive permeability accounts for the observation that Mg deficiency results in little or no measurable loss of cardiac Mg (Harrison, Wakim & Brown, 1971).

If Mg transport in heart muscle is carrier-mediated, it is essential to determine the electrochemical gradient for this ion in order to decide whether and in what direction Mg is actively transported. The definition

of the electrochemical gradient for Mg under the conditions of our experiments is problematical for two reasons: first, the chemical activity of Mg at the cytoplasmic face of the sarcolemma is unknown; and secondly, the membrane potential is varying cyclically with each action potential. By dividing the total cellular content of Mg by the cellular water content it is possible to obtain a nominal value of $17{\cdot}3 \pm 0{\cdot}3$ m-mole Mg/kg cell water for the intracellular Mg concentration. The chemical activity of Mg is probably much lower because most of the Mg is most likely present as salts of adenine nucleotides and inorganic phosphate. Chemical analyses of perfused rat hearts by Williamson (1966) have shown the following values for the adenine nucleotide and inorganic phosphate contents of this tissue (in m-mole/kg dry weight): ATP, 24·0; ADP, 2·62; and inorganic phosphate, 13·3. The distribution of these compounds within the cell is not known. Moreover, an exact calculation of the concentrations of ionized Mg in the presence of adenine nucleotides, of cellular binding sites for Mg, and of K ion is very complicated (Botts, Chashin & Schmidt, 1966). The large discrepancies in the reported binding constants of divalent cations and various ligands when different analytical procedures are used further complicate estimation of intracellular free Mg (Leeuwen, 1964).

These difficulties notwithstanding, it seems more probable that if there is an active transport of Mg in the rat heart, this transport is directed outward. At an external Mg concentration of 0·56 mM the equilibrium potential for Mg is -46 mV and -8 mV for internal Mg concentrations of 17 mM and 1 mM, respectively. The true value of the ionized Mg concentration is probably less than 1 mM. This conclusion is based on the observations of Ebashi & Endo (1968) in 'skinned' skeletal muscle fibres. These investigators found that the presumably physiological relation between developed tension and ionized Ca concentration was shifted to improbably high Ca concentrations when the ionized Mg concentration substantially exceeded 0·05 mM. If the effective membrane potential is indeed more negative than -8 mV, the outward transport of Mg would be against the electrochemical gradient. According to this interpretation, the observed dependence of cellular Mg exchange on $[Mg]_o$ would represent counter transport (Wilbrandt & Rosenberg, 1961). The active transport of Mg out of the cell would serve to keep the internal concentration of ionized Mg from rising to levels which might adversely affect the Ca dependence of the contractile mechanism.

A transport of Mg showing saturation kinetics has also been reported for cultured mammalian cells (Beauchamp, Silver & Hopkins, 1971) and for bacteria (Silver & Clark, 1971).

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