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Topical Review

Magnesium Transport across Cell Membranes

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Introduction

Magnesium is the second or third most abundant intracellular cation. It is a necessary co-factor for hundreds of enzymes and plays an essential role in protein synthesis. It stabilizes the structures of ribosomes and membranes and may be necessary for the insertion of proteins into membranes (Wacker & Vallee, 1964; Walser, 1967; Ebel & Günther, 1980; Wacker, 1980). Its important and wide-ranging effects within the cell have led to the suggestion that magnesium may be a second messenger co-ordinating cellular responses to changes in the environment (Rubin, 1977). The concentrations of ionized magnesium inside and outside the cell and the chemistry of magnesium make it unlikely that magnesium can have a 'trigger' function like calcium. However, slow small magnitude changes in concentration could be important in fine control and co-ordination of cell activity.

The concentration of magnesium inside cells is kept within narrow limits in spite of wide changes in magnesium concentration in the external medium under experimental conditions. This implies the existence of specialized magnesium transport systems, since magnesium can penetrate membranes, albeit slowly, and since magnesium concentration is kept well below electrochemical equilibrium. This review will focus on magnesium transport across cell membranes, with a brief look at magnesium transport in the kidney and gut.

Some biologically relevant chemical features of magnesium have been summarized by Williams (1970). Magnesium ions are small and highly polarizing and have a large hydrated size in solution. They pass with difficulty through small water-filled channels (pores), hence the low magnesium permeability of some membranes and epithelia. The existence of a highly specific, high-affinity magnesium binding site is unlikely because of the small size of the unhydrated ion, its strongly polarizing nature and its co-ordination number of 6. No high-affinity magnesium binding sites have been reported which do not bind calcium even better (e.g. EDTA). This lack of specific high-affinity binding immediately puts constraints on magnesium transport systems. However, there are binding sites with moderate affinities (K_d greater than 10^{-5} M) which select magnesium over calcium (e.g. ATP). The range of ionized magnesium concentrations in biological systems is normally greater than 10^{-5} M, so selective magnesium transport should be possible, and some examples will be discussed (I–VII).

Abbreviations

ADP: adenosine 5′-diphosphate; ATP: adenosine 5′-triphosphate; cAMP: adenosine 3′:5′-cyclic monophosphate; CCCP: carbonylcyanide *m*-chlorophenyl-hydrazone; 2,4-DNP: 2,4-dinitrophenol; *E:* membrane potential in volts; EDTA: ethylenediaminetetraacetic acid; EGTA; ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; *F:* Faraday constant; FCCP: carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; IAA: iodoacetic acid; K_d: dissociation constant; NEM: N-ethylmaleimide; *R:* gas constant; *T:* absolute temperature; TEA: tetraethylammonium; TTX: tetrodotoxin; $[X_o]$: concentration of X in medium; $[X_i]$: concentration of X inside cell.

I. Squid Axons

The concentration of ionized magnesium in squid axoplasm is about 3 mM (Table 1) which is far below the electrochemical equilibrium level of 1.6 M expected with a membrane potential of −60 mV and with 15 mM ionized magnesium in the haemolymph. Squid axons must therefore have active magnesium

Key Words magnesium transport · ionized magnesium · membrane transport

Table 1. Magnesium concentrations in squid giant axons

Species	Mg in axoplasm		Mg in haemolymph		Reference
	Total (mmol/kg axoplasm)	Ionized (mM)	Total (mmol/kg haemolymph)	Ionized (mM)	
Loligo forbesi	6.4 ± 0.8	2.3 – 3.5[a]	—	—	Baker & Crawford, 1972
Loligo pealei	6.7 ± 0.5	3 – 4	43.5 ± 1.9	—	De Weer, 1976
Loligo pealei	—	3 – 3.5	—	—	Brinley & Scarpa, 1975
Doryteuthis plei	4.2 ± 0.2	—	57 ± 1.0	≈15	Caldwell-Violich & Requena, 1979

[a] Calculated from the data of Baker and Crawford assuming that the water content of axoplasm is about 87% (value given for *Loligo pealei* by Koechlin, 1955).

transport systems, since magnesium can cross axon membranes. The brief reversals of the electrochemical gradient during the peak of the action potential (the magnesium equilibrium potential E_{Mg}, is about +20 mV) do not help maintain the low internal concentration since squid axons gain a small quantity of magnesium averaged over the entire action potential (*see* Caldwell-Violich & Requena, 1979 and later).

The amount of energy required to expel 1 mol magnesium ions from axons is about 15.5 kJ when the membrane potential is −60 mV. This energy could be provided either by the breakdown of some high energy compound (the hydrolysis of ATP under physiological conditions yields about 50 kJ mol^{-1}) or by coupling the movement of magnesium to the movement of another molecule down its electrochemical gradient.

Magnesium Efflux

^{28}Mg effluxes from injected *Loligo forbesi* and *Loligo pealei* axons are 1 pmol cm^{-2} sec^{-1} (Baker & Crawford, 1972) and 3 pmol cm^{-2} sec^{-1} (De Weer, 1976), respectively. ^{28}Mg efflux from *Loligo pealei* axons dialyzed with a solution containing physiological concentrations of ATP, magnesium and sodium is also about 3 pmol cm^{-2} sec^{-1} (Mullins et al., 1977). The Q_{10}s of the fluxes range between 3 and 5 (Baker & Crawford, 1972; De Weer, 1976) suggesting facilitated transport rather than passive diffusion. The measured flux is unlikely to reflect magnesium-magnesium exchange since it increased when external magnesium was removed (Baker & Crawford, 1972). It is inhibited by D600 and lanthanum (De Weer, 1976) but not by high doses of ouabain (Baker & Crawford, 1972).

Depolarization of the membrane potential by increasing the external potassium concentration does not affect either magnesium efflux (De Weer, 1976) or the magnesium content of axons (Caldwell-Violich & Requena, 1979). These findings suggest that magnesium transport is electroneutral, although some complex interaction between membrane potential and potassium binding cannot be ruled out.

^{28}Mg efflux is not affected by removal of calcium or potassium from the medium. Removal of external sodium inhibits magnesium efflux by 70 to 80% in injected axons (Baker & Crawford, 1972; De Weer, 1976) but by only 33% in perfused axons (Mullins et al., 1977). This difference cannot as yet be explained. The sensitivity of efflux to sodium removal is not affected by changes in the concentrations of internal ATP or magnesium (Mullins et al., 1977). Baker and Crawford (1972) have plotted the sodium-dependent magnesium efflux as a function of external sodium concentration. Their data were well described by Michaelis-Menten kinetics if sodium stimulates efflux by binding to a single external site at which magnesium could compete. 100 mM sodium half-maximally stimulates efflux when the external magnesium concentration is 55 mM. De Weer (1976) reports similar findings and has shown that the concentrations of sodium needed for half-maximal efflux are 39, 210 and 270 mM, with magnesium concentrations of 0, 50 and 100 mM, respectively. Increasing the internal sodium concentration by injection (Baker & Crawford, 1972) or perfusion (Mullins et al., 1977) markedly reduces magnesium efflux, and the sensitivity of this response is independent of internal ATP concentration (Mullins et al., 1977).

The findings described above suggest that magnesium efflux depends on the size of the sodium gradient across the membrane. The simplest explanation is that magnesium efflux is driven by sodium influx down its electrochemical gradient. The kinetic studies of Baker and Crawford (1972) and De Weer (1976) further suggest that a single sodium ion exchanges for each magnesium ion. However, this latter suggestion is contrary both to the apparent electroneutrality of magnesium efflux and to ther-

modynamic predictions that more than one sodium ion must enter for each magnesium ion expelled. It can be shown that if magnesium movement is driven solely by the energy of the sodium gradient then

$$\left(\frac{[Mg_o^{2+}]}{[Mg_i^{2+}]}\right) \leq \left(\frac{[Na_o^+]}{[Na_i^+]}\right)^r \exp\left(\frac{EF(2-r)}{RT}\right)$$

where r is the number of sodium ions exchanging with each magnesium ion (coupling ratio). The ratio $[Mg_o^{2+}]/[Mg_i^{2+}]$ is about 5 (*see* Table 1) and the ratio $[Na_o^+]/[Na_i^+]$ is normally taken as about 10, though estimates range between 7 and 15 (Koechlin, 1955; Hinke, 1961; Caldwell-Violich & Requena, 1979). Thus, if the sodium gradient alone is to drive magnesium transport, r must have a value of at least 1.4 when the membrane potential is −60 mV. The apparent contradiction between kinetic behavior and thermodynamics has still to be resolved. However, Baker and Crawford's (1972) data would be equally compatible with a system in which magnesium efflux is activated by sodium binding to two identical sites with apparent affinities of about 30 mM.

The sodium influx which should accompany the observed magnesium efflux of about 3 pmol cm^{-2} sec^{-1} is 6 pmol cm^{-2} sec^{-1} if the electroneutral model is accepted. Brinley and Mullins (1968) have described an increased sodium influx of about 12 pmol cm^{-2} sec^{-1} when ATP is added to a sodium-free fluid perfusing squid axons. Part of this sodium influx may well be associated with magnesium efflux, facilitated by ATP.

In the absence of external sodium, increasing the external magnesium concentration stimulates magnesium efflux (Baker & Crawford, 1972; De Weer, 1976) suggesting that Mg/Mg exchange may occur in sodium-free media. In the absence of both sodium and magnesium, removal of calcium produces a marked stimulation (10- to 20-fold) of magnesium efflux which is inhibited by small concentrations of lanthanum (De Weer, 1976). The magnesium efflux seen in the absence of external sodium, magnesium and calcium could be passive leakage from the axon or may represent the expulsion of magnesium by a calcium transport system.

Magnesium efflux is reduced by 85 to 95% when internal ATP is reduced by treating squid axons with cyanide (Baker & Crawford, 1972; Mullins et al., 1977) or apyrase (De Weer, 1976). Magnesium efflux is independent of internal magnesium concentration when the axons are perfused with 3 mM ATP but increases linearly with increasing magnesium concentration in the absence of ATP, reaching the same level as in controls (normal ATP) when internal magnesium concentration is 16 mM (Mullins et al., 1977). Mullins et al. (1977) have suggested that ATP acts catalytically to increase the affinity of the transport system for internal magnesium and have shown that ATP stimulates magnesium efflux both in the presence and absence of external sodium. In both cases 0.35 mM ATP is required to produce half-maximal stimulation. Thus ATP must stimulate not only Na/Mg exchange but also some other magnesium transport system. Their work demonstrates that Na/Mg exchange can occur in the absence of ATP and that magnesium efflux seen in the absence of ATP is not mere leakage, since increasing the sodium concentrations in the ATP-free perfusate decreases magnesium efflux. It has not yet been discovered whether ATP hydrolysis accompanies magnesium efflux.

Magnesium Influx

^{28}Mg influx into *L. forbesi* axons bathed in artificial seawater is about 0.62 pmol cm^{-2} sec^{-1} (Baker & Crawford, 1972). The higher figure of 3 pmol cm^{-2} sec^{-1} has been measured for *L. pealei* axons (Mullins & Brinley, 1978). Influx into perfused axons is critically dependent on the sodium and ATP contents of the perfusate (Mullins & Brinley, 1978). Influx is about 1 pmol cm^{-2} sec^{-1} when the perfusate contains no ATP or sodium, it rises to 2.5 pmol cm^{-2} sec^{-1} in the presence of 3 to 4 mM ATP and further increases to 3 pmol cm^{-2} sec^{-1} when 80 to 100 mM sodium is added to the perfusate. This latter figure is close to that found in intact axons. The low value of 0.12 pmol cm^{-2} sec^{-1} obtained by Rojas and Taylor (1975) may be attributed to the absence of sodium and ATP in their perfusion fluids. Magnesium influx increases slightly when external sodium is replaced by choline (Baker & Crawford, 1972) but increases five- to 10-fold when sodium is replaced by lithium (Baker & Crawford, 1972; Mullins & Brinley, 1978).

Magnesium influx, like efflux, thus depends on the size of the sodium gradient across the membrane and a component of it probably occurs through Na/Mg exchange. ATP markedly accelerates magnesium influx both through Na/Mg exchange and through some other route.

Net Magnesium Fluxes

Many of the conclusions reached above have been corroborated by Caldwell-Violich and Requena (1979) in their studies of net magnesium fluxes in axons from *Doryteuthis plei*. These axons excrete a magnesium load against a steep electrochemical gradient only when sodium is present in the me-

dium. This is also found in axons depleted of ATP by IAA and FCCP. However, in these axons the final magnesium level is higher than in axons containing ATP. The rates of magnesium movement are similar in depleted and nondepleted axons. In the absence of sodium the axons gain rather than lose magnesium. The amount gained is greater when sodium is replaced by lithium rather than by choline or sucrose. Similar behavior is seen in ATP-depleted axons but the magnesium content is always 30% lower in the presence of ATP. The larger magnesium uptake seen in the presence of lithium might be explained as follows. In the absence of external sodium magnesium efflux is strongly inhibited but influx is also inhibited because of the fall in internal sodium. Lithium, however, enters the axons and may activate magnesium influx, perhaps by replacing sodium on the Na/Mg exchanger. Replacement of external sodium by lithium also causes a larger stimulation of calcium influx into squid axons than does replacement by dextrose (Baker et al., 1969). In this case the data were interpreted as indicating that lithium binds to an external activation site.

Magnesium Fluxes during Action Potentials

Just as squid axons show a net gain of calcium during the action potential, so do they also show a net gain of magnesium. The extra magnesium influx during an action potential is about 0.007 pmol cm^{-2} from media containing 55 mM magnesium (Baker & Crawford, 1972). It rises steeply with magnesium concentration, reaching about 0.1 pmol cm^{-2} when axons are bathed in 112 mM magnesium. This is comparable to the extra calcium influx seen in axons bathed in 112 mM calcium (Hodgkin & Keynes, 1957). 55 mM manganese blocks magnesium influx just as it blocks calcium entry via the late calcium channel (Baker, Hodgkin & Ridgway, 1971). Rojas and Taylor (1975) have shown that magnesium influx increases during depolarizing voltage clamp pulses, to reach a maximum after about 1 msec. Influx is not blocked by external TTX or internal TEA. Thus magnesium, unlike calcium, does not appear to move through the fast TTX-sensitive sodium channel. This same study shows that magnesium influx is about 3 times greater than calcium influx under comparable conditions. There is also an increase of about 0.01 pmol cm^{-2} per impulse in magnesium efflux during the action potential (De Weer, 1976). The net effect of these changes is that axons show an overall gain of about 0.3 pmol magnesium cm^{-2} per impulse when the external magnesium concentration is 250 mM (Caldwell-Violich & Requena, 1979). The net change in the magnesium content of axons bathed in physiological media (55 mM magnesium) during the action potential has not yet been measured.

Summary

Magnesium fluxes across squid axons are about 3 pmol cm^{-2} sec^{-1} under physiological conditions. This is about 30 times greater than calcium fluxes under similar conditions (*see* Di Polo & Beaugé, 1983). Internal magnesium is kept well below electrochemical equilibrium by active magnesium extrusion and the axon uses considerably more energy to maintain its magnesium content than to maintain its calcium content (*see*, for instance, Brinley, 1973). Na/Mg exchange accounts for the majority of magnesium efflux and for some of the influx. Other, sodium-independent magnesium transport systems also exist. ATP appears to activate both Na/Mg exchange and sodium-independent systems. ATP hydrolysis associated with magnesium transport has not been demonstrated and it is not clear whether an ATP driven "magnesium pump" exists.

Na/Mg exchange is strikingly similar to Na/Ca exchange and perhaps shares the same carrier (Brinley, Spangler & Mullins, 1975; Mullins, 1981). If this is so, in the presence of physiological calcium concentrations (70 nM) the carrier would mainly be involved in magnesium transport. Increasing the calcium concentration to unphysiologically high levels (as in many perfusion experiments) would allow calcium to compete effectively with magnesium for the carrier, resulting in Na/Ca exchange.

It is interesting to note that although Na/Mg exchange seems likely, no one has yet identified a magnesium-dependent component of sodium influx or sodium efflux.

II. Barnacle Muscle

Active magnesium extrusion might be expected in barnacle muscle fibers. The membrane potential of these fibers is between −50 and −60 mV (Ashley & Ellory, 1972) and internal ionized magnesium concentration is about 4 to 6 mM (Ashley & Ellory, 1972; Brinley, Scarpa & Tiffert, 1977). External ionized magnesium concentration would have to be 0.2 mM to be at electrochemical equilibrium under these conditions. It is certainly higher than this (commonly used crustecean Ringer's solution contains about 24 mM magnesium; Fatt & Katz, 1953), which suggests that magnesium is kept well below electrochemical equilibrium in the fibers.

Magnesium efflux has been measured from fibers of *Balanus nubilus* injected with ^{28}Mg (Ashley

& Ellory, 1972). Efflux is 6 to 12 pmol cm^{-2} sec^{-1} (assuming cylindrical fibers). This represents about 0.4 to 0.8 pmol cm^{-2} sec^{-1} if the area of the complex cleft system is taken into account. Efflux has a Q_{10} of about 3 to 4 and is not blocked by ouabain, D600 or by depolarizing the membrane potential with 100 mM external potassium. Efflux is reversibly inhibited by increasing external magnesium concentration to 100 mM, suggesting that it does not merely represent Mg/Mg exchange (*see* Ashley, Ellory & Hainaut, 1974). However, Brinley (1973) and Vogel and Brinley (1973) reported that 50% of magnesium efflux from perfused fibers may be attributed to Mg/Mg exchange. Efflux is inhibited by adding any of the following to the medium: 100 mM calcium, 32 mM manganese, 32 mM cobalt, 2 mM gadolinium or 1 mM lanthanum (Ashley & Ellory, 1972). This may be attributed to a nonspecific effect of external di- or trivalent cations on cation transport (Ellory, Flatman & Stewart, 1983). Replacement of external sodium by lithium, choline or sucrose causes a large, reversible inhibition of magnesium efflux, but increasing the internal sodium concentration more than twofold has no consistent effects on efflux. Reduction of internal sodium concentration to 2 mM by perfusion causes a 350% stimulation of magnesium efflux (Vogel & Brinley, 1973).

One explanation of these data is that internal magnesium is controlled by electroneutral Na/Mg exchange with two sodium ions entering for each magnesium ion leaving. The transport system is thought to be similar to, but distinct from, the Na/Ca exchange system which also exists in these fibers, since NEM has opposite effects on calcium and magnesium transport (Ashley et al., 1974). Electroneutral exchange is compatible with a membrane potential of −60 mV and a sodium gradient of 33 (Ashley & Ellory, 1972). In fact, the minimum coupling ratio is about 1.5 sodium ions per magnesium ion under these conditions. Brinley (1973) objects to the idea that all magnesium efflux is via Na/Mg exchange, since about 30% of the energy available from the sodium pump would then be utilized in controlling the magnesium content of muscle fibers. The existence of a separate magnesium transport system is certainly compatible with the observations of Ashley and Ellory (1972).

III. Vertebrate Muscle

Magnesium content is kept below electrochemical equilibrium in skeletal, smooth and cardiac muscle. The total magnesium concentration in all these tissues is about 17 mM (Fenn & Haege, 1942; Gilbert, 1960; Walser, 1967; Sparrow, 1969; Moawad & Daniel, 1971; Palatý, 1971; O'Donnell &-Kovács, 1974) of which about 0.5 to 3 mM is ionized (Table 2). The concentration of ionized magnesium in interstitial fluid is probably about 0.5 mM (Walser, 1961, 1967; Heaton, 1967) so that E_{Mg} is 0 to −20 mV. The membrane potential is far more negative than this in resting muscle, so that magnesium extrusion must occur against an electrochemical gradient. In cardiac muscle, however, the membrane potential can be more positive than E_{Mg} during the plateau of the action potential, and this may help to drive magnesium efflux and help to maintain the muscle's low magnesium content. Between 12 and 17 kJ mol^{-1} of energy are required to expel magnesium from muscle fibers when the membrane potential is −90 mV. This is similar to the amount of energy contained in the sodium gradient across the muscle membrane and is about $\frac{1}{3}$ of the energy available from the hydrolysis of 1 mol of ATP.

The complex structure of vertebrate muscle makes it difficult to interpret the net and unidirectional fluxes of magnesium which have been observed. Measurements of magnesium uptake may represent the movement of magnesium into the extracellular space and perhaps binding there, or the movement of magnesium into the muscle cell itself. Movement into the cell is further complicated by uptake by cytoplasm, mitochondria and nucleus.

Fenn and Haege (1942) have shown that frog skeletal muscle takes up magnesium when incubated in media containing more than 4 mM magnesium and loses magnesium when incubated in media containing less than 1 mM magnesium. With solutions containing between 1 and 4 mM magnesium, the small gain in tissue magnesium content could be accounted for by magnesium trapped in the extracellular space. The finding that magnesium can move across muscle membranes is supported by the work of Brandt, Glaser and Jones (1958) and Rogers and Mahan (1959). Both groups have examined the uptake of ^{28}Mg after intravenous injection. Heart muscle takes up magnesium faster than skeletal muscle. The uptake by skeletal muscle occurs in two equal components (Rogers & Mahan, 1959), one rapid ($t_{1/2} \cong 1.2$ hr) and one slow ($t_{1/2} \cong 25$ hr). Stimulation of skeletal muscle by a pacemaker increases the rate of uptake in rat (Rogers & Mahan, 1959) but not in dog (Brandt et al., 1958).

Magnesium transport in rat ventricle muscle has been carefully examined by Page and Polimeni (1972). ^{28}Mg injected into the animal equilibrates with ventricle muscle in about 20 hr. This *in vivo* finding has been confirmed with *in vitro* measurements of ^{28}Mg uptake by ventricle perfused with Krebs-Henseleit solution containing 0.56 mM magnesium. 98% ventricular magnesium exchanges

Table 2. Concentration of ionized magnesium in muscle

Tissue	Value (mM)	Method	Reference
Frog skeletal	3 – 4.4	Characteristics of ^{31}P NMR spectrum of phosphocreatine. Value depends on value chosen for the dissociation constant between phosphocreatine and magnesium. Measurement made at 4°C.	Cohen & Burt, 1977
Frog skeletal		Absorbance of light by metallochromic dyes at wavelengths chosen to minimize interference from Ca^{2+} and H^+. Measurement made at room temperature.	Baylor, Chandler & Marshall, 1982
	0.5 – 1.2	Arsenazo III	
	0.2 – 0.3	Dichlorophosphonazo III	
	3.0 – 6.0	Arsenazo I	
Frog skeletal	3.3 ± 0.4	Magnesium-selective microelectrode at room temperature	Hess, Metzger & Weingart, 1982
Frog skeletal	0.6	Characteristics of ^{31}P NMR spectrum of ATP. Value depended on the dissociation constant between ATP and magnesium. Measurement made at 25°C.	Gupta & Moore, 1980
Frog skeletal	3.4	Calculated from the known concentrations and dissociation constants of the identified Mg chelators in muscle and total Mg concentration.	Nanninga, 1961
Rabbit heart	1.5 – 3.6	Membrane of heart muscle made permeable to small molecules by exposing it to solutions with low Ca content for 12 min and then perfusing with solution containing Ca.	Paradise, Beeler & Visscher, 1978
Rat heart	<1	Examination of properties of many Mg-dependent processes in the cell	Page & Polimeni, 1972 Polimeni & Page, 1973
Rat heart	2.5	Magnesium content and transport properties of mitochondria isolated from tissue. Measurements made at 25°C.	Crompton, Capanno & Carafoli, 1976
Sheep purkinje	3.5 ± 0.3	Magnesium-selective microelectrode at room temperature	Hess, Metzger & Weingart, 1982
Sheep ventricle	3.1 ± 0.4		
Ferret ventricle	3.0		
Rat tail artery smooth muscle	<0.1	Magnesium efflux from arteries incubated at 37°C in magnesium-free media containing 2 deoxyglucose	Palatý, 1971

with ^{28}Mg with a rate constant of 0.0038 min^{-1} (= 0.15 nmol mg dry wt^{-1} min^{-1}) which is equivalent to a flux of about 0.21 pmol cm^{-2} sec^{-1}. Exchange rate is unaffected by the external load against which the heart is pumping (Polimeni & Page, 1973) but magnesium uptake is stimulated by increasing the external magnesium concentration. Uptake could be explained by Michaelis-Menten kinetics in which a saturable magnesium carrier has an affinity for external magnesium of 0.57 mM and is able to carry a maximum flux of 0.3 nmol mg dry wt^{-1} min^{-1}. The same workers have shown that ^{28}Mg efflux also occurs. It has a rate constant of 0.0038 min^{-1} and is stimulated by increasing the external magnesium concentration, suggesting that some Mg/Mg exchange occurs here. These tracer studies indicate a low magnesium permeability and this is confirmed by the observation that ventricular magnesium concentration is identical whether perfused with solution containing 0 or 0.56 mM magnesium. In both cases a small fall in magnesium content occurs.

Späh & Fleckenstein (1979) have shown that partially depolarized cardiac muscle can produce action potentials when incubated in media containing high concentrations of magnesium. They suggest that in cardiac muscle a separate magnesium channel exists which allows magnesium movements and thus current flow during the action potential. The channel is blocked by β-adrenergic agonists. However, Kiyosue et al. (1982) challenge the idea that these action potentials are due to current flow through a magnesium channel. They have shown that high external magnesium concentrations increase the depolarization needed to inactivate the fast sodium channels so that inward current may still flow through these in partially depolarized muscle.

There have been several confirmations of Fenn and Haege's (1942) demonstration that magnesium can enter or leave frog skeletal muscle depending on the external magnesium concentration (Gilbert, 1960; O'Donnell & Kovács, 1974; Ling, Walton & Ling, 1979). Gilbert (1960) has demonstrated rapid net fluxes. Uptake is about 0.25 nmol mg dry wt^{-1} min^{-1} from a solution containing 10 mM magnesium and a new steady state is attained after 3 hr. Steady-

state fiber magnesium concentration increases linearly with external magnesium concentration. These gains are at least 3 times larger than would have been expected if magnesium were confined to the extracellular space, suggesting that magnesium does indeed enter the cells. Similar results are reported by Ling et al. (1979). Starvation leads to the loss of 40% internal magnesium probably due to depletion of intracellular magnesium chelators such as ATP. ^{28}Mg uptake by frog muscle from a solution containing 2 mM magnesium occurs in three phases (Gilbert, 1960). Two small rapid phases probably represent equilibration of magnesium into the extracellular space and binding to the cell surface. The third and slowest phase may be attributed to ^{28}Mg exchanging with intracellular magnesium with a rate constant of 0.0085 min^{-1}. Only 10% of intracellular magnesium is exchangeable so the influx is about 0.03 nmol mg dry wt^{-1} min^{-1}. Assuming each gram of wet muscle has 300 cm^2 of surface area (Bianchi & Shanes, 1959) this is equivalent to a flux of 0.33 pmol cm^{-2} sec^{-1}. The high rate of turnover of magnesium chelating compounds (ATP, phosphocreatine) at 37°C in beating mammalian heart, and the higher rate of magnesium entry, probably account for the rapid and complete equilibration of ^{28}Mg in these tissues (Page & Polimeni, 1972). Gilbert (1960) has used frog muscles in a quiescent unfed state at 22°C, so that magnesium entry is much slower and turnover of chelators would be much lower. Gilbert (1961) has also shown that magnesium uptake is increased in alkaline solution and depressed in acid solution. This may reflect an effect of pH on the transport system or the fact that magnesium chelation is increased in alkaline solutions. Gilbert (1960) has also estimated that 1% muscle energy is utilized in controlling its magnesium content.

O'Donnell (1973) has shown that ^{28}Mg influx into frog skeletal muscle is similar to that of calcium influx under the same conditions. The low values obtained for magnesium influx are probably due to the prolonged wash periods used in his experiments.

There have been several studies of magnesium transport in smooth muscle. Sparrow (1969) has shown that in guinea pig taenia coli all intracellular magnesium exchanges with ^{28}Mg with a time constant of 0.0017 min^{-1} (1.2 mM external magnesium). Transmembrane flux is therefore 0.04 nmol mg dry wt^{-1} min^{-1} which is equivalent to about 0.04 pmol cm^{-2} sec^{-1} assuming that taenia coli has a surface area of 3.3×10^3 cm^2 per gram wet wt (Goodford, 1967). Magnesium content of muscles incubated in media containing 1.2 mM magnesium rises from 6 to 16 mmol kg wet wt^{-1} when external sodium is replaced by sucrose. It falls again quickly when the sodium is put back. Thus these muscles are unable to maintain a low magnesium content in the absence of an inward sodium gradient, suggesting that Na/Mg exchange may control magnesium content. Calcium may compete with magnesium at the carrier, since ^{28}Mg exchange is slower and magnesium content lower in the presence of high concentrations of external calcium.

Rat uterine smooth muscle loses magnesium when incubated in magnesium-free media (Moawad & Daniel, 1971). The rate of loss is increased by the poisons 1AA and 2,4-DNP which also cause the loss of magnesium from muscles incubated in solutions containing physiological magnesium levels. The effects of these poisons are difficult to assess since they could either directly affect the transport system, or reduce the levels of fuels for transport, or else increase the concentration of intracellular ionized magnesium by reducing the levels of magnesium chelators and by causing release from organelles. An increased level of ionized magnesium seems the most likely explanation of the increased efflux in these experiments. An increase in external calcium concentration increases magnesium loss from poisoned axons into magnesium-free media. This may be the result of a direct effect of calcium on the transport system or perhaps of Ca/Mg exchange. Alternatively, calcium may have entered the cells and displaced bound magnesium and also caused a more rapid depletion of magnesium chelators such as ATP. The resulting increase in ionized magnesium concentration could then have stimulated efflux. Treatment of uterine muscle with 1 mM ouabain does not affect magnesium content. ^{28}Mg equilibration across the membrane is slow, reaching about 25% in 6 hr.

The magnesium content of rat vascular smooth muscle rises with external magnesium concentration both in the presence (Palatý, 1971) and absence (Palatý, 1974) of external calcium. However, with external magnesium levels below 0.6 mM, internal magnesium concentrations are much lower in the absence of external calcium than in its presence. This might indicate either an interaction between calcium and magnesium transport or more likely an increase in the leakiness of the membrane in the absence of divalent cations. Poisoning the fibers with 2,4-DNP and 1AA increases the rate at which magnesium is lost into magnesium-free media, perhaps because of an increase in the concentration of intracellular free magnesium by any of the mechanisms previously described.

Removal of all but 21 mM sodium from media containing 1.2 mM magnesium causes a large increase in fiber magnesium content which returns to

control levels when sodium is replaced (Palatý, 1971). Treatment of vascular muscle with ouabain causes an increase in both sodium and magnesium concentrations and both return to normal when ouabain is washed away (Palatý, 1974). The entry of magnesium into ouabain-treated muscle is drastically reduced by increasing the external calcium concentration. Palatý (1974) has also brought about changes in the sodium content of muscle by manipulating the external potassium concentration. The magnesium content of these muscles increases with internal sodium concentration at fixed sodium and magnesium concentrations in the medium. Unfortunately the effects on magnesium content of changes in the membrane potential resulting from the changes in potassium concentration have not been assessed. Palatý's data, however, strongly suggest that Na/Mg exchange plays a role in the control of magnesium content of vascular smooth muscle and that calcium may compete with magnesium for transport.

IV. Mitochondria

Mitochondria from rat liver and heart contain about 20 to 40 nmol magnesium mg protein^{-1} (Johnson & Pressman, 1969; Bogucka & Wojtczak, 1971; Binet & Volfin, 1974; Schuster & Olson, 1974; Crompton, Capano & Carafoli, 1976; Kun, 1976; Åkerman, 1981) and bovine vascular smooth muscle mitochondria contain about 70 nmol magnesium mg protein^{-1} (Sloane, Scarpa & Somlyo, 1978). Between 40 and 80% of the magnesium is found in the matrix and the majority of the rest in the intermembrane space (Bogucka & Wojtczak, 1971; Kun, 1976; Åkerman, 1981). Assuming that in all cells mitochondrial water content is about 1 μl mg protein^{-1}, the magnesium concentration in mitochondria is in some tissues (e.g. skeletal muscle) similar to the cytosolic concentration and in others several times higher. Thus, the fraction of cell magnesium inside mitochondria is similar to the fraction of cell volume occupied by mitochondria. The latter varies widely. For instance about 18% of the volume of rat liver cells is occupied by mitochondria (Loud, 1962) whereas the proportion in rat ventricular muscle exceeds 36% (Page, 1978). It is not yet known whether this mitochondrial pool of magnesium is more or less labile than the bound cytoplasmic pool. The large size of the pool means, however, that mitochondria could be important in controlling cytoplasmic magnesium concentration.

Detailed thermodynamic analysis of magnesium transport in mitochondria is not possible since the concentration of ionized magnesium in the matrix is not known. Mitochondria have very large potentials across their inner membranes, the matrix being 150 to 180 mV negative with respect to the cytosol (Åkerman & Nicholls, 1983). The concentration of magnesium in the matrix would have to be about 10^5 to 10^6 times greater than the cytosolic ionized concentration if magnesium were to come into electrochemical equilibrium. This would predict a matrix concentration in excess of 50 M which is far greater than the total magnesium concentration. Magnesium is thus kept well below electrochemical equilibrium, with passive influx and active efflux.

Mitochondria take up magnesium by a respiration-dependent mechanism (Brierley et al., 1963; Carafoli, Rossi & Lehninger, 1964; Judah et al., 1965; Johnson & Pressman, 1969; Schuster & Olson, 1974; Crompton, Capano & Carafoli, 1976; Kun, 1976; Sloane et al., 1978; Diwan et al., 1979) which is associated with efflux of protons (Brierley et al., 1963; Judah et al., 1965) and probably requires phosphate in the medium (Brierley et al., 1963; Crompton et al., 1976). Uncoupling agents such as FCCP which cause the collapse of both the proton gradient and membrane potential inhibit influx (Johnson & Pressman, 1969; Schuster & Olson, 1974) and can cause the release of accumulated magnesium (Kun, 1976). The amount of magnesium accumulated increases with the magnesium concentration in the medium (Crompton et al., 1976; Kun, 1976; Diwan et al., 1979). ^{28}Mg influx into rat liver mitochondria appears to occur through a carrier which is half-maximally activated by 0.7 mM magnesium and has a maximal transport rate of 0.9 nmol mg protein^{-1} min^{-1} (Diwan et al., 1979). A similar rate of 0.3 to 0.4 nmol mg protein^{-1} min^{-1} is reported by Johnson and Pressman (1969). Kun (1976), however, reports a high rate of about 8 nmol mg protein^{-1} min^{-1} in rat liver mitochondria treated with digitonin to reduce lysosomal contamination. This treatment may have also damaged the mitochondria. High rates of net magnesium movement (5 nmol mg protein^{-1} min^{-1}) have been observed in rat heart mitochondria (Crompton et al., 1976).

Magnesium uptake by mitochondria is inhibited by adding ADP to the medium and this inhibition is prevented by oligomycin (Brierley et al., 1963; Crompton et al., 1976). This suggests that mitochondria undergoing oxidation-phosphorylation cannot take up magnesium. Magnesium uptake by liver mitochondria is stimulated by parathormone (Johnson & Pressman, 1969).

The movements of calcium and magnesium into mitochondria are probably by different routes. Lanthanum, which blocks calcium entry, has little effect on magnesium entry (Kun, 1976; Diwan et al., 1979). ATP, which supports calcium uptake, has

only a weak effect on magnesium uptake, and acetate, which can replace phosphate in supporting calcium uptake, is ineffective in magnesium uptake (Crompton et al., 1976). Various other factors, such as the relationship between the amount of magnesium transported and respiration rate, the kinetics of transport, and the effects of NEM on transport, also suggest separate magnesium and calcium entry routes (Carafoli et al., 1964; Diwan et al., 1979). Diwan et al. (1979) have pointed out that magnesium transport in fact parallels to potassium transport under a variety of conditions. The potassium analog thallium is a competitive inhibitor of magnesium transport, and magnesium is known to be a competitive inhibitor of potassium transport. Crompton et al. (1976) have shown that ruthenium red, a potent blocker of calcium uptake, does not affect succinate-supported magnesium uptake. These results contrast with those of Kun (1976) who has shown that ATP can support magnesium uptake in digitonin-treated mitochondria and that ruthenium red blocks this uptake. Calcium uptake is affected in the same way. The discrepancy is difficult to resolve but may result from the digitonin treatment, or tissue-dependent differences in mitochondria, or perhaps the high levels of magnesium used by Kun allowed competition for the calcium transport system.

Magnesium Efflux from Heart Mitochondria

Rat heart mitochondria suspended in magnesium-free media lose magnesium at a rate of about 1 to 2 nmol mg protein^{-1} min^{-1} (Crompton et al., 1976; Åkerman, 1981). Increasing the external magnesium concentration reduces the loss so that none occurs when the medium contains 2.5 mM magnesium and a net magnesium gain occurs at higher concentrations (Crompton et al., 1976). Efflux is energy-dependent and is blocked by respiratory inhibitors such as antimycin A and by uncouplers such as FCCP (Crompton et al., 1976). It is unaffected by ruthenium red and by low doses of lanthanum (Crompton et al., 1976; Åkerman, 1981) but is stimulated by phosphate and by oligomycin (Crompton et al., 1976). ADP is a potent inhibitor of efflux and this effect may be prevented by adding phosphate to the medium or by pretreating the mitochondria with oligomycin or with the adenine nucleotide translocase inhibitor atractyloside. Efflux can thus occur during oxidative phosphorylation and requires internal phosphate. The latter idea is supported by the finding that magnesium efflux is blocked by calcium only when phosphate is absent from the medium. The calcium effect is itself blocked by ruthenium red which prevents calcium from entering the mitochondria and thus from lowering internal phosphate concentration (Crompton et al., 1976). These data also suggest that magnesium and calcium have separate transport pathways in heart mitochondria.

Åkerman (1981) has shown that magnesium efflux is inhibited by nigericin which abolishes the proton gradient across the mitochondrial membrane but does not affect membrane potential. On the other hand it is stimulated by valinomycin which enhances the proton gradient while reducing the membrane potential. These results suggest that the energy to drive magnesium efflux is provided by the proton gradient and that transport may occur via Mg/H exchange.

Magnesium Efflux from Smooth Muscle Mitochondria

Respiration-dependent magnesium release has also been seen in vascular smooth muscle mitochondria (Sloane et al., 1978). The efflux is about 10 times faster than that in heart mitochondria and is stimulated by magnesium in the medium. It is inhibited by FCCP and antimycin A.

Magnesium Efflux from Liver Mitochondria

Rat liver mitochondria, suspended in a medium containing phosphate but no magnesium, lose magnesium at a rate of about 1 to 2 nmol mg protein^{-1} min^{-1}. Efflux is energy-dependent and is blocked by antimycin A and by FCCP. It is also blocked by EGTA, ruthenium red and lanthanum, suggesting a link between calcium and magnesium transport in liver mitochondria. One possibility is that calcium is released and taken back up by mitochondria in a futile cycle and that magnesium release is associated with the uptake of calcium (Siliprandi et al., 1977, 1979; Zoccarato et al., 1981). Magnesium efflux is blocked by external magnesium, ADP and bongkrekate which prevents the loss of endogenous ADP from mitochondria. Magnesium loss is accompanied by a parallel loss of adenine nucleotides, implying perhaps that they share a common transport system (Zoccarato et al., 1981). Atractylate, which blocks the entry of adenine nucleotides, stimulates net magnesium loss.

Binet and Volfin (1974) have also reported that liver mitochondria lose magnesium when calcium is present in the medium. They claim that this loss may be prevented by a cytoplasmic factor (CMF) in the presence of ADP. This might be expected in the presence of ADP alone, so it is hard to assess the

importance of CMF. Furthermore, Kun (1976) was unable to repeat the isolation of CMF.

Summary

Magnesium uptake by mitochondria appears to be driven by the electrical gradient across the mitochondrial membrane, whereas some efflux probably occurs by Mg/H exchange using the energy stored in the proton gradient. Magnesium and calcium appear to be transported by separate mechanisms in heart mitochondria but may be linked in liver mitochondria where magnesium transport may also be associated with adenine nucleotide transport. Internal phosphate appears to stimulate efflux and external phosphate appears to stimulate influx. Magnesium transport by mitochondria is slow and it would probably take several minutes for a maximal change in transport rate to cause a significant change in cytoplasmic magnesium concentration.

V. Bacteria

Magnesium is an essential requirement for bacterial growth (Lusk, Williams & Kennedy, 1968; Jasper & Silver, 1977). Most culture media provide at least 40 μM magnesium for optimal growth and *Escherichia coli* grown in such media contain about 15 to 30 μmol magnesium per 10^7 cells, which is equivalent to about 20 to 40 mM magnesium in the cell water (Lusk et al., 1968; Silver, 1969; Silver & Clark, 1971). About 90% magnesium is bound within the cytoplasm, mainly to ribosomes, and the concentration of ionized magnesium is about 1 to 4 mM (Lusk et al., 1968; Jasper & Silver, 1977). The magnesium content of *E. coli* only doubles when the concentration of magnesium in the medium is increased several thousandfold from 4×10^{-5} to 10^{-1} M, suggesting the existence of powerful magnesium regulating systems in the membrane (Silver & Clark, 1971).

Magnesium transport in *E. coli* has been described by Silver (1969), Lusk and Kennedy (1969), Silver and Clark (1971) and Nelson and Kennedy (1971). ^{28}Mg uptake is stimulated by external magnesium, conforming to Michaelis-Menten kinetics. The concentration of magnesium which half maximally activates uptake depends on the medium but is normally about 15 to 60 μM (*see also* Park, Wong & Lusk, 1976). Maximum transport rate is about 10 nmol mg protein^{-1} min^{-1}. Uptake is energy-dependent and is inhibited in the absence of glucose or by growing the *E. coli* mutant strain T28 at 40°C at which temperature its ATP levels fall to very low levels (Nelson & Kennedy, 1971). It is inhibited by metabolic poisons such as 2,4-DNP, azide and cyanide and also by cooling the wild-type strain. Uptake is not affected by changes in the concentrations of sodium, potassium, calcium or strontium in the medium, but is competitively inhibited by manganese and cobalt which are probably transported into the cell on the magnesium transport system.

Magnesium efflux is also energy-dependent and is inhibited by CCCP, 2,4-DNP, cyanide, azide or cooling. ^{28}Mg efflux is stimulated by magnesium, cobalt or manganese in the medium. This implies that the magnesium transport system performs some Mg/Mg exchange and that cobalt and manganese can also activate this system.

Magnesium transport in wild-type *E. coli* is constitutive and is not repressed by growing the bacteria in media containing high magnesium concentrations (Silver & Clark, 1971). However, studies with mutant strains of *E. coli* indicate that at least two transport systems exist (Nelson & Kennedy, 1972; Park et al., 1976). System I is constitutive and can transport manganese, nickle and cobalt as well as magnesium. On the other hand system II is repressible when bacteria are grown in high magnesium concentrations (10 mM) and can transport only magnesium. The kinetic parameters of the two systems are similar and both are present in wild type *E. coli*. At least 3 genes control the expression of these magnesium transport systems (Park et al., 1976). Mutants *cor A* lack system I and are thus resistant to poisoning by cobalt. Mutants *cor B* express system I only when grown in high magnesium concentrations. Mutants *Mgt* lack system II. Mutants *corAMgt* lack both systems and have very high magnesium requirement for growth.

Phototrophically or heterotrophically grown *Rhodopseudomonas capsulata* accumulate magnesium by mechanisms which obey Michaelis-Menten kinetics (Jasper & Silver, 1978). Maximal transport rates are 1.8 nmol mg dry weight^{-1} min^{-1} in phototrophically grown bacteria and 0.6 nmol mg dry weight^{-1} min^{-1} in aerobically grown bacteria. 55 μM magnesium half-maximally activates transport in both states. Uptake is energy-dependent and is inhibited by 2,4-DNP, CCCP, cyanide and cooling. Cobalt, manganese and iron competitively inhibit magnesium uptake.

Scribner, Eisenstadt and Silver (1974) have observed specific magnesium uptake in *Bacillus subtilis*. It is inhibited by CCCP and cyanide and competitively inhibited by manganese. Magnesium uptake during log growth phase can be described by Michaelis-Menten kinetics. Maximal transport rate is 4.4 nmol mg dry weight^{-1} min^{-1} and 0.25 mM magnesium half-maximally stimulates transport. Magnesium transport capacity declines during sporulation but the affinity of the transport system rises

sharply. There may be two magnesium transport systems, one with a low affinity and high capacity and the other with a low capacity but high affinity. Only the latter system is present during sporulation.

B. subtilis may also contain a separate magnesium transport system which is inducible by citrate (Willecke, Gries & Oehr, 1973). 0.45 mM magnesium half-maximally activates this system in the presence of saturating concentrations of citrate (7 mM). The concentration of ionized magnesium available to activate the system may have been considerably less than 0.45 mM due to chelation by citrate. The maximal transport rate is 145 nmol mg dry weight^{-1} min^{-1}. Magnesium stimulates citrate transport to a maximum rate of 123 nmol mg dry weight^{-1} min^{-1} and 0.55 mM magnesium half-maximally activates the system. This, together with other evidence, suggests that magnesium and citrate are cotransported with 1 : 1 stoichiometry. Cobalt, nickle and manganese but not calcium also stimulate citrate transport in *B. subtilis*. CCCP inhibits citrate-dependent magnesium transport as does lowering the internal potassium concentration by exposing mutant or valinomycin-treated wild-type cells to media containing low potassium concentrations.

Magnesium transport in *Staphylococcus aureus* also obeys Michaelis-Menten kinetics. Uptake is half-maximally stimulated by 70 μM magnesium and the maximal rate is 9 nmol mg^{-1} min^{-1} (Jasper & Silver, 1977). Transport is inhibited by CCCP, cyanide and cooling.

VI. Isolated Mammalian Cells

Human KB cells equilibrate with ^{28}Mg with a half-time of about 3 hr (Beauchamp, Silver & Hopkins, 1971). Uptake appears to be carrier-mediated and obeys Michaelis-Menten kinetics. The maximal transport rate is 660 pmol 10^7 cells^{-1} min^{-1} and half-maximal rate is achieved when the media contain 0.1 mM magnesium. Uptake is energy-dependent and is blocked by CCCP and cyanide. Ouabain also blocks transport, indicating that uptake required a functioning sodium pump.

Magnesium transport in S49 mouse lymphoma cells has been described by Maguire and Erdos (1978, 1980) and Erdos and Maguire (1983). Only 3% of cellular magnesium is available to equilibrate with ^{28}Mg. Uptake is stimulated by external magnesium and is described by Michaelis-Menten kinetics. Maximum transport rate is 360 pmol 10^7 cells^{-1} min^{-1} (2.9 nmol mg protein^{-1} min^{-1} or 0.12 pmol cm^{-2} sec^{-1}), and half-maximal rate is produced by 0.35 mM magnesium. Uptake is reduced by cyanide, azide and 2,4-DNP, suggesting that it is energy-dependent. It is not inhibited by 10^{-5} M ouabain or by changes in external sodium or potassium. It is, however, stimulated by alkalinization of the medium so that Erdos and Maguire (1983) have speculated that magnesium may be countertransported with protons, perhaps by a proton-stimulated ATPase. An alternative hypothesis is that stimulation is due to changes in cellular magnesium buffering. Uptake is not affected by the calcium blockers nifedipine and verapamil in S49 cells. β-adrenergic agonists and prostaglandin E_1 inhibit magnesium uptake but have no effect on magnesium efflux or on the transport of sodium, potassium, calcium or manganese. Work with mutant strains of S49 have shown that the β-effect is not mediated by changes in cAMP levels but that it does require an intact β-receptor adenylcyclase complex (Maguire & Erdos, 1978, 1980). β-adrenergic agonists have also been shown to inhibit magnesium uptake by GM86 Friend erythroleukemia cells and G8 muscle cells. Adenosine may modulate magnesium transport in NG10815 neuroblastoma-glioma hybrid cells (Erdos & Maguire, 1983).

VII. Other Work on Magnesium Transport Systems

Net and tracer magnesium fluxes have been seen in rat liver slices. Magnesium and calcium transport show similar characteristics although separate routes may be involved. Glucose depletion, iodoacetate and changes in the concentrations of sodium and potassium do not affect magnesium transport. Cyanide and alkalinization of the medium increase transport and cooling decreases it. Calcium inhibits magnesium transport just as magnesium inhibits calcium transport, suggesting that the transport of these ions is linked (Wallach, Gamponia & Ahmed, 1970). However, parathormone, thyroxine and cAMP affect magnesium transport differently from calcium transport (Wallach et al., 1972).

Magnesium transport has been observed in sheep brain synaptosomes (Carvalho, 1979), rat pancreatic islet cells (Henquin et al., 1983), fat cells and vesicles (Elliot & Rizack, 1974), sheep placenta (Care et al., 1979) and cat choroid plexus (Reed & Yen, 1978). In sheep synaptosomes magnesium is probably transported by a different route from calcium and involves the countertransport of sodium or potassium. In pancreatic islet cells its transport may be linked to insulin release and is stimulated by glucose but not by 3-0-methylglucose. The effects of glucose are more pronounced in the absence of calcium. In fat cells magnesium transport is stimulated

by adrenaline, noradrenaline and ACTH. Active magnesium transport across the choroid plexus has been demonstrated.

Magnesium transport in the gut has been reviewed by Walser (1967) and Ebel & Günther (1980). All regions of gut can transport magnesium, though the nature of transport varies according to region, species and stage of development (*see* Meneely, Leeper & Ghishan, 1982). There are at least two components of magnesium uptake. One increases linearly with mucosal magnesium concentration whereas the other saturates (Ross, 1962; Brannan et al., 1976; Brown, Care & Pickard, 1977; Martens, Harmeyer & Michael, 1978; Roth & Werner, 1979). Whether magnesium transport is active is still controversial (*see* Ebel & Günther, 1980), though active transport seems likely in the sheep rumen (Brown et al., 1977; Martens et al., 1978). Behar (1974) has shown that manipulations which affect water transport cause a parallel change in magnesium transport, suggesting that magnesium might be taken up by bulk transport via an intercellular route. However, Ross (1962) and Martens et al. (1978) have observed magnesium transport which appears to be independent of water transport and may occur via a transcellular route.

Magnesium and calcium transport in the gut share many characteristics. Orally administered calcium depresses absorption of magnesium, and magnesium depresses calcium absorption (Walser, 1967). Humans with endstage renal disease who absorb calcium poorly also show severely reduced magnesium absorption (Brannan et al., 1976). These findings suggest a link between calcium and magnesium transport. However, tube studies on the jejunum and ileum of normal subjects have shown that calcium does not affect magnesium uptake although magnesium depresses calcium uptake. In addition, patients with hypercalciuria show increased calcium absorption but normal magnesium absorption (Brannan et al., 1976).

Magnesium transport in kidney has been reviewed recently by Quamme and Dirks (1983). 70 to 80% plasma magnesium is filtered and of this 90% is reabsorbed by the nephron. Only 20 to 30% of the filtered load is reabsorbed in the proximal tubule, probably because of the low magnesium permeability of the tight junctions. Little reabsorption occurs in the descending limb of Henle's loop, though this may be a major site for magnesium secretion. Most reabsorption occurs in the thick ascending limb. Magnesium absorption here depends on sodium chloride reabsorption and maneuvers which reduce sodium chloride reabsorption greatly reduce magnesium reabsorption. In rabbit kidney magnesium transport in isolated ascending limbs depends on the potential difference across the cells (Shareghi & Agus, 1982). Under normal perfusion conditions the lumen is 9 mV positive with respect to the serosa, and net magnesium reabsorption occurs. Magnesium reabsorption is increased by maneuvers which increase the potential and net magnesium secretion is observed when the potential is reversed. Serosal calcium has little effect on magnesium transport and serosal magnesium has little effect on calcium transport. Thus calcium and magnesium transport in the thick ascending limb are similar but probably use separate routes. Parathormone and dibutyryl cAMP both stimulate magnesium transport in the thick ascending limb (Shareghi & Agus, 1982). Quamme and Dirks (1983) have suggested that magnesium enters the cells down the electrochemical gradient across the luminal membrane and is then pumped out of the cell across the serosal membrane, perhaps by Mg/Na exchange. Changes in luminal potential probably affect the ease with which magnesium enters the cells at the lumen and thus control the rate and direction of magnesium transport.

2 to 5% filtered magnesium is reabsorbed in the distal convoluted tubule and 1 to 3% in the collecting duct (Quamme & Dirks, 1983). Little is known about the nature of transport in these regions. The terminal nephron may be a site of magnesium secretion during magnesium loading.

Use of Ionophores in Magnesium Transport Studies

Several ionophores can carry magnesium across cell membranes. These are useful for controlling the magnesium content of cells and for investigating magnesium transport at the molecular level. The most useful magnesium ionophore is A23187, but X537A (Pressman, 1976) and ionomycin (Liu & Hermann, 1978) can also transport magnesium.

The ionophore A23187 selectivity transports divalent cations under most conditions. It carries calcium slightly better than magnesium (Reed & Lardy, 1972; Pfeiffer, Reed & Lardy, 1974; Pfeiffer, Taylor & Lardy, 1978). It can transport sodium when the concentrations of divalent cations in the medium are low (Flatman & Lew, 1977). A23187 electroneutrally exchanges one divalent cation for two protons (Reed & Lardy, 1972; Case, Vanderkooi & Scarpa, 1974; Kafka & Holz, 1976; Pressman, 1976). Thus unless the cell has some means of maintaining the proton gradient across its membrane, such as the anion exchanger in red cells, this can limit the amount of magnesium transported. Addition of a protonophore such as FCCP alleviates this problem. A23187 has been used to selectively

alter magnesium concentration in red cells (Reed & Lardy, 1972; Flatman & Lew, 1980; Flatman, 1982), chloroplasts (Sokolove, 1979) and mitochondria (Reed & Lardy, 1972; Åkerman, 1981).

Conclusions

This review has shown that magnesium moves across cell membranes and that the size of these fluxes is similar to or even greater than resting calcium fluxes. Magnesium fluxes are too large to be accounted for by passive diffusion through the lipid of the membrane. This suggests that there are specific magnesium transport systems. Magnesium transport across certain membranes (muscle, nerve) shows features in common with calcium transport and may utilize some of the same carriers. However, in other instances (mitochondria, bacteria) magnesium transport shows very different features from calcium transport, implying separate magnesium and calcium transport systems.

Many cells (and mitochondria) keep their magnesium contents well below electrochemical equilibrium, indicating that they possess active magnesium transport systems. The source of energy for magnesium transport may be the coupling of magnesium exit to the obligatory entry of either sodium (nerve, muscle), protons (mitochondria, bacteria, ionophores, S49 cells) or potassium (synaptosomes, yeast cells—*see* Lichko, Okorokov & Kulaev, 1980) which travel down their electrochemical gradients. There is some evidence that there may also be a separate magnesium pump which uses energy from ATP hydrolysis. However, in at least one instance (squid axon) ATP may also regulate Na/Mg exchange. The size of magnesium fluxes means that cells may actually spend as much energy (even more in squid axons and barnacle muscle) on maintaining magnesium content as on maintaining resting calcium content.

In mammals it appears that cellular magnesium can be adjusted to accommodate the demands of the whole body. Certain hormones are known to affect magnesium transport. Parathormone stimulates magnesium transport into some cells and into mitochondria. Noradrenaline and adrenaline stimulate magnesium uptake into fat cells, but β-adrenergic agonists inhibit magnesium uptake by S49 and perhaps heart cells. These findings tend to favor the interesting idea that intracellular magnesium may act as a second messenger in some systems.

This review is intended to provide a selective and critical, rather than exhaustive account of magnesium transport. However, current controversies have been introduced and considerable reference to the major body of data is supplied in order to outline areas where further exploration is likely to prove most rewarding. It is hoped that the review will thus stimulate fresh work in the field.

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