Magnesium Equilibrium in Muscle

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ABSTRACT Isolated frog sartorii were immersed in Ringer's solution, which had a 2 mM magnesium concentration containing Mg^{28}. The uptake of the radioactive magnesium was measured under steady state conditions. Although the biological variability was fairly large, it was observed that the uptake proceeded in three stages lasting respectively about 0.5, 30, and 300 minutes and accounting respectively for about 0.21, 0.71, and 0.67 millimole magnesium/kg. muscle. It was assumed that the first stage represents surface adsorption, the second stage represents extracellular water and connective tissue phases, and the third stage entry inside the cell. It is estimated that the maximum intracellular magnesium concentration is about 1.1 mM and that only about 0.6 millimole magnesium/liter intracellular water is exchanged per hour. The maximum energy required per hour to pump the magnesium out of the cell against the electrochemical gradient is calculated to be only 1.5 cal./kg. muscle. About 75 to 80 per cent of the magnesium in muscle is non-exchangeable and difficult to remove by diffusion. It appears from previous work that the exchangeable magnesium behaves similarly to exchangeable calcium.

INTRODUCTION

Because of interrelationships between divalent ions, it is highly desirable in studying one divalent ion to take into account other divalent ions. Until recently, it has not been possible to study the permeability of the muscle cell membrane to magnesium, since there had not been available any long lived radioactive magnesium isotope. However, when Mg^{28} was discovered with a half-life of 21.3 hours (1), it became possible to study the membrane permeability to magnesium and determine the possible similarity to calcium permeation in muscle (2). This was the purpose of the present investigation. A preliminary report of this study has already been presented (3).

METHODS

GENERAL PROCEDURE FOR EQUILIBRATING MUSCLES Frog muscles were dissected out and were maintained overnight in Ringer's solution at 5°C. Generally, a muscle

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set consisted of the tibialis anticus longus, ileofibularis, semitendinosus, and the sartorius (occasionally the peroneus). Then one muscle set and its contralateral set were transferred, respectively, to the experimental and the control flasks, each of which contained 50 ml. of solution. Unless otherwise stated, the control Ringer's solutions were composed of 2 mM of $CaCl_2$, 111 mM of NaCl, 2.68 mM of KCl, 2 mM of $MgCl_2$, and 2.38 mM $NaHCO_3$. After 5 hours with gentle agitation at a temperature of about 23–26°C., the individual muscle sets were blotted on filter paper moistened with Ringer's solution and weighed on a torsion balance. Each muscle set was placed in a platinum crucible, dried at about 100°C. for approximately 1 hour, and ashed overnight at 500°C. The muscle sets usually weighed between 250 and 350 mg. The magnesium content was determined by an EDTA (ethylenediaminetetraacetic acid)

TABLE I

METAL DETERMINATIONS*

No. of determinations	Micromoles in sample		Average micromoles metal recovered*	Standard deviation of sample
	Mg	Ca		
2	0.00	2.00	1.97	0.01
11	1.00	1.00	0.96	0.06
2	1.00	10.00	9.80	0.02
6	2.00	0.00	0.00	0.00
5	10.00	1.00	0.88	0.13
4‡	7.59	3.30	2.04	0.04
5‡	8.59	2.30	1.51	0.31
5‡	9.59	1.30	0.97	0.26

* Metal as used here approximates calcium (see text).

‡ These recovery experiments were carried out on 0.5 ml. aliquots of a muscle ash solution to which known amounts of calcium and/or magnesium had been added. The endogenous amounts of magnesium and calcium in these samples were respectively, 7.59 and 1.30 micromoles.

titration with an error of less than 3 per cent (4). In this procedure, after precipitating with oxine, EDTA was added in the presence of a calcium indicator. The moles of EDTA are equivalent to the moles of a divalent metal and it can be seen from Table I that this metal determination (using a calcium indicator) corresponds as a first approximation to the calcium. The calcium values reported in this paper were obtained in such a manner, and therefore are only very approximate estimates of the actual calcium values. The magnesium is titrated with additional EDTA using a magnesium indicator.

PROCEDURE USING Mg^{28} To assure a steady state of the magnesium, the isolated tissues were kept in Ringer's solution at 23–26°C. for 3 hours. Thereafter they were soaked for various periods of time in Ringer's solution containing Mg^{28} and were then weighed and ashed in the usual manner. (The Mg^{28}, containing 50 to 100 microcuries Mg^{28}, was obtained from Brookhaven National Laboratory.) The ash was dissolved in water, transferred to planchets with a diameter of 2.54 cm., and dried. The radioactivity in C.P.M. (counts per minute) was determined using an end-window

thin mica (1.4 mg./cm.^{2}) Geiger-Müller tube. The C.P.M. were corrected for radioactive decay by calculating the C.P.M. at a reference time. The exchangeable magnesium in the sample was obtained by dividing the C.P.M. by the specific activity (approximately $2 \cdot 10^6$ C.P.M./millimole magnesium at time of shipment arrival). All the radioactive muscle experiments were performed on the sartorii. For these experiments, other muscles from the same frog were immersed in non-radioactive Ringer's solution for the necessary times and subsequently analyzed for magnesium. These determinations permitted calculation of the per cent of magnesium which exchanged in the radioactive muscles.

A tendon study was also performed. After immersion in Ringer's solution, several tendons were grouped together and analyzed for magnesium. From this average value the per cent of magnesium which exchanged in the radioactive tendons was calculated.

For the washout experiments, the sartorii were placed in Ringer's solution containing Mg^{28} for 10 hours at 5°C. followed by 3 hours at 23–26°C. Thereafter, they were put for various periods of time in planchets containing 2 ml. of non-radioactive Ringer's solution and were gently agitated. The muscles were transferred at designated periods of time to other planchets containing non-radioactive Ringer's solutions. This procedure was repeated until 5 hours had elapsed. The muscles were ashed and both the 2 ml. volumes and muscles were analyzed for radioactivity. Chemical analyses were also performed on other muscles similarly treated and from this data the per cent of magnesium which exchanged was calculated. (A correction was also made for initial radioactive contamination.) However, due to the low specific activity and the relatively short half-life, the actual counts measured were very low; and therefore not too much reliance can be placed on these values obtained from the washout experiment.

RESULTS

The uptake of magnesium in muscle immersed in Ringer's solution containing 10 mM of magnesium for various periods of time is tabulated in Table II and illustrated in Fig. 1. The control muscles were immersed in the same solution for 5 hours. The average magnesium value for all the 102 controls in this series was 11.49 millimoles/kg. In Fig. 1 this average value was plotted as 100 per cent of the control. Within 3 hours, equilibrium was reached and was maintained even up to 72 hours, at which time the solutions became cloudy due to tissue breakdown.

The effect of the magnesium concentration in Ringer's solution on the magnesium uptake in muscle after an equilibration time of 5 hours is shown in Table III and Fig. 2. The average magnesium content of the 30 control muscles (immersed in 2 mM Mg) was 6.75 millimoles/kg. This value was plotted as equivalent to 100 per cent in Fig. 2. When muscles were immersed in a magnesium-free solution, the amount of magnesium in the muscle was decreased about 20 per cent. As the external magnesium concentration was increased, the magnesium uptake increased in approximately a linear manner.

TABLE II

EFFECT OF TIME ON THE UPTAKE OF MAGNESIUM IN MUSCLE IMMERSED IN RINGER'S SOLUTION CONTAINING 10 mM MAGNESIUM

Time		No. of experiments	Millimoles Mg/kg. weight		Experimental / Control
Control	Experimental		Control	Experimental	
hrs.	*hrs.*				*per cent*
5	0	6	11.31 ± 0.29*	6.82 ± 0.53	61.2 ± 5.42
5	0.5	6	10.69 ± 0.07	9.43 ± 0.16	88.3 ± 1.21
5	1	6	11.27 ± 0.18	10.92 ± 0.16	97.0 ± 1.70
5	3	6	11.18 ± 0.18	12.39 ± 0.04	110.9 ± 1.46
5	5	6	12.14 ± 0.10	11.98 ± 0.26	98.8 ± 2.50
5	10	18	11.30 ± 0.14	11.42 ± 0.09	101.1 ± 0.71
5	24	6	12.66 ± 0.14	14.05 ± 0.24	113.2 ± 3.02
5	48	6	11.68 ± 0.27	12.68 ± 0.09	108.7 ± 1.45
5	72	42	11.17 ± 0.16	11.05 ± 0.11	99.3 ± 0.91
Average			11.49 ± 0.08		

* As used in this paper, the ± deviations refer to standard errors of the mean.

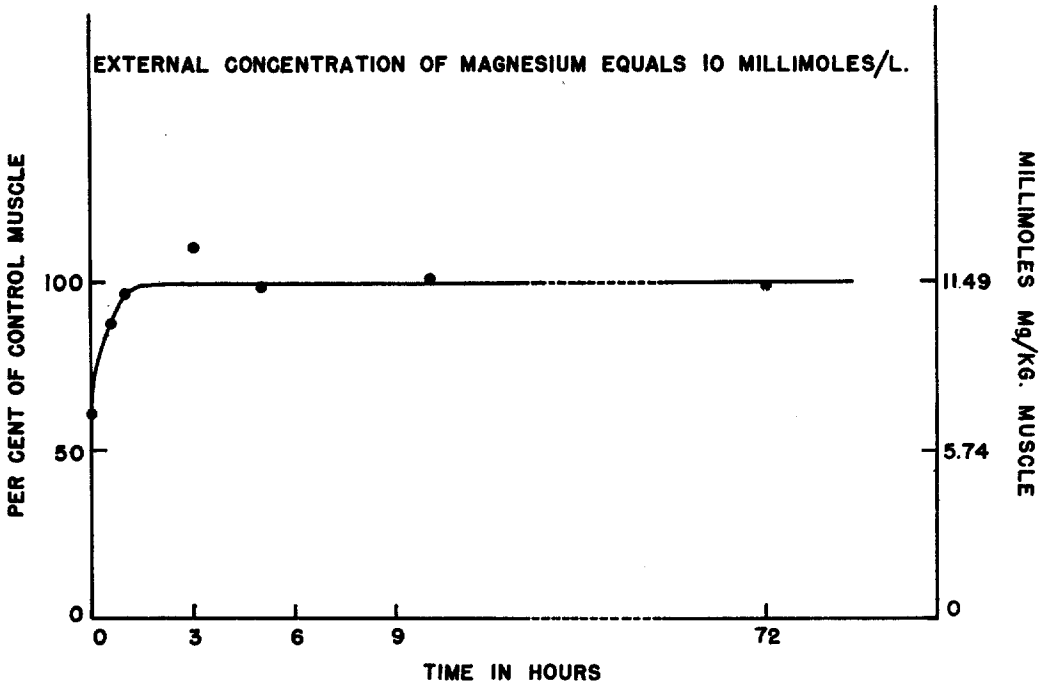

FIGURE 1. Effect of equilibration time on the magnesium uptake by muscle immersed in Ringer's solution containing 10 mM magnesium. Per cent of control muscle was calculated using the average value of 11.49 millimoles Mg/kg. as equivalent to 100 per cent of the control muscle (see Table II).

Table IV gives the effects of other procedures on the magnesium content. Muscles which were dissected and analyzed without previous immersion in Ringer's solution contained 6.22 ± 0.63 millimole/kg. which agreed quite well with the muscles which were immersed in the Ringer's solution containing 2

TABLE III

EFFECT OF MAGNESIUM CONCENTRATION IN RINGER'S SOLUTION ON THE MAGNESIUM CONTENT IN MUSCLE

mM Mg in Ringer's		No. of experiments	Millimoles Mg/kg. weight		Experimental / Control
Control	Experimental		Control	Experimental	
					per cent
2	0	6	7.39 ± 0.07	6.00 ± 0.32	81.8 ± 1.78
2	2	6	6.69 ± 0.25	6.55 ± 0.29	97.9 ± 2.77
2	5	6	6.17 ± 0.06	8.42 ± 0.11	136.5 ± 2.41
2	10	6	6.99 ± 0.10	12.39 ± 0.25	177.4 ± 4.47
2	17.5	6	6.53 ± 0.10	16.76 ± 1.99	257.6 ± 15.56
Average			6.75 ± 0.06		

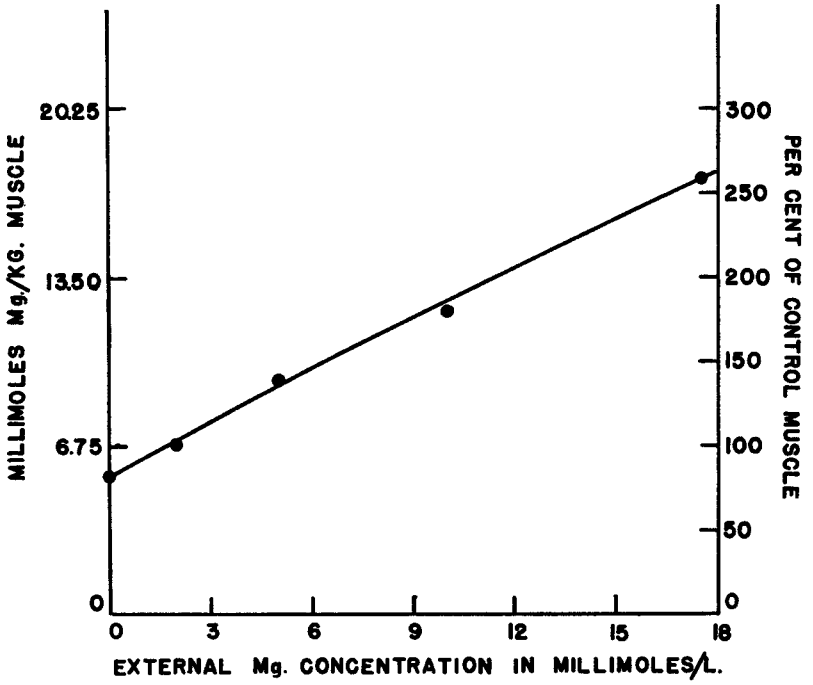

FIGURE 2. Relationship between magnesium concentration and amount of magnesium in muscle after an equilibration period of 5 hours. Per cent of control muscle was calculated using the average value of 6.75 millimoles Mg/kg. as equivalent to 100 per cent of the control muscle (see Table III).

mM magnesium. Muscles which were cut into small pieces had only about 75 per cent of the magnesium present in the control muscles. Immersing the muscles for a prolonged period of 24 hours also produced a decreased magnesium content. There seemed to be a slight tendency for muscles from summer

TABLE IV

EFFECT OF VARIOUS CONDITIONS ON THE MAGNESIUM CONTENT IN MUSCLE

Condition		mM Mg in Ringer's	No. of experiments	Millimoles Mg/kg. weight		Experimental / Control
Control	Experimental			Control	Experimental	
						per cent
Immersed*	Fresh dissected	2	12	6.17 ± 0.10	6.22 ± 0.63	100.9 ± 1.53
Not cut*	Cut	2	18	6.07 ± 0.51	4.90 ± 0.13	76.7 ± 1.74
No EDTA	EDTA	0	12	5.94 ± 0.10	4.49 ± 0.06	75.8 ± 1.08
5 hrs.*	24 hrs.	2	12	6.10 ± 0.08	3.66 ± 0.19	59.8 ± 2.41
Winter*	Summer*	2	‡	6.24 ± 0.07	6.69 ± 0.06	107.2 ± 1.58

* The muscles were treated identically.
‡ There were 30 muscles analyzed from winter frogs and 101 muscles analyzed from summer frogs.

TABLE V

EFFECT OF VARIOUS CONDITIONS ON THE CALCIUM CONTENT IN MUSCLE

Condition		mM Ca in Ringer's	No. of experiments	Millimoles Ca/kg. weight		Experimental / Control
Control	Experimental			Control	Experimental	
						per cent
Immersed*	Fresh dissected	2	12	2.70 ± 0.14	1.66 ± 0.12	62.4 ± 4.72
Not cut*	Cut	2	18	2.54 ± 0.14	6.75 ± 0.10	264.7 ± 11.02
No EDTA	EDTA	0	12	2.23 ± 0.11	1.71 ± 0.10	77.1 ± 3.47
5 hrs.*	24 hrs.	2	12	2.60 ± 0.21	4.04 ± 0.31	167.7 ± 19.80
Winter*	Summer*	2	‡	2.52 ± 0.08	2.47 ± 0.05	98.3 ± 3.81

* The muscles were treated identically.
‡ There were 30 muscles analyzed from winter frogs and 101 muscles analyzed from summer frogs.

frogs when immersed in Ringer's solution for 5 hours to have more magnesium than muscles from winter frogs.

Table V gives the effects of these same conditions on the calcium content. However, it should be emphasized that the method used gives only a very approximate calcium content and therefore small differences would not be detectable. It is clear though that the freshly dissected muscle contains less

calcium than muscle immersed in Ringer's solution. Cutting the muscle or immersing the muscle for 24 hours increased the calcium content. EDTA also decreased the calcium content. No difference by this approximate method was observed in the calcium content of muscles from winter and summer frogs when immersed in Ringer's solution for 5 hours.

Table VI gives the exchange of Mg^{28} in sartorius muscle and Achilles tendon when these tissues were immersed in Ringer's solution containing 2 mM Mg. The average muscle concentration of magnesium was 7.95 ± 0.08 millimole/kg. The washout study may be interpreted as a confirmatory set of data, since the reliability of the samples was not great due to the small amount of radioactivity present. The tendon contained 2.42 ± 0.04 millimole Mg/kg. (7 determinations).

TABLE VI

THE EXCHANGE OF Mg^{28} BY FROG SARTORIUS MUSCLE AND ACHILLES TENDON

Time	Muscle uptake study		Muscle washout‡ study		Tendon uptake study	
	No. of experiments	C*	No. of experiments	C*	No. of experiments	C*
min						
0.01	8	1.3 ± 0.2			3	4.5 ± 0.5
0.1	8	2.0 ± 0.2	12	0.2 ± 0.9	3	7.8 ± 2.2
0.5	5	3.2 ± 0.4	12	4.0 ± 1.1		
1.0	8	3.7 ± 0.3	12	6.3 ± 1.7	3	7.6 ± 0.7
2.5	5	5.7 ± 0.5				
5	9	7.5 ± 0.9	12	10.8 ± 1.0	3	28.6 ± 1.3
10	5	9.1 ± 0.7				
15	9	9.6 ± 0.8	12	15.2 ± 0.7	3	49.4 ± 2.0
30	8	13.4 ± 1.6			3	65.7 ± 2.1
60	9	16.1 ± 1.9	12	17.4 ± 0.5	3	68.2 ± 4.9
120	8	15.8 ± 1.3			3	84.7 ± 3.2
180	6	19.7 ± 3.0	12	18.7 ± 0.3		
300	14	19.7 ± 1.2	12	19.4 ± 0.2	3	76.2 ± 3.9

* C = Per cent magnesium exchanged.

‡ Corrected for contamination made in the initial transfer from the radioactive solution to the non-radioactive solution.

DISCUSSION

ANALYSIS OF Mg^{28} DATA. In these experiments, the volume of the Ringer's solution was so much larger than the tissue volume that the radioactive concentration of the Ringer's solution was constant throughout the experiment. The muscles were soaked previously in non-radioactive Ringer's solution long enough to assure a condition close to a steady state (see Fig. 1 and Table II).

The equation of the radioactive exchange was estimated employing a minimum number of exponential terms, which turned out to be three. The equation of the uptake by the sartorius muscle is as follows:—

$$C = -8.9\, e^{-0.143t} - 8.5\, e^{-0.0085t} - 2.6\, e^{-13.1t} + 20.0 \qquad (1)$$

(a) C is the per cent magnesium exchanged with the Mg^{28}.

(b) t is the time in minutes.

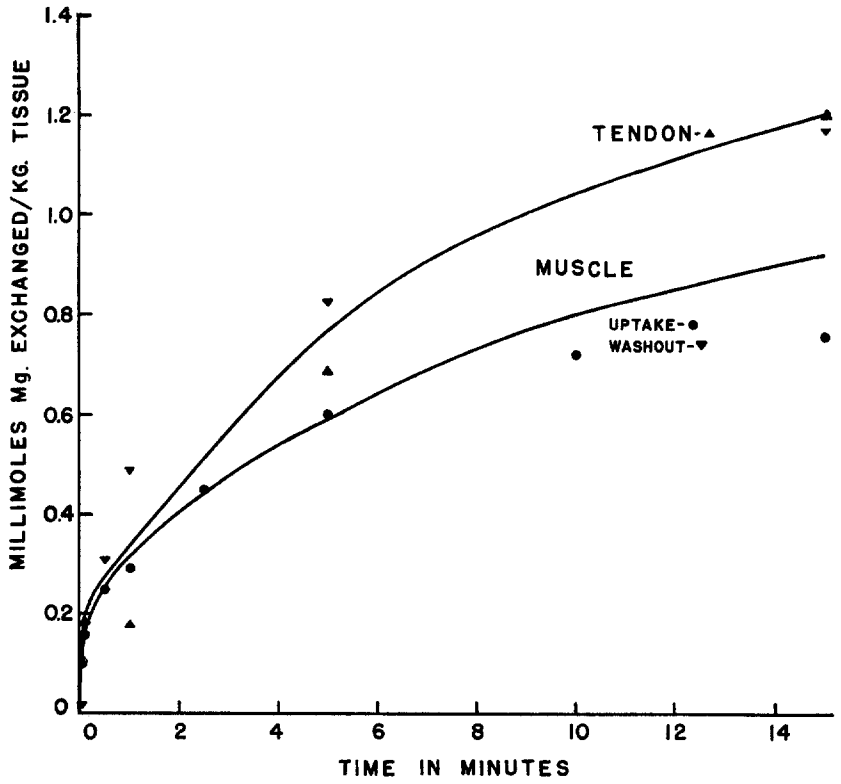

FIGURE 3. Calculated Mg^{28} exchange curves of sartorius muscle (using both uptake and washout techniques) and Achilles tendon (using uptake technique) up to 15 minutes. Symbols mark observed points.

The average magnesium content of the muscles used in the radioactive experiments was 7.95 millimoles/kg. To obtain the millimoles exchangeable Mg/kg., it is only necessary to multiply the per cent Mg exchanged by 7.95 millimoles/kg. and divide by 100 per cent. Thus, Equation 1 becomes

$$M = -0.71\, e^{-0.143t} - 0.67\, e^{-0.0085t} - 0.21\, e^{-13.1t} + 1.59 \qquad (2)$$

(a) M is the exchangeable magnesium in millimoles/kg.

(b) t is the time in minutes.

Figs. 3 and 4 illustrate the calculated uptake curve and the experimental points for the sartorius muscle. The washout experimental points are also included as confirmatory evidence. With so much variability in the data as illustrated in these figures, it is evident that Equations 1 and 2 represent only very rough approximations.

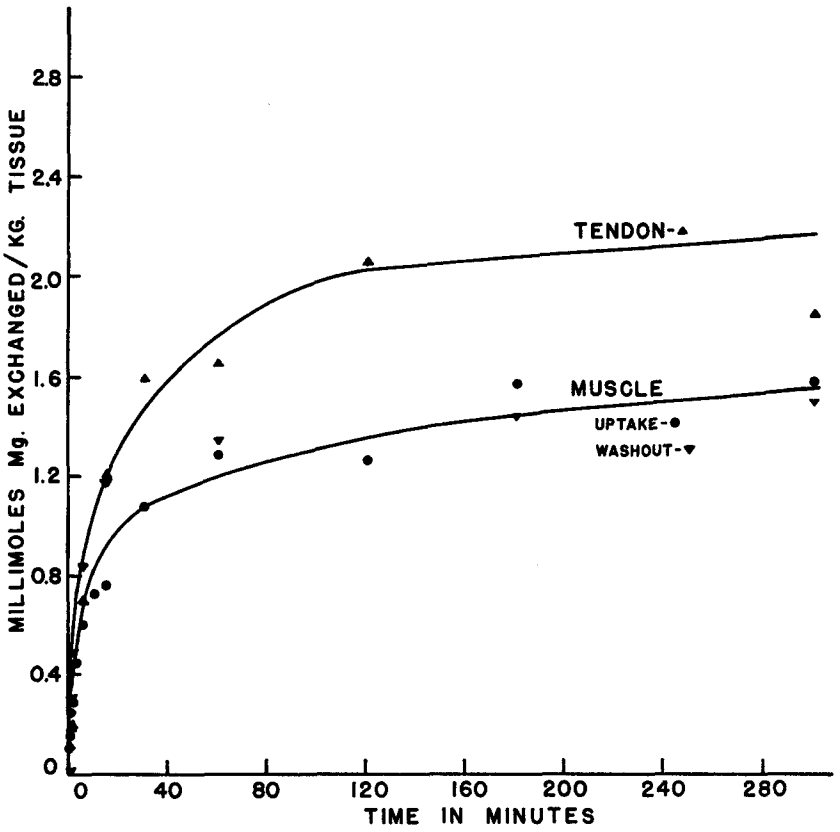

FIGURE 4. Calculated Mg^{28} exchange curves of sartorius muscle (using both uptake and washout techniques) and Achilles tendon (using uptake technique) up to 300 minutes. Symbols mark observed points.

Since the kinetics of the uptake of Ca^{45} into muscle (2) is quite similar to the Mg^{28} uptake, it was decided to analyze the magnesium data in a similar manner. It was assumed that each exponential corresponded to a phase of exchange. This assumption implies that during the time in which each phase becomes equilibrated, there is no significant change in the other phases. This appears to be a reasonable assumption (2). The rapid phase of exchange lasted only about half a minute, and was assumed to represent surface adsorption, the second phase of exchange lasted about 30 minutes and was assumed to represent the effective extracellular (extracellular water plus connective

tissue) phase, and the slow phase of exchange lasted about 300 minutes and was assumed to represent the intracellular exchange. The meaning and assumptions regarding these phases have previously been discussed (2). The exchange of these compartments is given by the following equations and is shown graphically in Fig. 5

$$M_s = -0.21\, e^{-13.1t} + 0.21 \quad (3)$$

$$M_p = -0.71\, e^{-0.143t} + 0.71 \quad (4)$$

$$M_n = -0.67\, e^{-0.0085t} + 0.67 \quad (5)$$

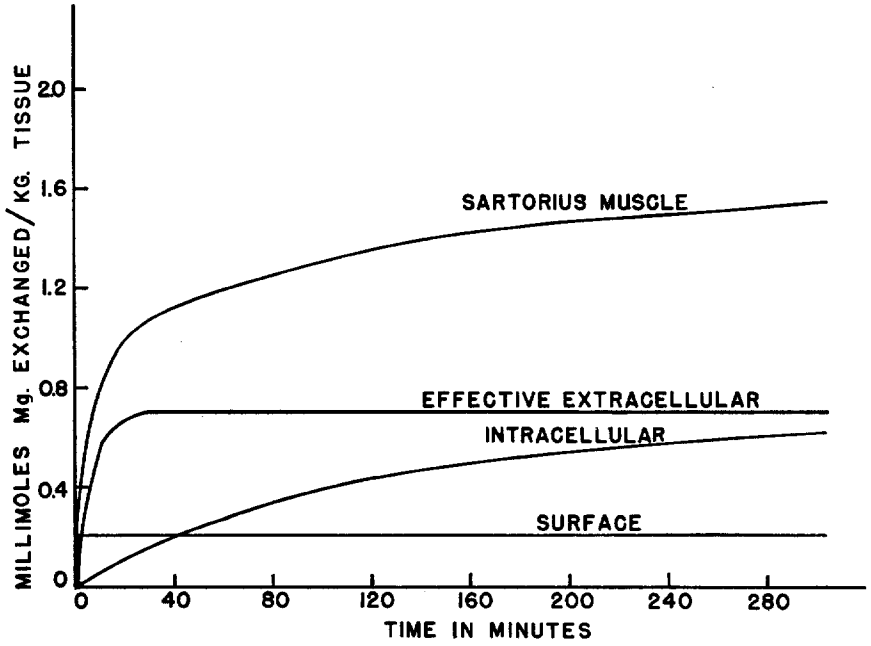

FIGURE 5. Calculated Mg^{28} exchange curves of the effective extracellular, intracellular, and surface phases of sartorius muscle.

(a) M_s, M_p, M_n are exchangeable Mg amounts (millimoles Mg/kg. muscle) in the surface, effective extracellular, and intracellular phases, respectively.

(b) t is the time in minutes.

Frater *et al.* (5) have also considered a three phase system in muscle for divalent ions.

Assuming that the extracellular water phase contains 0.18 liter H_2O/kg. muscle and the intracellular phase contains 0.62 liter H_2O/kg. muscle, it is possible to obtain estimates of the concentrations, exchange constants, and amounts of magnesium in the different phases of the sartorius muscle in the steady state. Fig. 6 illustrates these calculated values.

The effective extracellular phase is equal to the equilibrium M_p (see Equation 4) divided by the magnesium concentration in the Ringer's solution. It equals 0.36 liter H_2O/kg. which is larger than the assumed extracellular space of 0.18 liter H_2O/kg. Therefore, it was assumed that connective tissue accounted for a part of this Mg^{28} exchange. To test this hypothesis, it was de-

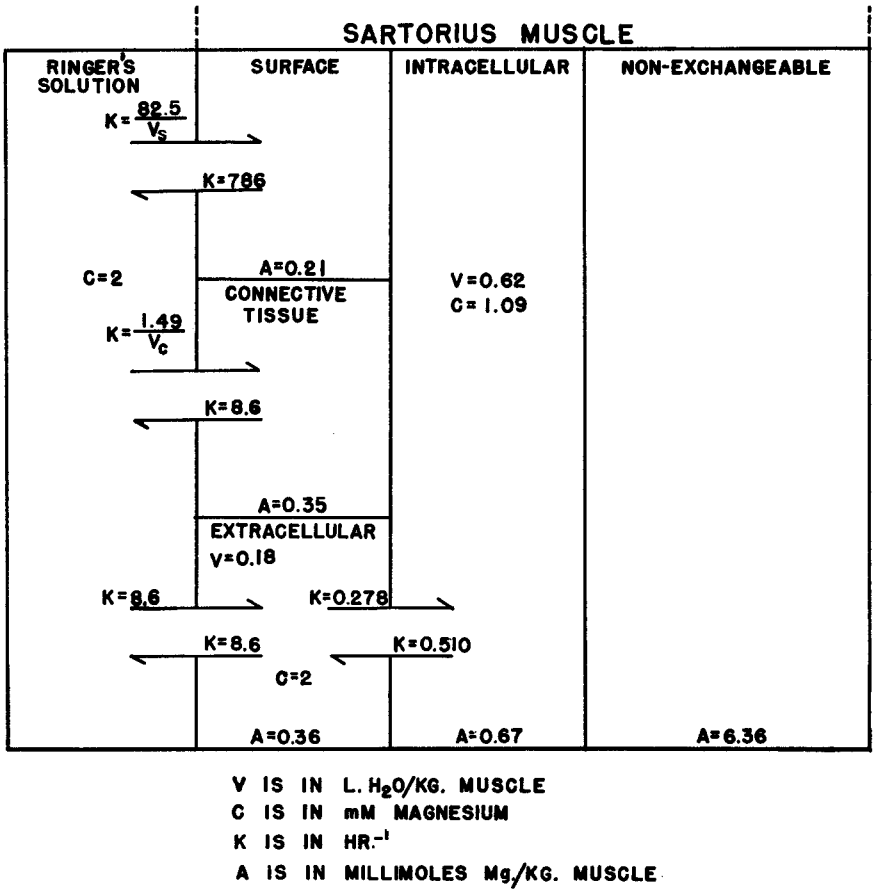

FIGURE 6. Volumes, concentrations, exchange constants, and amounts of magnesium in the different phases of the sartorius muscle. The symbols V_s and V_c refer respectively to the surface phase volume and connective tissue phase volume.

cided to determine whether the Achilles tendon could concentrate Mg^{28}. It appears from Table VI and Figs. 3 and 4 that tendon contains significant amounts of exchangeable magnesium. The same is true for calcium (2, 6). Thus, it seems reasonable to suspect that connective tissue can take up Mg^{28}.

The surface phase contained 0.21 millimole magnesium/kg. If the slow phase represents the intracellular phase, then the muscle cell membrane must be permeable to magnesium. It has been pointed out before that high con-

centrations of magnesium cause a penetration of magnesium into the cell (7–9), but that permeation of magnesium is slow (7, 8). The amounts in the surface, connective tissue, and intracellular phases were very similar to the amounts obtained for calcium in these phases (2). About 80 per cent of the magnesium was in a non-exchangeable form (6.36 millimoles/kg.). Rogers and Mahan (10) have reported that the rat gastrocnemius after 7 hours is only 20 per cent equilibrated with Mg^{28}, and this agrees with the value obtained for frog sartorius muscle reported here. Addition of EDTA to muscle decreases the magnesium content by about 25 per cent (Table IV), which indicates that the chelating action of this substance did not influence the non-exchangeable magnesium. Conway and Cruess-Callaghan (7) have pointed out that a considerable amount of magnesium is not in the diffusible form. Due to the large biological variability these calculations concerning the various phases should be taken only as rough approximations. A greater reliance should be placed on the values for the surface and non-exchangeable phases than on the values for the extracellular and intracellular phases.

ENERGY EXPENDITURE FOR MAINTAINING MAGNESIUM IN THE STEADY STATE At equilibrium the concentration of ions between two compartments should be distributed according to the following equation (2)

$$\frac{C_1}{C_2} = r^z q \tag{6}$$

(a) $$\ln r = \frac{F}{RT} E_m$$

(b) $$\ln q = \frac{W_q}{RT}$$

(c) Subscripts 1 and 2 refer to compartments 1 (intracellular) and 2 (extracellular) respectively.

(d) C is the concentration of the ion.

(e) z is the valence.

(f) F is the Faraday constant.

(g) R is the gas constant.

(h) T is the absolute temperature.

(i) E_m is the membrane potential (compartment 2 minus compartment 1). The value of r is a function of E_m as defined above.

(j) W_q is the net potential due to forces other than electrical and chemical ones (compartment 2 minus compartment 1), and can be considered as an active transport potential. The membrane work factor q is a function of W_q as defined above.

Assuming a value of r about 32.9, then the membrane work factor q is calculated to be $5.04 \cdot 10^{-4}$. Being less than one, this indicates that there is some active force pushing the magnesium out of the cell. Actually, the intracellular

concentration should only refer to the ionized magnesium, and if any is chemically bound, then q would deviate even further from 1. The value for the active transport potential for magnesium was calculated to be -4.47 cal./millimole, which is approximately the same for calcium and sodium (2). Thus the electrochemical gradients pushing magnesium, calcium, and sodium ions into the cell are roughly about the same. The energy consumed by the active transport mechanism can be obtained by multiplying the active transport potential by the outward flux. The outward flux for magnesium is equal to the intracellular exchange constant times the intracellular concentration or to 0.51 hour^{-1} times 1.09 mM (see Fig. 6). This flux would therefore amount to 0.556 mM/hr., and the active transport energy would amount to 1.54 cal./kg.-hr. which is only about 1 per cent of the resting cell energy. One point to be emphasized is that unless the mechanism of transport is known, it is not possible to determine the active transport energy. If the energy which is released as the magnesium enters the cell is utilized to force the magnesium out of the cell, the active transport energy would equal zero. However, if none of the energy which is released by magnesium entry is utilized by the active pump forcing the magnesium out, then the active pump would require the calculated 1.54 cal./kg.-hr. Thus, the maximum energy required to keep the magnesium out of the cell is relatively small no matter what the mechanism is.

From the data given in Table III and Fig. 2, it was possible to determine the effect of the external magnesium concentrations on the intracellular magnesium concentrations. The equation used for the determination of the equilibrium intracellular concentrations was

$$C_i = \frac{A_i}{A_s + A_c + A_i} \frac{A_T - A_n - EC_e}{I} \tag{7}$$

(a) A_i is the amount of exchangeable magnesium in the intracellular space.
(b) A_s is the amount of exchangeable magnesium in the surface phase.
(c) A_c is the amount of exchangeable magnesium in the connective tissue.
(d) A_T is the total amount of magnesium in the muscle.
(e) A_n is the non-exchangeable magnesium.
(f) E is the extracellular water space (0.18 liter/kg.).
(g) C_e is the extracellular magnesium concentration.
(h) I is the intracellular space (0.62 liter/kg.)
(i) C_i is the intracellular magnesium concentration.

It was assumed that both A_n and the ratio $\frac{A_i}{A_s + A_c + A_i}$ remained constant with any change in C_e. This would occur if the exchangeable magnesium phases are not close to saturation. If these assumptions are approximately

correct, then by substituting the amounts obtained from Fig. 6 into Equation 7, it becomes possible to calculate C_i.

$$C_i = 0.879\ (A_T - A_n - 0.18\ C_e) \tag{8}$$

It was assumed that when C_e was equal to zero, then C_i was equal to zero, so that $A_T = A_n = 5.52$ millimoles/kg. The value of A_n probably depends upon

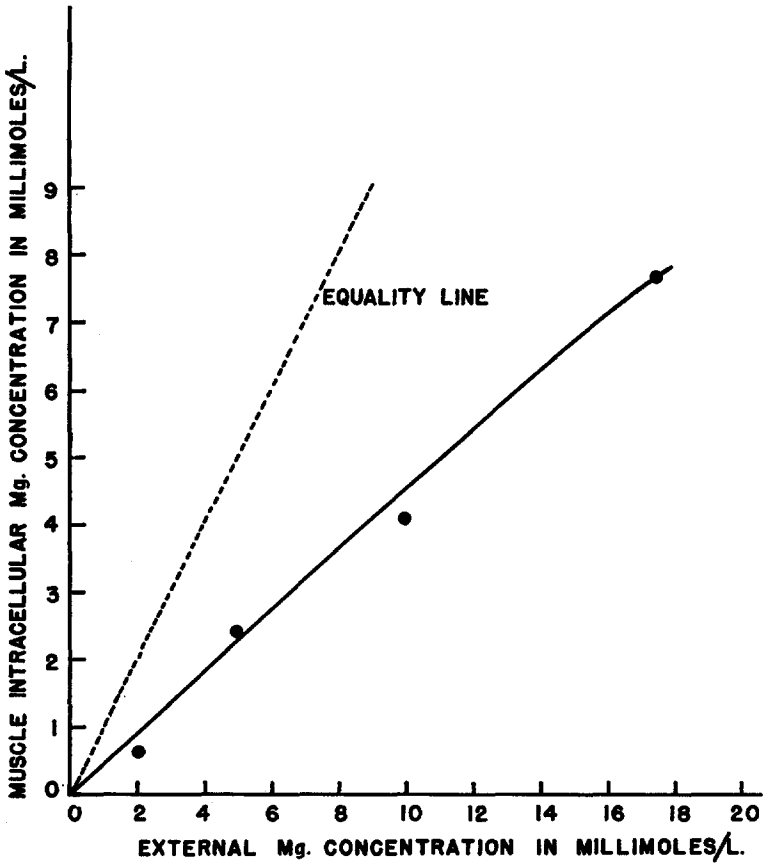

FIGURE 7. Relationship between external magnesium concentration and muscle intracellular magnesium concentration after an equilibration period of 5 hours.

several factors. Thus, muscles from summer frogs contain more magnesium than muscles from winter frogs, and it would also appear likely that the non-exchangeable magnesium differs. Fig. 7 shows the relationship between the extracellular and intracellular magnesium concentrations. As the external magnesium concentration is increased, it appears that the intracellular concentration increases in an approximately linear fashion. Thus, as C_e is increased, the ratio of C_i to C_e remains constant. The membrane potential of

muscle is not affected by an increase in C_e (11) so the value of r^2 remains constant. Thus, it can be seen from Equation 6 that the membrane work factor q remains constant.

The value of 2 mM magnesium used consistently in these experiments appears to be a good value to use, since the magnesium content of the immersed muscles is the same as in the freshly dissected. This observation was previously reported by Fenn and Haege (9). It has been noted that muscles from summer frogs have higher calcium contents than muscles from winter frogs (12).

Implication of a calcium pump pushing the ion out of the muscle cell (2) and squid giant axon (13) has been given. This work also implicates a magnesium pump. Active transport across the yeast cell for magnesium has been described (14, 15). In fact the reabsorption of magnesium in the kidney appears to occur in the distal tubule at the same site as for calcium (16, 17).

The binding of magnesium appears to be greater than that of calcium within the cell as evidenced by the greater amount of magnesium within the cell. Adenosinephosphates have a greater affinity for magnesium than for calcium (18). The binding of calcium and magnesium to proteins seems to be the same for proteins in serum (19). The binding of calcium and magnesium to deoxyribonucleic and ribonucleic acids seems to be the same (20). When there is cell destruction produced either by cutting or prolonged immersion in Ringer's solution, there is a definite tendency for the magnesium to decrease (Table IV) and the calcium to increase (Table V) (2). If the magnesium concentration is high as seen in Table II and Fig. 1, then it is more difficult to observe the decrease. Perhaps, the binding groups are changed, so that the calcium replaces magnesium at certain sites. It is interesting to note that in magnesium deficiency, muscle magnesium decreases and muscle calcium increases (21, 22). The same occurs in muscular dystrophy (23).

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