CALCIUM EQUILIBRIUM IN MUSCLE

By DANIEL L. GILBERT* AND WALLACE O. FENN

(From the Department of Physiology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

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INTRODUCTION

The present investigation was undertaken to determine the permeability of the muscle cell membrane to calcium and to calculate any energy requirement to keep the calcium in a steady state. A preliminary report of this study has already been presented (1).

Methods

General Procedure for Equilibrating Muscles.—The muscles were dissected out of the frogs, and were maintained overnight in Ringer's solution at 5°C. Generally, a set of muscles consisted of the tibialis anticus longus, peroneus, ileofibularis, sartorius, and the semitendinosus. Then one muscle set and its contralateral set were transferred, respectively, to the experimental and the control flasks, each of which contained 100 ml. of solution. Unless otherwise stated, the control solutions were composed of 1.8 mM/liter of $CaCl_2$, 111 mM/liter of NaCl, and 1.34 mM/liter of KCl. 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 500 and 1000 mg. This technique was essentially the same as described by Reilly (2). The calcium content was determined by either of the following methods:—

Calcium Analysis by Method 1.—The muscle ash was dissolved in 1 N HCl, neutralized with NH_4OH, and then acidified with 0.1 N acetic acid until the color of the solution was orange using methyl red as an indicator. Calcium oxalate was formed with the addition of a saturated solution of ammonium oxalate. Precipitation of calcium oxalate in acetic acid solution has been effective in separating calcium from magnesium (3). The calcium in the calcium oxalate samples was determined by Clark and Collip's modification (4) of the Kramer and Tisdall method (5). In this procedure, the oxalate in the sample was determined by titration with 0.01 N $KMnO_4$. One ml. of $KMnO_4$ solution was equivalent to 0.005 mM calcium.

Calcium Analysis by Method 2.—In this procedure, the calcium in the calcium oxalate was determined by titration with EDTA (ethylenediaminetetraacetic acid) ((ethylenedinitrilo)-tetraacetic acid disodium salt, Eastman Organic Chemicals,

* Present address: Department of Physiology, Albany Medical College, Albany, New York.

Distillation Products Industries, Rochester, New York) using the procedure of Flaschka and Holasek (6). The standard procedure was as follows:—

The calcium oxalate precipitate was dissolved in two to five drops of 1 N HCl. One ml. of approximately 0.01 N Mg EDTA was added (or more if necessary). (The stock solution of Mg EDTA was prepared by mixing $MgCl_2 \cdot 6H_2O$ and EDTA together. The pH was adjusted to about 7.0, and the resulting solution was tested to make sure there was no excess Mg or EDTA). Next 2 ml. of buffer (3.55 gm. NH_4Cl plus 22.4 ml. concentrated NH_4OH plus enough H_2O to make a final volume of 100 ml.) was added to bring the pH to about 10, and finally one or two drops of a nearly

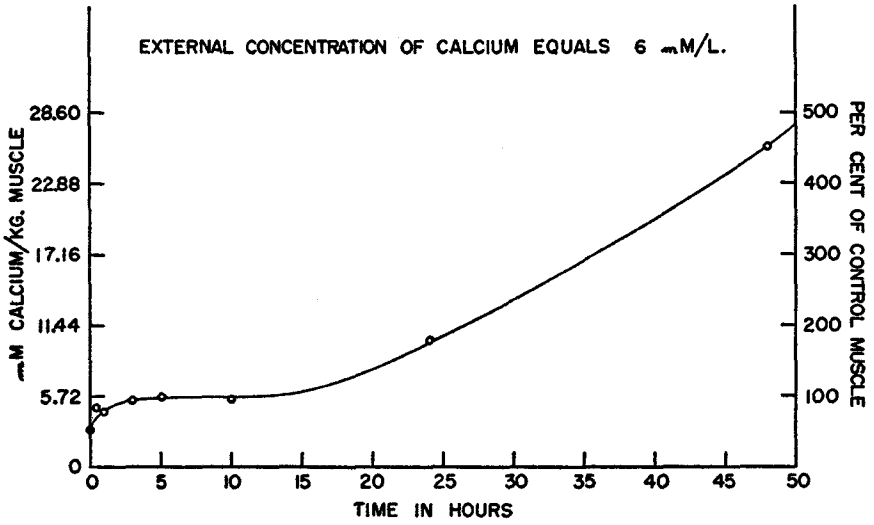

FIG. 1. Effect of equilibration time on the calcium uptake by muscle immersed in Ringer's solution containing 6 mM/liter calcium. Per cent of control muscle was calculated using the average value of 5.72 mM Ca/kg. as equivalent to 100 per cent of the control muscle (see Table I).

saturated, freshly prepared solution of eriochrome black T (1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid sodium salt (practical), Eastman Organic Chemicals) was added. The samples were titrated with 0.002 M EDTA until the color changed from red to blue. One ml. of the EDTA solution was equivalent to 0.002 mM of calcium. The EDTA solution was made up approximately and standardized daily against a calcium chloride standard. An additional 0.12 μM calcium was added to each sample, since in actual determinations on small and large standard calcium samples this small average amount could not be recovered.

Procedure Using Ca^{45}.—For these experiments the Ringer's solution was composed of 2 mM $CaCl_2$, 111 mM/1 NaCl, 2.68 mM/1 KCl and 2.38 mM/1 $NaHCO_3$. Before use the tissues were kept overnight in Ringer's solution at 5°C., followed by 2.5 to 3.0 hours at room temperature. Thereafter they were soaked for various periods of time in Ringer's solution containing Ca^{45} and were then weighed and ashed in the usual

manner. (The Ca^{45} was obtained from Oak Ridge National Laboratory.) The ash was dissolved in water, transferred to planchets with a diameter of 3.7 cm. and dried. The radioactivity in C.P.M. (counts per minute) was determined using an end-window thin mica (3.5 mg./cm.^{2}) Geiger-Müller tube. The radioactivity for the sample was expressed in per cent of the radioactivity in the Ringer's solution.

RESULTS

The uptake of calcium in muscle immersed in Ringer's solution containing 6 mM/liter of calcium for various periods of time is tabulated in Table I and illustrated in Fig. 1. The control muscles were immersed for 5 hours. The average calcium value for all the 51 controls in this series was 5.72 mM/kg.

TABLE I

Effect of Time on the Uptake of Calcium in Muscle Immersed in Ringer's Solution Containing 6 mM/Liter Calcium

Time		No. of experiments	mM Ca/kg. wt.		Exp./Control	P
Experimental	Control		Experimental	Control		
hrs.	*hrs.*				*per cent*	*per cent*
0	5	7	2.65 ± 0.26	5.09 ± 0.51	53.1 ± 4.2	0.0
0.5	5	6	4.06 ± 0.26	4.97 ± 0.48	83.8 ± 5.5	3.1
1	5	6	4.58 ± 0.18	5.97 ± 0.56	79.3 ± 5.9	1.7
3	5	5	5.14 ± 0.24	5.40 ± 0.20	95.2 ± 3.3	21.8
5	5	6	5.29 ± 0.42	5.51 ± 0.36	97.6 ± 4.5	61.3
10	5	6	5.86 ± 0.69	6.26 ± 0.54	95.8 ± 8.5	64.5
24	5	9	11.3 ± 1.1	6.49 ± 0.47	179 ± 17	0.2
48	5	6	25.1 ± 2.1	5.72 ± 0.40	453 ± 55	0.1
			Average.....	5.72 ± 0.17		

In Fig. 1 this average value was plotted as 100 per cent of the control. After 1 hour, the experimental value was about 80 per cent of the control value. By 3 hours equilibrium was reached and was maintained until 10 hours. When the time increased to 24 and 48 hours, the experimental values increased to about 180 and 450 per cent respectively. At these times definite signs of tissue breakdown were observed and the Ringer's solution became cloudy.

The effect of the calcium concentration in Ringer's solution on the calcium uptake in muscle after an equilibration time of 5 hours is shown in Table II and Fig. 2. The average calcium content of the 71 control muscles (immersed in 1.8 mM Ca/liter) was 2.84 mM/kg. This value was plotted as equivalent to 100 per cent in Fig. 2. When muscles were immersed in a calcium-free solution, the amount of calcium in the muscle was decreased to 1.24 mM/kg. The calcium uptake increased as the external calcium concentration was increased from 0 to 24 mM/liter.

Eight sets of six sartorii each (800 mg. total weight) immersed in a Ringer's solution containing 2 mM/liter of $CaCl_2$, 111 mM/liter of NaCl, 2.68 mM/liter of KCl, and 2.38 mM/liter of $NaHCO_3$ for 5 hours had a calcium content of 3.32 ± 0.22 mM/kg. A better estimate of the calcium content of sartorii under

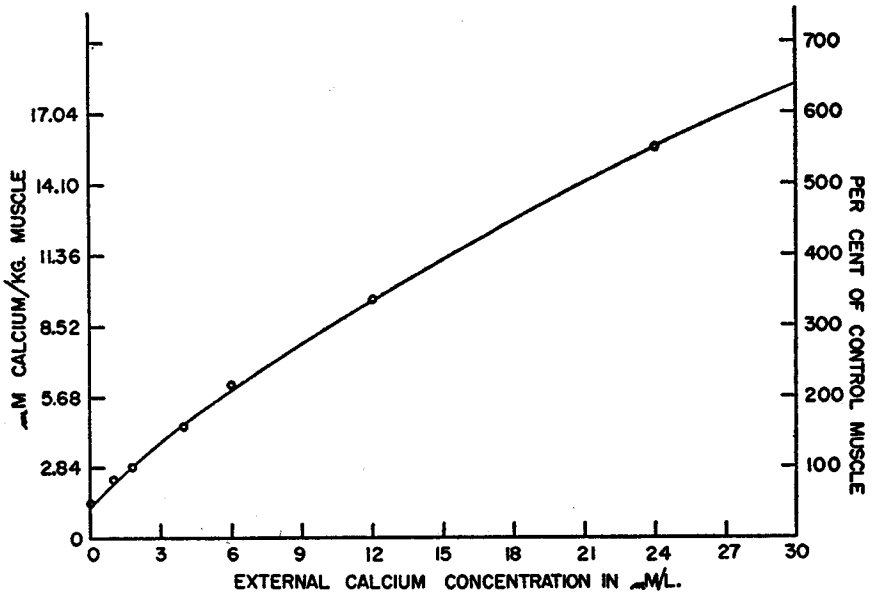

FIG. 2. Relationship between calcium concentration and amount of calcium in muscle after an equilibration period of 5 hours. Per cent of control muscle was calculated using the average value of 2.84 mM Ca/kg. as equivalent to 100 per cent of the control muscle (see Table II).

TABLE II

Effect of Calcium Concentration in Ringer's Solution on the Calcium Content in Muscle

mM/liter Ca in Ringer's		No. of Experiments	mM Ca/kg. wt.		Exp./Control
Experimental	Control		Experimental	Control	
					per cent
0	1.8	12	1.24 ± 0.03	2.53 ± 0.07	49.2 ± 1.7
1	1.8	12	2.41 ± 0.10	2.95 ± 0.13	82.3 ± 3.1
4	1.8	12	3.91 ± 0.18	2.46 ± 0.05	159 ± 6
6	1.8	12	6.03 ± 0.30	2.79 ± 0.11	217 ± 12
12	1.8	12	10.1 ± 0.44	3.04 ± 0.10	337 ± 18
24	1.8	12	17.4 ± 0.62	3.28 ± 0.21	552 ± 39
			Average.....	2.84 ± 0.05	

In all cases, the differences between experimental and control values were significant (P = 0.0 per cent).

these conditions can be obtained from Fig. 2 for which a large number of muscle sets were analyzed. This graphical estimate was about 3.0 mM/kg.

Table III gives the effects of a few conditions on the calcium content. Muscles which were dissected and analyzed without previous immersion in

TABLE III

Effect of Various Conditions on the Calcium Content in Muscle

Condition		mM/liter Ca in Ringer's	No. of Experiments	mM Ca/kg. wt.		Exp./Control
Experimental	Control			Experimental	Control	
						per cent
Freshly dissected	Immersed	1.8	12	1.63 ± 0.07	2.84 ± 0.12	57.8 ± 2.5
Cut	Not cut	1.8	11	5.29 ± 0.10	2.84 ± 0.12	188 ± 5
Cut	" "	6.0	6	11.6 ± 0.4	5.75 ± 0.32	204 ± 8
10 mM/liter EDTA	No EDTA	0.0	11	0.91 ± 0.03	1.12 ± 0.03	81.4 ± 3.1

In all cases the differences between experimental and control values were significant (P = 0.0 per cent).

TABLE IV

The Uptake of Ca^{45} by Frog Sartorius Muscle, Gastrocnemius Muscle, and Achilles Tendon

Time	Sartorius muscle		Gastrocnemius muscle		Achilles tendon	
	No. of experiments	C*	No. of Experiments	C*	No. of experiments	C*
min.						
0.01	8	4.4 ± 0.5	5	2.8 ± 0.4	3	8.6 ± 0.2
0.1	10	8.2 ± 0.6	5	3.2 ± 0.3	3	12.5 ± 3.9
0.5	10	13.7 ± 0.9				
1	10	18.5 ± 1.6	5	5.5 ± 0.5	3	20.9 ± 2.5
2.5	10	36.2 ± 3.6				
5	10	32.6 ± 1.8	5	10.9 ± 2.0	4	37.7 ± 6.1
10	10	44.4 ± 2.5	5	13.1 ± 0.9	3	56.3 ± 5.5
15	10	59.4 ± 3.8	5	15.6 ± 0.6	3	63.4 ± 4.2
30	10	63.5 ± 2.2	5	19.5 ± 3.2	3	66.4 ± 2.0
45	10	68.8 ± 7.9				
60	10	81.9 ± 5.2	5	27.9 ± 3.4	3	87.9 ± 6.3
90	10	68.9 ± 4.8	5	30.9 ± 3.1	3	107 ± 11
120	10	95.4 ± 7.0				
150	10	68.6 ± 3.4				
180	10	73.5 ± 6.0	5	40.9 ± 6.0	3	106 ± 14
300	10	99.1 ± 12.4	4	36.2 ± 3.8	3	102 ± 9
420			2	61.0 ± 10.6	1	130
600	10	93.1 ± 11.7	4	50.8 ± 3.8	3	116 ± 16

* $C = \frac{\text{C.P.M. per 100 mg. of tissue}}{\text{C.P.M. per 0.1 ml. of Ringer's solution}} 100$

Ringer's solution contained 1.63 ± 0.07 mM/kg. which was significantly lower than the calcium in muscle immersed in Ringer's solution. Muscles which were cut into small pieces took up about twice as much calcium as their control muscles. A 10 mM/liter solution of EDTA added to calcium-free Ringer's solution removed an additional 0.21 mM Ca/kg.

Table IV gives the uptake of Ca^{45} in sartorius muscle, gastrocnemius muscle, and Achilles tendon when these tissues were immersed in Ringer's solution containing 2 mM/liter. The total uptake in the sartorius muscle appeared to be greater than in the gastrocnemius muscle and less than in tendon.

DISCUSSION

Analysis of Ca^{45} Data.—If a membrane separates two compartments, then the general permeability equation will be:—

$$\frac{dC_2}{dt} = K_1 C_1 - K_2 C_2 \tag{1}$$

(a) Subscripts 1 and 2 refer, respectively, to compartments 1 and 2.
(b) Units of exchange constants K_1 and K_2 are in time^{-1}.

In order to integrate Equation 1, it is necessary to know the relationships between time and K_1, K_2, and C_1. The exchange constants may be functions of the chemical concentrations, and if the chemical concentration changes with time, then the exchange constants may also change with time. However, if the chemical concentrations are constant, then it may be assumed that the exchange constants are also constant. This latter situation would, of course, be true during a steady state condition in which there is no net transfer of any chemical substance from one compartment to another. If a trace of a radioactive isotope is introduced into a steady state system, and if C_1 and C_2 represent concentrations of the radioactive isotope, then C_1 and C_2 may vary without affecting either the chemical concentrations in compartments 1 and 2 or the exchange constants K_1 and K_2. For a simple system in which C_1 is constant and $C_2 = 0$ when $t = 0$, the integrated form of Equation 1 is:—

$$C_2 = -\frac{K_1}{K_2} C_1 e^{-K_2 t} + \frac{K_1}{K_2} C_1 \tag{2}$$

In the Ca^{45} 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 I). If the muscle could be considered as a single homogeneous compartment, then the Ca^{45}

uptake should correspond to Equation 2 which involves only a single exponential term. The equation of the radioactive uptake was estimated employing a minimum number of exponential terms, which turned out to be three. The method of calculating this type of equation is similar to that described by Solomon (7). The equation of the uptake by the sartorius muscle is as follows:—

$$C = -38.9\, e^{-0.114\, t} - 41.7\, e^{-0.0107\, t} - 14.4\, e^{-0.56\, t} + 95.0 \tag{3}$$

(a) C is the Ca^{45} concentration of muscle.
(b) t is time in minutes.

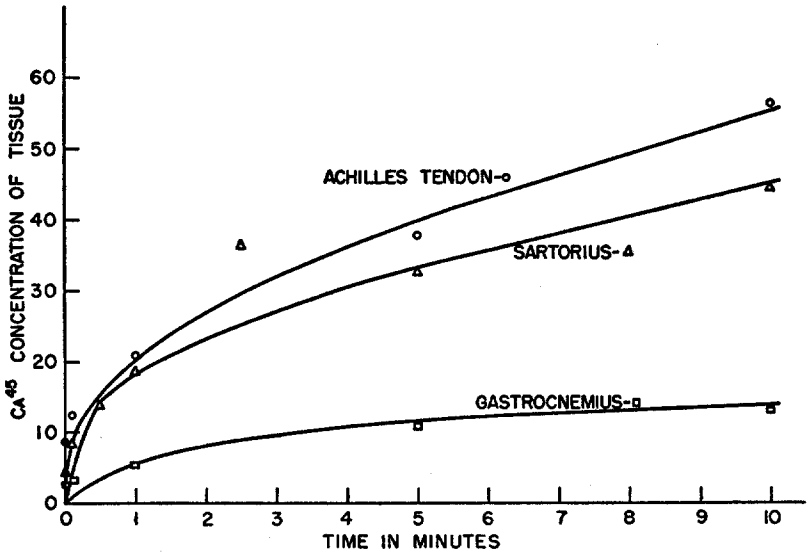

FIG. 3. Calculated Ca^{45} uptake curves of sartorius muscle, gastrocnemius muscle, and Achilles tendon up to 10 minutes. Symbols mark observed points.

The constant 95 was determined graphically. No mathematical analysis is given for the gastrocnemius muscle since diffusion would be slower owing to the thickness of the muscle itself (8) and would more seriously complicate this type of analysis. However, it can be pointed out that the rate of uptake by the gastrocnemius was slower than for the sartorius, probably owing to the diffusion factor. Figs. 3 and 4 illustrate the calculated uptake curve and the experimental points for the sartorius muscle. With so much variability in the data (as illustrated in Figs. 3 and 4) it is evident that Equation 3 represents only a very rough approximation.

Since there were three exponential terms in Equation 3, it was important to determine what type of compartment arrangements could correspond to

such an equation. For a system of N compartments the permeability equation is a single linear differential equation of order N-1 with constant coefficients.

$$\frac{d^{N-1}C}{dt^{N-1}} + a_1 \frac{d^{N-2}C}{dt^{N-2}} + \cdots a_{N-2}\frac{dC}{dt} + a_{N-1}C = a_n \tag{4}$$

(a) C is the concentration in one particular compartment.
(b) Constants a are functions of the exchange constants.
(c) a_n is also a function of the amount in the system.

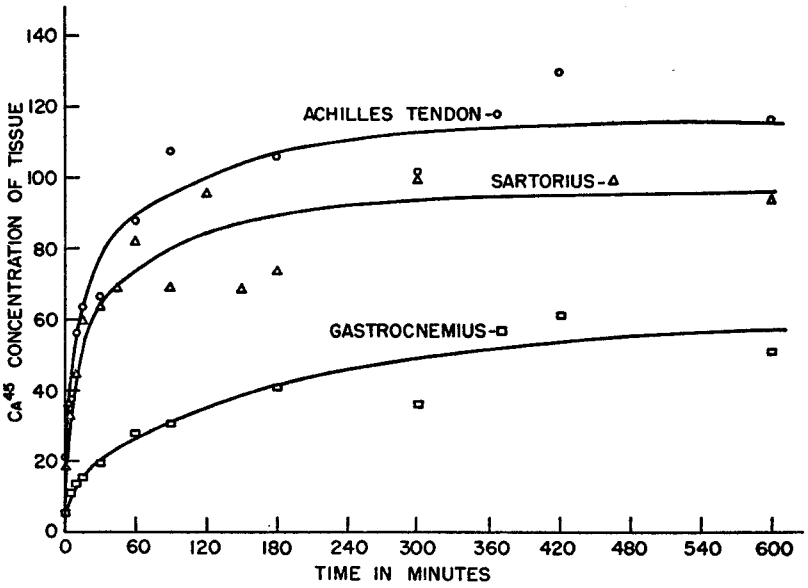

FIG. 4. Calculated Ca^{45} uptake curves of sartorius muscle, gastrocnemius muscle, and Achilles tendon up to 600 minutes. Symbols mark observed points.

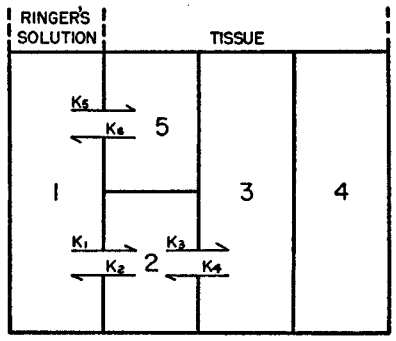

FIG. 5. Diagram showing compartments of tissue. Compartment 1 is the Ringer's solution; compartment 2 is the extracellular space; compartment 3 is the intracellular space; compartment 4 is the non-exchangeable space; and compartment 5 is the surface space.

The integrated form will have a maximum of (N-1) exponentials and since each exponential contains 2 constants, there will be 2(N-1) constants in the integrated equation. Thus, if the number of exchange constants exceed 2(N-1), then all of the exchange constants cannot be determined unless additional assumptions are made. Since the Ca^{45} uptake curve was described by an equation with three exponentials, a four compartment system containing six exchange constants was chosen as illustrated in Fig. 5. Compartment 1 is the Ringer's solution, compartment 2 is the extracellular space, compartment 3

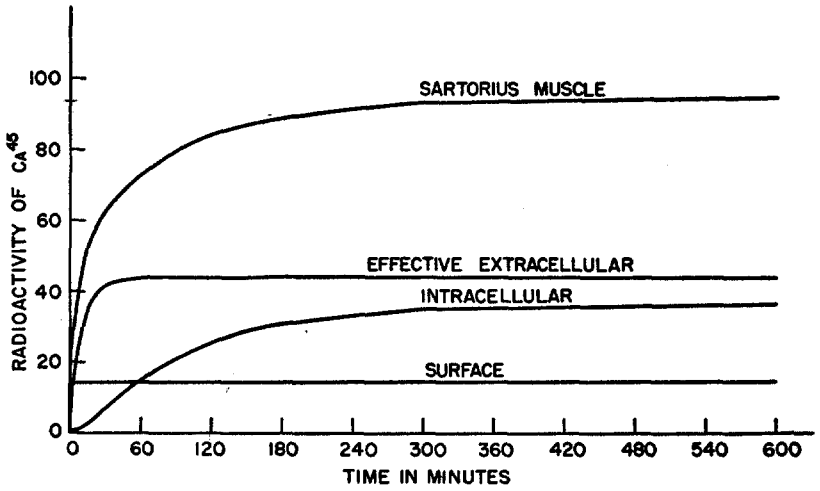

FIG. 6. Calculated Ca^{45} uptake curves of the effective extracellular, intracellular, and surface phases of sartorius muscle.

is the intracellular space, compartment 5 is the surface space, and compartment 4 is the non-exchangeable space. It was found that there were six solutions which would fit these conditions. Each solution contained six exchange constants, but three solutions were disgarded since they contained negative constants. Two other solutions were regarded as improbable because they required the assumption that the extracellular space would become equilibrated before the surface space. The solution finally chosen was the one in which the calcium became equilibrated first with the surface phase and last with the intracellular phase. The kinetics of the uptake of the three phases is illustrated in Fig. 6 and described by the following equations:—

(5) $$C_s = -14.4e^{-3.56t} + 14.4$$

(6) $$C_p = -42.7e^{-0.114t} - 1.3e^{-0.0107t} + 44.0$$

(7) $$C_n = 3.8e^{-0.114t} - 40.4e^{-0.0107t} + 36.6$$

(a) C_s, C_p, and C_n are the Ca^{45} amounts in the compartments 5, 2, and 3, respectively.
(b) t is time in minutes.

The extracellular space as determined by this method is equal to the value of C_p after equilibrium has been obtained. From Equation 6 it appears that the measured extracellular calcium space amounted to 44 per cent. However, this value appeared to be too large and therefore it was assumed that the effective extracellular space actually included some calcium bound in the connective

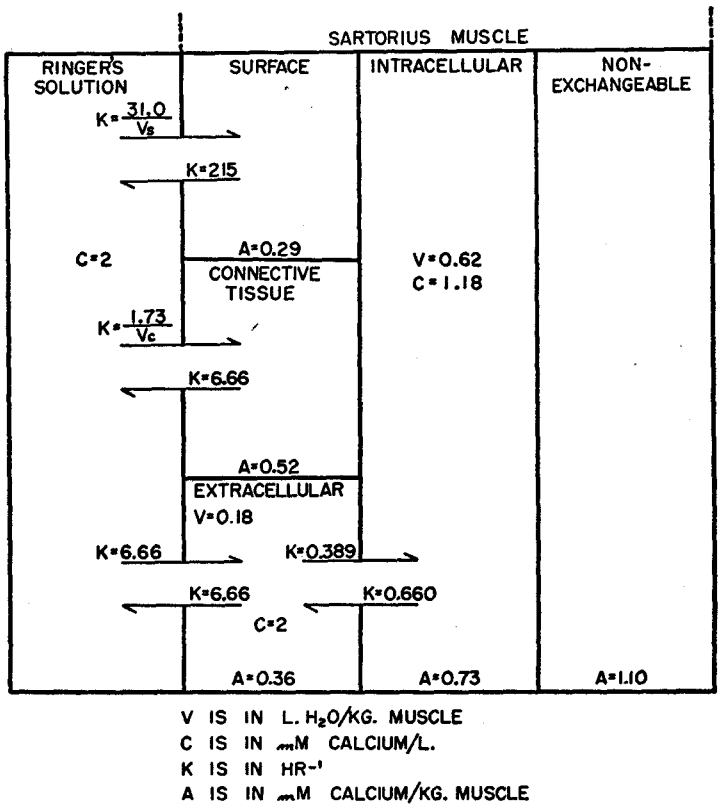

FIG. 7. Volumes, concentrations, exchange constants, and amounts of calcium in the different phases of the sartorius muscle.

tissue or not free in solution in the same concentration as in the outside solution. Fig. 7 illustrates the chemical amounts of calcium in the several compartments of the sartorius muscle in the steady state. For these calculations it was assumed that the muscle water was equal to 80 per cent (9) and that the extracellular water space was equal to 18 per cent (10). Any water in the surface and connective tissue phases was arbitrarily included in the extracellular water phase.

If it be assumed that the surface phase is completely equilibrated (and re-

mains so) before any appreciable Ca^{45} enters the extracellular phase and that the extracellular phase is equilibrated (and remains so) before the Ca^{45} enters the intracellular phase, then the mathematics becomes easier and almost identical values are found. Thus, using this approximation, Equations 5, 6, and 7 become:—

$$C_e = -14.4e^{-3.68t} + 14.4 \tag{8}$$

$$C_p = -38.9e^{-0.114t} + 38.9 \tag{9}$$

$$C_n = -41.7e^{-0.0107t} + 41.7 \tag{10}$$

Calcium Localization in Muscle.—It appears that the surface phase became equilibrated in about a minute and contained 0.29 mM calcium/kg. muscle or about 10 per cent of the total muscle calcium. This phase certainly cannot be the extracellular water phase since it equilibrated so rapidly and may be considered perhaps as the epimysium. The extracellular water and connective tissue phases become equilibrated in about 30 minutes, which seems a reasonable period of time. The extracellular water phase was calculated to contain 12 per cent of the total calcium and the connective tissue about 17 per cent. To test the hypothesis that the connective tissue may take up a sizable portion of the Ca^{45}, Achilles tendon was immersed in radioactive calcium solution, and appeared to be able to concentrate the Ca^{45} (Table IV and Fig. 3). Manery (11) has previously pointed out the similarities between tendon and the connective tissue phase in skeletal muscle, and therefore, it seems reasonable to suspect that connective tissue could account for the 17 per cent. It is interesting to note that Harris (12) has reported that the initial loss of Ca^{45} in frog muscle is rapid and exceeds the amount in the extracellular space. Of the total calcium of the muscle 24 per cent was in compartment 3 and exchanged in about 300 minutes. If the assumption is correct that this compartment represents intracellular space then this observation constitutes proof that the cell membrane is permeable to calcium. If it is not intracellular however, then it must be in some slowly exchangeable portion of the extracellular space which seems improbable. The non-exchangeable calcium amounted to 1.1 mM/kg. muscle or about 37 per cent of the muscle calcium. The observation was also made that when muscles were immersed in Ringer's solution containing no calcium, the amount of calcium in the muscle after 5 hours' equilibration varied from 1.12 (Table III) to 1.24 (Table II) mM/kg. muscle. It appears therefore that this non-exchangeable calcium is still in the muscle even after soaking in calcium-free solution. EDTA, a known calcium-chelating agent was added to the Ringer's solution in order to determine whether this non-exchangeable calcium could be removed. However, Table III shows that 0.9 mM/kg. still remained in the muscle even after treatment with 10 mM/liter EDTA. It was calculated from seven experiments of Reilly (2) that 1.78 ±

0.11 mM/kg. of calcium was still present in muscle after immersion in a calcium-free solution.

Energy Expenditure for Maintaining Calcium in the Steady State.—At equilibrium, the total free energy of an ion on both sides of a permeable membrane must be the same. This condition is expressed in Equation 11.

$$RT \ln C_1 + zFV_1 + W_1 = RT \ln C_2 + zFV_2 + W_2 \tag{11}$$

(a) Subscripts 1 and 2 refer, respectively, to compartments 1 and 2.
(b) C is the concentration of the ion (actually the activity should be used instead of the concentration).
(c) R is the gas constant.
(d) T is the absolute temperature.
(e) V is the electrical potential.
(f) z is the valence of the ion.
(g) F is the Faraday constant.
(h) W is the potential due to other forces besides electrical and chemical ones.

The membrane potential may be designated as $V_2 - V_1$ or E_m and the work potential may be designated as $W_2 - W_1$ or W_q. Equation 11 then becomes

$$\ln \frac{C_1}{C_2} = \frac{zF}{RT} E_m + \frac{W_q}{RT} \tag{12}$$

It is finally possible to simplify Equation 12 even further, if some additional definitions are made.

$$\frac{C_1}{C_2} = r^z q \tag{13}$$

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

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

If these equilibrium states are used for the steady state in muscle, then the work potential might be considered as an active transport potential, which would be equal and opposite to the passive electrochemical potential. Subscripts 1 and 2 would then refer respectively to the intracellular and extracellular compartments. The value of r equals about 32.9 if the membrane potential is assumed to be 88 mv. (13). If q equals 1, then there exists just a passive Donnan equilibrium; and if q is less than 1, then there is an electrochemical gradient forcing the ion into the cell, which might be counterbalanced by an active transport mechanism forcing the ion out of the cell. If the potassium equilibrium obeys the Donnan equilibrium, then the reciprocal of q for sodium would be identical with Dean's sodium pump factor "e" (14). Using an intracellular concentration of 1.18 mM calcium/liter and an extra-

cellular concentration of 2mM/liter (see Fig. 7), the work factor q was found to equal $5.54 \cdot 10^{-4}$. Being less than 1, this indicates that there is some active force pushing the calcium out of the cell. Actually, the intracellular concentration should only refer to the ionized calcium, and if any is chemically bound, then q would deviate even further from 1. The value for the work potential for calcium was calculated to be -4.37 cal./mM. Landahl (15) calculated that the work function for sodium was $-5.2/RT$ (about -3 cal./mM). Thus the electrochemical gradient pushing the 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 work potential by the outward flux. The outward flux for calcium is equal to the intracellular exchange constant times the intracellular concentration or to 0.66 $hour^{-1}$ times 1.18 mM/liter (see Fig. 7). This flux would therefore amount to 0.779 mM/(liter hour), and the active transport energy would amount to 2.1 cal./(kilogram hour) which is only about 1 per cent of the resting energy, assuming that the resting energy output is about 175 cal./(kilogram hour) (16). It should be emphasized that unless the mechanism of transport is known, it is not possible to determine the active transport energy. Thus, in the steady state the energy which is released as the calcium enters the cell may be utilized to force the calcium out of the cell. Then the active transport energy would equal zero. Ussing's exchange diffusion (17) would be an example of this phenomenon. However, if none of the energy which is released by calcium entry, is utilized by the active pump forcing the calcium out, then the active pump would require the calculated 2.1 cal./(kilogram hour). If there is a net transfer of ions, then the minimum active transport energy would equal the net flux times the work potential. It appears from these experiments, however, that the maximum energy required to keep the calcium out of the cell is relatively small no matter what the mechanism is.

From the data given in Table II and Fig. 2, it was possible to determine the effect of the external calcium concentrations on the intracellular calcium concentrations. These data agree roughly with values obtained by Reilly (2). The equation used for the determination of the intracellular concentrations was:—

$$C_i = \frac{A_i}{A_s + A_c + A_i} \cdot \frac{A_T - A_n - EC_e}{I} \tag{14}$$

(a) A_i is the amount of calcium in the intracellular space.
(b) A_s is the amount of calcium in the surface phase.
(c) A_c is the amount of calcium in the connective tissue.
(d) A_T is the total amount of calcium in the muscle.
(e) A_n is the non-exchangeable calcium (1.10mM/kg.).
(f) E is the extracellular water space (0.18 liter/kg.).
(g) C_e is the extracellular calcium concentration.

(h) I is the intracellular space (0.62 liter/kg.).
(i) C_i is the intracellular calcium 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. If these assumptions are correct, then by substituting

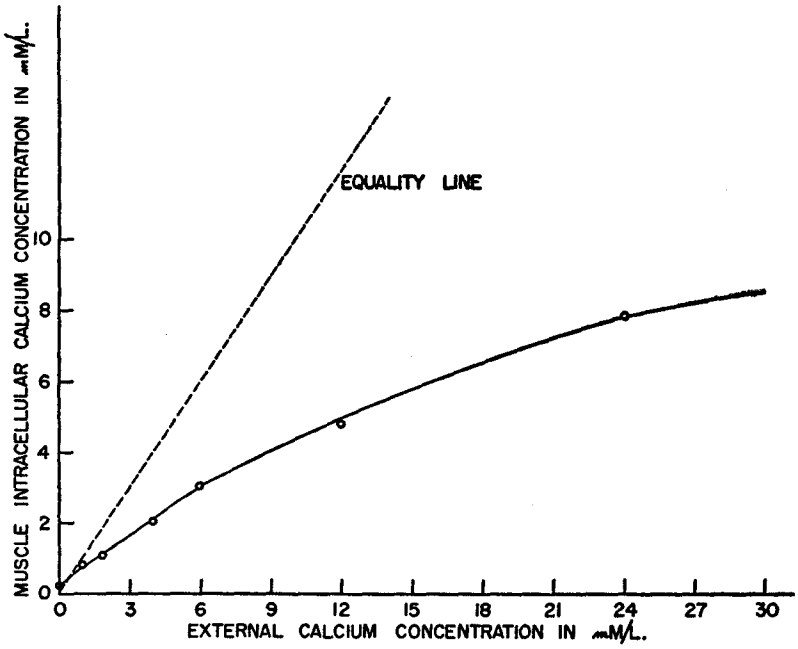

FIG. 8. Relationship between external calcium concentration and muscle intracellular calcium concentration after an equilibration period of 5 hours.

the amounts obtained from Fig. 7 into Equation 14, it becomes possible to calculate C_i.

$$C_i = 0.765\ (A_T - 1.10 - 0.18 \cdot C_e) \tag{15}$$

Fig. 8 shows the relationship between the extracellular and intracellular calcium concentrations. As the external calcium concentration is increased, it appears that the intracellular concentration only slightly increases. Thus as C_e is increased, the ratio of C_i to C_e is decreased. From Equation 13, it can be seen that the product $r^s q$ must also decrease with an increase in C_e. However, the value of r^s probably increased, since the membrane potential increases with increased external calcium concentration (18). Therefore, the

membrane factor q would definitely decrease as C_e is increased; which would mean that the calcium pump forcing the ion out of the cell is able to do more work as the external calcium concentration is increased.

Further evidence for a calcium pump is given in Table III, in which it is noted that merely cutting the muscle into small pieces increased the calcium content, presumably because of some interference with the activity of the pump. Another factor which would explain part of this observed increase might be calcium-binding by the exposed muscle protein. Weimar (19) has found that frog muscle brei can bind a large amount of calcium, and Weise (20) could not detect any ultrafilterable calcium from rat muscle finely cut up. Fig. 1 and Table I also show that prolonged immersion in Ringer's solution produces a tremendous increase in calcium, with visible signs of tissue breakdown. Again this calcium increase can be interpreted to mean that the calcium pump decreases its efficiency as the cell dies, and that protein breakdown exposes an increased number of binding sites for calcium. Other conditions, in which it is possible to suspect a deterioration of the calcium pump and in which there is observed an increased calcium content are in muscular dystrophy (21, 22), in muscular atrophy produced by denervation, tenotomy, or fasting (23) and in old age (24, 25). Malignant tissues contain low amounts of calcium, but there was observed an inability to exchange calcium (26) which might indicate an impermeability of the membrane.

It should be added that due to the large variability in the data, the various calculations should be taken only as rough approximations. Variation in muscle calcium contents has been previously reported (27). Perhaps experiments in which the loss of radioactive calcium from Ca^{45}-equilibrated muscles is measured, may give better results, since only one muscle would be needed to obtain a complete curve. It would be interesting to observe the effects of changing the external calcium on the exchange constants. Such data might prove very valuable in elucidating the transport mechanism.

SUMMARY

1. A study of the calcium equilibrium in isolated frog muscle has been attempted.

2. When sartorius muscles were immersed in Ca^{45} Ringer's solution, the surface phase took up the Ca^{45} in about 1 minute; the extracellular water space and connective tissue in about 30 minutes; and the intracellular space in about 300 minutes.

3. The percentages of total calcium in the whole muscle immersed in Ringer's solution was as follows: 10 per cent in the surface phase; 12 per cent in the extracellular water space; 17 per cent in the dry connective tissue; 24 per cent in the intracellular space; and 37 per cent as non-exchangeable calcium.

4. The exchange constants of isolated frog sartorius muscle to calcium has been determined. The flux of intracellular calcium in the steady state was approximately 0.8 mM/(liter hr).

5. It appears that there is a calcium pump pushing calcium out of the cell against an electrochemical gradient of about 4 cal./mM of calcium. However, since the flux is low, the maximum energy required per hour to pump calcium out of the cell against this high gradient is only about 2 cal./kg. muscle or about 1 per cent of the resting energy.

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