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SODIUM AND WATER CONTENTS OF SARCOPLASM AND SARCOPLASMIC RETICULUM IN RAT SKELETAL MUSCLE: EFFECTS OF ANISOTONIC MEDIA, OUABAIN AND EXTERNAL SODIUM

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SUMMARY

1. During the first 2 hr washout of ^{24}Na from rat extensor digitorum longus muscle fits a sum of two exponentials, neither of which represents loss of extracellular tracer. This implies a model with two intracellular components.

2. Results of suitably designed experiments indicate that the two components are bidirectionally connected to each other as well as to extracellular space. These results are incompatible with a model in which every fibre is homogeneous with respect to Na concentration and flux, but in which there is a distribution of these properties among fibres.

3. Results are consistent with identification of the more slowly exchanging component as sarcoplasm and the more rapidly exchanging component as sarcoplasmic reticulum (SR).

4. Parameters of the general model include six transport coefficients, two volumes, and contents of two Na pools. The number of equations is inadequate to yield unique solutions by which the values of these parameters can be calculated. However, we derive inequalities that place upper and lower limits on the parameters.

5. If the model is correct, the rate constant for Na efflux from SR to extracellular space is at least five times greater than that across sarcolemma. Under standard conditions flux (per muscle weight) from SR is at least 100 times greater than that from sarcoplasm.

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6. Under standard conditions, only 2–4% of intracellular Na, or 0·5–0·9 m-equiv/kg wet wt., is in sarcoplasm, and the rest is in SR.

7. Bounds on fluid volumes of sarcoplasm and SR under standard conditions are calculated with the assumption that Na concentration in SR is the same as in extracellular space. According to the calculations, fluid volume of sarcoplasm is 0·54 ml./g wet wt. Fluid volume of SR is about 0·124 ml./g wet wt., or 14·3% of fibre volume, in agreement with Peachey's estimate (1965) of volume of SR in frog muscle.

8. Three tests are applied to the model, with the following results: (*a*) volume of sarcoplasm increases in hypotonic solution and decreases in hypertonic solutions, as predicted for an osmometer. Volume of SR tends to change in the opposite direction, in agreement with results of Birks & Davey (1969) from electron microscopy on frog muscle; (*b*) the major effect of partial substitution of external Na by Li is a reduction in Na content of SR, with no significant change in that of sarcoplasm or in volume of either component; (*c*) the major effect of 10^{-5} M ouabain is an increase in Na content of sarcoplasm, with no demonstrable change in that of SR or in volume of either component.

9. These results support the model, particularly our identification of the slowly exchanging component as sarcoplasm, identification of the rapidly exchanging component as SR, and the assumption that Na concentration in SR is close to that in extracellular fluid.

INTRODUCTION

Washout of radiosodium from whole muscle is not a simple exponential function of time. Various interpretations have been put forth to account for the shape of the washout curve, among them models that retain the simplicity of first-order kinetics for Na exchange across the barriers present in the system. In one such model (e.g. Creese, 1968) the number of limiting barriers is supposed minimal and the washout curve viewed as a sum of two exponentials related to efflux of intracellular and extracellular tracer respectively. This view is supported by results of Hodgkin & Horowicz (1959) who showed that single fibres from frog muscle exhibited monoexponential washout, at least for the duration of their experiments. In another model, suggested by Keynes & Steinhardt (1968), the number of barriers is increased in keeping with anatomic evidence and the appearance of two exponentials on the washout curve considered compatible with exit of Na from two intracellular Na pools, namely sarcoplasm and sarcoplasmic reticulum, connected in a series-parallel arrangement. Provided sarcoplasmic reticulum is the more rapidly exchanging component, this view is compatible with earlier compartmental hypotheses concerning

Na flux and distribution in muscle, some of which preceded the rediscovery of the sarcoplasmic reticulum.

For example, Levi & Ussing (1948) observed two phases of ^{24}Na washout from frog muscle and, from the faster phase, calculated an apparent interspace volume that was about twice as large as the generally accepted 13 % of muscle weight. They suggested that a part of the muscle anatomically belonging to the fibres exchanges its Na^+ just as rapidly as do the interspaces proper. Carey & Conway (1954), to explain their observation that ^{42}K entry and Na exit from muscle fibres occur in two distinct phases, proposed that Na in muscle is not uniformly distributed throughout the fibre but that most of it is contained in a special region with a high permeability to both Na and K and with Na concentration higher and K concentration lower than in the general fibre mass. The special region they proposed is neither extracellular space, nor superficial fibres, nor fibres of small diameter. Harris (1963) also accepted the presence in muscle cells of a non-specific internal space associated with at least 15 % of intracellular water and having Na, K, and Cl concentrations essentially equal to those in bathing solutions. He suggested the endoplasmic reticulum as a possible site for the non-specific region.

We were led on empirical grounds to a two-component model for Na efflux from rat extensor digitorum longus. In washout experiments of 2 hr duration, under a variety of conditions, the loss of ^{24}Na from this muscle fits a sum of two exponentials, both apparently of intracellular origin.

There are three possible explanations for such an observation.

(*a*) If ^{24}Na efflux from a single fibre were in fact described by a single exponential until all ^{24}Na was washed out, then the curvature observed in our washout experiments might be attributable to a family of exponential decays distributed among the populations of muscle fibres arranged in parallel with one another. This possibility can be tested, and we show it is not the explanation.

(*b*) It may be that the fit to two exponentials is just empirical. Presumably Na efflux is by diffusion and active transport, and Na distribution inside the fibre and its organelles is by diffusion and perhaps by other means, in space of irregular and complicated shape. Perhaps we deal really with a single mass transfer function which might be fitted by an infinite series of exponential terms, weighted heavily by the first two terms. In this case, individual terms would have no relation to separate structural components of a muscle fibre.

(*c*) A third possibility, the one we adopt, is that there may be in fact two components in muscle, each relatively homogeneous with respect to Na, and that the rate-limiting events in ^{24}Na washout occur at barriers

surrounding the two intracellular Na pools. If so, we can begin by supposing that one component might be sarcoplasmic Na and the other might be Na in sarcoplasmic reticulum. This model can be tested by experimental conditions that change volume or Na content of sarcoplasm and sarcoplasmic reticulum. Results of these tests are consistent with the proposed two-component model.

METHODS

Glucose-Krebs–Ringer-bicarbonate buffer (GKRHCO$_3$), pH 7·4, gassed with 95 % O_2, 5 % CO_2, was used throughout. The standard medium had the following composition (mM): NaCl, 117; $NaHCO_3$, 28; KCl, 3·5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $CaCl_2$, 2·5; glucose, 11.

To prepare anisotonic solutions we changed the standard NaCl concentration, 117 mM, by factors of 0·5, 1·5 or 2 while leaving the other salt concentrations unaltered. Osmolality of loading solutions was not measured but was calculated as if solutions were ideal and as if discrepancies between measured and expected Na concentrations (average 4–8 %) were due solely to evaporation during the loading period. Expressed as multiples of osmolality of the standard Ringer solution at the end of the loading period, R, osmolalities of the anisotonic solutions used were 0·63, 1·36, and $1·66 \times R$.

In solutions with reduced Na concentration, called 80 % Li, 117 mM-LiCl was substituted for NaCl in the standard medium.

Ouabain, when used, was added to the standard medium as the octahydrate (Sigma Chemical Company, St Louis, Mo.).

Both male and female rats, Charles River CD strain, were obtained from the Charles River Breeding Laboratories, Inc., Brookline, Mass. Body weight ranged from 50 to 100 g; extensor digitorum longus muscles ranged in weight from 20 to 50 mg.

The entire experiment was conducted at room temperature, approximately 25° C. Rats were sacrificed by cervical fracture. An extensor digitorum longus muscle was exposed, ligatures were attached to both its tendons and rest length was measured before the tendons were severed and the muscle was removed to a chamber for loading with ^{24}Na. Loading was accomplished with the muscle submerged in 3 ml. gassed buffer containing $^{24}NaCl$. Both extensor digitorum longus muscles of a rat were loaded simultaneously for 1 hr, one muscle to serve as a zero time control for ^{24}Na and electrolyte contents, and the other to be transferred with attached strings to the washout chamber and bathed with freshly gassed inactive buffer flowing at approximately 10 ml./min through a volume of about 1·2 ml. During transfer the muscle and strings were rinsed in inactive buffer and the strings blotted before the muscle was fixed at rest length in the washout chamber. Transferring a muscle from loading to washout chamber required 1·5–2 min. Zero washout time was taken as the time a muscle was first exposed to inactive buffer.

The floor of the washout chamber was made of 1/1000 in. thick Teflon. It rested on a base of $\frac{1}{4}$ in. thick aluminium through which was cut an oval window, approximately $\frac{1}{8} \times \frac{3}{4}$ in., above which the muscle was centred. Strings and most of the tendons were shielded by the metal frame.

The floor of the washout chamber constituted the window of a gas-flow Geiger tube, output of which is a measure of radioactivity remaining in the muscle plus contaminant activity contributed by strings, sides of washout chamber, etc. Output was registered continually through a count-rate meter and printed out after a pre-set count had been accumulated, usually over intervals of less than 5 min. Washout time was standardized to 2 hr, although muscle radioactivity was often

statistically indistinguishable from contaminant radioactivity at this time, after which the muscle was removed. Tendons and strings were left in place in the washout chamber for counting as contaminant activity.

Data were handled as follows. True background was subtracted from each point. Remaining radioactivity was point-for-point corrected for ^{24}Na decay, further corrected by subtraction of contaminant radioactivity contributed by strings, tendons, etc. and plotted semilogarithmically against washout time. The abscissa of each point was taken as the time at which the counting interval began. A straight line was drawn by eye through the tail of the curve and the retropolation of that line subtracted point-for-point from the rest of the curve. The difference was plotted semilogarithmically and a second straight line was fitted visually through these difference points. This technique is known as curve-peeling.

Electrolytes of both the zero time muscle and the washed muscle were extracted into 5 ml. water in plastic test tubes by overnight refrigeration. ^{24}Na content was measured in a well counter. Extracts were frozen for at least 2 weeks while ^{24}Na decayed, after which Na content was determined either by flame photometry or by atomic absorption spectrometry. The same analyses were done on aliquots of loading solutions.

Inulin space was determined in separate experiments using inulin [^{14}C] of molecular weight range 5000–5500 obtained from New England Nuclear, Boston, Mass. Muscles were tied at rest length to a Lucite holder and bathed for 1 hr in 75 ml. buffer containing [^{14}C]inulin and no carrier inulin. In order to estimate inulin spaces of those muscles that had been incubated for a total of 3 hr (1 hr loading, 2 hr washout), a 2 hr pre-incubation without inulin preceded the 1 hr exposure to [^{14}C]inulin. After exposure to [^{14}C]inulin, muscles were blotted, weighed and extracted by overnight refrigeration in 5 ml. water. ^{14}C concentrations of extracts and loading media were determined by scintillation counting. Measured by this technique, inulin space of muscles bathed in $GKRHCO_3$ for 1 or 3 hr was 0·13 ml./g wet wt. This standard procedure was used despite the fact that values 10% lower were obtained when [^{14}C]inulin was extracted into 1 ml. $GKRHCO_3$ with vigorous shaking for 2 hr at room temperature and values up to 30% higher were obtained if exposure to [^{14}C]inulin was extended to 3 hr. The higher value reported previously (Zierler, Rogus & Hazlewood, 1966), 0·2 ml./g wet wt., had been measured with [^{14}C]inulin that was probably polydisperse.

THEORY

Glossary

Parameters of the washout curve

- B_1 fractional intercept of slower component,
- B_2 fractional intercept of faster component,
- r_1 rate constant of slower component, in hr^{-1},
- r_2 rate constant of faster component, in hr^{-1}.

Subscripts in the model

- 1 component 1 (sarcoplasm),
- 2 component 2 (sarcoplasmic reticulum, SR),
- i intracellular, sum of 1 and 2,
- 0 extracellular,
- T total, sum of intracellular and extracellular.

Symbols in the model

N Na content, in m-equiv/kg wet wt.,
N^* ^{24}Na content,
C Na concentration, in m-equiv/l.,
C^* ^{24}Na concentration,
α specific activity relative to that of loading solution,
V fluid volume, as fraction of wet weight,
V_D dry weight, as fraction of wet weight,
$k_{jj'}$ rate coefficient for Na movement into j from j', in ml./hr,
$\lambda_{jj'}$ $[= k_{jj'}/V_{j'}]$ rate constant for Na movement into j from j', in hr^{-1}.

Derived rate constants, in hr^{-1}

$$\lambda_1 = \lambda_{01} + \lambda_{21},$$
$$\lambda_2 = \lambda_{02} + \lambda_{12},$$
$$a = r_2 - \lambda_1 = \lambda_2 - r_1,$$
$$b = r_2 - \lambda_2 = \lambda_1 - r_1.$$

Notation for time

$t = m + t'$ total experimental time,
m ^{24}Na loading time,
t' washout time.

The model

The two-component model is based on the empirical observation that washout of intracellular ^{24}Na from rat extensor digitorum longus fits a sum of two exponentials and thus satisfies eqn. (1)

$$\frac{N_i^*(t')}{N_i^*(0)} = B_1 e^{-r_1 t'} + B_2 e^{-r_2 t'}, \tag{1}$$

where N_i^* is intracellular tracer content and t' is washout time. The most general model allows two avenues for Na exchange between each component and the bathing solution, one directly through extracellular space and one through the other Na pool. This system is diagrammed in Fig. 1 where components 1 and 2 are the two intracellular Na pools, identified as sarcoplasm and sarcoplasmic reticulum, respectively, and 0 refers to extracellular space. The last is not shown as a separate compartment because it exchanges so rapidly with the bathing solution that in the model it may be considered an extension thereof.

The k's are non-negative constants relating Na flux to concentration and the convention for subscript jj' is 'into j from j''.

Each component has Na content N and concentration C, ^{24}Na content N^* and concentration C^*, and fluid volume V. Components 1 and 2 comprise the total intracellular pool, given subscript i, so that

$$N_1 + N_2 = N_i, \tag{2}$$
$$N_1^* + N_2^* = N_i^*, \tag{3}$$
$$V_1 + V_2 = V_i. \tag{4}$$

With constant k's, the set of differential equations describing the model is

$$dN_1/dt = k_{10} C_0 + \lambda_{12} N_2 - \lambda_1 N_1, \tag{5a}$$
$$dN_2/dt = k_{20} C_0 + \lambda_{21} N_1 - \lambda_2 N_2, \tag{5b}$$

where substitutions $\lambda_{jj'} = k_{jj'}/V_{j'}$, $\lambda_1 = \lambda_{01} + \lambda_{21}$, and $\lambda_2 = \lambda_{02} + \lambda_{12}$ have been made. If the V's are constant, the λ's are rate constants with dimension time^{-1}. It is not implied that the k's and λ's may not be concentration-dependent.

In tracer experiments the differential equations for the model are

$$dN_1^*/dt = k_{10}C_0^* + \lambda_{12}N_2^* - \lambda_1 N_1^*, \tag{6a}$$

$$dN_2^*/dt = k_{20}C_0^* + \lambda_{21}N_1^* - \lambda_2 N_2^*. \tag{6b}$$

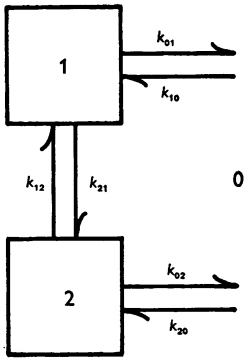

Fig. 1. Two-component model for Na flux and distribution in skeletal muscle. Component 1 represents sarcoplasm, component 2 sarcoplasmic reticulum (SR) and 0 extracellular space which connects with bathing solution. Rate coefficients k_{01} and k_{10} are for Na movements across sarcolemma, k_{12} and k_{21} across walls of SR, k_{02} and k_{20} across triadic junction into T system which is considered extracellular.

Eqns. (5) and (6) hold for any experimental time, t. Let $t = m + t'$, where m is loading time and t' is washout time. Then $t = m$ and $t' = 0$ describe the same experimental time.

In washout experiments, since extracellular tracer is so rapidly depleted, $C_0^*(t')$ is taken as zero. Solution of eqn. (6a,b) then yields the quantity of tracer remaining in each component as a function of washout time, t':

$$N_1^*(t') = \left[\frac{[\lambda_2 - r_1]N_1^*(0) + \lambda_{12}N_2^*(0)}{r_2 - r_1}\right] e^{-r_1 t'} + \left[\frac{[r_2 - \lambda_2]N_1^*(0) - \lambda_{12}N_2^*(0)}{r_2 - r_1}\right] e^{-r_2 t'}, \tag{7a}$$

$$N_2^*(t') = \left[\frac{\lambda_{21}N_1^*(0) + [\lambda_1 - r_1]N_2^*(0)}{r_2 - r_1}\right] e^{-r_1 t'} - \left[\frac{\lambda_{21}N_1^*(0) - [r_2 - \lambda_1]N_2^*(0)}{r_2 - r_1}\right] e^{-r_2 t'}, \tag{7b}$$

where r_1 and r_2 are related to the model by

$$r_1 + r_2 = \lambda_1 + \lambda_2, \tag{8a}$$

$$r_1 r_2 = \lambda_1\lambda_2 - \lambda_{12}\lambda_{21}. \tag{8b}$$

In practice $N_1^*(t')$ and $N_2^*(t')$ are not observed separately. Addition of eqns. (7a) and (7b) and division through by the constant $N_t^*(0)$ gives the fraction of initial

intracellular radioactivity remaining at any washout time. The result is identical with eqn. (1), the empirical equation for the normalized washout curve, with

$$B_1 = \frac{1}{r_2 - r_1}[[r_2 - \lambda_{01}]N_1^*(0)/N_i^*(0) + [r_2 - \lambda_{02}]N_2^*(0)/N_i^*(0)], \tag{9}$$

$$B_1 + B_2 = 1, \quad 0 < B_1 < B_2 \quad \text{and} \quad 0 < r_1 < r_2.$$

Calculation of bounds on model parameters

The model is a problem with seven equations (eqns. 2, 3, 4, 5*a*, 5*b*, 6*a* and 6*b*) in twelve unknowns ($2N$'s, $2N^*$'s, $2V$'s and $6\,k$'s) and cannot be solved uniquely. However, the problem is worth pursuing in the form of inequalities, for symmetry of the model imposes certain conditions on relationships among the unknowns, and the data permit imposition of further restrictions. Together these enable us to place upper and lower bounds on each of the unknowns in terms of measurable quantities.

First, we seek bounds on the λ's. Given the two-component model with $r_1 < r_2$ and $B_1 < B_2$, symmetry admits two sets of relations among the unknowns in eqn. (9), in either of which the component having the smaller initial ^{24}Na content also has the smaller rate constant for efflux directly to extracellular space. To show this we combine eqns. (3) and (9) to give

$$\frac{N_1^*(0)}{N_2^*(0)} = \frac{\lambda_{02} - (B_1 r_1 + B_2 r_2)}{(B_1 r_1 + B_2 r_2) - \lambda_{01}}, \tag{10}$$

where $(B_1 r_1 + B_2 r_2)$ has a positive value known from measurements on the washout curve. In order that the right side of eqn. (10) be positive,

either $$\lambda_{01} < B_1 r_1 + B_2 r_2 < \lambda_{02}, \quad N_1^*(0) < N_2^*(0), \tag{11}$$

or $$\lambda_{02} < B_1 r_1 + B_2 r_2 < \lambda_{01}, \quad N_2^*(0) < N_1^*(0).$$

Both λ_{01} and λ_{02} cannot equal $(B_1 r_1 + B_2 r_2)$ because their sum is less than twice this quantity. We have adopted the notation of eqn. (11), whence $\lambda_{01} < \lambda_{02}$ and $N_1^*(0) < N_2^*(0)$.

From eqn. (8*a*, *b*) it follows that $r_1 < \lambda_1 < r_2$ and $r_1 < \lambda_2 < r_2$.
From eqns. (8) and (11) and $\lambda_{02} < \lambda_2$,

$$B_1 r_1 + B_2 r_2 < \lambda_2,$$

$$\lambda_1 = r_1 + r_2 - \lambda_2 < B_2 r_1 + B_1 r_2.$$

With $B_1 < B_2$, this upper bound on λ_1 is smaller than the lower bound on λ_2, or $\lambda_1 < \lambda_2$.

Introduce the rate constants

$$a = \lambda_2 - r_1 = r_2 - \lambda_1 \tag{12a}$$

and $$b = \lambda_1 - r_1 = r_2 - \lambda_2, \tag{12b}$$

where $0 \leqslant b < a$ and, with eqn. (8*a*, *b*),

$$a + b = r_2 - r_1, \tag{12c}$$

$$ab = \lambda_{12}\lambda_{21}. \tag{12d}$$

But $\lambda_{21} \leqslant \lambda_1$ and $\lambda_{12} = \lambda_2 - \lambda_{02} < \lambda_2 - r_2 + (B_1 r_1 + B_2 r_2)$, whence

$$\lambda_{12} + \lambda_{21} < (B_2 r_1 + B_1 r_2).$$

In the last inequality if $(1+B_2)/B_2 < r_2/r_1$ as it did in all our experiments (e.g. if $B_1 < B_2$ and $3r_1 < r_2$)

then
$$\lambda_{12}+\lambda_{21} < r_2-r_1 = a+b. \tag{12e}$$

From eqns. (12*d*) and (12*e*) it follows that

$$b < \lambda_{12} < a, \tag{12f}$$

$$b < \lambda_{21} < a. \tag{12g}$$

With the restrictions $B_1 < B_2$ and $3r_1 < r_2$, eqns. (11) and (12*f*,*g*) and the definitions of a, b and the λ's lead to bounds on the efflux rate constants:

$$0 \leqslant b < \lambda_{12}, \tag{13a}$$

$$0 \leqslant b < \lambda_{21} \leqslant r_1+b, \tag{13b}$$

$$0 \leqslant \lambda_{01} \leqslant r_1, \tag{13c}$$

$$\lambda_{02} \leqslant r_2-2b \leqslant r_2. \tag{13d}$$

An upper bound on λ_{12} and on b and a lower bound on λ_{02} will be derived as we consider equations for loading as well as for washout.

We may combine expressions describing events during loading, at $t \leqslant m_-$, and those describing events during washout, at $t \geqslant m_+$ or $t' \geqslant 0$, if the system is stationary. Experimentally this means that loading and washout conditions are the same, so that none of the parameters can change at the onset of washout.

To this end, return to eqns. (6*a*) and (6*b*) and solve for N_1^*, N_2^* and their sum N_i^* during loading. The results, for m sufficiently large that $C_0^*(m)$ may be considered constant, is

$$N_1^*(m) = \frac{A_{11}C_0^*}{r_1}[1-e^{-r_1 m}]+\frac{A_{12}C_0^*}{r_2}[1-e^{-r_2 m}]. \tag{14a}$$

$$N_i^*(m) = \frac{(A_{11}+A_{21})C_0^*}{r_1}[1-e^{-r_1 m}]+\frac{(A_{12}+A_{22})C_0^*}{r_2}[1-e^{-r_2 m}], \tag{14b}$$

where
$$(r_2-r_1)\,A_{11} = k_{10}a+k_{20}\lambda_{12},$$
$$(r_2-r_1)\,A_{12} = k_{10}b-k_{20}\lambda_{12},$$
$$(r_2-r_1)\,A_{21} = k_{20}b+k_{10}\lambda_{21},$$
$$(r_2-r_1)\,A_{22} = k_{20}a-k_{10}\lambda_{21}.$$

Treat loading eqns. (14*a*) and (14*b*) as a pair of simultaneous equations from which k_{20} is eliminated. The result is

$$\frac{N_1^*(m)}{N_i^*(m)} = \frac{(r_2-b-\lambda_{02})\beta_1}{(r_1+r_2-\lambda_{02})\beta_1+r_1\beta_2}+G.k_{10}.\frac{C_0^*}{N_i^*(m)}, \tag{15}$$

where the β's are functions of r_1, r_2, and m, and where G is a function of r_1, r_2, b, λ_{02}, and m, and $G > 0$. Rearrange washout eqn. (9) and, with substitutions, obtain

$$\frac{N_1^*(0)}{N_i^*(0)} = \frac{(r_2-b-\lambda_{02})\,(\lambda_{02}-B_1r_1-B_2r_2)}{(\lambda_{02}-r_1)\,(r_2-\lambda_{02})}. \tag{16}$$

For $k_{10} \geqslant 0$, combination of eqns. (15) and (16) leads to a lower bound on λ_{02}

$$\lambda_{02} \geqslant r_2-\rho, \tag{13e}$$

where the equality holds for $k_{10} = 0$

and
$$\rho = \frac{(r_2-r_1)B_1[\gamma(m)-r_2]}{\gamma(m)-(r_1B_2+r_2B_1)}$$

with
$$\gamma(m) = \frac{r_2^2(1-e^{-r_1 m})-r_1^2(1-e^{-r_2 m})}{r_2(1-e^{-r_1 m})-r_1(1-e^{-r_2 m})}.$$

Note that ρ and $\gamma(m)$ are defined totally by quantities measurable experimentally. If $k_{10} = 0$, λ_{02} is fixed in terms of observables.

With definitions of λ_2 and b, eqn. (13*e*) yields an upper bound on λ_{12},

$$\lambda_{12} \leqslant \rho - b, \tag{13f}$$

where the equality holds for $k_{10} = 0$.

We now seek limits on the ratio N_1^*/N_i^* at $t = m$ or at $t' = 0$ in terms of measurable quantities. First, recast eqn. (9):

$$\frac{N_1^*(0)}{N_i^*(0)} = \frac{\lambda_{02} - (r_1 B_1 + r_2 B_2)}{\lambda_{02} - \lambda_{01}}. \tag{17}$$

On the right-hand side of eqn. (17) only λ_{01} and λ_{02} are not measured. Therefore, a range of permissible values of $N_1^*(m)/N_i^*(m)$ is generated as the estimates of λ_{01} and λ_{02} are allowed to vary through their respective ranges given by eqns. (13*c*, *d*, *e*).

It can be shown by differentiation of eqn. (17) with respect to λ_{01} and λ_{02} that the upper limit on $N_1^*(m)/N_i^*(m)$ coexists with the upper limits of both λ_{01} and λ_{02}. This occurs in the parallel configuration of the model, designated by subscript P, in which there are no interconnexions between the two components. In this form $\lambda_{12} = \lambda_{21} = b = 0$, $\lambda_{01} = r_1$ and $\lambda_{02} = r_2$, whence

$$\left[\frac{N_1^*(m)}{N_i^*(m)}\right]_P = B_1. \tag{18a}$$

The lower limit on $N_1^*(m)/N_i^*(m)$ coexists with the lower limits of both λ_{01} and λ_{02}. This occurs in a mammillary configuration of the model with $k_{10} = 0$ and $\lambda_{01} = 0$, designated by subscript M. In this form $\lambda_{02} = r_2 - \rho$, $\lambda_{12} = \rho - b_M$, $\lambda_{21} = r_1 + b_M$, where

$$b_M = r_1\rho/(r_2 - \rho) \tag{13g}$$

is the upper bound on b, and

$$\left[\frac{N_1^*(m)}{N_i^*(m)}\right]_M = \frac{(r_2 - r_1)\, B_1 - \rho}{r_2 - \rho}. \tag{18b}$$

Analytically, the value of the unknown $N_1^*(m)/N_i^*(m)$ lies between the upper limit given by eqn. (18*a*) and the lower limit given by eqn. (18*b*).

Fig. 2 illustrates a graphic solution for bounds on $N_1^*(m)/N_i^*(m)$, λ_{02} and b under standard conditions. The range of permissible values of each of these unknowns lies within the triangular area bounded by the lines $k_{10} = 0$, $\lambda_{01} = 0$ and $b = 0$. The value of $N_1^*(m)/N_i^*(m)$ lies between the upper limit, eqn. (18*a*) at the point $(0, r_2)$, and the lower limit, eqn. (18*b*) at the intersection of $k_{10} = 0$ and $\lambda_{01} = 0$.

To calculate $N_1(m)/N_i(m)$ when $N_1^*(m)/N_i^*(m)$ is known, corrections for specific activities are made,

$$\frac{N_1(m)}{N_i(m)} = \frac{N_1^*(m)}{N_i^*(m)} \cdot \frac{\alpha_i(m)}{\alpha_1(m)}, \tag{19}$$

where the α's are specific activities relative to that of the loading solution, and $\alpha_i(m)$ is observable.

In order to find the general expression for $\alpha_1(m)$, hold C_0^* and C_0 constant, solve the set of loading eqns. (6) for $N_1^*(m)/C_0^*$ and the set (5) for $N_1(m)/C_0$, and obtain their ratio, $\alpha_1(m)$. Bounds on $\alpha_1(m)$ depend on the sign of $\dot{N}_1(m)$:

with $$\dot{N}_1(m) \leqslant 0, \quad \alpha_1(m) \leqslant 1 - e^{-r_1 m} = [\alpha_1(m)]_P, \tag{20a}$$

with $$\dot{N}_1(m) \geqslant 0, \quad \alpha_1(m) \geqslant 1 - \frac{r_2 e^{-r_1 m} - r_1 e^{-r_2 m}}{r_2 - r_1} = [\alpha_1(m)]_M. \tag{20b}$$

Whatever the sign of $\dot{N}_1(m)$, the magnitude of $\alpha_1(m)$ is restricted by

$$\alpha_1(m)\, N_1(m) + \alpha_2(m)\, N_2(m) = \alpha_i(m)\, N_i(m), \qquad (20c)$$

$$\alpha_1(m) \leqslant 1, \quad \alpha_2(m) \leqslant 1.$$

When $\dot{N}_1(m) \leqslant 0$, a solution for $N_1(m)/N_j(m)$ in the mammillary configuration is

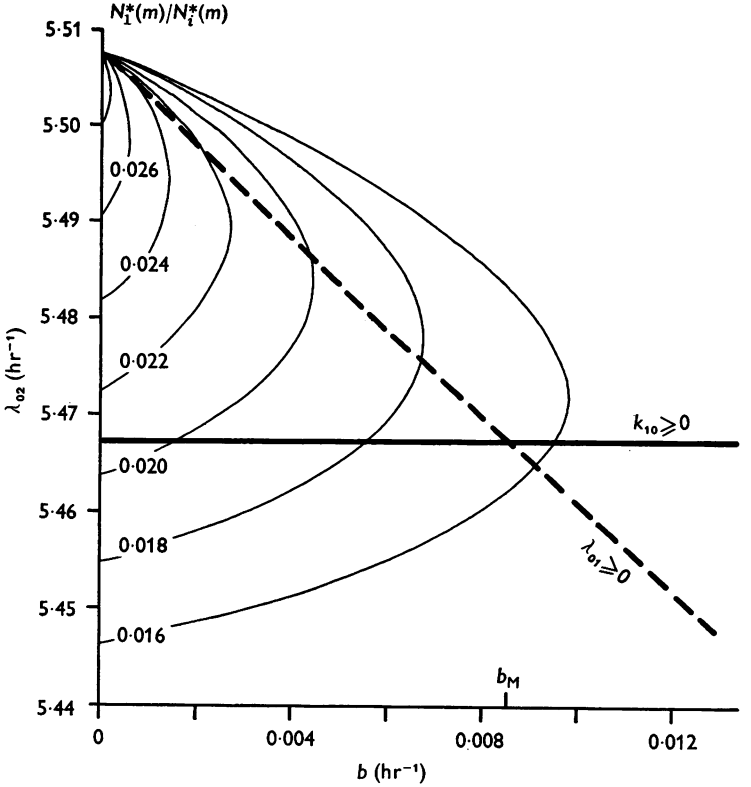

Fig. 2. Graphic solution for bounds on $N_1^*(m)/N_i^*(m)$, λ_{02} and b calculated with means of B_1, B_2, r_1 and r_2 from experiments done under standard conditions (Table 3). Family of thin continuous curves from eqn. (16) shows λ_{02} *vs.* b at fixed values of $N_1^*(m)/N_i^*(m)$, the last given in decrements of 0·002 from $B_1 = 0{\cdot}03$ which is a point at $\lambda_{02} = r_2 = 5{\cdot}508$ hr^{-1}. Region on or above heavy continuous line represents points for which $k_{10} \geqslant 0$. Region on or below heavy dashed line represents points for which $\lambda_{01} \geqslant 0$. Region bounded on left by axis of ordinates represents points for which $b \geqslant 0$, i.e. for which $\lambda_{12}\lambda_{21} \geqslant 0$. Triangular area bounded by these three lines thus includes all values of λ_{02}, b and $N_1^*(m)/N_i^*(m)$ consistent with the model. Parallel solution of the model, at point of convergence of the family of thin curves, gives upper limit of $N_1^*(m)/N_i^*(m)$ $(= B_1)$, upper limit of λ_{02} $(= r_2)$ and lower limit of b $(= 0)$. Mammillary solution of the model, at intersection of the two heavy lines, gives lower limit of $N_1^*(m)/N_i^*(m)$, eqn. (18*b*), lower limit of λ_{02}, eqn. (13*e*), and upper limit of b $(= b_M)$, eqn. (13*g*).

obtained directly. From eqn. (6*a*) with $\dot{N}_1(m) \leqslant 0$ and $k_{10} \geqslant 0$, it follows that $\lambda_1 N_1(m) \geqslant \lambda_{12} N_2(m)$ or, with eqns. (3) and (12*b*), that

$$N_1(m)/N_i(m) \geqslant \lambda_{12}/(r_1+b+\lambda_{12}). \tag{21}$$

With $k_{10} = 0$ and $\lambda_{01} = 0$, insertion of values of λ_{12} and b_M from eqn. (13*f*, *g*) leads to

$$N_1(m)/N_i(m) = \rho(r_2-r_1-\rho)/(r_1+\rho)\,(r_2-\rho). \tag{22}$$

Since this expression is identical to that given by eqn. (18*b*) if loading had been to uniform specific activity, eqn. (22) gives a lower bound on $N_1(m)/N_i(m)$.

From eqns. (18*a*), (19), (20*a*) and (22) for the case $\dot{N}_1(m) \leqslant 0$, bounds on $N_1(m)$ are known in terms of observables:

$$\frac{\rho(r_2-r_1-\rho)\,N_i(m)}{(r_1+\rho)\,(r_2-\rho)} \leqslant N_1(m) \leqslant \frac{B_1\alpha_i(m)\,N_i(m)}{[\alpha_1(m)]_P}. \tag{23}$$

Bounds on $N_2(m)$ are obtained from the difference between the measured $N_i(m)$ and bounds calculated for $N_1(m)$.

To calculate fluid volume of SR, V_2, we make the assumption that Na concentration in SR, C_2, is the same as in extracellular fluid, C_0, whence

$$V_2 = N_2(m)/C_0(m). \tag{24}$$

Fluid volume of sarcoplasm is $V_1 = V_i - V_2$.

RESULTS

Empirical basis for the model

Description of the washout curve as a sum of two exponentials

For the first 2 hr, washout of ^{24}Na from rat extensor digitorum longus muscle fits a sum of two exponentials and thus satisfies the form of eqn. (1). This description excludes the first few minutes of washout time during which a steep slope, attributed to loss of extracellular tracer, was sometimes seen. This description is based on the fact that a fit to two exponentials was observed in some 200 experiments of 2 hr washout duration run under nineteen different experimental conditions (i.e. different combinations of loading and washout solutions).

A ^{24}Na washout curve obtained under standard conditions (i.e. loaded for 1 hr and washed for 2 hr in $GKRHCO_3$) is shown in Fig. 3. On the average in eleven such experiments B_1, the intercept of the slower component, was only 3 % of the sum of the two intercepts. The smaller rate constant, r_1, was one fifth the size of the larger, r_2. The 2 hr point on the washout curve was $0{\cdot}28 \pm 0{\cdot}05$ (S.E. of mean) % of the initial (*ca.* 2 min) point as recorded by the Geiger tube beneath the washout chamber. Radioactivity remaining in a muscle washed for 2 hr was $0{\cdot}08 \pm 0{\cdot}02$ % of that in the paired, unwashed muscle as measured in a well counter. There does not seem to be enough ^{24}Na remaining in the system after 2 hr of washing to influence the shape of the washout curve appreciably. Yet one might ask whether the washout curve, if extended even farther, would better fit a new sum of exponentials different from those drawn at 2 hr.

To answer this question the washout period was extended beyond 8 hr in three experiments done under otherwise standard conditions. One of these is shown in Fig. 4. In each case the washout curve, which continued to flatten with time, could be viewed as a sum of three exponentials, of which the two steepest were similar in slope and intercept to those resolved from 2 hr experiments and the flattest was negligible by comparison.

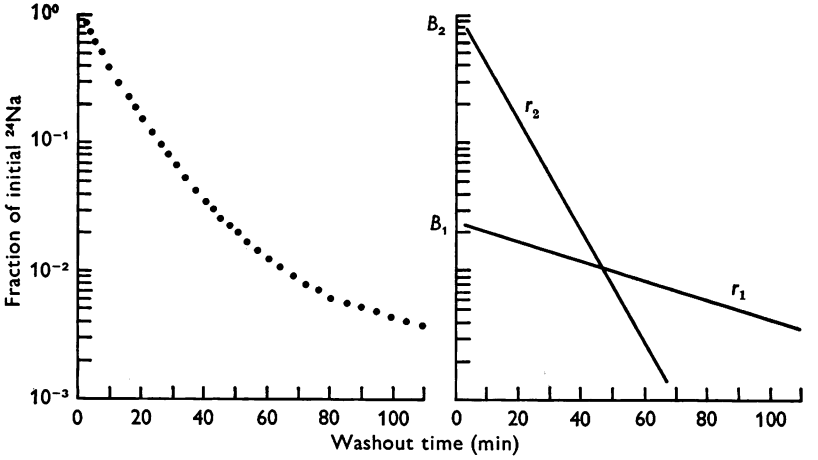

Fig. 3. Washout of ^{24}Na from rat extensor digitorum longus muscle under standard conditions. Left panel shows experimental points. In right panel the experimental curve has been resolved by curve-peeling into two exponentials, with fractional intercepts B_1 and B_2 and rate constants r_1 and r_2. Initial ^{24}Na was taken as the sum of the two zero time intercepts. Note that the earliest point on curve is about 2 min after zero washout time.

In Table 1 rate constants and fractional intercepts of the three exponentials resolved from the extended washout curves are compared with those of the two exponentials resolved from the same washout data of which only the first 2 hr have been plotted, as if the experiment had been terminated at 2 hr (inset, Fig. 4). Neglect of all but the first 2 hr of washout tended to overestimate r_2, underestimate r_1 and alter B_1 compared to the corresponding values derived from the longer washout curves, but not grossly.

No tail comparable to that seen under standard conditions was observed in a single long experiment conducted in the presence of 10^{-5} M ouabain (Fig. 5), although one might be present under these conditions, concealed when B_1 is elevated or perhaps contributing to the elevation of B_1. Nevertheless, in this case interpretation of the washout curve as a sum of two exponentials is not affected by length of the observation period.

Thus our empirical fit to two exponentials is an approximation. However, extension of the washout curve failed to alter the facts upon which the model is based: that there are two major exponentials on the washout curve, that r_1 is small compared to r_2, and that B_1 is very small compared to B_2 under standard conditions.

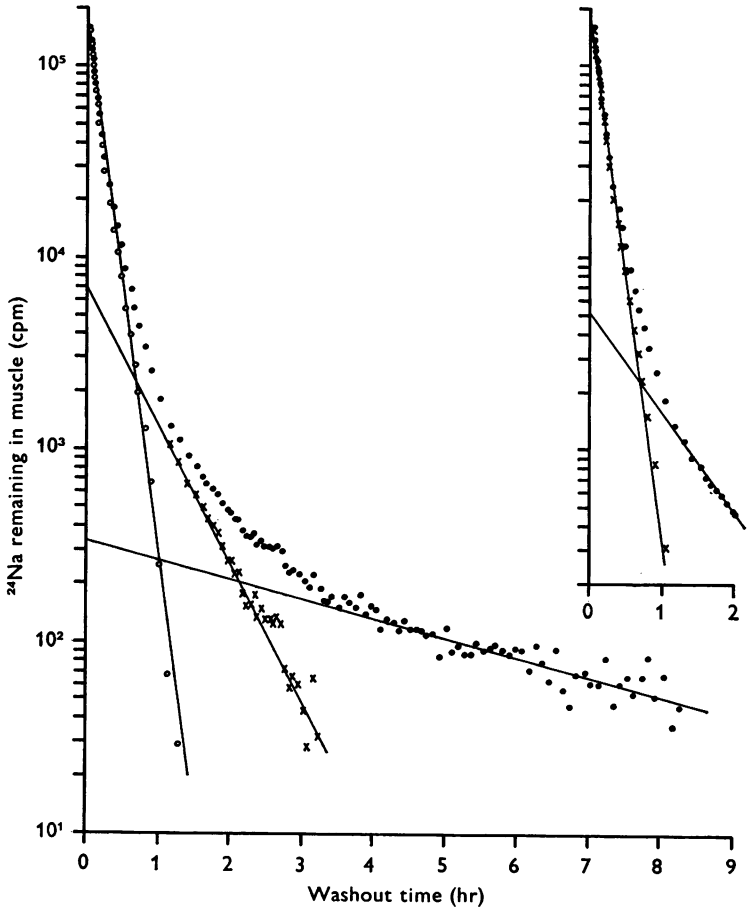

Fig. 4. ^{24}Na washout curve under standard conditions (filled circles) and its resolution into sum of three exponentials (straight lines) by curve-peeling. Inset shows first 2 hr of experimental curve and its resolution into two exponentials.

Whether or not the tail of the extended washout curve reflects a time-dependent change in muscle fibres, washout from a distinct cell population, washout from connective tissue or an artifact of washout from chamber and string has not been determined unequivocally. Possibly it

represents the small fraction of inexchangeable Na that Harris & Steinbach (1956) suggested likely to be associated with muscle connective tissue. In this regard, we have found that the curve of ^{24}Na washout from rat tendon has a tail comparable to that seen in whole muscle (rate constant 0·25, 0·24, 0·16 hr^{-1}, fractional intercept 0·2, 0·4, 0·8 % of initial radioactivity, in three 7–8 hr washouts from rat plantar fascia). We have also found that

TABLE 1. Comparison of long (8–9 hr) and short (2 hr) washout curves, standard conditions

Extent of washout curve	8–9 hr			2 hr	
	Fast	Medium	Slow	2	1
r	4·159	1·094	0·107	4·726	0·956
B	0·967	0·028	0·005	0·962	0·038
r	3·781	1·066	0·375	4·521	1·040
B	0·887	0·096	0·017	0·864	0·136
r	5·941	1·664	0·231	6·301	1·182
B	0·960	0·038	0·002	0·970	0·030

First three columns of data: rate constants, r, and fractional intercepts, B, of the three exponentials resolved from three washout experiments of 8–9 hr duration. Last two columns: rate constants (hr^{-1}) and fractional intercepts of the two exponentials resolved from plots of only the first 2 hr of data points from same experiments. Column headed 2 presents r_2 and B_2, and column headed 1 presents r_1 and B_1 analogous to the parameters labelled in Fig. 3.

washout from a piece of ^{24}Na-loaded string of the type used routinely (black braided surgical silk, 4–0) is not complete after 5·5 hr. That is, at least part of the tail of the extended curve for ^{24}Na washout from whole muscle, as we have measured it, is due to sources other than muscle fibres. If all of it were contributed by non-muscular sources, the tail of the curve would have no theoretic importance and description of the washout curve as a sum of the two exponentials seen at 2 hr would indeed be adequate. Whatever its origin, the tail on the extended washout curve under standard conditions has been neglected in development of the model.

Extracellular washout

In roughly one third of the experiments remnants of an additional slope, much steeper than the two major slopes, were evident during the first few minutes of washout. Appearance of this steep slope raised the possibility that loss of tracer from extracellular space is not usually observed on ^{24}Na washout curves because the extracellular pool has been virtually depleted of tracer during the 1·5–2 min required to transfer a muscle from loading to washout chamber and commence counting.

To test this hypothesis it was necessary to examine the washout curve during times earlier than 1·5 min. Accordingly, thirteen muscles were tied at rest length, each to a portable holder, loaded with ^{24}Na for 1 hr and washed by dipping through a series of tubes, each containing 10 ml. inactive buffer, for a total washout time ranging from 5 to 90 sec. Muscles

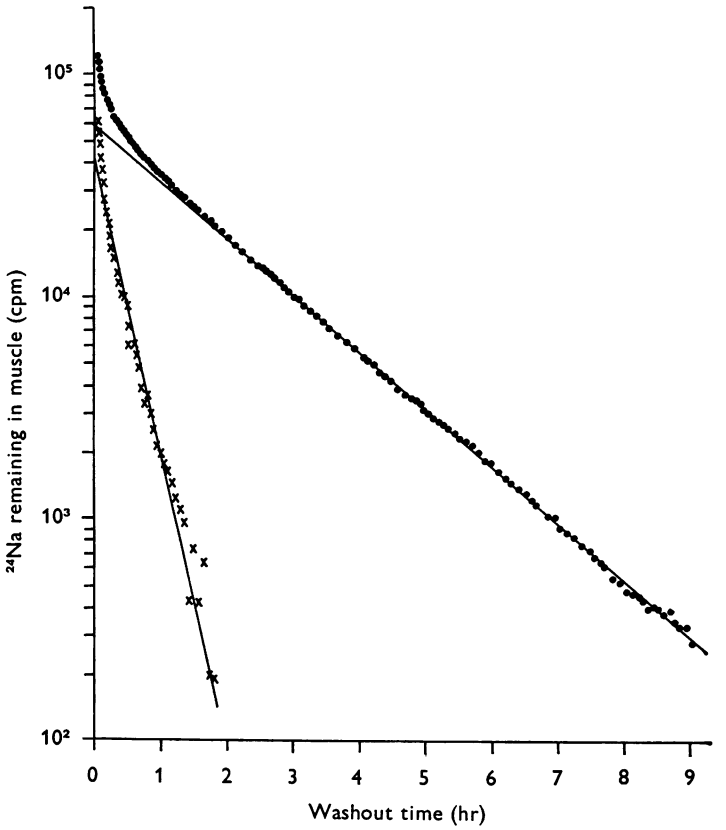

Fig. 5. Semilogarithmic plot of radioactivity remaining in muscle against washout time. 10^{-5} M ouabain was present during the 1 hr loading period and during washout. Filled circles, experimental points; crosses, points obtained by curve-peeling; straight lines, resolution of washout curve into two exponentials. Flatter line has rate constant r_1, steeper line has rate constant r_2. Fractional intercept of slow component, B_1, is much larger than under standard conditions.

were blotted and weighed and residual ^{24}Na was counted in a well counter. Fig. 6 shows ^{24}Na space – that is, residual ^{24}Na normalized for muscle weight and ^{24}Na concentration of loading solution – plotted semilogarithmically against washout time. The time course fits a linear plot almost as well as a semilogarithmic one, but the latter was chosen for ease of comparison

with the other data. The curve for whole muscle falls to half its initial value in 1·4 min. This is equivalent to a rate constant of 30 hr^{-1}.

Since inulin space, our measure of extracellular fluid volume, is 0·13 ml./g wet wt., and since ^{24}Na space fell from 0·232 ml./g at zero time to 0·106 ml./g after 90 sec of washout, we have accounted for all or nearly all the initial extracellular ^{24}Na in this time. It is, therefore, likely that the extracellular component of the washout curve was not detected under our experimental conditions in which measurement of washout did not begin until about 2 min of actual washout has occurred.

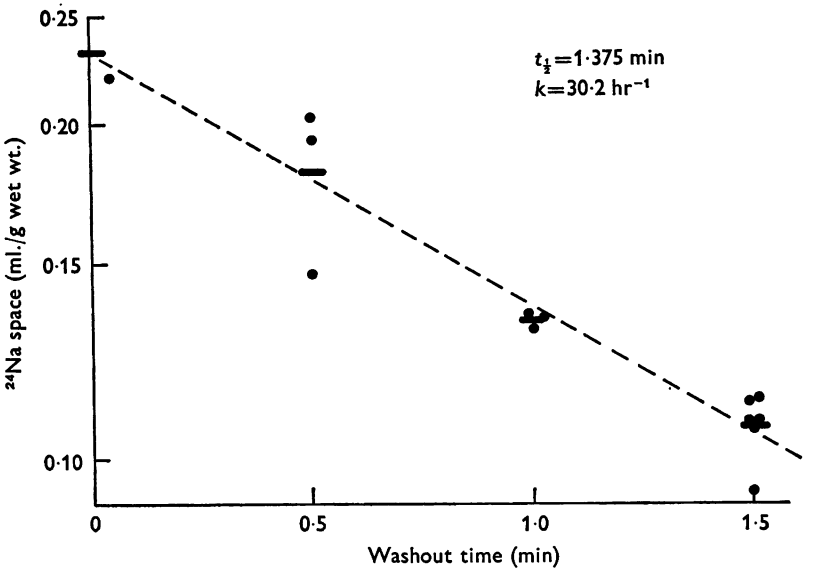

Fig. 6. Washout of ^{24}Na from whole muscle under standard conditions at times earlier than 1·5 min. ^{24}Na space is the volume of muscle, in ml./g wet wt., that ^{24}Na would occupy if it were present at the same concentration as in the loading solution. Each circle represents one muscle. Bars indicate means of the values shown except for the bar at zero time which is the mean of forty-seven values.

In a second series of experiments, 80 % of Na^+ in the bathing solution was replaced by Li^+ and loss of unlabelled Na^+ was measured. Fourteen to sixteen muscles were tied at rest length to the same Lucite rack and presoaked for 30–90 min in 75 ml. buffer containing 145 m-equiv Na^+/l. At zero time the rack of muscles was transferred without rinsing to a stirred bath containing 75 ml. buffer initially having 28 m-equiv Na^+/l. At various intervals from 2 to 120 min muscles were removed from the rack, blotted, weighed and extracted in 5 ml. water overnight in the cold.

Results of eight such experiments are shown in Fig. 7 where whole muscle Na^+ content is plotted against time of exposure to low Na^+ solution.

The loss of Na^+ from extracellular space expected on changing solutions was calculated as inulin space × difference in Na^+ concentrations, or 0·13 ml./g × (145–28) m-equiv/l. = 15·2 m-equiv/kg muscle. This is an over-estimate because Na^+ concentration of the low Na^+ buffer was slightly

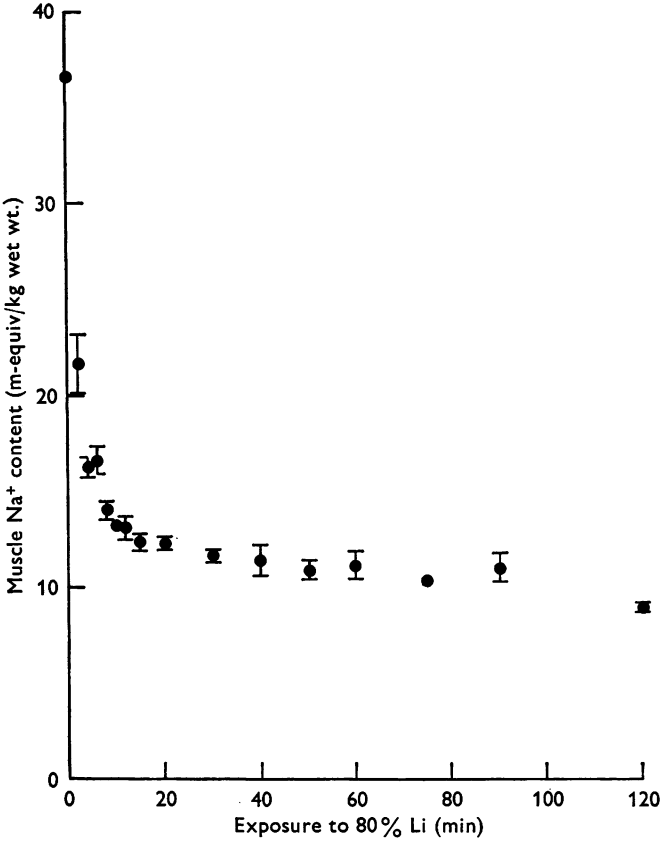

Fig. 7. Washout of Na^+ from muscles after transfer to medium in which 80 % of Na^+ had been replaced by Li^+. The zero time point is the mean Na^+ content of 109 muscles and each point thereafter is the mean Na^+ content of at least six muscles. Height of each vertical bar is 2 S.E. of mean.

higher than 28 m-equiv/l. owing to the small volume of standard buffer introduced during transfer. Fig. 7 shows that 36·7 − 21·7 = 15·0 m-equiv Na^+/kg muscle was lost during the first 2 min. That is, virtually all extracellular Na^+ was in equilibrium with the bathing solution within 2 min.

From these results it was concluded that the contribution of extracellular

^{24}Na to the two slopes regularly seen on the washout curve may be neglected. The great bulk of extracellular ^{24}Na, present at true zero time of washout, has been washed out during the time in which we are arranging the muscle in the chamber for detection of radioactivity.

Existence of interconnexions between the two components

In eqns. (13*a*, *b*), the rate constants λ_{12} and λ_{21} have zero as their lower limits. That is, theory alone provides no compelling reason for retaining either of them in the model. Data in Table 2, however, provide evidence for the existence of at least one of these rate constants.

TABLE 2. Parameters of ^{24}Na washout curves under various conditions

			hr^{-1}		
Load	Wash	n	r_1	r_2	B_1
1. $GKRHCO_3$	Same as load	11	1·17 ± 0·12	5·51 ± 0·34	0·030 ± 0·003
2. $GKRHCO_3$	80 % Li	5	1·79 ± 0·15	7·04 ± 1·11	0·137 ± 0·019
3. $GKRHCO_3$	$GKRHCO_3$ + ouabain 10^{-5} M	8	0·85 ± 0·10	7·10 ± 0·26	0·202 ± 0·011
4. $GKRHCO_3$ + ouabain 10^{-5} M	Same as load	6	1·08 ± 0·09	7·33 ± 0·59	0·421 ± 0·028
5. $GKRHCO_3$ + ouabain 10^{-5} M	80 % Li + ouabain 10^{-5} M	7	0·93 + 0·05	5·62 ± 0·52	0·644 ± 0·044

First column indicates composition of the solution containing ^{24}NaCl in which muscles were loaded for 1 hr. Second column indicates composition of ^{24}Na-free medium with which muscles were washed for 2 hr. $GKRHCO_3$ is the standard medium, glucose-Krebs–Ringer-bicarbonate; 80 % Li solution has 80 % of Na^+ substituted by Li^+. All experiments done at room temperature, about 25° C. n is the number of efflux experiments. r_1 is the rate constant and B_1 the fractional intercept of the slower component on the washout curve. r_2 is the rate constant of the faster component, derived by curve-peeling. Data are means ± S.E. of mean.

To prove that the two components of the model are interconnected, that is, to prove that λ_{12} and λ_{21} are not both zero, we use the following rationale. Suppose two sets of muscles have been loaded with ^{24}Na under identical conditions. Then $N_1^*(0)/N_i^*(0)$ (and $N_2^*(0)/N_i^*(0)$) is the same in both cases. Suppose that the two sets of muscles are now washed out under dissimilar conditions and parameters of the washout curves determined. If the two components of the model are arranged in parallel, that is, if $\lambda_{12} = \lambda_{21} = 0$, then, according to eqn. (18*a*), $B_1 = N_1^*(0)/N_i^*(0)$, and B_1

must be the same in both sets of experiments. If the two components are connected, that is, if λ_{12} and λ_{21} are not both zero, then B_1 is given by eqn. (9). In eqn. (9) recall that r_1 and r_2 are both functions of λ_{01}, λ_{21}, λ_{02} and λ_{12} (see eqn. (8) and definitions of λ_1 and λ_2), whence B_1 is also a function of these four λ's. Then, although $N_1^*(0)/N_i^*(0)$ has been fixed by the loading conditions, B_1 has freedom to change in response to changes in one or more λ's induced by the washout conditions. In our two sets of experiments if B_1 does change, despite fixed $N_1^*(0)/N_i^*(0)$, we must conclude that λ_{12} and λ_{21} are not both zero.

Table 2 shows results of experiments which may be compared for this purpose. In each case B_1 changed significantly when washout conditions were changed, although loading conditions had been identical (compare lines 1 and 2, lines 1 and 3, lines 4 and 5 of Table 2). The conclusion is that the two components of the model are interconnected, that λ_{12} and λ_{21} are not both zero. This result is particularly important because it rules out identification of the two components of the model with anatomical structures that are likely to be arranged purely in parallel, for instance, two distinct fibre populations, and forces us to consider identification of the two components with structures that are interconnected, for instance, subcellular components that are common to every fibre such as sarcoplasm and sarcoplasmic reticulum.

Calculation of volumes and Na contents of sarcoplasm and SR under standard conditions

Table 3 presents data from eleven experiments done under standard conditions (i.e. loaded for 1 hr and washed for 2 hr in $GKRHCO_3$).

The change in intracellular Na content that occurs during washout is ΔN_i. Since ΔN_i was not significantly different from zero, we supposed that N_1 and N_2 were both constant during washout, particularly at the start of washout, and used eqn. (23) to calculate the mean upper and lower bounds on N_1/N_i shown in Table 4. Bounds on Na contents and fluid volumes of the two components were subsequently calculated from equations given in Theory, and their means are also given in Table 4.

The upper and lower bounds calculated for any given parameter in Table 4 are very close to each other, certainly not different from each other in the case of N_2, V_2 and V_1. Under standard conditions, only 2–4 % of intracellular Na is in component 1, sarcoplasm, and the rest is in component 2, sarcoplasmic reticulum or SR. With the assumption that Na concentration in SR, C_2, is the same as in extracellular fluid or bathing solution, C_0, we estimate that the fluid volume of SR, V_2, lies between 12·3 and 12·5 % of wet weight. The remaining intracellular water content, 54·2–54·4 % of wet weight, is fluid volume of sarcoplasm, V_1.

TABLE 3. Parameters of ^{24}Na washout curves and results of muscle analyses, standard conditions

B_1	$0{\cdot}030 \pm 0{\cdot}003$
r_1, hr^{-1}	$1{\cdot}17 \pm 0{\cdot}12$
r_2, hr^{-1}	$5{\cdot}51 \pm 0{\cdot}34$
$N_T(t')$, m-equiv/kg	$43{\cdot}4 \pm 0{\cdot}8$
$N_T(m)$, m-equiv/kg	$41{\cdot}1 \pm 2{\cdot}2$
$C_0(m)$, m-equiv/l.	$156{\cdot}7 \pm 2{\cdot}6$
$\alpha_T(m)$	$0{\cdot}940 \pm 0{\cdot}026$
α_T	$0{\cdot}975 \pm 0{\cdot}007$ ($n = 50$)
V_0, ml./g	$0{\cdot}132 \pm 0{\cdot}003$ ($n = 25$)
$1-V_D$, ml./g	$0{\cdot}800 \pm 0{\cdot}005$ ($n = 13$)
α_i	$0{\cdot}947 \pm 0{\cdot}003$
$N_i(m)$, m-equiv/kg	$20{\cdot}4 \pm 1{\cdot}9$
$N_i(t')$, m-equiv/kg	$24{\cdot}2 \pm 0{\cdot}8$
ΔN_i, m-equiv/kg	$+3{\cdot}8 \pm 1{\cdot}9$

Data are means $\pm$ S.E. of mean. n is the number of determinations and, unless otherwise stated, $n = 11$, from eleven pairs of muscles used in washout experiments.

First block of data was obtained from one muscle of each pair used in washout experiments: B_1, fractional intercept of slower component on the washout curve; r_1 and r_2, rate constants of the two exponentials on the washout curve; $N_T(t')$, total Na content of muscle at end of washout, where t' is washout time, 2 hr.

Second block of data was obtained from contralateral muscles at end of loading period where m is loading time, 1 hr. $N_T(m)$, total Na content of loaded muscle; $C_0(m)$, Na concentration of loading solution; $\alpha_T(m)$, specific activity of loaded muscle relative to specific activity of loading solution, calculated

$$\alpha_T(m) = [N^*_T(m)/C^*_0(m)]/[N_T(m)/C_0(m)].$$

Occasional values of $\alpha_T(m)$ exceeded unity.

Third block of data includes measurements from separate experiments of 1 hr duration: α_T, same as $\alpha_T(m)$ above but with thirty-nine additional, similarly treated muscles; V_0, inulin space, and $1-V_D$, total water content, where V_D is dry weight, all from separate experiments. S.E. of mean of α_T, V_0 and V_D were ignored in subsequent calculations.

Fourth block of data was derived from preceding measurements: $\alpha_i(m)$, average intracellular specific activity relative to that of loading solution,

$$\alpha_i(m) = [\alpha_T(m)\, N_T(m) - V_0\, C_0]/N_i(m),$$

calculated with mean value of α_T from larger series, mean V_0 and individually measured $N_T(m)$ and $C_0(m)$; $N_i(m)$, intracellular Na content at end of loading period, $N_i(m) = N_T(m) - V_0 C_0$, calculated with mean V_0 and individual $N_T(m)$ and $C_0(m)$; $N_i(t')$, intracellular Na content at end of washout, calculated with individual $N_T(t')$, mean V_0, and with $C_0(t')$ taken as 145 m-equiv/l.; ΔN_i, paired difference $[N_i(t') - N_i(m)]$.

Some corollaries of Table 4 are given in Table 5. From the mean estimate of N_1 and V_1 are calculated C_1, the Na concentration in sarcoplasmic water, and the ratio of Na concentrations on either side of sarcolemma, C_0/C_1. The latter leads to an estimate of the Na equilibrium potential, E_{Na}, of $+116$ to

TABLE 4. Calculation of bounds on N_1, N_2, V_1 and V_2, standard conditions

$$0{\cdot}016 \pm 0{\cdot}001 \leqslant N_1^*(m)/N_i^*(m) \leqslant 0{\cdot}030 \pm 0{\cdot}003$$
$$0{\cdot}58 \pm 0{\cdot}04 \leqslant \alpha_1(m) \leqslant 0{\cdot}66 \pm 0{\cdot}04$$
$$0{\cdot}027 \pm 0{\cdot}002 \leqslant N_1/N_i \leqslant 0{\cdot}043 \pm 0{\cdot}004$$
$$0{\cdot}56 \pm 0{\cdot}07 \leqslant N_1 \leqslant 0{\cdot}88 \pm 0{\cdot}12 \text{ m-equiv/kg}$$
$$19{\cdot}54 \pm 1{\cdot}84 \leqslant N_2 \leqslant 19{\cdot}86 \pm 1{\cdot}86 \text{ m-equiv/kg}$$
$$0{\cdot}123 \pm 0{\cdot}011 \leqslant V_2 \leqslant 0{\cdot}125 \pm 0{\cdot}011 \text{ ml./g}$$
$$0{\cdot}542 \pm 0{\cdot}011 \leqslant V_1 \leqslant 0{\cdot}544 \pm 0{\cdot}011 \text{ ml./g}$$

Upper and lower bounds are means ± S.E. of mean of quantities calculated individually from eleven experiments done under standard conditions. $N_1^*(m)/N_i^*(m)$: ^{24}Na content of sarcoplasm as a fraction of intracellular ^{24}Na content at end of loading period, eqns. (18*a*, *b*). $\alpha_1(m)$: specific activity of sarcoplasm relative to that of loading solution, eqn. (20*a*, *b*). N_1/N_i: sarcoplasmic Na content, N_1 from eqn. (23), as a fraction of intracellular Na content, N_i. N_2: Na content of sarcoplasmic reticulum, $N_2 = N_i - N_1$. V_2: fluid volume of sarcoplasmic reticulum, eqn. (24). V_1: fluid volume of sarcoplasm, $V_1 = V_i - V_2$ where $V_i = 1 - V_0 - V_D$. Na contents and fluid volumes are expressed per unit of muscle wet weight. Muscle density is taken as unity.

TABLE 5. Estimates of sarcoplasmic Na concentration, Na equilibrium potential, and fluxes from sarcoplasm and sarcoplasmic reticulum, standard conditions

$$1{\cdot}03 \leqslant C_1 \leqslant 1{\cdot}61 \text{ m-equiv/l.}$$
$$90 \leqslant C_0/C_1 \leqslant 152$$
$$+116 \leqslant E_{Na} \leqslant +130 \text{ mV}$$
$$0{\cdot}62 \leqslant \lambda_1 N_1 \leqslant 1{\cdot}02 \text{ m-equiv.kg}^{-1}\text{.hr}^{-1}$$
$$107 \leqslant \lambda_2 N_2 \leqslant 109 \text{ m-equiv.kg}^{-1}\text{.hr}^{-1}$$
$$0 \leqslant \lambda_{01} N_1/\lambda_{02} N_2 \leqslant 0{\cdot}009$$

Values calculated from means of data in Table 4. C_1: Na concentration in sarcoplasm, in m-equiv/l. of sarcoplasmic water. C_0/C_1: ratio of external to sarcoplasmic Na concentrations. E_{Na}: Na equilibrium potential. $\lambda_1 N_1$ and $\lambda_2 N_2$: Na effluxes from sarcoplasm and sarcoplasmic reticulum, respectively, expressed per kg of muscle wet weight. $\lambda_{01} N_1/\lambda_{02} N_2$: ratio of Na efflux across sarcolemma to efflux through T system.

+130 mV. Unidirectional Na efflux from sarcoplasm via both routes, i.e. across sarcolemma or into SR, is $\lambda_1 N_1$. It amounts to about 1m-equiv/hr.kg wet wt. of muscle. Efflux from SR via both routes, i.e. into sarcoplasm or into the *T* system, is $\lambda_2 N_2$, about 110 m-equiv/hr. kg wet wt. of muscle. Fluxes by way of direct routes from sarcoplasm and SR are compared in the last line of Table 5. Na efflux across sarcolemma, $\lambda_{01} N_1$, is less and perhaps much less than 1 % of that through the *T* system, $\lambda_{02} N_2$.

Tests of predictions of the model

Effects of anisotonic solutions

If the two components of the model are sarcoplasm and SR, they should exhibit the differential osmotic behaviour predicted by Birks & Davey

TABLE 6. Effects of osmotic strength on ^{24}Na washout curves, muscle Na and water contents

Relative osmolality ($\times R$)	n	$C_0(m)$ (m-equiv/l.)	B_1	hr^{-1}		m-equiv/kg			ml./g	
				r_1	r_2	$N_T(m)$	$N_i(m)$	ΔN_i	V_0	$1-V_D$
1·66	10	272	0·19 ± 0·07	1·80 ± 0·21	7·88 ± 0·70	116·0 ± 3·5	53·2 ± 3·3	+1·6 ± 2·8	0·230 ± 0·004 (15)	0·752 ± 0·002 (14)
1·36	6	215	0·18 ± 0·05	2·09 ± 0·33	8·23 ± 1·04	72·8 ± 2·9	25·7 ± 2·6	−8·5 ± 2·6	0·219 ± 0·009 (8)	0·778 ± 0·002 (8)
0·63	6	94	0·25 ± 0·04	0·70 ± 0·06	4·59 ± 0·28	17·0 ± 1·1	9·3 ± 1·3	−2·2 ± 1·2	0·085 ± 0·002 (17)	0·828 ± 0·003 (8)

Osmotic strength was varied by addition or subtraction of NaCl from standard Ringer solution and is expressed as a multiple of R, the estimated osmolality of standard Ringer solution at the end of the ^{24}Na loading period. n is the number of ^{24}Na washout experiments, from which first seven columns of data were obtained. See legend to Table 3 for description.

Inulin space, V_0, and total water content, $1-V_D$, were measured separately in muscles soaked for 1 hr. Number of observations is in parentheses.

Values are means ± S.E. of mean. Error in V_0 was ignored in calculation of S.E. of mean of N_i and ΔN_i. Values of N's and V's are expressed per unit of muscle wet weight.

(1969). To study volume changes in the two components we conducted ^{24}Na washout experiments in solutions made hypertonic or hypotonic by addition or subtraction of NaCl from the standard glucose-Ringer-bicarbonate medium. In a given experiment loading and washout solutions had the same Na concentration, at least within 8 %. Data from these experiments are presented in Table 6 together with inulin spaces, V_0, and total water contents, $1 - V_D$, measured separately in muscles soaked for one hour in the anisotonic solutions.

TABLE 7. Effects of osmotic strength on fluid volumes of sarcoplasm and sarcoplasmic reticulum

Relative osmolality × R		ml./g wet weight			
		$\leqslant V_1 \leqslant$		$\leqslant V_2 \leqslant$	
1·66	10	0·348 ± 0·011	0·367 ± 0·014	0·154 ± 0·014	0·173 ± 0·011
1·36	6	0·453 ± 0·012	0·464 ± 0·014	0·095 ± 0·014	0·106 ± 0·012
1·00	11	0·542 ± 0·011	0·544 ± 0·011	0·123 ± 0·011	0·125 ± 0·011
0·63	6	0·671 ± 0·011	0·688 ± 0·010	0·055 ± 0·010	0·073 ± 0·011

Osmotic strength is expressed as a multiple of R, the estimated osmolality of the standard isotonic Ringer's solution. n is the number of ^{24}Na washout experiments. Bounds on V_1 and V_2, fluid volumes of sarcoplasm and sarcoplasmic reticulum, respectively, under anisotonic conditions were estimated by the sequential calculations described in legend to Table 4. Data are means ± S.E. of mean. Errors in inulin space and total water content were ignored in calculation of S.E. of mean of limits of V_1 and V_2.

Mean specific activity of loaded muscles relative to specific activity of loading solution, $\alpha_T(m)$, exceeded 0·97 in each set of experiments.

ΔN_i, the change in intracellular Na content that occurred between the first and third hours of exposure to the anisotonic solutions, was not different from zero in solutions with relative osmolalities of $1{\cdot}66 \times R$ or $0{\cdot}63 \times R$. At relative osmolality $1{\cdot}36 \times R$, ΔN_i is significantly different from zero when errors in V_0 are ignored ($\Delta N_i = 8{\cdot}5 \pm 2{\cdot}6$ m-equiv/kg, Table 6) but not when they are taken into account ($\Delta N_i = -8{\cdot}4 \pm 5{\cdot}2$ m-equiv/kg). We calculated bounds on V_1 and V_2 under all three anisotonic conditions by the same procedure used for standard conditions, which requires $\dot{N}_1(m) \leqslant 0$.

Upper and lower bounds of V_1 and V_2 in the three anisotonic solutions are shown in Table 7. Values in isotonic solution (Table 4) have been included for comparison.

Notice in Table 7 that V_1, expressed in ml. per gram of muscle wet weight, decreased in hypotonic solution and increased progressively in the

hypertonic solutions while V_2 changed in the opposite direction except at $1{\cdot}36 \times R$. But muscle wet weight is a poor reference base for these comparisons because total water content, $1-V_D$, varies with tonicity of the bathing solution (Table 6). A comparison using dry weight as the reference base, justified on the grounds that muscles used in the four sets of experiments were obtained from rats of approximately the same size, follows.

Birks & Davey (1969) constructed a curve to predict the volume behaviour of frog muscle SR under different osmotic conditions. Their prediction was based on data of Blinks (1965), who used an optical technique to measure fibre volume more or less directly, and on data of Dydyńska & Wilkie (1963), who subtracted sucrose space from muscle weight to obtain fibre volume, and on the hypothesis that these two sets of data did not coincide because the fluid volume of SR had been included in the fibre volume measured by Blinks but excluded from that measured by Dydyńska & Wilkie. The continuous segment of the straight line with negative slope in Fig. 8 is that constructed by Birks & Davey. Its slope was determined by the above considerations, and its position with respect to the volume axis was fixed such that the volume of SR at normal osmotic pressure is that estimated by Peachey (1965), 13 % of cell volume. Birks & Davey confirmed that the slope of this line describes the volume behaviour of SR by measuring dimensions of certain elements of SR on electron micrographs of frog sartorius muscle that had been soaked in anisotonic solutions.

In order to compare our estimates of fluid volume of SR, V_2, with the line of prediction in Fig. 8, we first normalized the data of Table 7 according to dry weight, V_D, from Table 6 and then expressed V_2 as percentage of standard cell volume, that is, as percentage of the normalized value of $V_1+V_2+V_D$ in standard isotonic solution. (V_D = 0·2 wet weight in isotonic solution.) Results are shown as horizontal bars near the lower line in Fig. 8. Heights of the bars include the upper and lower limits of the quantity expressed, $[V_2/V_D].100/[V_1^0+V_2^0+V_D^0]$, where the superscripts indicate values in isotonic solution.

The upper line in Fig. 8 was drawn for comparison with sarcoplasmic volume, V_1+V_D if all muscle solids are assigned to sarcoplasm. It describes the behaviour of a perfect osmometer which contains osmotically inactive material equal to 27 % of its volume at normal osmotic pressure ($V_D/[V_1+V_D]$ = 0·27). For this line, cell volume on the vertical axis is V_1+V_D, normalized according to dry weight, and standard cell volume is the value of normalized V_1+V_D in isotonic solution. Values of V_1 from Table 7 have been so expressed and plotted near the upper line in Fig. 8 as horizontal bars, heights of which include upper and lower limits of the quantity expressed, $[(V_1+V_D].100/(V_1^0+V_D^0)]$.

In Fig. 8, predicted and calculated volumes agree qualitatively in that our estimates of V_1+V_D fall along a line with positive slope, as predicted for sarcoplasm, and estimates of V_2 fall along a line with negative slope, as predicted for SR.

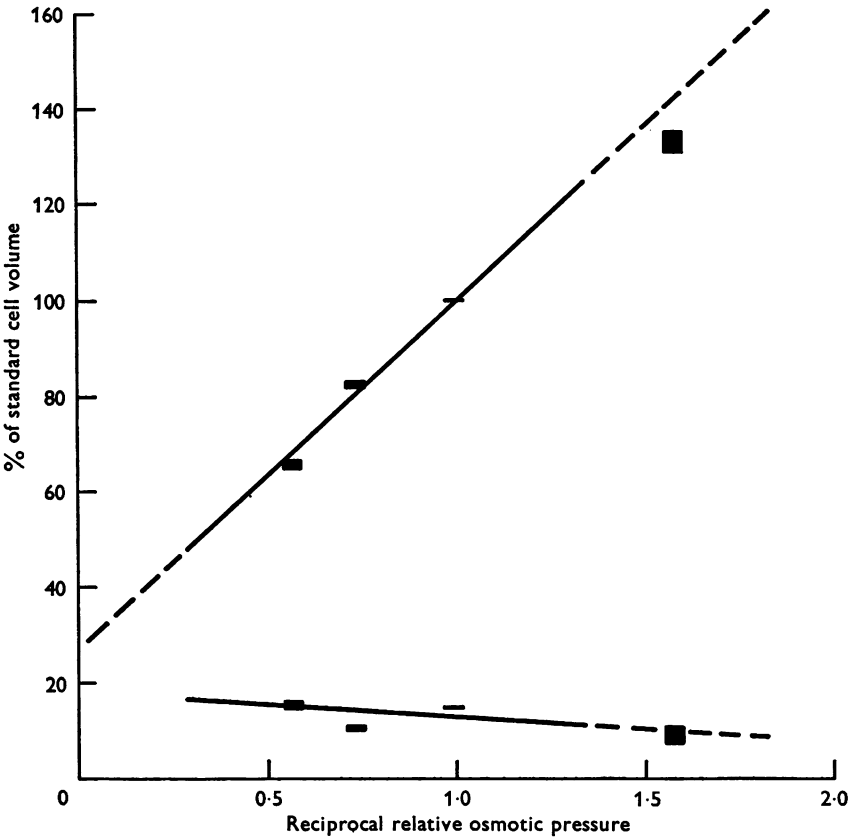

Fig. 8. Upper line describes volume behaviour of perfect osmometer containing osmotically inactive material equal to 27 % of its volume in medium of unit relative osmotic pressure. Upper bars are volumes of sarcoplasm calculated from two-component model and ^{24}Na washout data, V_1+V_D, where V_1 is fluid volume of sarcoplasm and V_D is dry weight, all normalized to the same V_D and expressed as per cent of standard cell volume, that is, as % normalized V_1+V_D at unit relative osmotic pressure.

Lower line, taken from Birks & Davey (1969), describes volume behaviour of sarcoplasmic reticulum, SR. Lower bars are fluid volumes of SR calculated from two-component model and ^{24}Na washout data, V_2, all normalized to the same V_D and expressed as % standard cell volume, that is, as percentage of normalized $V_1+V_2+V_D$ at unit relative osmotic pressure.

Heights of bars include upper and lower limits of normalized V_1+V_D or V_2. All muscle solids were assigned to sarcoplasm, and muscle density was taken as unity.

Slopes of the predicted and calculated relationships in Fig. 8 are similar largely because we assumed that $C_2 = C_0$, where C_2 and C_0 are Na concentrations in SR and in bathing solution, respectively. Had we assumed instead that C_2 was some constant fraction of C_0, $C_2 = \eta C_0$ where $0 < \eta < 1$, each estimate of V_2 would have been elevated by the factor $1/\eta$, hence the points representing normalized V_2 would have been better fitted by a steeper line with negative slope. Estimates of V_1 would have been changed, although less regularly and less perceptibly than estimates of V_2, such that points representing normalized $V_1 + V_D$ would have been better fitted by a steeper line with positive slope. However, the slope of the line representing volume behaviour of the perfect osmometer would have been reduced. Thus there is quantitative agreement between slopes of the predicted and observed relationships in Fig. 8 only when it is assumed that η is approximately one, or C_2 is approximately equal to C_0.

Whether our estimates of V_2 can be compared with the absolute volumes of SR shown as the lower line in Fig. 8 is uncertain. It has already been pointed out that the intercept of this line was fixed such that total volume of SR at normal osmotic pressure would coincide with 13 % of cell volume. Of this, according to Birks & Davey, 10 % of cell volume is fluid volume of SR and the remainder is volume of solid material in SR. On this basis, our estimates of fluid volume of SR, V_2, cannot be compared absolutely with the lower line in Fig. 8, which includes volume of solids in SR. Blinks (1965), however, from his data and the data of Dydyńska & Wilkie (1963), estimated that the aqueous fraction of the cell exceeds the apparent solvent volume by about 13 % of fibre volume at normal osmotic strength. That is, by the same data from which Birks & Davey subsequently drew their predicted slope, fluid volume of SR is 13 % of fibre volume at normal osmotic strength. On this basis, our values of V_2 can be compared absolutely with the lower line in Fig. 1, for both estimate fluid volume of SR.

Measurements of sucrose space in rat extensor digitorum longus support the hypothesis on which the lines of prediction in Fig. 8 were based, that sucrose enters SR as well as extracellular space. That is, V_S is larger than V_0 and almost as large as $(V_0 + V_2)$, where V_S is [^{14}C]sucrose space measured by the same technique as that used for inulin space determination, V_2 is fluid volume of SR estimated from the model and ^{24}Na washout data, and V_0 is extracellular volume measured as inulin space. Table 8 compares V_S with upper and lower limits of $(V_0 + V_2)$ in muscles soaked for 1 hr in two of the anisotonic solutions as well as under standard conditions. [^{14}C]sucrose space in the $1{\cdot}66 \times R$ medium was not measured. According to Table 8, V_S was always smaller than $(V_0 + V_2)$ under the conditions tested, as if SR is not wholly accessible to sucrose, but the difference between

V_S and (V_0+V_2) was statistically significant only for muscles soaked in the $1{\cdot}36 \times R$ solution, in which other anomalous results have been noted.

Ling, Neville, Will & Shannon (1969) have shown that single fibres of bullfrog semitendinosus muscle have a sucrose space or mannitol space of about 10% of fibre volume. Their observation supports the view that sucrose enters the muscle fibre and agrees with our finding that $(V_S - V_0)$ is equal to 10·6% of wet weight, or 12·2% of fibre volume, under standard conditions.

TABLE 8. Comparison of sucrose space, V_s, with (V_0+V_2)

		ml./g wet weight		
		V_S	$\leqslant \Delta[(V_0+V_2)-V_S] \leqslant$	
Relative osmolality $\times R$	n			
1·36	7	0·262	0·051	0·062
		± 0·010	± 0·024	± 0·022
1·0	8	0·238	0·018	0·020
		± 0·009	± 0·018	± 0·018
0·63	9	0·131	0·008	0·026
		± 0·006	± 0·012	± 0·013

Data are means ± S.E. of means. n is the number of determinations of V_S. Bounds on V_2 are from Table 7. Values of V_0 in the anisotonic solutions are from Table 6. In the isotonic medium, $V_0 = 0{\cdot}132 \pm 0{\cdot}003$ ml./g.

In summary, the volumes of the two components, estimated from the model and data from ^{24}Na washout studies on rat muscle, changed as predicted in anisotonic solutions.

Effects of reduced external Na

The two component model predicts that, regardless of any other effects it might have, substitution of external Na by a suitable ion should reduce N_2, the Na content of SR. This follows from the postulate that the triadic junction is freely permeable to Na so that, soon after a change in external Na concentration has been made, $C_2 = C_0$ where C_2 and C_0 are Na concentrations in SR and in extracellular fluid, respectively. Since the barriers about sarcoplasm are relatively impermeable to Na, the model predicts that substitution of external Na by a suitable ion should produce little or no change in N_1, the Na content of sarcoplasm. A suitable ion in this context is one which can enter muscle fibres from bathing solution at the same rates and by the same routes as Na. Li seems to meet the requirements, for the loss of muscle Na induced by partial substitution of external Na by Li (Fig. 7) is compatible with a very rapid replacement of extracellular Na by Li, a less rapid replacement of Na in SR by Li and a slow replacement of sarcoplasmic Na by Li. If the presence of Li does not

alter the inward and outward movements of water differently, the model predicts no alteration in V_1 or V_2, fluid volumes of sarcoplasm and SR, respectively.

To test these predictions, experiments were done with Li^+ substituted for 80% of external Na^+. Partial data from these experiments have already been presented (Table 2) to argue that the two components are interconnected, not arranged in parallel. In order to obtain an upper bound on $N_1^*(m)/N_i^*(m)$ smaller than that given by the parallel solution of the model, we did the following.

First, five experiments were performed in which muscles were loaded under standard conditions but washed out in 80% Li. Mean values of the fractional intercept of the slow component and of rate constants of the two exponentials observed on ^{24}Na washout curves under these conditions are shown as B_1', r_1' and r_2' in Table 9. There is no reason why tracer content of sarcoplasm as a fraction of intracellular tracer content at the end of the loading period, $N_1^*(m)/N_i^*(m)$, should be different in these muscles than in muscles loaded similarly but washed under standard conditions. Hence, $N_1^*(m)/N_i^*(m)$ under these conditions is known within limits, $0{\cdot}016 \leqslant N_1^*(m)/N_i^*(m) \leqslant 0{\cdot}030$ (Table 4).

Secondly, five experiments were done in which muscles were both loaded and washed in 80% Li. The fractional intercept of the slow component is given as B_1 and rate constants of the two exponentials on the washout curve are given as r_1'' and r_2'' in Table 9. Comparison with results obtained under standard conditions shows that substitution of Li for 80% of external Na greatly increased B_1, from 0·03 to 0·41, and practically doubled the observed rate constants.

In the model all rate constants, including the observed rate constants, r_1 and r_2, and the elements of which they are composed (λ_{01}, λ_{21}, λ_{02}, λ_{12}, a and b) are true constants under a given set of conditions. The model therefore requires that any change in rate constant produced by a sudden change in experimental conditions (e.g. a change in composition of bathing solution) must be, in effect, a step change. That is, although rate constants for loading may be different in the two sets of experiments described above, the two sets of rate constants for washout should be identical. The data suggest that this requirement has been met, for the rate constants r_1' and r_1'', or r_2' and r_2'', observed in the two sets of experiments in Table 9 were not different. Moreover, neither the paired differences, $r_2'-r_1'$ and $r_2''-r_1''$, nor the paired products, $r_1'r_2'$ and $r_1''r_2''$, were different. Therefore we combined the data on rate constants from the two sets of experiments for use in subsequent calculations and give the means as r_1 and r_2 in Table 9.

Total Na content of the loaded muscle, $N_T(m)$ in Table 9, fell roughly in

TABLE 9. Parameters of ^{24}Na washout curves and results of muscle analyses, reduced external Na concentration

B_1'	$0{\cdot}137 \pm 0{\cdot}019$
r_1', hr^{-1}	$1{\cdot}79 \pm 0{\cdot}15$
r_2', hr^{-1}	$7{\cdot}04 \pm 1{\cdot}07$
B_1	$0{\cdot}407 \pm 0{\cdot}031$
r_1'', hr^{-1}	$2{\cdot}20 \pm 0{\cdot}12$
r_2'', hr^{-1}	$9{\cdot}33 \pm 1{\cdot}03$
r_1, hr^{-1}	$2{\cdot}00 \pm 0{\cdot}11$ ($n = 10$)
r_2, hr^{-1}	$8{\cdot}18 \pm 0{\cdot}81$ ($n = 10$)
N_T (m), m-equiv/kg	$8{\cdot}46 \pm 0{\cdot}44$
N_T (t'), m-equiv/kg	$9{\cdot}44 \pm 0{\cdot}25$
$C_0(m)$, m-equiv/l.	$29{\cdot}1 \pm 1{\cdot}2$
α_T (m)	$0{\cdot}847 \pm 0{\cdot}030$
α_i (m)	$0{\cdot}668 \pm 0{\cdot}048$
V_0, ml./g	$0{\cdot}160 \pm 0{\cdot}003$ ($n = 41$)
$1 - V_D$, ml./g	$0{\cdot}812 \pm 0{\cdot}002$ ($n = 12$)
ΔN_i, m-equiv/kg	$+1{\cdot}16 \pm 0{\cdot}28$
N_i (m), m-equiv/kg	$3{\cdot}78 \pm 0{\cdot}40$
N_i (t'), m-equiv/kg	$4{\cdot}95 \pm 0{\cdot}25$

The test solution is 80 % Li, prepared by substitution of Li for 80 % of the Na in standard $GKRHCO_3$.

B_1' is fractional intercept of slow component, and r_1' and r_2' are rate constants of the two exponentials observed on ^{24}Na washout curves from five muscles loaded in standard solution but washed in test solution.

B_1 is fractional intercept of slow component, and r_1'' and r_2'' are rate constants of the two exponentials observed on ^{24}Na washout curves from five muscles both loaded and washed in test solution.

Value of r_1 is mean of data for r_1' and r_1''. Value of r_2 is mean of data for r_2' and r_2''.

Remaining measurements are from ^{24}Na flux experiments with test solution used throughout the 1 hr load and 2 hr wash, or from muscles soaked in test solution for 1 hr: $N_T(m)$ and $N_T(t')$, total Na contents of loaded muscle and washed muscle, respectively; $C_0(m)$, Na concentration of loading solution; $\alpha_T(m)$, whole muscle specific activity relative to specific activity of loading solution after 1 hr of ^{24}Na-loading; $\alpha_i(m)$, average intracellular specific activity relative to that of loading solution; V_0, inulin space, and $(1 - V_D)$, total water content, after 1 hr exposure to test solution; ΔN_i, paired differences in intracellular Na content that occurred during washout; $N_i(m)$, intracellular Na content of loaded muscle, calculated with mean V_0 given in table and with individually measured $N_T(m)$ and $C_0(m)$; $N_i(t')$, intracellular Na content of washed muscle, calculated with V_0 measured in muscles soaked for 3 hr in test solution and $C_0(t')$ taken as 28 m-equiv/l.

Data are means ± S.E. of mean. Errors about means of α_T and V_0 were ignored in calculations of S.E. of mean of derived quantities. The number of observations is n and, unless otherwise indicated, $n = 5$. Na contents, N, and volumes, V, are expressed per unit of muscle wet weight.

proportion to the reduction in C_0, from 41·1 m-equiv/kg wet wt. under standard conditions to 8·46 m-equiv/kg in 80 % Li.

Specific activity of the loaded muscle relative to that of the loading solution, $\alpha_T(m)$, was significantly lower in the 80 % Li experiments than under standard conditions, 0·85 compared to 0·98. $\alpha_T(m)$ is calculated as the ratio of ^{24}Na space ($= N_T^*(m)/C^*$) to Na space ($= N_T(m)/C$, where C* and C are concentrations of ^{24}Na and Na in loading solution). The reduction in $\alpha_T(m)$ was due not to reduction in ^{24}Na space (0·245 ± 0·006 ml./g in five muscles in 80 % Li, compared to 0·237 ± 0·004 in forty-eight muscles under standard conditions) but to an increase in Na space (0·292 ± 0·015 ml./g in 80 % Li compared to 0·245 ± 0·005 under standard conditions). As a result, calculated $\alpha_i(m)$, the average intracellular specific activity relative to specific activity of loading solution, was lower than under standard conditions, 0·67 compared to 0·95.

V_0 and $1-V_D$ were measured in separate experiments with muscles soaked for 1 hr in 80 % Li. Both were larger than under standard conditions.

From the value of ΔN_i in Table 9, which was calculated from paired data and for which errors in inulin space, V_0, were ignored, it would appear that muscles had gained intracellular Na between the first and third hours in 80 % Li solution. However, the apparent gain is not significantly different from zero when errors in V_0 are taken into account. Therefore we assumed N_i, and N_1, constant during washout, or $\dot{N}_1(m) = 0$.

From the data now at hand and equations derived previously we can find upper and lower bounds for N_1, N_2, V_1, and V_2 in muscles exposed for 1 hr to 80 % Li. We begin by finding upper and lower limits for

$$N_1^*(m)/N_i^*(m).$$

With substitutions, eqn. (9) for the fractional intercept of the slow component on the washout curve, may be written

$$B_1 = \{[(a+\lambda_{21})N_1^*(m)/N_i^*(m)] + [(b+\lambda_{12})N_2^*(m)/N_i^*(m)]\}/[r_2-r_1].$$

Substitution for $N_2^*(m)$ from eqn. (3) and for λ_{21} from eqn. (12*d*) yields the quadratic

$$(1-x)\lambda_{12}^2 - [(B_1-x)(r_2-r_1) - (1-2x)b]\lambda_{12} + abx = 0, \qquad (25)$$

where x has been written for $N_1^*(m)/N_i^*(m)$.

We can assign values to x in eqn. (25) and generate families of curves relating λ_{12} and b. Two such families are shown in Fig. 9 to illustrate a graphic solution for x in 80 % Li-treated muscles.

The family of thin continuous curves describing λ_{12} as a double-valued function of b is a plot of eqn. (25) for the 80 % Li-load-and-wash experiments with values of r_1, r_2 and B_1 from Table 9. Fixed values of x decrease from $x = B_1$, which is a point at the origin.

We know from definitions that $\lambda_{12} = ab/\lambda_{21}$ and $\lambda_{01}+\lambda_{21} = r_1+b$. The heavy dashed line in Fig. 9 shows the relation between λ_{12} and b when $\lambda_{01} = 0$; all points below it relate to negative values of λ_{01}. Thus values of λ_{12} and b are restricted to the region on or above the heavy dashed line, where $\lambda_{12} \geqslant ab/(r_1+b)$.

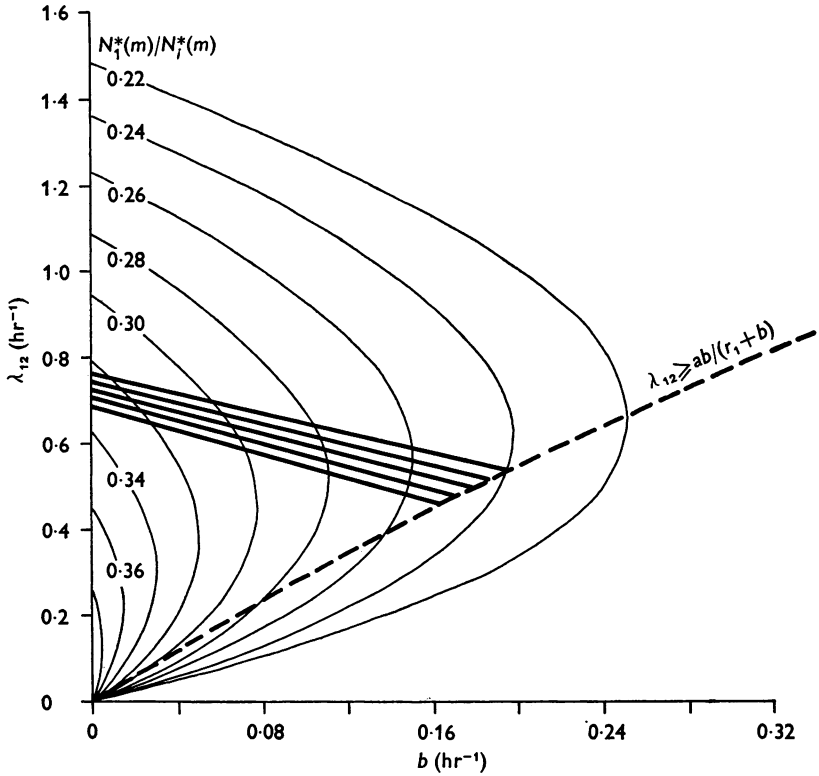

Fig. 9. Graphic solution for bounds on $N_1^*(m)/N_i^*(m)$ in muscles exposed to 80 % Li during the 1 hr ^{24}Na loading period. Upper bound is at the point (0, 0·683); lower bound is at the point (0·196, 0·535). See text for details.

The second family of curves represents the standard-load, 80 % Li-wash experiments with values of r_1, r_2 and B_1' from Table 9. For muscles loaded under standard conditions $N_1^*(m)/N_i^*(m)$ is not unknown but lies between 0·016 and 0·030. This family, shown in Fig. 9 as a band of close-packed, almost parallel curves, is bounded from above when $N_1^*(m)/N_i^*(m) = 0{\cdot}016$ ($= x'$) and from below when $N_1^*(m)/N_i^*(m) = 0{\cdot}030$ ($= x''$). It too is double-valued in λ_{12} but the second values lie below the line $\lambda_{12} = ab/(r_1+b)$ and are not shown.

Superposition of the two families of curves is justified by the model's requirement that all rate constants for washout, including λ_{12} and b, be

identical in the two sets of experiments. Superposition yields a graphic solution of upper and lower limits for $N_1^*(m)/N_i^*(m)$ in muscles loaded in the presence of 80 % Li. The upper limit, 0·334, occurs where the line for $x'' = 0{\cdot}03$ intersects $b = 0$. The lower limit, 0·240, is found where the permissible value of b is greatest, at the intersection of the curves for $x' = 0{\cdot}016$ and $\lambda_{12} = ab/(r_1+b)$.

The analytic solution for $N_1^*(m)/N_i^*(m)$ in the muscles loaded in 80 % Li is

$$\frac{B_1-B_1'+x'\,[r_2/(r_2-r_1)-B_1]}{r_2/(r_2-r_1)-B_1'} \leqslant \frac{N_1^*(m)}{N_i^*(m)} \leqslant \frac{B_1-B_1'+x''(1-B_1)}{1-B_1'}, \qquad (26)$$

where x' is the lower and x'' the upper bound of $N_1^*(m)/N_i^*(m)$ under standard conditions.

TABLE 10. Calculation of bounds on N_1, N_2, V_1, and V_2; reduced external Na concentration

$$0{\cdot}240 \leqslant N_1^*(m)/N_i^*(m) \leqslant 0{\cdot}334$$
$$\alpha_1(m) \geqslant 0{\cdot}856$$
$$0{\cdot}196 \leqslant N_1/N_i \leqslant 0{\cdot}261$$
$$0{\cdot}742 \leqslant N_1 \leqslant 0{\cdot}987 \text{ m-equiv/kg}$$
$$2{\cdot}798 \leqslant N_2 \leqslant 3{\cdot}038 \text{ m-equiv/kg}$$
$$0{\cdot}096 \leqslant V_2 \leqslant 0{\cdot}104 \text{ ml./g}$$
$$0{\cdot}548 \leqslant V_1 \leqslant 0{\cdot}556 \text{ ml./g}$$

Data were calculated from means in Table 9 and equations in the text. Bounds are appropriate at zero washout time, i.e. after muscles have been exposed to ^{24}Na and to 80 % Li for 1 hr.

$N_1^*(m)/N_i^*(m)$: ^{24}Na content of sarcoplasm as a fraction of intracellular ^{24}Na content, eqn. (26) or determined graphically in Fig. 9. $\alpha_1(m)$: specific activity of sarcoplasm relative to that of loading solution; lower bound from eqn. (20*b*). N_1/N_i: sarcoplasmic Na content as fraction of intracellular Na content; lower bound from eqn. (21) with values of rate constants determined graphically in Fig. 9; upper bound from eqn. (19) with upper bound on $N_1^*(m)/N_i^*(m)$ and lower bound on $\alpha_1(m)$ given above. N_1: Na content of sarcoplasm, product of N_1/N_i and N_i. Na content of SR, N_2, and fluid volumes of sarcoplasm and SR, V_1 and V_2 respectively, calculated as in Table 4. N's and V's are expressed per unit of muscle wet weight.

Results of the calculations are shown in Table 10. $N_1^*(m)/N_i^*(m)$ was greater than under standard conditions (Table 4); that is, after 1 hr of loading in 80 % Li, sarcoplasmic ^{24}Na made a relatively greater contribution to intracellular ^{24}Na content than it did under standard conditions. The upper bound was 18 % smaller than B_1, the estimate of $N_1^*(m)/N_i^*(m)$ in the parallel solution of the model.

Notice that $\alpha_1(m) > \alpha_i(m)$ where the value of $\alpha_i(m)$ was given in Table 9. The implication is that after 1 hr of loading in 80 % Li specific activity of sarcoplasm is greater than average intracellular specific activity, and that

of SR is less. The lower limit of $\alpha_1(m)$, from eqn. (20*b*), was used in eqn. (19) to calculate an upper limit on N_1/N_i.

In Fig. 9, the lower bound on $N_1^*(m)/N_i^*(m)$ occurs at the co-ordinates $b = 0{\cdot}196$, $\lambda_{12} = 0{\cdot}535$. These values of b and λ_{12} were used in eqn. (21*a*) to find a lower bound on N_1/N_i directly. The value of $\alpha_1(m)$ required to relate this to the lower bound on $N_1^*(m)/N_i^*(m)$ in eqn. (18*b*) is $\alpha_1(m) = 0{\cdot}805$, which is not significantly less than the lower bound on $\alpha_1(m)$, 0·856.

Bounds on N_1/N_i were greater than under standard conditions; that is, the contribution of sarcoplasmic Na to intracellular Na content was increased because the Na content of SR was reduced. Comparison of estimates of N_1, N_2, V_1 and V_2 in 80 % Li with those under standard conditions shows that the major effect of external Na reduction was a decrease in N_2, as predicted by the model, from about 20 to 3 m-equiv/kg. Since the decrease in $N_i(m)$, from $20{\cdot}4 \pm 1{\cdot}9$ m-equiv/kg under standard conditions to $3{\cdot}8 \pm 0{\cdot}4$ m-equiv/kg in 80 % Li, was highly significant and since the ranges of N_1 in the two sets of experiments overlap, the decrease in N_2 is statistically significant. Calculated V_2 was slightly, but not significantly, lower than under standard conditions, about 0·10 compared to 0·125 ml./g. If real, the decrease in V_2 matches the increase in extracellular volume, V_0, from 0·13 ml./g under standard conditions to 0·16 ml./g in 80 % Li. The ranges of V_1 under the two experimental conditions overlap.

In summary, substitution of Li for 80 % of external Na reduced N_2, the Na content of SR, with no significant change in Na content of sarcoplasm or in volume of either component, as predicted by the model.

Effects of 10^{-5} M *ouabain*

The two-component model predicts that exposure to ouabain should increase the Na content of sarcoplasm, N_1, but not of SR, N_2, and should have no effect on the volumes V_1 and V_2. To test these predictions, ^{24}Na washout experiments were done in glucose–Ringer-bicarbonate medium containing 10^{-5} M ouabain.

Eight experiments were performed in which muscles were loaded under standard conditions but washed out with 10^{-5} M ouabain. Mean parameters of these washout curves are given as B_1', r_1', and r_2' in Table 11.

Six experiments were performed in which muscles were both loaded and washed in the medium containing 10^{-5} M ouabain. Washout parameters from these experiments are given as B_1, r_1'' and r_2'' in Table 11. Notice that B_1 was 14 times higher than under standard conditions. The rate constant of the slower component was not different whereas that of the faster component was higher than under standard conditions. Na contents of the loaded and washed muscles are also shown. One loaded muscle was damaged so that there are only five values for measurements at time m.

N_T and N_i were higher than under standard conditions at the end of the loading period, and they continued to rise during the washout period.

Bounds on $N_1^*(m)/N_i^*(m)$ were found by the graphic method described in the previous section. This method assumes that all of the rate constants in the model are determined by the presence of 10^{-5} M ouabain and are independent of the duration of exposure to ouabain. This requirement has been met in so far as we can demonstrate. For one thing, in the presence of 10^{-5} M ouabain r_1 is constant, independent of time and presumably of muscle Na content, for many hours (Fig. 5). For another, the rate constants r_1' and r_1'' or r_2' and r_2'' observed in the two sets of experiments reported in Table 11 were not different. Neither were the paired differences, $r_2'-r_1'$ and $r_2''-r_1''$, nor the paired products, $r_1'r_2'$ and $r_1''r_2''$. Therefore we combined the data on rate constants and give the means as r_1 and r_2 in Table 11.

TABLE 11. Parameters of ^{24}Na washout curves and results of muscle analyses, 10^{-5} M ouabain

B_1'	0·202 ± 0·011 (8)
r_1', hr^{-1}	0·85 ± 0·10 (8)
r_2', hr^{-1}	7·10 ± 0·26 (8)
B_1	0·421 ± 0·028 (6)
r_1'', hr^{-1}	1·08 ± 0·09 (6)
r_2'', hr^{-1}	7·33 ± 0·59 (6)
r_1, hr^{-1}	0·95 ± 0·08 (14)
r_2, hr^{-1}	7·20 ± 0·28 (14)
N_T (m), m-equiv/kg	48·0 ± 2·4 (5)
N_T (t'), m-equiv/kg	63·2 ± 1·6 (6)
C_0 (m), m-equiv/l.	148 ± 4 (5)
α_T (m)	0·982 ± 0·011 (26)
α_i (m)	0·969 ± 0·001 (5)
V_0, ml./g	0·145 ± 0·004 (17)
$1-V_D$, ml./g	0·783 ± 0·004 (10)
ΔN_i, m-equiv/kg	+15·7 ± 1·4 (5)
N_i (m), m-equiv/kg	26·5 ± 2·1 (5)
N_i (t'), m-equiv/kg	42·1 ± 1·6 (6)

See legend to Table 9 for description of symbols.

The test solution is 10^{-5} M ouabain in Ringer solution. Data are means ± S.E. of mean. Number of observations is in parentheses.

In calculation of N_i (t'), value of C_0 (t') was taken as 145 m-equiv/l.

A graphic solution for $N_i^*(m)/N_i^*(m)$ in ouabain-treated muscles is illustrated in Fig. 10. The family of thin continuous curves describing λ_{12} as a double-valued function of b is a plot of eqn. (25) for muscles loaded and washed in ouabain with values of r_1, r_2 and B_1 from Table 11. Fixed values of x decrease from $x = B_1$, which is a point at the origin. The family of almost parallel curves is a plot of eqn. (25) for muscles loaded under

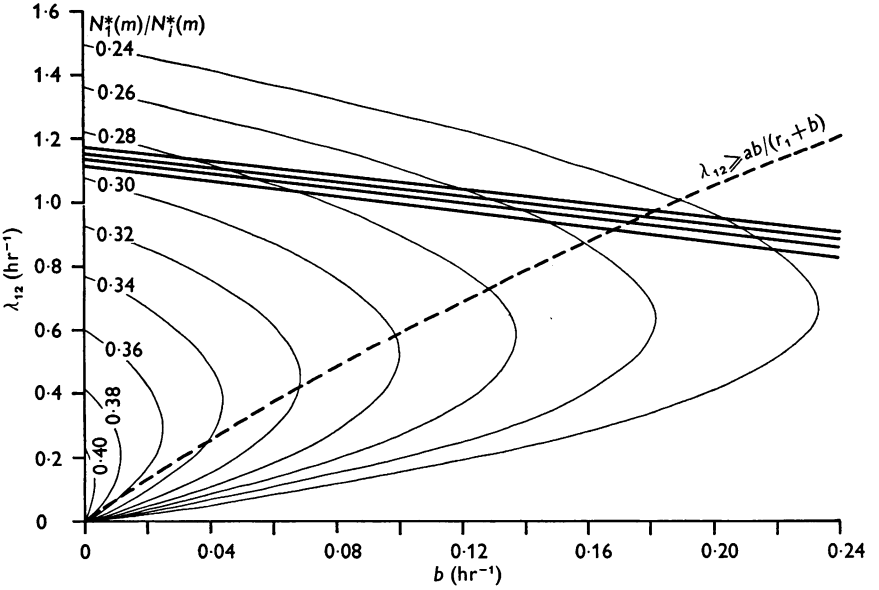

Fig. 10. Graphic solution for bounds on $N_1^*(m)/N_i^*(m)$ in muscles exposed to 10^{-5} M ouabain during the 1 hr ^{24}Na loading period. Upper bound is at the point (0,1·11); lower bound is at the point (0·185, 0·985). See text for details.

TABLE 12. Calculation of bounds on N_1, N_2, V_1 and V_2; 10^{-5} M ouabain

$$0{\cdot}242 \leqslant N_1^*(m)/N_i^*(m) \leqslant 0{\cdot}296$$
$$0{\cdot}883 \leqslant \alpha_1(m) \leqslant 0{\cdot}902$$
$$0{\cdot}266 \leqslant N_1(m)/N_i(m) \leqslant 0{\cdot}318$$

$$7{\cdot}06 \leqslant N_1(m) \leqslant 8{\cdot}44 \text{ m-equiv/kg}$$
$$18{\cdot}10 \leqslant N_2(m) \leqslant 19{\cdot}48 \text{ m-equiv/kg}$$
$$0{\cdot}122 \leqslant V_2 \leqslant 0{\cdot}132 \text{ ml./g}$$
$$0{\cdot}506 \leqslant V_1 \leqslant 0{\cdot}516 \text{ ml./g}$$

Bounds are appropriate at zero washout time, i.e. after muscles have been exposed to ^{24}Na and to 10^{-5} M ouabain for 1 hr.

Data were calculated from means in Table 11 and equations in the text. Bounds on $N_1^*(m)/N_i^*(m)$ were determined analytically from eqn. (26) or graphically from Fig. 10. Bounds on $\alpha_1(m)$ obey eqn. (20c). With these values, bounds on $N_1(m)/N_i(m)$ were obtained from eqn. (19). See legend to Table 10 for description of symbols and calculation of N_1, N_2, V_1 and V_2.

standard conditions but washed in ouabain with values of r_1, r_2 and B_1' from Table 11. This family is bounded from above when $x = 0{\cdot}016$ and from below when $x = 0{\cdot}030$. Superposition yields bounds on $N_1^*(m)/N_i^*(m)$ in muscles loaded in the presence of 10^{-5} M ouabain.

Table 12 summarizes the results leading to bounds on Na content and volumes of sarcoplasm and SR.

$N_1^*(m)/N_i^*(m)$ was at least 8 times larger than under standard conditions. Its upper bound was 30 % smaller than B_1, the estimate of $N_1^*(m)/N_i^*(m)$ in the parallel solution of the model.

Bounds on $\alpha_1(m)$ follow from the restriction that $\alpha_2(m) \leqslant 1$ in eqn. (20*c*). They were used in eqn. (19) to determine bounds on $N_1(m)/N_i(m)$.

With respect to Na contents and volumes of the two components, only one effect of 10^{-5} M ouabain was demonstrated. That was an increase in sarcoplasmic Na content, $N_1(m)$, from less than 0·9 m-equiv/kg under standard conditions to greater than 7 m-equiv/kg, an increase by a factor of at least 8 and perhaps 15. The bounds on N_2 and V_2 under standard conditions or after 1 hr of exposure to 10^{-5} M ouabain are not different, and, if errors about the estimated upper and lower limits of V_1 in Table 12 are of magnitude comparable to those under standard conditions, neither are the bounds on V_1.

That the effect of 10^{-5} M ouabain on $N_1(m)$ is statistically significant was established as follows. If the mean value of every measurement that enters the calculation of $N_1(m)$ in muscles exposed to ouabain (and this includes the lower limit of $N_1^*(m)/N_i^*(m)$ under standard conditions, B_1', B_1, r_1, r_2, $N_T(m)$, V_0 and C_0, all considered independent) were changed simultaneously by 3 S.E. of mean in the direction appropriate to minimize $N_1(m)$, then $N_1(m)$ after ouabain would still be greater than 1·64 m-equiv/kg, which is greater than the upper bound on N_1 under standard conditions, 0·88 ± 0·12 m-equiv/kg.

We conclude that exposure to 10^{-5} M ouabain for 1 hr did increase Na content of component 1, sarcoplasm, with no demonstrable effect on Na content of component 2, SR, or on volume of either component, as predicted by the model.

Effect of ouabain on rate constants

It is generally agreed that ouabain, by specifically inhibiting a $Na^+ + K^+ -$ ATPase, decreases a rate constant for efflux from sarcoplasm. The model therefore predicts that ouabain should decrease at least one of the rate constants λ_{01} or λ_{21} compared to its value under standard conditions.

To test this prediction, experiments were done with 10^{-3} M ouabain present in loading and washout solutions. Under these conditions r_1 was reduced significantly to 37 % of its value under standard conditions (Table 13). This result is consistent with a reduction in λ_{01} and/or λ_{21} and with the prediction that ouabain decreases a rate constant for efflux from sarcoplasm.

With 10^{-5} M ouabain, however, we cannot prove that a reduction in λ_{01} or λ_{21} occurred. Table 14 gives numerical bounds on the four rate constants for efflux under standard conditions. Notice that separation of

λ_{01} and λ_{21} has not been achieved. Table 14 also gives bounds on the four λ's in muscles exposed to 10^{-5} M ouabain. Bounds on the rate constants for efflux from sarcoplasm are the same after 10^{-5} M ouabain as under standard conditions. However, the upper bound on either of these rate constants, λ_{01} or λ_{21}, is calculated from eqns. (13*b*, *c*) as their sum ($= \lambda_1 = r_1 + b$, where b is small).

TABLE 13. Parameters of washout curve, 10^{-3} M ouabain

r_1, hr^{-1}	$0{\cdot}43 \pm 0{\cdot}04$
r_2, hr^{-1}	$6{\cdot}49 \pm 1{\cdot}44$
B_1	$0{\cdot}717 \pm 0{\cdot}040$
B_2	$0{\cdot}283 \pm 0{\cdot}040$

Means ± S.E. of mean of rate constants, r_1 and r_2, and fractional intercepts, B_1 and B_2, from five ^{24}Na washout experiments with 10^{-3} M ouabain present during loading and washout.

TABLE 14. Rate constants, hr^{-1}, for ^{24}Na efflux

Standard conditions	10^{-5} M ouabain
$0 \leqslant \lambda_{01} \leqslant 1{\cdot}17$	$0 \leqslant \lambda_{01} \leqslant 0{\cdot}95$
$0 \leqslant \lambda_{21} \leqslant 1{\cdot}18$	$0 \leqslant \lambda_{21} \leqslant 1{\cdot}14$
$5{\cdot}47 \leqslant \lambda_{02} \leqslant 5{\cdot}51$	$6{\cdot}02 \leqslant \lambda_{02} \leqslant 6{\cdot}15$
$0 \leqslant \lambda_{12} \leqslant 0{\cdot}04$	$0{\cdot}87 \leqslant \lambda_{12} \leqslant 1{\cdot}18$
$0 \leqslant b \leqslant 0{\cdot}008$	$0 \leqslant b \leqslant 0{\cdot}18$

Bounds on rate constants under standard conditions calculated from eqns. (13 *a–g*) with mean values of B_1, r_1 and r_2 from Table 3. Bounds on λ_{12} and b after exposure to 10^{-5} M ouabain determined graphically (Fig. 10); bounds on remaining λ's from definitions, with values of B_1, r_1 and r_2 from Table 11.

It is possible that ouabain has a dual effect. It may not only decrease Na efflux by inhibiting a Na pump but it may increase permeability of membrane barriers to Na. Thus, at small concentrations of ouabain, with less inhibition of the ouabain-sensitive Na pump, the action on passive permeability may mask the contribution of ouabain to reduction of rate constants. At larger concentrations, the predominant effect may be on the Na pump, and r_1 is reduced.

Table 14 shows that ouabain increased at least one of the rate constants for efflux from SR. The most striking effect of 10^{-5} M ouabain was in elevation of λ_{12} from less than $0{\cdot}04\ hr^{-1}$ under standard conditions to greater than $0{\cdot}87\ hr^{-1}$.

DISCUSSION

Our results show that the time course of ^{24}Na washout from rat extensor digitorum longus muscle viewed between 2 min and 2 hr fits a sum of two exponentials. The fit holds for curves obtained under a variety of experi-

mental conditions and thus seems an adequate basis for proposing a two-component model for Na efflux from rat skeletal muscle fibres.

The model ignores the fact that in long experiments under standard conditions, in addition to two major exponentials, the washout curve has a tail (Fig. 4 and Table 1). However, it seems unlikely that the model could have survived the tests presented in this report if the tail of the curve seen under standard conditions represents an important third component, or a serious distortion of ^{24}Na flux from two components, or a signal that the total curve represents non-exponential washout from one component.

In the model intrafibre Na is contained in two interconnected pools which exchange with the bathing solution at different rates. The model is thus at odds with the observation of Hodgkin & Horowicz (1959) that ^{24}Na loss from single fibres of frog muscle is monoexponential and with their conclusion that fibre Na may be treated as an homogeneous distribution. However, in these experiments of Hodgkin & Horowicz, the amount of radiosodium introduced into each single fibre during loading was necessarily small, only a few hundred counts per minute, and its washout was followed for only one time constant, perhaps because the residual counts were close to background. This may have been not long enough to reveal curvature on a semilogarithmic plot or a slowly exchanging fraction of Na if one were present. Furthermore, as these authors point out, tracer loss was not monitored during the first few minutes of washout, so that a rapidly exchanging fraction of sodium, if present, would have passed unnoticed in their experiments.

Horowicz & Gerber (1965) found that ^{24}Na loss from bundles of two or more fibres rarely followed a single exponential time course over periods exceeding one or two time constants but, in view of the single fibre results cited above, accepted this as due to monoexponential washout from individual fibres, each having a slightly different rate constant for ^{24}Na loss. Such components of efflux would reasonably be arranged in parallel and, according to eqn. (18*a*), the intercept of each component a direct measure of its initial ^{24}Na content. Our data are incompatible with a model having two distinct fibre populations or any two Na pools simply arranged in parallel because the intercepts change in response to changes in washout solution even when loading conditions, and presumably initial ^{24}Na distribution, are unchanged (Table 2).

In the model, under standard conditions less than 4 % of intracellular Na is contained in the slowly exchanging, ouabain-sensitive component 1 identified as sarcoplasm and more than 96 % in the rapidly exchanging component 2 designated sarcoplasmic reticulum. In this division the model is consistent with Conway's evidence (1957) that sodium in frog muscle

fibres is distributed in two fractions. His slowly exchanging fraction, comprising perhaps 7 % of the exchangeable fibre Na in fresh muscle, is temperature-sensitive and is apparently held inside fibre membranes. This corresponds well with our component 1. Conway asisgned the rapidly exchanging fraction, containing the bulk of fibre Na, to a special region external to the fibre membrane, possibly sarcolemma. He considered the special region as one freely permeable to Na^+, not requiring active Na^+ excretion and having a free Na^+ concentration equal to that in the interspaces with surplus Na^+ held by fixed anions. These are more or less the characteristics of our component 2 which we associated with sarcoplasmic reticulum. First, the large value of λ_{02}, within 1 % of r_2 under standard conditions, implies that component 2 is in relatively free communication with the bathing solution. Secondly, our results suggest that component 2 is insensitive to ouabain in so far as inhibitory effects on Na excretion are concerned, hence that Na excretion here is not likely to be active. Thirdly, when we assume that Na concentration in component 2 is equal to that in the interspaces just as the free Na^+ concentration in Conway's special region was considered to be, we calculate a volume of component 2 that is close to Peachey's estimate (1965) of volume of SR in frog muscle. In short, our model is in excellent agreement with Conway's division of muscle fibre Na into two fractions, and our component 2, sarcoplasmic reticulum, corresponds well with his special region.

Keynes & Steinhardt (1968) proposed a model for Na efflux from frog muscle identical in circuitry to that shown in Fig. 1, and they suggested that one component was sarcoplasm, and the other sarcoplasmic reticulum. However, they associated with sarcoplasm the component of the washout curve having the larger rate constant and larger intercept during exposure to Li Ringer. This is different from our proposal that the evidence is consistent with identification of the rapid component with the large intercept as Na in sarcoplasmic reticulum.

Another important detail in which our model differs from that of Keynes & Steinhardt has to do with the location of the Na pump or, more precisely, with the site of ouabain sensitivity. Keynes & Steinhardt considered that ouabain acts at sarcolemma in preference to supposing that ouabain acts on the internal membranes to which, they suggested, it presumably does not have ready access. According to our data, calculated from the model, it is at least possible that ouabain does have access to the internal membranes, for the rate constant for Na movement from SR to sarcoplasm, λ_{12}, was increased by ouabain (Table 13).

In the formalism of the model, ouabain can act either at sarcolemma or at the walls between sarcoplasm and SR or both, and our data are consistent with any one of the possibilities. However, we are inclined to place

the Na pump in the walls of SR. Perhaps it does not reside there exclusively, but there is evidence that neither does it reside exclusively in sarcolemma. First, activity of the $Na^+ + K^+$-activated, ouabain-inhibited ATPase thought to be employed in the active transport of Na from cells was found only in the microsomal fraction of skeletal muscle, which is presumably enriched in fragmented SR, and not distributed in all fractions, as fragments of sarcolemma might be expected to be distributed (Rogus, Price & Zierler, 1969). Secondly, in glycerol-treated frog muscle, in which T tubules have been disrupted and the ion movements between SR and extracellular space interrupted in at least some of the fibres presumably, K influx was reduced more than was K efflux, as if active K transport had suffered (Henderson, 1970). Thirdly, skinned muscle fibres, which lack sarcolemma, are responsive to strophanthidin (Costantin & Podolsky, 1967). All of these suggest that ouabain-sensitive Na-K transport can take place across the internal membranes separating sarcoplasm from SR.

Within the framework of the model, we have derived expressions for estimation of upper and lower bounds for Na contents and fluid volumes of the two components. Under standard conditions the upper bound and the lower bound calculated for each of these quantities are so close to each other, usually within experimental error, that the solutions are effectively unique. The two sets of limits correspond to two specific configurations of the model. The set associated with the upper limit of N_1 applies to the parallel configuration and the set associated with the lower limit of N_1 applies to a mammillary configuration in which routes across sarcolemma are absent.

If the model is correct, under standard conditions only 2 to 4 % of intracellular Na is in sarcoplasm and the rest is in sarcoplasmic reticulum, SR.

The assumption that Na concentration in SR is the same as in extracellular fluid or bathing solution leads to an estimate of fluid volume of SR of about 12·4 % of wet weight or 14·3 % of fibre volume, which agrees well with Peachey's (1965) estimate from electron microscopy of volume of SR in frog sartorius muscle, 13 % of fibre volume. If this assumption is correct, the calculated ratio of Na concentrations on either side of sarcolemma leads to an estimate of the equilibrium potential for Na of at least +116 mV, much higher than conventional estimates of about +50 mV based on the supposition that all non-extracellular Na is in sarcoplasm.

Calculated efflux from sarcoplasm, both into SR and across sarcolemma into extracellular space, is about 1 m-equiv/hr.kg wet wt. Calculated efflux from SR, both into sarcoplasm and into the T system, is about 110 m-equiv/hr.kg wet wt. To express these fluxes per unit of fibre surface area for ready comparison with fluxes reported by others, we consider the

muscle to be composed of cylindrical fibres having $1{\cdot}05 \times 10^6$ cm^2 of cylinder surface per kg muscle wet weight (Zierler *et al.* 1966). Then the fluxes from sarcoplasm and SR are about 0·3 and 30 p-mole.cm^{-2}.sec^{-1}, respectively. Each of these figures differs by an order of magnitude from the Na flux into a resting frog muscle fibre or bundle, about 3·6 p-mole.cm^{-2}.sec^{-1}, reported by Horowicz & Gerber (1965). If the model is correct, the custom of expressing fluxes per unit of cylindrical surface has little meaning in the case of muscle since the surface area is not simply that of the cell envelope and its size is unknown.

There are two routes by which fibre Na can reach interstitial fluid. One is across sarcolemma. The magnitude of this efflux is given by $\lambda_{01}N_1$. The other is across the triadal junction into the T system, and its magnitude is given by $\lambda_{02}N_2$. Since N_2/N_1 is over 20, and $\lambda_{02}/\lambda_{01}$ is no less than 5 and may be infinite, Na efflux is at least 100 times greater by way of the T system than by translocation through sarcolemma, and the ratio may be unbounded at its upper value.

A major result under standard conditions is that our values of V_1 and V_2, calculated as volumes of two compartments hypothesized because the ^{24}Na washout curve fit a sum of two exponentials, agree with the estimates of volumes of sarcoplasm and sarcoplasmic reticulum made from the data of Peachey (1965), who used an entirely different technique. This agreement argues that our interpretation of the tracer washout curve is not seriously in error, for if curvature of the washout function were not due to existence of two effectively homogeneous Na pools from which flux obeys first-order kinetics, but were due to some other cause, such as diffusional delays through extracellular space (Keynes, 1954), one might calculate parameters with dimension of volume, but one might not expect quantitative agreement with volumes of real compartments in muscle.

Three tests have been applied to the two-component model. Results support the model, particularly our claim that component 1 represents sarcoplasm and component 2 represents sarcoplasmic reticulum.

First, the volume of component 1, sarcoplasm, increased in hypotonic solution and decreased in hypertonic solutions, as predicted for an osmometer. The volume of component 2 decreased in the hypotonic solution and tended to increase in the more hypertonic solution tested, in general agreement with the changes in volume of SR observed by Birks & Davey (1969).

Secondly, the major effect of substitution of 80 % of external Na by Li, was a reduction in Na content of component 2, SR. This result is independent of our assumption that $C_2 = C_0$ but would be expected if SR and extracellular fluid are in relatively free communication with respect to Na movement and have Na concentrations that are similar. Indeed, when

volume of SR, V_2, was calculated on the assumption that $C_2 = C_0$, the estimate of V_2 in 80 % Li solution was not significantly different from the value of V_2 calculated under standard conditions. There is a possibility that after exposure to 80 % Li solution V_2 is smaller than under standard conditions, but errors in the calculations are too large to prove that such a reduction occurred. In any case, we did not over-estimate V_2 in the 80 % Li experiments, as we would have done if after 1 hr the Na concentration in SR, C_2, were still falling, approaching external Na concentration, C_0.

Other effects of partial substitution of Na by Li were noted. The rate constants of both exponentials observed on the ^{24}Na washout curve, r_1 and r_2, were increased by at least 50 % of their respective values under standard conditions. These results do not suggest that an exchange diffusion mechanism of the type proposed by Ussing (1949) is operating in this preparation. The fact that the rate constants were increased could, however, be interpreted as support for the proposal made by Beaugé & Sjodin (1968) and Beaugé & Ortiz (1970) that Li has a direct, stimulating effect on the Na pump.

In the third test of the model, the only effect of 10^{-5} M ouabain demonstrated on Na contents and volumes of the two components was an increase in Na content of component 1, sarcoplasm. This result reinforces the notion that SR lies external to Na pumping mechanisms. If changes in V_1, V_2 or N_2 were produced by 10^{-5} M ouabain, they were beyond our limits of detection. The fact that V_1 and V_2 were not appreciably altered by ouabain supports our assumption that C_2 and C_0 are similar.

Caldwell (1968) has reviewed evidence for attributing the apparently complex kinetics of Na flux and the low activity coefficients for Na in muscle to a distribution of Na between two structural components having different characteristics. The model and the data we have presented are in accord with that interpretation.

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