Muscle: A Three Phase System

The partition of monovalent ions across the cell membrane

SHIRLEY E. SIMON, B. M. JOHNSTONE, K. H. SHANKLY, and F. H. SHAW

ABSTRACT The partition of Li+, Br−, and I− across the membrane of the sartorius muscle of the toad Bufo marinus has been investigated both at the steady state and with kinetic methods. Li+ was found to have access to an amount of muscle water similar to that of Na+. Br− and I− could be regarded as being interchangeable with cellular Cl−. None of the foreign ions caused significant losses of cellular K+.

Li+ efflux from the cell was slower in muscles which were equilibrated for long periods in Li+ than in short equilibrated muscles. Na+ efflux from Li+-treated muscles was similar in rate to normal controls, but the amount of Na+ in the slow fraction was increased by Li+.

I− efflux was extremely rapid, and it was not possible to differentiate kinetically between intra- and extracellular material.

These results have been found to be consistent with the hypothesis of a three phase system for muscle.

The authors have recently (22) proposed a three phase system in amphibian muscle to account for the distribution of Na+, K+, and Cl− between the cell and the extracellular fluid. The tissue is assumed to consist of an extracellular phase, and two intracellular phases, the “free intracellular phase” and the “ordered phase.” The greater part of the intracellular compartment will be occupied by the ordered phase, which will include all the highly organized cellular structures in a semirigid space lattice. K+, inorganic phosphate, and all the cellular constituents which are accumulated by the cell will be incorporated in this phase, and their adsorption will depend on the maintenance of the integrity of the system, for which a constant supply of metabolic energy will be necessary. These accumulated constituents will also be found in the free intracellular phase in a concentration similar to that existing in

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the external medium. It has been shown that increases in the external level of \( K^+ \) (22), and inorganic phosphate (2) result in increases in the level of these ions in the free phase in a manner similar to that found for \( \text{Cl}^- \). Other inorganic ions of the extracellular phase will be excluded from the ordered phase, and will be confined to the free intracellular phase. This exclusion from the major part of the cell will result in an apparent concentration gradient for these substances across the cell membrane, when the concentrations are expressed in terms of the total cell volume (22).

In this paper no attempt will be made to describe the exact nature of the ordered phase except to say that it may have many of the properties of a highly ordered polyelectrolyte gel, or an ion exchange resin. It is not to be visualized, however, as a purely physicochemical system as its exact nature will depend on the metabolic state of the cell at any instant. We are purely concerned here with a study of the partition of some monovalent ions across the cell membrane in an attempt to define the nature of the free intracellular phase. If it could be shown that these partially excluded ions are in a diffusion equilibrium with the extracellular fluid then such a system would render unnecessary the concept of active transport as applied to the \( \text{Na}^+ \) "pump."

It has previously been shown (23) that movements of \( \text{Na}^+ \) and \( \text{Cl}^- \) are closely correlated in the muscle cell, and are largely unrelated to \( K^+ \) movements. From considerations such as these it has been inferred that \( \text{Na}^+ \) and \( \text{Cl}^- \) are confined to the free intracellular phase. The ratio of external concentration to internal concentration for an ion confined to the free intracellular phase should define the dimensions of this phase, if one assumes that these ions are in a simple diffusion equilibrium with the external solution, and are not also contained in the ordered phase.

The experiments outlined below have been designed to test the validity of these assumptions. It would appear that the partition of monovalent ions between the cell and the external medium can only be described approximately in terms of such a simple system. In this paper it will be shown that the distribution of the monovalent anions agrees with the above hypothesis. Whilst the partition of \( \text{Li}^+ \) also confirms the theory the release of \( \text{Li}^+ \) is apparently anomalous.

**Methods**

The sartorius muscle of the toad, *Bufo marinus*, was used throughout this study. The partition of monovalent ions across the cell membrane was investigated both at the steady state and with kinetic methods. The data obtained at the steady state have permitted a study of the effects of "foreign" ions on the normal ionic constituents of the cell, whilst the kinetic studies have given information about the effect of alteration of the ionic environment on the fluxes of these ions.

The methods used in the steady state studies are similar to those reported previ-
ously (23). The mathematical approach to the kinetic studies will be described in the text.

A Beckman flame spectrophotometer was used for the cation analyses, due care being taken to obviate interference errors by the "doping" of standards with salts in equivalent proportions to those present on the test solutions. Cl\textsuperscript{−}, Br\textsuperscript{−}, and I\textsuperscript{−} were determined potentiometrically by a modification of the method reported previously (23).

Unless otherwise stated the extracellular volume of each muscle was determined with radioiodinated serum albumin (RISA), since it has been shown that the extracellular space may vary considerably between toads (24). Intracellular ionic levels have been calculated on the basis of the found space. Some data obtained from muscles in which the RISA space was not determined have, however, been included, since the analytical methods are such that all the relevant ions can be determined in the same muscle. Thus it is possible to obtain an exact comparison of the distribution of the ions by this approach, although the absolute amounts may be in error. Data obtained from paired muscles will also be used for comparative purposes, since the extracellular space of paired muscles has been found to be similar (24).

RISA,\textsuperscript{32}NaCl, and Na\textsuperscript{131}I were obtained from the Atomic Energy Research Establishment, Harwell. Radiations were counted with an EKCO scintillation counter and rate meter, and a Philips scaler. The isotopes were added to the test Ringer to give an activity of from 25 to 50 microcuries per ml.

**Solutions** Ringer solutions used were modifications of those reported previously (20) and will be described in the text. All Ringers were analyzed for Na, K, Li, etc., since the amount of these ions present was altered to a varying extent by the addition of RISA to determine the extracellular space. All Ringers had 10 mg. per liter aureomycin added to ensure sterility, as it was found that bacterial contamination may be considerable after 6 to 8 hours' soaking.

**RESULTS**

**The Partition of Lithium across the Cell Membrane of the Sartorius Muscle**

Li\textsuperscript{+} was substituted for Na\textsuperscript{+} in various proportions in the Ringer solution, the total cation content being kept constant. Muscles were soaked for 4 hours in the solutions, without previous equilibration in normal Ringer. All results were obtained from paired muscles, one soaked in normal bicarbonate-buffered Ringer, the other in the test solution. The experiments were carried out in two series. In one the extracellular volume of each muscle was estimated with RISA; in the other series this was not done, and intracellular contents were estimated by using a mean extracellular space of 20 per cent which is the average of our values (24). The results of the first series are presented in Table I, and will be considered in conjunction with the second, larger series.
1. The amount of Li⁺ entering the cell was proportional to the external level of the ion over the entire range of concentrations used, which varied from 20 to 110 m.eq per liter Li⁺. The exact value of the ratio Li⁺_{out}/Li⁺_{in} could be obtained when the extracellular volume was known, and was found to vary, after 4 hours' soaking, between the extreme range of 4.5 to 10, with a mean value of 7.1. Thus, if one assumes the intracellular Li⁺ to be in a diffusion equilibrium with the extracellular fluid, then the intracellular "Li⁺ space" is approximately one-seventh of the total cell volume.

2. The partition of Li⁺ was compared with the Na⁺ distribution in the same muscle, and was also compared with the Na⁺ distribution in the companion muscle soaked in normal Ringer. Here a difference was found between the two series of experiments which cannot be ascribed to an incorrect estimate of the extracellular volume, when this was not measured. In the larger group of results obtained during the summer the relative proportions of Na⁺ and Li⁺ in the cell were not significantly different. That is the intracellular

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Ratio</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li in Ringer, m.eq. per liter</td>
<td>34</td>
<td>58</td>
</tr>
<tr>
<td>Na in Ringer, m.eq. per liter</td>
<td>90</td>
<td>70</td>
</tr>
<tr>
<td>Li, m.eq. per kg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na, m.eq. per kg.</td>
<td>27.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>22.9*</td>
<td>3.9*</td>
</tr>
<tr>
<td>Cl, m.eq. per kg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Treated</td>
<td>26.1</td>
<td>4.2</td>
</tr>
<tr>
<td>K, m.eq. per kg.</td>
<td>108</td>
<td>102</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>100*</td>
<td>97.5</td>
</tr>
<tr>
<td>Extracellular space</td>
<td>20.5</td>
<td>20.9</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>17.9*</td>
<td>18.0*</td>
</tr>
</tbody>
</table>

Figures given are the mean of five observations. The ionic contents are intracellular levels calculated on the found extracellular space. Only figures marked with an asterisk are significantly different from controls. (P ≥ 0.05). "Ratio" refers to external concentration/internal concentration.
level of these ions varied with the external level to the same extent, and the “space” available to each was similar. This equality of ratios out/in would not be affected by an incorrect estimate of the extracellular space, although the absolute values of the ratios may be held in doubt. In the series of experiments carried out in the winter, which are set out in Table I, this equality was not found. The Na⁺ ratio was significantly lower than the Li⁺ ratio, and consequently in these toads more of the muscle water was available to Na⁺ than to Li⁺.

The partial substitution of Li⁺ for Na⁺ in the Ringer brought about a loss of Na⁺ from the cell in all muscles, although this loss was less than proportional to the external decrease in the Na⁺ level, and consequently the ratio Na⁺<sub>out</sub>/Na⁺<sub>in</sub> was lower in Li⁺-treated muscles than in controls in normal Ringer. It could be inferred from this that more of the cell water is available to Na⁺ under these conditions. It may be that part of the Na⁺ is “bound” either in the free phase or the ordered phase, and is consequently unable to diffuse out of the cell when the external level is lowered. A variation in the amount of bound Na⁺ between winter and summer toads could account for differences in the partition of Li⁺ if one assumes that the Li⁺ is only able to displace the bound Na⁺ very slowly. This question will be discussed further in the section on kinetics.

3. The Li⁺-treated muscles tended to have a lower K⁺ content than those held in normal Ringer, but the losses were small, and in only a few experiments were they statistically significant. It was not possible to correlate the losses of K⁺ with the Li⁺ content of the Ringer.

4. In none of the experiments was the Cl⁻ content of the muscles significantly different from that of the controls, despite the consistent difference in the Na⁺ ratio.

5. The muscles all contracted on stimulation; also there was no significant volume change on immersing the muscles in Li-Ringer. There was, however,
a slight but significant decrease in the volume of the RISA extracellular space in Li solutions, despite the fact that the over-all weight of the muscle did not change. This would imply a swelling of the fibres which was not sufficient to distend the whole muscle. A reduction of the original extracellular space by less than 15 per cent would not be great enough to invalidate the comparison of values obtained from paired muscles, when this space was not estimated. (See Table I.)

Thus the only ionic interaction shown by these experiments would appear to be between Na⁺ and Li⁺. Li⁺ can replace part of the Na⁺ in the cell, but there appears to be a Na⁺ fraction which is more firmly held, and which is not displaced by Li⁺ over the 4 hour soaking period.

The Effect of Reduction in the Ringer-Li⁺ Level on the Cellular Li⁺

The authors have previously reported (21) the apparent extrusion of Na⁺ from muscles found on transferring them from normal Ringer to a low Na⁺-Ringer. This apparent extrusion is also found for Cl⁻ (23), and is believed to result from the passive movement of the ions with the concentration gradient, into or out of the free intracellular phase. If Li⁺ is contained in this region in a fashion similar to Na⁺, then moving the muscle from a high Li-Ringer to one with a lower Li⁺ content should result in a reduction of the intracellular Li⁺.

Ten muscles were equilibrated in Ringer containing 59 m.eq. per liter Li⁺ for 3 hours, then one of each pair was transferred to Ringer containing 31 m.eq. per liter Li⁺ for a further 3 hours. The control muscle was left in the original Li⁺ solution for this second 3 hour period. It was found (Table II), by comparing the intracellular Li⁺ content of the muscles calculated on the RISA space at the end of the 6 hours soaking, that only a small and non-significant amount of Li⁺ had moved out of the cell. There was, however, an increase in cellular Na⁺ as was to be expected, as the external level of this ion had been increased, and the ratio Na⁺out/Na⁺in was not significantly altered. Thus the size of the "Na⁺ space" was not changed, but the movement of Li⁺ out of the cell was in some way impeded. Confirming the results of the previous section, the Li ratio of the control muscles was higher than that of the Na.

The Rate of Entry of Li⁺ into the Cell

As it is not possible to obtain a radioisotope of Li⁺ the rate of entry of the ion into the cell can only be found by analyzing groups of muscles which have been held in Li⁺ for varying periods of time. This procedure is open to the obvious error that muscles take up Li⁺ at different rates, there being often a
marked difference between different batches of toads. However, by using groups of from ten to sixteen muscles at each time considered it was possible to draw an influx curve. Data were obtained at 0.5, 1.5, 3, 4, and 18 hours, and it was obvious that there was an initial rapid gain of intracellular Li⁺, which was complete in 1.5 hours. The slower rate of entry during the subsequent 18 hours was estimated with more accuracy using paired muscles. One of each pair was soaked in Li-Ringer for 18 hours prior to analysis, while the companion muscle was soaked in normal Ringer. The latter muscle was then transferred to Li-Ringer for 1.5 hours. The soaking in normal Ringer was carried out in an attempt to have the muscles in a comparable metabolic condition, if one assumes that this will vary only with the time of removal of the tissue from the animal. The intracellular Li⁺ content of the muscles rose from 10.3 m.eq. per kg. at 1.5 hours to 19.0 m.eq. per kg. after 18 hours.

Na⁺, Cl⁻, and K⁺ movements were followed at the same time as the Li⁺ entry, and were found to agree with those reported in the previous section. The Na⁺-Li⁺ movements were essentially reciprocal, and Cl⁻ and K⁺ were not significantly different from control levels, even over the 18 hour soaking time.

### The Rate of Loss of Lithium from Muscle.

**Lithium Efflux into Normal Ringer**

Muscles were equilibrated for varying periods of time in Ringer containing 40 to 60 m.eq. per liter Li⁺ and RISA. They were then lightly blotted, weighed, and transferred serially to photometer buckets containing 2 ml. aliquots of Ringer. The muscles were left in the buckets for timed intervals, then drained against the side of the bucket for 10 seconds, and transferred to the next bucket. The radiation due to RISA was counted in each bucket, then the Li⁺ was estimated photometrically. At the end of the run the residual radioactivity and cation content were assayed.

It was hoped that the efflux of RISA from the extracellular space would provide us with a “muscle parameter” taking into account the geometry of the packed fiber mass, the rate of diffusion from it, and the dimension of the extracellular space. This approach was only partially successful, presumably because of the difference in molecular size and possible adsorption effects (24). However, we were able to determine the extracellular space by this method and use it in the analysis of the lithium efflux.

The Li⁺ efflux was plotted as the logarithm of Li⁺ remaining in the muscle against time, the shape of the curve being shown in Fig. 1. The curve was analyzed by extrapolating the slow, straight portion back to zero time, then subtracting this from the curve to give a residue (the first component). This first component, in 80 per cent of the muscles, was sufficiently straight in its
latter part to be similarly split into two components, a medium portion, and a fast component, which has approximately the shape of a diffusion curve from a plane sheet. The results were also graphed against the square root of time (Harris (7)). However, this method of plotting was not so fruitful as the logarithmic approach. The amount of Li\(^+\) in each component at zero time was expressed as a space (the percentage of the muscle volume which would be occupied by that amount if at Ringer concentration). The spaces and half-times of the components averaged, slow 22 per cent, 550 minutes; medium 14 per cent, 11 minutes; fast 14 per cent, 1 minute.

If all the intracellular Li\(^+\) is assumed to belong to the slow component as would occur if the efflux were membrane-limited and the movement of Li\(^+\) through the cytoplasm were fast compared with its movement through the membrane, then the amount in the faster component should be extracellular. Comparison with RISA space measured on the same muscles shows the space of the faster component to exceed the RISA space in every instance, there being no correlation and the means of 28 per cent and 19 per cent respectively being significantly different on a "t" test at the 0.1 per cent level. The size of the extracellular space will obviously be altered by the amount of pressure used in blotting the muscle. In these experiments the difference between the RISA space and the apparent extracellular space for Li\(^+\) cannot be affected.

**Figure 1.** The efflux of Li\(^+\) from muscle into Ringer.
by the blotting error, since the influx Ringer contained both RISA and Li+, and consequently both spaces would be altered similarly by the one blotting procedure. Therefore some part of the faster compartment must be intracellular or bound in the extracellular phase.

The fastest component may be expected to be a free extracellular component, and was therefore compared with the residue left after subtracting the slow straight portion from the RISA efflux; the space occupied by this fast component of the RISA efflux was 11 per cent mean, and was correlated with the space of Li+ fast component at the 0.1 per cent level.

A small part of the medium component must also be extracellular to make up the difference between the space of the fast fraction and RISA space. The remainder of the medium fraction is likely to be intracellular, as a partial correlation, significant at the 1.0 per cent level, exists between the amount in this component and the amount in the slow component of the Li+ efflux, but no correlation exists with the amount in the fast component of the Li+ efflux, nor with the amount in the slow phase of the RISA efflux.

Thus the efflux from the intracellular space must be described as a sum of exponentials, rather than the single exponential to be expected if membrane resistance were the only cellular property controlling efflux. Further than this a consideration of the fast component does not affect the conclusions of this paper and will be dealt with more extensively in another paper.

The mathematical methods of treatment of this type of information have been reviewed by Robertson (18), who has pointed out the difficulties inherent in the interpretation of such data.

Variation in the Half-Time of the Slow Component of the Efflux

The rate at which intracellular Li+ leaves the cell appears to vary considerably with the time of equilibration of the tissue in the Li-Ringer. Muscles which were equilibrated for 90 minutes in Li-Ringer showed a faster rate of efflux than those which were equilibrated overnight. The half-time for the slow component of these effluxes was 105 ± 17 minutes. After 18 hours' equilibration the figures for the slow components had risen to 513 ± 50 minutes (14 observations). Three muscles were effluxed after 3 hours' equilibration, and the half-time was 570 minutes which is not significantly different from that of the 18 hour muscles.

It will be seen that the standard error for the slow component is fairly great. Different batches of toads tended to give different half-times. Paired muscles usually yielded closely similar results, but there were occasional differences without apparent cause. It is of interest in this respect that when the Li+ efflux and the Na+ efflux were both followed into sucrose, the Li+ rate varied from animal to animal, but the Na+ rate remained relatively constant (see below).
The effect of time of influx on the rate of efflux would indicate that Li$^+$ influx can be divided into a permeation process, and a process of combination with the cellular constituents. The first process is faster than the second, and it is the desorption of Li$^+$ from this complex that is the principal rate-limiting step in the efflux.

The assumption that Li$^+$ is adsorbed in the cell would resolve the paradox that little Li$^+$ escaped into a Li$^+$-containing solution (previous section) whilst there was a definite efflux into a Li$^+$-free solution. This would result from the form of the adsorption isotherm.

The Effect of Change of External Environment on the Rate of Lithium Efflux

1. Li$^+$ Efflux into Na$^+$-Free Solutions If the rate of loss of Li$^+$ from the cell is assumed to depend on a desorption from sites, then it would seem possible that it is dependent on an exchange of Li$^+$ for Na$^+$. It was shown in the steady state section that Li$^+$ exchanges with the Na$^+$ of the cell, and it could therefore be reasoned that Li$^+$ exit would be slowed in a Na$^+$-free medium.

Muscles were loaded with Li$^+$ and RISA in the usual way, then one of each pair was effluxed into Ringer, and the companion was effluxed into an isoosmotic sucrose solution. Na$^+$ loss from the cell was also followed into the sucrose solution. Experiments were carried out with muscles influxed for 1.5, 3, and 18 hours. In all, eight pairs of muscles were used. The results of four typical experiments are set out in Table III.

| TABLE III |

A COMPARISON OF Li$^+$ EFFLUX INTO RINGER AND SUCROSE

<table>
<thead>
<tr>
<th>Efflux solution</th>
<th>Time of influx</th>
<th>Time to slow component</th>
<th>Apparent extracellular space</th>
<th>RISA space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hrs.</td>
<td>min.</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Li$^+$ into Ringer</td>
<td>3</td>
<td>800</td>
<td>21.4</td>
<td>14.0</td>
</tr>
<tr>
<td>Li$^+$ &quot; sucrose</td>
<td>3</td>
<td>820</td>
<td>23.9</td>
<td>15.3</td>
</tr>
<tr>
<td>Na$^+$ &quot;</td>
<td>3</td>
<td>120</td>
<td>28.5</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>490</td>
<td>33.4</td>
<td>19.9</td>
</tr>
<tr>
<td>Li$^+$ into Ringer</td>
<td>3</td>
<td>520</td>
<td>34.6</td>
<td>17.8</td>
</tr>
<tr>
<td>Na$^+$ &quot;</td>
<td>3</td>
<td>116</td>
<td>40.8</td>
<td>22.6</td>
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<tr>
<td></td>
<td>18</td>
<td>410</td>
<td>24.6</td>
<td>19.6</td>
</tr>
<tr>
<td>Li$^+$ into Ringer</td>
<td>18</td>
<td>450</td>
<td>27.7</td>
<td>22.6</td>
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<tr>
<td>Na$^+$ &quot;</td>
<td>18</td>
<td>108</td>
<td>33.7</td>
<td>25.3</td>
</tr>
<tr>
<td>Li$^+$ into Ringer</td>
<td>18</td>
<td>420</td>
<td>26.6</td>
<td>23.8</td>
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<tr>
<td>Li$^+$ &quot; sucrose</td>
<td>18</td>
<td>410</td>
<td>39.4</td>
<td>25.3</td>
</tr>
<tr>
<td>Na$^+$ &quot;</td>
<td>18</td>
<td>96</td>
<td>43.0</td>
<td>25.3</td>
</tr>
</tbody>
</table>
We were unable to demonstrate any significant difference between the rate of efflux of Li$^+$ into Ringer or sucrose, or in the shape of the curves, at any influx time tested. It would therefore seem unlikely that Li$^+$ efflux is primarily dependent on an exchange with Na$^+$.

The Na$^+$ efflux curve from Li$^+$-treated muscles into sucrose resembled in form the Li$^+$ curve. In muscles which were equilibrated for 1.5 hours the rate of Na$^+$ loss from the muscle was similar to the rate of Li$^+$ loss, both effluxes had half-times for the slow component of the order of 100 minutes. Muscles which were equilibrated for 3 or 18 hours showed much slower rates of Li$^+$ loss, although the rate of Na$^+$ loss remained essentially constant. Thus the mechanism causing the retention of Li$^+$ in the long soaked muscles has not affected the Na$^+$ movement.

The amount of Na$^+$ in the fast component of the efflux was obtained, as it was in the Li$^+$ efflux, by extrapolation of the slow component to zero time, and then by subtraction of this figure from the total. This apparent extracellular space for Na$^+$ was compared with the RISA space, and was found to exceed it in every instance (Table III). The apparent extracellular space for Na$^+$ was greater than this space calculated from the Li$^+$ efflux, from the same muscles. The RISA efflux was followed in these same muscles, and we noted a profound slowing of the rate of movement of RISA out of the interfiber space into sucrose compared with movement into Ringer. It might be expected that the movement of sucrose into the extracellular region could affect the rate of movement of material outwards, although we were unable to detect any difference in the Li$^+$ curve. However, the rate of RISA efflux was reduced to approximately one-sixth. It is possible that there may be an aggregation of the serum protein molecules in a non-electrolyte medium, as we did not observe this phenomenon when RISA was effluxed into choline chloride.

The finding that Li$^+$ efflux is not dependent on the presence of Na$^+$ in the external medium was confirmed in experiments in which the muscles were effluxed into isoosmotic choline chloride. Four pairs of muscles were used, with an influx time of 18 hours. There was a statistically insignificant decrease in the half-time of the slow component in choline chloride, the times falling from 630 to 510 minutes.

2. THE EFFECT OF Li$^+$ ON Na$^+$ EFFLUX These experiments suggested that Na$^+$ and Li$^+$ movements out of the cell are not interdependent. Thus the slower efflux of Li$^+$ after long equilibration cannot be ascribed to a non-specific decrease in membrane permeability, or to the poisoning of an extrusion mechanism, since the half-time for the slow component of Na$^+$ efflux is not altered by the time of equilibration of the muscle in Li$^+$. Since this point seems of considerable theoretical interest it was checked in two further experiments.

First, two muscles were equilibrated in Li-Ringer for 18 hours, while their
companion muscles were held in normal Ringer. Both sets were then effluxed into sucrose, and Li\(^+\) and Na\(^+\) movements were followed photometrically.

Li\(^+\) and Na\(^+\) movement out of the cell into sucrose must be accompanied by an anion. Our previous work on the correlation of Na\(^+\) and Cl\(^-\) levels in the muscle (23) would suggest that Cl\(^-\) is the anion. Shanes (19) has shown that Na\(^+\) movement out of vertebrate nerve into sucrose is accompanied by Cl\(^-\). The outward movement of an ion pair may complicate the kinetic picture. The steady state exchange of Na\(^+\) such as can be followed in tracer studies could possibly show differences from the effluxes into sucrose. Consequently our second experiment on the effect of Li\(^+\) on Na\(^+\) efflux was carried out with \(^{23}\)Na. Two muscles were loaded with Li\(^+\) and \(^{23}\)Na for 18 hours, while their companions were loaded with \(^{23}\)Na in normal Ringer. The isotope content of the two Ringers was approximately the same. The results of the two experiments, \(i.e.\) efflux into sucrose, and \(^{23}\)Na exchange, were, however, similar, and will be considered together.

The shape of the \(^{23}\)Na efflux curve may be seen in Fig. 2. Both the curve of
22Na efflux from normal Ringer and that from Li+-treated muscles can be described as the sum of at least three exponentials. The curves differ, however, in that the efflux of Na+ from Li+-treated muscles appears to straighten whilst the curve obtained for Na+ movement from muscles soaked in normal Ringer is not quite linear even after 6 hours' efflux. This departure from linearity, which is shown in Fig. 2, is small but it has been obtained in three experiments out of four. Consequently the analysis of this curve as the sum of three exponentials must be regarded as an approximation.

The efflux curves obtained for the loss of Na+ into sucrose resembled those obtained for the exchange studies with 22Na. Na+ efflux from Li+-treated muscles tended to follow even more the general form of the Li+ curve (Fig. 1) than did 22Na exchange from Li+-treated muscles, while Na+ movement from

<table>
<thead>
<tr>
<th>Material in Material in Material in Space as Space as per cent of per cent of per cent of total mass total mass total mass total mass</th>
<th>Half-time</th>
<th>Half-time</th>
<th>Half-time</th>
<th>Half-time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li+</td>
<td>44.4</td>
<td>100</td>
<td>7.95</td>
<td>16</td>
</tr>
<tr>
<td>22Na</td>
<td>26.6</td>
<td>6.9</td>
<td>96</td>
<td>16</td>
</tr>
<tr>
<td>22Na</td>
<td>35.4</td>
<td>4.58</td>
<td>15.7</td>
<td>16</td>
</tr>
<tr>
<td>Li+</td>
<td>30.6</td>
<td>6.72</td>
<td>112</td>
<td>20</td>
</tr>
<tr>
<td>22Na</td>
<td>38.5</td>
<td>3.40</td>
<td>76</td>
<td>18</td>
</tr>
</tbody>
</table>

untreated muscles showed a curvature similar to that shown by the isotope study. Thus one of the effects of Li+ on the Na+ efflux is a tendency to straighten the efflux curve.

The amount of Na+ leaving the muscle per unit time was less for the Li+-treated muscles than for the controls. Consequently it can be said that Li+ has slowed the Na+ efflux. If, however, it be granted that Na+ efflux can be divided into three fractions with different time constants, then an inspection of Fig. 2 will reveal that Li+ has increased the quantity of material in the slowest component. This increase has occurred chiefly at the expense of the medium component, and the rate constant for Na+ movement from the three fractions has not been significantly altered.

An estimate has been made of the amounts of material in the three components of the efflux in terms of the total mass, and these data are presented in Table IV. It must be stressed that we do not wish to equate these quantities with any compartment of the cell. The spaces given in Table IV were ob-
tained assuming curve A in Fig. 2 is the sum of three exponentials. As has been mentioned above this is not quite exact. Although the results in the table may not be absolute, nevertheless the alterations in the spaces brought about by Li+ treatment, relative to each other, are meaningful.

Listed in the left-hand column of Table IV is the total space available in the muscle to Li+ and 22Na. It is obvious that the Li+ space exceeds the Na+ space under these conditions. It was shown in the steady state section that the Li+ space is equal to or less than the Na+ space after 4 hours' treatment. A comparison was made of the Li+ and Na+ spaces at 18 hours, and it was found that they tended to be similar in magnitude, but occasionally one exceeded the other. The results were the same for any one animal, but there were differences between animals.

The total space available to 22Na is less in the Li+-treated muscles than in the controls. It was shown in the steady state section that Li+ tends to diminish the size of the extracellular space, and this would reduce the total Na+ if there were a constant intracellular level of the ion. If, however, one regards the fast component of the efflux as an index of the extracellular space (column 4 in Table IV), then the reduction in this space is too small to account for the difference in the total Na+ space. It is possible that Li+ has prevented the entry of 22Na to part of the cell, but this point must be checked in a differently designed experiment.

Thus the chief effect of Li+ on the Na+ efflux was to increase the amount of material in the slowest moving fraction, without significantly altering the rate at which it moved out of the cell. The form of the Na+ curve is similar to that found by other authors. Harris and Steinbach (9) presented evidence for a third component in the Na+ efflux from frog muscles and Troshin (26) also described this efflux as the sum of three exponentials.

The Effect of High Potassium Chloride-Ringer on Lithium Efflux

Since the movement of a cation out of the cell will be hindered by the membrane potential the partial depolarization of the membrane could be expected to facilitate Li+ efflux. High KCl levels in the Ringer have been found to depolarize the membrane (22), and to increase the membrane conductance (11). Further, the flux rates of both K+ and Na+ are increased under these conditions (4, 13).

Two pairs of muscles were equilibrated in Li-Ringer for 18 hours, then one of each pair was effluxed into Ringer containing 30 m.eq. per liter KCl in addition to the usual constituents, and the controls were effluxed into normal Ringer. We were unable to detect any change between the treated and control muscles, and the efflux curves for each pair of muscles were virtually superimposable.
The Effect of Plasma on the Rate of Lithium Efflux

It has been shown in this laboratory (unpublished data) that soaking excised muscles in toad plasma prevents the gain of Na⁺ and loss of K⁺ which is usually found during the first hour's immersion in Ringer. This confirmed the findings of Fenn (6) and Carey and Conway (3) for frog muscle. We investigated whether this stabilizing action of plasma would alter the rate of Li⁺ efflux. Muscles were equilibrated in Li-Ringer in the usual way, then one of each pair was effluxed into normal Ringer. We were unable to detect any difference between the two sets of muscles. It may well be that the changes found on soaking a muscle in Ringer are irreversible, and consequently transferring the muscle to plasma after it has been equilibrated in Ringer would be without effect. However, we were unable to influx the muscle in plasma, since the addition of Li⁺ to plasma would have rendered it hyperosmotic.

The Effect of Low Temperature on the Rate of Lithium Efflux

We have concluded from the experiments reported above that the principal rate-limiting step in the Li⁺ efflux involves the desorption of Li⁺ from some complex in the cell. If this process involves chemical reaction then it should be temperature-dependent, and we have therefore endeavoured to determine the Q₁₀ of the efflux.

Muscles were equilibrated in Li-Ringer and RISA for 3 or 18 hour periods, then one of each pair was effluxed in the cold room at 2°C., and the companion muscle was effluxed at 18°C. The half-time for the slow component of the Li⁺ efflux was longer at the lower temperature, and the RISA efflux was also retarded. There was no detectable effect on the half-time of the fast component of Li⁺ efflux.

The results, however, were erratic, and the calculation of an accurate Q₁₀ proved impossible. Since the difficulty seemed to reside in the difference in response of paired muscles we tried to determine the Q₁₀ in the one muscle by transferring it to the cold room half way through the efflux. The expected slowing took place, but we were balked here by the limitations of the method of Li⁺ assay. We have, however, calculated a mean temperature coefficient from the data to hand, which yielded a Q₁₀ of 1.8. This would seem rather low for a chemical reaction. The question will be considered at a later date in connection with the effect of metabolic inhibitors on Li⁺ efflux.

The Partition of Bromide and Iodide across the Cell Membrane

It was shown in the previous section that Li⁺ substitutes for Na⁺ in the muscle cell, and is virtually without effect on the internal levels of Cl⁻ or K⁺. Some
mutual interaction between Li⁺ and Na⁺ was seen. In this section experiments on the substitution for Cl⁻ in the Ringer with Br⁻ or I⁻ will be reported. Since the results obtained for the two ions were very similar they will be considered together.

The foreign anion was added to Ringer at levels of approximately 40 and 60 m.eq. per liter, a similar amount of Cl⁻ being omitted. One muscle of each pair was held in the test Ringer, the companion acted as the control, in the usual manner. Six pairs of muscles were used in each experiment, and the soaking time was 4 hours. The results of one experiment in which the RISA space was estimated in each muscle are shown in Table V. The justification for using for comparative purposes results obtained without an estimate of the extracellular space was made in the Li⁺ section.

1. The Na⁺ level of the cell was not affected by Br⁻ or I⁻ in any experiment.

2. Cellular Cl⁻ fell as the extracellular level was lowered, but the ratio of Cl⁻ out/Cl⁻ in remained constant. Thus the size of the Cl⁻ space was not altered by Br⁻ or I⁻.

3. Br⁻ and I⁻ entered the cells in proportion to the external level, and the ratio of Br⁻ or I⁻ out/in was similar in magnitude to the Cl⁻ ratio in the same muscle. In the experiment in which the extracellular space was estimated the absolute value of the iodide ratio may be obtained. The mean value was 6.5, the range being from 10.0 to 4.3. These values are similar to those found for the Li⁺ ratio.

4. There were occasional small K⁺ losses in muscles in the test Ringer, but these were not a constant finding, nor could they be attributed more to one anion than to the other.

5. The test Ringer produced no volume change, and all muscles responded to electrical stimulation. The contracture produced tended to be unduly prolonged (10, 16).
The Effect of the Reduction in the Ringer Level of Iodide or Bromide on the Cellular Anions

As was mentioned in the Li⁺ section, it is possible to demonstrate an apparent extrusion of Na⁺ and Cl⁻ from the muscle cell against the concentration gradient, when the tissue is transferred from a solution containing a high concentration of the substance in question to a solution containing a low level. If the Na⁺ and Cl⁻ of the muscle were entirely extracellular then the reduction in the external level would be expected to result in a proportionate decrease in the ionic assay of the muscle. If, however, the muscle contains these ions within the membrane (Tables I, II, and V), then a proportional decrease in the total assay must involve the loss of intracellular as well as extracellular material. It was seen in the Li⁺ section that the loss of Li⁺ from the muscle was not proportional to the external decrease in this type of experiment, and calculations based on the found extracellular space showed that there was no significant loss of Li⁺ from the cell.

We have repeated this experiment, by first equilibrating muscles in Ringer in which all the Cl⁻ has been substituted with Br⁻ or I⁻, and then transferring one of each pair to Ringer containing half Cl⁻, half Br⁻, or I⁻. The control muscles were analyzed at the end of the equilibration period. It was shown in these experiments that the total amount of muscle water available to the anion in question was the same in either Ringer. That is, the loss of Br⁻ or I⁻ from the cell was, within the limitations of the assay method, exactly proportional to the decrease in external level. The entry of Cl⁻ was also proportional to the external level. It would seem from this that, whatever the magnitude of the intracellular Cl⁻-Br⁻-I⁻ space, it appears to be in a diffusion equilibrium with the external medium.

Further experiments were carried out in which muscles were equilibrated in Br⁻ or I⁻ containing Ringer, and then one of each pair was transferred to normal Ringer for a further 2 hours. Here also the total amount of muscle water available to the three anions appeared to be identical, and we were unable to detect any Br⁻ or I⁻ in the muscles after their transfer to Cl⁻-Ringer.

It would appear from these studies that Br⁻ and I⁻ are interchangeable with cellular Cl⁻, and produce no significant change in the Na⁺ and K⁺ levels of the cell.

The Rate of Movement of Iodide across the Cell Membrane

\(^{131}\)I was added as the carrier-free Na salt to normal Ringer to give an activity of from 30 to 50 microcuries per ml. The rate of entry of the isotope was determined by immersing the muscle in the “hot” Ringer for increasing
time intervals, and counting the activity of the muscle between each im- 
mersion. The muscle was blotted lightly after removal from the active solution, 
as it was found that this gave more consistent results than either short washes 
in inactive Ringer or merely draining the muscle against the side of the vessel 
for a standard time. The rate of loss of $^{125}\text{I}$ was determined in a fashion similar 
to that used in the Li$^+$ efflux experiment. The isotope-loaded muscle was 
serially transferred to pots of inactive Ringer, and the activity lost into the 
Ringer was counted under the scintillation counter. Due care was taken when 
constructing standard curves of the active Ringer to duplicate the geometry 
of the counting system used in the actual experiment.

A Comparison of Influx and Efflux Rates

Influx and efflux were compared in the same muscle by following the rate of 
entry of $^{125}\text{I}$ into the muscle, and when uptake of the isotope had ceased, 
transferring it to inactive Ringer. It was found that over a total period of 6 
hours the two processes appeared to be reciprocal.

Since the late part of the influx curve cannot be determined with the same 
degree of precision as the late part of the efflux curve we have attempted a 
careful appraisal of the latter. Muscles were influxed in $^{125}\text{I}$ in the usual way, 
then the efflux was followed for a period of 6 hours. Since the loss of iodide 
from the cell is very rapid it is necessary to use high initial levels of radio-
activity in order that the late part of the curve will give readings which are 
significantly above background. We found that a minimum level of 50 micro-
curies per ml. in the influx solution using muscles weighing from 200 to 300 
mg. was necessary to ensure reasonable data. The data used in the early parts 
of the curve were calculated from the time for 4,000 counts, the later points 
were obtained from the time for 1,000 counts. The background was also timed 
for 1,000 counts at least three times during the efflux. At the end of the efflux 
the muscles were transferred to a Ringer solution containing RISA, and 
after 3 hours' equilibration the extracellular space was determined.

The shape of the efflux curve for two paired muscles determined in this 
way is shown in Fig. 3. It will be seen that paired muscles yield similar curves, 
the difference being of the order of accuracy of the counting procedure. Fur-
ther, the curve appears to be the sum of an infinite number of exponentials. 
There is no single rate-limiting step in the efflux such as would be expected 
if the membrane offered a significant barrier to diffusion. These efflux curves 
resemble those for Cl$^-$ and Br$^-$ found for frog muscle by Harris (7), but ap-
pear to be faster than he found for the loss of inactive iodide by an analytical 
procedure. Harris has assumed that his Cl$^-$ and Br$^-$ curves (Fig. 3 of his 
paper) refer to ion movement largely from the extracellular space, which 
would be a diffusion-limited process. However, by estimating the extra-
cellular space in the same muscle we have been able to show that the expected amount of isotopic iodide had penetrated the cell, yielding a ratio of external level/internal level of 8.2 (mean of four observations). This means that approximately one-third of the total $^{131}$I count was intracellular. It can be seen from Fig. 3 that 90 per cent of the total activity was lost in the first 20 minutes, which must include intracellular as well as extracellular material.

It will be obvious that this extremely rapid $I^-$ movement imposes practical difficulties in drawing up a curve. We have found that if the efflux is terminated too soon the curve may be described as the sum of two or three exponentials which cannot be related to any dimension of the muscle. To follow the efflux for the necessary time requires, as was mentioned above, the use of high activity Ringer, and if the disintegrations are counted with a conventional G.M. tube this will lead to difficulties with paralysis time corrections.

**The Effect of Change of Environment on the Rate of Iodide Efflux**

We have attempted to alter the rate of $I^-$ efflux by altering the nature of the efflux solution, as was done in the Li$^+$ section. These attempts met with singularly little success, but our failure to alter the rate is of itself significant.
The Effect of Low Temperature

After equilibration one muscle of each pair was effluxed at 18° and the companion was effluxed at 2°. The difference between the two sets of curves was so slight as to be within the error of the method.

The Effect of Time of Equilibration

The rate of loss of Li⁺ from the cell was found to be profoundly altered by the length of time the muscle was equilibrated in the Li⁺ solution. We were unable to observe any difference between efflux curves for iodide obtained after 90 minutes' equilibration, or after 18 hours in the active Ringer.

Efflux into Chloride versus Efflux into Iodide

Muscles were loaded in normal Ringer containing ¹³¹I, then one of each pair was effluxed into normal Cl⁻-Ringer, and the companion was effluxed into Ringer in which all the Cl⁻ had been substituted with I⁻. The efflux into I⁻ tended to straighten after approximately 120 minutes, while, as was seen in Fig. 3, the efflux into Cl⁻ was continuously curved. It would be difficult to ascribe any significance to this finding, since the muscles in the I⁻ solution tended to go into prolonged contracture every time they were handled. The rate of movement of ¹³¹I was the same into both solutions at times earlier than 120 minutes.

The Effect of High KCl-Ringer on Iodide Efflux

As was found in the Li⁺ section the addition of 30 m.eq. per liter KCl to normal Ringer did not alter the efflux rate for I⁻, although the high K circulation would have altered the membrane potential (which according to current concepts should have altered the passage of an anion), conductance, and respiration. Thus efflux of iodide cannot be influenced by these changes, as has been found for the Li⁺ cation.

DISCUSSION

The results arising out of the investigation reported here would indicate that as a first approximation, Li⁺ can substitute for Na⁺ in the muscle cell, and Br⁻ or I⁻ can substitute for Cl⁻. The partition of these ions across the cell membrane follows a pattern similar to that found for Na⁺ and Cl⁻ previously (23), and may be considered to support the concept of muscle as a three phase system. The differences between the behaviour of these ions and Na⁺ and
Cl\(^{-}\), and the changes in the distribution of the normal ionic constituents brought about by them are of great interest.

It is an a priori assumption that these ions will be excluded from the ordered phase. Consequently they would not be expected to displace K\(^{+}\) from this phase unless they have a toxic effect on the metabolic processes which maintain the K\(^{+}\) sites. The K\(^{+}\) losses which could be ascribed to the foreign ions were small and irregular, and could not be regarded as an ionic interchange with the free phase ions.

The only interaction of Li\(^{+}\) with the normal ions of the cell was found to occur with Na\(^{+}\), and a study of these effects may throw light on the mechanism of Na\(^{+}\) exclusion from the cell. It was noted in the steady state section that the ratio of Na\(^{+}\)\(_{\text{out}}\)/Na\(^{+}\)\(_{\text{in}}\) was consistently less in the Li\(^{+}\)-treated muscles than in the controls. It could be that part of the Na\(^{+}\) in the free phase is loosely bound and during 4 hours' soaking Li\(^{+}\) was not able to displace this "bound" Na\(^{+}\). This latter suggestion is strengthened by a comparison of the Li\(^{+}\) space and the Na\(^{+}\) space in the same muscle. It was noted that in summer toads at 4 hours' soaking the two spaces were identical, but that in winter toads the amount of muscle water available to Li\(^{+}\) was less than that for Na\(^{+}\). Spaces which were compared at 18 hours showed equality, with slight variations in either direction. It would seem that there is a competition between the two ions for adsorption sites in the free phase. Na\(^{+}\) may be more firmly bound in winter toads, and is consequently more slowly displaced by Li\(^{+}\).

It is obvious from the data in Table II, and the kinetic information, that Li\(^{+}\) is more tightly held in the cell than Na\(^{+}\). Since the main rate-limiting step in the Li\(^{+}\) efflux does not seem to be operative at the membrane, we have regarded the Li\(^{+}\) retention as being due to adsorption onto some cellular material. The rate of desorption of Li\(^{+}\) from this complex would then determine the efflux rate. If this rate is slow compared to the passage of the ion through the protoplasm then one may interpret the efflux curve as consisting of a fast component which contains extracellular Li\(^{+}\) and unbound intracellular Li\(^{+}\), and a slow component governed by a first order law which reflects the tightness of the Li\(^{+}\) binding. The variation in the rate constant of the slow component between different animals may arise from a different degree of interaction of Li\(^{+}\) with the binding site. The increasing length of efflux time with increasingly long influxes seems to indicate a slow interaction between Li\(^{+}\) and the binding site. It is likely that all binding sites in biological systems are to some extent cyclically broken down and regenerated. Thus it is impossible to tell from kinetic data whether one is dealing with a loosely bound ion, or whether the ion is very firmly held, but there is a fast turnover rate. It has been suggested that the size of the Q\(_{10}\) may help to differentiate between these two types (4). The datum we have obtained for the Q\(_{10}\) of the Li\(^{+}\) efflux is
of the same order of magnitude as that found by Harris (5) for the steady state exchange of Na⁺. This figure seems to be in between that which would be expected from either of the two situations mentioned above.

The insensitivity of the rate of Li⁺ efflux to changes in the external environment must reflect an insensitivity of the rate of desorption to exchange with Na⁺, or to changes in the membrane potential and metabolism of the cell. An investigation of the effect of changes in the ionic environment on the metabolic status of the sartorius muscle has been carried out in parallel with this work, and will be reported elsewhere (17). It may, however, be noted here that Li⁺-treated muscles do not show a significantly different rate of oxygen uptake compared with paired controls in normal Ringer, but there is a depression in the aerobic lactic acid production both over a 4 hour period in the Li⁺ solution, and over an 18 hour period. There was no difference in the level of creatine phosphate and adenosinetriphosphate between control and treated muscles over either time interval.

The failure of Li⁺ to alter the high energy phosphate bond level is in agreement with its failure to cause K⁺ loss, since we have shown that breakdown of ATP is a concomitant of K⁺ loss (1). The effect of metabolic inhibitors on the Li⁺ efflux will be dealt with at a future date, but it may be inferred from our work (1) and that of Van der Kloot (28) that Na⁺ extrusion is dependent on normal glycolysis, and this may also hold true for Li⁺. See also reference 13.

It has been shown that the amount of Na⁺ in the slow fraction of the efflux is increased by Li⁺, but the rate of movement of Na⁺ from this fraction is not significantly altered. If one envisages the intracellular Na⁺ as being in an equilibrium between a free and bound form, then the effect of Li⁺ would be to alter the position of the equilibrium in favour of the bound form. This could result from a decreased rate of turnover of the Na⁺ site. It would seem unlikely from the data in Table IV that this bound Na⁺ fraction can be regarded as deriving from the ordered phase, since the total amount of muscle water available to Na⁺ was less in the Li⁺-treated muscles than in the controls. If the increase in Na⁺ binding in these muscles were due to an increased entry of Na⁺ into the ordered phase this would imply that a greater amount of the muscle water should be accessible to Na⁺.

It may be concluded from the foregoing that Na⁺ and Li⁺ are not in a diffusion equilibrium with the external medium, but that a variable fraction is more tightly held within the cell. There may be a species difference in the amount and degree of binding of Na⁺ in the free intracellular phase, which would explain the different results obtained with different amphibia. Harris (5) has noted that the sum of intracellular Na⁺ and K⁺ tends to be constant in the frog. We have been notably unable to confirm this for *Bufo marinus* (23). If part of the cell Na⁺ is, as it were, removed from solution, this could explain the difference in our findings.
In measuring the rate of loss of isotopic Na\(^+\) from heart Johnson (12) has also been unable to equate the amount of Na\(^+\) in the extracellular space (measured with sucrose) with the amount of material in the fast component of the efflux. A mechanism similar to that which we find in the sartorius may be operative.

It has been suggested (14) that ions such as Na\(^+\) may be accumulated in the extracellular space by a process such as binding onto connective tissue fibers. In steady state studies when the intracellular concentration is calculated from the observed extracellular space on the assumption that the ions in this phase are at the same concentration as in the external medium, this would lead to an erroneous overestimation of the intracellular level. We have at present no evidence to either refute or confirm this. Ions which are bound in the extracellular phase would tend to move out of this phase more slowly than free ions, and so would be identified kinetically with the slower fractions of the efflux. It would therefore seem unlikely that a mechanism such as this is responsible for our finding that the RISA space cannot be identified with the apparent extracellular space calculated from the fast component of the efflux.

Our finding that Li\(^+\) is a tolerable Na\(^+\) substitute in muscle confirms the results of other workers using different tissues. Zerahn (29) found that Li\(^+\) substitutes for Na\(^+\) in producing a short circuit current in the isolated frog skin, and he showed that the ion is transported in the skin, in a manner similar to Na\(^+\). However, pure Li-Ringer caused a deterioration of the skin (27), and Li\(^+\) tends to be retained in the skin to a greater extent than Na\(^+\).

A number of workers have studied the effect of Li\(^+\) on the red blood cell. For bibliography see reference 25. It would appear that Li\(^+\) competes with Na\(^+\) on equal terms in influx experiments, and the substitution of Li\(^+\) for Na\(^+\) in the Ringer results in a proportionate lowering of the intracellular Na\(^+\). No evidence has, however, been presented to indicate that Li\(^+\) can be expelled from the red blood cell.

Li\(^+\) also appears to be a Na\(^+\) substitute in maintaining the resting potential in nerve. For bibliography see reference 19.

The distribution of Li\(^+\) which we have found in this type of acute experiment using an isolated muscle preparation does not give a true indication of Li\(^+\) distribution in the chronically treated animal. We have substituted half of the external Na\(^+\) with Li\(^+\) without producing any gross deleterious affect on the muscle. Plasma levels above 4 m.eq. per liter appear to be toxic for the intact animal. Further, over a period of 3 to 4 days' treatment Li\(^+\) penetrates all the cell water of the red blood cell, although in vitro experiments indicate that at up to approximately 18 hours it enters only a small part of the red cell.

It would appear from the experiments listed in the second part of this
paper that Br⁻ and I⁻ may be considered to be virtually interchangeable with Cl⁻ in the muscle cell. The pattern of the results conforms closely with that reported previously for Cl⁻ (23), and indicates that the distribution of these anions between the free intracellular phase and the external medium comes near to the postulated condition of a diffusion equilibrium. The lack of effect of the foreign anions on the normal cations of the cell confirms the findings of Edwards et al. (5) for frog muscle, and is in distinction to the profound effect on mechanical response and the electrical properties of the membrane (5, 10, 16).

Before considering the kinetic data for I⁻ we must point out that we have purposely avoided using the method of loading the muscle in the tracer I⁻ in a high KCl-Ringer, as Harris did. We have confirmed his finding that muscles loaded in this way have an efflux curve shaped differently from those loaded in normal Ringer, but feel that these effluxes, which involve a net loss of both cation and anion from the cell, follow a different law from the steady state exchanges reported here. The movement of isotopic I⁻ out of the cell appears to be a diffusion-limited rather than a membrane-limited process. Movement across the membrane is fast, and is insensitive to change in temperature and a variety of environmental differences. It is doubtful whether the tailing of the efflux curve can be ascribed to the binding of a small fraction of the internal I⁻, and it may be that this fraction is hindered in its movement out of the cell by having to pass through a maze of fixed positive charges.

Harris (8, 9) has proposed what is in effect a three phase system for muscle, which seems very similar to our hypothesis. He has discussed the possibility that the bioelectric potential may be the potential difference between the inner K-rich region of the cell and the solution. Unless one takes some such view as this it is difficult to reconcile the very fast movement of Na⁺ and I⁻ (and from Harris’ data also of Cl⁻) across membrane with the relative impermeability for these ions required by the Goldman equation. We have never regarded the free intracellular phase as an annular region outside the ordered phase, nor would this be a necessary concomitant of Harris’ suggestion. Ling (15) has postulated that the potential may be closely related to that of the glass electrode, rather than to that of a Donnan membrane system. The cell, as a charged conductor, is devoid of electric field in the interior. One may therefore envisage an intermingling of the free and ordered phases without invalidating Harris’ suggestion as to the seat of the potential.

In conclusion one may say that Br⁻ and I⁻ appear to be interchangeable with cellular Cl⁻, and so support the three phase theory in its original simple form. The finding that Li⁺ and Na⁺ are to some extent bound in the free intracellular phase invalidates the assumption that all ions in this phase are in a diffusion equilibrium with the external medium. It is therefore
necessary to postulate that this region is not entirely randomized, and that some interaction between the ions and the cellular constituents may occur. The matter will be discussed further in another paper when data obtained for the partition of divalent ions will be presented.

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