THE EFFECT OF TEMPERATURE ON POTASSIUM EQUILIBRIA IN CHICK EMBRYO MUSCLE*

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Cohn and Brues (2) have described a technique employing radioactive isotopes by means of which the rate of exchange of potassium and phosphate ions between the intra- and extracellular phases of tissue cultures could be measured. In the present paper, this technique is employed to study rates of exchange of potassium in chick embryo muscle cultures at different temperatures, and also to study the effect of lowering temperature on the total amount of potassium contained within the cells of cultures.

Methods and Calculations

Minced leg muscle taken from chick embryos of 9 to 11 days’ incubation was explanted in a thin plasma clot on the window of a roller bottle, as described by Shaw, Kingsland, and Brues (3). The medium consisted of 4 ml. of modified Baker’s peptone medium (4). The explant was allowed to incubate overnight at 37°C. to permit injured cells to repair or disintegrate. At the start of an experiment, the medium was exchanged for one containing radioactive potassium (K⁴⁺), but otherwise of identical composition. Radioactivity in the culture was measured at 15 to 60 minute intervals by means of a Lauritsen electroscope or Geiger-Muller counter placed opposite the window of the culture bottle. The direct measurements of radioactivity external to the window were used to determine the actual amount of K⁴⁺ in the cultured cells during the course of the experiment, by a method described previously (2). At the end of the experiment, the medium was withdrawn and, when necessary, activity measurements were made and total potassium determined on the medium. When tissue analyses were desired, the tissue was washed twice in situ by gentle rocking with 0.85 per cent saline solution for 15 seconds, then removed, ashed with nitric acid, and analyzed for radioactivity and potassium content. Total potassium was determined according to the method of Shohl and Bennett (5) as modified by Hald (6). From these final analyses, the relative specific activity (SA) of the tissue mass at the termination of the experiment was determined, and its specific activity during the course of the experiment was calculated from the readings. Determinations of the rate of radioactive potassium uptake were made at 37°, 26°, 15°, 10°, and 5°.

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In order to study the effect of lowering temperature on total cell potassium, cultures were first allowed to reach an approximate radioactive equilibrium with the medium at 37°. Subsequent losses or gains in activity as the culture is shifted to a lower temperature and returned to 37° represent mass movement of potassium out of or into the cells. Cultures at equilibrium at 37° were lowered to 26°, 15°, 10°, and 5°; readings were continued over a period of hours at the lower temperature and while they were subsequently returned to 37°.

Throughout the experiments, the relative specific activity (SA) of tissue is expressed as a per cent of the specific activity of the entire system of culture plus medium, so that, using cultures of approximately equal mass, all results can be expressed on the same scale. From calculations in the previous paper (2),

\[ SA = \frac{100 \cdot S_t}{S}, \]

where \( S_t = \frac{K^t}{K} \) in cells, and \( S = \frac{K^t}{K} \) in the entire system.

Since it was shown previously that within the limits of experimental error (about 10 per cent), all the cell potassium is exchangeable at 37°, a further simplification is possible. Analyses of tissue and medium have shown that, under the conditions employed, this exchange is virtually complete (\( SA = 96 \)) at about 9 hours, and 85 per cent complete (\( SA = 85 \)) at 5 hours. Subsequent uptake curves could then be fitted by setting equal to 85 per cent the level of activity attained at 5 hours at 37°. Similarly, at the end of experiments at low temperatures (15°, 10°, 5°), the cultures were warmed to 37° for several hours in order to establish a reference level of essentially complete exchange.

RESULTS

The loss of potassium from tissue on changing cultures from 37° to 10° or to 5° is illustrated in Fig. 1. Cultures which have reached radioactive equilibrium at 37° show no apparent loss of activity when lowered to 15°, but show a loss of about 12 per cent when lowered to 10° and a 36 per cent loss when lowered to 5°. This loss can be explained only on the basis of a mass shift of potassium from cells to medium at the lower temperatures. The lost activity is rapidly regained on restoring the cultures to 37°, indicating that potassium has reentered the cells. None of the cultures were followed longer than 9 to 11 hours at low temperatures.

Uptake curves of radioactive potassium by the tissue cultures at 26°, 15°, 10°, and 5° are drawn in Fig. 2. All curves are drawn from pooled data. Because of the method of attainment of equilibrium, that is by warming the cultures to 37° at the end of each experiment, the curves at 10° and 5° are given in terms of the amount of potassium present in the cells at 37° rather than in terms of the
Fig. 1. Cold loss at 10° and 5° and recovery on restoration to 37°, from pooled data. Cultures first brought to approximate equilibrium with radioactive potassium at 37°. Loss at 10°, (●); loss at 5°, (○).
Fig. 2. Uptake of radioactive potassium by chick embryo muscle at 26°, 15°, 10°, 5°, drawn from pooled data. 26° (○); 15° (●); 10° (○); 5° (○). The curves at 10° and 5° are given in terms of the amount of potassium present in the cells at 37°.
Fig. 3. Uptake of radioactive potassium at 37°, drawn from pooled data. Broken lines represent uptake curves at 10° and 5° after correction has been made for cold loss.
amounts present within the cells at the lower temperatures as estimated from the data on cold loss (Fig. 1). If correction is made for estimated cold loss so that the uptake curves reflect the approximate rate of attainment of equilibrium of the potassium which is actually present in the cell at the lower temperature,

![Figure 4](image)

Fig. 4. Logarithmic plots of the uptake curves at 37°, 15°, 10°, 5°. Plots at 10° and 5° are drawn from the curves in Fig. 3. The linear portion is extrapolated to \( t = 0 \) (broken line).

the curves in Fig. 3 (broken line) are obtained. The uptake curves at 15° and at 37° drawn from pooled data are included in Fig. 3 for comparison. No significant difference was found between the rates of uptake at 37° and 26°.

The potassium uptake curves illustrated in Fig. 3 are plotted semilogarithmically in Fig. 4. All curves are angulated, showing an initial portion with a large slope and a later portion with a smaller slope.

In a system in which an ion behaves as a single molecular species, the uptake
of an introduced radioactive tracer should behave according to Fick's equation, which in this case takes the form (2):

\[ \text{SA} = 100 \left(1 - e^{-k \cdot \frac{P}{P_s} \cdot t}\right) \]

where \( k \) = turnover rate, or proportion of cell potassium exchanged per hour,

\( t = \) time, in hours, and

\( \frac{P}{P_s} = \) ratio of total potassium to that in the medium, which, as determined under the present experimental conditions, is approximately 1.15.

The factor \( k \cdot \frac{P}{P_s} \) can be obtained from experimental results by plotting \( \ln(100 - \text{SA}) \) against time. If Fick's equation describes the data, this semilogarithmic plot should be a straight line, with \( k \cdot \frac{P}{P_s} \) equal to the negative slope. Our data as plotted in Fig. 4, do not, however, yield such a straight line. This suggests that two potassium components are involved, one of which (\( P_d \)) turns over much more rapidly than the other (\( P_s \)).

Two potassium components may be related in either of two ways: (a) \( P_d \) and \( P_s \) may be simultaneously in equilibrium with \( P_t \); or (b) only \( P_d \) may be in equilibrium with \( P_t \), while \( P_s \) is in equilibrium with \( P_a \). Case (b) is more complicated, and the mathematical equations which describe it have been calculated for phosphate (2), in which case the components can be estimated by chemical analysis, and where there is good reason to believe that the conditions are correctly described by it. It has been assumed here that the two hypothetical states for potassium are described by case (a), although there is no reason to believe a priori that this should be the case. In any event, direct measurement of specific fractions of cell potassium is not possible at present and calculations under case (b) can therefore not be made. Moreover, theoretical uptake curves according to either case (a) or case (b) may so closely resemble one another that either could fit the present data.

Assuming that exchange between \( P_d \) and \( P_s \) can be neglected as a rate-determining factor, a system containing two potassium fractions exchanging with the medium may be described by the following equation:

\[ \text{SA} = 100 \left(1 - \frac{P_d}{P_t} \cdot e^{-1.15 k t} - \frac{P_s}{P_t} \cdot e^{-1.15 k t}\right) \quad (1) \]

where \( k_1 \) = turnover rate of the "fast" fraction, \( P_d \),

\( k_2 \) = turnover rate of the "slow" fraction, \( P_s \),

\( P_t = P_d + P_s \).

\( \frac{P}{P_s} \) has been taken as 1.15, which is the approximate mean value under the experimental conditions. When \( k_1 \) is much greater than \( k_2 \), and \( t \) is large, the first exponential term of equation (1) may be ignored, so that:

\[ \ln(100 - \text{SA}) = \ln \left(\frac{P_s}{P_t}\right) - 1.15 k_2 \cdot t \quad (2) \]
From the graph of \( \ln (100 - SA) \) against time (Fig. 4), the slope of the later portion of the curve equals \(-1.15 k_2\), and the extrapolated intercept at \( t = 0 \) is equal to \( \ln \frac{P_n}{P_i} \), whence the magnitudes of \( P_n \) and \( P_d \) may be derived. Differentiating (1), the slope of the curve at \( t = 0 \) is given by:
\[
\frac{dSA}{dt} (t = 0) = 1.15 \left( \frac{P_d}{P_i} \cdot k_2 - \frac{P_n}{P_i} \cdot k_1 \right)
\]

Since \( P_d, P_n, \) and \( k_2 \) are known, and \( \frac{dSA}{dt} \) may be estimated graphically, \( k_1 \) can be calculated.

### Table I

**Per Cent of Tissue Potassium in the Slow \((P_n)\) and Fast \((P_d)\) Fractions and Their Estimated Turnover Rates at Various Temperatures**

Values are calculated from Fig. 4 and Equation 3.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>No. of cultures</th>
<th>( P_n )</th>
<th>( P_d )</th>
<th>( k_2 )</th>
<th>( k_1 )</th>
<th>No. of cultures</th>
<th>Per cent lost in cooling from 37°</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>16</td>
<td>84</td>
<td>16</td>
<td>0.29</td>
<td>1.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>81</td>
<td>19</td>
<td>0.11</td>
<td>2.0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>78</td>
<td>22</td>
<td>0.075</td>
<td>1.2</td>
<td>9</td>
<td>11.5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>85</td>
<td>15</td>
<td>0.026</td>
<td>1.1</td>
<td>9</td>
<td>36.0</td>
</tr>
</tbody>
</table>

\( P_n = \) slow fraction, per cent of total.  
\( P_d = \) fast fraction, per cent of total.  
\( k_2 = \) turnover rate of the slow fraction per hour.  
\( k_1 = \) turnover rate of the fast fraction per hour.

The size and rate constants of the fast and slow fractions as determined from the curves in Fig. 4 and calculated from Equation 3 are summarized in Table I. The rate constant of the fast fraction appears to change little with temperature whereas the rate of the slow fraction increases by a factor of 10 or more between 5° and 37°.

**Discussion**

The data presented in this paper strongly support two conclusions: that potassium is lost from cells of chick embryo muscle on cooling; and that the rate of exchange of cell potassium has a large temperature coefficient. Quantitative values, the order of accuracy of which is still uncertain, can be given for both types of potassium movement. On the other hand, the interpretation of the observed deviation of the radioactivity uptake curves from the simple form of
Fick's equation is less clear. The following possible interpretations may be considered: (1) the initial rapid exchange represents an untraced experimental error; (2) the uptake curves represent exchange with two phases of potassium ion, differing in their rate of exchange with the radioactive isotope present in the medium. With regard to the first possibility, there is little that can be said. Contaminating sodium was probably eliminated by two reprecipitations of the radioactive potassium chloride from concentrated sodium perchlorate solution. The analysis of the data according to the assumption that two different potassium fractions exist in the tissue cultures has been presented above. Although the existence or the location of cell membranes is not essential to this analysis, the bulk of the tissue potassium and probably all the slow fraction are intracellular. Part of the fast fraction is probably extracellular, representing incomplete exchange of the potassium of the interstitial fluid with that of the medium in the bottle. Until uncertainties regarding the existence and nature of the fast fraction can be clarified, further conclusions will be hazardous.

There is little information in the literature on the temperature coefficient of the rate of uptake of radioactive potassium, which the present study shows to be quite large. Overstreet and Broyer (7) using radioactive potassium calculated that 10 per cent of the potassium in their "low salt" barley roots was exchangeable in 3 hours at 0°C. This was compared with a rapid and complete exchange at higher temperatures. In this respect potassium is analogous to phosphate, which penetrates erythrocytes little or not at all at 7°, but enters rapidly at higher temperatures (8). Cardiac muscle also shows a large temperature coefficient for the uptake of phosphate (9), and the movements of this ion are in general believed to be closely related to cellular metabolism (10).

In the case of phosphorus, many compounds are known whose turnover is linked with cell metabolism. Little is known, however, concerning the existence or nature of intracellular potassium compounds.

It is possible that the loss of potassium from cells at low temperature is a general phenomenon. Several investigators have shown that erythrocytes lose potassium to plasma when stored at 4-7°C. (11-14). The loss is independent of the previous history of the blood, and amounts to about 30 per cent of the cell potassium in a few days (11, 12). The lost potassium is partially regained on rewarming the cells. Conway and his coworkers (15) report that frog muscle loses 50 per cent of its potassium at 3° although they do not state how rapidly this loss takes place. Krogh (16), however, reports that although the chick chorionic membranes which he studied lost potassium at 3-7°, the calculated rate of loss was very slow, being equal to or less than 1 microequivalent per gram of wet membrane per hour. Here also potassium and phosphate behave in a similar manner, for Halpern (17) reports that phosphate is lost from erythrocytes at 3°C. and reenters the cells at 37°.
Thus the intracellular potassium of tissue cultures, like phosphate ion, has a turnover rate which is highly sensitive to temperature changes. It exchanges very slowly with the potassium of the medium at 5°, and rapidly at 37°. A considerable portion of it is no longer held in the cell at reduced temperature, but the loss is not proportional to the temperature, for none appears to be lost at or above 15°. Further, there is evidence that it may be divided into two fractions: the larger fraction having a high temperature coefficient and a slow turnover rate, the smaller having a low temperature coefficient and a more rapid turnover rate. Brooks (18), working with paramecia, found similarly that radioactive potassium exchanged most rapidly at the beginning of an experiment. He felt that this was due to "successive gains or losses of K⁺" during the experiment. Nevertheless, available data are equally consistent with the possibility that we are dealing with a system in which the potassium is present in the cell in two different chemical states.

Two opposing theories concerning the nature of the concentration of potassium ion within cells are widely held at the present time. One of these is the theory that intracellular potassium is largely "bound," the other is that the intracellular potassium is "free" but that losses due to outward diffusion of intracellular potassium are repaired by a constant active transport of the ion inward again by the cell. Neither theory is yet wholly satisfactory.

The concept of intracellular binding of potassium has appeared in both the American and European literature for many years, and has been restated recently (19–22). According to these authors, a large fraction if not most of the intracellular potassium should be considered as being present in chemical combination with non-diffusible organic elements of the cell, or more generally, has a very low apparent activity coefficient. A relatively small portion is considered to be freely ionized, or with an activity coefficient approaching that in a simple aqueous solution of the same concentration. The "bound" potassium is in equilibrium with the "free" form, explaining the ready exchangeability with radioactive isotope. This theory ignores the difficulty of conceiving the nature of the osmotic equilibrium of animal cells between protoplasm and environment when the bulk of the potentially osmotically active intracellular substance is rendered inactive by binding. Our data accord well with the "bound" potassium hypothesis, and, considered in toto, seem difficult to explain in any other way. At 5°, the membrane is freely permeable, as is evident from the continued outward mass movement of potassium across the cell membrane for several hours after the cell has been chilled. Nevertheless, 50 per cent of saturation with radioactive potassium has not been reached in 12 hours. One could explain this either by postulating the existence of a group of intracellular organic potassium compounds whose dissociation constants fall rapidly with temperature, or by a decline in the rate of metabolic turnover of certain organic potas-
sium compounds. It may be supposed further that some of these organic potassium compounds require metabolic activity to maintain their stability, and hence are degraded at low temperatures, resulting in loss of intracellular potassium.

The alternative viewpoint has recently been expressed by Krogh (23) and Hald et al. (24). They consider that the potassium within cells is free, and that its concentration there is not an equilibrium but a "steady state." The cell must continually expend energy in order to transport potassium to replace that lost by outward diffusion. Similarly the cell is kept relatively free from sodium by an outward transport of that ion. The picture has been simplified by Dean (25) who shows that, theoretically, an outward transport of sodium alone can account for potassium accumulation. The observation that cooling produces a loss of cell potassium accords well with this theory, since it may be presumed that at lower temperatures the depressed cell metabolism no longer supplies sufficient energy to continue potassium or sodium transport. The low rate of exchange of added isotope with the remaining cell potassium at low temperatures is more difficult to explain. One must assume that the membrane becomes less permeable at low temperatures or else that the barrier to diffusion which it offers has a high energy coefficient. Either possibility is difficult to reconcile with the observed rapidity of outward movement during cold loss. Also difficult to reconcile is the non-linear logarithmic slope of the radioactivity uptake curve, suggestive of the possible existence of two intracellular potassium fractions with different rates of exchange.

SUMMARY

The effect of temperature upon the exchange rates between intra- and extracellular potassium in chick embryo muscle was determined by the use of radioactive potassium. The temperature coefficient of at least four-fifths of the cell potassium is large. At temperatures below 15°C., potassium is lost from the cell and is regained on warming. The results suggest the possibility that 20 per cent or less of the cell potassium may differ from the rest by being more rapidly exchangeable with the medium.

BIBLIOGRAPHY

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