Diffusion and Osmotic Transfer in Corn Mitochondria

A. R. Overman, G. H. Lorimer, and Raymond J. Miller
Department of Agronomy, University of Illinois, Urbana, Illinois 61801

ABSTRACT

An equation based on the assumption of solute diffusion into an expanding sink is derived and describes the passive swelling of corn shoot (Zea mays L.) mitochondria. The experimental data show that corn mitochondria not only swell due to diffusive forces but also contract according to osmotic phenomena, provided sufficient impermeant solute is included to prevent lysis.

The behavior of isolated animal mitochondria as osmometers has been described by Tedeschi and Harris (14) and, more recently, by Bentzel and Solomon (1). Lorimer and Miller (6) have demonstrated that mitochondria from etiolated corn shoots behave similarly. This suggests that passive swelling and contraction may be described by dynamic osmosmetry (4). Observations on volume changes over short time periods in animal mitochondria (13, 15) appear to be consistent with this concept. Likewise, osmotically induced volume changes in sea urchin eggs are related to Staverman reflection coefficients for various solutes (5).

Plant mitochondria undergo a respiration-independent "large amplitude" swelling in electrolyte solutions (12). This process may take as long as 20 min to reach an equilibrium volume and may be reversed by the addition of an oxidizable substrate such as NADH or ATP (12). Plant mitochondria also undergo a similar swelling when transferred from a sucrose medium to isomolar solutions of monosaccharide sugars such as glucose and ribose (6). However, this cannot be reversed by the institution of respiration or upon the addition of ATP (Lorimer and Miller, unpublished results). This long-time swelling is not predicted by the equations presently in the literature (4).

It has been suggested (2, 9, 10, 12) that the respiration-independent swelling of mitochondria in solutions of electrolytes represents the dissipation of a high energy bond associated with the relaxation of a contractile mechanism regulating mitochondrial volume. The ATP or respiration-dependent contraction is considered to be a reversal of this process.

An alternative viewpoint is that the changes in the volume of the mitochondria merely reflect osmotic adjustments that occur as a result of the movement of solutes (6).

In an attempt to resolve this question, and to obtain equations that would account for the long-time swelling, equations were derived based on the assumption of solute diffusion into an expanding sink or out of a contracting source, which describe respiration-independent movements of water into and out of corn shoot mitochondria.

THEORY

The existence of two compartments in rat liver mitochondria, the sucrose-accessible space and the sucrose-inaccessible space, has been demonstrated (1, 8). The former was equated with the volume between the inner and outer membranes and the latter with the volume enclosed by the inner membrane. Measurements of the ratio of the sucrose-accessible space to the total volume of the mitochondria range in value from 0.65 to 0.80 (1, 8). Measurements with plant mitochondria fall within this range (6). The sucrose-accessible space was found to increase as the volume of the mitochondria decreased in response to increasing external osmotic pressure (8). These data are consistent with the events diagrammatically represented in Figure 1. Electron micrographs of animal mitochondria fixed in various concentrations of osmoticant support this interpretation (3, 11, 16) as do micrographs of corn shoot mitochondria fixed before and after swelling in KCl (12).

Assuming the validity of this interpretation, then (a) sucrose acts as an osmoticant only for the inner compartment, the outer membrane being permeable to sucrose, and (b) the surface area of the inner membrane remains constant during swelling and contraction, since it folds and unfolds rather than stretching.

Water movement across mitochondrial membranes in response to changes in the osmolarity of the external medium occurs in a matter of seconds, whereas passive solute transfer occurs on a time scale of minutes (14). Thus, the system maintains a state of virtual osmotic equilibrium, described by an osmotic equation of state. Solute transport is described by a rate equation, Fick's law of diffusion. Combination of these relations provides correlations between changes within the mitochondria of solute concentration and volume as a function of time.

Since the outer membrane is permeable to sucrose, the model developed below treats the mitochondrion effectively as if it were bounded by a single membrane, although, from the experimental standpoint, this approach has certain shortcomings (6).

The osmotic equation of state using the Van't Hoff approximation may be written as

\[ C_o + C_1 = C_i + \frac{S}{V} \]  

(1)

where \( C_o \) and \( C_1 \) are the concentrations of permeant solute outside and inside respectively; \( C_i \) is the concentration of the external impermeant solute; \( S \) is the quantity of internal impermeant solute; and \( V \), the internal solution volume. Both \( C_o \) and \( C_1 \) are treated as constants, since there is a large excess of bathing solution.

In neutral permeant solutes, the final equilibrium state of the system \( (C_i \rightarrow C_o) \) depends upon the value of \( C_o \). Since mitochondria behave as osmometers, the final equilibrium volume also depends upon the value of \( C_o \). It has been demonstrated that this is indeed the case (6).
For the rate equation we employ Fick's law (difference form) and mass balance (without chemical reaction) obtaining for the rate of solute transport

\[
\frac{DA}{l} (C_o - C_i) = \frac{d(VC_i)}{dt}
\]  

(2)

where \( D \) is the membrane diffusion coefficient, \( A \) the membrane surface area, \( l \) the membrane thickness, and \( t \) is time. We assume that \( DA/l \) is constant, the area being treated as a constant for the reasons given above. It is apparent that net diffusion will cease when \( C_i = C_o \).

Combination of equations 1 and 2 yields

\[
\frac{DA}{l} \left( \frac{S - C_i}{V} \right) = (C_o + C_i) \frac{dV}{dt}
\]  

(3)

As \( t \rightarrow \infty \), then \( C_i \rightarrow C_o \) and \( V \rightarrow V_e \), so that \( C_e = S/V_e \) from equation 1. For the initially contracted state, \( C_i = 0 \) and \( V = V_s \), so that \( C_o + C_s = S/V_s \), from equation 1. With these equalities, equation 3 becomes, after rearrangement,

\[
\frac{dV}{dt} = \frac{DA V_o}{l} \left( \frac{V_s}{V_e} \right)^{1/2} \left( \frac{V - V_s}{V_s} \right)
\]  

(4)

Integration of equation 4 yields

\[
\frac{V}{V_e} + \ln \left( \frac{1 - \frac{V}{V_s}}{\frac{V}{V_s}} \right) = \frac{DA V_o}{l} \left( \frac{V_s}{V_e} \right)^{1/2} t + I
\]  

(5)

where \( I \) is the constant of integration. Using the initial condition \( V = V' \) at \( t = t' \), we have

\[
\frac{V - V'}{V_s} + \ln \left( \frac{V_s - V}{V_s - V'} \right) = \frac{DA V_o}{l} \left( \frac{V_s}{V_e} \right)^{1/2} (t - t')
\]  

(6)

or, in equivalent form

\[
-\frac{\Delta V_e}{V_e} \frac{\Delta V}{\Delta V_e} - \ln \left( 1 - \frac{\Delta V}{\Delta V_e} \right) = k \Delta t
\]  

(7)

\( k \) is a constant of proportionality.

**Fig. 1.** Schematic representation of the probable events occurring during osmotic swelling of a mitochondrion. \( P \): Osmotic pressure; \( V_{sa} \): sucrose-accessible volume; \( V_{si} \): sucrose-inaccessible volume.

\( V_{sa1} > V_{sa2} \)

\( V_{si1} < V_{si2} \)

**Fig. 2.** Typical plots of equations 7 and 8. Curves for \( V_s/V_e = \frac{3}{4} \) and \( \frac{1}{2} \) correspond to expansion, those with \( V_s/V_e = 2/1 \) and \( 4/1 \) represent contraction.
Fig. 3. The time course for the swelling (Δ%T) of mitochondria suspended in 0.350 M ribose, 0.05 M sucrose, 0.02 M tris-HCl.

Fig. 4. The plot of (1 - ΔV/ΔVe) against time for mitochondria swollen in 0.350 M ribose and 0.05 M sucrose. The circles and the solid line represent the experimental data and the predicted response, respectively. The extrapolation to zero time yields an intercept value of $e^{-\Delta V/\Delta Ve}$ equal to 0.60. Hence, $\Delta V_e/\Delta Ve$ equals 0.51. The rate constant, $k$, computed from the slope of the linear portion of the plot has a value of 0.22 min$^{-1}$. 
with the definitions, \( \Delta V = V - V', \Delta V_e = V_e - V', \Delta t = t - t' \) and \( k_e = D A V_e / V_e^2 \). Equation 7 applies to the swelling process since the condition \( C = C_e \) has been used.

Contraction can be treated in an analogous manner. For the case where \( C_e = 0 \), noting that \( C_e \to 0 \) as \( t \to \infty \), we obtain for the contraction process

\[
\frac{-\Delta V_e}{\Delta V_e} - \ln \left( 1 - \frac{\Delta V_e}{\Delta V_e} \right) = k_e \Delta t
\]

where \( \bar{V} \) is used to denote the contracting volume, and \( k_e = D A / \bar{V}^2 \). Here \( \Delta V_e \) will assume a negative value, since \( \bar{V}_e < \bar{V}' \) for contraction.

It will be noted that the equations contain no term for the elasticity of the membrane. This was omitted as its contribution is considered to be negligible (1).

The utility of equations 7 and 8 rests in the fact that only relative volume changes need be measured with time, rather than absolute volumes. Furthermore, the choice of a reference time is not critical for the subsequent analysis. These factors are of primary importance with regard to data acquisition, where we record changes in the optical transmission with time. A short period (approximately 1 min) is required for optical adjustment of the mitochondrial suspension within the spectrophotometer; i.e., the initial segment of the optical trace must be discarded in the analysis.

Examination of equations 7 and 8 reveals that at long times (i.e., as the equilibrium volume is approached), the term \( \Delta V / \Delta V_e \) approaches unity. Then the equation reduces to

\[
\frac{-\Delta V_e}{\Delta V_e} - \ln \left( 1 - \frac{\Delta V_e}{\Delta V_e} \right) = k_e \Delta t
\]

which corresponds to the linear portion of the semilogarithmic plot. Extrapolation of the linear portion of the plot to zero time thus yields an exponential value for \( \Delta V_e / \Delta V_e \). Since \( \Delta V_e / \Delta V_e = 1 - V' / V_e \), a value for the relative change in volume \( V' / V_e \) can be determined. Figure 2 shows typical plots for equations 7 and 8.

An explicit relation between the rates of contraction and of swelling may be obtained for the case where the final volume of contraction and the initial volume of expansion are the same \( (\bar{V}_e = V_e) \). If the time constant is defined as \( \tau = 1/k_e \), then the ratio for the time constant for contraction, \( \tau_c \), to that for swelling, \( \tau_s \), is given by

\[
\frac{\tau_s}{\tau_c} = k_e \frac{V_e}{\bar{V}_e} = \frac{DA}{V_e^2} \left( \frac{\bar{V}_e}{\bar{V}} \right)^2
\]

where \( V_e \) and \( \bar{V}_e \) are the contracted and expanded volumes, respectively. It follows that the contraction process proceeds more rapidly than swelling, since for swelling \( V_e > \bar{V}_e \), whereas for contraction \( V_e > \bar{V}_e \). That is, in contraction diffusion occurs from a contracting volume, concentrating the internal solutes; in swelling diffusion occurs into an expanding volume, diluting the internal solutes.

**MATERIALS AND METHODS**

Mitochondria were isolated by a procedure similar to that of Stoner and Hanson (12). All procedures were conducted between 0 and 4°C. Approximately 100 g, fresh weight, of etiolated 3-day-old corn shoots (Zea mays L., WF9 × M14) were ground in an ice-cold mortar with 25 ml of 0.4 M sucrose; 0.02 M tris-HCl, pH 7.5; and 0.005 M EDTA. The slurry was filtered through cheesecloth, and the filtrate was centrifuged at 1,000 g for 10 min to remove cell debris. The supernatant was centrifuged at 10,000 g for 10 min. The pellet was resuspended with 0.4 M sucrose in 0.02 M tris-HCl, pH 7.5, and the suspension further centrifuged at 10,000 g for 10 min. The supernatant was discarded and the pellet was resuspended with 2 ml of 0.4 M sucrose in 0.02 M tris-HCl, pH 7.5, (about 10 mg of protein/ml), and the suspension was stored on ice.

This procedure is essentially the same as that used by Stoner and Hanson (12) working with identical tissue, except that the 0.05 M malate used by these workers was omitted in the present work. Electron micrographs of their preparation revealed that there was little contamination with other cell organelles and that the outer membranes of the mitochondria were intact. Our preparation gave values for the respiratory control ratio greater than 1.7, sometimes as high as 3.0, suggesting that the integrity of the mitochondria had been preserved.

The gravimetric and optical methods for determining mitochondrial volume have previously been described (6). A linear relationship exists between the volume of mitochondria as determined gravimetrically with centrifuged pellets, and the change in percentage transmittance at 520 m,u induced by altering the concentration of sucrose. Earnshaw (personal communication) has found a similar relationship for volume changes of bean hypocotyl mitochondria, induced by suspension of the mitochondria in solutions of KCl.

All experiments were performed at 25°C in solutions buffered with 0.02 M tris-HCl, pH 7.5. Protein was determined by the procedure of Lowry et al. (7), using bovine serum albumin standards. Additional experimental information is provided in the text and in the figure captions.

**RESULTS**

**Swelling and Contraction in Isotonic Solutions.** In order to perform an analysis of the volume changes undergone by mitochon-
Fig. 6. The plot of $(1 - \Delta V/\Delta V_0)$ against time for mitochondria, previously swollen in 0.350 M ribose + 0.05 M sucrose, contracted in 0.4 M sucrose, and resuspended in 0.350 M ribose + 0.05 M sucrose. The circles and the solid line represent the experimental data and the predicted response, respectively. The extrapolation to zero time yields an intercept value of $e^{-\Delta V_e/\Delta V_e}$ equal to 0.60. Hence, $\Delta V_e/\Delta V_e$ equals 0.51. The rate constant, $k$, computed from the linear portion of the plot has a value of 0.27 min⁻¹.

Fig. 7. The plot $(1 - \Delta V/\Delta V_0)$ against time for mitochondria swollen in 0.22 M KCl, 0.05 M sucrose, pH 6.9. The circles and solid line represent the experimental data and the predicted values, respectively. The extrapolation yields an intercept of $e^{-\Delta V_e/\Delta V_e}$ equal to 0.75. Hence, $\Delta V_e/\Delta V_e$ equals 0.29. The rate constant, $k$, computed from the slope of the linear portion of the plot has a value of 0.26 min⁻¹.
In order to prevent lysis of the mitochondria, osmotic support was provided in the form of sucrose, an impermeant solute. Thus, a stable volume was reached at equilibrium.

Swelling in Ribose. A 0.135-ml aliquot of mitochondrial solution was suspended in 6.9 ml of 0.350 M ribose and 0.05 M sucrose, and the changes with time were recorded. The transmittance changes are plotted in Figure 3. The transformation $\Delta V / \Delta T$, $\Delta T / \Delta V$ between relative changes in volume and transmittance follows from the linear relationship established previously (6). Figure 3 shows the time course of expansion. Parameters $k$ and $\Delta V / V$, in equation 7 are evaluated from the slope and intercept, respectively, of the linear segment of Figure 4. Predicted response based upon these parameters is shown as the solid line. Analysis of these data, Figure 4, shows that there is good agreement between the experimental values and those predicted from equation 7.

Osmotic Contraction of Ribose-swollen Mitochondria. A 0.5-ml aliquot of mitochondrial solution was suspended in 4.0 ml of 0.350 M ribose in 0.05 M sucrose and allowed to equilibrate for 30 min. The mitochondria were reisolated by centrifugation for 10 min at 10,000 g and resuspended in 0.5 ml of 0.350 M ribose in 0.05 M sucrose. A 0.135-ml aliquot of the reisolated mitochondria was suspended in 6.9 ml of 0.4 M sucrose, and the response was recorded.

Figure 5 shows that for contraction there is good agreement between experimental and predicted results. It should be noted that the curvature is reversed from that for expansion, as predicted by equation 8. Again the parameters were evaluated from the linear segment of the data. Note also that the rate constant is considerably larger for contraction than for expansion, which is consistent with the mathematical model developed above.

The rate constant (Fig. 5) for osmotic contraction is some 18 times larger than for swelling. This is a consequence of the dependency of the rate constants upon the ratio $(V_0/V)^2$. For contraction, this ratio is greater than one while for swelling the ratio is less than 1.

Reswelling of Osmotically Contracted Mitochondria. A 0.5-ml aliquot of mitochondrial solution was suspended in 4 ml of 0.350 M ribose in 0.05 M sucrose and allowed to equilibrate for 30 min. The concentration of sucrose was then increased to 0.4 M by the addition of 0.6 M sucrose. The mitochondria were reisolated by centrifugation for 10 min at 10,000 g and resuspended in 0.5 ml of 0.4 M sucrose. A 0.135-ml aliquot of reisolated mitochondria was suspended in 0.35 M ribose in 0.05 M sucrose, and the events were recorded. The experimental values are in good agreement with those predicted from equation 7 (Fig. 6). The rate constant, $k$, for the reswelling process is somewhat larger than that for the primary swelling, 0.27 min⁻¹ as compared with 0.22 min⁻¹, respectively.

Swelling in KCI. The above experiments were performed with nonelectrolytes, and so there should have been no Donnan effects. Since many experiments with mitochondria use KCI for which Donnan effects would be expected, swelling was carried out in KCI. A 0.05-ml aliquot of mitochondrial solution was suspended in 2.55 ml of 0.224 M KCI in 0.05 M sucrose, pH 6.9, and the events were recorded. Experiments performed at pH 7.5 gave similar results. The experimental and calculated curves are in good agreement (Fig. 7).

DISCUSSION

The agreement between experimental and calculated results is good (Figs. 4, 5, 6, and 7). The shape of the curves for swelling and osmotically induced contraction of the mitochondria (Figs. 4, 5, 6, and 7) is in agreement with the theoretical curves (Fig. 2), constructed on the basis of equations 7 and 8.

### Table I. Relative Mitochondrial Volume Changes Expected When the Volume of Sucrose-inaccessible Space Not Free to Participate in Osmotic Phenomena Is Taken into Consideration

<table>
<thead>
<tr>
<th>Sucre Concentration</th>
<th>Volume of Sucre-inaccessible Space, Not Free to Participate in Osmotic Events</th>
<th>Volume of Sucre-inaccessible Space, Free to Participate in Osmotic Events</th>
<th>Total Volume of Sucrose-inaccessible Space</th>
<th>Volume Change Relative to That in 0.4 M Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.400</td>
<td>0.272</td>
<td>0.192</td>
<td>0.464</td>
<td>1.0</td>
</tr>
<tr>
<td>0.272</td>
<td>0.272</td>
<td>0.283</td>
<td>0.555</td>
<td>1.19</td>
</tr>
<tr>
<td>0.050</td>
<td>0.272</td>
<td>1.48</td>
<td>1.75</td>
<td>3.77</td>
</tr>
</tbody>
</table>

That $k_e > k_r$ is also predicted from equation 10. However, quantitative examination of the experimentally determined values of $\tau_e$ and $\tau_r$ reveals an apparent discrepancy. The experimentally determined ratio $\tau_e/\tau_r = 14.8$ according to equation 10 corresponds to a relative volume change, $V/V_e$, of 3.84. However, for an 8-fold change in the concentration of external impermeant solute, one expects, for a perfect osmometer, an 8-fold change in volume.

This disagreement at first seems unreasonable and would tend to discredit the theoretical development. But none of these measurements or calculations takes into account the osmotic dead space. In the determination of absolute mitochondrial volumes by gravimetric analysis of wet and dry centrifuged pellets, the osmotic dead space includes three components: the extramitochondrial water; the sucrose-accessible space, free to undergo volume changes; and the water of hydration of the matrix macromolecules, which is not free to participate in osmotic phenomena. The first two components probably do not play a significant role in the optical determination of the relative volume, whereas the nonosmotic water may play a significant role.

Bentzel and Solomon (1) found that in a 272-millimolar sucrose solution, the volume of the sucrose-inaccessible space is 0.555 µl/mg, dry weight, of which 0.272 µl/mg, dry weight, was apparently not free to participate in osmotic phenomena. Using these values of Bentzel and Solomon (1) and assuming that this nonosmotic volume is constant and that the sucrose-inaccessible space that does participate in osmotic phenomena behaves as a perfect osmometer, the values in Table I are calculated. From Table I it is seen that the theoretical volume change for an 8-fold change in concentration is 3.77 when the nonosmotic water is taken into account. This compares very favorably with the value of 3.84 above.

From equation 9 and the intercept a value for $V/V_e$ can be obtained. But unlike the slope, the intercept does depend on the reference time, since these were not constant or time zero, this ratio has not been calculated.

The principal purpose of this study was to elucidate the mechanism of passive swelling of mitochondria. The results clearly indicate that the volume changes represent a diffusion-controlled osmotic flow of water. Three facts point to this conclusion. First, the equilibrium volume of the mitochondria suspended in ribose depends not on the quantity of ribose that has entered the mitochondria but upon the concentration of impermeant solute present (6). This result is predicted from equation 1, which is based upon the equilibrium of the permeant solute across the membrane. Second, the swelling in ribose is osmotically reversible by increasing the concentration of impermeant solute in the external medium (Fig. 5). Again equation 1 predicts this result. Third, the kinetics of the primary swelling in ribose (Fig. 4), those of the osmotically induced contraction (Fig. 5), and those of the reswelling of the mitochondria following osmotically in...
duced contraction (Fig. 6) are consistent with this view. Finally, since for swelling \( V_o < V_e \) while for contraction \( V_o > V_e \), one predicts that the rate constant for contraction will be larger than that for swelling. This has been shown to be the case (Figs. 4 and 5).

The results for KCl-induced swelling are similar to those for ribose. Even though there are Donnan effects in this system, the passive swelling is controlled by the diffusion of solute.

While these experiments were performed in the absence of an additional source of metabolic energy, it is probable that changes in the volume of mitochondria, observed upon the addition of an oxidizable substrate or of ATP, are due to osmotic adjustments in response to solute transport.

LITERATURE CITED