A SIMPLE, INEXPENSIVE METHOD FOR TEACHING HOW MEMBRANE POTENTIALS ARE GENERATED

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We have developed a simple laboratory exercise that uses an inexpensive dialysis membrane (molecular weight cutoff = 100) to illustrate the generation of membrane potentials ($V_m$) across plasma membranes of animal cells. A piece of membrane ~2.0 cm² is mounted in an Ussing-like chamber. One chamber half is designated cytosol and the other half external. Chamber sidedness helps students relate their findings to those of real cells. As in real cells, outward directed K⁺ concentration gradients [high cytosolic K⁺ concentration ([K⁺]c) and low extracellular K⁺ concentration] generate cytosol electrically negative $V_m$ with a slope of approximately ~45 mV/decade change in [K⁺]c. The polarity of $V_m$ reflects the outward flow of potassium ions because flow of the larger counterion, H₂PO₄⁻, is restricted by the pores in the membrane. A slope less than Nernstian (<59 mV/decade) suggests that the membrane is slightly permeable to H₂PO₄⁻. Importantly, this facilitates teaching the use of the Nernst equation to quantify the relationship between ion concentration ratios across membranes and magnitude of $V_m$. For example, students use their data and calculate a permeability ratio $P_K/P_{H2PO4}$ that corresponds to a slope ~24% less than Nernstian. This calculation shows that Nernstian slopes are achieved only when permeability to the counterion is zero. Finally, students use the concept of membrane capacitance to calculate the number of ions that cross the membrane. They learn where these ions are located and why the bulk solutions conform to the principle of electroneutrality.

Key words: diffusion potentials; Nernst equation; dialysis membranes
ents responsible for generating \( V_m \). There are several reasons for the confusion. 1) In most cells, the plasma membrane electrogenic \( \text{Na}^+ \) pump, whose activity maintains the high intracellular \( \text{K}^+ \) concentrations, contributes little to \( V_m \) in part because the pump behaves as a source of constant current; it has an infinitely high resistance (5). 2) The high concentration side of the membrane has an electrical polarity opposite that of the charge carried by the membrane permeant ion. 3) Even though ions cross the membrane to generate \( V_m \), bulk solution electroneutrality is maintained (3, 4, 9). Therefore, before students can appreciate how the interaction between membrane pumps and leaks generate membrane potentials in real cells, they must understand how the flow of ions across membranes generates membrane potentials. For a current, detailed analysis of the origin of resting membrane potentials, see Sperelakis (13). A description of the interaction between plasma membrane ion pumps and ion channels in generating membrane potentials in animal cells is found in Byrne and Schultz (3).

Accordingly, we developed a simple, hands-on exercise that employs a mock cell to generate membrane potentials. The apparatus and approach presented here follow, in part, a laboratory exercise developed at California State University at Hayward (CSUH) that used cation- and anion-selective membranes to generate membrane potentials (8). It is also similar to the experiment published by Manalis and Hastings in 1974 (11). In both of those laboratory exercises, \( \text{K}^+ \) gradients were imposed across the cation-selective membrane and \( V_m \) were generated with nearly Nernstian slopes, 58 mV/decade. These exercises revealed the Nernst equilibrium potential for \( \text{K}^+ \) and showed that sustained \( \text{K}^+ \) gradients maintain \( V_m \) constant. Unfortunately, the ion-selective membranes used are no longer available, and the description of the CSUH experiment (8) is no longer in print. We searched for a suitable alternative membrane and found a new, low molecular weight cutoff (MWCO = 100) membrane, manufactured by Spectrum, that served our purpose. With the dialysis membrane, if one ion of a dissociable salt can pass through the membrane while the counterion is restricted because of its larger size, then a membrane potential will be generated when the two solutions bathing the membrane have different salt concentrations (see Fig. 1).

Compared with teaching the concept of membrane potentials with microelectrode techniques and the above-mentioned ion-selective membranes, the Spectrum dialysis membrane offers several advantages. 1) It is relatively inexpensive; an 8 \( \times \) 10-in. sheet costs about $62.00 (Spectrum part no. 133080), and ~40 pieces of experimental membrane can be obtained from a single sheet. 2) The membrane is shipped ready to use and requires no time-consuming cleaning procedure. 3) Because the relationship between transmembrane ion concentration ratio and \( V_m \) is less than Nernstian, a permeability ratio can be calculated for the permeant ion and the less permeant counterion. Importantly, this helps students relate the magnitude of \( V_m \) to that predicted by the Nernst equation. 4) Expensive equipment such as oscilloscopes and elec-
trometers is not required; rather, a simple pH meter operated in voltage mode suffices. 5) The dialysis membrane chamber is relatively inexpensive and can be purchased from Jim’s Instrument Manufacturing (Iowa City, IA). 6) The entire experiment is designed around a mock cell with cytosol and extracellular fluid compartments to help students relate their results to conditions found in real cells. 7) Because membrane thickness is known, a value for membrane capacitance can be derived with a simple assumption. Hence, students employ the concept of membrane capacitance and calculate the number of potassium ions that cross the membrane to generate $V_m$. 8) The approach used here employs the scientific method such that students formulate hypotheses about polarity and magnitude of $V_m$ before they conduct experiments and analyze data. 9) Changes in ion activities in different concentrations of the salt solutions are of no concern because dilute (0.1–10 mM) solutions are employed. Change in ion activity is a particularly difficult concept for students to grasp and adds to the complexity of data analysis when salt concentrations approach 100 mM.

The experiments described here give students a hands-on experience with the generation of membrane potentials. In addition, students calculate the number of ions that cross the membrane to generate $V_m$, and they are introduced to the Nernst equation as an equilibrium expression and learn how this important electrochemical equation is used in electrophysiological studies. The second experiment illustrates the role of the transmembrane Na$^+$ gradient in reversal of $V_m$ during an action potential in excitable cells (7).

**EXPERIMENTAL APPARATUS**

The experimental apparatus is relatively simple (Fig. 2). An ~2-cm$^2$ piece of membrane is cut from the sheet of dialysis membrane and placed in 0.1 mM KH$_2$PO$_4$ for ~10 min to allow antibacterial agents to diffuse from the membrane pores. We have found that placing the piece of membrane in distilled water gives anomalous results. After a light layer of silicone grease is applied around the chamber opening on each chamber half, the membrane is mounted by gently forcing the membrane down over the chamber’s membrane holding pins with stainless steel forceps. The two chamber halves are slipped together, guided by two large stainless steel guide pins (Fig. 2). The two chamber halves are held together in a utility vise (jaw width 2.5 in., jaw opening 2$^\frac{3}{4}$ in., jaw depth 1.5 in., and overall length 7$^\frac{3}{4}$ in.).

The two chamber halves are filled with appropriate salt solutions, and KCl-agar bridges are inserted into each chamber half to make an electrical connection to the calomel electrodes (catalog no. 13-620-258, Fisher Scientific). The agar bridges are prepared by boiling agar (3% bacteriological; catalog no. A-5306, Sigma) in 3 M KCl dissolved in distilled water. Polyethylene (PE) tubing is then filled with the hot agar-KCl mixture from a plastic syringe fitted with a large stainless steel needle. To comply with electrophysiological convention, the extracellular electrode is connected to ground on the pH meter (Digital Chemcadet model 5984-50, Cole-Parmer Instrument), and the other electrode is connected to the cytosol side of the chamber. In our studies, the output of the pH meter (i.e., $V_m$) is fed to a Vernier analog-to-digital converter. The digitized signal is displayed on the screen of a Macintosh computer, employing Vernier’s Data Logger software.

**SOLUTIONS**

We have experimented with several salts and found three that give reliable and reproducible results: K$_2$HPO$_4$, KH$_2$PO$_4$, and Na$_2$HPO$_4$, all dissolved in distilled water to give stock concentrations of 10 mM. Salts that failed to give reliable results were KCl, potassium tetrathionate, potassium pyrosulfate, potassium nitrate, potassium sulfate, potassium gluconate, sodium diatrizoate, and sodium citrate. The stock solutions of K$_2$HPO$_4$, KH$_2$PO$_4$, and Na$_2$HPO$_4$ are diluted to give concentrations of 1 and 0.1 mM for use in the experiments. To obtain additional data points, concentrations of 0.5 mM and 5 mM may be used. Although identical results are obtained with the two K$^+$ phosphate salts, it is best to use KH$_2$PO$_4$ to calculate the permeability ratio $P_K/P_{H_2PO_4}$ as described in EXPERIMENTS.

**Streaming potentials do not contribute to $V_m$**. Ion concentration gradients are established across the dialysis membrane by placing salt solutions of different concentrations in the cytosol and extracellular compartments. Thus osmotic gradients are also generated that could cause streaming potentials due to water flow “dragging” ions across the membrane.
To test for streaming potentials, we added mannitol to the salt solutions on the low salt concentration side of the membrane to achieve osmolarities equal to those on the high salt concentration side. We then compared $V_m$ to measurements made in the absence of mannitol and found no difference between the two. Therefore, streaming potentials do not make measurable contributions to $V_m$ in these experiments.

EXPERIMENTS

Effect of Increasing Cytosolic $K^+$ Concentration on $V_m$

In the first experiment, students explore the relationship between transmembrane $K^+$ gradients and $V_m$. To mimic $K^+$ gradients present across plasma membranes of real cells, students consult Table 1 and determine in which compartment they increase $K^+$ concentration. Importantly, this gives students insight into the nature of the $K^+$ concentration gradient. Next, they develop a hypothesis, which includes statements about magnitude and polarity of $V_m$ to predict what will happen to $V_m$ when they increase the cytosolic $K^+$ concentration ([K$^+$]$_c$) while holding the extracellular $K^+$ concentration ([K$^+$]$_e$) constant. In keeping with convention (see Figs. 5–14B, Ref. 7), instructors may choose to begin the experiment with high [K$^+$]$_c$ and increase [K$^+$]$_e$, which depolarizes $V_m$.

The experiment is started by placing 0.1 mM KH$_2$PO$_4$ in each chamber half. The potential displayed on the pH meter represents zero $V_m$. A 3-ml syringe with a
A piece of PE tubing attached is used to empty and refill
the chamber halves when changing salt solutions.
When the salt concentration is increased in a chamber
half, the chamber should be rinsed at least once with
the new salt solution before $V_m$ is recorded. After
$V_m$ is recorded with the higher KH$_2$PO$_4$ concentrations, 0.1
mM phosphate salt should be returned to the cytosolic
chamber to check for drift. Usually little drift occurs.
However, in some cases drift of a few millivolts
occurs, and either this must be added to or subtracted
from $V_m$ or the experiment must be repeated. With
the recording arrangement illustrated in Fig. 2, in-
creases in [K$^+$]$_e$ generate a cytosol negative
$V_m$. After $V_m$ is recorded with 0.1, 1, and 10 mM KH$_2$PO$_4$
(students should also include concentrations of 0.5
and 5 mM) in the cytosol compartment, the students
plot a graph of $V_m$ versus [K$^+$]$_c$ (Fig. 3A) and then plot
$V_m$ versus the ratio of [K$^+$]$_e$ to [K$^+$]$_c$, that is, ratios of 1,
0.1, and 0.01 (Fig. 3B). This graph shows that the ratio
of potassium ion concentrations across the membrane
is the important factor in generation of $V_m$ because a
ratio of 1.0 gives a $V_m$ of zero. With this plot, $V_m$
becomes increasingly more negative as a function of
[K$^+$]$_e$/[K$^+$]$_c$, but not in a linear manner.

The next objective is to establish a linear relationship
between $V_m$ and [K$^+$]$_e$/[K$^+$]$_c$. When doing this, students
will be introduced to the Nernst equation and to
the reason for taking the log [K$^+$]$_e$/[K$^+$]$_c$ to establish a
linear relationship between $V_m$ and the ratio of K$^+$
concentrations on the two sides of the membrane.
Ideally, measurements of $V_m$ are made when diffusion
forces (concentration gradient) and electrical forces
($V_m$) are equal, that is, at equilibrium. Thus we can
equate these two forces. We can write $E$ for electrical
force and $C_e/C_c$ for concentration ratio (extracellular
to cytosolic) or concentration force in the K$^+$
transmembrane chemical gradient, where C represents concentration (in mol/l). At equilibrium we then have

$$E = C_e/C_c \quad (1)$$

The problem with Eq. 1. At this point the students
should be asked what the problem is with this
equation. Clearly, the units of the two forces are not
the same. To rectify this situation, the concentration
ratio ($C_e/C_c$) is multiplied by a constant $k$ that must
have units of volts. Now the units on the two sides of

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cytosolic Concentration, mM</th>
<th>External Concentration, mM</th>
<th>$C_e/C_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid nerve</td>
<td>Na$^+$ 49</td>
<td>K$^+$ 410</td>
<td>Na$^+$ 440</td>
</tr>
<tr>
<td>Frog sartorius muscle</td>
<td>Na$^+$ 10</td>
<td>K$^+$ 140</td>
<td>Na$^+$ 120</td>
</tr>
</tbody>
</table>

Data are from Eckert et al. (6). $C_e/C_c$, ratio of external to cytosolic ion concentration.
the equation are the same, and we can rewrite Eq. 1 as

\[ E = k(C_e/C_c) \]  

(2)

Next, the constant \( k \) should be explored. In other words, where does \( k \) come from and why does it have units of volts? \( k \) is equal to \( RT/zF \), where \( R \) is the gas constant (2 cal/mol \( \times \) Kelvin), \( T \) is the absolute temperature (Kelvin units), \( z \) is the valence of the membrane permeant ion, and \( F \) is the Faraday constant (23,062 cal/mol \( \times \) V). The Faraday constant is the conversion factor that converts energy in a chemical concentration gradient (cal/mol) into equivalent energy in an electrical gradient (volts). At this point we have found it useful to have the students derive the units of \( k \) to convince themselves that it has units of volts. Now Eq. 2 can be rewritten to include \( RT/zF \) as

\[ E = (RT/zF)(C_e/C_c) \]  

(3)

However, a plot of \( E \), or \( V_m \), against \((RT/zF)(C_e/C_c)\) still gives a nonlinear relationship. To establish a linear relationship between \( E \) and \((RT/zF)(C_e/C_c)\), the students should be asked how the numbers on the x- and y-axes differ. On the y-axis the numbers represent an arithmetic progression, such as 10, 20, 30, 40, etc., because the difference between any two successive numbers is constant. On the x-axis the numbers represent a geometric progression in which \( C_e/C_c \) decreases as 1, 0.1, 0.01 (12). In this progression, the ratio between any two successive numbers is constant and the numbers decrease exponentially. If we take negative logarithms of the numbers on the x-axis, we obtain numbers that represent an arithmetic progression of 0, 1, 2, etc. Now, if \(-\log(C_e/C_c)\) is plotted against \( V_m \), one arithmetic progression is plotted against another arithmetic progression and a linear relationship is established between the two variables (Fig. 4).

If we expand Eq. 3 to include \( \log(C_e/C_c) \) as follows

\[ E = (RT/zF) \log(C_e/C_c) \]  

(4)

then we have the familiar Nernst equation. This is usually written as the \( \log(C_e/C_c) \)

\[ E = (2.3RT/zF) \log(C_e/C_c) \]  

(5)

\( E \) is now referred to as the equilibrium \( V_m \). The value of 2.3\( RT/zF \) is 59 mV at 23°C. With a monovalent salt, a 10-fold ratio for \( C_e/C_c \) will generate a \( V_m \) of 59 mV if the membrane is permeable to one ion only. Accordingly, the slope of the relationship between \( V_m \) and \(-\log(C_e/C_c)\) has a value of 59 mV/decade change in \( C_e/C_c \), and is referred to as a Nernstian slope. The significance of the Nernst equation is that it describes the mathematical relationship between an ion chemical concentration difference (or gradient) across a membrane and \( V_m \) under equilibrium conditions, i.e., when there is no net flow of ion across the membrane and \( V_m \) is constant (2, 3).

**Why is the slope less than Nernstian?** We suggest that the following analysis be performed by the students as a homework assignment.

At this point, the students should be asked why the slope of their relationship between \( V_m \) and \(-\log(C_e/C_c)\) is less than Nernstian. We suggest that the following analysis be performed by the students as a homework assignment.

The slope is less than Nernstian because the dialysis membrane is slightly permeable to the counterion \( \text{H}_2\text{PO}_4^{-} \). The Nernstian relation was calculated with the Nernst equation for a monovalent cation in which the salt concentration was increased in the external solution. We assume that less-than-Nernstian slopes result from a slight permeability of the dialysis membrane to the counterion \( \text{H}_2\text{PO}_4^{-} \).
\[(\text{C}_D/\text{C}_i)\text{ for } \text{KH}_2\text{PO}_4\text{ is less than Nernstian. As noted above, a Nernstian slope is obtained only if the membrane is permeable to one ion. This suggests that the dialysis membrane is somewhat permeable to the counterion, } \text{H}_2\text{PO}_4^-/-\text{. A small permeability to } \text{H}_2\text{PO}_4^-/-\text{ is not unreasonable, given that } \text{H}_2\text{PO}_4^-/-\text{ has a molecular weight of 96 and the dialysis membrane has an MWCO of 100. Hence, we assume that the membrane is slightly permeable to } \text{H}_2\text{PO}_4^-/-\text{. These data allow the students to calculate a permeability ratio } \frac{\text{P}_{\text{H}_2\text{PO}_4}}{\text{P}_K}\text{ and to demonstrate how permeability to the counterion generates less-than-Nernstian slopes. Furthermore, this calculation reveals that Nernstian slopes are achieved only when the membrane is permeable to one ion. For a membrane permeable to both ions of a dissociable salt, such as } \text{KH}_2\text{PO}_4, V_m\text{ is given by (3)}\]

\[V_m = \frac{(\text{P}_K - \text{P}_{\text{H}_2\text{PO}_4})}{(\text{P}_K + \text{P}_{\text{H}_2\text{PO}_4})} \times 59 \text{ mV-log} \left(\frac{[\text{KH}_2\text{PO}_4]_e}{[\text{KH}_2\text{PO}_4]_i}\right) \quad (6)\]

This expression is derived from the equation that gives the diffusion potential arising from the diffusion of a salt that dissociates into monovalent cations and anions (3). Because permeability is proportional to the diffusion coefficient, we can substitute permeabilities for diffusion coefficients (3). Furthermore, the students should be aware that this equation reduces to the Nernst equation for } K^+ \text{ if membrane permeability to } \text{H}_2\text{PO}_4^-/-\text{ approaches zero. From Fig. 4, the slope is } -45 \text{ mV/decade change in } [K^+], \text{ (we use the absolute value to simplify the calculation). We have consistently found slopes ranging from 40 to 50 mV/decade change in } [K^+],. \text{ However, in the students’ hands, slopes are sometimes less, } 30-45 \text{ mV/decade. For a 10-fold concentration gradient we have}\]

\[45 \text{ mV} = (\text{P}_K - \text{P}_{\text{H}_2\text{PO}_4})/(\text{P}_K + \text{P}_{\text{H}_2\text{PO}_4}) \times 59 \text{ mV}\]

To facilitate calculation of a permeability ratio, we arbitrarily set } \text{P}_K \text{ to 1.0. We will solve for the membrane permeability ratio } \frac{\text{P}_{\text{H}_2\text{PO}_4}}{\text{P}_K}. \text{ Dividing each side of the above equation by } 59 \text{ mV gives}\]

\[45 \text{ mV}/59 \text{ mV} = 0.76 = (\text{P}_K - \text{P}_{\text{H}_2\text{PO}_4})/(\text{P}_K + \text{P}_{\text{H}_2\text{PO}_4})\]

When we solve for } \frac{\text{P}_{\text{H}_2\text{PO}_4}}{\text{P}_K}, \text{ we have}\]

\[-0.23/ -1.76 = 0.13 = \frac{\text{P}_{\text{H}_2\text{PO}_4}}{\text{P}_K}\]

With } \text{P}_K \text{ = 1.0, then } \frac{\text{P}_K/\text{P}_{\text{H}_2\text{PO}_4}} = 1/0.13 = 7.7. \text{ The dialysis membrane is 7.7 times more permeable to } K^+ \text{ than to } \text{H}_2\text{PO}_4^-/-\text{, and the permeability to } \text{H}_2\text{PO}_4^-/-\text{ drives the slope of the relationship between } V_m \text{ and } -\log ([K^+]_i/[K^+]_e) \text{ to a value } <59 \text{ mV/decade change in } [K^+].\]

**How Many Ions Cross the Membrane to Generate } V_m?**

One of the most confusing aspects for students in understanding membrane potentials is that relatively few ions cross the membrane to generate } V_m. \text{ Furthermore, the ions that cross the membrane do not enter the pool of ions present in bulk solution because the solution is a conductor (4). These ions are present in a layer adjacent to, or just beneath, the membrane (Fig. 5) (4, 7). The following calculation reveals the number of ions needed to generate } V_m \text{ across the dialysis membrane:}\]

\[\text{FIG. 5. Flow of cations from cytosol to external solution causes charge separation across the dialysis membrane because the membrane behaves as an electrical capacitor. Cations that cross the membrane leave behind an equal number of anions. These cations and anions are present in thin layers adjacent to the membrane, and they interact electrostatically across the thickness of the membrane (7). This interaction holds these ions in the thin layers adjacent to the membrane. Thus the number of cations and anions in bulk cytosol and external solution are the same, and both solutions conform to the principle of electrical neutrality. [Modified from Eckert et al. (7)]}\]
membrane, and Fig. 5 shows where these ions accumulate. To calculate the number of ions that cross the dialysis membrane to generate $V_m$, students employ the concept of membrane capacitance ($C_m$) (4). Biological membranes behave as electrical capacitors because they separate and store charge (4, 7, 10). The relationship between $C_m$ and $V_m$ is given by

$$C_m = \frac{Q}{V_m} \quad (7)$$

where $Q$ is the amount of charge stored by the membrane. Assuming that the cellulose ester dialysis membrane has a dielectric constant similar to that of biological membranes, we can estimate the capacitance of the dialysis membrane from the difference in thickness of the two membranes (4). Plasma membranes are ~5 nm thick, whereas the dialysis membrane is ~20 µm thick, a difference of 4,000. Plasma membranes have a capacitance of 1 µF/cm² (4, 9). Thus the dialysis membrane has a capacitance of $~2.5 \times 10^{-4}$ µF/cm². For the KH$_2$PO$_4$ and Na$_2$HPO$_4$ salts, the slope of the relationship between $V_m$ and $C_e/C_i$ is ~45 mV/decade change in ion concentration.

Therefore,

$$Q/C_m = 45 \text{ mV}$$

and

$$Q = (2.5 \times 10^{-10} \text{ F/cm}^2)(0.045 \text{ V})$$

$$= 1.1 \times 10^{-11} \text{ C/cm}^2$$

where C is coulomb (unit of electrical charge). To calculate the number of ions ($N$) that cross the membrane, we divide $Q$ by the charge per ion ($e$) (4)

$$N = \frac{Q}{\text{membrane area}/e} \quad (8)$$

so that

$$N = (1.1 \times 10^{-11} \text{ C/cm}^2)(~1.0 \text{ cm}^2)/1.6 \times 10^{-19} \text{ C}$$

$$= \sim 6.9 \times 10^7$$

Approximately 7 million ions cross the ~1.0-cm² area of dialysis membrane to generate a $V_m$ of 45 mV. This seems like a large number of ions. However, these ions are stored near the membrane (see Fig. 5) and interact electrostatically with the layer of anions left behind. As noted above, they do not change the number of ions in the bulk solution; hence, bulk electroneutrality is maintained (4). Furthermore, this number of ions, compared with the number of ions in bulk solution, represents a tiny fraction, as the following calculation shows. There are $6 \times 10^{23}$ ions per mole, and the 3 ml of 0.1 mM phosphate (0.2 mM potassium and sodium ions) salt solutions have $3.6 \times 10^{17}$ ions. The ratio of ions that crossed the membrane to those present in bulk solution is

$$6.9 \times 10^7/3.6 \times 10^{17} = 1.9 \times 10^{-10}$$

This calculation shows that, relative to the number of ions in bulk solution, very few ions cross the membrane to charge $C_m$ and generate $V_m$.

**Effect of Increases in Extracellular Na$^+$ Concentration on $V_m$**

In the second experiment the students explore the relation between $V_m$ and transmembrane Na$^+$ gradients. This experiment will give students insight into the ionic basis of action potentials in excitable cells. Again, students consult Table 1 and determine the compartment in which they must increase Na$^+$ concentration to mimic the Na$^+$ gradient found in animal cells. Next, they develop a hypothesis about magnitude and polarity of $V_m$ that result from increases in external Na$^+$ concentration ([Na$^+$]$_e$). This experiment is conducted in the same manner as with increases in [K$^+$]$_c$. After conducting the experiment, students should answer the following questions.

1) Why is the absolute value of the slope (see Fig. 4) obtained with Na$_2$HPO$_4$ similar to that obtained with KH$_2$PO$_4$?

2) Why is the polarity of $V_m$ generated with Na$_2$HPO$_4$ opposite to that obtained with KH$_2$PO$_4$?

3) How do the results with Na$_2$HPO$_4$ relate to the change in $V_m$ that occurs during an action potential in excitable cells?

**Major Points of Lab Exercise**

By the end of this laboratory exercise, the students should have grasped the following major points.
A \( V_m \) develops when there is an ion gradient across a membrane if one ion of a dissociable salt is restricted from crossing the membrane while the other ion is freely permeant.

2) The electrical polarity of \( V_m \) reflects flow of the membrane permeant ion down its concentration gradient.

3) In real cells and as demonstrated in the mock cell, \( V_m \) results from potassium ions from flowing down their concentration gradient from the cytosol to the cell exterior.

4) The slope of the relation between \( C_e/C_c \) for the membrane permeant ion and \( V_m \) is Nernstian only if the membrane is predominantly permeable to one ion of a salt that dissociates into monovalent cations and anions.

5) Relatively few ions cross the membrane to charge \( C_m \) and generate \( V_m \); bulk electroneutrality is maintained.

6) Increases in external \( \text{Na}^{+} \) concentration reveal the concentration gradient responsible for reversal of \( V_m \) during an action potential in excitable cells.

**SUMMARY**

This laboratory exercise provides a simple set of experiments that can be reliably and reproducibly conducted by students in a three-hour laboratory period. The experiments reveal how membrane potentials are generated and show the ion gradient responsible for action potentials in excitable cells, and the students are introduced to the Nernst equation as an equilibrium expression.

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