Measurement of the Electrical Potential Difference and the Distribution of Ions in the Shay Chloroleukemic Tumor Cell

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SUMMARY

The electrolyte pattern and the potential difference (PD) across the membrane of the Shay chloroleukemic tumor cell was examined. The potassium ion concentration was 122 ± 9 mEq/liter of cell water; sodium was 48 ± 4 mEq/liter; chloride was 72 ± 7 mEq/liter; and water was 77.5 ± 0.5% of cell wet weight. The average PD was 9.2 ± 0.4 mV, inside negative to ground potential outside. Raising the external potassium concentration from 7 to 120 mEq/liter by replacement of external sodium did not change the internal potassium concentration significantly after equilibration for 1 hr at 23°C; the PD was reduced by 2 mV. Replacement of chloride ion with sulfate reduced intracellular chloride significantly and depolarized the membrane to zero. However, the Nernst equilibrium potential for chloride ion in normal Ringer's medium was —21.3 mV, more negative than the measured values. Since the steady-state flux of sodium ion exceeded that for chloride ion, sodium may contribute to the deviation of the measured PD from a true chloride equilibrium potential by providing a low-resistance shunt.

INTRODUCTION

Few studies have been made on the electrical PD3 or membrane potential, or on the distribution of ions across the membranes of cells in tissues other than nerve and muscle. Even less information is available on malignant tissues, yet the membrane potential may influence the behavior of malignant cells. Cone (4, 5) predicted that the malignant cell would have a membrane potential much lower than that of the corresponding normal cell and that the source of this lower PD may be related to the molecular character of the cell surface and the degree of its interaction with other cells. Since both intracellular and membrane functions are influenced by ions, the aberrant behavior seen in cancer may have as its basis the altered permeability characteristics of the cell membrane as reflected in the electrical PD.

The uniform population of leukemic myeloblasts available from rats bearing the Shay chloroleukemia provides an ideal source of leukemic cells for these electrophysiological studies. In this investigation, the PD of the Shay chloroleukemic tumor cell was measured and compared with the associated electrolyte pattern.

MATERIALS AND METHODS

Maintenance of the Tumor. The Shay chloroleukemia has been maintained in our laboratory by means of s.c. or i.p. transplants of leukemic cells into young, male Long-Evans rats. The method of transplantation has been previously described (11). Pieces of 10-day-old i.p. tumors, about 0.4 to 0.5 g wet weight, were used in this study.

Electrical Recording Apparatus. Impalement of tumor cells was accomplished with the use of 3 M KCl-filled glass microelectrodes. Typical microelectrodes had tip resistances of 10 to 30 megohms and tip potentials of —1 to —3 mV. The microelectrode, held by micromanipulators, was connected to the input of a Bioelectric NF1 preamplifier via a 3 M KCl-agar bridge and an Ag-AgCl wire. The preamplifier was connected to a Keithley electrometer and recorder. In some experiments, the output of the preamplifier was connected to a Tektronix storage oscilloscope, and a record of the PD measured was made with a Polaroid camera attached to the screen of the oscilloscope. The chamber for holding the tissue was constructed by pouring paraffin into the bottom of a Petri dish and carving out an area for the tissue at one end and the reference electrode containing Ringer's agar at the other. This chamber, as well as the micromanipulators, was supported on lead bricks to minimize vibration. The whole setup was kept within a copper mesh Faraday cage to minimize pickup. In general, the surface cells of a piece of tumor were impaled. The microelectrode or tissue was moved during a series of impalements to cover all areas of the tissue.

Measurement of the Effect of Increased External Potassium on the PD. For determination of the role of potassium in maintaining the PD of the tumor cell, the concentration of potassium in the external medium was increased and the procedure described below was used. The Ringer's solutions were prepared as described by Aull (1). Potassium was increased at the expense of sodium in order to maintain isosmolarity. An i.p. tumor was removed from a rat, cut into pieces, and trimmed of any connective tissue. One piece was placed in each of 4 paraffin chambers containing 10 ml of either the normal (7 mM), 20, 80, or 120 mM potassium
Ringer's medium with added $^{22}\text{Na}$. After about 1 hr at room temperature ($23^\circ$), PD measurements were made, the tissues were removed, and the remaining supernatant Ringer's medium was saved for analysis. The tissues were extracted by the ashing procedure described below. Concentrations of sodium and potassium in the extracts and media were measured by means of a Baird-Atomic Flame photometer. A previously determined value of 0.10 ml inulin water per g wet weight of tissue was used to calculate the ECS. The equation used to calculate the intracellular concentration (Subscript $i$) of an ion was:

$$\text{(ion)}_i = \frac{\mu\text{moles in tissue extract} - \mu\text{moles in ECS}}{\text{ml tissue water} - \text{ml ECS water}}$$

$$\mu\text{moles in cells} = \frac{\text{ml cell water}}{\text{liter cell water}}$$

The extent of equilibration of the tissue with the medium was determined from the distribution of $^{22}\text{Na}$, by comparison of the specific activity of the tissue with that of the medium. This experiment was performed 4 times.

At the completion of an experiment, the tissues were blotted well on filter paper, placed in a preweighed platinum boat, and weighed. The boats were then placed in an oven at $110^\circ$ overnight, and the dry weight was measured. In this way, the weight of water lost by the tissue was determined. For ashing, the platinum boats containing the dried tissues were placed in a muffle furnace at $550^\circ$ overnight. The resulting ash was dissolved by the addition of 0.2 ml of 0.1 N nitric acid to the boat, followed by 5 ml of distilled water. Appropriate dilutions of this extract were made in order to measure the sodium and potassium concentrations with the flame photometer. The amount of $^{22}\text{Na}$ was measured with a well-type scintillation counter. It was found that 98% of a sodium or potassium standard was recovered by the ashing procedure.

Measurement of the Effect of Decreased External Chloride on the PD. To examine the role of chloride in the maintenance of the PD, we performed 4 experiments in which PD measurements were made in pieces of tumor bathed in media containing sulfate in place of chloride ions. These solutions were prepared as described by Aull (1). The experiment was performed as follows. Ten ml of each medium, i.e., normal (160 mM chloride), sulfate (7 mM chloride), and high potassium sulfate (chloride-free) Ringer's medium, with $^{36}\text{Cl}$ were placed in paraffin chambers. A piece of tumor was placed in each chamber and allowed to equilibrate at room temperature ($23^\circ$) for about 120 min. PD measurements were made, and the tissues were then extracted by the acetic acid procedure described below.

The ECS of the tumor was determined from the distribution of inulin-$^{14}\text{C}$ by the following procedure. A piece of the tumor was incubated at room temperature in normal Ringer's medium containing inulin-$^{14}\text{C}$ for 120 min. The incubation time of 120 min was previously found to be sufficient for equilibration by experiments in which the uptake of inulin-$^{14}\text{C}$ by the tissue was examined as a function of time.

After incubation, the tissue was removed, blotted, and extracted with acetic acid. The remaining medium was saved, and the $^{14}\text{C}$ label in the medium and in the tissue extract was counted. The ECS was determined from the equation:

$$\text{ECS} = \frac{\text{cpm in extract}}{\text{g wet weight of tissue}} - \frac{\text{cpm in medium}}{\text{ml of medium}} = \frac{\text{ml medium}}{\text{g wet weight}} - \frac{\text{ml inulin water}}{\text{g wet weight of tissue}}$$

Acetic acid was used to extract chloride and inulin-$^{14}\text{C}$ from pieces of tumor used in these experiments. In this extraction procedure, 5 ml of dilute acetic acid reagent (2 drops of glacial acetic acid plus 10 ml of distilled water) were added to the glass vial containing the dried tissue. The vial was covered, placed in a boiling water bath for about 2 hr and then was allowed to stand overnight. This procedure was adapted from that of Dunham and Gainer (7). The resulting extract was clear, and the extracted tissue remained behind as an intact, rubber-like mass. The acetic acid extraction was satisfactory, recovering at least 95% of the inulin-$^{14}\text{C}$ and the chemical chloride from pieces of tumor. Chloride concentrations in the extracts and Ringer's media were measured with a Buchler-Cotlove chloridometer. The $^{34}\text{Cl}$ and $^{14}\text{C}$ labels were counted in a Packard liquid scintillation counter.

Measurement of the Uptake of Labeled Sodium and Labeled Chloride by the Tumor. A kinetic analysis of the data from the uptake of $^{22}\text{Na}$ and $^{36}\text{Cl}$ by pieces of tumor was made to determine the fluxes of these ions across the cell membrane. The procedure used for these uptake experiments was as follows. Pieces of tumor were incubated in normal Ringer's medium containing either $^{22}\text{Na}$ or $^{36}\text{Cl}$ for time intervals ranging from zero to 150 min. The tissues were then extracted with acetic acid, ion concentrations were measured, and the labels were counted in the extracts and supernatant media. The acetic acid extraction described above was found to recover 99% of the $^{22}\text{Na}$ and 98% of the $^{34}\text{Cl}$ from pieces of tumor.

RESULTS

Effects of Increased External Potassium on the PD. Typical recordings of the PD are shown in Chart 1. Since it is not clearly understood how the tip potential of a microelectrode changes when inside a cell, in this study the PD was taken as that potential which exists when the microelectrode is believed to be inside a cell relative to the ground potential (zero), regardless of the previously measured tip potential. The histograms in Chart 2 show the distribution of the PD measurements from tissues bathed in the 7.0, 20, 80, or 120 mM potassium Ringer's medium. In normal Ringer's medium (7.0 mM potassium), there was a peak in the distribution between $-7$ and $-8$ mV, and the mean PD was $-9.2 \pm 0.4$ mV (Table 1). A slight shift in the peak and in the mean PD occurred when external potassium was increased; measure-
ments between -4 and -5 mV were most often obtained in 80 mM potassium Ringer's medium, and the mean PD was -6.7 ± 0.4 mV (Table 1). However, with an even higher concentration of external potassium (120 mM), the mean PD was -7.3 ± 0.4 mV (Table 1), and the greatest number of PD recordings were obtained between -7 and -8 mV.

If potassium were the ion solely responsible for maintaining the PD across the tumor cell membrane, then the measured PD would be expected to be equivalent to the Nernst equilibrium potential for potassium. The calculated potassium equilibrium potential for cells in normal Ringer's medium is -72.8 mV. The measured PD was about -9 mV, and this changed only slightly with an increase in external potassium. However, it cannot be concluded that the membrane is impermeable to potassium. If potassium and sodium permeabilities are comparable, then one would not expect to find any effect on the PD when extracellular potassium was increased at the expense of sodium.

<table>
<thead>
<tr>
<th>Ringer's medium</th>
<th>[K]_i</th>
<th>[Na]_i</th>
<th>% cell water</th>
<th>[K]_o</th>
<th>[Na]_o</th>
<th>% equilibration</th>
<th>PD (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>121.6 ± 9.2</td>
<td>48.4 ± 3.9</td>
<td>77.5 ± 0.5</td>
<td>7.0 ± 0.1</td>
<td>165.8 ± 4.9</td>
<td>82.3 ± 4.1</td>
<td>9.2 ± 0.4 (49)</td>
</tr>
<tr>
<td>20 mM potassium</td>
<td>115.5 ± 5.6</td>
<td>42.8 ± 7.7</td>
<td>78.1 ± 0.5</td>
<td>20.8 ± 0.4</td>
<td>158.2 ± 7.2</td>
<td>89.3 ± 2.3</td>
<td>8.2 ± 0.4 (40)</td>
</tr>
<tr>
<td>80 mM potassium</td>
<td>120.1 ± 8.7</td>
<td>32.9 ± 4.0</td>
<td>78.0 ± 0.3</td>
<td>80.7 ± 1.6</td>
<td>107.4 ± 5.0</td>
<td>81.3 ± 5.2</td>
<td>6.7 ± 0.4 (52)</td>
</tr>
<tr>
<td>120 mM potassium</td>
<td>137.7 ± 2.2</td>
<td>26.2 ± 4.5</td>
<td>78.5 ± 0.4</td>
<td>119.2 ± 1.4</td>
<td>75.3 ± 3.3</td>
<td>82.0 ± 5.9</td>
<td>7.3 ± 0.4 (37)</td>
</tr>
</tbody>
</table>

*a % equilibration = (s.a. _tissue_)/(s.a. _medium_) X 100, where s.a. = specific activity in units of cpm/μmole.
*b Mean ± S.E.
*c No. in parentheses, no. of PD recordings.
Table 2
Effects of decreased external chloride on the PD and on the chloride concentration of Shay chloroleukemic tumor cells

<table>
<thead>
<tr>
<th>Ringer's medium</th>
<th>[Cl]₀</th>
<th>[Cl]₀</th>
<th>% cell water</th>
<th>Measured PD (mV)</th>
<th>Calculated $E_{Cl}^a$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>71.7 ± 6.7b</td>
<td>163.1 ± 3.4</td>
<td>77.3 ± 0.6</td>
<td>96.9 ± 7.3</td>
<td>-7.4 ± 0.2 (46)c</td>
</tr>
<tr>
<td>Sulfate</td>
<td>20.5 ± 1.7</td>
<td>10.0 ± 1.0</td>
<td>77.2 ± 0.6</td>
<td>44.1 ± 8.2</td>
<td>+0.7 ± 0.4 (51)</td>
</tr>
<tr>
<td>High potassium sulfate</td>
<td>19.3 ± 1.9</td>
<td>5.9 ± 0.3</td>
<td>76.1 ± 0.4</td>
<td>42.3 ± 3.8</td>
<td>+0.2 ± 0.2 (50)</td>
</tr>
</tbody>
</table>

Where

$$E_{Cl} = \frac{RT}{zF} \ln \frac{[Cl]₀}{[Cl]₀}$$

b Mean ± S.E.

c No. in parentheses, no. of PD recordings.

As seen in Table 2, the difference between the mean PD for cells in the normal Ringer's medium (−7.4 ± 0.2 mV) and that of cells in sulfate Ringer’s medium (+0.7 ± 0.4 mV) or in the high potassium sulfate Ringer’s medium (+0.2 ± 0.2 mV) was significant (with $p < 0.0005$). The tumor cells lost chloride to the external medium and reached a concentration of 20.5 and 19.3 mEq/liter cell water in the sulfate and high potassium sulfate media, respectively. These values differ significantly ($p < 0.0005$) from the intracellular chloride concentration of tumors bathed in normal Ringer's medium (71.1 mEq/liter cell water).

Since the cells were depolarized or even showed a reversal of the sign of the PD, the distribution of chloride ions across the tumor cell membrane was considered to be of significance. However, when the Nernst equilibrium potentials for chloride were calculated for the intra- and extracellular concentrations of chloride in each medium (Table 2), they were found to differ significantly from the corresponding mean-measured PD. This suggests that some other ion, perhaps sodium, is also important in the maintenance of the PD.

Determination of the Flux of Sodium and Chloride across the Membrane. To assess the contribution of sodium to the PD, the flux of sodium across the cell membrane relative to that of chloride was determined. Fluxes were calculated from the equation: flux = rate constant $\times$ compartment size. Since whole pieces of tissue were used in all experiments, the model proposed for the system consists of 3 compartments: the medium in which the tissue was bathed, the ECS, and the ICS. The rate constants for exchange of either sodium or chloride among these compartments were determined from a kinetic analysis of the data for the uptake of either ion by the tissue. Only the rate constants pertaining to the exchange of sodium or chloride between the ECS and ICS, i.e., across the cell membrane, are important in the discussion. The results of the uptake of $^{22}$Na and $^{36}$Cl are shown in Charts 4 and 5, in which a graph of the relative specific activity (specific activity of the tissue/specific activity of the medium) versus time is plotted. An analog computer was used to generate the best fit to the experimental points. In the program, the potentiometer settings used to generate the curve are analogous to the rate constants. The lines drawn were started so as to intercept the Y-axis, because the beginning or “zero” time sample actually represents a few seconds, which was the time it took to dip the

Unpublished program prepared by H. G. Hempling and available on request.

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Chart 3. The effect of decreased concentrations of external chloride on the PD. Decreasing the chloride concentration from 160 mM (normal Ringer’s medium) to 7 mM by replacement with sulfate (sulfate Ringer’s medium) caused a depolarization. In chloride-free medium (high potassium sulfate Ringer’s medium), as well as in the sulfate Ringer’s medium, a few positive PD recordings were made, but most attempts to measure a PD failed (indicated as a zero PD).

Effects of Decreased External Chloride on the PD. The histograms in Chart 3 show that, in the media in which chloride was replaced by sulfate, small positive PD recordings were made, but most cells showed no measureable PD (zero).
Chart 4. A typical curve fitted to the $^{22}\text{Na}$ uptake data by the analog computer. Relative specific activity is defined as $[\text{specific activity of the tissue (in cpm}/\mu\text{mole of sodium)}/\text{specific activity of the medium (in cpm}/\mu\text{mole of sodium)}]$. After 120 min, about 85% of the tissue sodium had equilibrated with the sodium in the medium.

tissue in the $^{22}\text{Na}$ or $^{36}\text{Cl}$ medium. The 1st part of each curve (the sharp rise) represents the uptake of sodium or chloride by the ECS compartment and appears to be slightly faster for chloride than for sodium.

In the calculation of the compartment sizes based on the chemical determinations of chloride or sodium in the medium, ECS, and ICS, all values were normalized to per ml of medium, extracellular fluid, or cell water, respectively. The resulting compartment sizes are in dimensions of μmole of sodium or chloride. The rate constants resulted from the graphs of relative specific activity versus time. The specific activity of the medium was normalized to per ml of medium. Since the compartment sizes are in dimensions of μmole of sodium or chloride and the rate constant has the dimensions of reciprocal time in min, the resulting flux of sodium or chloride across the cell membrane is given in terms of μmole/min, previously based on per ml of water. Thus the flux of sodium across the cell membrane was 0.4 to 0.8 μmole/min, while that of chloride was 0.33 μmole/min.

DISCUSSION

The results of the PD measurements on the Shay chloroleukemic tumor in external media containing decreased concentrations of chloride indicate that the chloride ion distribution plays a significant role in the maintenance of the PD. However, this PD is not entirely predicted by the Nernst equilibrium potential for chloride. Since the measured PD is lower, it was proposed that sodium ions may be contributing to the PD. The data for the flux of sodium and chloride ions across the cell membrane indicate that these cells have a relatively high permeability to sodium. If we accept the measurements of the PD as a valid measure of the membrane potential, the permeability of the sodium ion ($P_{Na}$) relative to that of chloride can be calculated with the following form of the Goldman equation (1.8):

$$-9.2 \text{ mV} = -59 \log_{10} \left[ \frac{[54.0 + P_{Na}(54.4)]/45.1 + P_{Na}(162.2)}{54.4} \right].$$

In this equation, the values for the concentration of intracellular sodium and chloride represent the exchangeable components derived from the kinetic analysis of the uptake of $^{22}\text{Na}$ and $^{36}\text{Cl}$. These concentrations were 54.4 mEq sodium and 45.1 mEq chloride per liter cell water and represent the intracellular compartment sizes of these ions derived from the flux studies. Since this equation was used as part of the analysis of the flux data, it was decided that these values are
Chart 5. Curve fitted to the mean $^{35}\text{Cl}$ uptake data by the analog computer. After 120 min, about 93% of the tissue chloride had equilibrated with the chloride in the medium.

more relevant. They differ somewhat from the values obtained from direct analysis of tissues in which the intracellular ion concentrations were calculated as the difference between the total concentration of the ion in the tissue and the concentration of that ion in the ECS. These values were 48.4 mEq sodium per liter cell water (Table 1) and 71.7 mEq chloride per liter cell water (Table 2). In the above equation, the values of 154.0 and 162.2 mEq/liter are those for chloride and sodium, respectively, in the external medium. The mean PD in normal Ringer’s medium was -9.2 mV. The $P_{Na}$ thus calculated is 0.50. This is close to the value of 0.34 calculated by Aull (1) for the $P_{Na}$ of Ehrlich mouse ascites cells. A high passive permeability to sodium was found for the Ehrlich ascites cells, and it was hypothesized that sodium contributes significantly to the PD (1). A comparable situation may be present in the Shay chloroleukemic cell. Further experiments designed to examine the active and passive fluxes of sodium, chloride, and potassium are needed to determine the true nature of the membrane potential of these leukemic cells.

Membrane potentials of some other malignant cells have been measured. In the case of mouse fibroblasts (L-cells) in culture, Lamb et al. (9) observed that the measured PD, -15.36 mV, was in close agreement with the Nernst equilibrium potential for chloride, -17.45 mV. In addition, a PD of -17 mV for HeLa cells (2) and -12 mV for KB cancer cells (10) has been found. From these studies it would appear that malignant cells tend to have a relatively low PD.

Cone (3-5) advanced the hypothesis that different levels of the PD play a role in the control of cell division. He pointed out that cells that maintain a very high PD, e.g., nerve and muscle, seldom, if ever, enter mitosis, while cells with a low PD routinely divide. On the basis of Cone’s theories, the following model can be proposed for the malignant cell. The low membrane potential associated with a particular ion distribution acts to influence cellular metabolic activity concerned with DNA synthesis, surface polymer production, etc. As a result, DNA synthesis occurs, leading to cell division. At the cell surface, i.e., membrane and surface polymers, there is high permeability to sodium which in turn helps to maintain the low negative PD. Cone and Tongier (6) found that ionic conditions designed to impose a relatively high PD (-70 mV) blocked DNA synthesis while at a level of -10 mV maximum proliferation rates were observed in naturally synchronized Chinese hamster cells. However, Cone and Tongier did not make measurements of the actual PD of the cells in these experiments. While this is an attractive hypothesis for the relationship of the membrane potential to the proliferative activities of the cell, the experimental data upon which the
hypothesis is based are scant. More definitive experiments are needed to establish the relationship, if any, between the membrane potential and the aberrant behavior seen in cancer.

REFERENCES

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