Chapter 10

Gibbs–Donnan Equilibrium Potentials

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I. SUMMARY

A Gibbs–Donnan (G-D) equilibrium becomes established and a G-D potential is developed across the cell membrane of cells under conditions in which metabolism and energy production have been inhibited or the Na^+-K^+ pump has been inhibited by digitalis. The G-D equilibrium occurs because of the large impermeant charged macromolecules, such as proteins, inside the cell. The G-D equilibrium does not require energy for its establishment, i.e. it is passive. This contrasts with the normal resting potential (RP) of the cell, which requires active ion transport and use of metabolic energy to establish large ionic electrochemical gradients.

The G-D potential is usually less than −20 mV, whereas the RP is considerably greater. In the G-D equilibrium, all permeable ions are in equilibrium across the membrane, whereas this is not true for the normal RP. The equilibrium potentials for all permeant ions (e.g. E_K, E_{Cl}) are of equal magnitude and polarity. The G-D potential is developed even if the cell membrane had equal permeability or conductance for all small ions, whereas the normal RP requires different permeabilities for Na^+ and K^+, namely a low P_{Na}/P_K ratio. In the G-D equilibrium, the osmolarity of the cell becomes higher than the interstitial fluid bathing the cell and so the cell tends to gain water and swell (unless prevented from doing so by a rigid cell wall, such as in plant cells).

When equilibrium is established, the product of the concentrations of the permeant ions inside the cell is equal to that outside the cell. The gains in cations and anions inside the cell also must be equal to each other. From these required conditions, an equation can be solved to give the final concentrations at equilibrium and, from this, the calculated potential difference across the membrane.

II. INTRODUCTION

Because intracellular cytoplasm contains many colloids, including large non-diffusible polyvalent electrolytes, a *Donnan equilibrium* can be established across the cell membrane with an accompanying transmembrane *Gibbs–Donnan (G-D) potential*. The resting potential (RP) of most cells in the body, including nerve and muscle cells, however, is not due to a Donnan equilibrium and the normal RP is not a Gibbs–Donnan potential, as is erroneously stated in some textbooks (Sperelakis, 1995; Sperelakis, 2001). In the true Donnan equilibrium, all diffusible ions are in equilibrium across the membrane. But many ions, like Na^+, K^+, Ca^{2+} and H^+, in nerve and muscle cells are not in equilibrium; i.e.

$$E_{Na} \neq E_m$$
$$E_K \neq E_m$$
$$E_{Ca} \neq E_m$$

and

$$E_H \neq E_m$$

On the other hand, Cl^- is at equilibrium (i.e. passively distributed) in many vertebrate cells; namely,

$$E_{Cl} = E_m$$

In addition, a large internal pressure and concomitant swelling of animal cells would occur if a Donnan

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equilibrium were allowed to become established. The action of two types of cation pumps keeps the Donnan osmotic pressure from developing and keeps certain cations out of equilibrium. Thus, a second important function of the Na^+-K^+ pump is the *regulation of cell volume*. The Na^+-K^+ pump actively pumps three Na^+ ions out (to two K^+ ions pumped in) with each cycle. The pump action decreases the osmotic pressure of the cytoplasm and prevents cell swelling. Inhibition of active ion transport by any means leads to osmotic swelling because of the establishment of the Donnan equilibrium. Under such conditions, the cells gain Na^+, Cl^-, Ca^{2+} and H_2O and they lose K^+.

Thus, the G-D potential is *passive*; i.e. energy is not necessary to its establishment. In contrast, the RP is actively generated (indirectly or directly) by the action of the Na^+-K^+ pump. The G-D potential is usually less than -20 mV, whereas the RP is -40 to -100 mV, depending on the cell type (and its ratio of P_{Na} to P_K).

III. MECHANISM FOR DEVELOPMENT OF THE GIBBS–DONNAN POTENTIAL

In the Gibbs–Donnan equilibrium, a small membrane potential is established even though the biological membrane involved, or the artificial membrane used in a laboratory experiment, may be equally permeable to the small diffusible ions used. For the example illustrated in Fig. 10.1, where aqueous solutions of 0.1 M $(Na^+)_n$-proteinate^{n-} (side 1) and 0.1 M NaCl (side 2) are initially placed on the two sides of a two-compartment chamber separated by a membrane and, if $g_{Na} = g_{Cl}$ in this membrane then, at equilibrium, $E_{Na} = E_{Cl} = -18$ mV. The side containing the protein anion becomes negative with respect to the other side. Thus, because both diffusion potentials have the same polarity (as well as magnitude), a potential difference (PD) occurs across the membrane, even though conductances for Na^+ and Cl^- across the membrane may be equal. The osmotic pressure of the

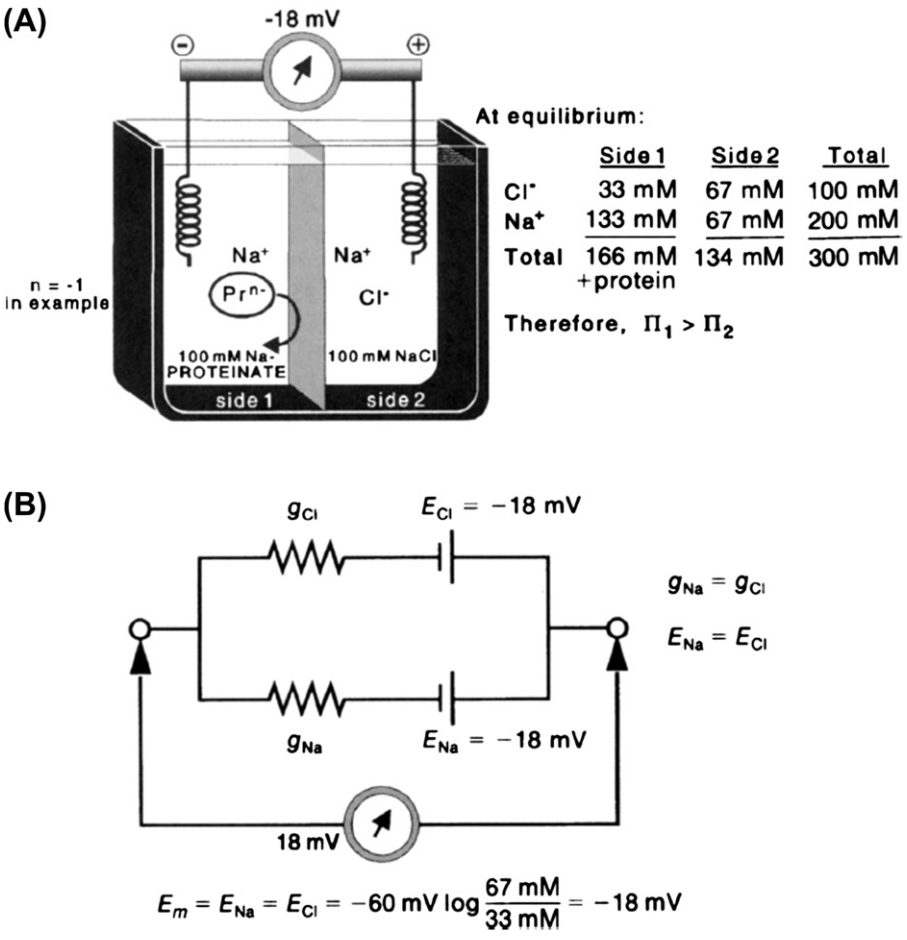

FIGURE 10.1 Gibbs–Donnan potential. (A) Gibbs–Donnan experiment. Diagram depicts the experimental arrangement for obtaining G-D potential. A membrane freely permeable to all small ions, but impermeable to the large protein molecules, is used to separate two solutions, only one of which (side 1) contains protein. Side containing the protein becomes negative, with respect to the other side, by a small voltage (−18 mV in example). This membrane potential (E_m) does not depend on active ion transport or on selective permeability properties of the membrane, as normal cell RP does. The diffusible ions (Na^+ and Cl^- in example), however, become unequally distributed across the membrane and it is their diffusion potentials ($E_{Na} = E_{Cl}$) that produce the G-D potential. (B) Equivalent circuit for experiment depicted in panel A, demonstrating that $E_m = E_{Na} = E_{Cl}$. The Na^+ and Cl^- batteries are of equal magnitude and of the same sign. Therefore, the relative conductances of the membrane to Na^+ and Cl^-, whether equal or not, are irrelevant to the potential (g_{Cl} and g_{Na} are conductances for Cl^- and Na^+, respectively).

solution on side 1 containing the non-permeant protein is greater than that on side 2. In the G-D equilibrium, all permeant ions are in electrochemical equilibrium across the membrane, i.e. they are passively distributed and there is no net electrochemical driving force:

$$(E_m - E_{Na}) = 0$$

$$(E_m - E_{Cl}) = 0$$

A more complete explanation for the development of the G-D potential follows. The G-D potential (which is an equilibrium PD) does not depend on metabolic energy. Therefore, this discussion applies to a cell that either has no ATP for pumping ions against electrochemical gradients or has had its Na^+-K^+ pump completely blocked by either ouabain or another agent. The G-D potential is passively produced by the concentration gradients for diffusible electrolytes (e.g. Na^+ and Cl^-) across a membrane. These ion gradients are caused by the presence of one or more large non-diffusible (with respect to the membrane) polyvalent electrolytes (e.g. *negatively-charged proteins*) on one side of the membrane, as is present in all biological cells. In essence, the negatively-charged protein molecules (at pH 7) inside the cell attract cations (e.g. Na^+ or K^+) and repel anions (e.g. Cl^-). Therefore, in the G-D situation, the inside of the cell has a higher concentration of Na^+ (or K^+) and a lower concentration of Cl^- than has the solution bathing the cell. The equilibrium potentials for Na^+ (E_{Na}) and for Cl^- (E_{Cl}) are equal in magnitude and are of the same sign, thereby producing a PD across the membrane. The PD is negative on the inside (side containing the protein) and usually is about −20 mV or less.

IV. GIBBS–DONNAN EQUILIBRIUM

To quantitate the ion distributions produced at equilibrium and the PD developed, let us examine the artificial system shown in Fig. 10.1A. In this system, a chamber is separated into two compartments by a collodion membrane, which has small uncharged pores that allow Na^+ and Cl^- ions, but not large protein molecules, to diffuse through. A 100 mM solution of Na^+ proteinate is added to one side (compartment 1) and a 100 mM solution of NaCl to the other side (compartment 2). An electrode is positioned on each side so that the PD across the membrane can be recorded (37°C). Let us assume that the Na^+ proteinate is completely ionized and, for simplicity, that the protein has a net negative charge of only one.

Thus, there is, at the first instant, no diffusion force for Na^+, but there is diffusion force for Cl^-, because Cl^- is 100 mM in compartment 2 and 0 mM in compartment 1. Na^+ must accompany the diffusion of Cl^- from side 2 to side 1, because the *principle of electroneutrality* in the bulk solution cannot be violated (i.e. there must be an equal number of cations and anions). So one relation that must be true when the system comes to equilibrium is that:

$$[Na^+]_2 = [Cl^-]_2 \tag{10.1}$$

In actuality, there is a small charge separation directly across the membrane to account for the PD; i.e. side 2 of the membrane has a small excess of Na^+ ions and side 1 has a small excess of Cl^- ions. Such a charge separation is very small, but is necessary to develop a PD across the membrane ($V = Q/C$) and is discussed in the preceding chapter on the RP generation.

The principle of electroneutrality also requires that the increase in Na^+ on side 1 must be exactly equal to the increase in Cl^- on side 1. Thus, the concentration difference of Na^+ that is built up at equilibrium must be exactly equal to the final concentration difference for Cl^-. This is because the large initial gradient for Cl^- is what drives the Na^+ to make its gradient. Therefore, it must also be true that:

$$\frac{[Na^+]_1}{[Na^+]_2} = \frac{[Cl^-]_2}{[Cl^-]_1} \tag{10.2}$$

Cross-multiplying gives:

$$[Na^+]_1[Cl^-]_1 = [Na^+]_2[Cl^-]_2 \tag{10.3}$$

Another way of considering this is that E_{Na} must equal E_{Cl} and, therefore, using the respective Nernst equations (see Chapter 9), we can write:

$$E_{Na} = E_{Cl} \tag{10.4}$$

$$\begin{aligned}\frac{-61\text{ mV}}{+1}\log\frac{[Na^+]_1}{[Na^+]_2} &= \frac{-61\text{ mV}}{-1}\log\frac{[Cl^-]_1}{[Cl^-]_2}\\ &= \frac{-61\text{ mV}}{+1}\log\frac{[Cl^-]_2}{[Cl^-]_1}\end{aligned} \tag{10.5}$$

Dividing both sides by −61 mV and removing the log gives Equation 10.2:

$$\frac{[Na^+]_1}{[Na^+]_2} = \frac{[Cl^-]_2}{[Cl^-]_1}$$

Equation 10.3 indicates that, at equilibrium, the product of the diffusible ions on side 1 must be equal to the product of the diffusible ions on side 2. From the Nernst equation, the relationships

$$E_{Na} = \frac{-RT}{zF}\ln\frac{[Na^+]_1}{[Na^+]_2} \tag{10.6}$$

$$= \frac{-61\text{ mV}}{+1}\log\frac{[Na^+]_1}{[Na^+]_2} \tag{10.7}$$

and

$$E_{Cl} = \frac{-61\text{ mV}}{-1}\log\frac{[Cl^-]_1}{[Cl^-]_2} \tag{10.8}$$

can be given because Cl^- is negative ($z = -1$), whereas Na^+ is positive ($z = +1$). Equation 10.8 is the same as (note that a negative sign in front of a log inverts the ratio)

$$E_{Cl} = -61 \text{ mV} \log \frac{[Cl^-]_2}{[Cl^-]_1} \quad (10.9)$$

because $[Na^+]_1/[Na^+]_2 = [Cl^-]_2/[Cl^-]_1$, as Equation 10.2 indicates and, from Equations 10.7 and 10.9, one obtains Equation 10.4: $E_{Na} = E_{Cl}$.

V. QUANTITATION OF THE GIBBS–DONNAN POTENTIAL

For quantitation, let us use x to indicate the amount (in mM) of Cl^- or Na^+ that shifted from side 2 to side 1 at equilibrium. Then the amount of Na^+ on side 2 is (100 mM$-x$) (the original amount minus the amount lost); Cl^- on side 2 is also (100 mM$-x$), because $[Na^+]_2 = [Cl^-]_2$. The Na^+ on side 1 at equilibrium is (100 mM$+x$) (the original amount plus the amount gained) and the Cl^- on side 1 is simply x. These parameters may be listed as follows:

$$[Na^+]_2 = 100 \text{ mM} - x$$
$$[Cl^-]_2 = 100 \text{ mM} - x$$
$$[Na^+]_1 = 100 \text{ mM} + x$$
$$[Cl^-]_1 = x$$

The value for x can be obtained by substituting these values into Equation 10.3:

$$[Na^+]_1 [Cl^-]_1 = [Na^+]_2 [Cl^-]_2 \quad (10.3)$$

$$(100 + x)x = (100 - x)(100 - x)$$

$$100x + x^2 = 10\,000 - 200x + x^2$$

$$300x = 10\,000$$

$$x = 33.3$$

Thus, at equilibrium

$$[Cl^-]_1 = 33 \text{ mM}$$
$$[Na^+]_1 = (100 + 33) = 133 \text{ mM}$$
$$[Cl^-]_2 = (100 - 33) = 67 \text{ mM}$$
$$[Na^+]_2 = (100 - 33) = 67 \text{ mM}$$

These values are also given in Fig. 10.1A. Note that all the equations and conditions are obeyed. The G-D potential produced then may be calculated by substituting into Equations 10.7 and 10.8:

$$E_{Na} = \frac{-61 \text{ mV}}{+1} \log \frac{133 \text{ mM}}{67 \text{ mM}} = -18 \text{ mV} \quad (10.10)$$

and

$$E_{Cl} = -\frac{-61 \text{ mV}}{-1} \log \frac{33 \text{ mM}}{67 \text{ mM}} = -18 \text{ mV} \quad (10.11)$$

Hence, $E_{Na} = E_{Cl}$ (Equation 10.4). That is, the two diffusion potentials are equal in magnitude and of the same sign. The PD across the membrane is −18 mV; side 1 containing the protein is negative. Therefore, relative permeability of the membrane to Na^+ and Cl^- is irrelevant. The equivalent circuit for this example at equilibrium is given in Fig. 10.1B.

VI. OSMOTIC CONSIDERATIONS

We should note that, at equilibrium, the sum of Na^+ and Cl^- on side 1 (166 mM) is greater than that on side 2 (134 mM). In addition, there is 100 mM protein on side 1. Thus, the total osmotic concentration on side 1 is 266 mOsm (milliosmolar), compared to 134 mOsm on side 2. Therefore, there is a large osmotic gradient between the two sides. Water moves from side 2 to side 1 (i.e. water accompanies the net movement of Na^+ and Cl^-) until the hydrostatic pressure head buildup is sufficient to oppose further net movement of water. As expected, the biological cell swells when a G-D equilibrium is allowed to develop following a blockade of active ion transport for long periods.

The total osmotic concentration, [osm], on each side, at equilibrium, may be summarized as follows:

$$[\text{osm}]_1 = [Na^+]_1 + [Cl^-]_1 + [\text{protein}]_1 \quad (10.12)$$

$$[\text{osm}]_2 = [Na^+]_2 + [Cl^-]_2 \quad (10.13)$$

Substitution gives:

$$[\text{osm}]_1 = 133 \text{ mM} + 33 \text{ mM} + 100 \text{ mM} = 266 \text{ mM}$$
$$[\text{osm}]_2 = 67 \text{ mM} + 67 \text{ mM} = 134 \text{ mM}$$

The osmotic pressure (Π, in atm) of each solution is equal to the osmotic concentration in osmol/L (C) times the osmotic coefficient (i) times the gas constant (R, 0.082 L·atm/mol·K) times the absolute temperature (T, in °K)

$$\Pi = iCRT \quad (10.14)$$

where C is the number of osmoles per liter of solution. In the example depicted in Fig. 10.1, a hydrostatic pressure of 3.17 atm would need to be applied to side 1 to prevent this compartment from gaining water from side 2 (at 20°C and assuming $i = 1.0$)

$$\begin{aligned}\Pi &= i\Delta CRT \\ &= (1.0)(266\ \text{mM} - 134\ \text{mM})\left(0.082\frac{\text{L}\cdot\text{atm}}{\text{mol}\cdot\text{K}}\right) \\ &\quad \times (273 + 20)\ \text{K} \\ &= \left(0.132\frac{\text{mol}}{\text{L}}\right)\left(0.082\frac{\text{L}\cdot\text{atm}}{\text{mol}\cdot\text{K}}\right)(293\ \text{K}) \\ &= 3.17\ \text{atm}\end{aligned} \tag{10.15}$$

The situation illustrated in Fig. 10.1 is actually more complex because the net water movement into side 1 acts to dilute the ion concentrations building up there and, therefore, a true G-D equilibrium can become established only if the net water movement is stopped, i.e. by allowing an osmotic pressure gradient to develop by making side 1 a closed, or rigid, system. Otherwise, theoretically all of the water and NaCl eventually would move out of side 2.

The example of a G-D equilibrium in Fig. 10.1 could have been illustrated using another salt, such as KCl, instead of NaCl, or two or more salts.

The extra osmotic pressure in side 1 (or inside a cell) produced by the presence of the negatively-charged proteins and other impermeant large charged molecules is known as the *colloid osmotic pressure* (COP). The COP is also important for water movement across the capillary wall, which separates the blood plasma (containing impermeant proteins) and the interstitial fluid (ISF). At the arterial end of the capillary, the intracapillary hydrostatic blood pressure exceeds the COP, so water moves out of the capillary into ISF space. At the venous end, the COP exceeds the capillary hydrostatic pressure, so water moves into the capillary. In the mid-region of the capillary, the two pressures are about equal and there is no net water flow. Thus, there is a circulation of fluid distributed along the length of the capillary and this idea is generally known as the *Starling hypothesis* (Davson, 1964; Sperelakis, 2001).

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