MEDICAL UNIVERSITY - PLEVEN
FACULTY OF MEDICINE
DEVISION OF PHYSICS AND BIOPHYSICS

Lecture № 13

GENERATION OF RESTING MEMBRANE POTENTIAL

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Physiological significance of the transmembrane electrical potential difference :

- It influences the transport of a vast array of nutrients into and out of cells;
- It is a key driving force in the movement of salt (and therefore water) across cell membranes and between organ-based compartments;
- It is an essential element in the signaling processes associated with coordinated movements of cells and organisms;
- It is ultimately the basis of all cognitive processes.

How does the electrical gradient across membranes arise?

It is the consequence of two physiological parameters:

- the presence of large gradients for K^+ and Na^+ across the plasma membrane;
- the relative permeability of the membrane to those ions.

The gradients for K^+ and Na^+ are the product of the activity of Na-K-ATPase, a primary active ion pump that is ubiquitously expressed in the plasma membrane of all animal cells.

The pump maintains

- the large outwardly directed K gradient, and
- the large inwardly directed Na gradient, that are hallmarks of animal cells.

The second parameter, the relative permeability of the plasma membrane to K^+ and Na^+ reflects **the open versus closed status of ion-selective membrane channels.**

Cell membranes display different degrees of permeability to different ions due to the **inherent selectivity of specific ion channels.**

The combination of

1) ***transmembrane ion gradients, and***
2) ***differential permeability to selected ions,*** **is the basis for generation of transmembrane potential difference.**

Consider the hypothetical situation of two solutions separated by a membrane selective to a single ion.

Side 1 (the "inside") contains 100 mM KCl and 10 mM NaCl.

Side 2 (the "outside") contains 100 mM NaCl and 10 mM KCl.

Hence, there is an "outwardly directed" K^+ gradient, and inwardly directed Na^+ gradient, and no transmembrane gradient for Cl^-.

Let this ideal membrane is permeable only to K^+.

Although intracellular ion concentrations generally do not change as a consequence of the downhill fluxes associated with transmembrane voltage changes, it is instructive to consider what would happen if the K gradient were to change.

Changes in the K gradient, typically the result of changes in $[K]_{out}$, are important, both physiologically and clinically.

The rule of thumb: any manipulation that reduces K gradient (i.e., either decreasing $[K]_{in}$ or increasing $[K]_{out}$), will decrease E_{mK}. In other words, if there is less energy in the chemical gradient, it will take less energy in an electrical gradient to balance it.

Cytoplasmic and extracellular K^+, Na^+ , Cl^- concentrations

Ion	C_{intra}, mM	C_{extra} [mM]	E_m [mV]	P [cm/s]
K^+	135	4	-92	1×10^{-7}
Na^+	12	140	+64	1×10^{-9}
Cl^-	4	116	-88	1×10^{-8}

E_m - calculated Nernstian equilibrium potentials for each ion gradient

P - typical permeability values for neurons

These values are only hypothetical for a mammalian cell. The listed concentrations of Cl^- in the cytoplasm and extracellular fluid do not add up to the total concentration of K^+ and Na^+.

However, electroneutrality is maintained in each compartment through the combined contribution of a diverse array of additional charged solutes.

Biological membranes do not show "ideal" permselectivity. Real membranes have a finite permeability to all the major inorganic ions in body fluids.

For most cells, the only ions that can exert any significant effect on bioelectrical phenomena are the "big three": K^+, Na^+, and Cl^- (Ca^{2+} also contributes to bioelectric issues in a few tissues, including the heart).

The Nernst equation, which represents an idealized situation, can be modified to represent the more physiologically realistic case in which the membrane shows a finite permeability to these three major players.

The new equation is called the "Goldman-Hodgkin-Katz Constant Field equation";or the "Goldman equation".

$$E_m = -60 \log_{10} \frac{P_K[K]_{in} + P_{Na}[Na]_{in} + P_{Cl}[Cl]_{out}}{P_K[K]_{out} + P_{Na}[Na]_{out} + P_{Cl}[Cl]_{in}}$$

"Goldman equation"

The Nernst equation is lurking within the Goldman equation:

if the membrane were to become permeable only to K^+, i.e. if P_{Na} and P_{Cl} were zero, then the equation simplifies to the Nernstian equation for K^+.

The calculated Nernstian potential for K, Na, and Cl establish the "boundary conditions" for the electrical potential difference across the cell membrane;
the cell cannot be more negative than 92 mV or more positive than 64 mV because there are no relevant chemical gradients sufficiently large to produce larger voltage across the membrane.

At rest, the membrane permeability of most cells **is greatest for K**, due to the activity of several distinct populations of K channels constitutively active under normal resting conditions. The relative contribution to the resting potential played by these channels varies with cell type.

In neurons relevant players include members of the family of inwardly rectifying K channels (KIR) and the K(2P) family of K "leak" channels.

An outwardly directed K gradient + a high resting permeability to K ⟹ electrically negative interior of animal cells with respect to the external solution.

The finite permeability of the membrane to Na^+ (and to Cl) prevents the membrane potential from ever actually reaching the Nernstian K^+ potential. The extent to which each ion gradient influences the potential difference is defined by the permeability of the membrane to each ion (Goldman equation).

Even very large concentrations exert little influence if the associated *P* value is small.

If the membrane were suddenly to become permeable only to Na^+, the result would be a Nernstian potential for Na.

In summary: the combination of an outwardly directed K gradient (due to Na-K ATPase activity) and a high resting P_K makes the interior of animal cells electrically negative with respect to the extacellular space.

The $[K]_{out}$ is particularly susceptible to changes since it is comparatively "small" (4 mM) and increases in $[K]_{out}$ of only a few mM can have large effects on resting membrane potential.

Such changes can occur as a consequence of, for e.g., crushing injuries that rapidly release into the blood stream large absolute amounts of K (from the K-rich cytoplasm in cells of the damaged tissue).

Failure of the Na-K ATPase during ischemia can result in local increases in $[K]_{out}$, a problem exacerbated by both the low starting K concentration and the low extracellular volume of "densely packed" tissues (e.g., in the heart or brain).

Alteration in membrane permeability to ions, can arise as a consequence of pathological defects in ion channel proteins (or "channelopathies").

Of particular relevance to the resting membrane potential are lesions in one or more subunits of the KIR channels. Mutations in these channels is linked to persistent hyperinsulinemic hypoglycemia of infancy, and to several polygenic CNS diseases, including white matter disease, epilepsy and Parkinson's disease.

What about the effect of Cl^-? The resting potential is as close to E_{mCl} as it is to E_{mK}.

Why don't we conclude that Cl is the dominant ion in defining the resting membrane potential?

Ans: The cell is spending its energy via Na-K-ATPase in establishing the gradients for K^+ and Na^+, not Cl^-.

The observed inwardly directed Cl^- gradient, with E_{mCl}= 89 mV is mainly due to the simple passive distribution of Cl^- in response to the electrical gradient that is effectively defined by the outwardly directed K gradient.

1) The cell actively builds transmembrane gradients of K and Na;
2) The outward flux of K down its gradient shifts the potential difference toward E_{mK};
3) Cl^-, which is high in the blood, moves into the cell in response to its chemical gradient;
4) But the inside negative potential established by K serves as a force to limit the buildup of Cl^-_i.

This is the case even in skeletal muscle cells in which the channel-mediated permeability to Cl exceeds that for K.

The fact that the Nernstian Cl potential is not exactly equal to the resting potential means that there are one or more "active" transport processes that keep Cl away from an equilibrium distribution (secondary active Cl/HCO_3 exchange).

Whereas in neurons Cl is a minor player in the resting membrane potential, there are several situations in which P_{Cl} (due to the activity of Cl channels) is very important.

- An increase in P_{Cl} is an effective way to "stabilize" the resting membrane potential by opposing changes in PD that would otherwise be produced by fluxes of K or Na. Thus P_{Cl} is modulated to influence synaptic transmission.

- A decrease in P_{Cl} makes it easier for the PD to shift away from its resting value. Thus, in the disease myotonia congenita, the observed hyperexcitability of skeletal muscle cells results from a decreased P_{Cl} (arising from defects in the ClCN1 Cl channel).

Factors contributing to the resting potential:

A. Gibbs-Donnan Equilibrium contributes less than **-10 mV** to the resting membrane potential.

B. The electrogenic Na-K ATPase:

In vertebrate skeletal muscle and many vertebrate nerve cells the contribution of the electrogenic Na-K ATPase to the resting membrane potential is small, less than **5 mV** or so. In contrast, in smooth muscle and some neurons, the electrogenic pump may make a major contribution to the resting membrane potential.

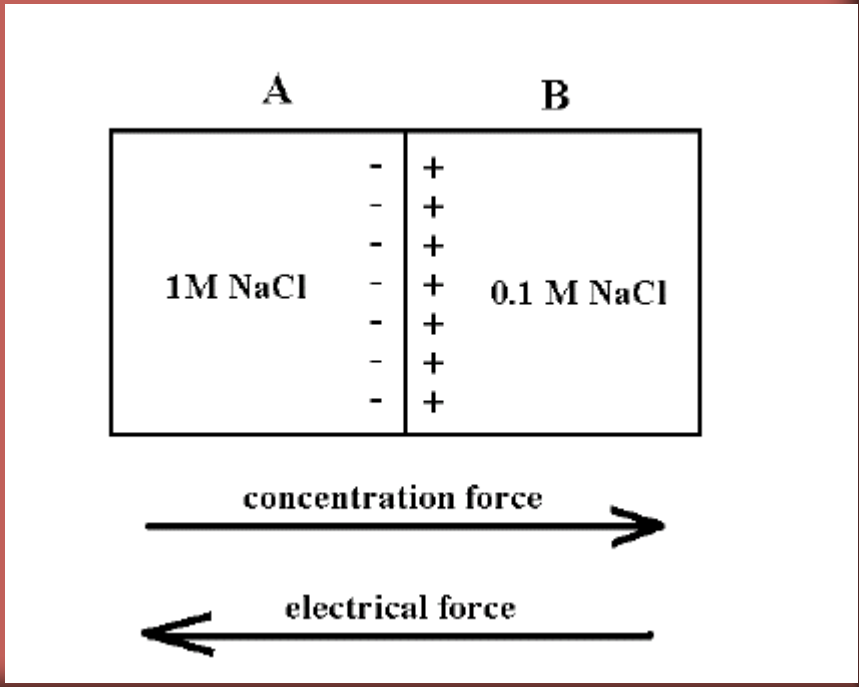
C. Electrodiffusion of ions: Each permeable ion "strives" to bring the transmembrane electrical potential difference toward its equilibrium potential.

Learning Goals

1. Which way will an ion flow spontaneously. Compare “electrical force” with “chemical force”.

Na^+ will diffuse from A to B because of the concentration gradient, but will tend to diffuse from B to A due to the electrical potential difference. Which way will Na^+ flow?

We need to be able to compare the "concentration force“ with the "electrical force"



What is the equation for the differerence of electrochemical potential $\Delta\mu$ of an ion across a membrane? What are the units for each term? What is the meaning of each term?

The electrochemical potential. Definition:

$$\mu^* = \mu_0 + RT \ln C + zF\varphi$$

μ_0 is the chemical potential in some reference state
R is the ideal gas constant,
T is the absolute temperature,
c is the concentration of the ion,
z is the valence of the ion,
F is Faraday's number,
φ is the electrical potential.

The dimentions of each term are energy/mole.

μ is the potential energy per mole of ions.
$RT \ln c$ is the energy possessed because of concentration.
$zF\varphi$ is the energy possessed by virtue of the electrical potential. The difference in μ^* across a membrane ($\Delta\mu$):

$$\Delta\mu^* = \mu_A - \mu_B = RT \ln \frac{C_A}{C_B} + zF(\varphi_A - \varphi_B)$$

$RT \ln \frac{C_A}{C_B}$

- is the difference in energy possessed by a mole of ions of side A compared to that on side B because of the concentration difference. This is the **"concentration force".**

$zF(\varphi_A - \varphi_B)$

- is the difference in energy possessed by a mole of ions of side A compared to that on side B because of the electrical potential difference. This is the **"electrical force"**.

Ions will flow spontaneously from where their μ* is higher to where their μ* is lower.

1. **Energy is required to make ions flow from lower μ to higher μ.**

   How much energy? **Δμ* per mole.**

2. **Energy is released when ions flow from higher μ to lower μ.**

   How much energy? **Δμ* per mole.**

Ionic equilibrium and the Nernst Equation

A. When an ion is in equilibrium across a membrane $\mu^*_A = \mu^*_B$, so that $\Delta\mu^*=0$.

Setting $\Delta\mu^*=0$ and solving for $\varphi_A - \varphi_B$ gives

$$\varphi_A - \varphi_B = -\frac{RT}{zF}\ln\frac{C_A}{C_B} = \frac{RT}{zF}\ln\frac{C_B}{C_A}$$

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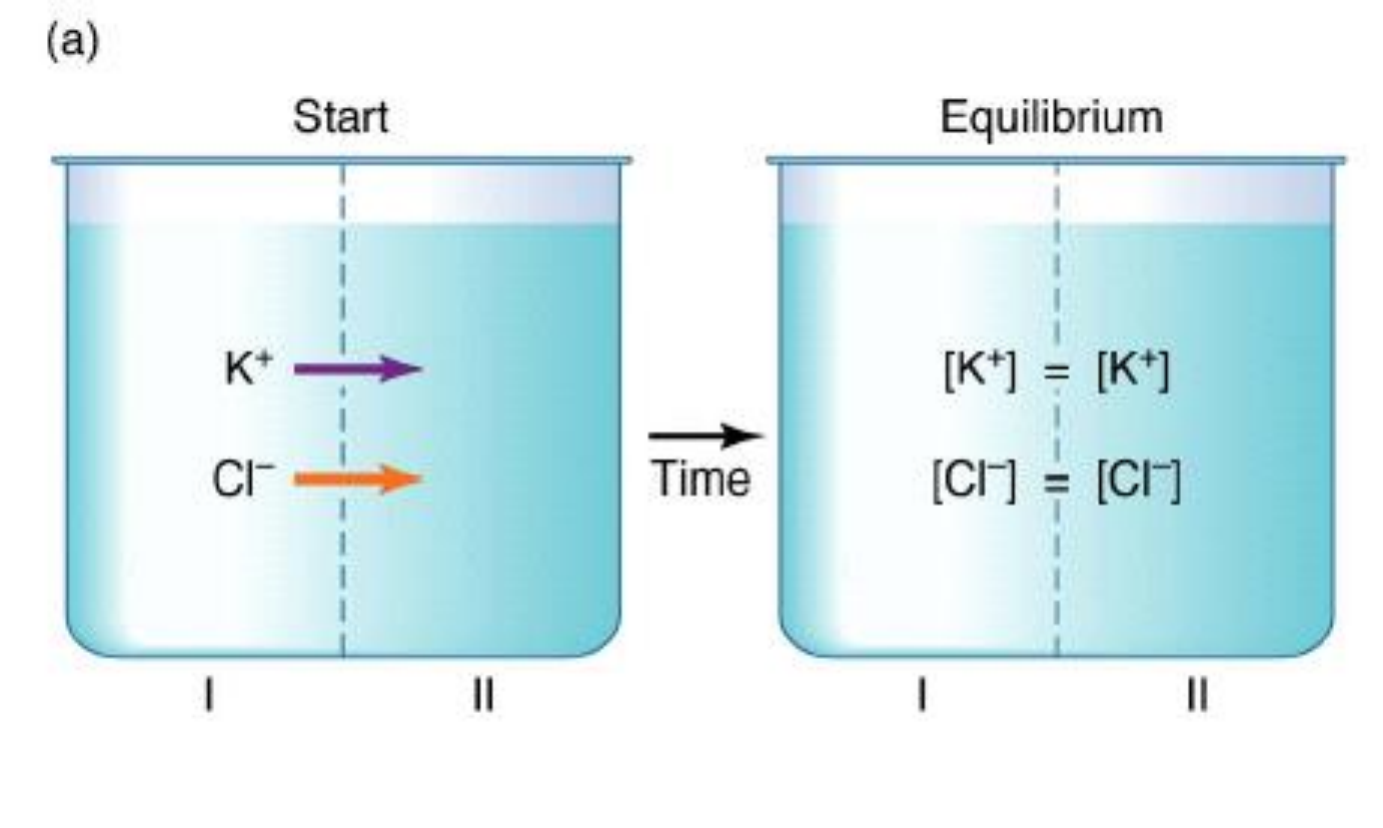

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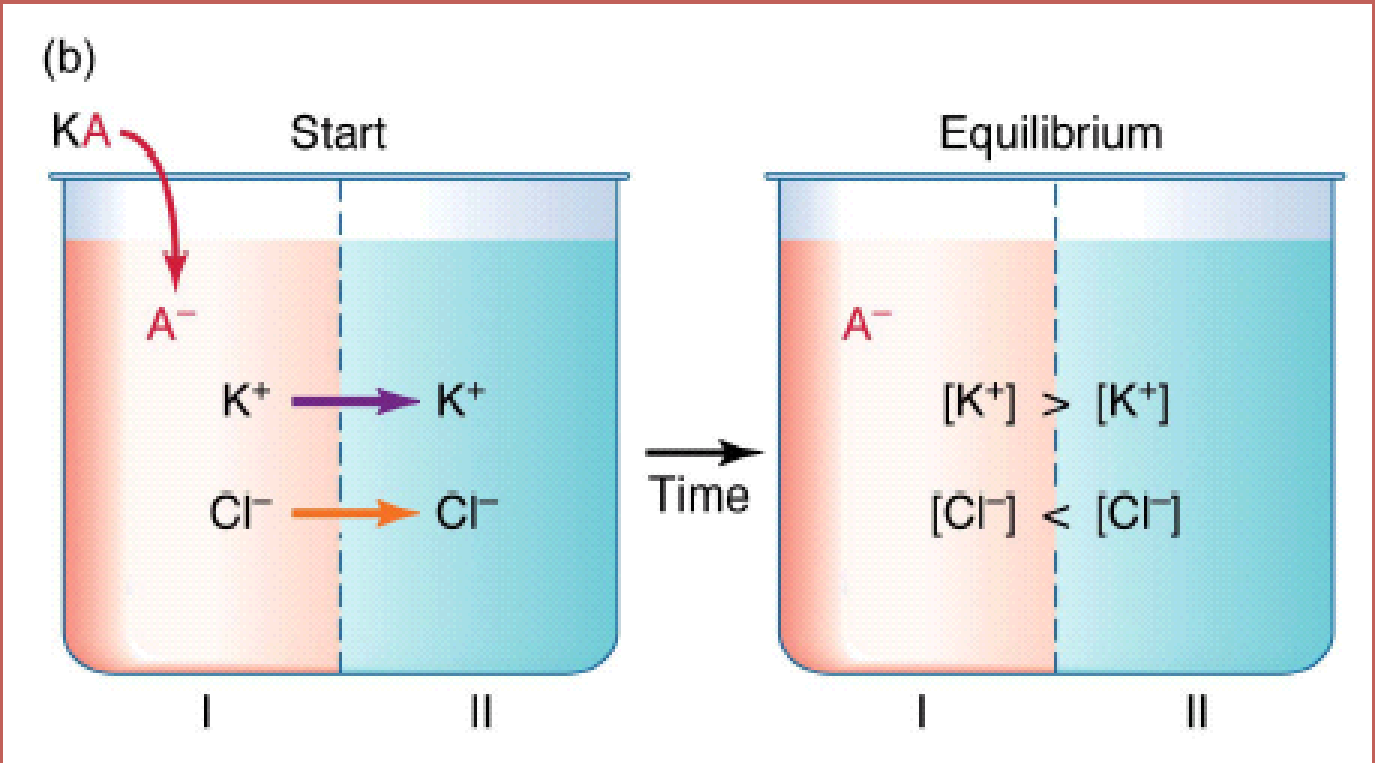

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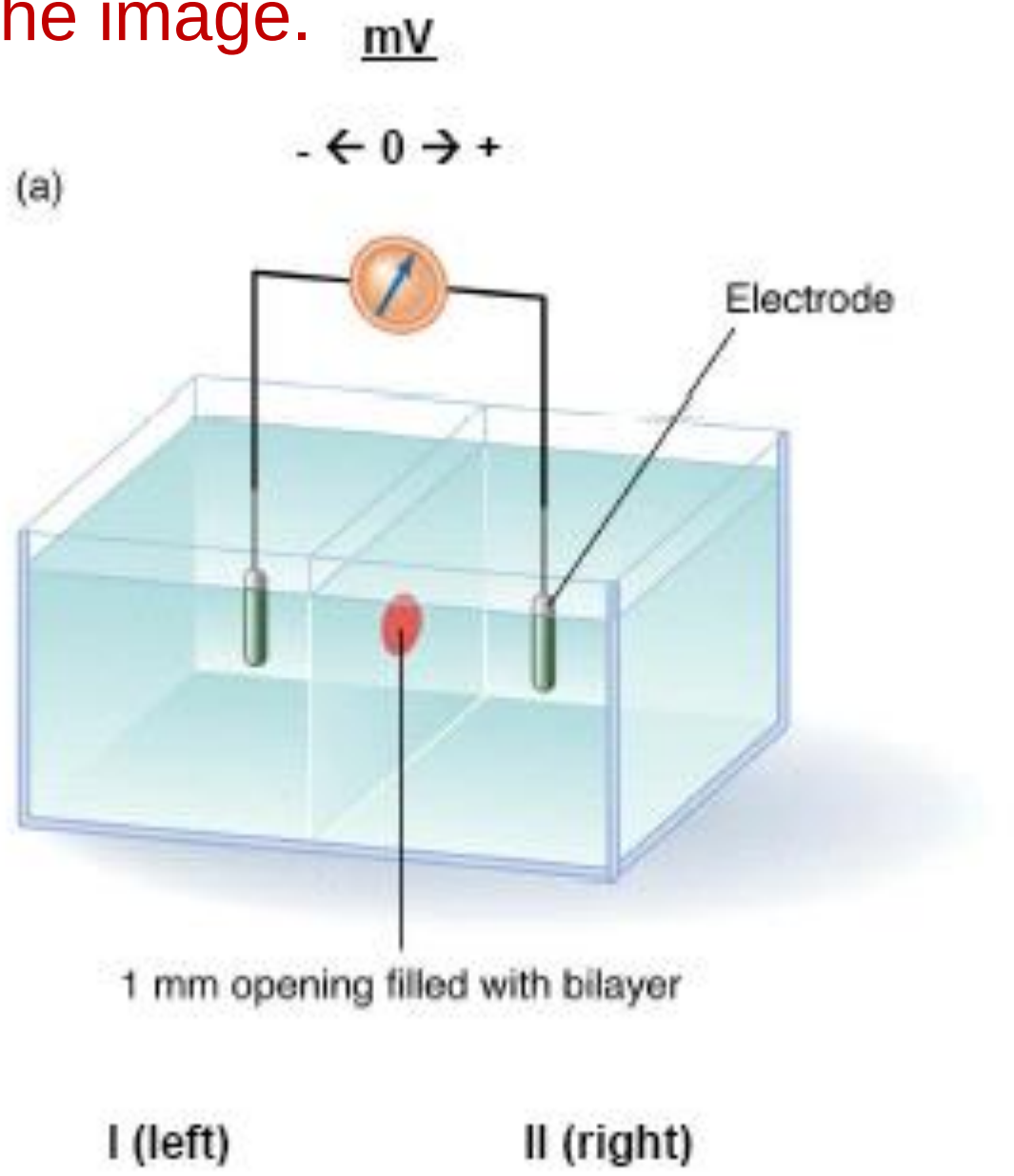


What happens to the membrane potential when the membrane is made permeable to only K^+:

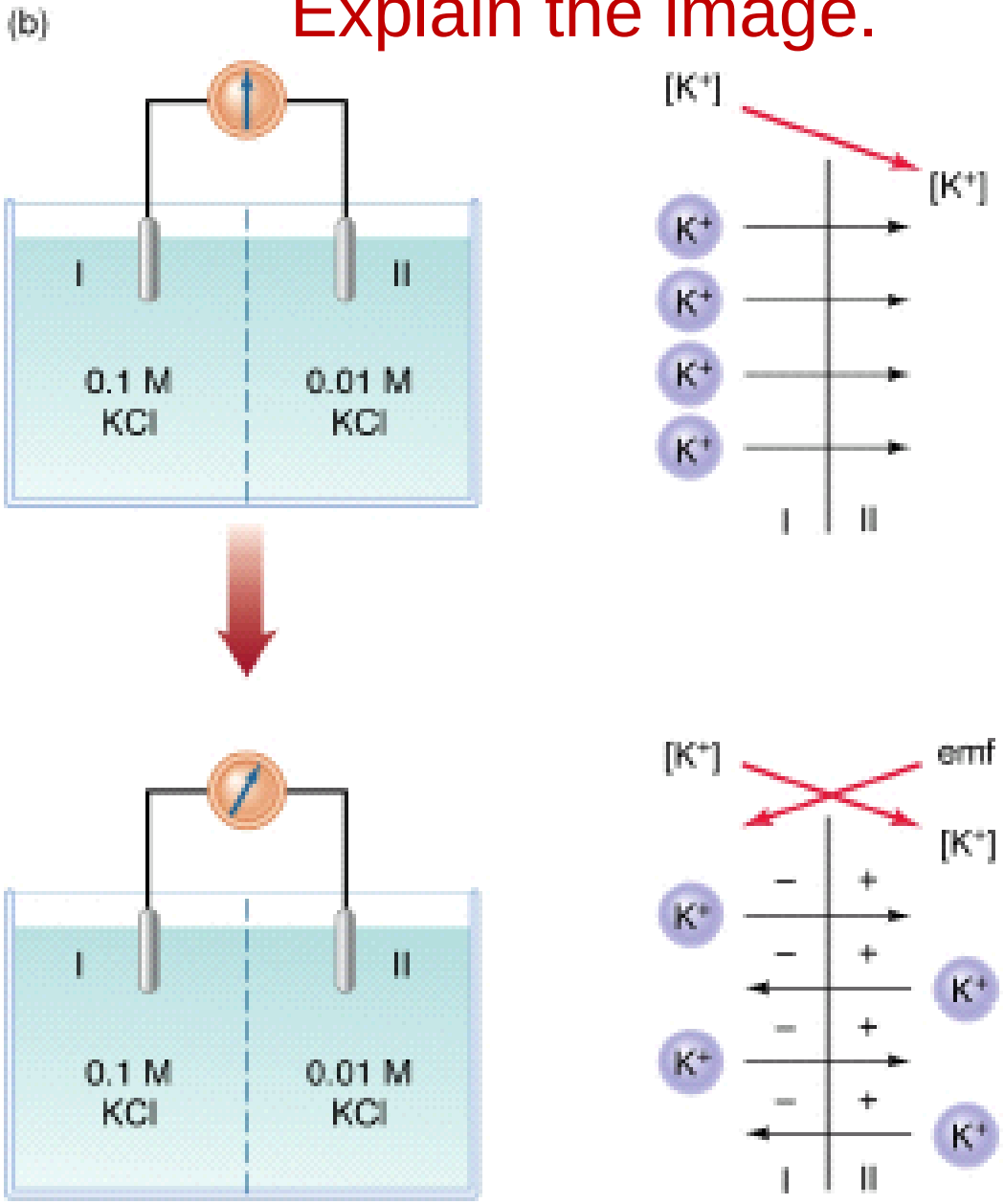
Left side

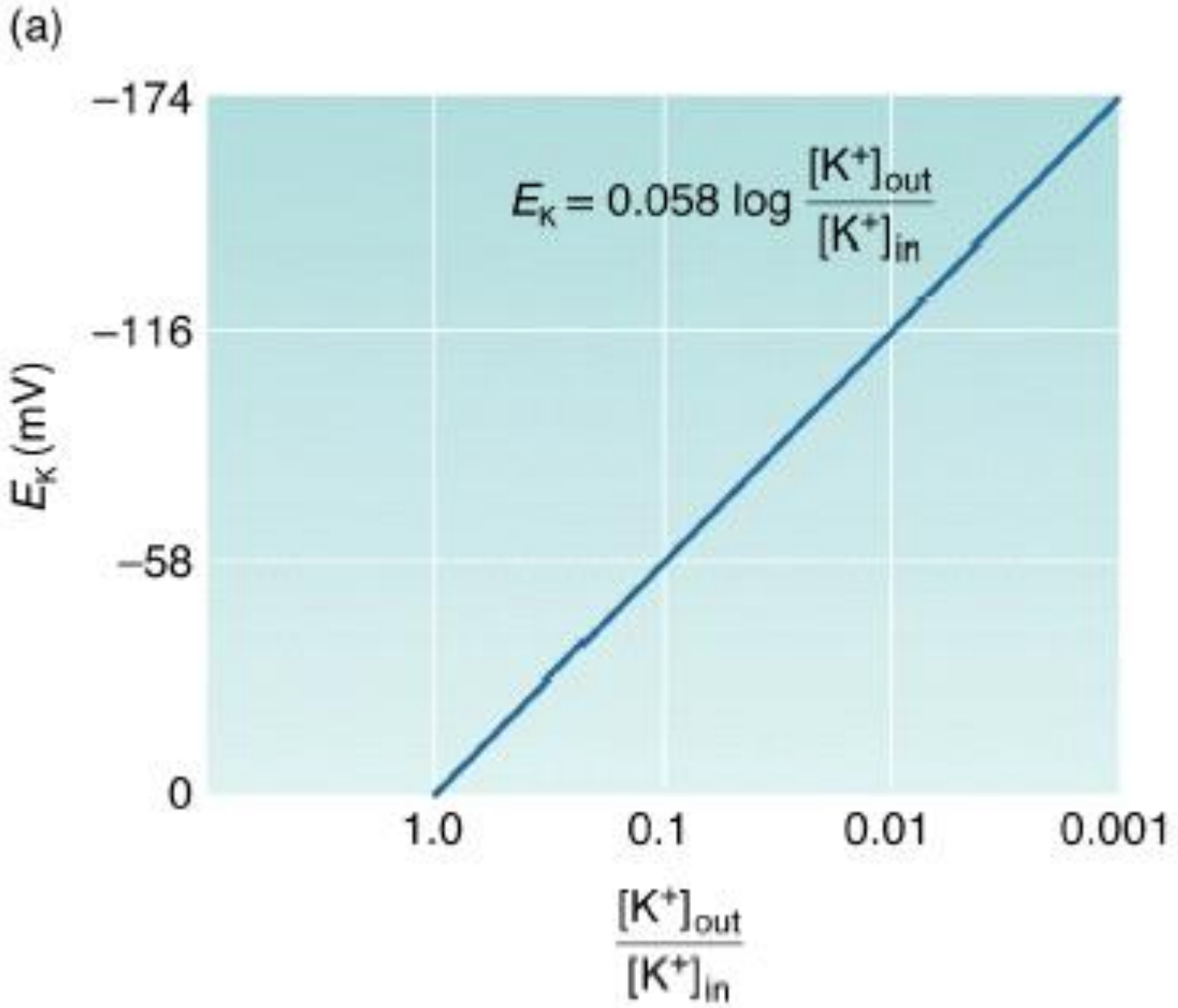
100 mM K^+
10 mM Na^+
110 nM Cl^-

Right side

100 mM K^+
10 mM Na^+
110 nM Cl^-

Explain the image.




(a)
−174
−116
−58
0
E_K (mV)
$E_K = 0.058 \log \frac{[K^+]_{out}}{[K^+]_{in}}$
1.0
0.1
0.01
0.001
$\frac{[K^+]_{out}}{[K^+]_{in}}$

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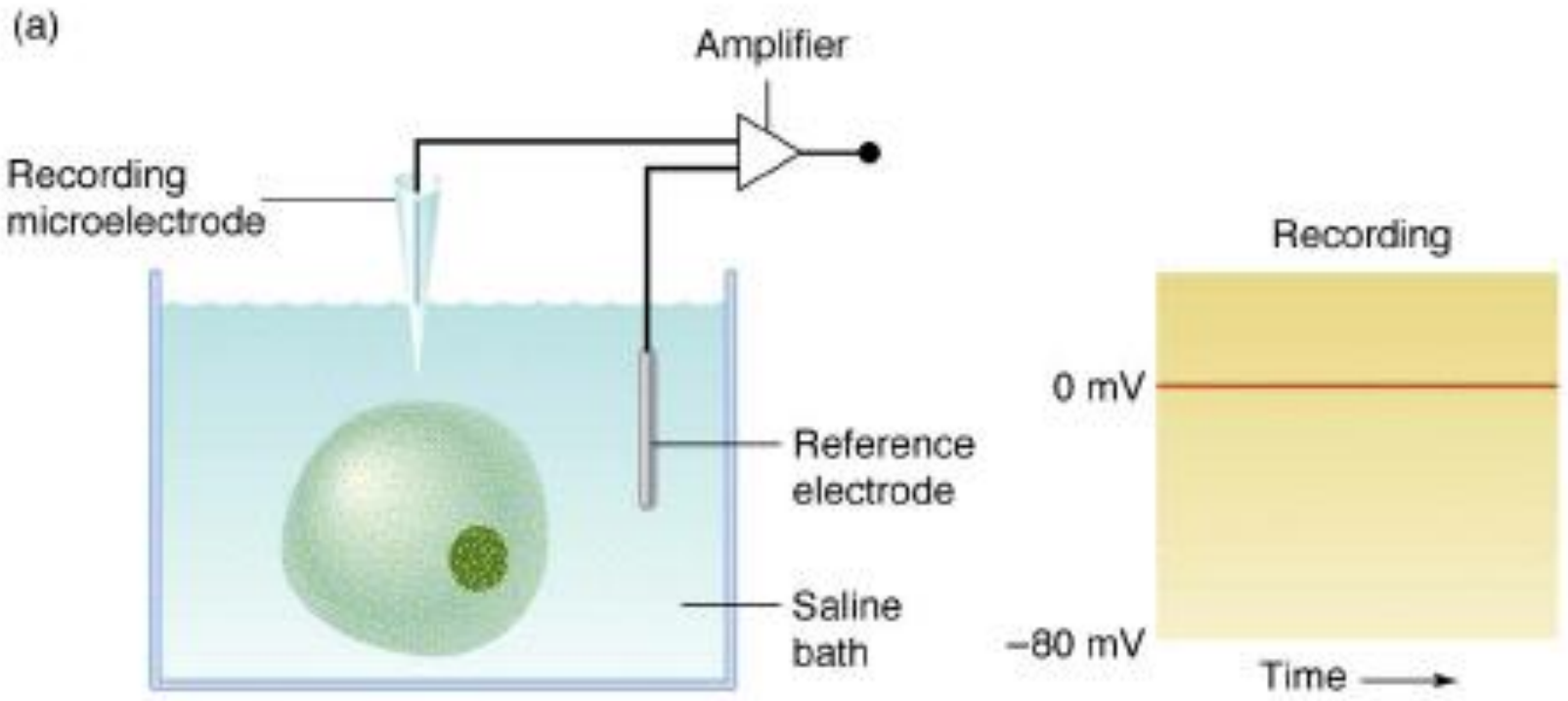

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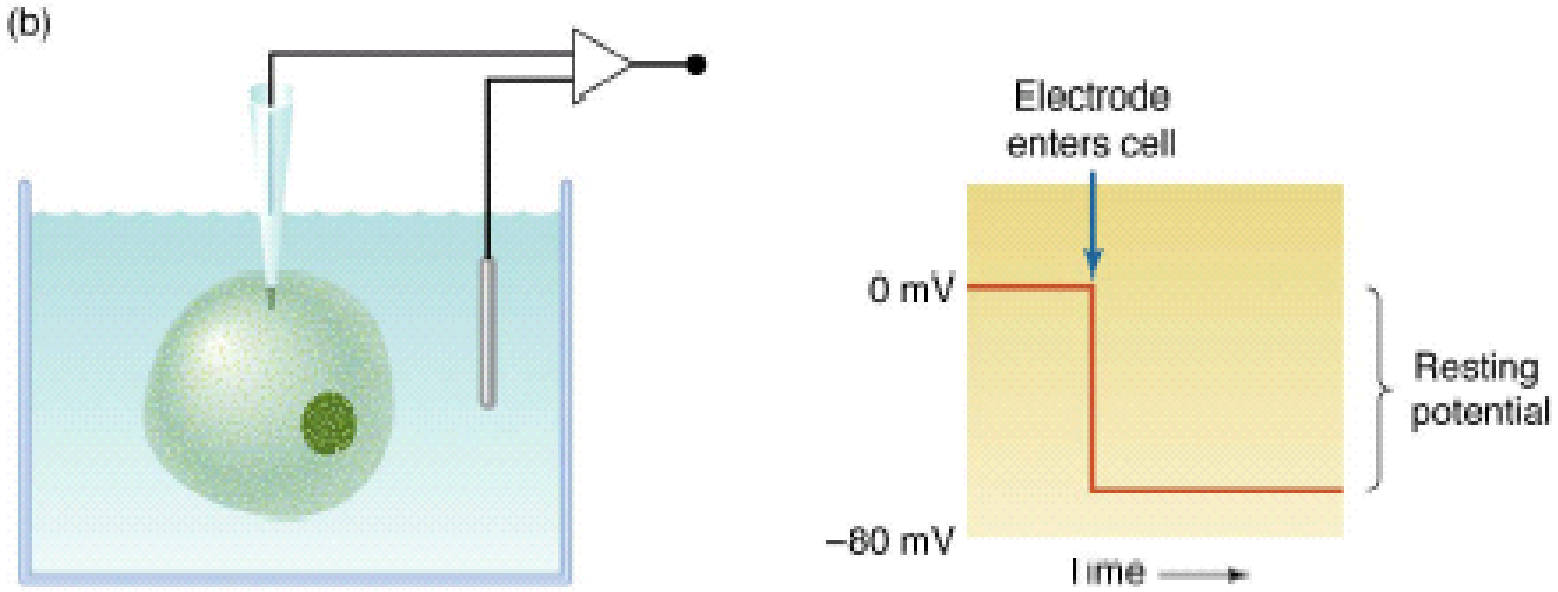


Every ion has its equilibrium potential.

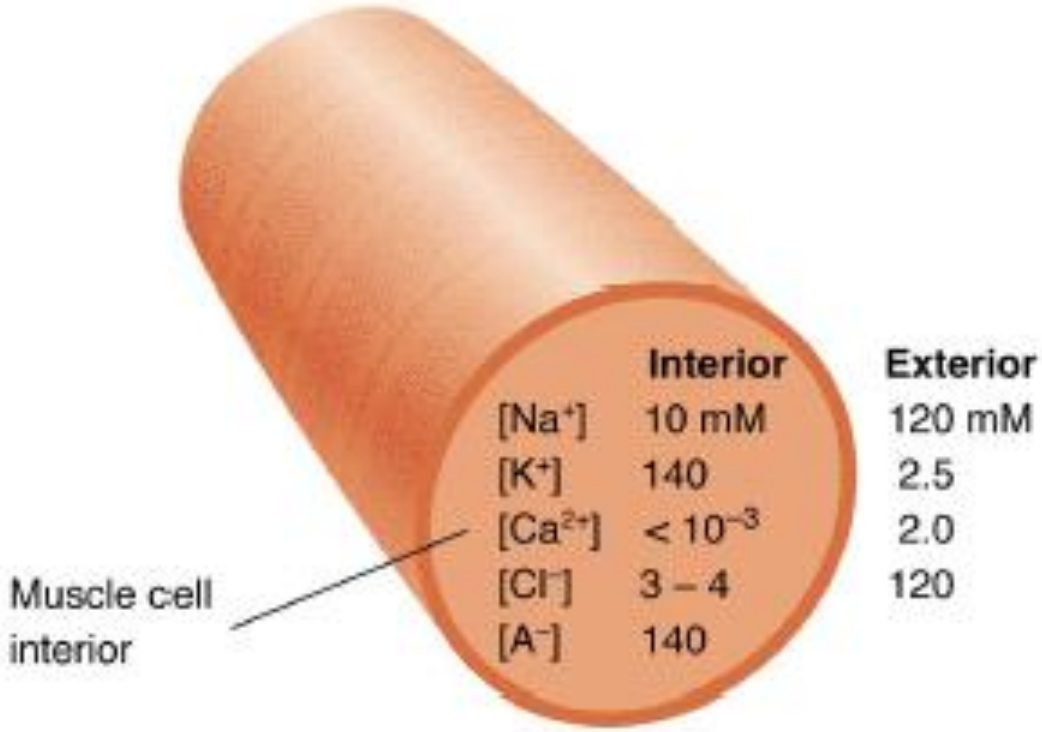
$$E_{mK} = \frac{RT}{F} \ln \frac{[K]_e}{[K]_i}$$

$$E_{mNa} = \frac{RT}{F} \ln \frac{[Na]_e}{[Na]_i}$$

$$E_{mCl} = \frac{RT}{F} \ln \frac{[Cl]_i}{[Cl]_e}$$

Cells have multiple ions to which membranes have varying permeabilities. Ions concentration and permeabilities are involved in establishing the membrane potential Emq that can be estimated using Goldman equation.

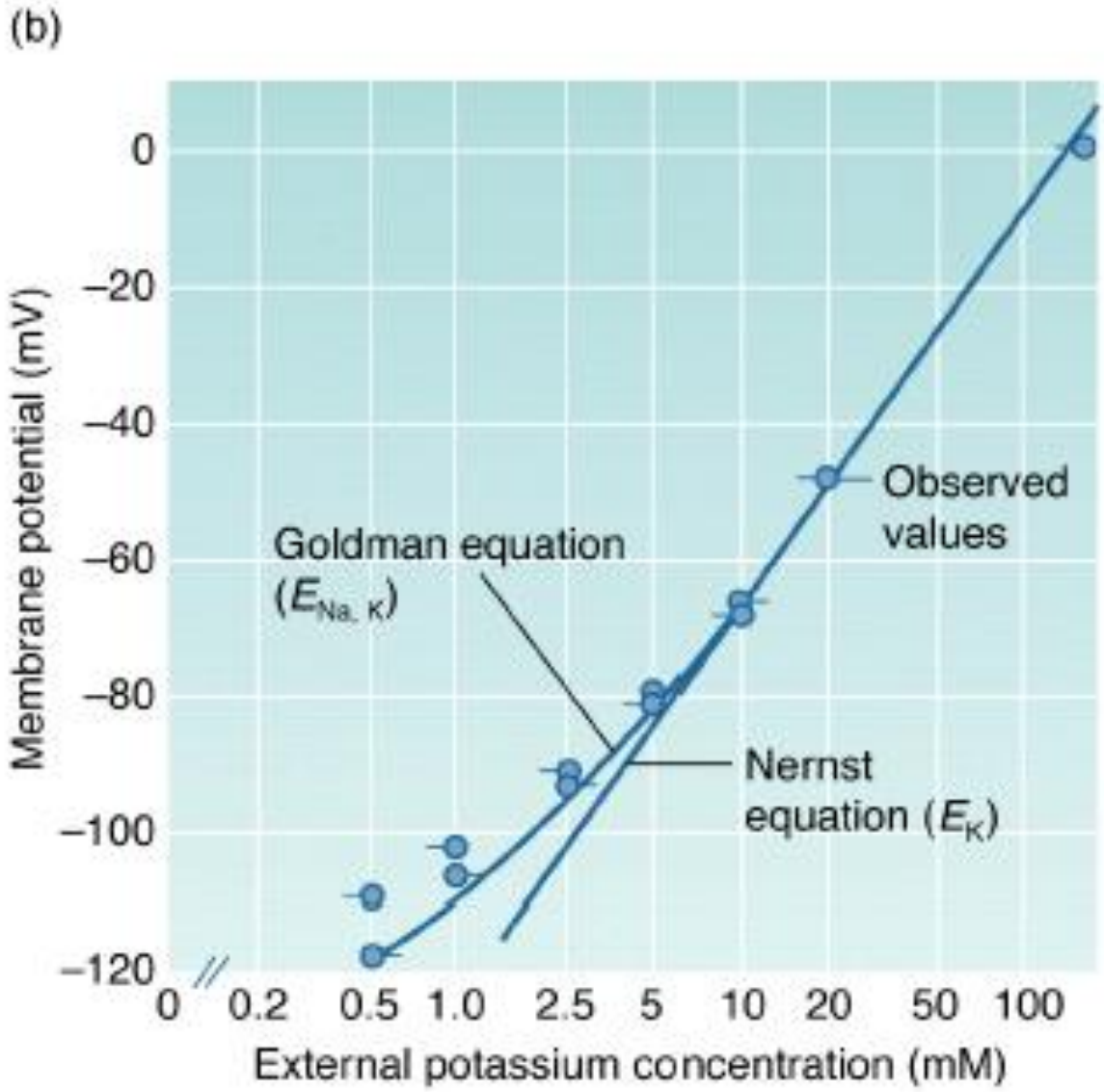
$$E_m = \frac{RT}{F} \ln \frac{P_K[K]_e + P_{Na}[Na]_e + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_e}$$



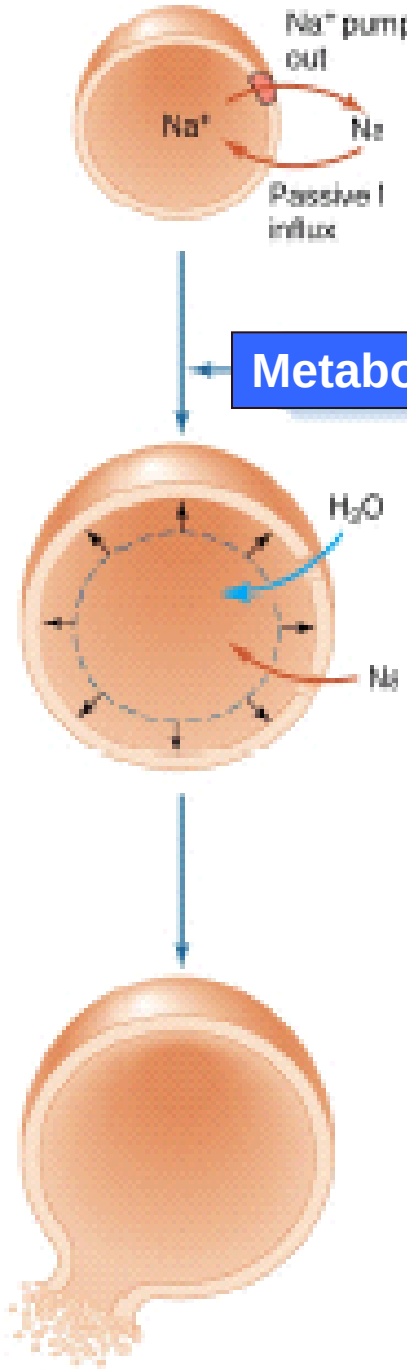
[A^-] = molar equivalent of negative charges carried by other molecules and ions.

$$E_m = \frac{RT}{F} \ln \frac{P_K[K]_e + P_{Na}[Na]_e + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_e}$$

$$E_{m=} 0.058 \lg \frac{1(2x5) + 0.01x120}{140 + 0.01x10} = -92 \text{ mV}$$


(b)
Membrane potential (mV)
0
–20
–40
–60
–80
–100
–120
0
0.2
0.5
1.0
2.5
5
10
20
50
100
External potassium concentration (mM)
Goldman equation
($E_{Na, K}$)
Nernst
equation (E_K)
Observed
values

Cells behave as if they were at Donnan equilibrium.



Normally, Na^+ levels are maintained at equilibrium as ion passively enters the cell and is pumped out

When inhibitor blocks active transport of Na^+ outward, the intracellular concentration of Na^+rises and water enters osmotically, increasing cell volume.

Eventually, increasing cell volume causes cell to burst.