Biophysics I (BPHS 3090)

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Diffusion Through Cell Membranes: History 101

Diffusion through Cell Membranes

Charles Ernest Overton (late 1800s): first systematic studies
- qualitative:
  - put cell in bath with solute
  - wait, rinse, squeeze
  - analyze to see how much got in (+ = some; +++ = a lot)
- 100's of solutes, dozens of cell types
- surprising results: previously cell membranes had been thought to be impermeant to essentially everything but water

Overton's Rules:
- cell membranes are semi-permeable
- relative permeabilities of plant and animals cells are similar
- permeabilities correlate with solubility of solute in organic solvents → membrane is lipid (specifically cholesterol and phospholipids)
- certain cells concentrate some solutes → active transport
- potency of anesthetics correlated with lipid solubility → Meyer-Overton theory of narcosis
- muscles don't contract in sodium-free media

Diffusion through Cell Membranes

Paul Runar Collander (1920-1950): first quantitative studies
- large cells (cylindrical algae cells, 1 mm diameter, 1 cm long)
- bathe cell in solute for time $t_1$, squeeze out cytoplasm, analyze
- repeat with new cell and new time $t_2$
- plot intracellular quantity versus time
- fit with exponential function of time (two-compartment theory)
- infer permeability from time constant
Membrane Diffusion: Two-Compartment Geometry

superscript indicates bath (inside or outside)

$c^i_n(t)$  $c_n(x,t)$  $c^o_n(t)$

inside  membrane  outside

$\phi_n(t)$

reference direction for flux is outward
Step 1: Dissolve

Equilibrium characterized by relative solubilities of solute $n$ in oil and water

partition coefficient $k_{oil:water} = \frac{c_{n}^{oil}}{c_{n}^{water}}$
Assume Dissolving is fast relative to diffusing

\[ k_{\text{membrane:bath}} = 2 \]
Step 2: Solute diffuses though membrane

$t = 0^+$

$t = 0^{++} > 0^+$

$t = \tau_{SS} >> 0^+$

steady state membrane time constant

$\tau_{SS} = \frac{d^2}{\pi^2 D}$
Step 3: Solute enters the cell

\[ c_n(x,t) = c_n(0,t) + \frac{x}{d} (c_n(d,t) - c_n(0,t)) \]
\[ = k_n c_n^i(t) + \frac{k_n x}{d} (c_n^o(t) - c_n^i(t)) \]

\[ k_n = k_{\text{membrane:bath}} = \frac{k_{\text{membrane:cytoplasm}}}{d} \]

Fick's law: \( \phi_n(t) = -D_n \frac{\partial c_n(x,t)}{\partial x} \)

\[ = -D_n \frac{c_n(d,t) - c_n(0,t)}{d} \]
\[ = D_n \frac{k_n}{d} (c_n^i(t) - c_n^o(t)) \]

\[ \phi_n(t) = P_n (c_n^i(t) - c_n^o(t)) \quad ; \quad P_n = \frac{D_n k_n}{d} \]

Fick's law for membranes

\( P_n \) = permeability of membrane to solute \( n \)
Step 4: Concentration in cell changes: two-compartment diffusion

Assume
- $V_i$ and $V_o$ constant
- well-stirred baths: $c_i^i(t)$, $c_o^o(t)$
- solute is conserved and membrane is thin: $c_i^i(t)V_i + c_o^o(t)V_o = N_n$
- membrane always in steady state: $\phi_n(t) = P_n(c_i^i(t) - c_o^o(t))$

By continuity,

$$A\phi_n(t) = -\frac{d}{dt}(c_i^i(t)V_i) = \frac{d}{dt}(c_o^o(t)V_o)$$

$$\frac{d}{dt}c_i^i(t) = -\frac{AP_n}{V_i}(c_i^i(t) - c_o^o(t)) = -\frac{AP_n}{V_i} \left( c_i^i(t) - \frac{1}{V_o} N_n + c_i^i(t) \frac{V_i}{V_o} \right)$$

$$\frac{d}{dt}c_i^i(t) + AP_n\left(\frac{1}{V_i} + \frac{1}{V_o}\right)c_i^i(t) = \frac{AP_n N_n}{V_i V_o}$$

First-order linear differential equation with constant coefficients, therefore

$$c_i^i(t) = c_i^i(\infty) + [c_i^i(0) - c_i^i(\infty)]e^{-t/\tau_EQ}$$

$$c_i^i(\infty) = \frac{N_n}{V_i + V_o}$$

$$\tau_EQ = \frac{1}{AP_n\left(\frac{1}{V_i} + \frac{1}{V_o}\right)}$$
Membrane Diffusion: Summary

Membrane diffusion

Dissolve and diffuse model
- solute outside cell dissolves into cell membrane
- solute diffuses through membrane
- solute dissolves into cytoplasm

Membrane time constant \( t_{SS} = \frac{d^2}{\pi^2 D_n} \)

Fick's law for membranes: \( \phi_n(t) = P_n (c^i_n(t) - c^o_n(t)) \); \( P_n = \frac{D_n k_n}{d} \)

Two-compartment diffusion

Cell time constant \( t_{EQ} = \frac{1}{A P_n \left( \frac{1}{V_o} + \frac{1}{V_i} \right)} \)
Dynamics of Membrane Diffusion

- Numerical solution to eqns.
- Arbitrary initial condition (top)
- Fast dynamics (middle)
- Steady-state set up (middle)
- Eventually, the two compartments change (bottom)

Figure 3.30
Effect of changing parameters on flux: What is being changed?

\[ \phi_n(t) = P_n (c_n^i(t) - c_n^o(t)) \quad ; \quad P_n = \frac{D_n k_n}{d} \]

Fick's law for membranes

\( P_n \) = permeability of membrane to solute \( n \)
\[ \phi_n(t) = P_n \left( c_n^i(t) - c_n^o(t) \right) ; \quad P_n = \frac{D_n k_n}{d} \]

Fick's law for membranes

\( P_n \) = permeability of membrane to solute \( n \)
Question(s)

→ What are cell membranes made of?

→ How does one go about determining such?

→ It is only relatively recently we had a picture such as this!!
Empirical means to estimate cell diffusion?

Figure 3.34

Flow cytometer

Figure 3.35
Diffusion of ethylene glycol through *Chara* membrane (Collander) (see Weiss eqns. 3.56, 3.58, 3.60)

- strong correlation between solute permeability and solute ether:water partition coefficient
- supports Overton’s rules & dissolve-diffuse mechanism
- relates in molecular weight (i.e., there is a strong ‘physical’ aspect to line of thought)

→ Raises question as to what solvent best resembles partitioning in actual membranes
These figures represent the key empirical observations leading up to the deduction of what constitutes the cell membrane.
- Diffusion is slow over long distances (e.g., neuron carrying information to and from the toe to the base of the spinal cord)

- So how else might things get across a cell membrane? Could such a mechanism speed up ‘transport’?

⇒ Specialized ion channels (permeability unique to different ions)
Figure 2.19

- Dissolution and diffusion through lipid bilayer
- Transport through water channels
- Transport through gated ion channels
- Carrier-mediated transport
- Pumps
Exercise

Two adjoining cells have closely apposed membranes. The concentrations of uncharged solutes $n$ are $c_n^1$ and $c_n^2$ inside cells 1 and 2, respectively, and $c_n^o$ in the intercellular space. The membrane permeabilities for this solute are $P_1$ and $P_2$ for the membranes of cell 1 and 2, respectively. Find the net permeability, $P$, between the inside of cell 1 and the inside of cell 2 in terms of $P_1$ and $P_2$, where

$$\phi_n = P \left( c_n^1 - c_n^2 \right)$$

and $\phi_n$ is the steady-state flux of $n$ in mol/(cm$^2$·s) across both membranes.