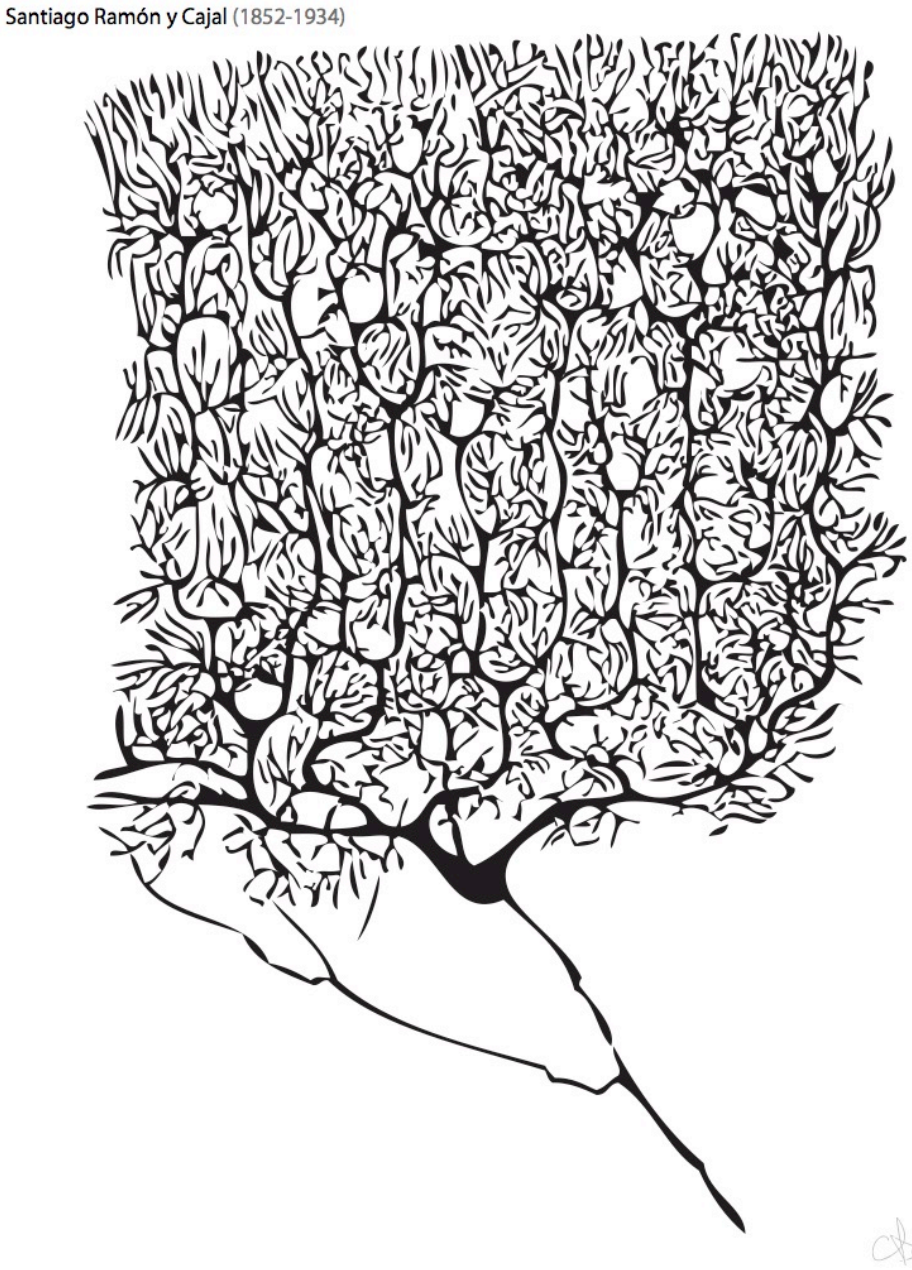
Cellular Electrodynamics



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York University
Winter 2020
BPHS 4080 Lecture 3

Reference/Acknowledgement:
- TF Weiss (Cellular Biophysics)
- D Freeman

Diffusion equation

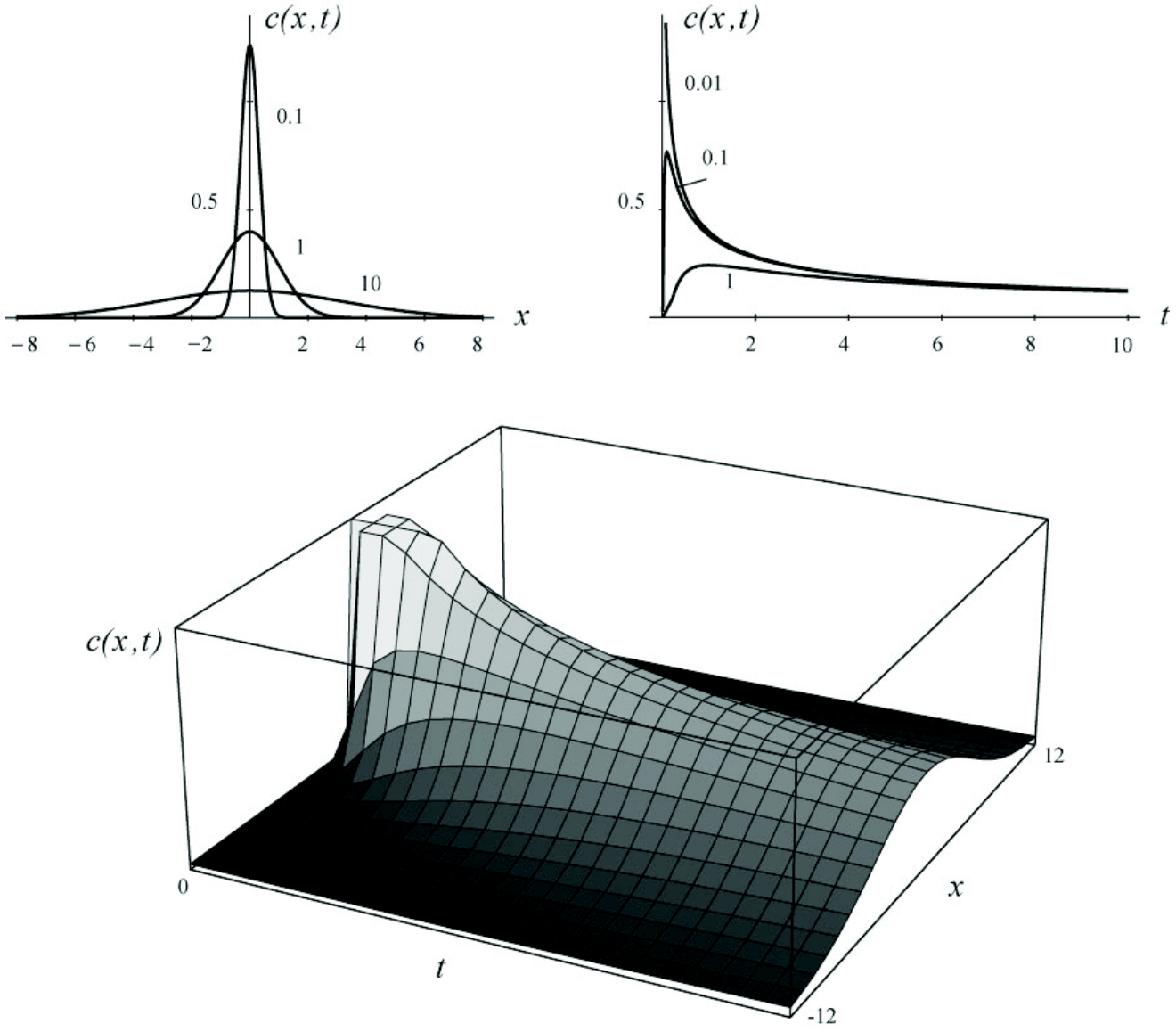
1. Fick's First Law: $$\phi = -D\frac{\partial c}{\partial x}$$

+

2. Continuity Equation: $$\frac{\partial \phi}{\partial x} = -\frac{\partial c}{\partial t}$$

$$\boxed{\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}}$$

(Fick's Second Law)



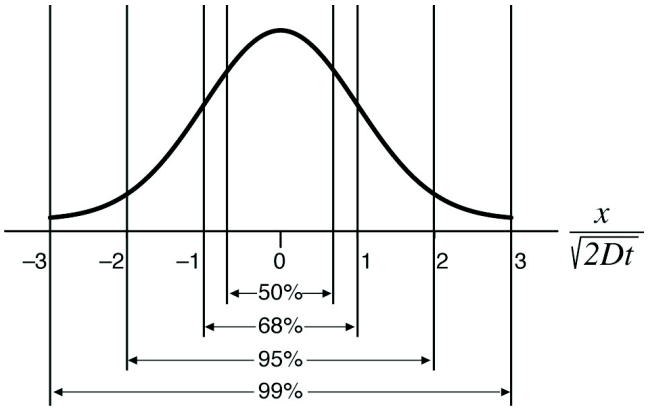
Weiss Fig.3.14 (modified)

Importance of Scale

$$c(x,t) = \frac{n_o}{\sqrt{4\pi Dt}} e^{-x^2/4Dt}$$

Gaussian function with zero mean and standard deviation:

$$\sigma = \sqrt{2Dt}$$



Question: How long does it take ($t_{1/2}$) for ~1/2 the solute to move at least the distance $x_{1/2}$?

$$\frac{x_{1/2}}{\sqrt{2Dt_{1/2}}} \approx \frac{2}{3} \quad \Longrightarrow \quad \boxed{t_{1/2} \approx \frac{x_{1/2}^2}{D}}$$

For small solutes (e.g. K^+ at body temperature) $D \approx 10^{-5} \ \frac{\text{cm}^2}{\text{s}}$

	$x_{1/2}$	$t_{1/2}$
membrane sized	10 nm	$\frac{1}{10}$ μsec
cell sized	10 μm	$\frac{1}{10}$ sec
dime sized	10 mm	10^5 sec ≈ 1 day

Freeman

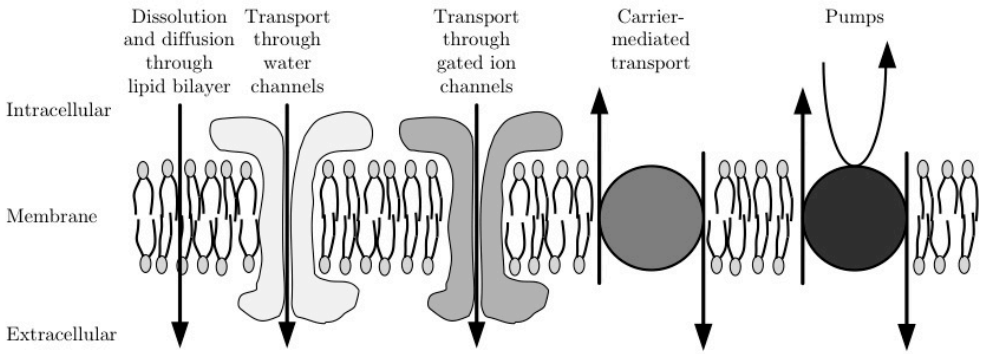
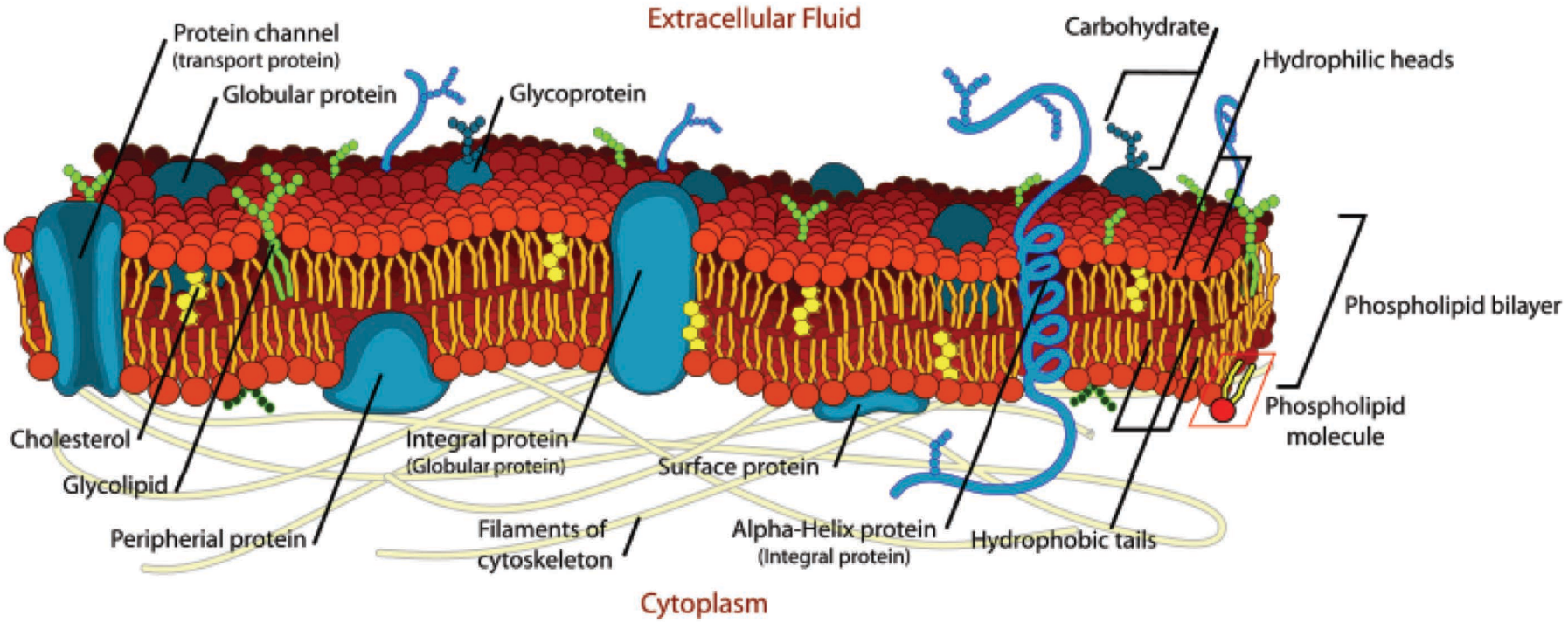
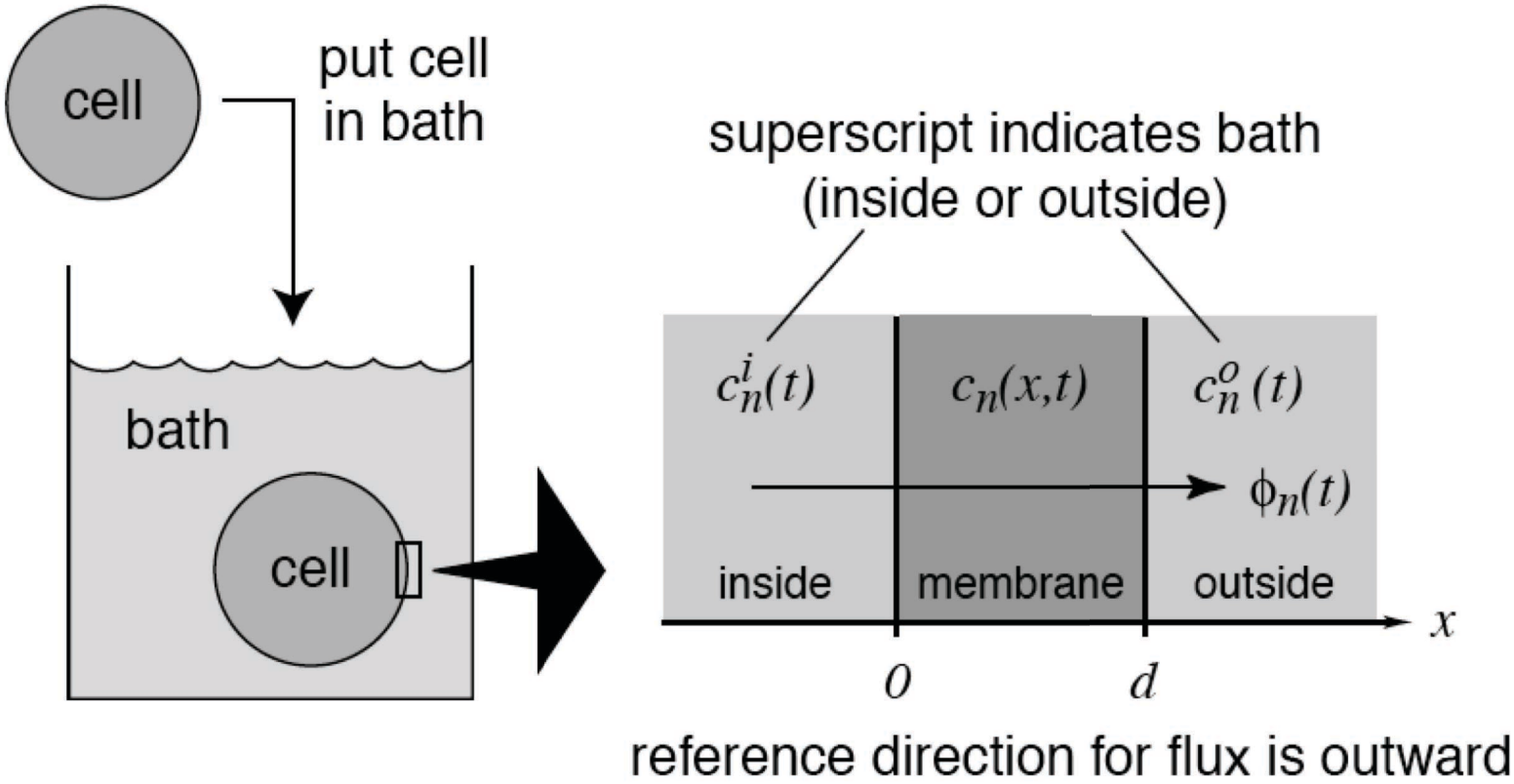


Figure 2.19



Wikipedia

Membrane Diffusion: Two-Compartment Geometry



reference direction for flux is outward

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:

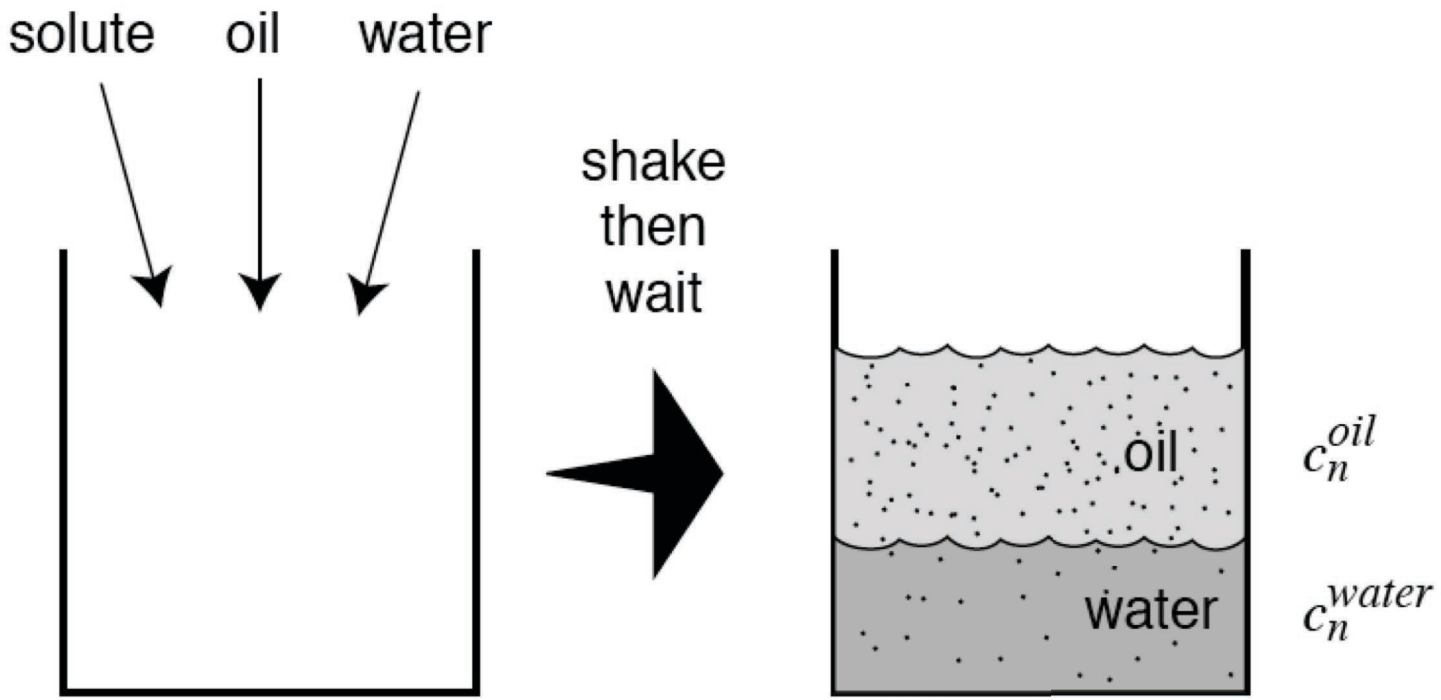
- cell membranes are semi-permeable
- relative permeabilities of plant and animals cells are similar
- permeabilities correlate with solubility of solute in organic solvents
  → membrane is lipid (specifically cholesterol and phospholipids)
- certain cells concentrate some solutes → active transport
- 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

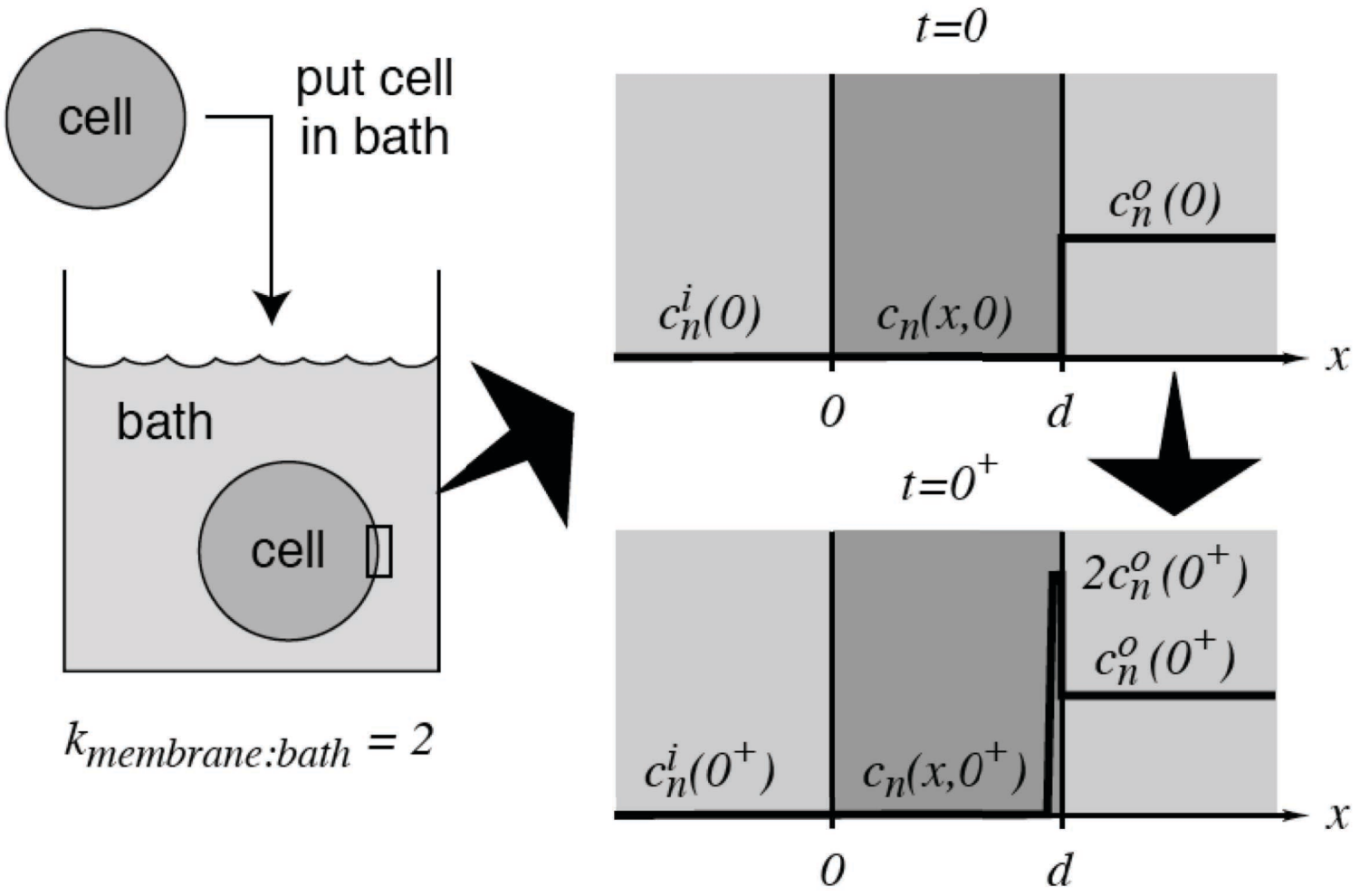
Step 1: Dissolve



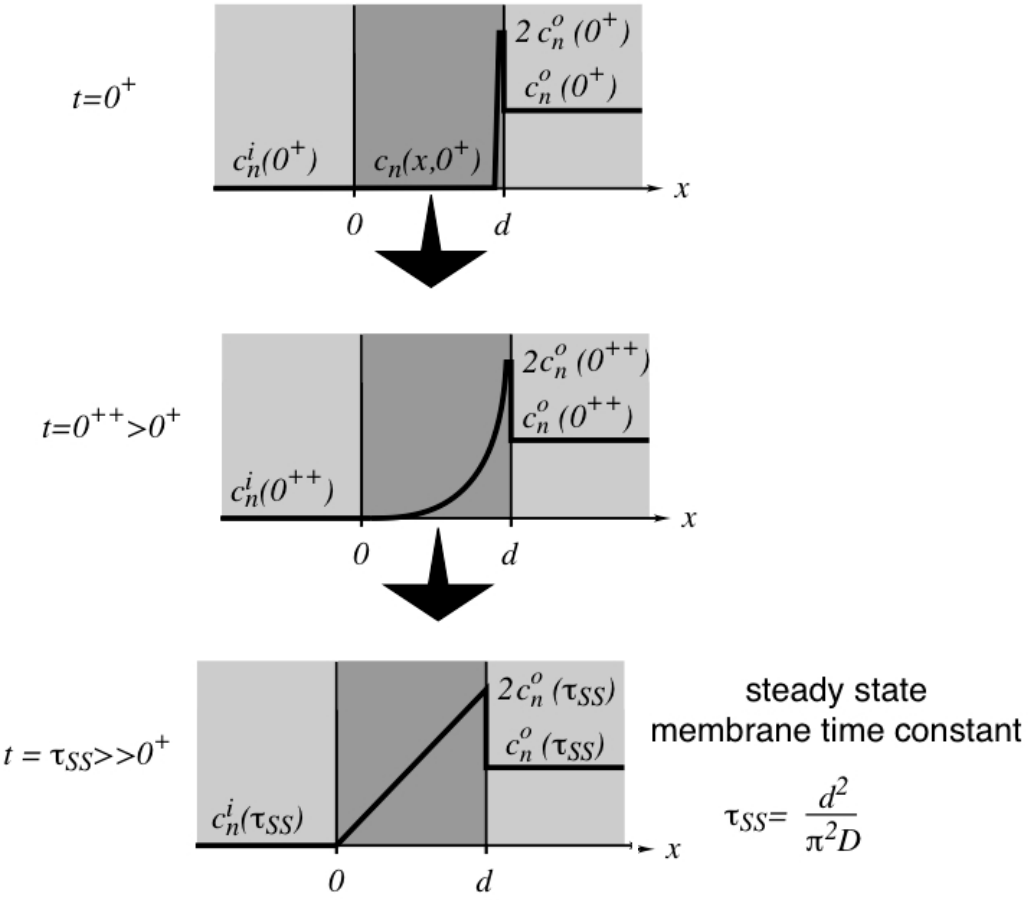
Equilibrium characterized by relative solubilities of solute n in oil and water

$$\text{partition coefficient } k_{oil:water} = \frac{c_n^{oil}}{c_n^{water}}$$

Assume Dissolving is fast relative to diffusing



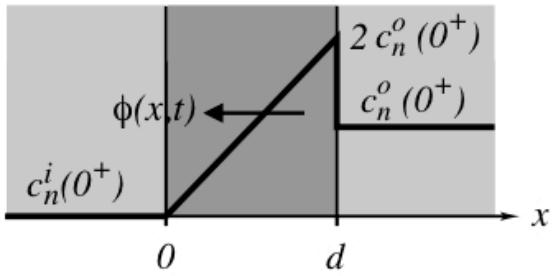
Step 2: Solute diffuses though membrane



steady state
membrane time constant

$$\tau_{SS}=\frac{d^2}{\pi^2 D}$$

Step 3: Solute enters the cell



$$c_n(x,t) = c_n(0,t) + \frac{x}{d}(c_n(d,t) - c_n(0,t)\,)$$

$$= k_n c_n^i(t) + \frac{k_n x}{d}(c_n^o(t) - c_n{}^i(t))$$

$k_n = k_{membrane:bath}$

$= k_{membrane:cytoplasm}$

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

$$= -D_n \frac{c_n(d,t) - c_n(0,t)}{d}$$

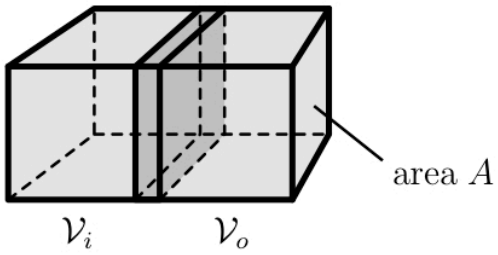
$$= \frac{D_n k_n}{d}(c_n{}^i(t) - c_n^o(t))$$

$$\boxed{\phi_n(t) = P_n\,(c_n{}^i(t) - c_n^o(t)) \;\; ; \;\; P_n = \frac{D_n k_n}{d}}$$

Fick's law for membranes

P_n = permeability of membrane to solute n

Step 4: Concentration in cell changes: two-compartment diffusion



Assume

- $\mathcal{V}_i$ and $\mathcal{V}_o$ constant
- well-stirred baths: $c_n^i(t)$, $c_n^o(t)$
- solute is conserved and membrane is thin: $c_n^i(t)\mathcal{V}_i + c_n^o(t)\mathcal{V}_o = N_n$
- membrane always in steady state: $\phi_n(t) = P_n(c_n^i(t) - c_n^o(t))$

By continuity,

$$A\phi_n(t) = -\frac{d}{dt}(c_n^i(t)\mathcal{V}_i) = \frac{d}{dt}(c_n^o(t)\mathcal{V}_o)$$

$$\frac{d}{dt}c_n^i(t) = -\frac{AP_n}{\mathcal{V}_i}(c_n^i(t) - c_n^o(t)) = -\frac{AP_n}{\mathcal{V}_i}\left(c_n^i(t) - \frac{1}{\mathcal{V}_o}N_n + c_n^i(t)\frac{\mathcal{V}_i}{\mathcal{V}_o}\right)$$

$$\frac{d}{dt}c_n^i(t) + AP_n(\frac{1}{\mathcal{V}_i} + \frac{1}{\mathcal{V}_o})c_n^i(t) = \frac{AP_nN_n}{\mathcal{V}_i\mathcal{V}_o}$$

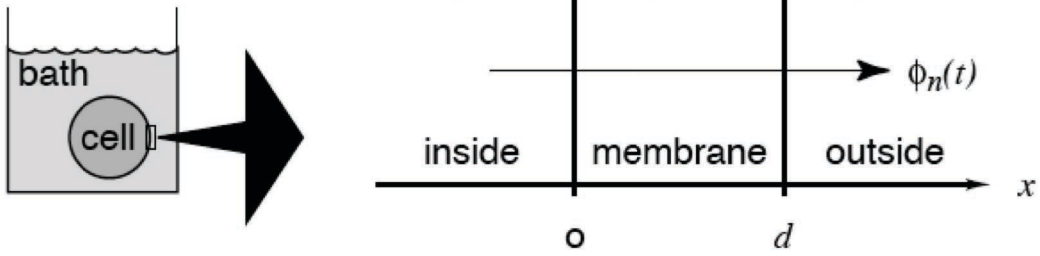
First-order linear differential equation with constant coefficients, therefore

$$c_n^i(t) = c_n^i(\infty) + [c_n^i(0) - c_n^i(\infty)]e^{-t/\tau_{EQ}}$$

$$c_n^i(\infty) = \frac{N_n}{\mathcal{V}_i + \mathcal{V}_o} \qquad \tau_{EQ} = \frac{1}{AP_n(\frac{1}{\mathcal{V}_i} + \frac{1}{\mathcal{V}_o})}$$

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 $t_{SS} = \dfrac{d^2}{\pi^2 D_n}$

Fick's law for membranes: $\phi_n(t) = P_n\,(c_n^i(t) - c_n^o(t))\;;\quad P_n = \dfrac{D_n k_n}{d}$

Two-compartment diffusion

Cell time constant $t_{EQ} = \dfrac{1}{AP_n\left(\dfrac{1}{V_o} + \dfrac{1}{V_i}\right)}$

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)

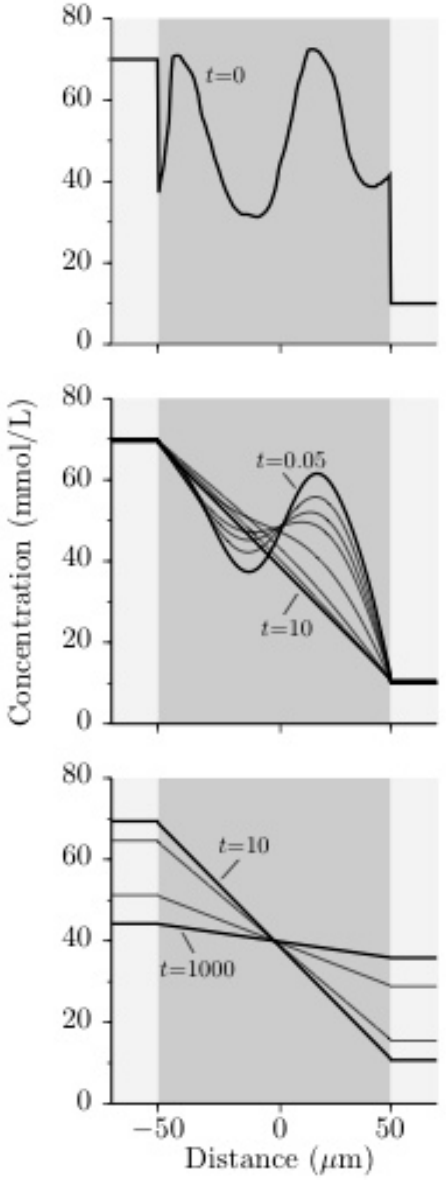
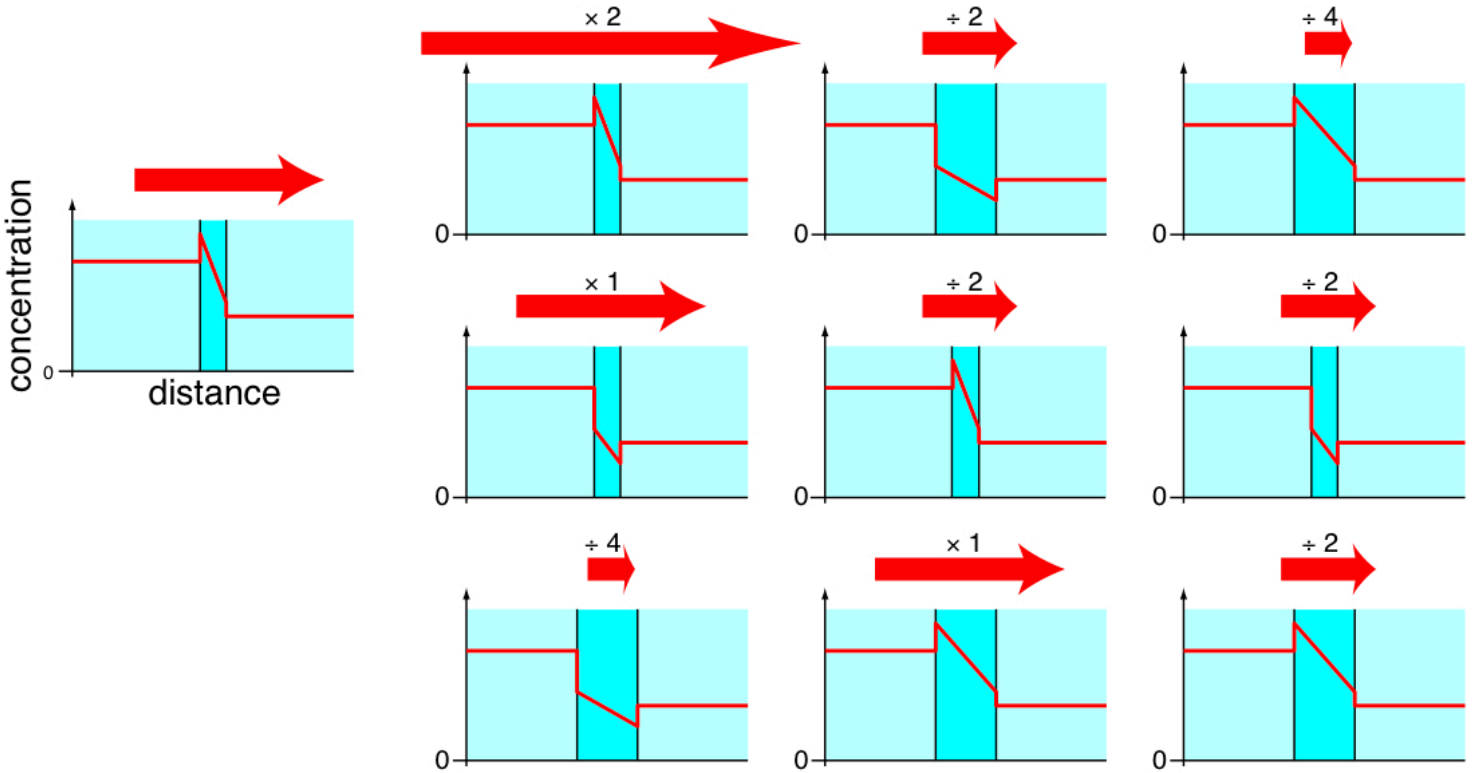


Figure 3.30

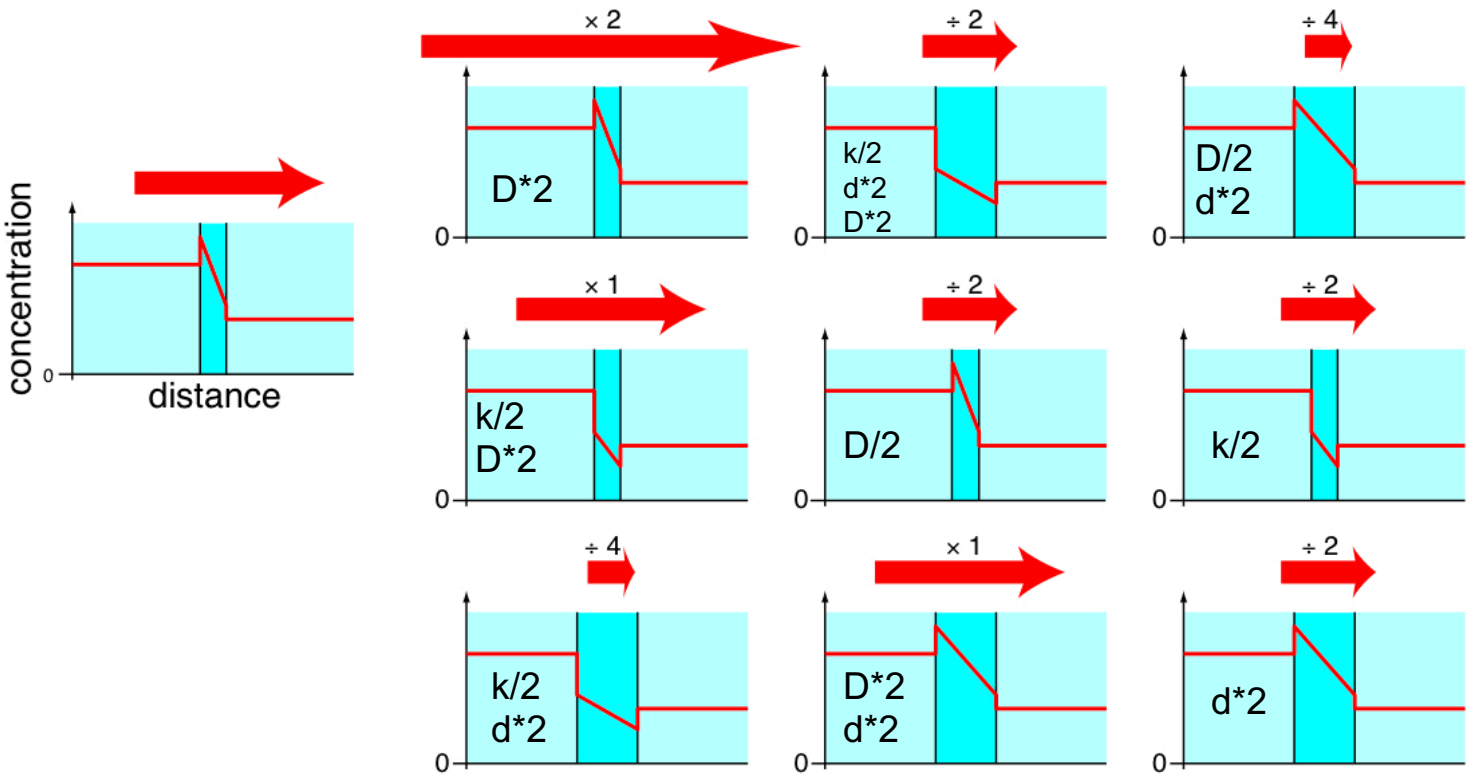
Effect of changing parameters on flux: What is being changed?



$$\phi_n(t) = P_n\,(c_n^{\,i}(t) - c_n^{o}(t)) \;\; ; \;\; P_n = \frac{D_n k_n}{d}$$

Fick's law for membranes

P_n = permeability of membrane to solute n



$$\phi_n(t) = P_n\,(c_n^{\,i}(t) - c_n^{o}(t)) \;\; ; \;\; P_n = \frac{D_n k_n}{d}$$

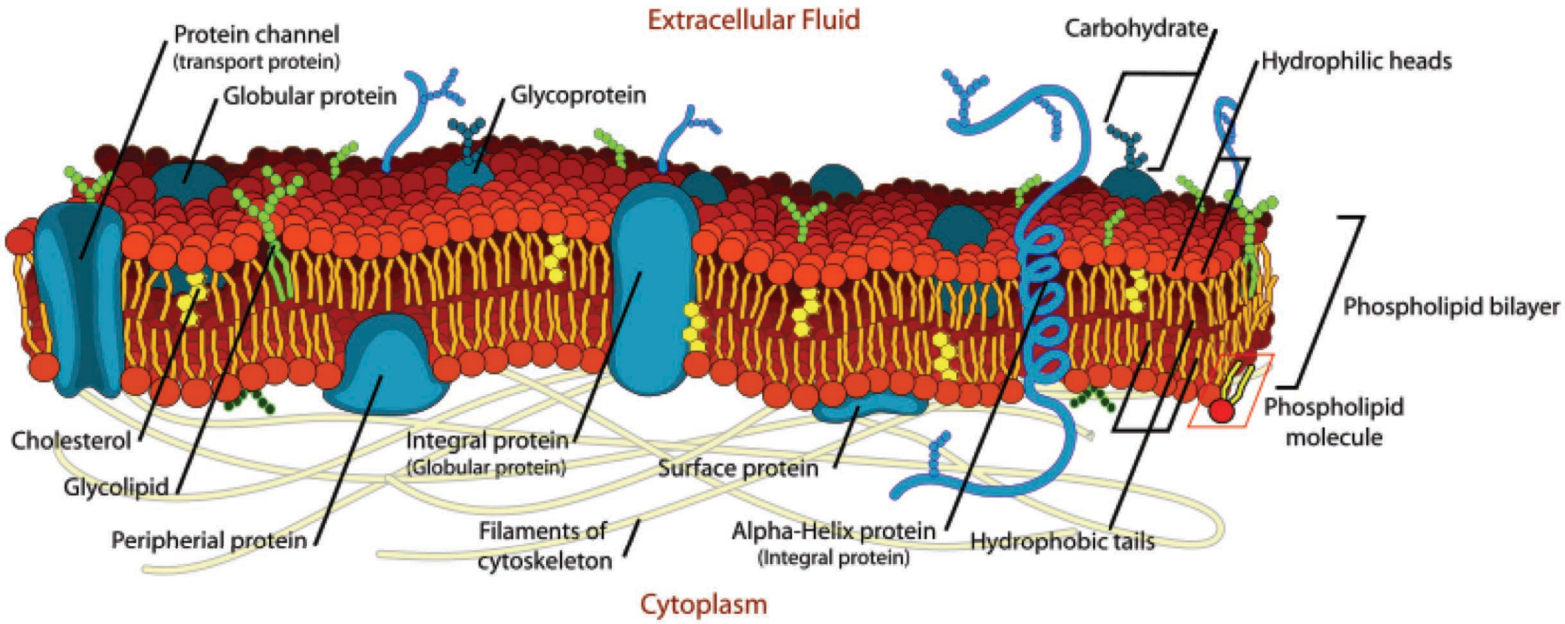
Fick's law for membranes

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!!

Wikipedia

Empirical means to estimate cell diffusion?

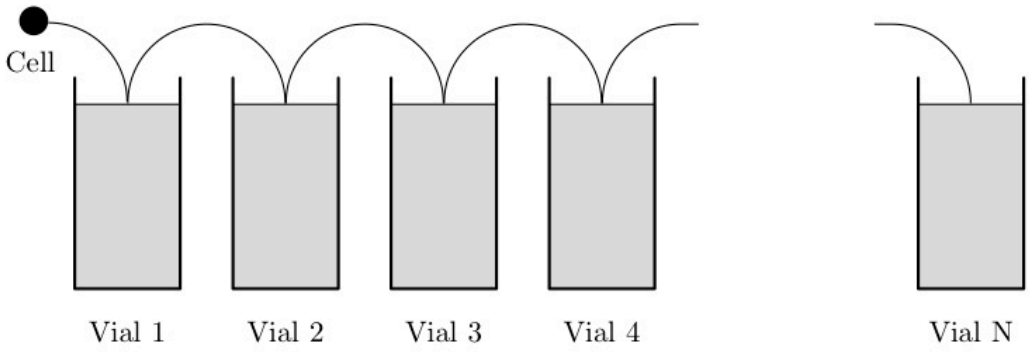


Figure 3.34

Flow cytometer

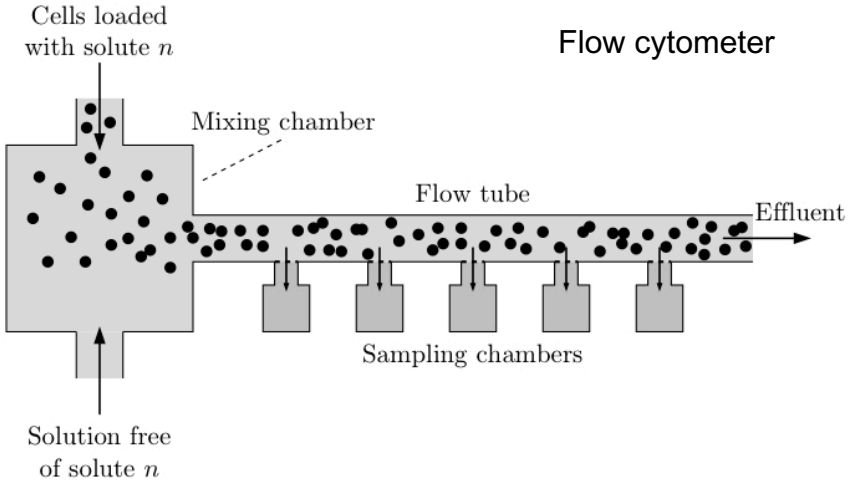


Figure 3.35

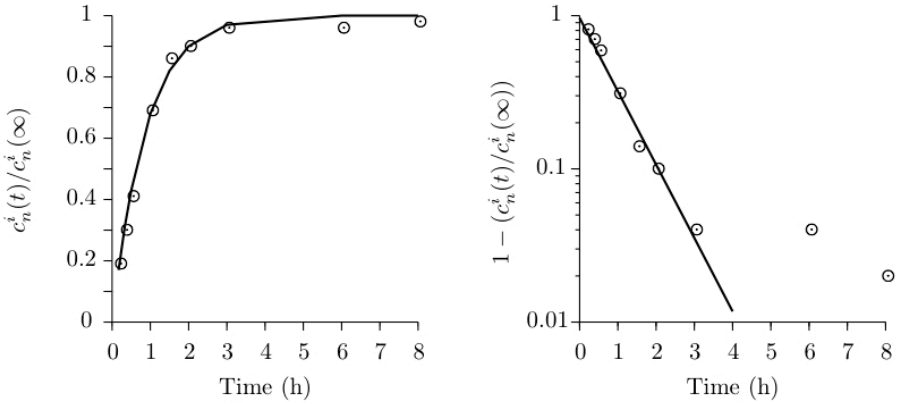


Figure 3.33

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

'Collander Plot'
(see Weiss sec.3.8.4)

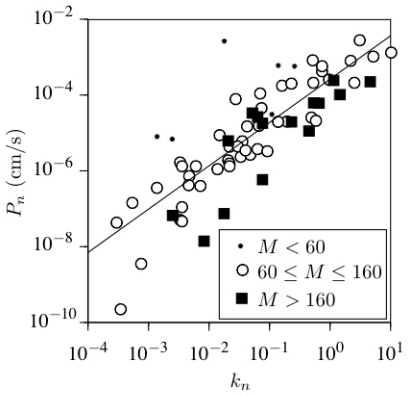


Figure 3.38

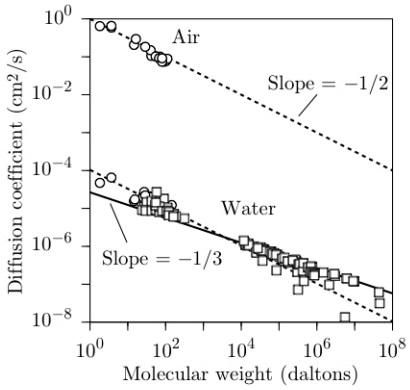


Figure 3.11

- 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)

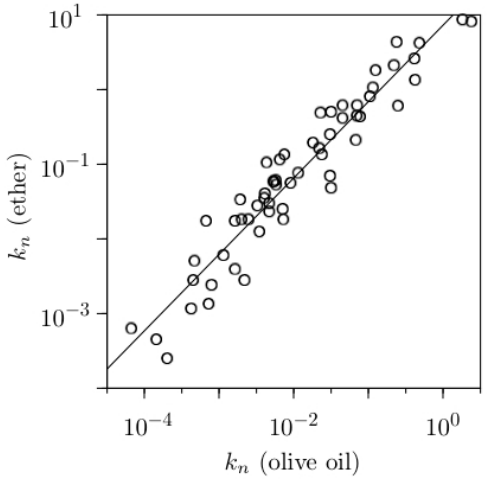


Figure 3.39

→ Raises question as to what solvent best resembles partitioning in actual membranes

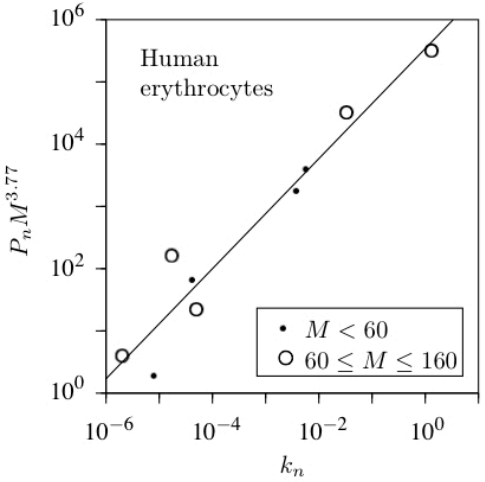


Figure 3.42

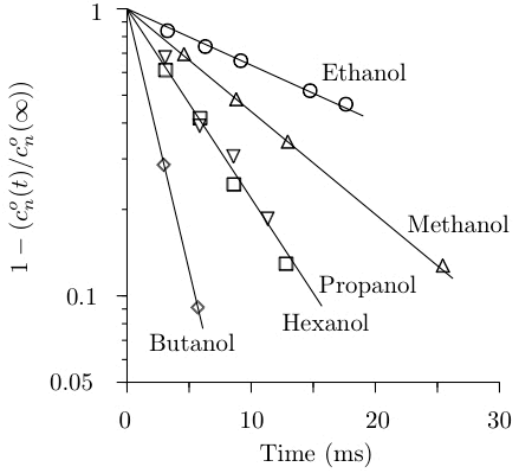


Figure 3.36

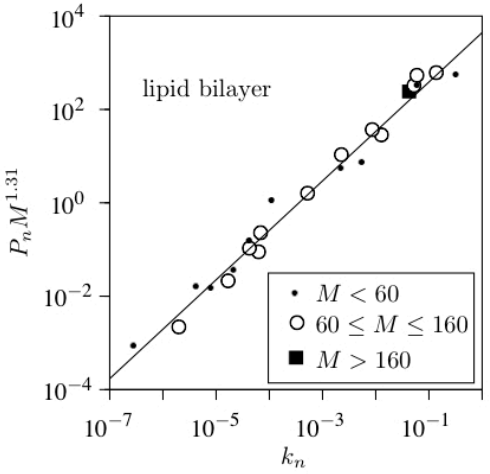


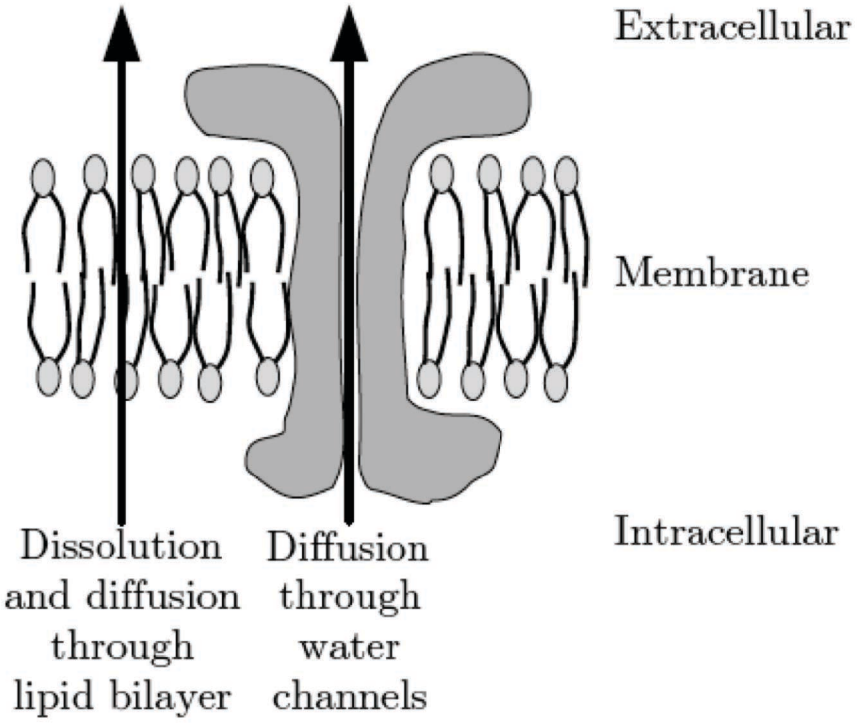
Figure 3.43

→ 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'?

$\Rightarrow$ Specialized ion channels (permeability unique to different ions)



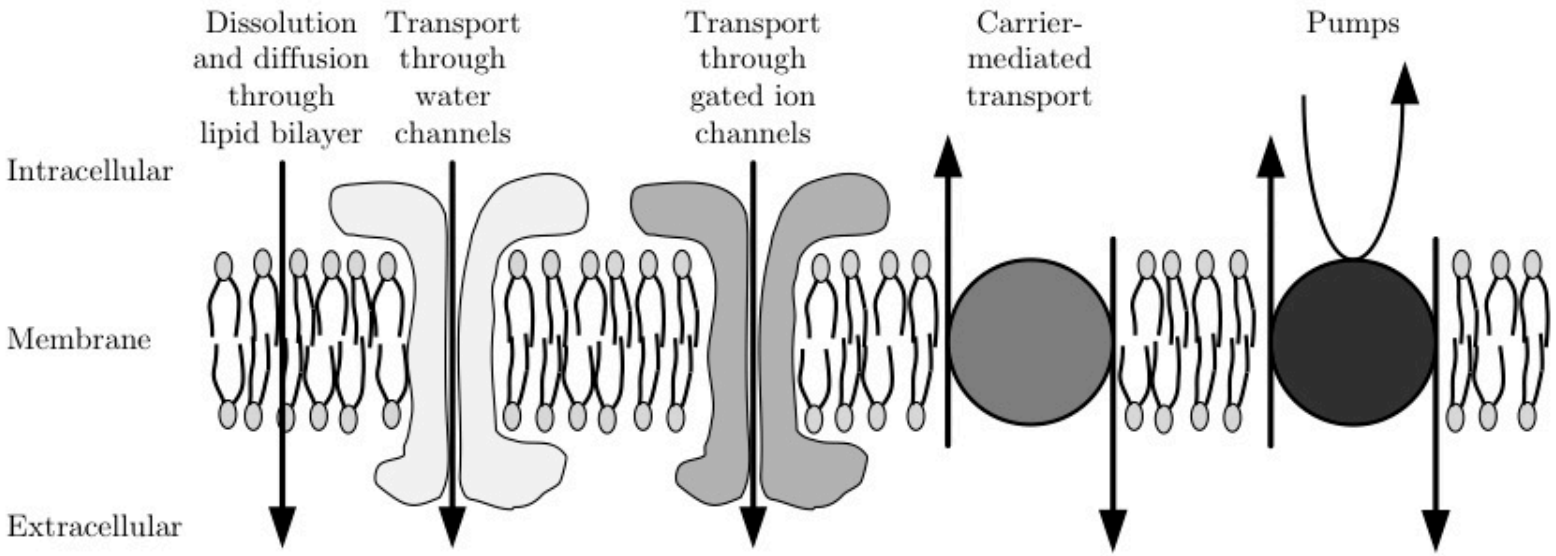
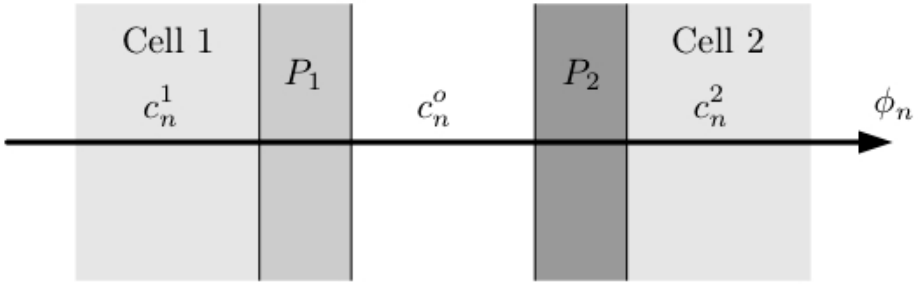

Dissolution and diffusion through lipid bilayer
Transport through water channels
Transport through gated ion channels
Carrier-mediated transport
Pumps
Intracellular
Membrane
Extracellular

Figure 2.19

Exercise



Two adjoining cells have closely apposed membranes. The concentrations of uncharged solutes n are $c^1{}_n$ and $c^2{}_n$ inside cells 1 and 2, respectively, and $c^o{}_n$ 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

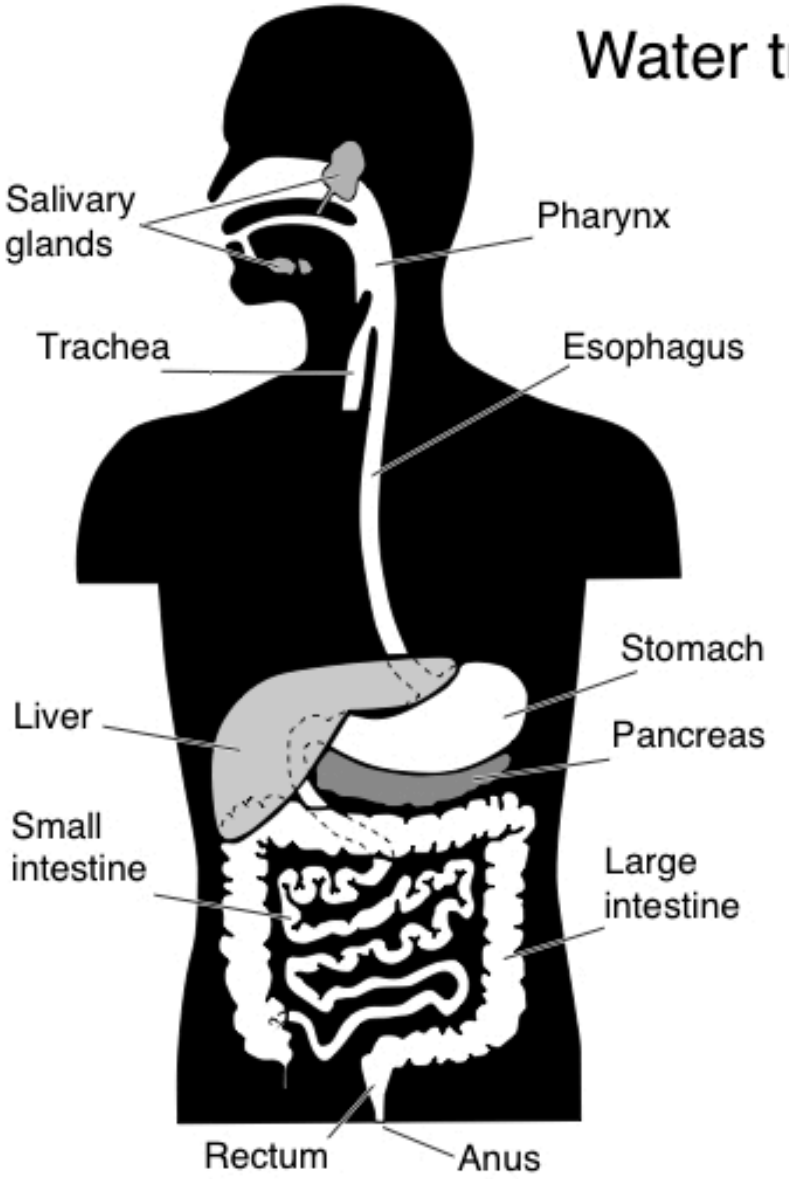
$$\phi_n = P\left(c_n^1 - c_n^2\right)$$

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

On a drizzly day toward the end of the week, Luce walked them in the woods, making water the topic of her ramble. It's what makes life so rampant around here, she said... All the moons from spring to early fall, everything plumps with water. Think jungle, and then go a degree onward in the direction of a deep green world so wet you could wring it out like a dishrag if you could get a good grip on either end of it. Giant hemlocks and sycamores and tulip trees. Rhododendrons. Moss and ferns. Understory too thick to see more than twenty feet into the woods, until killing frosts reveal the bones of the place. A steamy greenhouse of plants and creatures. Flip any rock or dead log, and myriad beings go crawling down individual vectors toward the darkness they crave. Sit in a yellow sunbeam, and the damp air around you thickens with myriad beings dancing up into the daylight they love. Life likes the wet and rewards it. Archaic forms incompatible with the modern world persist here. Hellbenders, deep in the creek beds. Panthers, high on the ridges. Even dead blighted chestnuts resurrect themselves out of the black forest floor, refusing to accept the terms of their extinction. Hope incarnate. All, Luce explained, due to moisture.

- Charles Frazier
(Nightwoods)

Water transport in digestive system



Daily traffic

- 800 g food + 1.2 L water ingested daily
- 1.5 L saliva
- 2 L gastric secretions
- 0.5 L bile
- 1.5 L pancreatic secretions
- 1.5 L intestinal secretions

} 7 L digestive fluids

15 pounds of water (10% of body weight) secreted and reabsorbed daily

Low Humidity Stimulates Epidermal DNA Synthesis and Amplifies the Hyperproliferative Response to Barrier Disruption: Implication for Seasonal Exacerbations of Inflammatory Dermatoses

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Shiseido Research Center, Skin Biology Research Laboratories, Yokohama, Japan; *Dermatology and †Medical (Metabolism Section) Services, Veterans Affairs Medical Center, San Francisco, and Departments of §Dermatology and ¶Medicine, University of California, San Francisco, U.S.A.

Although seasonal changes in humidity are thought to exacerbate various skin diseases, whether these flares can be attributed to prolonged exposure to extremes in environmental humidities has not been studied systematically. We recently showed that prolonged exposure to high *versus* low humidities induced profound changes in epidermal structure and permeability barrier homeostasis. Therefore, we asked here whether comparable extremes in humidity could initiate not only homeostatic, but also potentially pathophysiologic alterations. We showed first that exposure to low humidity increases epidermal DNA synthesis in normal murine epidermis. Moreover, exposure to a low humidity for 48 h further amplifies the DNA synthetic response to barrier disruption, resulting in marked epidermal hyperplasia. Additionally, exposure to a dry environment for 48 h prior to barrier disruption results in dermal mast cell hypertrophy, degranulation, as well as histologic evidence of inflammation. To demonstrate the role of changes in external moisture on these phenomena, we applied either an occlusive, water-impermeable plastic membrane, Petrolatum, or a nonocclusive humectant, both to nonperturbated and to perturbed skin. All three forms of treatment prevented the epidermal hyperplasia and dermal mast cell hypertrophy and degranulation induced by exposure to low humidity. These studies indicate that (i) exposure to changes in environmental humidity alone induces increased keratinocyte proliferation and markers of inflammation, and (ii) that these changes are attributable to changes in stratum corneum moisture content. Finally, these studies provide evidence that changes in environmental humidity contribute to the seasonal exacerbations/amelioration of cutaneous disorders, such as atopic dermatitis and psoriasis, diseases which are characterized by a defective barrier, epidermal hyperplasia, and inflammation. ***Key words: dry environment/humectant/mast cell occlusion. J Invest Dermatol 111:873–878, 1998***

The main function of the skin is to generate the epidermal permeability barrier at the level of the stratum corneum (SC), which allows life in a terrestrial environment. Acute barrier disruption by organic solvents, detergents, or tape stripping elicits a homeostatic repair response in the epidermis, which rapidly restores normal barrier function (Elias and Feingold, 1992). Repeated perturbations of the barrier induce cutaneous pathology, including epidermal hyperplasia and cutaneous inflammation (Denda *et al*, 1996).

Seasonal changes effect the condition of normal skin and may trigger various cutaneous disorders (Wilkinson and Rycroft, 1992; Sauer and Hall, 1996). In common dermatoses, such as atopic dermatitis or psoriasis, a decline in barrier function often parallels increased severity of clinical symptomatology (Grice, 1980; Pinnagoda *et al*, 1989). These conditions all tend to worsen during the winter season, when humidity is lower (Wilkinson and Rycroft, 1992; Sauer and Hall, 1996). Abundant indirect evidence suggests that decreased humidity precipitates these disorders (Rycroft and Smith, 1980), whereas in contrast, increased skin hydration appears to ameliorate these conditions (Chernosky, 1976; Rawlings *et al*, 1994). The mechanism(s) by which alterations in relative humidity might influence cutaneous function and induce cutaneous pathology are poorly understood. We recently showed that prolonged exposure of normal murine skin to a dry environment produced an increase in SC weight and thickness, with a commensurate reduction in basal transepidermal water loss (TEWL) (Denda *et al*, 1998).

Yet, whether exposure to a dry environment alone can also induce pathophysiologic changes is not yet known. In order to determine the possibility that changes in environmental humidity might initiate and/or aggravate cutaneous pathophysiology, we examined the effects of alterations in environmental humidities on epidermal DNA synthesis, epidermal hyperplasia, and mast cell number and degranulation in both normal hairless mice and mice with experimentally induced barrier defects. Our results show first, that changes in environmental humidity alone can modulate epidermal proliferation; and second, that a low humidity, when superimposed on a defective barrier, provokes further pathophysiologic changes. Together, these studies provide strong support for the hypothesis that seasonal exacerbations/aggravation of cutaneous dermatoses are attributable to decreased humidity.

MATERIALS AND METHODS

Animals Hairless mice, 7–10 wk old (HR-1, Hoshino, Japan), were used. Before experiments, animals were caged separately for at least 4 d. These cages

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Abbreviation: TEWL, transepidermal water loss.

0022-202X/98/$10.50 •

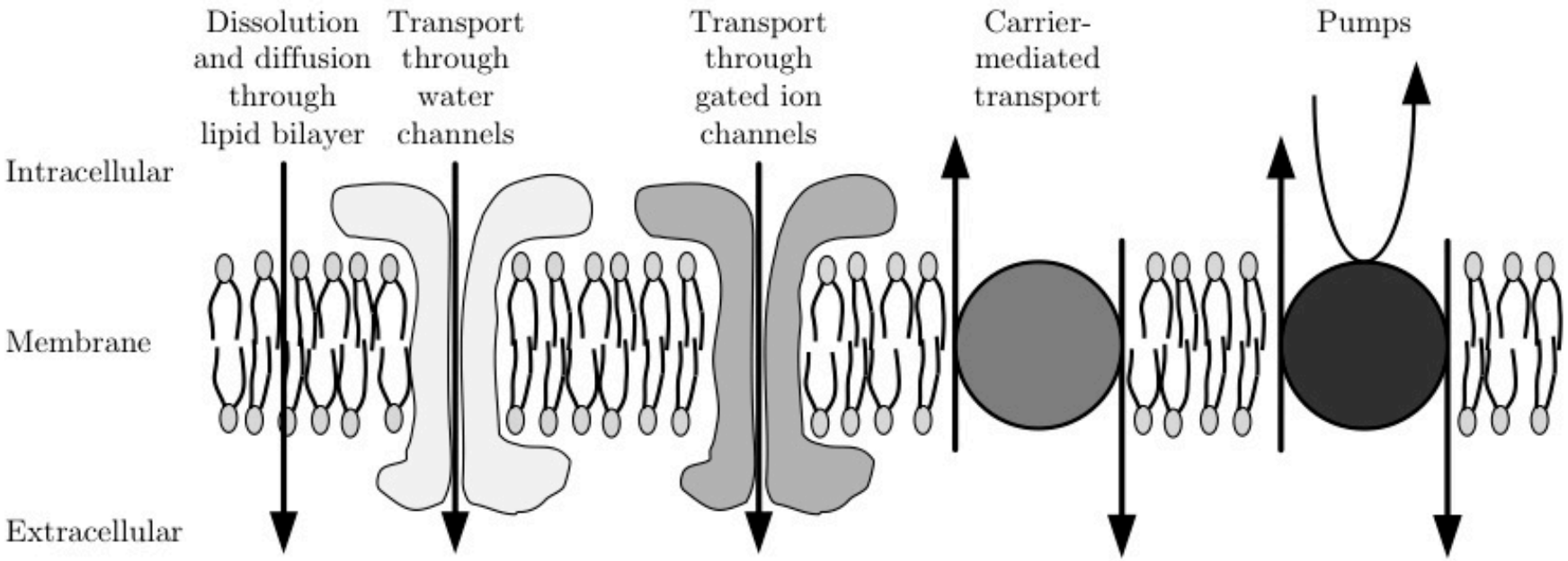
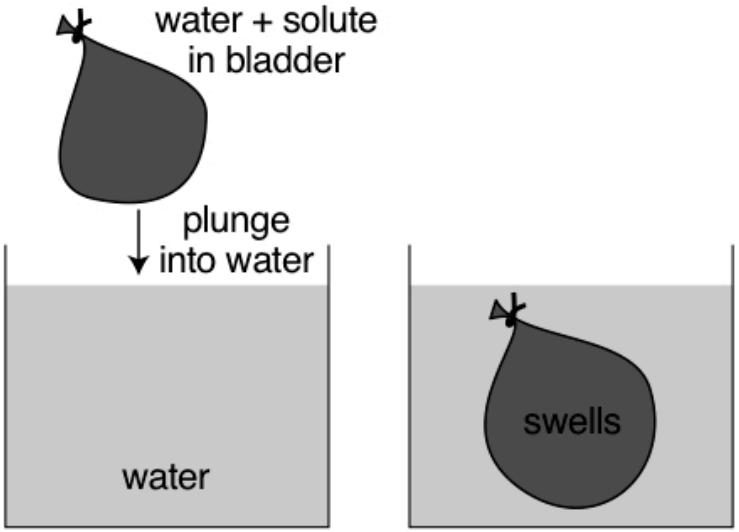

Dissolution and diffusion through lipid bilayer
Transport through water channels
Transport through gated ion channels
Carrier-mediated transport
Pumps
Intracellular
Membrane
Extracellular

Figure 2.19

Osmosis Observations

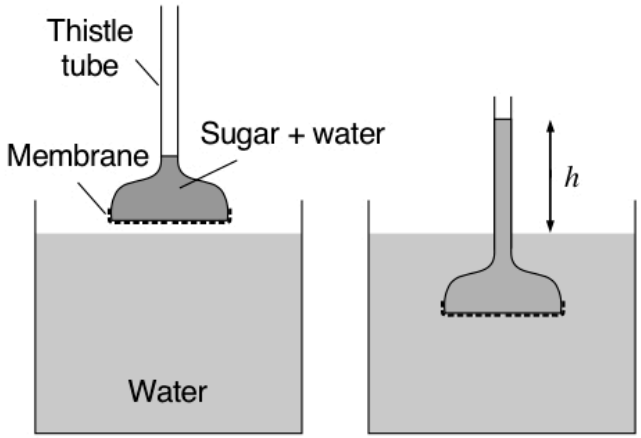
Henri Dutrochet (early 1800s)

- first described phenomenon and called it osmosis
- developed first osmometer: animal bladder filled with test solution, plunge into water, swells, turgid
- pressure greater for solutions with more solute



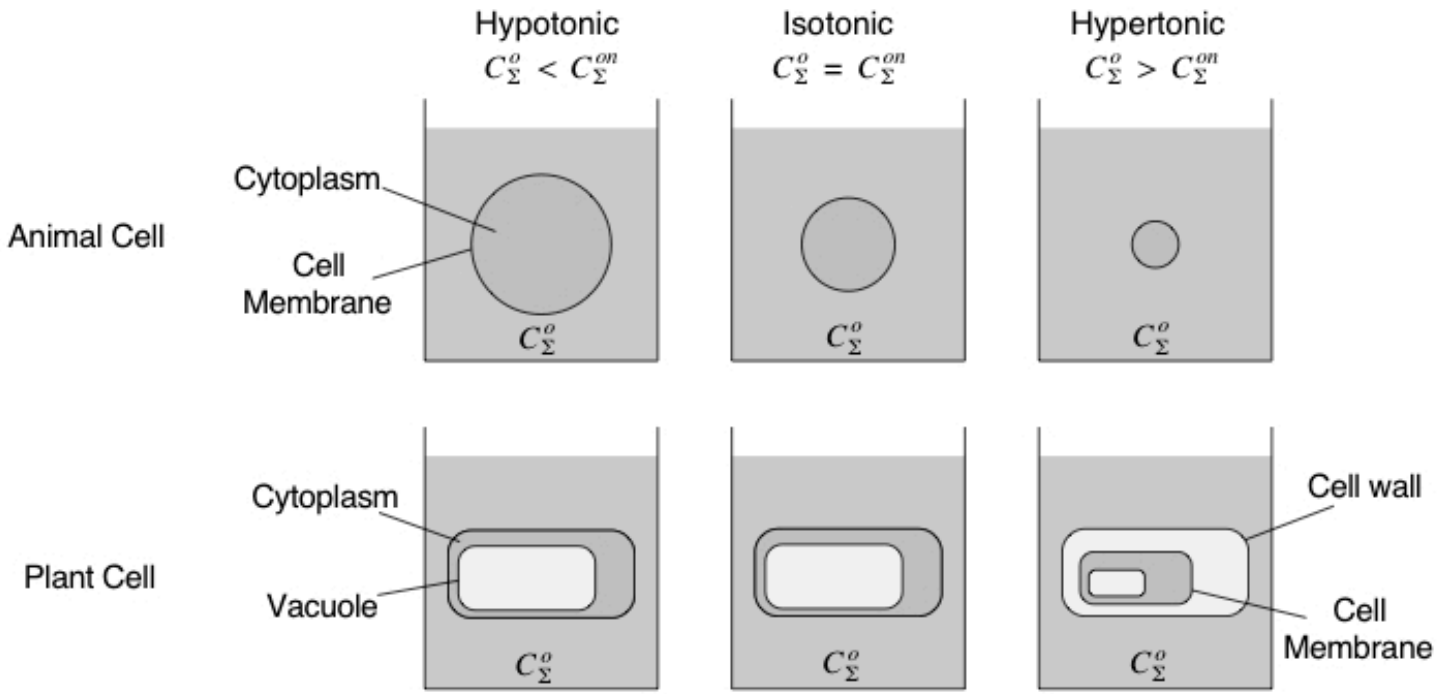
Wilhelm Pfeffer (mid 1800s)

- osmosis can be stopped with hydraulic pressure
- thistle tube + animal bladder (or artificial membrane by late 1800s)
  - water flows in direction to equalize sugar concentration
  - hydraulic pressure develops
  - flow stops when osmotic pressure = hydraulic pressure
- pressure proportional to concentration of solute
- pressure increases slightly with temperature



Hugo de Vries (late 1800s)

- studied osmosis in cells
- animal cell can shrink or swell depending on concentration
- isotonic (same "tension" as in cell's normal environment)
- plasmolysis – plant cell membrane separates from cell wall
- except for salts, plasmolysis occurs at same MOLAR concentration (does not depend on chemical properties of solute)
  → colligative property (freezing point depression, boiling point elevation)
- salts are different: ratios of small integers



Henricus van't Hoff (1886)

- formulated mathematical law
- count number of particles in volume V
- measure temperature T
- osmotic pressure = pressure produced by gas with same number of particles, same volume, and same pressure

van't Hoff's Law

$$\underbrace{\pi(x,t)}_{\text{osmotic pressure}} = \overbrace{R}^{\substack{\text{molar gas constant} \\ 8.314\ \text{J/(mol·K)}}}\ \overbrace{T}^{\text{absolute temperature}}\ \underbrace{\sum_n C_n(x,t)}_{\text{total solute concentration}}$$

- salts are different

Svante Arrhenius (1884)

- PhD (age 25): dissolution of salts into ions
- $NaCl \rightarrow Na^+ + Cl^-$ ($\therefore$ conducts electricity)
- count ions as separate particles
  → van't Hoff's law works for salts as well

$$\underbrace{\pi(x,t)}_{\substack{\text{osmotic pressure} \\ [\ \text{Pa} = \text{N/m}^2\ ]}} = R\,T\ \sum_n C_n(x,t) = R\,T\ \underbrace{C_\Sigma(x,t)}_{\substack{\text{osmolarity} \\ [\ \text{osmol/m}^3\ ]}}$$

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[Pa = N/m^2]

[$osmol/m^3$]

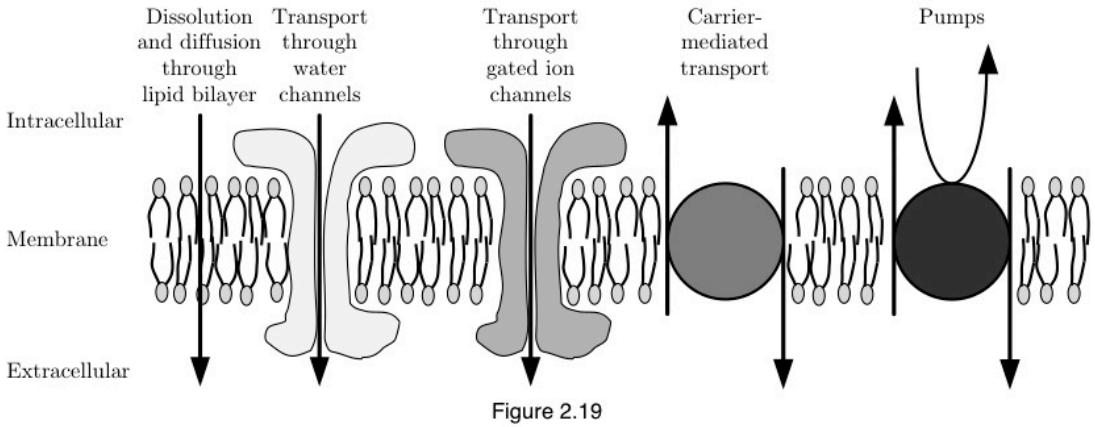

Figure 2.19

→ Notion of a *semi-permeable membrane*