FIRST ASSIGNMENT (27 SEP 07): Two Parts

Part One

In the equation measuring mobility/motion of a fluorescent probe of membrane 'fluidity':

$$(I_{\parallel} - I_{\perp})/(I_{\parallel} + 2 \bullet I_{\perp})$$

why is there a 2 in the term $2 \bullet I_{\perp}$?

Part Two

Phospholipase A_2 cleaves the $acyl_2$ chain of a di-acyl phospholipid to form a fatty acid and a lyso-phospholipid (mono-acyl phospholipid). Lyso-phospholipids are chaotropic, disrupting the integrity of bilayer membranes. Fatty acids are also chaotropic, and the major ingredient of soap. Why are these two products chaotropic, while di-acyl phospholipids are not?

GENERAL INSTRUCTIONS: Both answers should be short (no more than one-half of a page is required in either case). Diagrams are often helpful, and would be for this assignment. I expect that students may (or may not) work with each other on the assignment (depending on personal preference) and may come and ask me for help, but, the work you hand in should be your own.

Due Noon 01 OCT 07

Experimentally fluorescence anisotropy is the difference between fluorescence intensities emitted parallel to and perpendicular to the polarity of the exciting light, divided by the total emitted fluorescence[1]:

$$A = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2 \bullet I_{\perp})$$

where $2 \bullet I_{\perp}$ refers to the two perpendicular directions of emission (perpendicular to the y-axis, as shown below, and perpendicular to the x-axis (parallel to the y-axis).

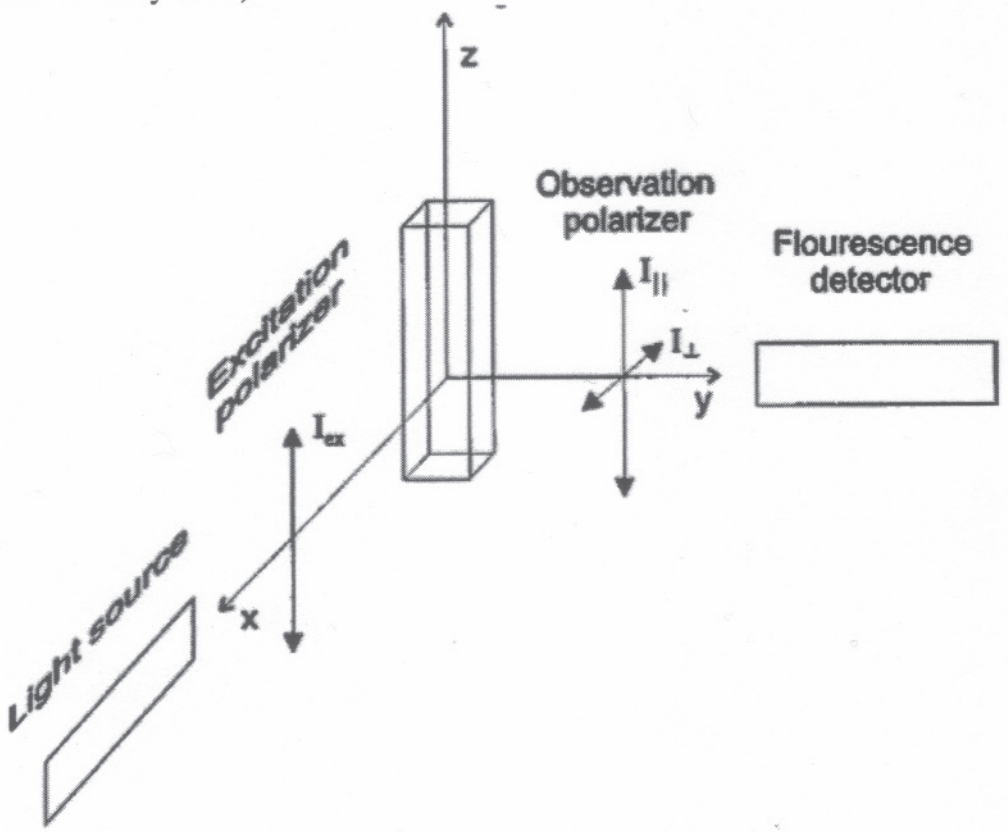


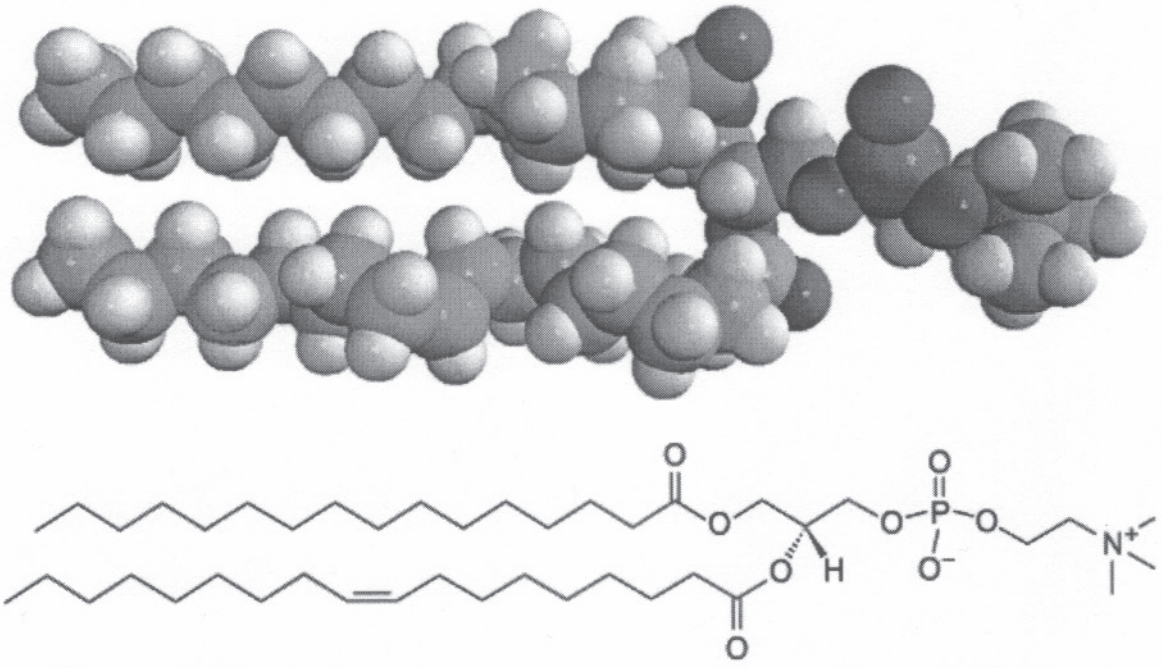
FIG. 1. Schematic diagram for measurement of fluorescence anisotropy of a cylindrically symmetrical emission field.

In the older literature, polarization was commonly used:

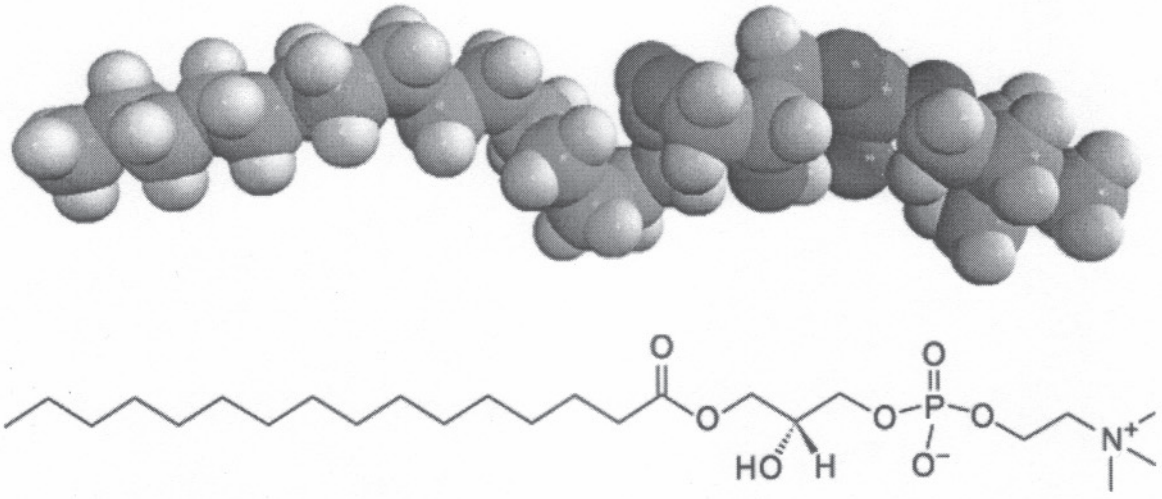
$$P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp}).$$

[1] Source: Bloomfield, VA (2000) Survey of biomolecular hydrodynamics. On-Line Biophysics Textbook Volume: Separations and Hydrodynamics (Todd M. Schuster, editor) Chapter 1

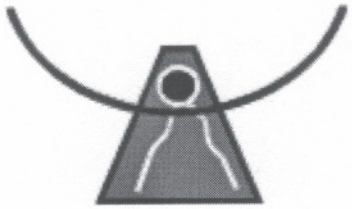
Phosphatidylcholine: Structure (both filled and wireframe structures)[2]



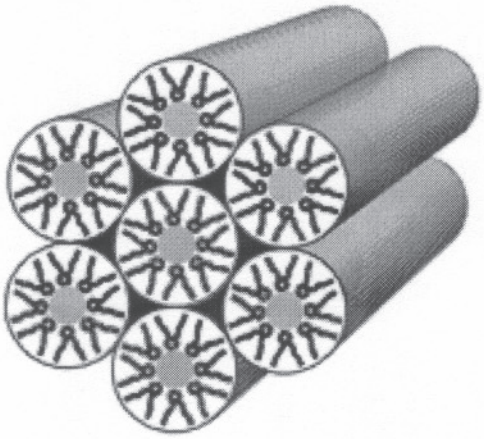
Lysophosphatidylcholine: Structure (both filled and wireframe structures)[3]

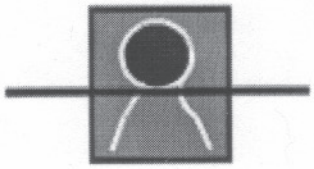


[2] Source: Avanti Polar Lipids Inc. (chicken egg)
(http://www.avantilipids.com/ProductStructures.asp?n=840051)
[3] Source: Avanti Polar Lipids Inc. (chicken egg)
(http://www.avantilipids.com/ProductStructures.asp?n=830071)

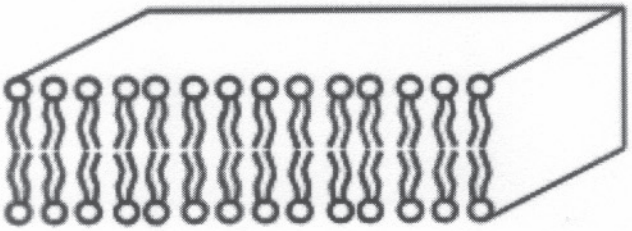


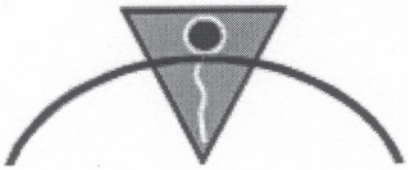
Cone



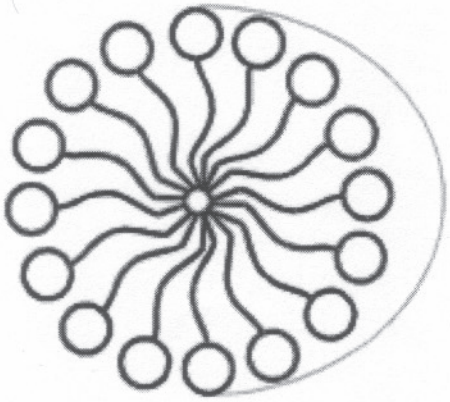


cylindrical





Inverted cone



First Term Test (04 October 2007) Membrane Transport

Question One

In addition to ATP production in oxidative phosphorylation, mitochondria can sequester Ca^{2+} at surprisingly high levels. Uptake is assuredly 'driven' by the negative inside electrical potential of the mitochondrial membrane, 'pulling' Ca^{2+} into the organelle. However, after inhibition of respiration, which depolarizes the potential to 'zero', much of this Ca^{2+} remains sequestered in the mitochondria.

With a membrane potential of about 150 mV, negative-inside, what would be the concentration of Ca^{2+} inside the mitochondria if the cytoplasmic $[Ca^{2+}]$ is 0.5 μM, and under steady state conditions (that is, flux equal to zero)?

Propose a mechanism by which mitochondria could retain this 'bound' Ca^{2+}, when the potential is 'zero', with reference to the unique lipid composition of mitochondria (see data sheets).

The mitochondrial cardiolipin is unlikely to form bilayer structures (the structure is shown in the data sheets). Explain and propose a model of a stable structure that it could form.

Question Two

The diffusion coefficient for glucose is $0.55 \cdot 10^{-5}$ cm^2 sec^{-1}; the permeability coefficient (through a lipid membrane) is $5.4 \cdot 10^{-6}$ cm sec^{-1}.

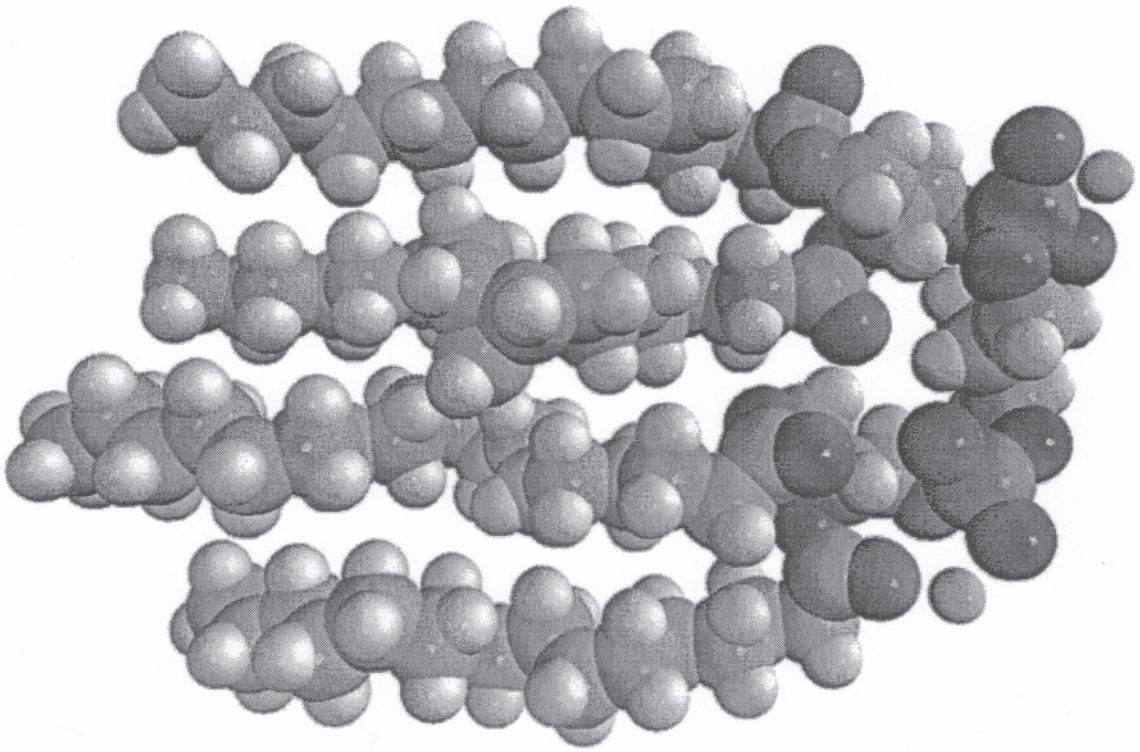
Calculate the partition coefficient (show all assumptions).

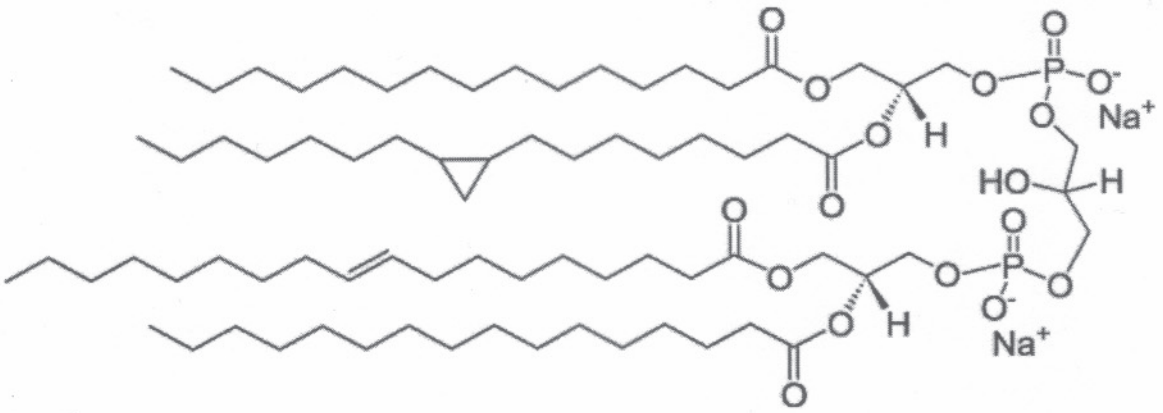
If a cell utilizes $1 \cdot 10^{-15}$ molecules sec^{-1} of glucose during respiration, and the internal glucose concentration is 2 mM, what extracellular concentration of glucose is required to maintain respiration? Assume (for the sake of simplicity) that the cell is a 10 μm by 10 μm cube.

Question Three

Compared to animals, plants tend to have a lower level of lipid saturation and lack C_{20} acyl chains (see data sheets) (nota bene: arachidonic acid is 20:4). Propose explanations for both phenomena.

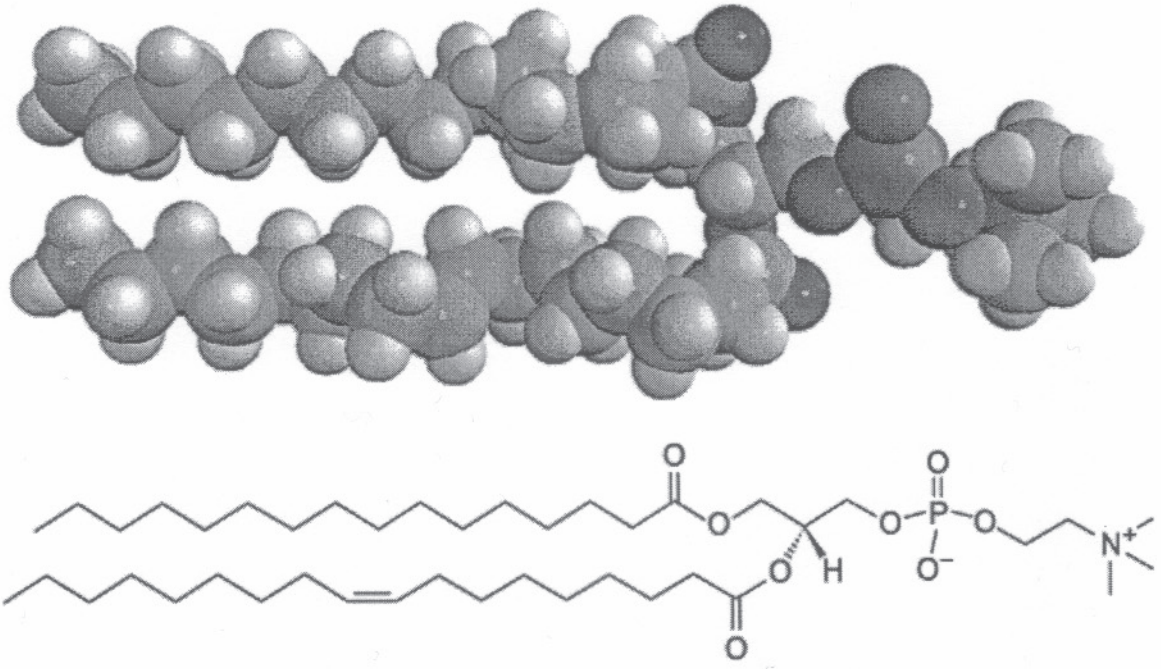
Cardiolipin: Structure (both filled and wireframe structures)[1]



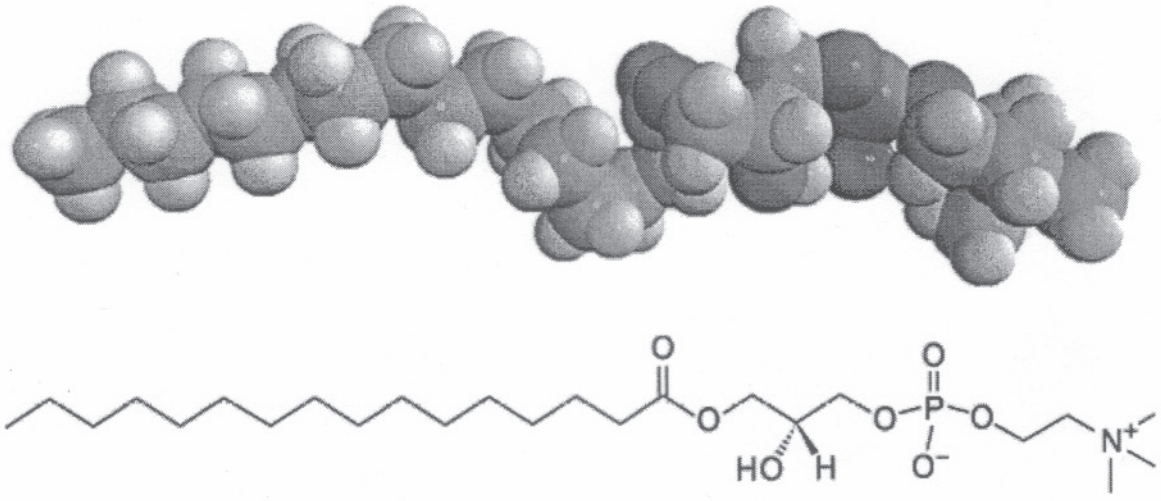


[1] Source: Avanti Polar Lipids Inc. (*E. coli* cardiolipin, sodium salt) (http://www.avantilipids.com/ProductStructures.asp?n=841199)

Phosphatidylcholine: Structure (both filled and wireframe structures)[2]

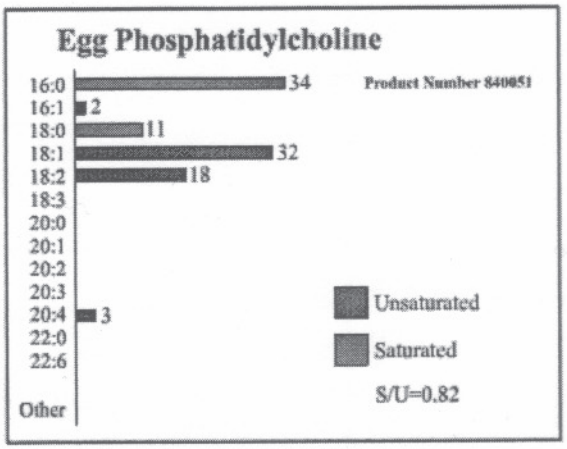


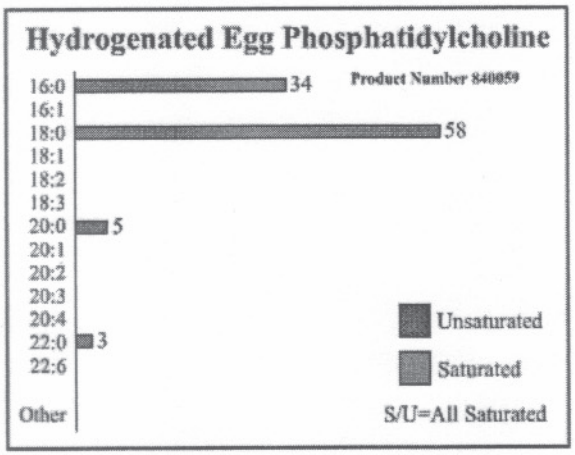
Lysophosphatidylcholine: Structure (both filled and wireframe structures)[3]

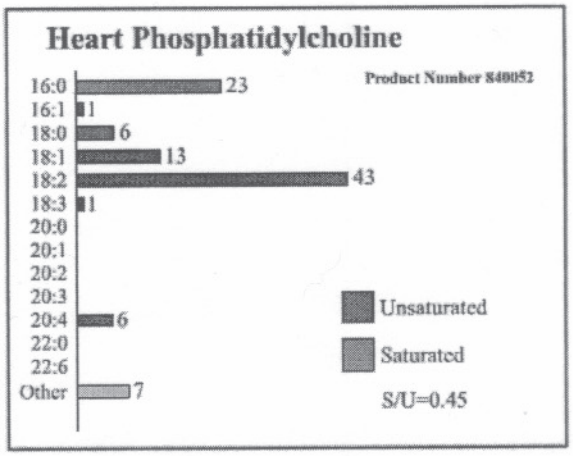


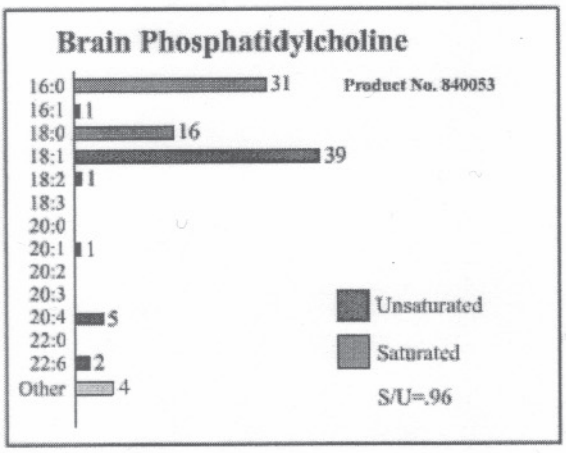
[2] Source: Avanti Polar Lipids Inc. (chicken egg)
(http://www.avantilipids.com/ProductStructures.asp?n=840051)
[3] Source: Avanti Polar Lipids Inc. (chicken egg)
(http://www.avantilipids.com/ProductStructures.asp?n=830071)

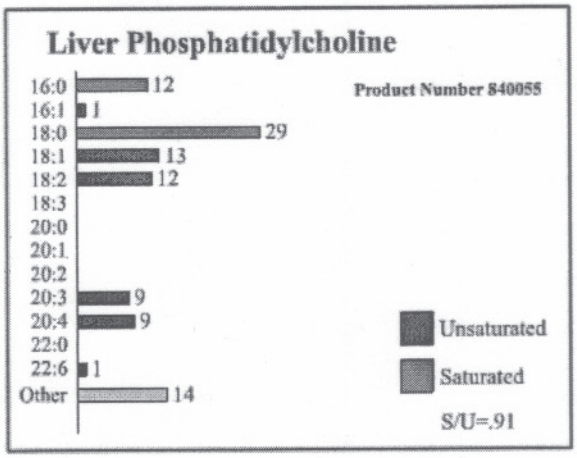
Fatty Acid Compositions of Phosphatidylcholine from Various Sources[4]

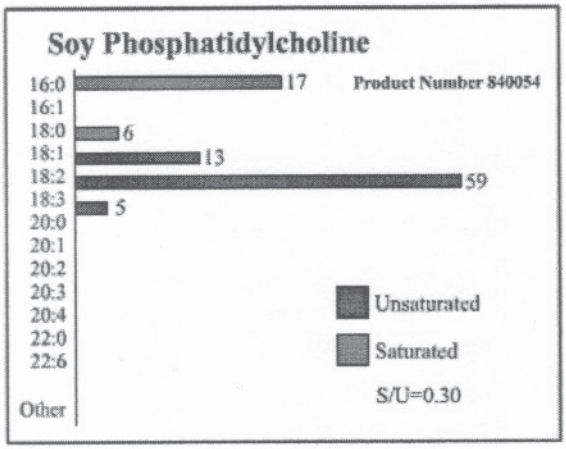












[4] Source: Avanti Polar Lipids Inc. http://www.avantilipids.com/FattyAcidContentOfTissue-DerivedPhosphatidylcholine.html

The composition of glycerolipids in various organelle membranes (from Heldt, H-W: Plant Biochemistry and Molecular Biology)

	% of total acyl lipid content		
Glycerolipids	chloroplast thylakoidal membrane	mitochondrial inner membrane	plasma membrane
monogalactosyldiglyceride (MGDG)	51	0	0
digalactosyldiglyceride (DGDG	26	0	0
sphingolipid (SL)	7	0	0
phosphatidylcholine (PC)	3	27	32
phosphatidylserine (PS)	0	25	0
phosphatidylethanolamine (PE)	0	29	46
phosphatidylglycerol (PG)	9	0	0
phosphatidylinositol (PI)	1	0	19
cardiolipin (CL)	0	20	0

Symbol	Value	Units	Comments
GAS CONSTANT			
R	8.314	$J\ mol^{-1}\ K^{-1}$	R is the Boltzmann's constant times Avogadro's number ($6.023 \cdot 10^{23}$)
	1.987	$J\ mol^{-1}\ K^{-1}$	
	8.314	$m^{-3}\ Pa\ mol^{-1}\ K^{-1}$	
RT	$2.437 \cdot 10^{3}$	$J\ mol^{-1}$	at 20°C (293°K)
	$5.833 \cdot 10^{2}$	$cal\ mol^{-1}$	at 20°C (293°K)
RT/F	25.3	mV	at 20°C (293°K)
2.303•RT	5.612	$kJ\ mol^{-1}$	at 20°C (293°K)
	1.342	$kcal\ mol^{-1}$	at 20°C (293°K)
FARADAY CONSTANT			
F	$9.649 \cdot 10^{4}$	$coulombs\ mol^{-1}$	F is the electronic charge times Avogadro's number
	$9.649 \cdot 10^{4}$	$J\ mol^{-1}\ V^{-1}$	
	23.06	$kcal\ mol^{-1}\ V^{-1}$	
CONVERSIONS			
kcal	4.187	J (joules)	Joule is an energy unit (equal to 1 Newton•meter)
Watt	1	$J\ sec^{-1}$	
Volt	1	$J\ coulomb^{-1}$	
Amp	1	$coulomb\ sec^{-1}$	
Pascal	1	$Newton\ m^{-2}$	Pascal is a pressure unit (equal to 10^{-5} bar)
Siemens	1	Ohm^{-1}	Siemens (S) is conductance, the inverse of resistance (Ω)
PHYSICAL PROPERTIES			
η_w	$1.002 \cdot 10^{-3}$	Pa sec	viscosity of water at 20°C. (Pa sec is $gm\ cm^{-1}\ sec^{-1}$)
ν_w	$1.004 \cdot 10^{-6}$	$m^2\ sec^{-1}$	kinematic viscosity of water at 20°C (viscosity/density)

Source: Noble, Park S. (1991) Physicochemical and Environmental Physiology

Sphere Area: $4 \bullet \pi \bullet r^2$	Sphere Volume: $\frac{4}{3} \bullet \pi \bullet r^3$
Cylinder Area: $4 \bullet \pi \bullet r \bullet h$	Cylinder Volume: $\pi \bullet r^2 \bullet h$
Cube Area: $6 \bullet h^2$	Cube Volume: h^3
Fick's Diffusion: $J = D \bullet \frac{dc}{dx}$	Fick's Diffusion: $\frac{dc}{dt} = D \bullet \frac{d^2c}{dx^2}$
Trans - Membrane Diffusion: $J = P \bullet (c_{outside} - c_{inside})$	
Trans - Membrane Diffusion: $J = -(uRT) \bullet \left(\frac{dc}{dx}\right) - (zFuc) \bullet \frac{d\psi}{dx}$	
Trans - Membrane Diffusion: $J = P(\frac{z \bullet F \bullet \psi}{R \bullet T}) \bullet \left(\frac{c_o - c_i \bullet e^{\left(zF\psi / R \bullet T\right)}}{1 - e^{\left(zF\psi / R \bullet T\right)}}\right)$	
Nernst Equation: $\Psi = \left[\frac{R \bullet T}{z \bullet F}\right] \bullet \ln\left[\frac{c_o}{c_i}\right]$	Zero Potential Flux: $J = P \bullet (c_o - c_i)$

$exp\left(\frac{zF\Psi}{RT}\right)$.

KEY: First Term Test (04 October 2007) Membrane Transport

ONE (20 pts)
question: –150 mV potential, calculate $[Ca^{2+}]_{inside}$ if $[Ca^{2+}]_{outside}$ is 0.5 μM (steady states, flux is zero)
answer: $[Ca^{2+}]_{inside}=[Ca^{2+}]_{outside}\bullet\exp(-zF\Psi/RT) = 0.5\ \mu M\bullet\exp(-(+2)\bullet -150/25) = 81.4$ mM
score breakdown: Nernst, 5/10; z = 2, 2.5/10; 81 mM, 2.5/10

question: mechanism of Ca^{2+} retention
answer: net negative charges of both cardiolipin and phosphatidylserine
score breakdown: 2.5/5 each

question: cardiolipin structure
answer: due to four acyl chains, inverted micelle, or inverted tubes
score breakdown: 5/5

TWO (20 pts)
question: partition coefficient
answer: $P\bullet d/D = K_p$, d is *ca* 4 nm, $K_p = 3.92\bullet10^{-7}$ very low for glucose.
score breakdown: equation, 6/10; d estimate, 2/10; answer, 2/10

question: extracellular glucose
answer:

$$J = P(c_o - c_i)$$

$$J = \frac{1\bullet10^{15}\ \text{molecules sec}^{-1}}{6\bullet(0.001\ \text{cm})^2\bullet6.023\bullet10^{23}\ \text{molecules mole}^{-1}} = 2.767\bullet10^{-4}\ \text{mole cm}^{-2}\ \text{sec}^{-1}$$

$$c_i = \left[\frac{2.767\bullet10^{-4}\ \text{mole cm}^{-2}\ \text{sec}^{-1}}{5.4\bullet10^{-6}\ \text{cm sec}^{-1}} + \frac{2\bullet10^{-3}\ \text{moles / liter}}{1000\ \text{cm}^3}\right]\bullet\left(\frac{1000\ \text{cm}^3}{1\ \text{liter}}\right) = 0.102\ \text{M (or 102 mM)}$$

score breakdown: equation(s), 8/10; answer, 2/10

THREE (10 pts)
question: fatty acid composition
answer: Unsaturation of plant lipids is likely due to their need to survive dramatic temperature ranges compared to some (warm blooded) animals. The absence of 20:4 lipids suggests that arachidonic acid signalling is absent in plants.
score breakdown: intelligibility, 10/10

SECOND ASSIGNMENT (25 OCT 07): Two Parts

The diffusion coefficient for glucose is $0.55 \bullet 10^{-5}$ cm^2 sec^{-1}.

During respiration, a cell utilizes $2.8 \bullet 10^{-4}$ moles cm^{-2} sec^{-1} of glucose.

Assume that glucose transporters are present so that glucose influx through the membrane is not a limiting step (*Nota bene* the permeability coefficient for a lipid membrane without transporters is small enough ($5.4 \bullet 10^{-6}$ cm sec^{-1}) that permeation would severely limit uptake of glucose if transporters were not present). An additional assumption that may prove helpful is that the glucose transporters have a very high affinity for glucose, so that the glucose concentration immediately outside the cell is very close to '0', while the glucose concentration far away from the cell is about 10 mM (not that far from blood glucose levels in humans)

What is the critical cell radius at which diffusion would limit glucose utilization?

At this cell size, If the external glucose concentration was 2 mM, what advective flow velocity would be required to allow cell utilization of glucose at $2.8 \bullet 10^{-4}$ moles cm^{-2} sec^{-1}?

GENERAL INSTRUCTIONS: Please be brief (no more than a page should be required, at least in theory). Diagrams are often helpful, and would be for this assignment. I expect that students may (or may not) work with each other on the assignment (depending on personal preference) and may come and ask me for help, but, the work you hand in should be your own.

Due Noon 29 OCT 07

SECOND ASSIGNMENT (25 OCT 07): **KEY**

The diffusion coefficient for glucose is $0.55 \cdot 10^{-5}$ cm^2 sec^{-1}.

During respiration, a cell utilizes $2.8 \cdot 10^{-4}$ moles cm^{-2} sec^{-1} of glucose.

Assume that glucose transporters are present so that glucose influx through the membrane is not a limiting step (*Nota bene* the permeability coefficient for a lipid membrane without transporters is small enough ($5.4 \cdot 10^{-6}$ cm sec^{-1}) that permeation would severely limit uptake of glucose if transporters were not present). An additional assumption that may prove helpful is that the glucose transporters have a very high affinity for glucose, so that the glucose concentration immediately outside the cell is very close to '0', while the glucose concentration far away from the cell is about 10 mM (not that far from blood glucose levels in humans)

What is the critical cell radius at which diffusion would limit glucose utilization?

$$a_{critical} = \frac{D \cdot C_\infty}{\beta} = \frac{0.55 \cdot 10^{-5} \text{ moles cm}^{-2} \text{ sec}^{-1} \cdot 0.01 \cdot 10^{-3} \text{ moles cm}^{-3}}{2.8 \cdot 10^{-4} \text{ moles cm}^{-2} \text{ sec}^{-1}} = 1.96 \cdot 10^{-7} \text{ cm}$$

At this cell size, If the external glucose concentration was 2 mM, what advective flow velocity would be required to allow cell utilization of glucose at $2.8 \cdot 10^{-4}$ moles cm^{-2} sec^{-1}?

From the Peclet number, incorporating the metabolic rate:

$$P_e = \frac{2 \cdot a \cdot v}{D} = \frac{2 \cdot \frac{D \cdot C_\infty}{\beta} \cdot v}{D} = \frac{2 \cdot C_\infty \cdot v}{\beta} \text{ or } v = \frac{\beta}{2 \cdot C_\infty} \text{ assuming } P_e = 1$$

assuming the Peclet Number remains 1, then the velocity required is

$$v_1 - v_2 = \frac{\beta}{2} \cdot \left(\frac{1}{0.002 \cdot 10^{-3}} - \frac{1}{0.01 \cdot 10^{-3}} \right) = \frac{2.8 \cdot 10^{-4} \text{ moles cm}^{-2} \text{ sec}^{-1}}{2} \cdot \left(4 \cdot 10^5 \ \frac{1}{\text{mole cm}^{-3}} \right)$$

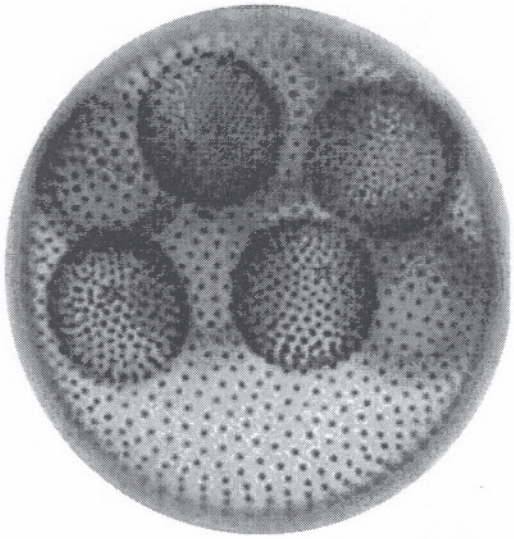
$\Delta v = 56$ cm sec^{-1}

Nota bene if Pe = 0.01
then $\Delta v = 0.56$ cm sec^{-1}

Second Term Test (01 November 2007) Membrane Transport

Question One

Although flagellar-driven advective transport may increase the uptake of nutrients at the surface of the *Volvox* colony, nutrients must still traverse the *inside* of the colony.



Given that *Volvox* is an autotroph, 'imbibing' carbon dioxide (CO_2) (which has a diffusion coefficient of about $1.4 \bullet 10^{-5}$ cm^2 sec^{-1} in aqueous solutions) to produce carbohydrate:

• Determine the time it would take for CO_2 to diffuse from the surface to the center of the colony (assume the colony diameter is 1 mm)

• If CO_2 was fixed into carbohydrate solely at the center of the colony, calculate and diagram the flux of $[CO_2]$ at steady state from the surface to the center of the colony. At equilibrium with air, $[CO_2]$ is between 12 (*ca* 30°C) and 16 μM (*ca* 4°C). Although the carboxylating enzyme has a $K_{1/2}$ of about 40 μM, assume that its affinity for CO_2 is much higher (*ca* '0' μM).

Question Two

Many cells and multicellular colonies with sizes similar to *Volvox* do not rely upon flagellar-driven flow. Beside lower metabolic demand or, possibly, cytoplasmic mass flow, another adaptive strategy is shape.

• Describe the most optimal shape for a large cell, that maximizes nutrient uptake per cell volume, with an explicit quantitative rationale.

Question Three

In a study on an endplate ion channel, Adams et al. (1980)[1] measured the permeability of the channel to various cations, both monovalent and divalent (Table I). Assuming that the pore radius and length are similar to gramicidin and the *Streptomyces* K^+ channel:

• Using the physical properties of the ions as a guide (Table I), explain the ionic selectivity of the endplate channel.

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[1] Adams, DJ, TM Dwyer, B Hille (1980) The permeability of endplate channels to monovalent and divalent metal cations. Journal of General Physiology 75:493–510.

Table I: Permeability ratios, atomic radii, hydration enthalpies and mobilities of selected ions.

Ion	P_X/P_{Na} (endplate channel)	Atomic Radius (Å)	Enthalpy of Hydration (kcal/mole)	Mobility (10^{-4}) (cm/sec)/(V/cm)
Tl^+	2.51	1.44	.	7.74
H^+	.	.	.	36.3
NH_4	.	1.48	.	7.52
Cs^+	1.42	1.69	-72	8.01
Rb^+	1.30	1.48	-79.2	8.06
K^+	1.11	1.33	-85.8	7.62
Na^+	1	0.95	-104.6	5.19
Li^+	0.87	0.6	-131.2	4.01
Cl^-	.	1.81	-82	7.92
F^-	.	1.36	-114	5.74
Br^-	.	1.95	-79	8.09
I^-	.	2.16	-65	7.96
NO_3^-	.	2.9	.	7.41
Mg^{2+}	0.25	0.65	-476	2.75
Ca^{2+}	0.22	0.99	-397	3.08
Sr^{2+}	0.18	1.13	-362	3.08
Ba^{2+}	0.21	1.35	-328	3.3

Symbol	Value	Units	Comments
GAS CONSTANT			
R	8.314	$J\ mol^{-1}\ K^{-1}$	R is the Boltzmann's constant times Avogadro's number ($6.023 \cdot 10^{23}$)
	1.987	$J\ mol^{-1}\ K^{-1}$	
	8.314	$m^{-3}\ Pa\ mol^{-1}\ K^{-1}$	
RT	$2.437 \cdot 10^{3}$	$J\ mol^{-1}$	at 20°C (293°K)
	$5.833 \cdot 10^{2}$	$cal\ mol^{-1}$	at 20°C (293°K)
RT/F	25.3	mV	at 20°C (293°K)
2.303•RT	5.612	$kJ\ mol^{-1}$	at 20°C (293°K)
	1.342	$kcal\ mol^{-1}$	at 20°C (293°K)
FARADAY CONSTANT			
F	$9.649 \cdot 10^{4}$	$coulombs\ mol^{-1}$	F is the electronic charge times Avogadro's number
	$9.649 \cdot 10^{4}$	$J\ mol^{-1}\ V^{-1}$	
	23.06	$kcal\ mol^{-1}\ V^{-1}$	
CONVERSIONS			
kcal	4.187	J (joules)	Joule is an energy unit (equal to 1 Newton•meter)
Watt	1	$J\ sec^{-1}$	
Volt	1	$J\ coulomb^{-1}$	
Amp	1	$coulomb\ sec^{-1}$	
Pascal	1	$Newton\ m^{-2}$	Pascal is a pressure unit (equal to 10^{-5} bar)
Siemens	1	Ohm^{-1}	Siemens (S) is conductance, the inverse of resistance (Ω)
PHYSICAL PROPERTIES			
η_w	$1.002 \cdot 10^{-3}$	Pa sec	viscosity of water at 20°C. (Pa sec is $gm\ cm^{-1}\ sec^{-1}$)
ν_w	$1.004 \cdot 10^{-6}$	$m^2\ sec^{-1}$	kinematic viscosity of water at 20°C (viscosity/density)

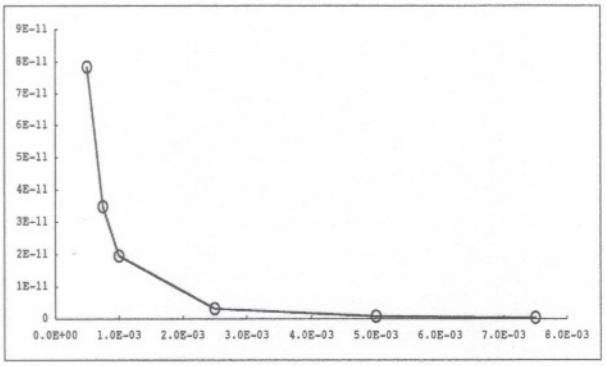
Source: Noble, Park S. (1991) Physicochemical and Environmental Physiology

Sphere Area: $4 \bullet \pi \bullet r^2$	Sphere Volume: $\frac{4}{3} \bullet \pi \bullet r^3$
Cylinder Area: $4 \bullet \pi \bullet r \bullet h$	Cylinder Volume: $\pi \bullet r^2 \bullet h$
Cube Area: $6 \bullet \mathrm{h}^2$	Cube Volume: h^3
Fick's Diffusion: $J = D \bullet \frac{dc}{dx}$	Fick's Diffusion: $\frac{dc}{dt} = D \bullet \frac{d^2c}{dx^2}$
Einstein's formalism for D: $D = \frac{1}{2} \bullet \frac{\Delta^2}{\tau}$	$\langle x^2 \rangle = 2 \bullet D \bullet t$ $\langle r^2 \rangle = 6 \bullet D \bullet t$
Trans - Membrane Diffusion: $J = P \bullet (c_{outside} - c_{inside})$	
Trans - Membrane Diffusion: $J = -(uRT) \bullet \left(\frac{dc}{dx}\right) - (zFuc) \bullet \frac{d\psi}{dx}$	
Trans - Membrane Diffusion: $J = P(\frac{z \bullet F \bullet \psi}{R \bullet T}) \bullet \left(\frac{c_o - c_i \bullet e^{\left(zF\psi / R \bullet T\right)}}{1 - e^{\left(zF\psi / R \bullet T\right)}}\right)$	
Nernst Equation: $\Psi = \left[\frac{\mathrm{R} \bullet \mathrm{T}}{\mathrm{z} \bullet \mathrm{F}}\right] \bullet \ln\left[\frac{c_o}{c_i}\right]$	Zero Potential Flux: $J = P \bullet (c_o - c_i)$
Ohm's Law: $\mathrm{V} = \mathrm{I} \bullet \mathrm{R}$, or $\mathrm{I} = \mathrm{g} \bullet \mathrm{V}$	$\mathrm{R} = \rho \bullet (\mathrm{l}/\mathrm{A})$ | $J = I/(z \bullet F)$
$C(r) = C_\infty \bullet \left(1 - \frac{a}{r}\right)$ | $J(r) = -D \bullet C_\infty \bullet \left(\frac{a}{r^2}\right)$	$I_m = 4 \bullet \pi \bullet a^2 \bullet \beta$ $I_D = 4 \bullet \pi \bullet a \bullet D \bullet C_\infty$
$P_e = \frac{2 \bullet a \bullet v}{D}$ | $R_e = \frac{\rho \bullet v \bullet l}{\eta}$	

KEY: Second Term Test (01 November 2007) Membrane Transport

ONE (20 pts)

• question: diffusion time over a distance of 0.05 cm with a diffusion coefficient of $1.4 \bullet 10^{-5}$ cm sec^{-1}.
answer: $\langle r^2 \rangle = 6 \bullet D \bullet t$; $t = (1/(6 \bullet D)\langle r^2 \rangle$; $(1/(6 \bullet 1.4 \bullet 10^{-5}$ cm $sec^{-1}))(0.05)^2 = 29.8$ sec
score breakdown: equation 6/10; answer 4/10.



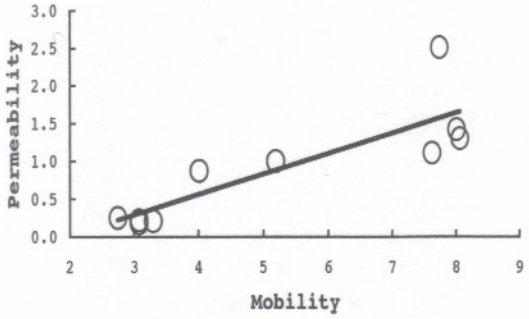
• question: Diagram of J(r) within the colony under boundary conditions of 14 μM and near '0' μM.
answer: $J(r) = -D \bullet C_{oo} \bullet (a/r^2)$
score breakdown: equation 6/10; graph / higher flux at center 4/10.

TWO (20 pts)

Most organisms differ greatly from the spherical shape of *Volvox*. Normally, under the constraint of large size, they are flat, sometimes dramatically so. A flat (leaf-like) shape confers two advantages: one is a greatly increased surface area per volume, the other is rapid diffusive supply. For a rectangular shape, the volume is equal to width • depth • length (w•d•l), and the surface area is equal to (2•w•d + 2•w•l + 2•d•l). If we assume a depth of about 50 μm, to assure rapid diffusion of nutrients within the cell (30 sec, see Question One), with a width and length of 1 cm, the volume is 0.05 cm^3, the area is 2.2 cm^2, and the ratio of surface area to volume is 44. Increasing the length to 10 cm yields a volume of 0.5 cm^3, an area of 21.1 cm^2, and a ratio of 42.2. By contrast, a sphere of the same volumes (0.05 and 0.5 cm^3) would have surface area to volume ratios of 13 and 6, respectively: far less area for a given volume.
score breakdown: quantitative comparison of shapes, 16/20; rectangle, 4/20.

THREE (20 pts)

question: Does the endplate channel match the selectivity of gramicidin, in which hydrated radius dominates, or is it selective like the K^+ channel.
answer: The best approach is to graph mobility (a measure of hydrated radius) versus relative permeabilities. It is self-evident that hydrated radius constrains access to the endplate channel pore, with no indication of selectivity that involves coordinated dipole bonding in a selectivity filter. Adams et al. (1980) do note that there is some indication of coordination for the divalents[1]



score breakdown: comparison of mobility to permeability, 12/20; absence of selectivity filter, 4/20; hydrated ion passage, 4/20.

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[1] "Alkali metal ions see the endplate channel as a water-filled, neutral pore without high-field-strength sites inside. Their permeability sequence is the same as their aqueous mobility sequence. Divalent ions, however, have a permeability sequence almost opposite from their mobility sequence and must experience some interaction with groups in the channel. In addition, the concentrations of monovalent and divalent ions are increased near the channel mouth by a weak negative surface potential."

THIRD ASSIGNMENT (22 NOV 2007)

Given a walled spherical cell (with modulus of elasticity of 1.4 MPa[1]) or non-walled spherical cell (with a modulus of elasticity of 6.0 kPa[2]), subjected to a hypo-osmotic treatment (a decrease in external concentration of 200 mM).

For initial cell sizes of either 1 mm or 10 μm, determine:

1) the changes in volume and surface area,

2) the fluxes required to counteract the hypo-osmotic shock (assuming the cell must respond within 10 seconds), and

3) the number of transporters required to allow this to occur (assume a molecule transport rate of 10^6 sec^{-1} per transporter).

Comment on the impact of the modulus of elasticity and cell size on osmo-sensitivity.

DUE NOON 28 NOVEMBER 2007

[1] Lew, RR, NN Levina, SK Walker, A Garrill (2004) Turgor regulation in hyphal organisms. Fungal Genetics and Biology 41:1007–1015.

[2] Matzke R, K Jacobsen, M Radmacher (2001) Direct, high-resolution measurement of furrow stiffening during division of adherent cells. Nature Cell Biology 3:607–610.

FINAL EXAM (13 DEC 2007)

Choose two of the following three questions

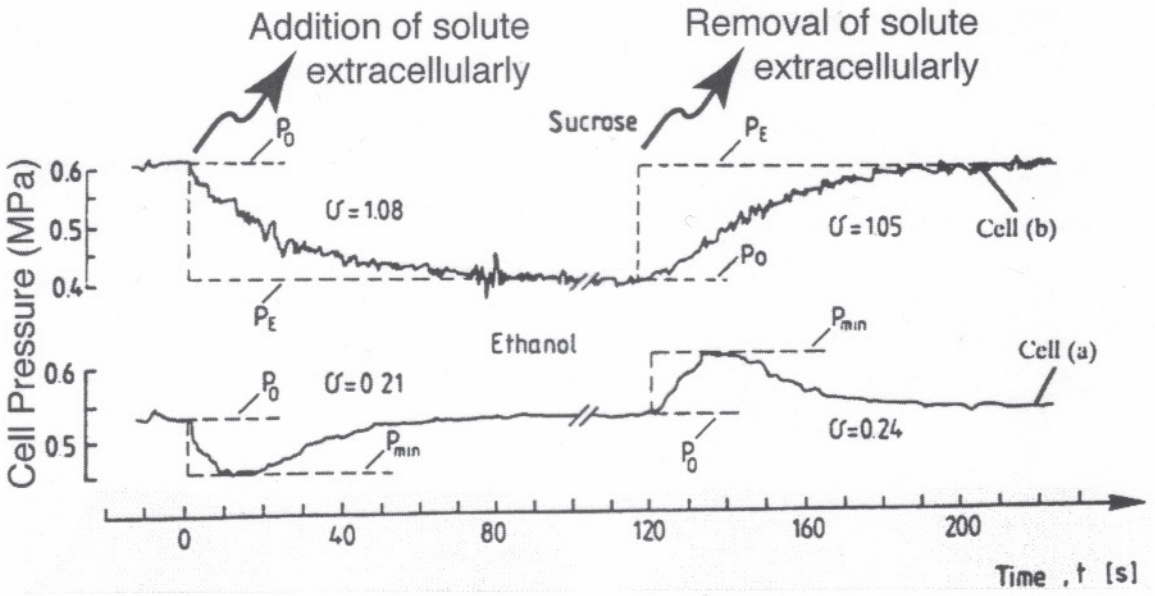
QUESTION ONE

The data shown below show the effect of extracellular addition of osmotically active solutes (upper trace, sucrose; lower trace, ethanol) on the hydrostatic pressure of a walled cell having a very high modulus of elasticity. The cell was an epidermal cell of *Tradescantia* that can be approximated as rectangular (30 μm wide by 80 μm long by 20 μm in depth)

• Determine the concentrations of sucrose and ethanol that were added to the external solution.

• Explain why removal of ethanol causes an increase in hydrostatic pressure to a level higher than the initial pressure, and why this does not occur when the sucrose is removed.

• Calculate the permeability coefficient of the cell for ethanol. For sucrose, explain why it is not possible to calculate the permeability coefficient based on the data shown below.



$J = \Delta C \cdot V/A \cdot \frac{1}{t}$

$\Delta C \cdot V/A = P \Delta C \cdot t \cdot$

$V/tA = P.$

0.0048
0.0012
0.0032
0.0092

ital.

6 pts

6 pts

6 pts

18

QUESTION TWO (PART ONE OF TWO)

From the data tabulated for atomic radii, enthalpies of hydration and mobilities, predict the enthalpy of hydration for the anion NO^{3-}. Show how you extrapolated the hydration value, and give supporting rationale for your extrapolation.

ATOMIC RADII, HYDRATION ENTHALPIES AND MOBILITIES			
Ion	Atomic Radii (Å)	Enthalpies of Hydration (kcal/mole)	Mobility (10^{-4}) (cm/sec)/(V/cm)
Tl^+	1.44	71	7.74
H^+	.	-269	36.3
NH_4^+	1.48	.	7.52
Cs^+	1.69	-72	8.01
Rb^+	1.48	-79.2	8.06
K^+	1.33	-85.8	7.62
Na^+	0.95	-104.6	5.19
Li^+	0.60	-131.2	4.01
Cl^-	1.81	-82	7.92
F^-	1.36	-114	5.74
Br^-	1.95	-79	8.09
I^-	2.16	-65	7.96
NO_3^-	2.90	.	7.41
Mg^{2+}	0.65	-476	2.75
Ca^{2+}	0.99	-397	3.08
Sr^{2+}	1.13	-362	3.08
Mn^{2+}	0.80	-458	.
Ba^{2+}	1.35	-328	3.30
Co^{2+}	0.74	-502	.
Ni^{2+}	0.72	-517	.
Zn^{2+}	0.74	-505	.

Source: Hille, B (1991) Ionic Channels of Excitable Membranes. Sinauer Associates. pp. 157 & 166.

QUESTION TWO (PART TWO OF TWO)

The data below show permeability ratios for a chloride channel from cardiac sarcoplasmic reticulum[1]. Explain how the permeability ratios were determined. Conductances, measured from current-voltage relations, were 116 picoSiemen (Cl^- and Br^-) or 40 picoSiemen (I^-, NO_3^-, and F^-). Is the anion channel selective, or is there permeation through a water-filled pore?

TABLE I

Reversal potentials and permeability ratios of the Cl^- channels in various anions

Anion	E_{rev}	n	P_{anion}/P_{Cl}
	mV		
Cl^-	60 ± 0.5	18	1.0
Br^-	75 ± 10	5	2.1
I^-	45 ± 10	4	0.59
NO_3^-	28 ± 6.8	5	0.30
F^-	23 ± 2.9	4	0.25

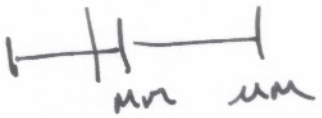
E_{rev}, reversal potential; P, permeability; n, numbers of experiments.

[1] Kawano S, A Kuruma, Y Hirayama, and M Hiraoka (1999) J Biol Chem, 274:2085-2092.

QUESTION THREE

Photosynthetic algal protists (for example, *Chlamydomonas*) can live either heterotrophically, or autotrophically. If heterotrophic, how fast would *Chlamydomonas* have to swim to take advantage of advective transport to import glucose (The diffusion coefficient for glucose is $0.55 \bullet 10^{-5}$ cm^2 sec^{-1}. *Chlamydomonas* is about 30 μm in diameter)?

protist swimming speeds are in the range of 30 $\mu m\ sec^{-1}$



Equations relevant to Membrane Transport: Geometry, Diffusion and Flux

Sphere Area: $4 \bullet \pi \bullet r^2$ Sphere Volume: $\frac{4}{3} \bullet \pi \bullet r^3$

Cylinder Area: $4 \bullet \pi \bullet r \bullet h$ Cylinder Volume: $\pi \bullet r^2 \bullet h$

Cube Area: $6 \bullet h^2$ Cube Volume: h^3

Fick's Diffusion: $J = D \bullet \frac{dc}{dx}$ Fick's Diffusion: $\frac{dc}{dt} = D \bullet \frac{d^2c}{dx^2}$

Einstein's Random Walks: $D = \frac{1}{2} \bullet \frac{\Delta^2}{\tau}$, $\langle x^2 \rangle = 2 \bullet D \bullet t$, and $\langle r^2 \rangle = 6 \bullet D \bullet t$

Membrane Diffusion: $J = P \bullet (c_{outside} - c_{inside})$

Membrane Diffusion: $J = -(uRT) \bullet \frac{dc}{dx} - (zFuc) \bullet \frac{d\Psi}{dx}$

Membrane Diffusion: $J = -P \bullet \left(\frac{zF\Psi}{RT}\right) \bullet \left(\frac{c_o - c_i \bullet e^{zF\Psi/RT}}{1 - e^{zF\Psi/RT}}\right)$

Nernst Equation: $\Psi = \left(\frac{RT}{zF}\right) \bullet \ln\left(\frac{c_o}{c_i}\right)$

Ohm's Law: $V = I \bullet R$, $I = g \bullet V$, $R = \rho \bullet \left(l/A\right)$, and $J = I/(zF)$

Radial Diffusion: $C(r) = C_\infty \bullet \left(1 - \frac{a}{r}\right)$, and $J(r) = -D \bullet C_\infty \bullet \left(\frac{a}{r^2}\right)$

Radial Currents: $I_m = 4 \bullet \pi \bullet a^2 \bullet \beta$, and $I_d = 4 \bullet \pi \bullet a \bullet D \bullet C_\infty$

Dimensionless relations $P_e = \frac{2 \bullet a \bullet v}{D}$ and $R_e = \frac{\rho \bullet v \bullet l}{\eta}$

Equations relevant to Membrane Transport: Water Fluxes

Volume Flow: $J_V \propto \frac{\partial P}{\partial x}$

Flow through a Pipe: $J_V = -\frac{r^2}{8 \bullet \eta} \bullet \frac{\partial P}{\partial x}$

Flow into / out of a cell:

$$J_V = -\frac{1}{A} \bullet \frac{\partial V}{\partial t}$$

$$J_V = L_p \bullet [P - RT(c_i - c_o)]$$

$$J_V = L_p \bullet \Delta\Psi$$

where $RT(c_i - c_o) = \pi_i - \pi_o$

when $J_V = 0$: $P = RT(c_i - c_o)$

Cell volume, pressure and osmotic relations

$$\frac{\partial P}{\partial V} = \frac{\varepsilon}{V} \approx \frac{\Delta P}{\Delta V} = \frac{P - P_0}{V - V_0}$$

$$\frac{\partial \pi_i}{\partial V} \approx \frac{\Delta \pi_i}{\Delta V} = \frac{\pi_i - \pi_{i,0}}{V - V_0}$$

$$P(t) = \left(P - P_e\right) \bullet e^{\left(-L_p \bullet A \bullet \frac{\varepsilon + \pi_i}{V} \bullet t\right)}$$

Symbol	Value	Units	Comments
GAS CONSTANT			
R	8.314	J mol^{-1} K^{-1}	R is the Boltzmann constant times Avogadro's Number ($6.023 \cdot 10^{23}$)
	1.987	cal mol^{-1} K^{-1}	
	8.314	m^{-3} Pa mol^{-1} K^{-1}	
RT	$2.437 \cdot 10^{3}$	J mol^{-1}	At 20 °C (293 °K)
	$5.833 \cdot 10^{2}$	cal mol^{-1}	At 20 °C (293 °K)
	$2.437 \cdot 10^{-2}$	liter MPa mol^{-1}	At 20 °C (293 °K)
RT/F	25.3	mV	At 20 °C (293 °K)
2.303 • RT	5.612	kJ mol^{-1}	At 20 °C (293 °K)
	1.342	kcal mol^{-1}	At 20 °C (293 °K)
FARADAY CONSTANT			
F	$9.649 \cdot 10^{4}$	coulombs mol^{-1}	F is the electric charge times Avogadro's Number
	$9.649 \cdot 10^{4}$	J mol^{-1} V^{-1}	
	23.06	kcal mol^{-1} V^{-1}	
CONVERSIONS			
kcal	4.187	J (joules)	Joules is an energy unit (equal to 1 Newton•meter)
Watt	1	J sec^{-1}	
Volt	1	J $coulomb^{-1}$	
Amperes	1	coulomb sec^{-1}	
Pascal (Pa)	1	Newton $meter^{-2}$	Pascal is a pressure unit (equal to 10^{-5} bars)
Siemens	1	Ohm^{-1}	Siemens (S) is conductance, the inverse of resistance (Ohm)
PHYSICAL PROPERTIES			
η_w	$1.004 \cdot 10^{-3}$	Pa sec	viscosity of water at 20 °C
ν_w	$1.004 \cdot 10^{-6}$	m^2 sec^{-1}	kinematic viscosity of water at 20 °C (viscosity/density)

incorrect constant

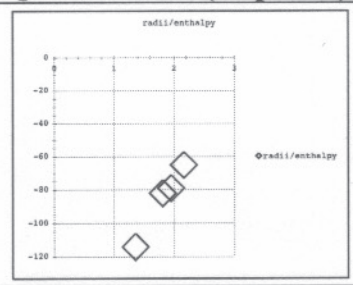
Source: Nobel, Park S (1991) Physicochemical and Environmental Physiology

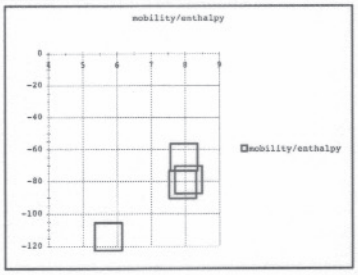
KEY

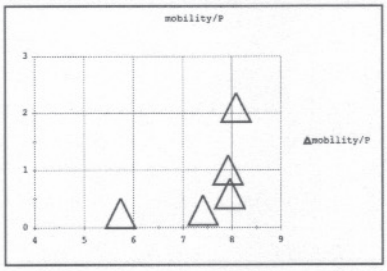
Question One (18 points)

A	$\Delta P = RT \cdot c$ 0.2 MPa / ($2.437 \cdot 10^{-3}$ liter MPa $mole^{-1}$) = 82 moles/liter (actually 82 mMoles/liter, because the given RT value was incorrect, should be 2.437)	3 points for sucrose, 3 points for ethanol
B	Ethanol permeates, thus pressure returns to the original value. When extracellular ethanol is removed, pressure jumps transiently, until ethanol permeates out.. Sucrose is impermeant.	6 points, partial points off
C	$J = P \cdot \Delta c$ $J = \Delta c \cdot (V/A) \cdot (1/t)$ So, $\Delta c \cdot (V/A) \cdot (1/t) = P \cdot \Delta c$, and $P = (V/A \cdot t)$ $(0.03 \cdot 0.08 \cdot 0.02\ cm3)/[(2 \cdot (0.03 \cdot 0.08) + 2 \cdot (0.03 \cdot 0.02) + 2 \cdot (0.08 \cdot 0.02)) \cdot 10\ sec] = 5.2 \cdot 10^{-4}$ cm/sec	6 points, partial points off

Question Two (18 points)







A. While extrapolation using atomic radii yields a enthalpy of about -20 kcal/mole, extrapolation with mobility yields an enthalpy of about -85 kcal/mole. Mobility yields better estimates of hydrated radius, thus the -85 extrapolation is more realistic.	6 points for extrapolation; 3 for justification by lower charge density leading to lower enthalpy.
B. Permeability is estimated from the reversal potentials. There is a relation between mobility and permeability: the higher the mobility, the higher the permeability. Thus, the channel behaves like a water-filled pore.	2 points for reversal potentials; 5 point for graphical/quantitative comparison; 2 points for water-filled channel conclusion

Question Two (18 points)

A. The Peclet Number (with 1 as the transition value) will assess the relative roles of advective versus diffusive transport directly: $Pe = (2 \cdot r \cdot v)/D$, or $v = 0.55 \cdot 10^{-5}\ cm^2\ sec^{-1}/(2 \cdot 0.003\ cm) = 9.2\ \mu m\ sec^{-1}$	18 points. Partial points were given for tortuosity if an attempt was made to compare diffusive times to swimming times.