Figure 2.19
Passive Transport: More than diffusion?

- Adding in other sugars affects things in a selective way.
- Saturation occurs.
Passive Transport: More than diffusion?

Structure of different solutes can have a big effect
Notion of a “carrier”

Carrier-Mediated Transport: glucose transporter as example

Distinguishing characteristics of glucose transport:
- facilitated -- i.e., faster than dissolve and diffuse
- structure specific -- different rates for even closely related sugars
- passive -- given a single solute, flow is down concentration gradient
- transport saturates -- solute-solute interactions
- transport can be inhibited -- solute-other interactions
- pharmacology (cytochalasin B)
- hormonal control (insulin)

similar to water channels
(Hg, vasopressin)
Possible ‘Carrier’ Mechanisms

Initial State

Binding

Translocation

Release

Reset
General Four-State Carrier Model

Binding & Unbinding

Translocation
First-order, reversible reaction

\[ R \xleftrightarrow{\alpha \beta} P \]

\[ \frac{dc_R(t)}{dt} = \beta c_P(t) - \alpha c_R(t) \quad \text{AND} \quad \frac{dc_P(t)}{dt} = \alpha c_R(t) - \beta c_P(t) \]

Equilibrium:

\[ \frac{dc_R(t)}{dt} = \frac{dc_P(t)}{dt} = 0 \rightarrow \beta c_P(\infty) = \alpha c_R(\infty) \]

\[ \frac{c_P(\infty)}{c_R(\infty)} = \frac{\alpha}{\beta} = K_a \quad \text{(association, equilibrium, affinity, stability, binding, formation constant)} \]

**Kinetics:** assume total amount of reactant and product is conserved

\[ c_R(t) + c_P(t) = C \]

\[ \frac{dc_R(t)}{dt} = \beta \left( C - c_R(t) \right) - \alpha c_R(t) \]

\[ \frac{dc_R(t)}{dt} + (\alpha + \beta) c_R(t) = \beta C \]
First-order, reversible reaction

\[ R \xrightarrow{\alpha} P \xrightleftharpoons{\beta} P \]

First-order linear differential equation with constant coefficients

\[ c_R(t) = c_R(\infty) - \left( c_R(\infty) - c_R(0) \right) e^{-t/\tau}, \text{ for } t > 0 \]

\[ c_R(\infty) = \frac{\beta}{\alpha + \beta} C = \frac{1}{1 + K_a} C \quad \text{AND} \quad \tau = \frac{1}{\alpha + \beta} \]

First-order, reversible reaction

\[ R \xrightarrow{\alpha} P \xrightleftharpoons{\beta} P \]

\[ C \]

\[ c_R(0) \]

\[ c_P(t) \]

\[ K_a C / (1 + K_a) \]

\[ c_P(0) \]

\[ c_R(t) \]

\[ C / (1 + K_a) \]

\[ t \]

\[ \tau = \frac{1}{\alpha + \beta} \]
Chemical Kinetics (v2)

Second-order reversible (binding) reaction

\[ S + E \xrightleftharpoons[\beta]{\alpha} ES \]

\[
\frac{dc_{ES}(t)}{dt} = \alpha c_S(t)c_E(t) - \beta c_{ES}(t), \\
\frac{dc_S(t)}{dt} = \frac{dc_E(t)}{dt} = \beta c_{ES}(t) - \alpha c_S(t)c_E(t),
\]

→ Law of mass action

Equilibrium:

\[
\frac{dc_{ES}(t)}{dt} = \frac{dc_S(t)}{dt} = \frac{dc_E(t)}{dt} = 0 \\
\alpha c_S(\infty)c_E(\infty) - \beta c_{ES}(\infty) = 0 \\
\frac{c_{ES}(\infty)}{c_S(\infty)c_E(\infty)} = \frac{\alpha}{\beta} = K_a \quad \text{(association constant)} \\
\frac{1}{K_a} = \frac{c_S(\infty)c_E(\infty)}{c_{ES}(\infty)} = K \quad \text{(dissociation constant)}
\]

Assume enzyme conserved: \( c_E(t) + c_{ES}(t) = C_E \)
How does \( c_{ES} \) depend on \( c_S \)? Eliminate \( c_E \).

\[ C_E = c_E(\infty) + c_{ES}(\infty) \]

\[ C_E = \frac{Kc_{ES}(\infty)}{c_S(\infty)} + c_{ES}(\infty) = \left( \frac{K}{c_S(\infty)} + 1 \right)c_{ES}(\infty) \]

\[ c_{ES}(\infty) = \left( \frac{c_S(\infty)}{K + c_S(\infty)} \right)C_E \]

→ Michaelis-Menten kinetics
Second-order reversible (binding) reaction

\[ S + E \xrightleftharpoons[\beta]{\alpha} ES \]

\[ C_{ET} = c_E(\infty) + c_{ES}(\infty) \]

\[ C_{ET} = \frac{K_{cES}(\infty)}{c_S(\infty)} + c_{ES}(\infty) = \left( \frac{K}{c_S(\infty)} + 1 \right) c_{ES}(\infty) \]

\[ c_{ES}(\infty) = \left( \frac{c_S(\infty)}{K + c_S(\infty)} \right) C_{ET} \]

Rectangular hyperbola: Michaelis-Menten Relation

Doubly-reciprocal coordinates: Lineweaver-Burk plot

\[ \frac{1}{c_{ES}(\infty)} = \left( 1 + \frac{K}{c_S(\infty)} \right) \frac{1}{C_{ET}} = \left( \frac{K}{C_{ET}} \right) \frac{1}{c_S(\infty)} + \frac{1}{C_{ET}} \]

\[ \frac{1}{C_{ET}} \]

\[ \frac{1}{c_S(\infty)} \]

\[ \frac{1}{c_{ES}(\infty)} \]

\[ K \]

\[ slope = \frac{K}{C_{ET}} \]

→ Linear way to plot nonlinear relationship!
Aside: Anscombe's Quartet

Anscombe (1973) Graphs in Statistical Analysis
General Four-State Carrier Model

Binding/Unbinding

Translocation
Possible ‘Carrier’ Mechanisms

**Initial State**
- Mechanism 1: Initial state with solute in the membrane.
- Mechanism 2: Initial state with solute in the membrane.
- Mechanism 3: Initial state with solute in the membrane.

**Binding**
- Mechanism 1: Binding process.
- Mechanism 2: Binding process.
- Mechanism 3: Binding process.

**Translocation**
- Mechanism 1: Translocation process.
- Mechanism 2: Translocation process.
- Mechanism 3: Translocation process.

**Release**
- Mechanism 1: Release process.
- Mechanism 2: Release process.
- Mechanism 3: Release process.

**Reset**
- Mechanism 1: Reset process.
- Mechanism 2: Reset process.
- Mechanism 3: Reset process.
Chemical Kinetics & ‘Carriers’

**Binding**

\[
S^i + E^i \xrightarrow{\alpha_1/\beta_1} ES^i
\]

\[
\frac{dC_{ES}^i}{dt} = \alpha_1 C_S^i C_E^i - \beta_1 C_{ES}^i
\]

\[
\frac{dC_S^i}{dt} = \frac{dC_E^i}{dt} = \beta_1 C_{ES}^i - \alpha_1 C_S^i C_E^i
\]

**Translocation**

\[
ES^i \xrightarrow{\alpha_2/\beta_2} ES^o
\]

\[
\frac{dC_{ES}^o}{dt} = \alpha_2 C_{ES}^i - \beta_2 C_{ES}^o
\]

\[
\frac{dC_E^i}{dt} = \beta_2 C_{ES}^o - \alpha_2 C_{ES}^i
\]
Chemical Kinetics & ‘Carriers’

Unbinding

\[ E S^o \overset{\beta_3}{\underset{\alpha_3}{\rightleftharpoons}} E^o + S^o \]

\[
\frac{dC_{ES}^o}{dt} = \alpha_3 C_S^o C_E^o - \beta_3 C_{ES}^o \\
\frac{dC_S^o}{dt} = \frac{dC_E^o}{dt} = \beta_3 C_{ES}^o - \alpha_3 C_S^o C_E^o
\]

Translocation

\[ E^i \overset{\alpha_4}{\underset{\beta_4}{\rightleftharpoons}} E^o \]

\[
\frac{dC_E^o}{dt} = \alpha_4 C_E^i - \beta_4 C_E^o \\
\frac{dC_E^i}{dt} = \beta_4 C_E^o - \alpha_4 C_E^i
\]
Numerous free parameters. Can we simplify?

\[
\begin{align*}
\frac{dC_{ES}^i}{dt} &= \alpha_1 C_S^i C_E^i - \beta_1 C_{ES}^i \\
\frac{dC_S^i}{dt} &= \frac{dC_E^i}{dt} = \beta_1 C_{ES}^i - \alpha_1 C_S^i C_E^i \\
\frac{dC_{ES}^o}{dt} &= \alpha_2 C_{ES}^o - \beta_2 C_{ES}^o \\
\frac{dC_E^o}{dt} &= \frac{dC_E^i}{dt} = \beta_3 C_{ES}^o - \alpha_2 C_{ES}^o \\
\frac{dC_E^i}{dt} &= \alpha_3 C_S^o C_E^o - \beta_3 C_{ES}^o \\
\frac{dC_E^o}{dt} &= \alpha_4 C_E^i - \beta_4 C_E^o \\
\frac{dC_E^i}{dt} &= \beta_4 C_E^o - \alpha_4 C_E^i
\end{align*}
\]
Simple, Symmetric Four-State Model

Binding/Unbinding (at equilibrium)

Translocation

Assumption: Steady-state
(i.e., carrier densities are independent of time)
Simple, Symmetric Four-State Model

1. Conservation of enzyme:

\[ \mathcal{N}_E^i + \mathcal{N}_E^o + \mathcal{N}_{ES}^i + \mathcal{N}_{ES}^o = \mathcal{N}_{ET} \]

2. Binding is fast (always in steady state):

\[ K = \frac{c_S\mathcal{N}_E^i}{\mathcal{N}_{ES}^i} = \frac{c_S\mathcal{N}_E^o}{\mathcal{N}_{ES}^o} \]

3. Translocation characterized by fluxes:

\[ \phi_{ES} = \alpha\mathcal{N}_{ES}^i - \beta\mathcal{N}_{ES}^o \]
\[ \phi_E = \alpha\mathcal{N}_E^i - \beta\mathcal{N}_E^o \]

4. Net flux of enzyme is zero:

\[ \phi_E + \phi_{ES} = 0 \]

→ Steady-state
(i.e., carrier densities are independent of time)
Simple, Symmetric Four-State Model

\[ \mathbf{n}_E^i + \mathbf{n}_E^o + \mathbf{n}_{ES}^i + \mathbf{n}_{ES}^o = \mathbf{n}_{ET} \]

\[ \phi_{ES} = \alpha \mathbf{n}_{ES}^i - \beta \mathbf{n}_{ES}^o \]

\[ \phi_E = \alpha \mathbf{n}_E^i - \beta \mathbf{n}_E^o \]

\[ \phi_E + \phi_{ES} = 0 \]

Combining equations...

\[ \mathbf{n}_{ES}^i = \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{c_S^i}{c_S^i + K} \right) \mathbf{n}_{ET} \]

\[ \mathbf{n}_E^i = \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{K}{c_S^i + K} \right) \mathbf{n}_{ET} \]

\[ \mathbf{n}_{ES}^o = \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{c_S^o}{c_S^o + K} \right) \mathbf{n}_{ET} \]

\[ \mathbf{n}_E^o = \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{K}{c_S^o + K} \right) \mathbf{n}_{ET} \]

Solving for the solute flux yields:

\[ \phi_S = \left( \frac{\alpha \beta}{\alpha + \beta} \right) \mathbf{n}_{ET} \left( \frac{c_S^i}{c_S^i + K} - \frac{c_S^o}{c_S^o + K} \right) \]
\[ K = \frac{c_s^i \mathcal{M}_E^i}{\mathcal{M}_{ES}^i} = \frac{c_s^o \mathcal{M}_E^o}{\mathcal{M}_{ES}^o} \]
\[ \phi_s = \left( \frac{\alpha \beta}{\alpha + \beta} \right) \mathcal{M}_{ET} \left( \frac{c_s^i}{c_s^o + K} - \frac{c_s^o}{c_s^i + K} \right) \]

Figure 6.22
→ Steady-state
(i.e., carrier densities are independent of time)

\[ c_s^i = 0, c_s^o = 0 \]
\[ c_s^i = K, c_s^o = 0 \]
\[ c_s^i = K, c_s^o = K \]
\[ c_s^i > K, c_s^o = 0 \]
\[ c_s^i = 0, c_s^o > K \]
\[ c_s^i \gg K, c_s^o \gg K \]

Figure 6.23

\[ K = \frac{c_s^i n_E^i}{n_{ES}^i} = \frac{c_s^o n_E^o}{n_{ES}^o} \]

\[ \phi_s = \left( \frac{\alpha \beta}{\alpha + \beta} \right) n_{ET} \left( \frac{c_s^i}{c_s^i + K} - \frac{c_s^o}{c_s^o + K} \right) \]
6.8 Consider the simple, symmetric, four-state carrier shown in Figure 6.21. For each of the following conditions, find $\eta^i_E$, $\eta^o_E$, $\eta^i_{ES}$, $\eta^o_{ES}$, and $\phi_S$. Explain the physical significance of each of your answers.

a. $\alpha = 0$.
b. $\beta = 0$.
c. $K = 0$.

6.9 For the simple, symmetric, four-state carrier shown in Figure 6.21, let $c^i_S = c^o_S = 0$. Sketch the carrier density in each of its four states as a function of $\alpha/\beta$. Give a physical interpretation of the results.
6.8 Consider the simple, symmetric, four-state carrier shown in Figure 6.21. For each of the following conditions, find $\eta^i_E$, $\eta^o_E$, $\eta^i_{ES}$, $\eta^o_{ES}$, and $\phi_S$. Explain the physical significance of each of your answers.

a. $\alpha = 0$.
b. $\beta = 0$.
c. $K = 0$.

6.9 For the simple, symmetric, four-state carrier shown in Figure 6.21, let $c^i_S = c^o_S = 0$. Sketch the carrier density in each of its four states as a function of $\alpha/\beta$. Give a physical interpretation of the results.

\[
\eta^i_{ES} = \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{c^i_S}{c^i_S + K} \right) \eta_{ET}
\]

\[
\eta^i_E = \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{K}{c^i_S + K} \right) \eta_{ET}
\]

\[
\eta^o_{ES} = \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{c^o_S}{c^o_S + K} \right) \eta_{ET}
\]

\[
\eta^o_E = \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{K}{c^o_S + K} \right) \eta_{ET}
\]

\[
\phi_S = \left( \frac{\alpha \beta}{\alpha + \beta} \right) \eta_{ET} \left( \frac{c^i_S}{c^i_S + K} - \frac{c^o_S}{c^o_S + K} \right)
\]
Practice problems (SOL)

Exercise 6.8

a. Since \( \alpha \) is zero, none of the enzyme can translocate to face the extracellular solution. Therefore the densities of outward facing enzymes \( \mathcal{N}_E^o \) and \( \mathcal{N}_E^i \) are zero. The inward facing densities partition in proportion to the intracellular concentration of solute and the dissociation constant for the binding reaction. Therefore,

\[
\mathcal{N}_E^i = \frac{c_S^i}{c_S^i + K} \mathcal{N}_{ET} \quad \text{and} \quad \mathcal{N}_E^o = \frac{K}{c_S^i + K} \mathcal{N}_{ET}.
\]

Since the enzyme cannot translocate, the flux of solute \( \phi_S \) is also zero.

b. The case \( \beta = 0 \) is similar to the case \( \alpha = 0 \) except that the enzyme can not face the intracellular solution. Therefore the densities of inward facing enzymes \( \mathcal{N}_E^i \) and \( \mathcal{N}_E^o \) are zero. The outward facing densities partition in proportion to the extracellular concentration of solute and the dissociation constant for the binding reaction. Therefore,

\[
\mathcal{N}_E^o = \frac{c_S^o}{c_S^o + K} \mathcal{N}_{ET} \quad \text{and} \quad \mathcal{N}_E^i = \frac{K}{c_S^o + K} \mathcal{N}_{ET}.
\]

Since the enzyme cannot translocate, the flux of solute \( \phi_S \) is also zero.

c. If \( K = 0 \), the enzyme cannot dissociate. Therefore, if there is any extracellular or intracellular solute, it will bind to the enzyme and never unbind. Therefore the unbound densities \( \mathcal{N}_E^i \) and \( \mathcal{N}_E^o \) will be zero. The bound densities will partition by the forward and reverse translocation rate constants, so that

\[
\mathcal{N}_E^i = \frac{\beta}{\alpha + \beta} \mathcal{N}_{ET} \quad \text{and} \quad \mathcal{N}_E^o = \frac{\alpha}{\alpha + \beta} \mathcal{N}_{ET}.
\]

Since the solute cannot unbind, there will be no transport, \( \phi_S \) will be zero.
Exercise 6.9 For $c_S^i = c_S^o = 0$ there is no carrier bound to enzyme. Therefore, on this basis and by inspection of Equations 6.55 and 6.57 (Weiss, 1996a) $\pi_{ES}^i = \pi_{ES}^o = 0$. However, from Equations 6.56 and 6.58 (Weiss, 1996a) it follows that

$$\pi_E^i = \frac{\beta}{\alpha + \beta} \pi_{ET} = \frac{1}{(\alpha/\beta) + 1} \pi_{ET},$$

$$\pi_E^o = \frac{\alpha}{\alpha + \beta} \pi_{ET} = \frac{(\alpha/\beta)}{(\alpha/\beta) + 1} \pi_{ET}. $$

These relations are plotted in Figure 6.2. If $\alpha/\beta = 1$ then half the carrier is in the inside configuration and the other half is in the outside configuration. As $\alpha/\beta$ is increased, more of the carrier is found in the outside configuration, whereas as $\alpha/\beta$ is decreased, more of the carrier is found in the inside configuration.

Figure 6.2: Density of carrier for a case when the solute concentration is zero on both sides of the membrane (Exercise 6.9).