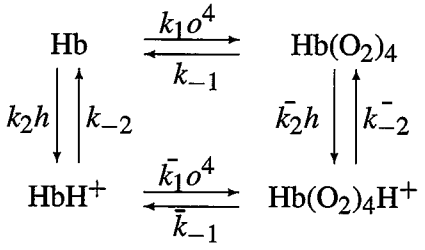
1 (a) **Allosteric regulation** corresponds to the regulation of an enzyme or protein activity on its main site by binding to another molecule, called an effector, on a secondary site called the allosteric site.

(b) The binding diagram corresponding to these reactions is the following.



Equilibrium constants: $K_1 = k_1/k_{-1}$, $K_2 = k_2/k_{-2}$, $\bar{K}_1 = \bar{k}_1/\bar{k}_{-1}$, $\bar{K}_2 = \bar{k}_2/\bar{k}_{-2}$,

(c) Assuming the equilibrium, we find that:

$$C_{bo} = K_1 o^4 C_b$$

$$C_{bh} = K_2 h C_b$$

$$C_{bho} = \bar{K}_1 o^4 C_{bh} = \bar{K}_1 K_2 o^4 h C_b$$

$$C = \left(1 + K_2 h + (K_1 + \bar{K}_1 K_2 h)\, o^4\right) C_b$$

The resulting saturation curve is:

$$Y = \frac{C_{bo} + C_{bho}}{C} = \frac{o^4}{o^4 + K_1^{-1}\phi(h)}, \text{ with } \phi(h) = \frac{(1 + K_2 h)}{1 + \frac{\bar{K}_1}{K_1} K_2 h} \tag{1}$$

The behaviour of the function ϕ controls the allostericity.

- ϕ large $\Longrightarrow$ late saturation $\Longrightarrow$ allosteric inhibitor
- ϕ small $\Longrightarrow$ early saturation $\Longrightarrow$ allosteric activator

ϕ can be rewritten as: $\phi(\zeta) = \frac{1+\zeta}{1+\kappa\zeta}$
where $\zeta = K_2 h$ and $\kappa = \bar{K}_1/K_1$.

(d) The figure shows that an increase in the hydrogen concentration, or decrease in the pH, shifts the saturation curve to the right. This means that $\bar{K}_1 < K_1$.

(e) The affinity for oxygen is decreased when an hydrogen ion is bound to the haemoglobin. The hydrogen ion therefore acts as an *allosteric inhibitor*.

Version: *cribs*

2 (a) With regard to models of ion channels, there are two voltage levels for which all sensible models should agree on the corresponding channel currents.

(i) What are these two voltage levels? Provide either a numerical value or a formula for each of them. If providing a formula, define the meaning of all variables in words.

Answer: The two voltage levels are $V = 0\,\text{mV}$ and the Nernst potential of the ion which is $V_{\text{Nernst}} = \frac{RT}{zF} \ln \frac{c_e}{c_i}$, where R is the universal gas constant, T is the absolute temperature, z is the valence of the ion, F is the Faraday constant, and c_e and c_i are the concentration of the ion outside and inside the cell, respectively. [20%]

(ii) Explain with reasons why any model should predict the same channel current at these voltage levels.

Answer: At $V = 0\,\text{mV}$ there is no external electric field acting on the ion, so the movement of the ion is solely determined by diffusion, for which the channel current can be computed without making further assumptions about the properties of the channel (which is where channel models differ). Ar $V = V_{\text{Nernts}}$ there is no current flowing through the channel (at steady state). [20%]

(iii) What are the current values predicted at these two voltage levels? Provide either a numerical value or a formula for each of them. If providing a formula, define the meaning of all variables in words.

Answer: At $V = 0\,\text{mV}$ the channel current is $I = PzF\,(c_i - c_e)$, where P is the permeability of the channel for the ion, z is the valence of the ion, F is the Faraday constant, and c_i and c_e are the concentration of the ion inside and outside the cell, respectively. At the Nernst potential $V = V_{\text{Nernst}}$, the channel current is $I = 0\,\text{nA}$. [20%]

(b) With regard to reducing the Hodgkin-Huxley model to a simpler dynamical system

(i) State the number of dynamical variables in the Hodgkin-Huxley model, and for each variable, describe its biophysical meaning.

Answer: There are 4 state variables in the Hodgkin-Huxley model, V, the membrane potential, m, the fraction of open activating gates of the Na^+ channel, h, the fraction of open inactivating gates of the Na^+ channel, and n, the fraction of open activating gates of the K^+ channel. [10%]

(ii) State the number of dynamical variables in the reduced dynamical system model. Explain how they relate to the original variables of the

Hodgkin-Huxley model.

Answer: There are 2 state variables in the reduced dynamical system model, V, the fast excitation variable, and W the slow recovery variable. V in this model is related to V and m in the Hodgkin-Huxley model, and W is related to n and h in the Hodgkin-Huxley model. [10%]

(iii) Explain the main logical steps in the reduction of the Hodgkin-Huxley model.

Answer: 1. We realise that during the generation of an action potential, the time constants of V and m ($C_{\mathrm{m}}/(g_{\mathrm{Na}^+} + g_{\mathrm{K}^+} + g_{\mathrm{L}})$ and τ_{m}) are about an order of magnitude faster ($< 1\,\mathrm{ms}$) that those of n and h (τ_{n} and $\tau_{\mathrm{h}} > 5\,\mathrm{ms}$ at their peak values). 2. We establish that V and m evolve along a one-dimensional manifold for most of the time. Thus, one of these variables (m) can be expressed as the instantaneous function of the other (V) without too much loss in model accuracy, $m(t) \simeq m_{\infty}(V(t))$. 3. Likewise, we establish that n and h evolve along a one-dimensional manifold (in fact, a straight line) for most of the time. Thus they can be expressed as the instantaneous (linear) function of the same variable (W) without too much loss in model accuracy, $h(t) \simeq 1 - W(t)$ and $n(t) \simeq W(t)/s$, where s is a suitable constant. 4. We are left with just two dynamical equations, describing the evolution of V and W. [20%]

3 (a) (i) At the equilibrium, the concentration of carbon dioxide is set by the partial pressure in the gas. $[CO_2] = \sigma P_{co2} = 2.51 \cdot 10^{-4}$ Molar.

H_2CO_3 is also at the equilibrium with CO_2, with a constant equal to K_a/K_1. $[H_2CO_3] = 4.3 \cdot 10^{-7}$ Molar.

(ii) No charges can be created by dissolving a neutral molecule in a neutral solution. The sum of all positive charges must equal the sum of all positive charges. Hence $[H^+] = [HCO_3^-] + 2\,[CO_3^{2-}] + [OH^-]$.

(iii) In order to find the concentrations of all species, a number of reasonable assumptions can be made. An acid is dissolved in water, so the pH is likely to be significantly acidic.

Assumption 1: $[H^+] \gg [OH^-]$, i.e. pH < 6

Additionally, since K_2 is very small, $[CO_3^{2-}]$ is probably negligible.

Assumption 2: $[HCO_3^-] \gg [CO_3^{2-}]$

These assumptions now enable us to solve rapidly for all concentrations.

The electro-neutrality provides: $[H^+] = [HCO_3^-]$.

Hence, $[H^+]^2 = [HCO_3^-]^2 = K_a\,[CO_2]$. This leads to $[HCO_3^-] = 1.04 \cdot 10^{-5}$ Molar and pH = 4.98, validating therefore assumption 1.

$[CO_3^{2-}]$ is obtained from the expression of K_2. Since $[H^+] = [HCO_3^-]$, we get $[CO_3^{2-}] = K_2$, which validates as well the assumption 2.

(b) (i) Since the gas pressure is the same, the concentrations of CO_2 and H_2CO_3 are unchanged. However, the electro-neutrality of the solution cannot be exploited any more. Although the solution is still neutral, we do not know what are the species present in the solution.

(ii) The pH being now known, the concentrations of HCO_3^- and CO_3^{2-} are respectively determined by K_1 and K_2.

$[H^+] = 3.98 \cdot 10^{-8}$ Molar, $[HCO_3^-] = 2.71 \cdot 10^{-3}$ Molar, $[CO_3^{2-}] = 3.82 \cdot 10^{-6}$ Molar.

(iii) If the pH is controlled, the concentrations of all species are proportional to $[CO_2]$, i.e. to the partial pressure in carbon dioxide.

4 (a) (i) When the radius changes by δR, the volume of the bubble changes (to the first order) by $A\delta R$. The corresponding work of pressure forces is $(P_e - P_i)A\delta R$. Concerning the surface energy, the variation is simple $\Gamma\delta A = \Gamma dA/dR\delta R$.

Putting these two expressions together, one gets:

$$\delta E = \left((P_e - P_i)A + \Gamma\frac{dA}{dr}\right)\delta R$$

(ii) The condition for the equilibrium is $dE/dR = 0$, ie: $(P_e - P_i)A + \Gamma\frac{dA}{dr} = 0$.

Since $A = 4\pi R^2$, $dA/dR = 8\pi R$.

$$(P_i - P_e) = \frac{2\Gamma}{R}$$

(b) (i) The tissue can be assumed to be linear elastic, i.e. that stresses are proportional to deformations with respect to the stress free state. The tangential stretch in any direction is $\varepsilon \propto (R - R_0)/R_0$. The tension in the tissue is essentially the stress integrated over the thickness of the layer. The stress itself is proportional to ε. Therefore, the tension is proportional to $(R - R_0)/R_0$. To the first order in $R - R_0$, $V - V_0 = 4\pi R_0^2(R - R_0) = 4\pi R_0^3\varepsilon$, hence the relationship.

(ii) The same reasoning can be used, replacing Γ by $\Gamma + T$.

(c) When using a saline solution, the part of the pressure difference due to surface tension can be neglected, suggesting that only the stretching of the tissue would contribute. Expansion of the tissue would therefore occur at lower pressure.

The experimental data confirms the reduction in pressure, but the most striking feature is the large hysteresis observed with air inflation, which cannot be explained with our model. These are mostly due to the highly dissipative and non-linear properties of the lung mucus, which is also washed away by the saline solution.

END OF PAPER

Version: *cribs*