Biophysics I (BPHS 4080)

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Website: http://www.yorku.ca/cberge/4080W2018.html

York University
Winter 2018
Lecture 15

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

Resting Potential: Model considering only a ***multiple*** *permeant ions*

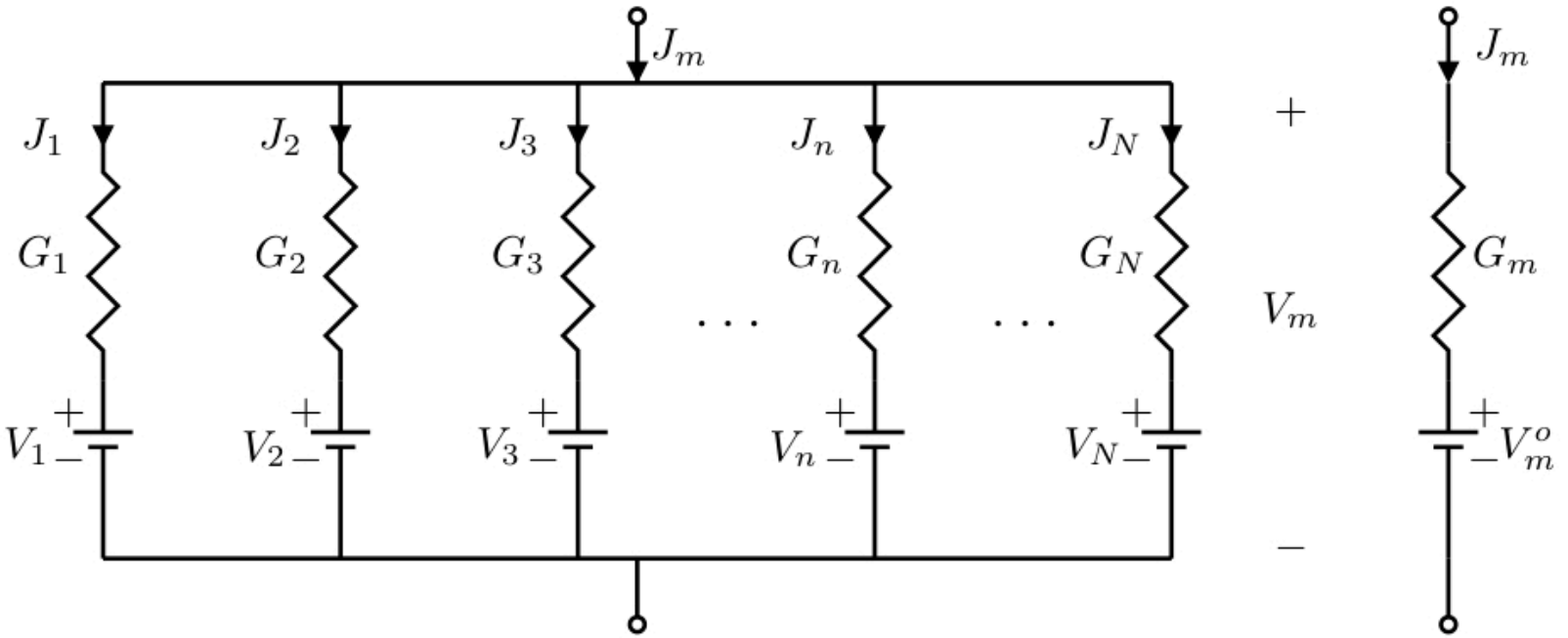


Figure 7.24

$$V_m^o = \frac{RT}{F}\left(\frac{G_K}{G_m}\right)\ln\left(\frac{c_K^o}{c_K^i}\right) + \sum_{n\neq K}\frac{G_n}{G_m}V_n$$

→ Still not entirely sufficient....

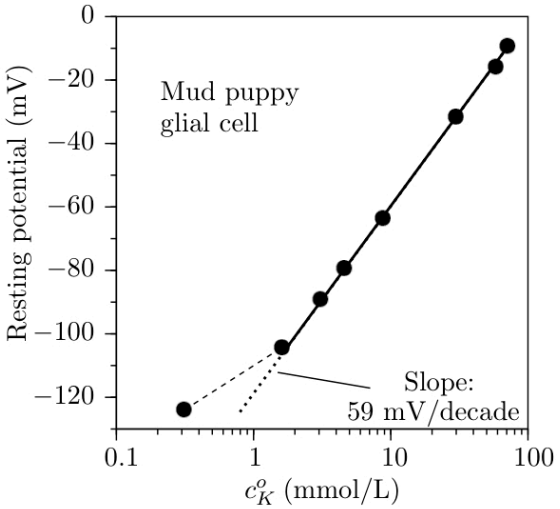


Figure 7.21

What if conductances were voltage-dependent?

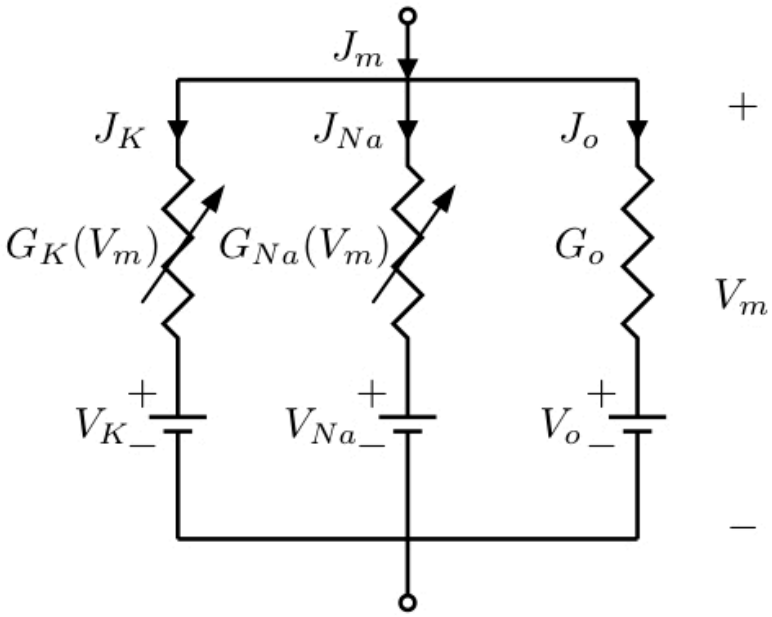


Figure 7.32

i.e., voltage-gated ion channels
(more detail in vol.2 ch.6)

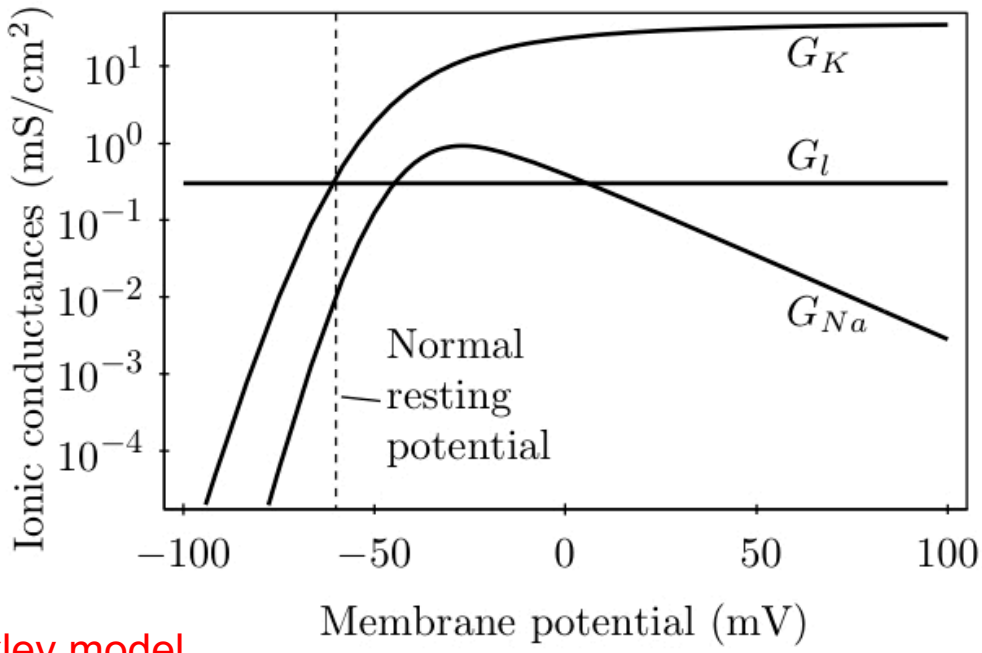


Figure 7.28

Hodgkin-Huxley model conductances

$$V_m^o = \frac{RT}{F}\left(\frac{G_K}{G_m}\right)\ln\left(\frac{c_K^o}{c_K^i}\right) + \sum_{n\neq K}\frac{G_n}{G_m}V_n$$

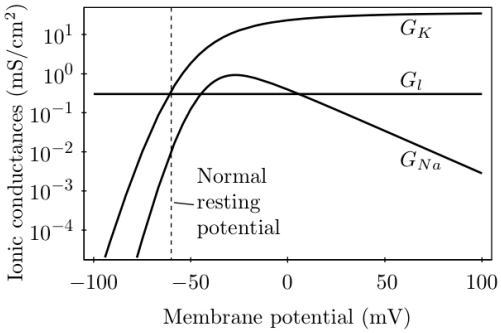


Figure 7.28

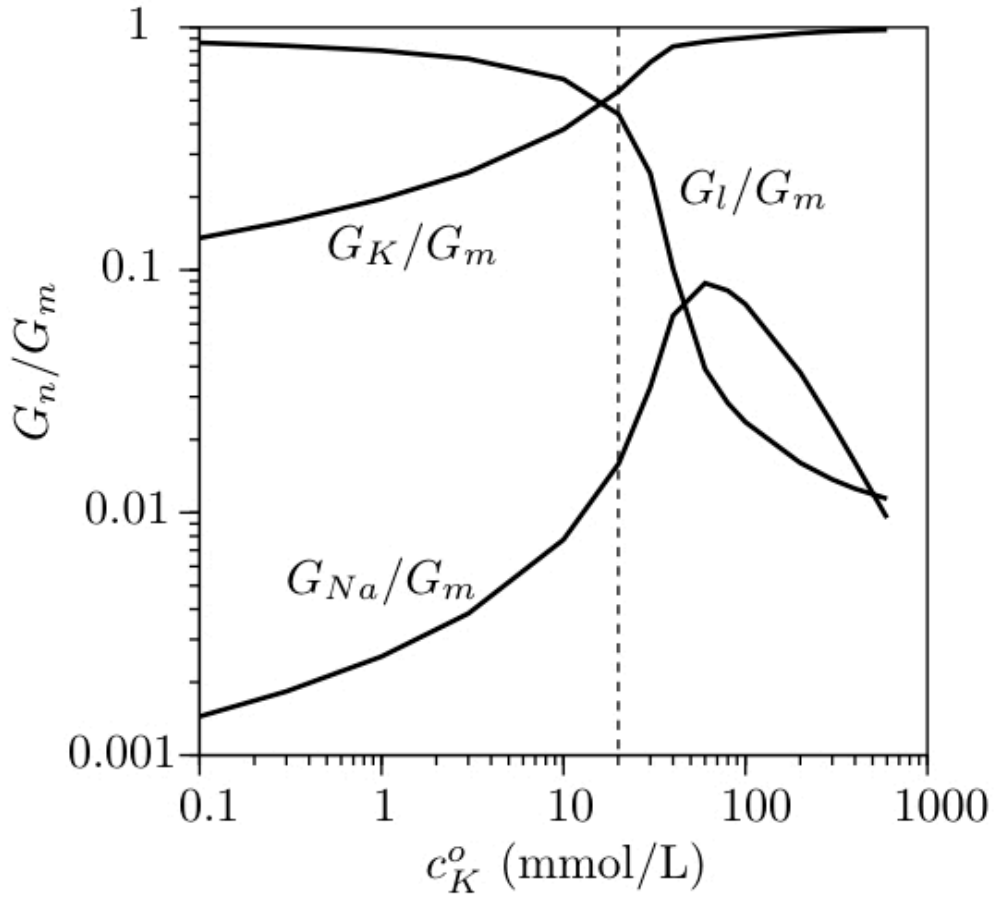


Figure 7.30

→ For physiological K+ concentrations, potassium is a dominant ion

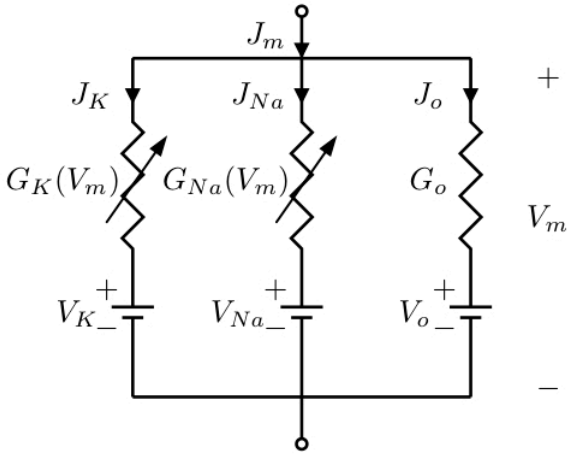


Figure 7.32

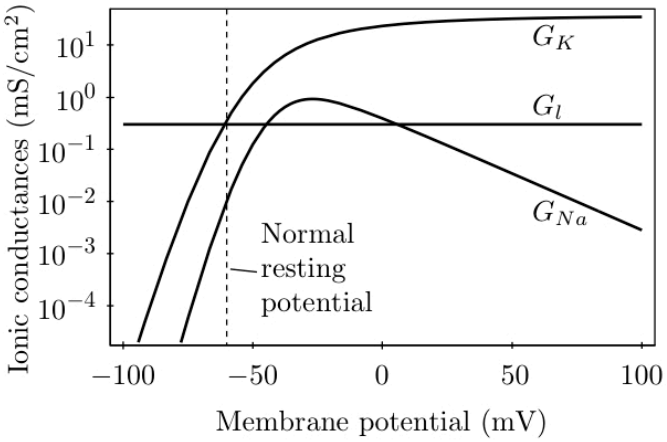


Figure 7.28

$$\sum_n G_n(V_m^o - V_n) = 0$$

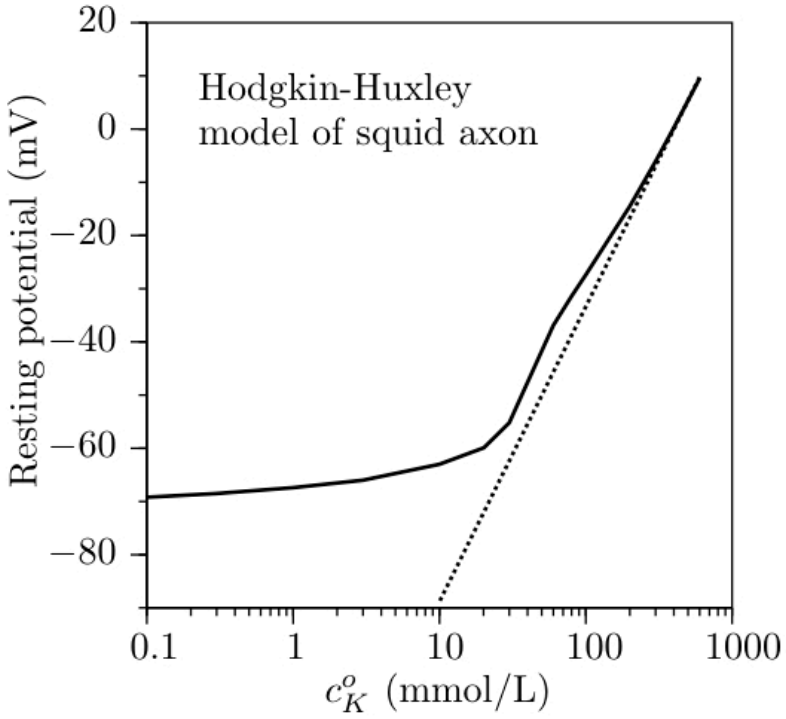
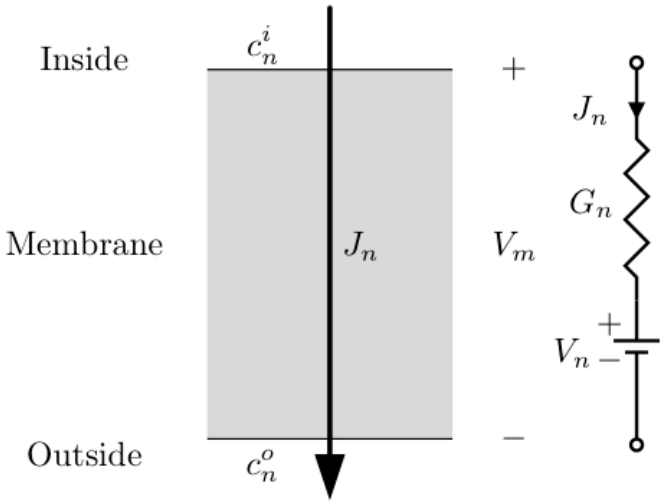


Figure 7.29

→ This looks like it might work!

Is a purely passive model adequate?

Inside cell: high [K⁺], low [Na⁺]
Outside cell: low [K⁺], high [Na⁺]



Ion	G_n (S/cm^2)	G_n/G_m	c_n^o/c_n^i	V_n (mV)
K^+	3.7×10^{-4}	0.55	0.05	−72
Na^+	1×10^{-5}	0.016	9.8	+55
leakage	3.0×10^{-4}	0.44	—	−49

$$J_K = G_K(V_m^o - V_K) = 0.37 \times 10^{-3}(-60 + 72) \times 10^{-3} \approx +4\,\mu\text{A/cm}^2$$

$$J_{Na} = G_{Na}(V_m^o - V_{Na}) = 1 \times 10^{-5}(-60 - 55) \times 10^{-3} \approx -1\,\mu\text{A/cm}^2$$

K⁺ efflux
Na⁺ influx

→ Passive case unsustainable!
(eventually leads to equilibrium)

$$\frac{RT}{z_1 F}\ln\left(\frac{c_1^o}{c_1^i}\right) = \frac{RT}{z_2 F}\ln\left(\frac{c_2^o}{c_2^i}\right) = \cdots = \frac{RT}{z_n F}\ln\left(\frac{c_n^o}{c_n^i}\right)$$

[osmotic effects also important here]

Table 7.4 Net flux of ions across the membranes of nerve axons during a propagated action potential (Cohen and De Weer, 1977). The ion fluxes are given per action potential.

Preparation	K^+ efflux	Na^+ influx
	(pmol/cm^2)	
Loligo forbesi axon	3.0	3.5
Loligo pealei axon	3.7	—
Sepia officinalis axon	3.6	3.8
Homarus nerve	4.1	5.2
Carcinus nerve	1.7–20	—
Rabbit vagus nerve	1	—

→ Action potentials make it even worse!

Ion Pumps

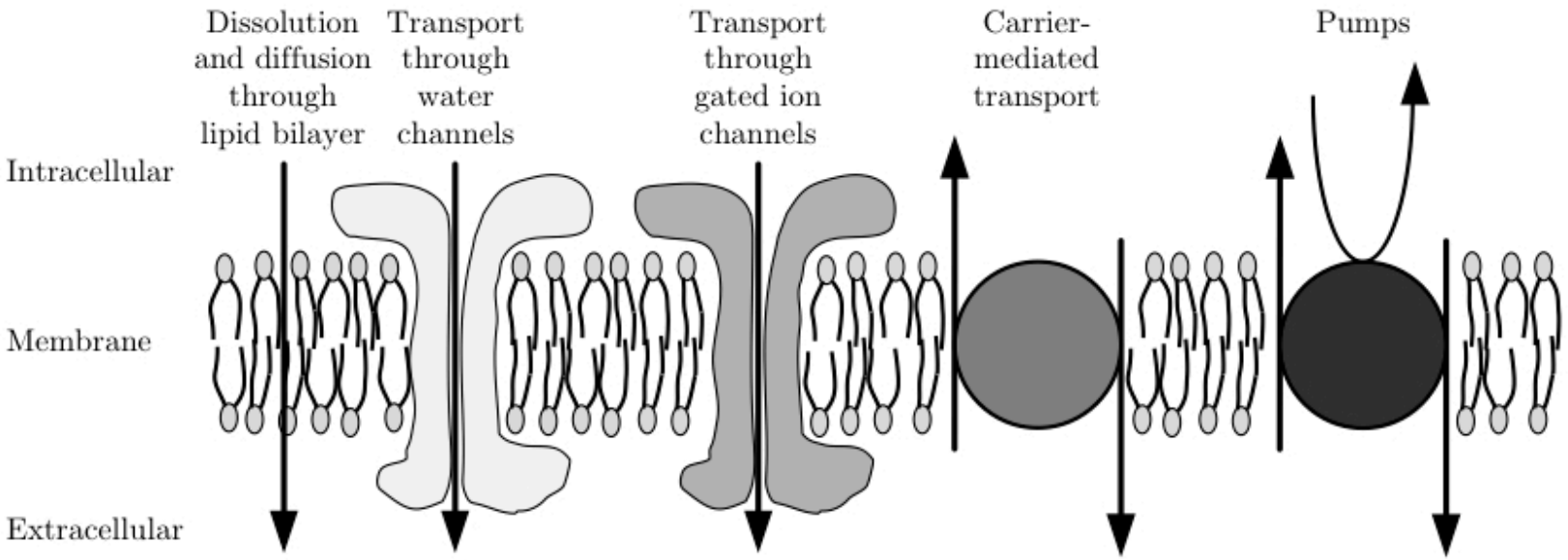


Figure 2.19

→ Need an 'active' (i.e., metabolically-dependent) mechanism to maintain normal physiological charge separation (e.g., Na^+/K^+ *pump*)

Ion Pumps

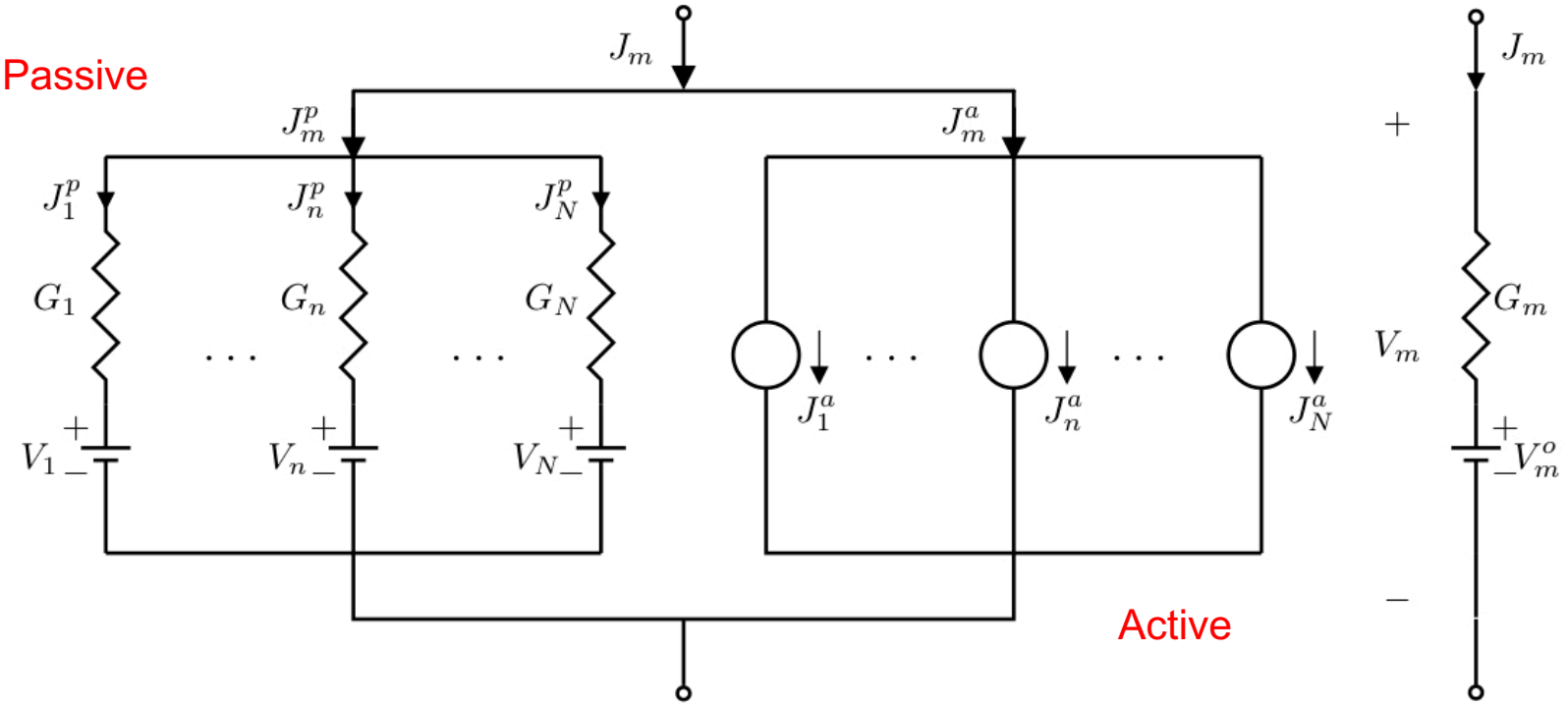


Figure 7.33

→ 'Active' implies energy is used to pump (i.e., create a current) *against* the electrochemical gradient

Why “active”?

[see Weiss sec.6.4.3]

Simple, Symmetric Four-State Model

- [Modify SS4 model] Suppose the two disassociation constants differ

$$\mathfrak{N}^i_{ES} = \frac{c^i_S}{c^i_S + K_i}\mathfrak{N}_{ET}, \text{ and } \mathfrak{N}^o_{ES} = \frac{c^o_S}{c^o_S + K_o}\mathfrak{N}_{ET}$$

$$\phi_S = (\phi_S)_{max}\left(\frac{c^i_S}{c^i_S + K_i} - \frac{c^o_S}{c^o_S + K_o}\right)$$

$$\phi_S = (\phi_S)_{max}\frac{K_o c^i_S - K_i c^o_S}{(c^i_S + K_i)(c^o_S + K_o)}$$

Now suppose $c^i_S > c^o_S$, but $K_o c^i_S < K_i c^o_S$: then $\phi_S < 0$

→ Why though would such (i.e., transport up the gradient) “require” energy?

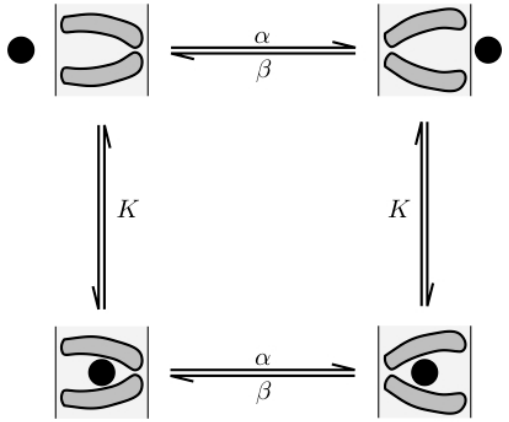


Figure 6.20

$$\mathfrak{N}^i_{ES} = \left(\frac{\beta}{\alpha+\beta}\right)\left(\frac{c^i_S}{c^i_S + K}\right)\mathfrak{N}_{ET}$$

$$\mathfrak{N}^i_{E} = \left(\frac{\beta}{\alpha+\beta}\right)\left(\frac{K}{c^i_S + K}\right)\mathfrak{N}_{ET}$$

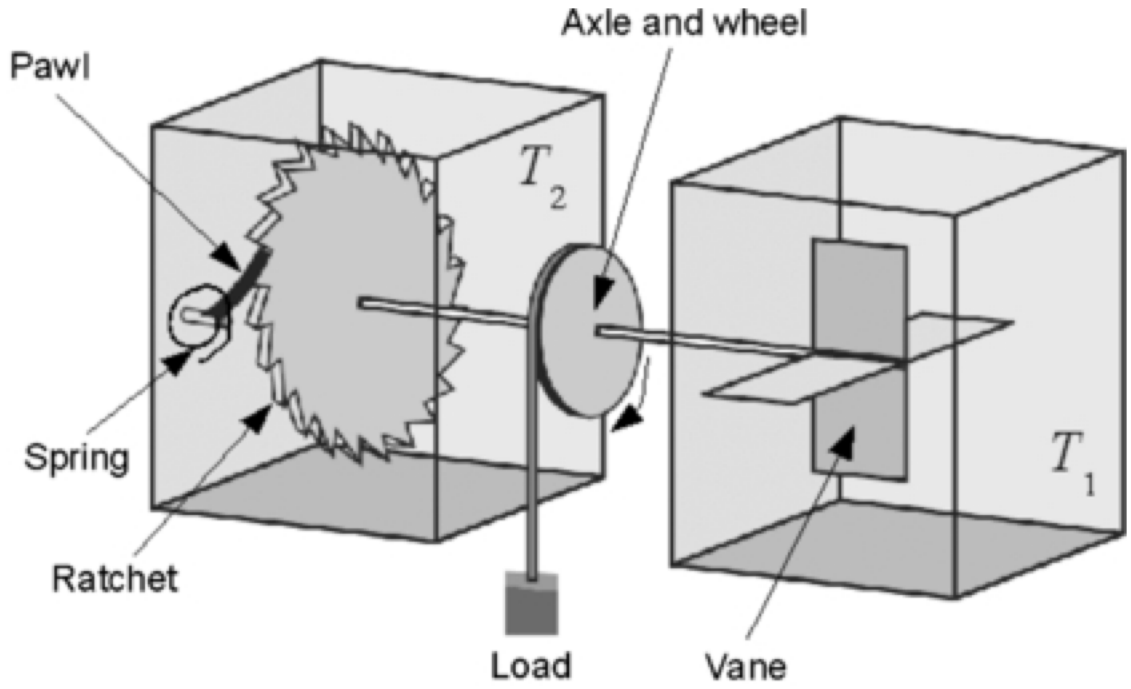
$$\mathfrak{N}^o_{ES} = \left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{c^o_S}{c^o_S + K}\right)\mathfrak{N}_{ET}$$

$$\mathfrak{N}^o_{E} = \left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{K}{c^o_S + K}\right)\mathfrak{N}_{ET}$$

$$\phi_S = \left(\frac{\alpha\beta}{\alpha+\beta}\right)\mathfrak{N}_{ET}\left(\frac{c^i_S}{c^i_S + K} - \frac{c^o_S}{c^o_S + K}\right)$$

Why “active”?

Brownian ratchet (Feynman-Smoluchowski ratchet)



- Version of “Maxwell’s demon”
- Violation of 2nd Law of Thermodynamics?

→ No

http://iopscience.iop.org/article/10.1088/1751-8113/41/31/312003

Why “active”?

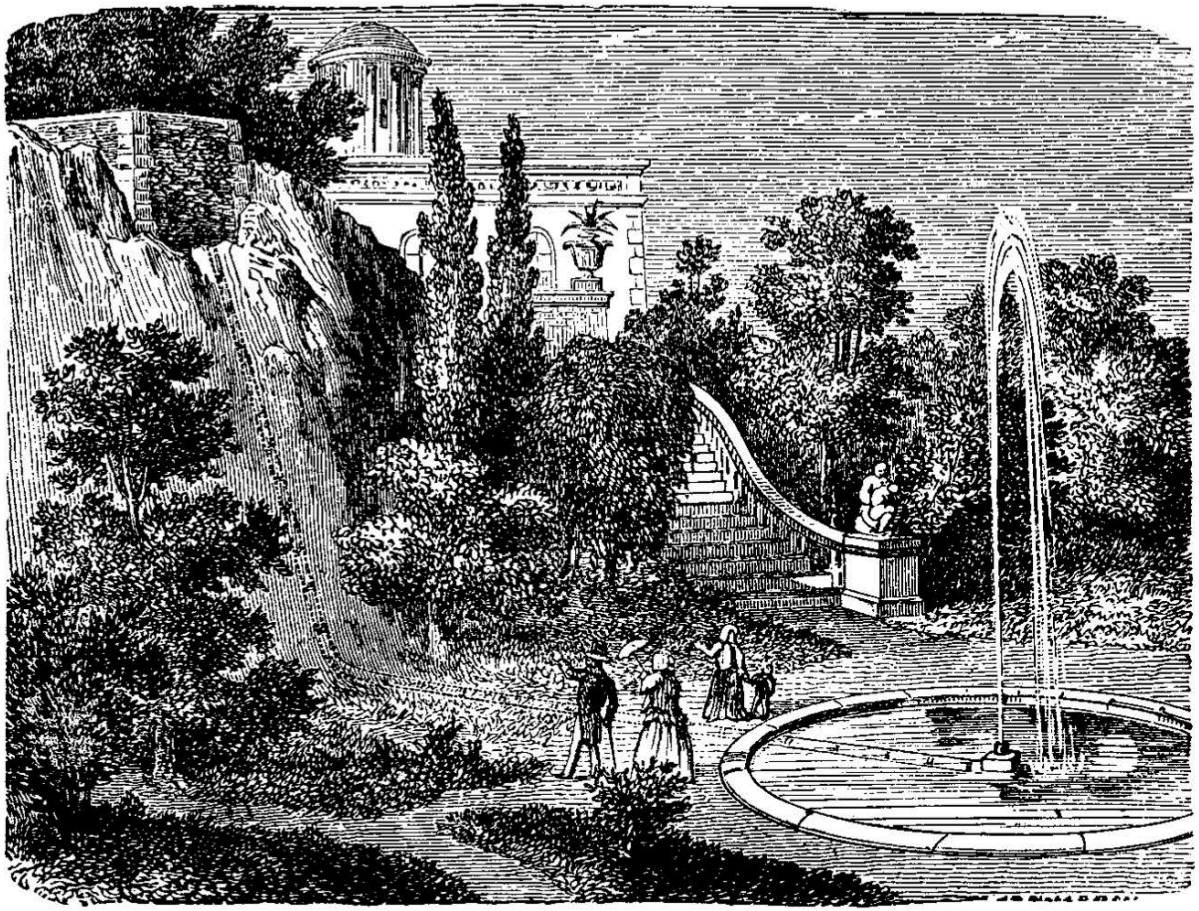


Figure 11.3: (Metaphor.) If water is continuously pumped to the upper reservoir, the fountain will come to a nonequilibrium steady state. If not, it will come to a quasisteady state, which lasts until the reservoir is empty.

Nelson (2002)

Why “active”?

Here is a mechanical analogy: Suppose you visit your friend and see in his garden a fountain. The fountain could be supplied by a high tank of water (Figure 11.3). In that case it flows, converting the gravitational potential energy of the water in the tank to kinetic energy (and ultimately heat), until the tank is empty; that is, it drives to equilibrium. But if you watch the fountain for many hours and it never stops, you may begin to suspect that your friend instead recirculates the water with a *pump,* using some external source of energy. In that case the fountain is in a steady, but nonequilibrium, state.

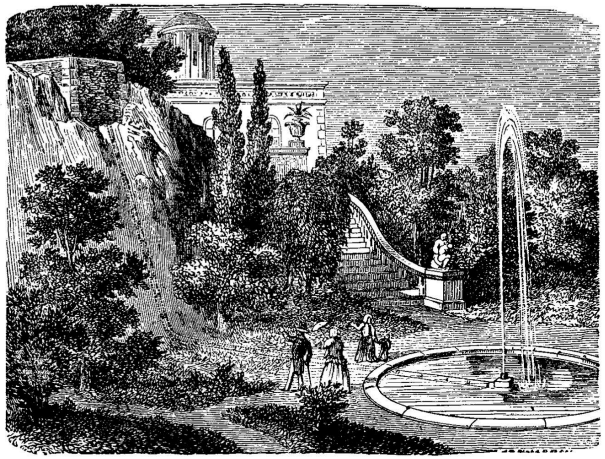


Figure 11.3: (Metaphor.) If water is continuously pumped to the upper reservoir, the fountain will come to a nonequilibrium steady state. If not, it will come to a quasisteady state, which lasts until the reservoir is empty.

Inside cell: high [K^+], low [Na^+]
Outside cell: low [K^+], high [Na^+]

$$\frac{RT}{z_1 F}\ln\left(\frac{c_1^o}{c_1^i}\right) = \frac{RT}{z_2 F}\ln\left(\frac{c_2^o}{c_2^i}\right) = \cdots = \frac{RT}{z_n F}\ln\left(\frac{c_n^o}{c_n^i}\right)$$

Idea: Cell is not strictly in equilibrium

Nelson (2002)

Ion Pumps

- Active pumps allow for *cellular quasi-equilibrium*

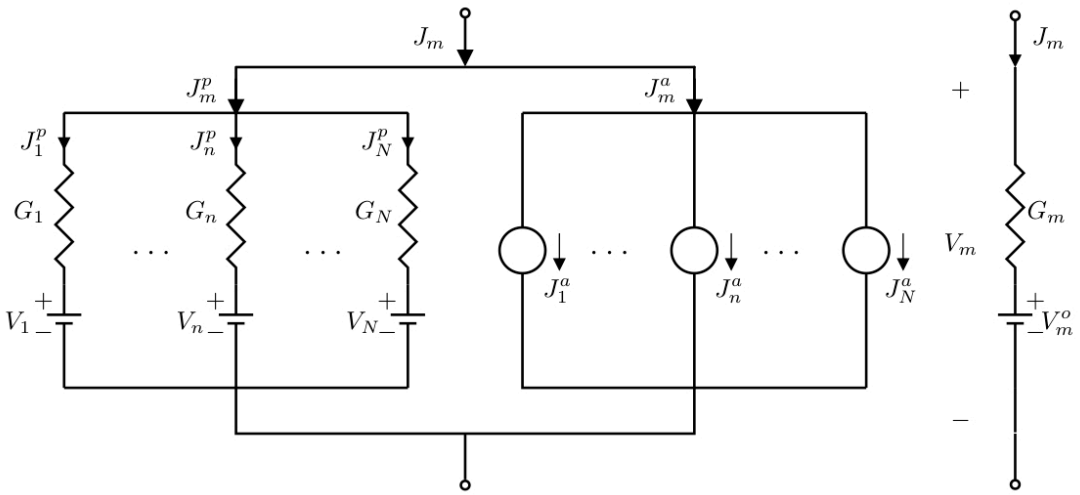


Figure 7.33

$$J_n^p + J_n^a = 0$$

Implies concentration on n does not change

(J^a is the *ion pump* term)

uniform cell at rest:

$$J_m = J_m^p + J_m^a = 0$$

$$\sum_n G_n(V_m^o - V_n) + \sum_n J_n^a = 0$$

$$V_m^o = \sum_n \frac{G_n}{G_m} V_n - \frac{1}{G_m} \sum_n J_n^a$$

$$J_m = \sum_n G_n(V_m - V_n) + \sum_n J_n^a = G_m \left(V_m - \left(\sum_n \frac{G_n}{G_m} V_n - \frac{1}{G_m} \sum_n J_n^a \right) \right)$$

Ion Pumps

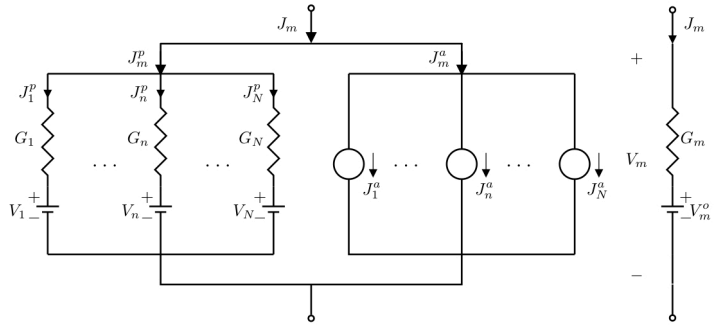
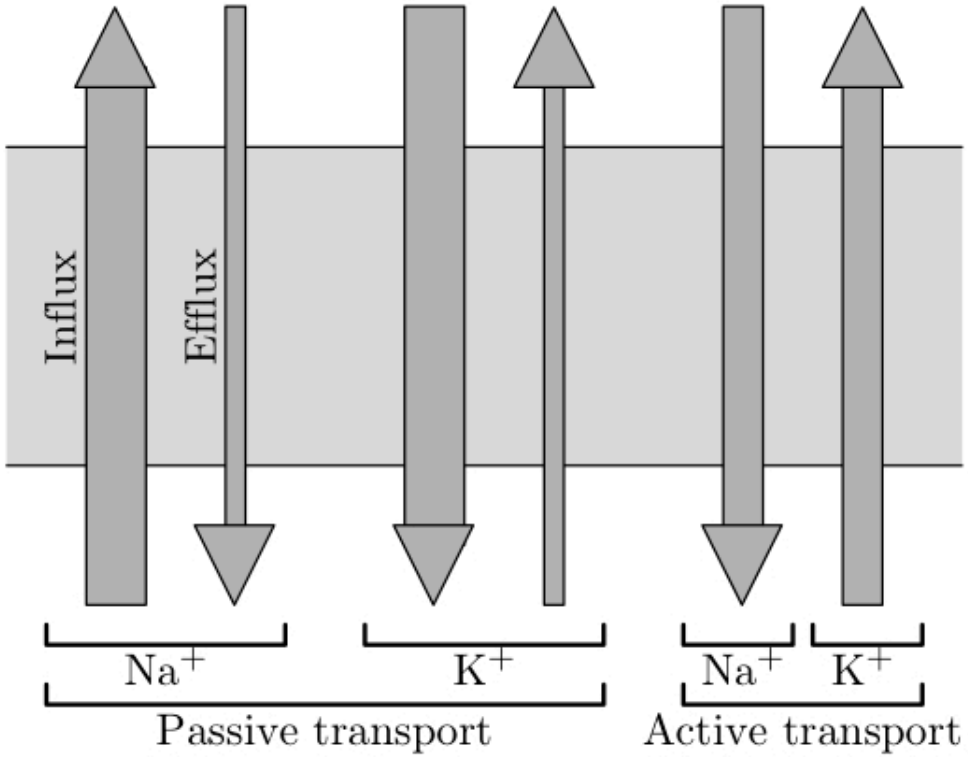


Figure 7.33

Passive:
K^+ efflux
Na^+ influx



Active:
Na^+ efflux
K^+ influx

Figure 7.35

→ Empirical means to differentiate: Examine Na+ efflux

Na+ efflux is metabolically dependent

- Axons pre-loaded w/ radioactive $^{24}Na^{+}$ (left, center) or perfused w/ dialysis tubing (right)
- Light grey bars indicate addition of metabolic poisons (left, center) or ATP (right) (“*the molecular unit of currency of intracellular energy transfer*”, Wikipedia)
- Plots use a proxy measure for Na^{+} efflux (“The change in count rate with time was proportional to efflux of radioactive Na”)
- Refs: Hodgkin & Keynes (1955), Mullins & Brinley (1967)

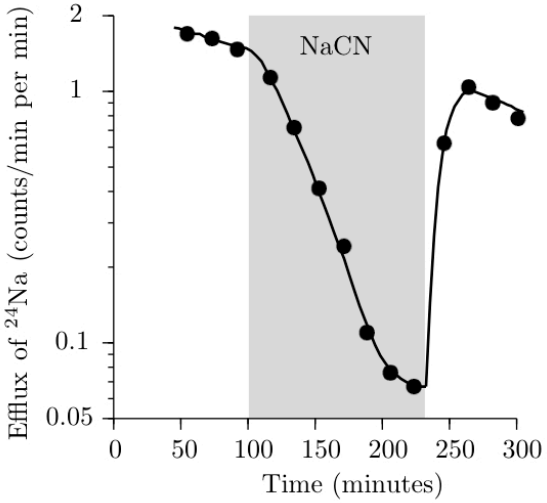


Figure 7.36

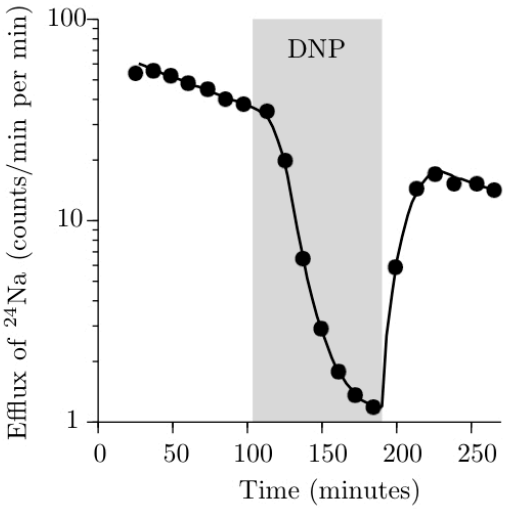


Figure 7.37

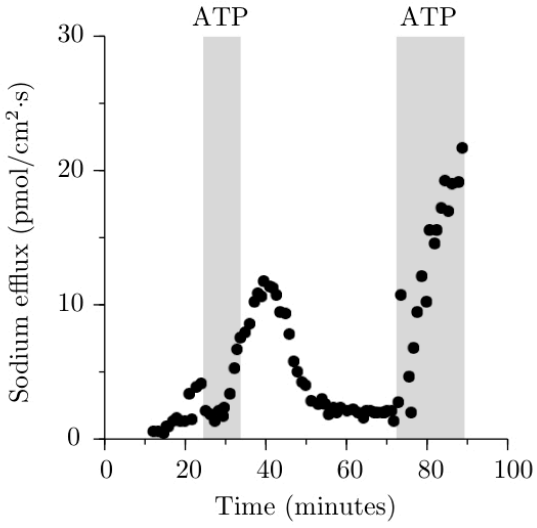


Figure 7.39

- Note decreasing in efflux before poisons (due to limited amount of tagged Na)
- Note distinction between “reversible” and irreversible” effects

→ Taken together, data are suggestive that efflux is metabolically-dependent

Cardiac Glycosides → Block Na+/K+ pump

- Ref: Baker & Willis (1972)

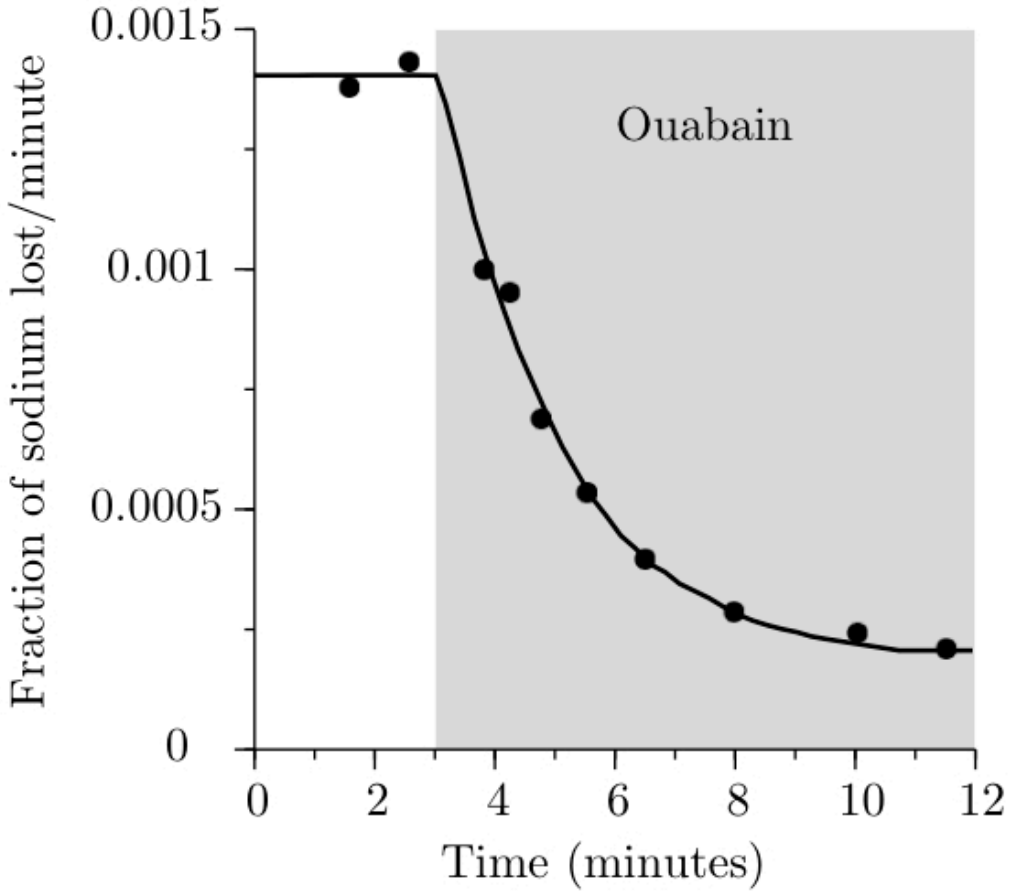


Figure 7.41

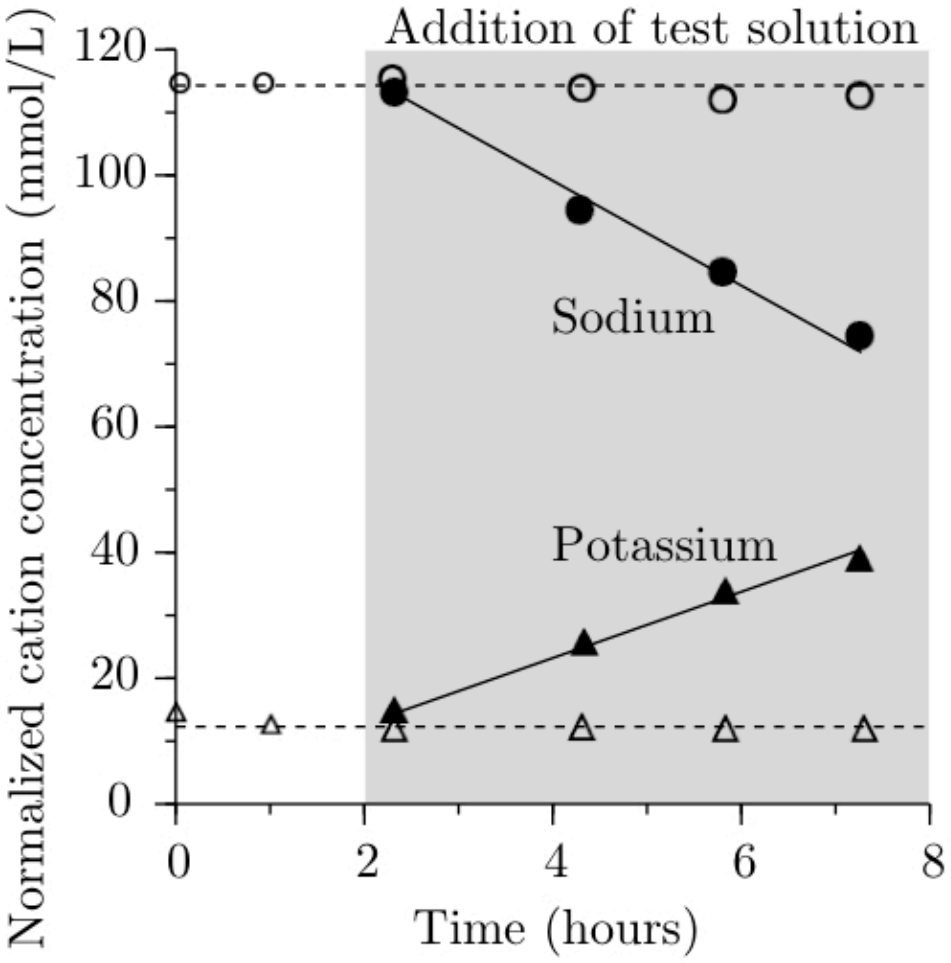
→ Cardiac glycosides (e.g., ouabain) inhibit/block Na^+/K^+ pump

(though these drugs had long been used to treat heart failure, it wasn't until ~1950s that it was understood how)

Pump Dependence Upon Ion Concentration

Are pumping of Na^+ and K^+ linked?

- Human erythrocytes (i.e., red blood cells) pre-loaded w/ high [Na^+] and low [K^+]
- Circles represent [Na^+] measurements
- Triangles represent [K^+] measurements
- Grey bar indicates change from low [Na^+]
- Filled symbols – 'test solution' containing K^+
- Open symbols – 'control solution' with no K^+
- Ref: Post & Jolly (1957)



No external K^+ means no Na^+ efflux

→ Pump activity linked to concentration of both Na^+ and K^+

Figure 7.42

Ion Pumps

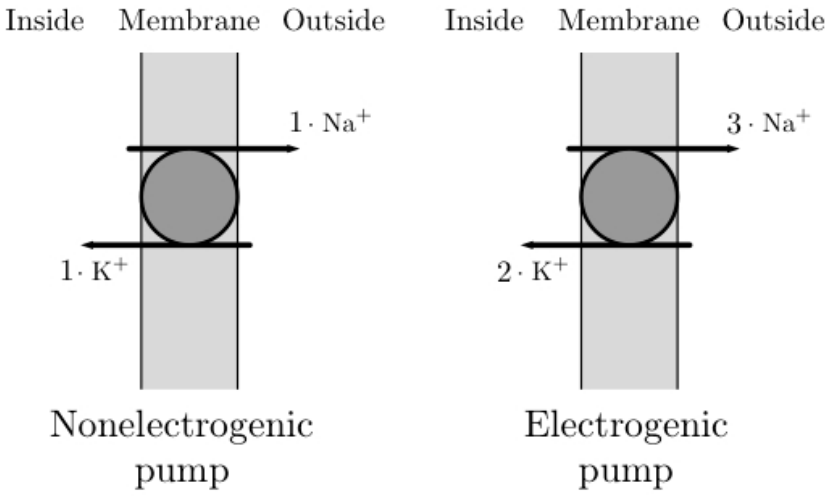


Figure 7.34

→ Does a pump contribute to the membrane potential?

(i.e., does active Na^+ efflux exceed active K^+ influx?)

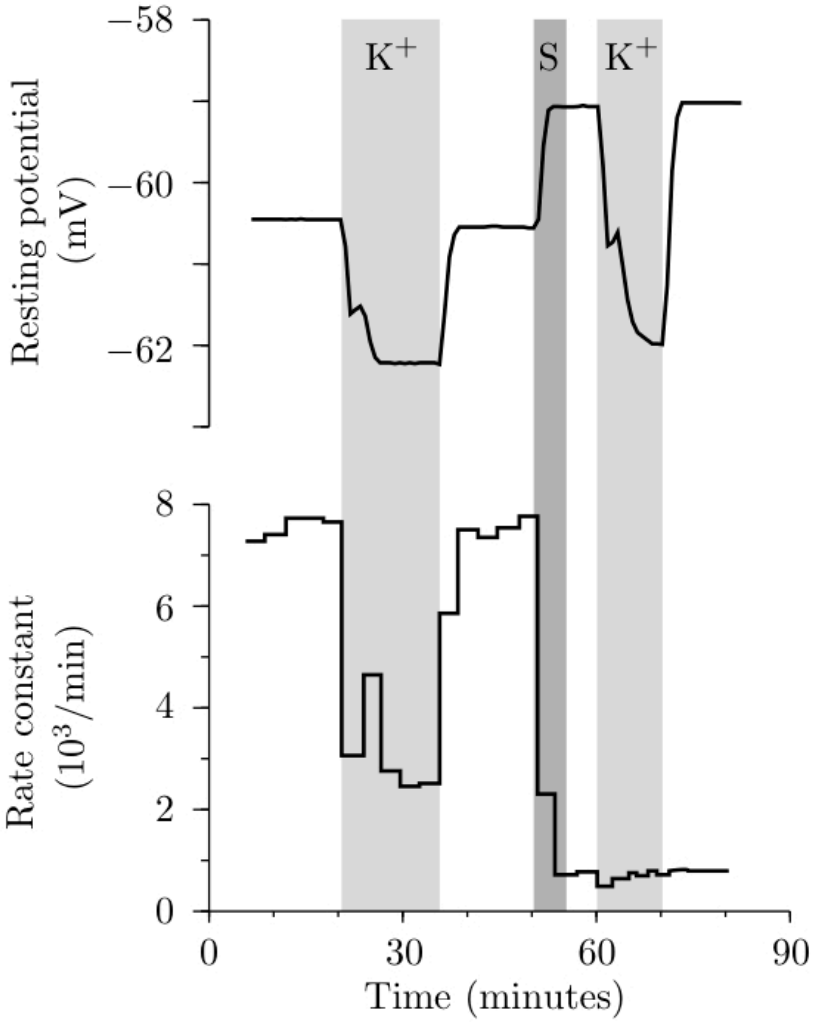


Figure 7.44

De Weer & Geduldig (Science, 1973)

- Axon pre-loaded w/ radioactive $^{22}Na^+$
- Light grey bars indicate reduced external K^+ concentration
- Dark grey bars indicate addition of strophanthidin (S) ["*a cardiac glycoside which mechanism of action is similar to Digitalis, Ouabain and digitoxin. It specifically inhibits the membrane protein Na+/ K+ ATPase*", wikipedia]
- Top shows resting potential, bottom a proxy measure for Na^+ efflux (via the radioactive rate count)
- Ref: De Weer & Geduldig (Science, 1973)

→ Pump inhibition (via strophanthidin or change in [K^+]) indicates pump causes several mV of *hyperpolarization*

→ Need to be careful experimentally when potentially (pun!) affecting both active and passive transport mechanisms

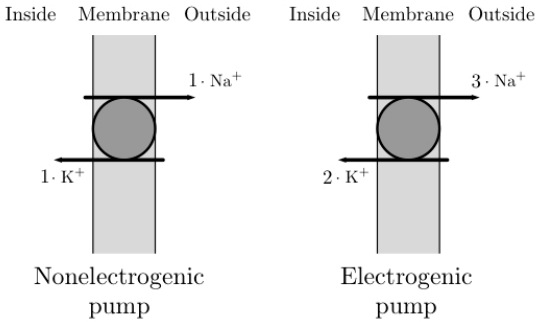


Figure 7.34

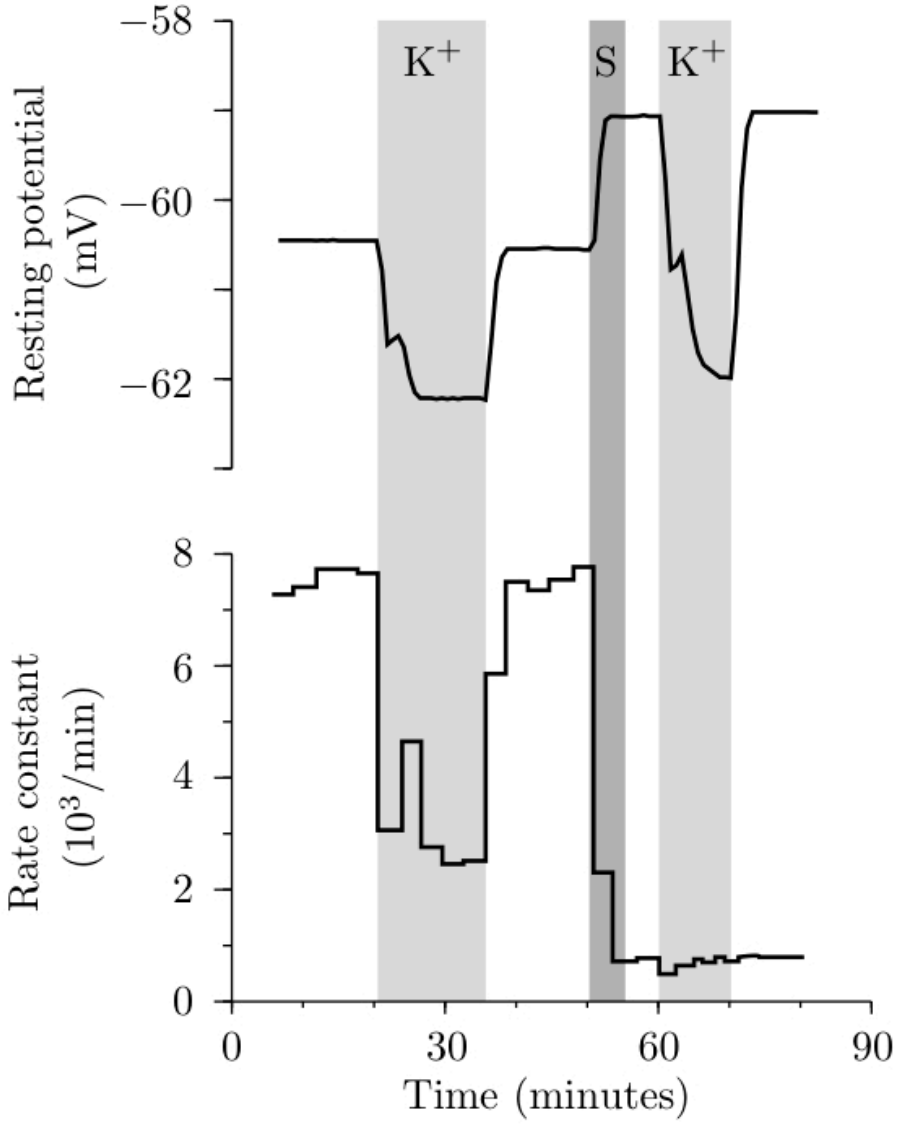


Figure 7.44

Electrogenicity of Na^+/K^+ pump?

Does active efflux of Na^+ exceed active influx of K^+?

$$V_m^o = \sum_n \frac{G_n}{G_m} V_n - \frac{1}{G_m} \sum_n J_n^a$$

Can stimulating pumps cause larger electrogenic effect?

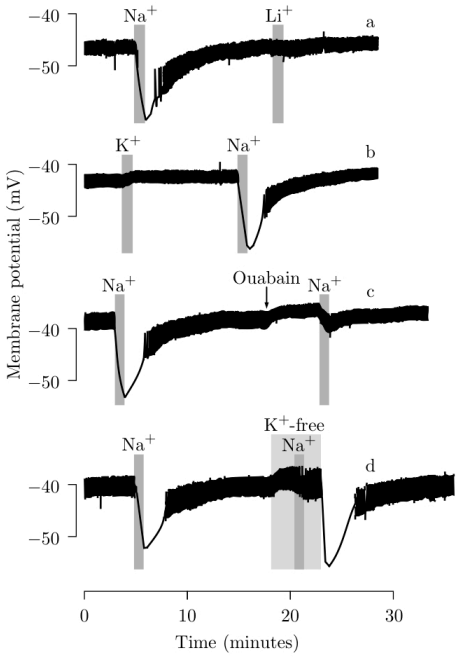


Figure 7.45

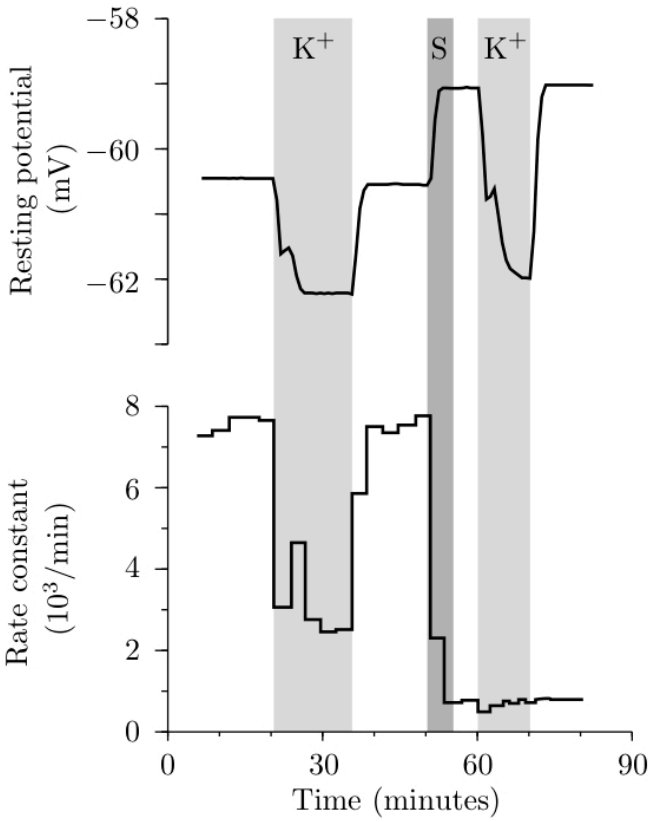


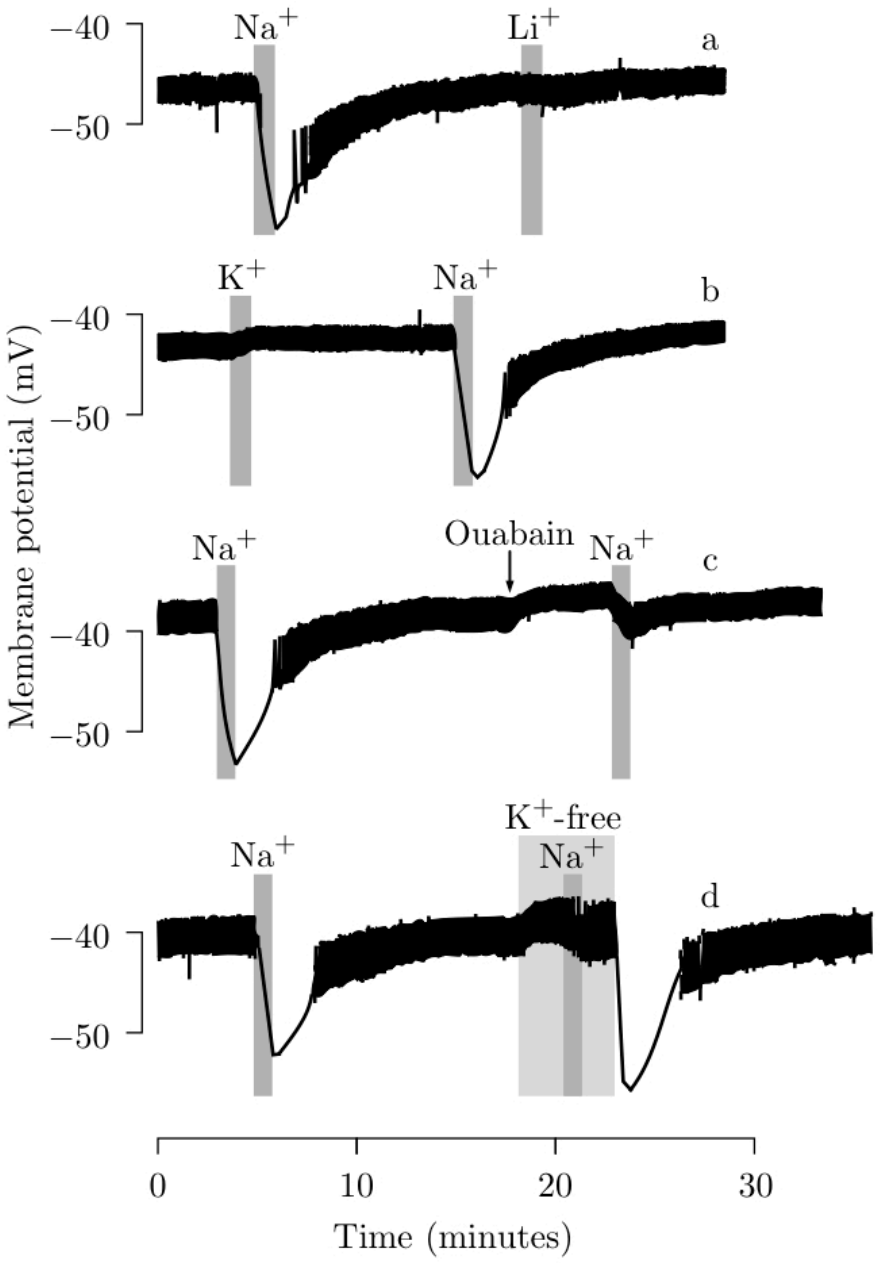
Figure 7.44

Electrogenicity of Na+/K+ pump?

- Snail neurons impaled by three pipettes: one to measure membrane potential and two to inject (intracellularly) a specific ion type
- Grey bars indicate ion injection
- Bottom traces also includes condition where external K^+ is removed
- Ref: Thomas (1969)

→ Injecting Na+ (but not K+ or Li+) intracellularly hyperpolarizes

→ Blocking the pump (via ouabain) or removing K^+ abolishes the effect



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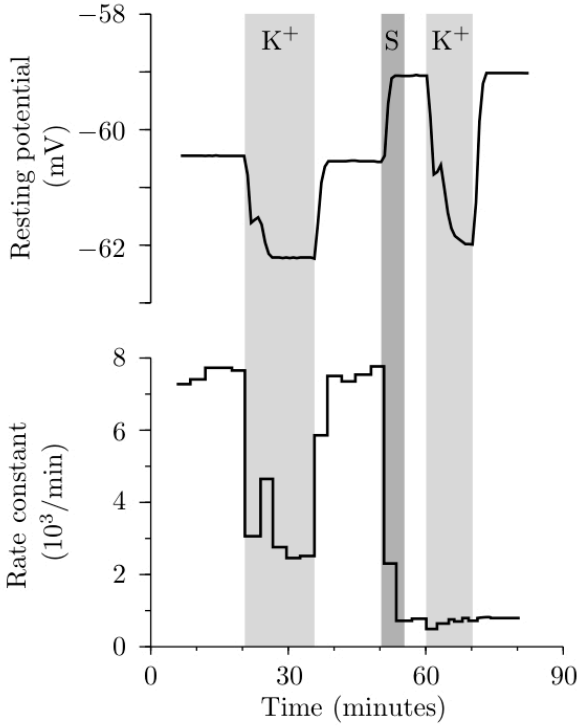

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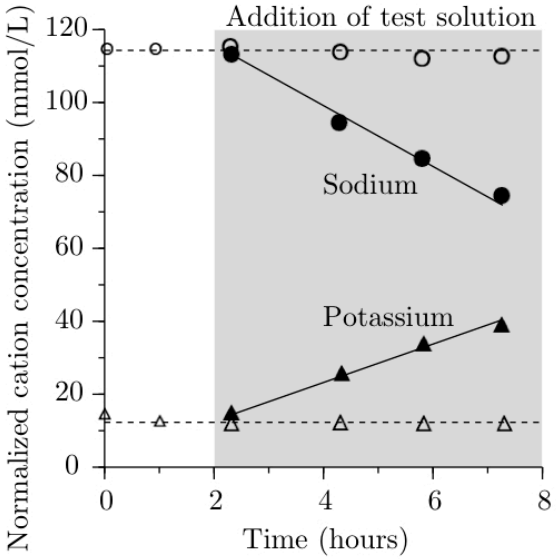


Figure 7.42

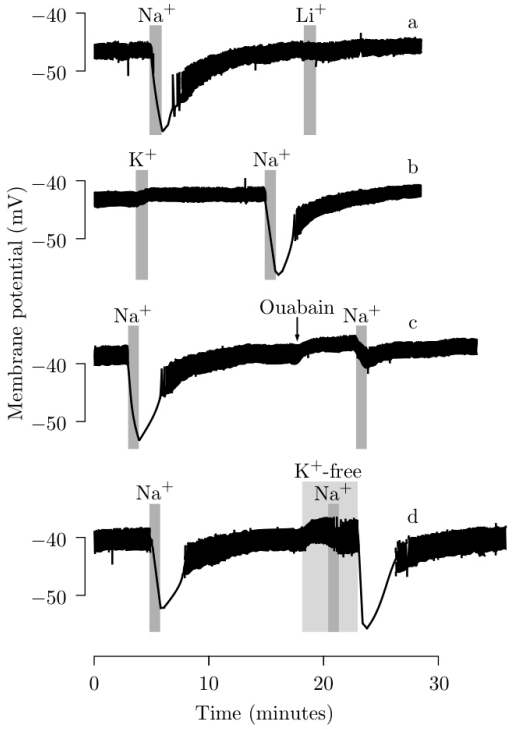


Figure 7.45

→ Evidence leading towards the notion of the Na⁺/K⁺ pump

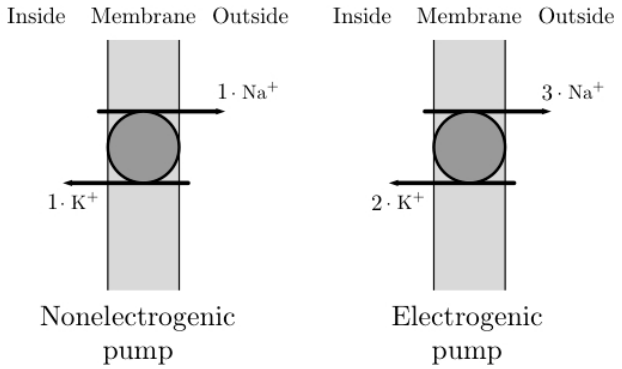


Figure 7.34

$(Na^+\text{-}K^+)$-ATPase

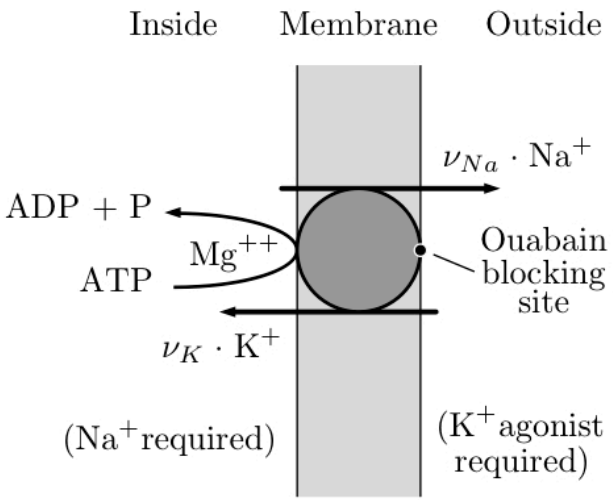


Figure 7.50

→ J.C. Skou (Nobel Prize in 1997)

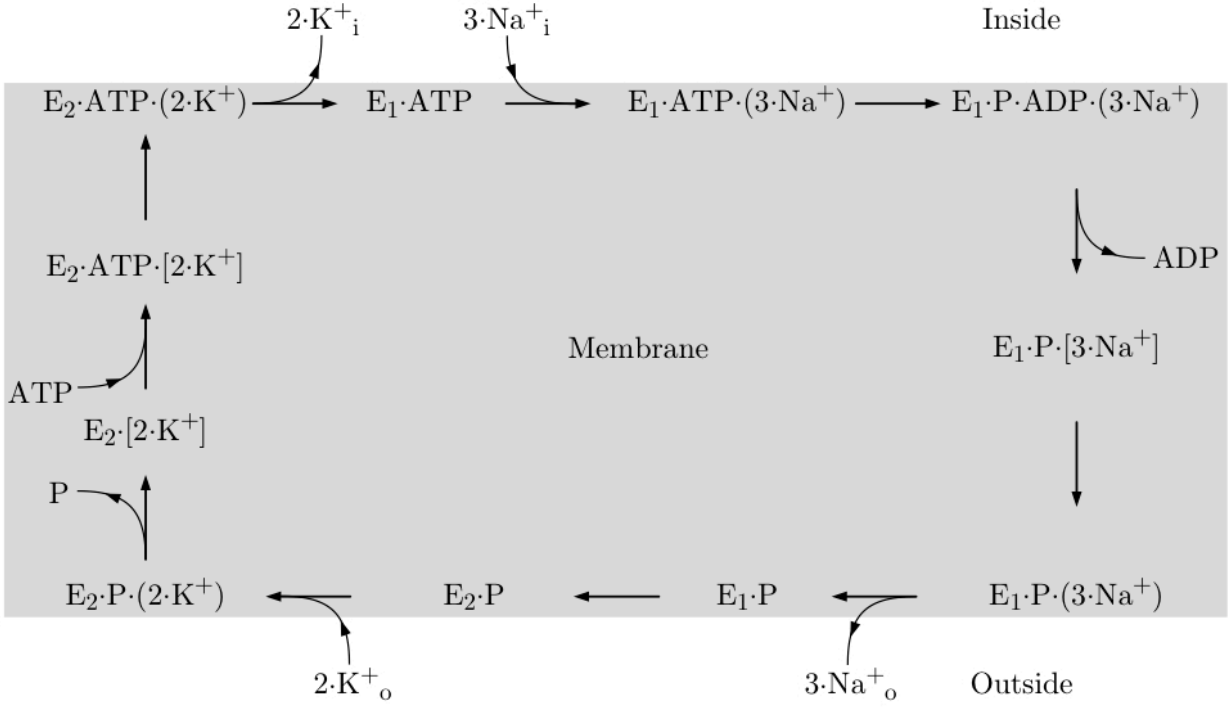
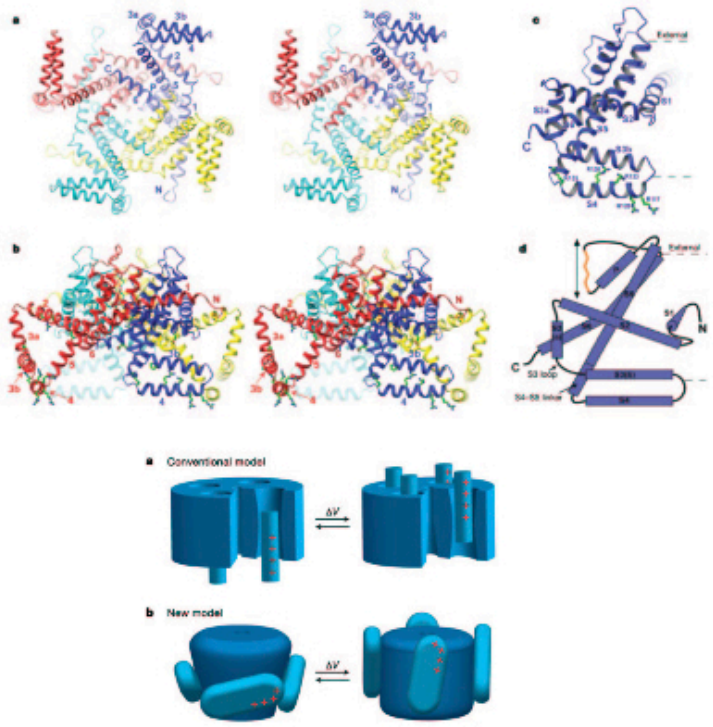


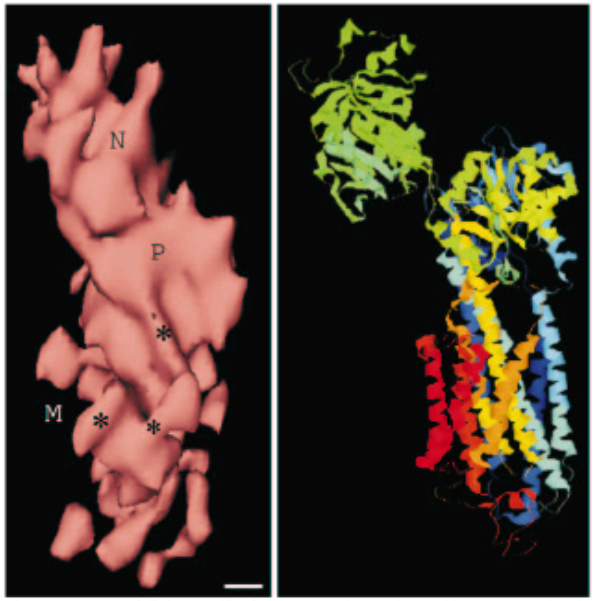
Figure 7.51

Family of related "channels"



Y. Jiang, A. Lee, J. Chen, V. Ruta, M. Cadene, B. Chalt, and R. MacKinnon (2003), Nature 423:33-41.

Family of related "pumps"



H. Hebert, P. Purhonen, H. Vorum, K. Thomsen, and A.BB. Maunsback (2001), J. Mol. Biol. 314:479-494.

→X-ray crystallography & electron microscopy can reveal structure of voltage-gated channels (i.e., *integral membrane proteins*)

[we'll discuss these techniques in BPHS 4090]

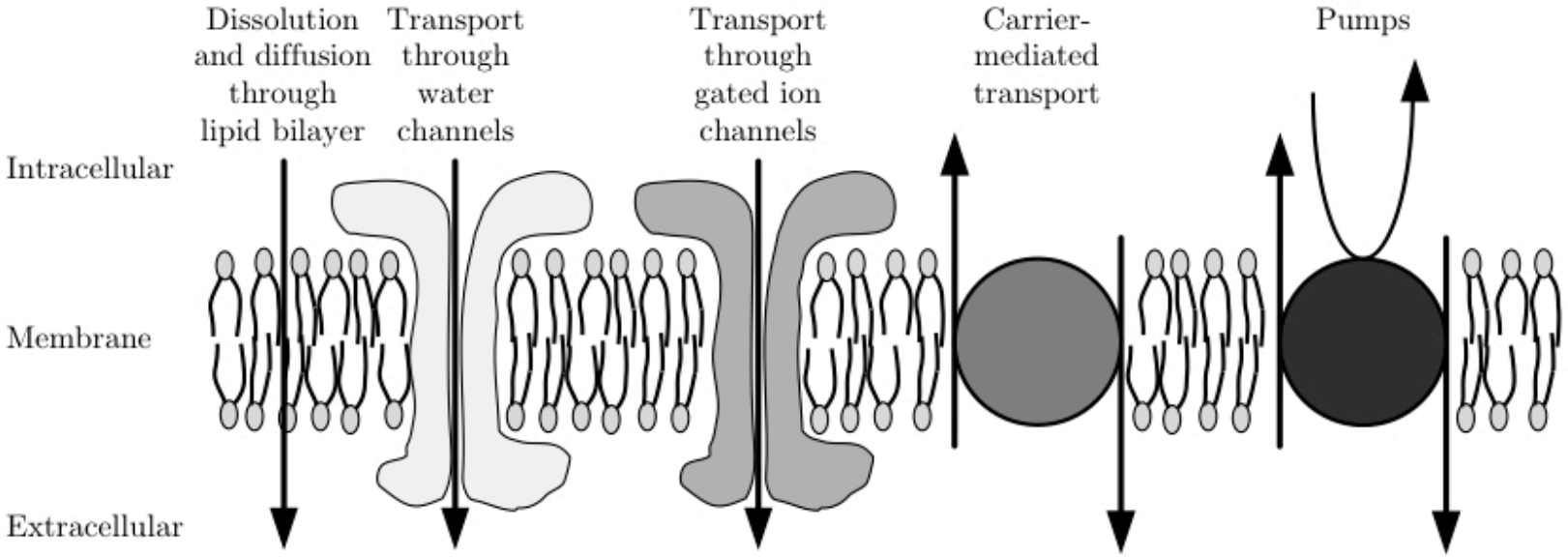

Dissolution and diffusion through lipid bilayer
Transport through water channels
Transport through gated ion channels
Carrier-mediated transport
Pumps
Intracellular
Membrane
Extracellular

Figure 2.19

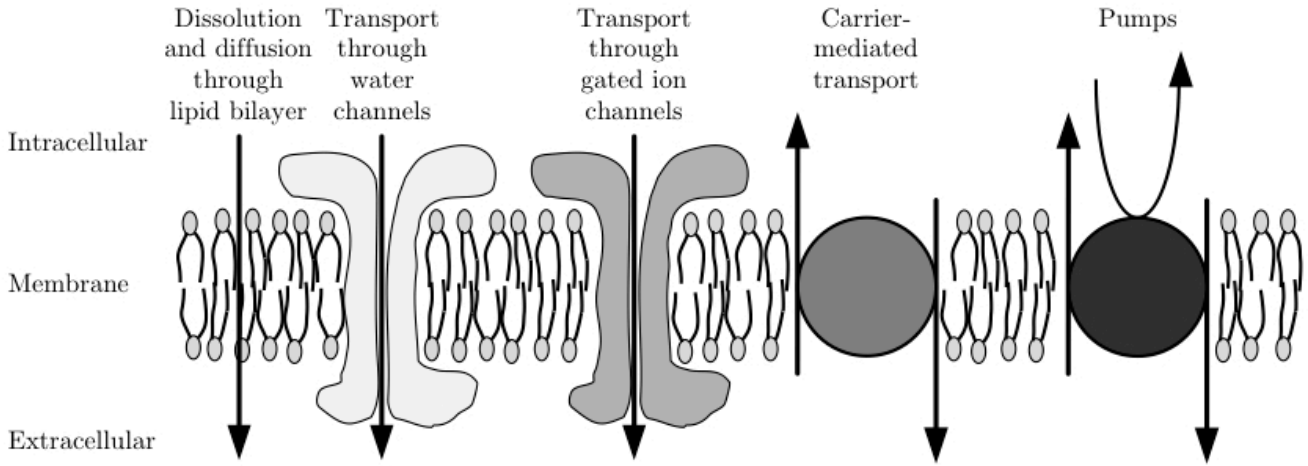


Figure 2.19

(Voltage-)gated ion channels

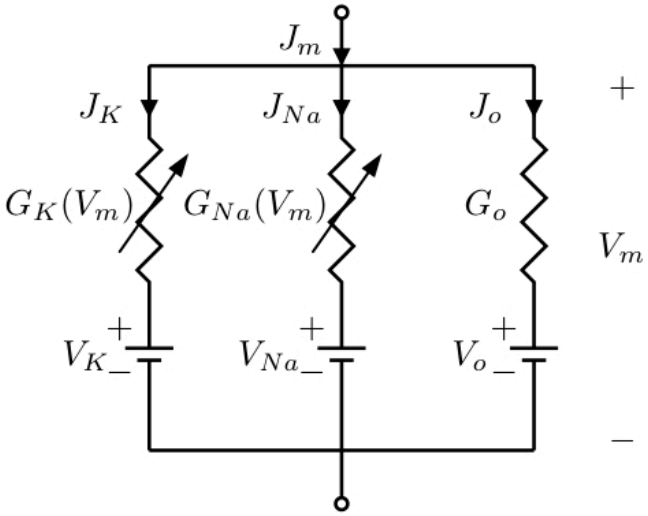


Figure 7.32

Pumps

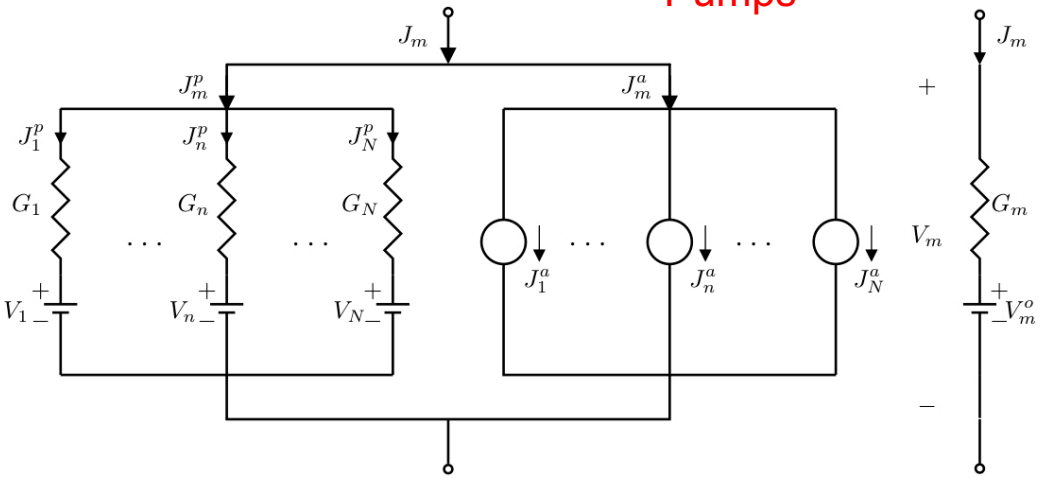


Figure 7.33

Looking Ahead: Hodgkin-Huxley network

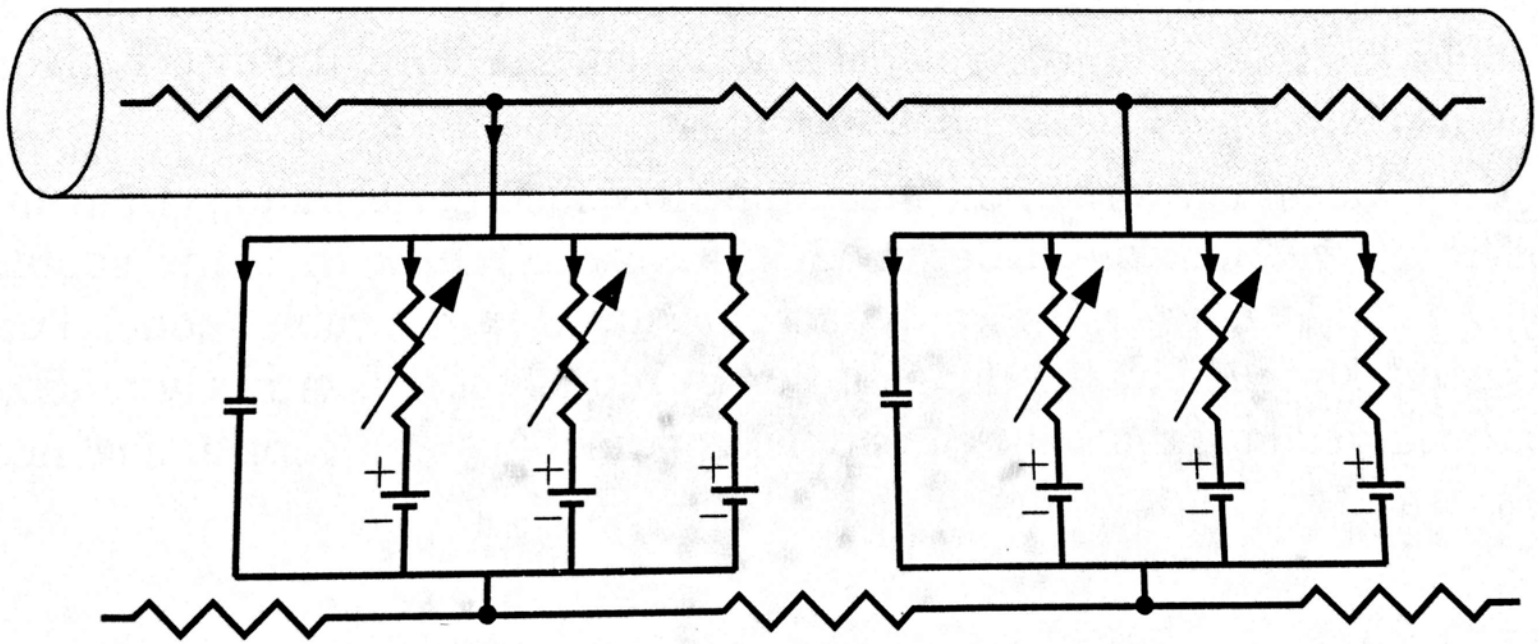


Fig.4.7 (vol.2)