Biophysics I (BPHS 3090)

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Website: http://www.yorku.ca/cberge/3090W2015.html

York University
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Lecture 16

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

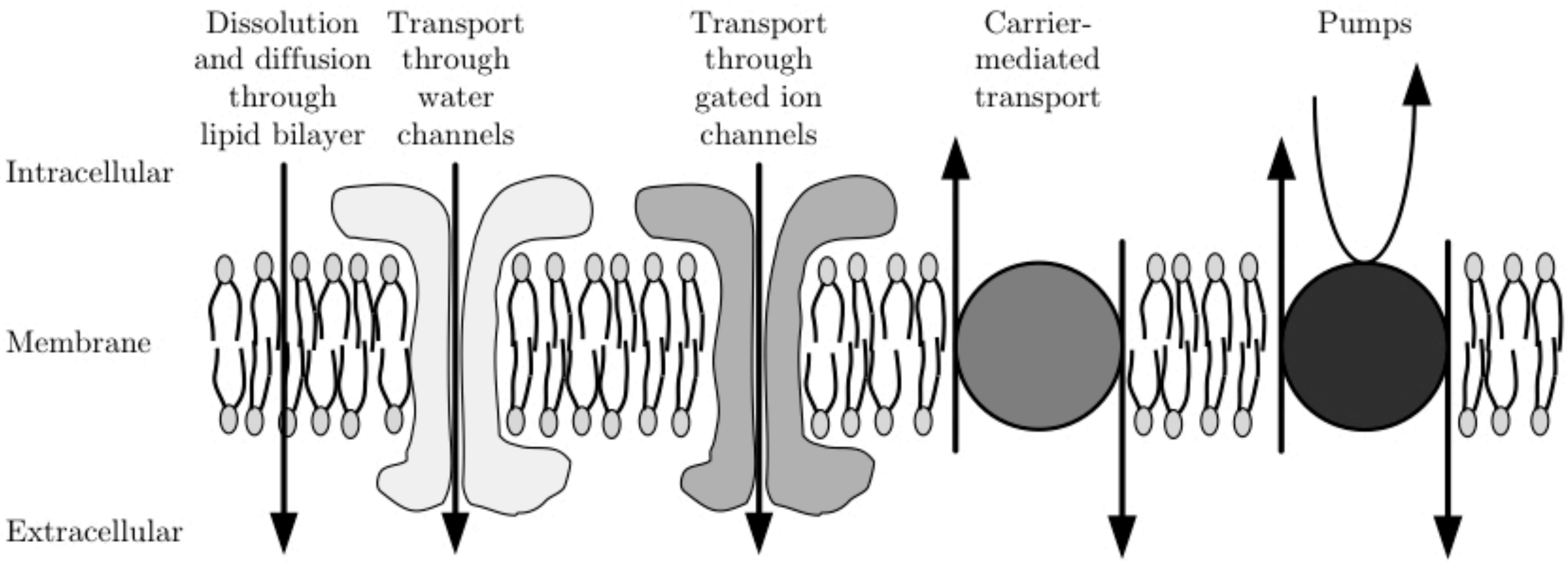

Dissolution and diffusion through lipid bilayer
Transport through water channels
Transport through gated ion channels
Carrier-mediated transport
Pumps
Intracellular
Membrane
Extracellular

Figure 2.19

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

→ What if different ions are able to diffuse?

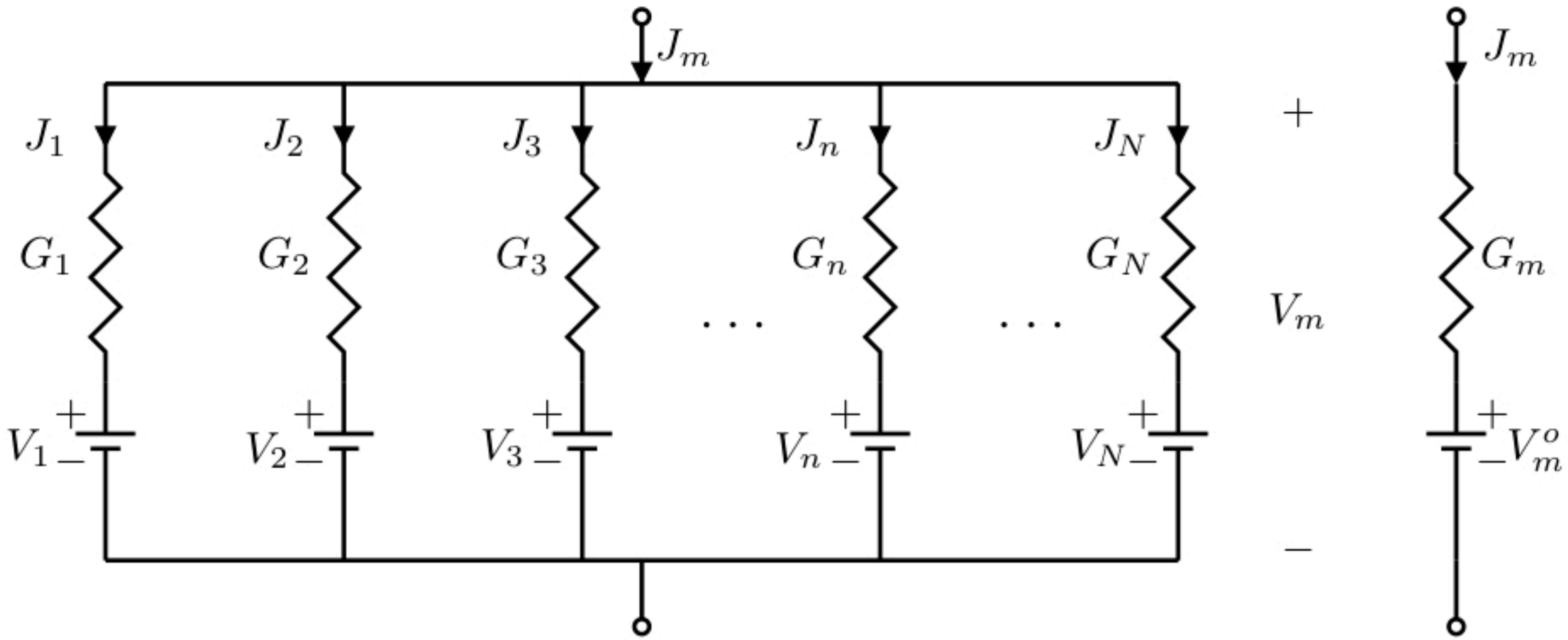


Figure 7.24

Voltage-dependent conductances?

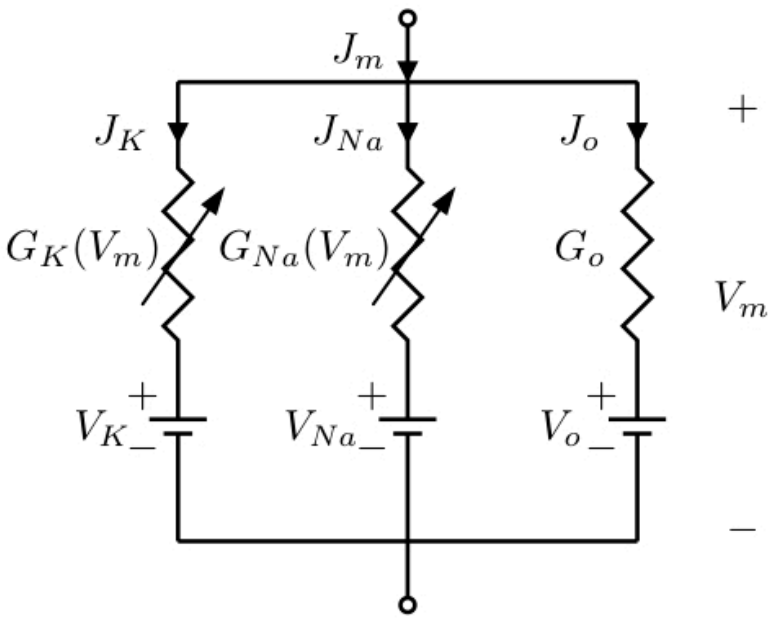


Figure 7.32

Voltage-gated ion channels
(more detail in vol.2 ch.6)

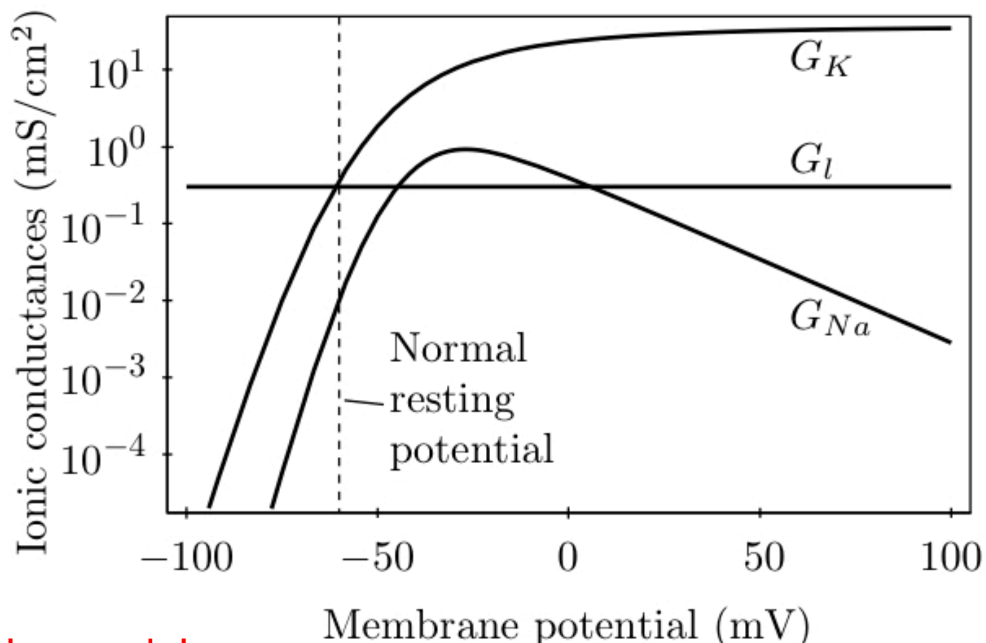


Figure 7.28

Note: How does a microscopic ion channel (which is either open or closed) give rise to "smooth" macroscopic conductances as shown here? [we'll come back to this later in the course]

Hodgkin-Huxley model conductances

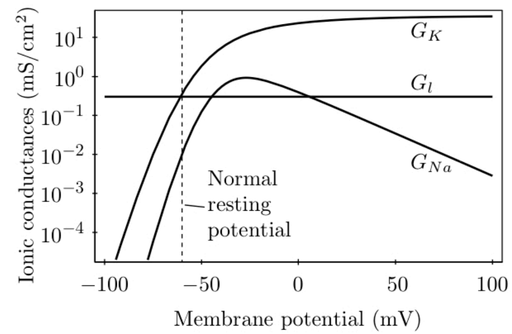


Figure 7.28

K⁺ contribuition?

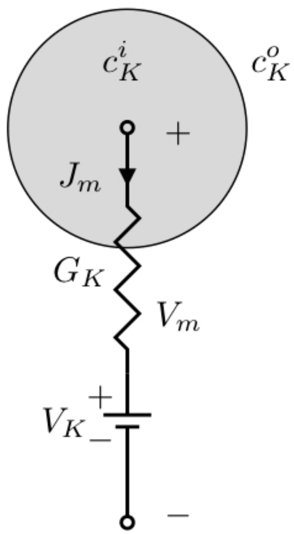


Figure 7.18

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

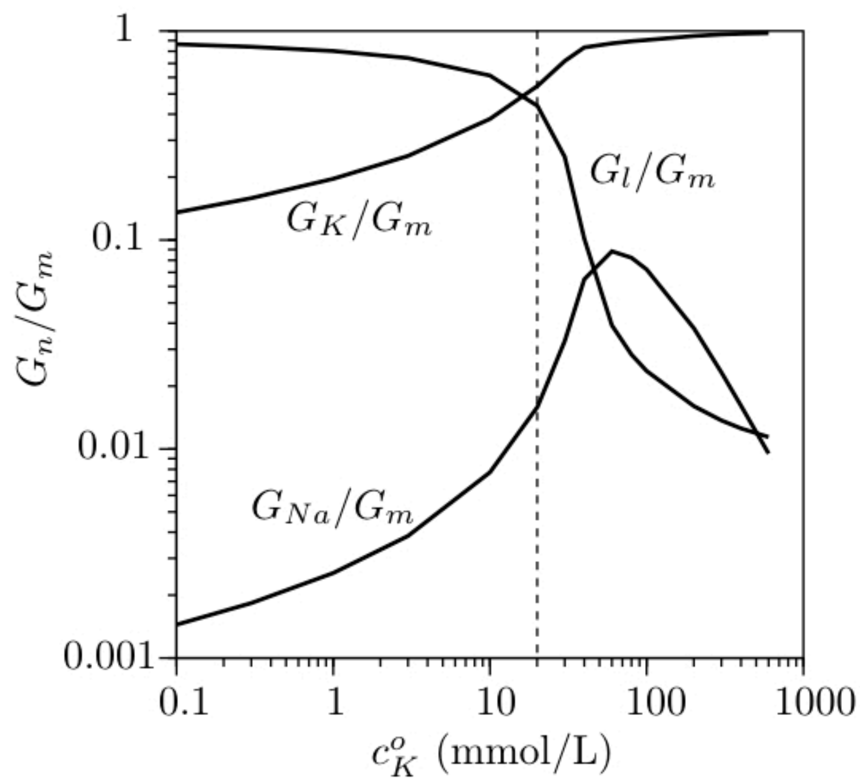
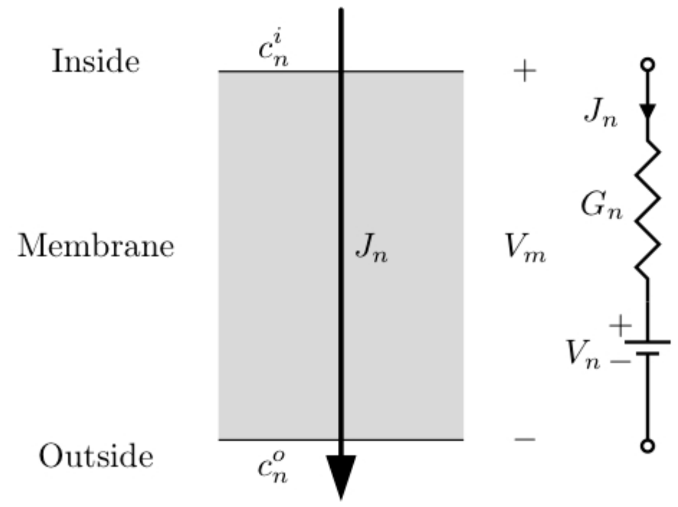


Figure 7.30

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

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)

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

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

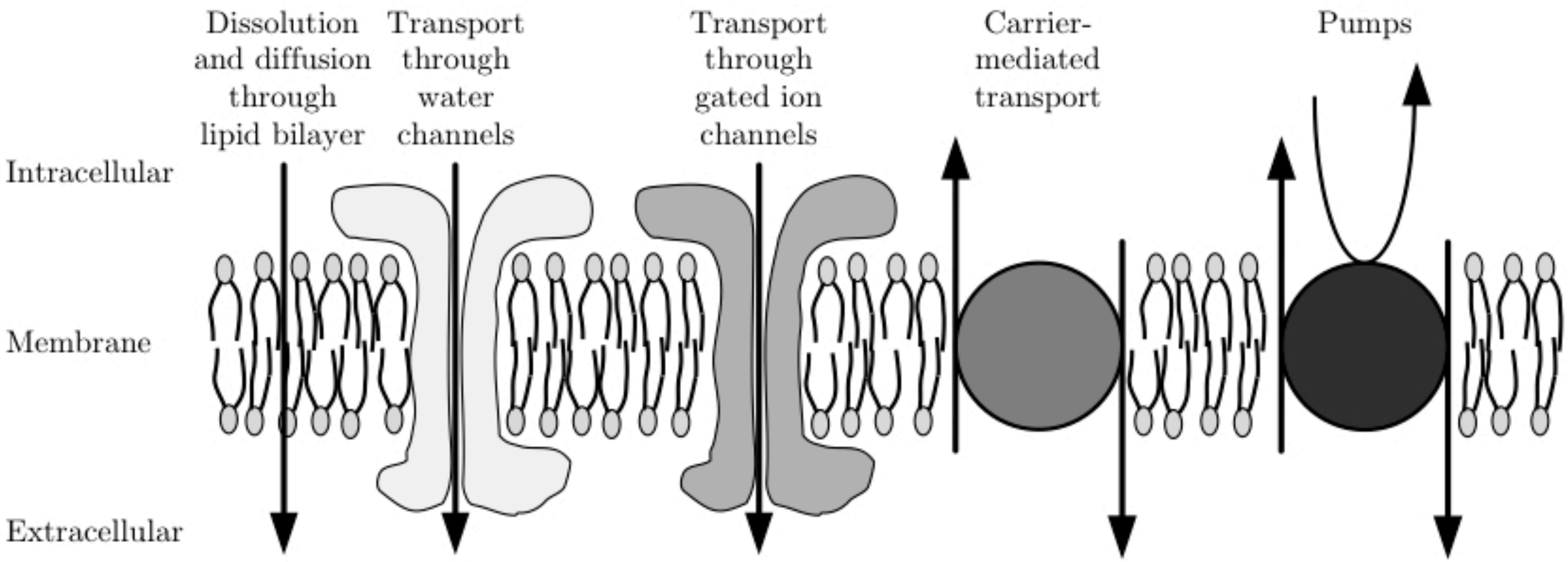


Figure 2.19

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

<u>Ion Pumps</u>

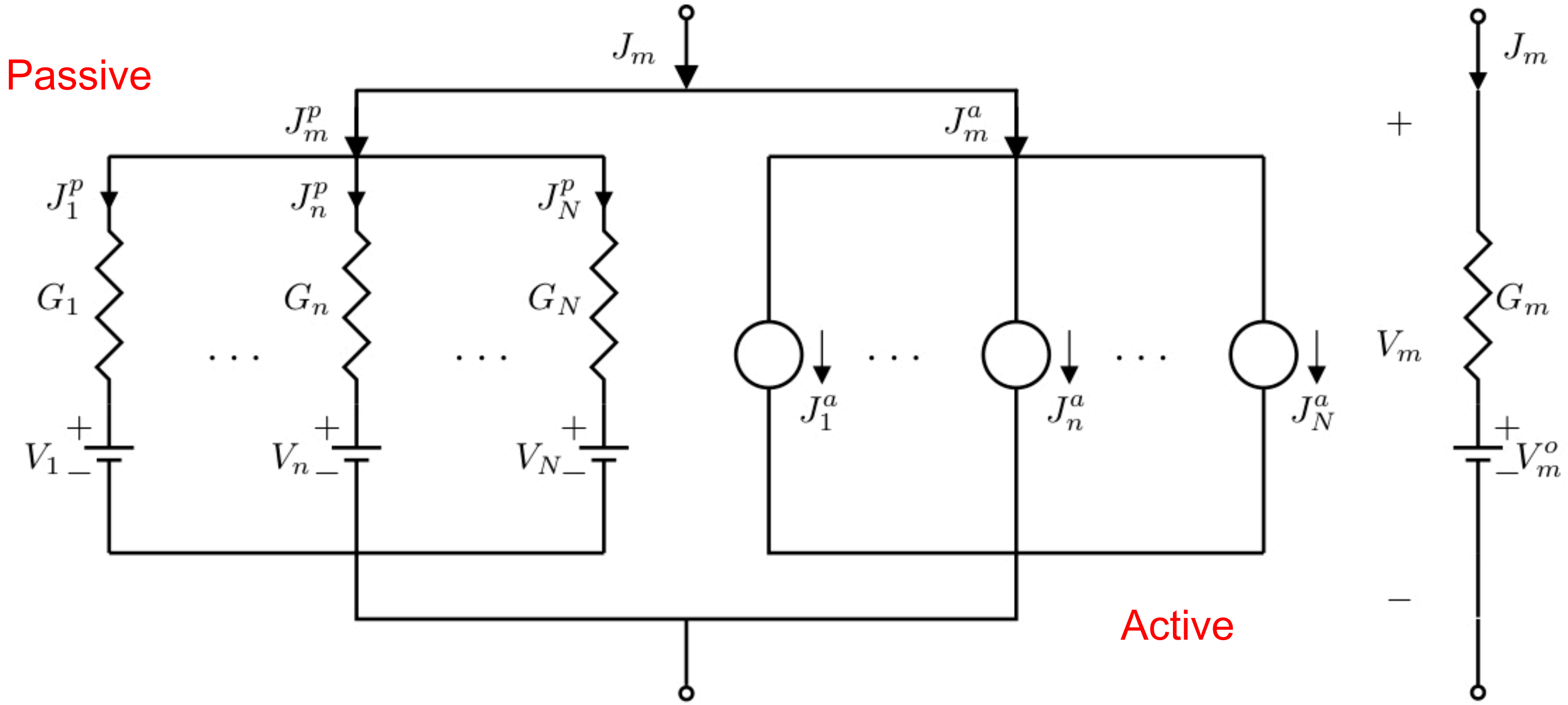


Figure 7.33

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

Ion Pumps

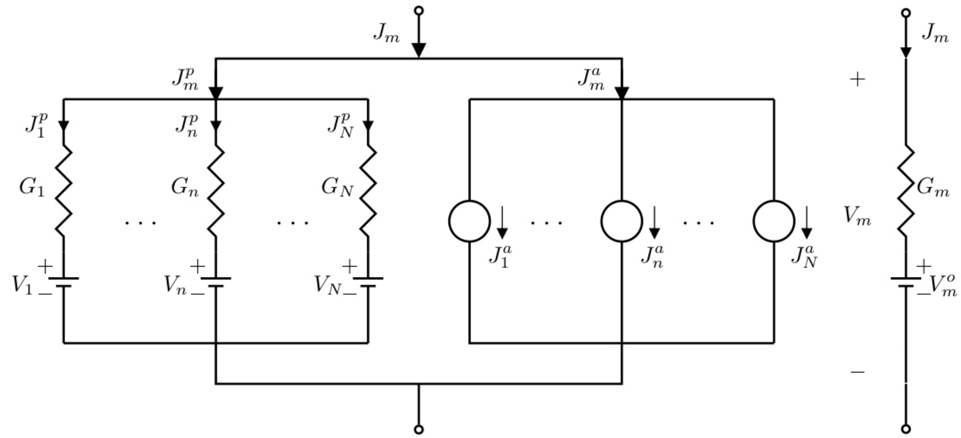
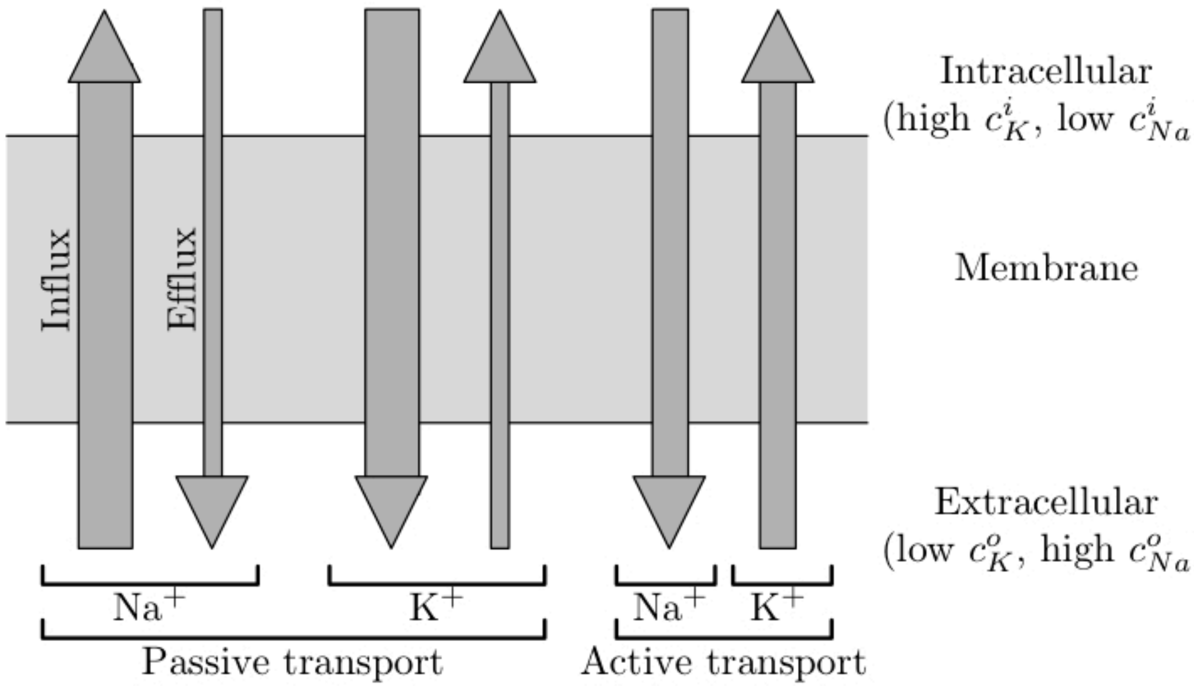


Figure 7.33

Passive:
K^+ efflux
Na^+ influx



Active:
Na^+ efflux
K^+ influx

Figure 7.35

<u>Ion Pumps</u>

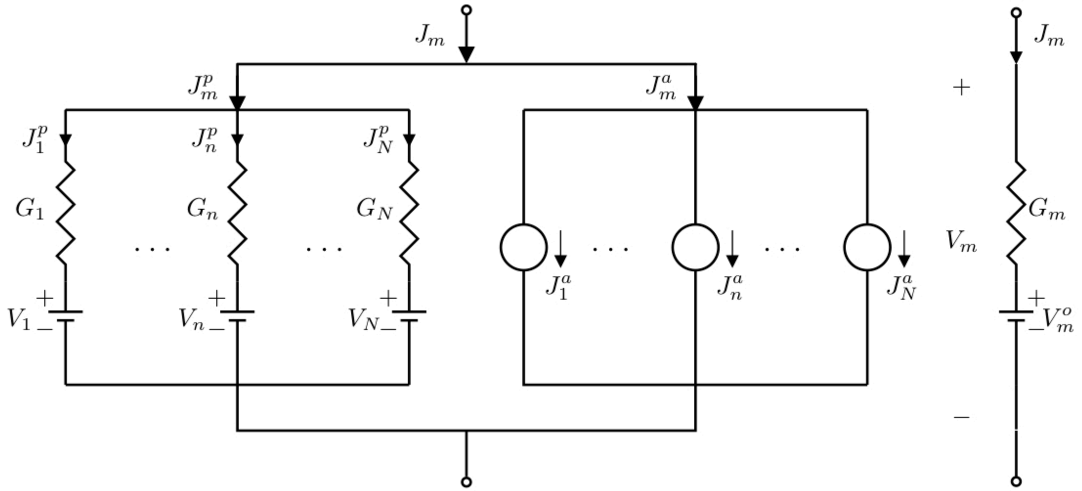


Figure 7.33

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

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

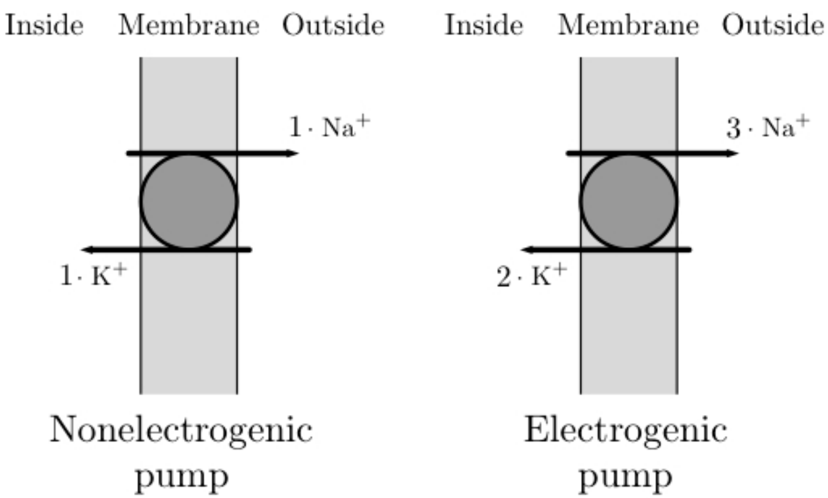


Figure 7.34

→ Does a pump contribute to the membrane potential?

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

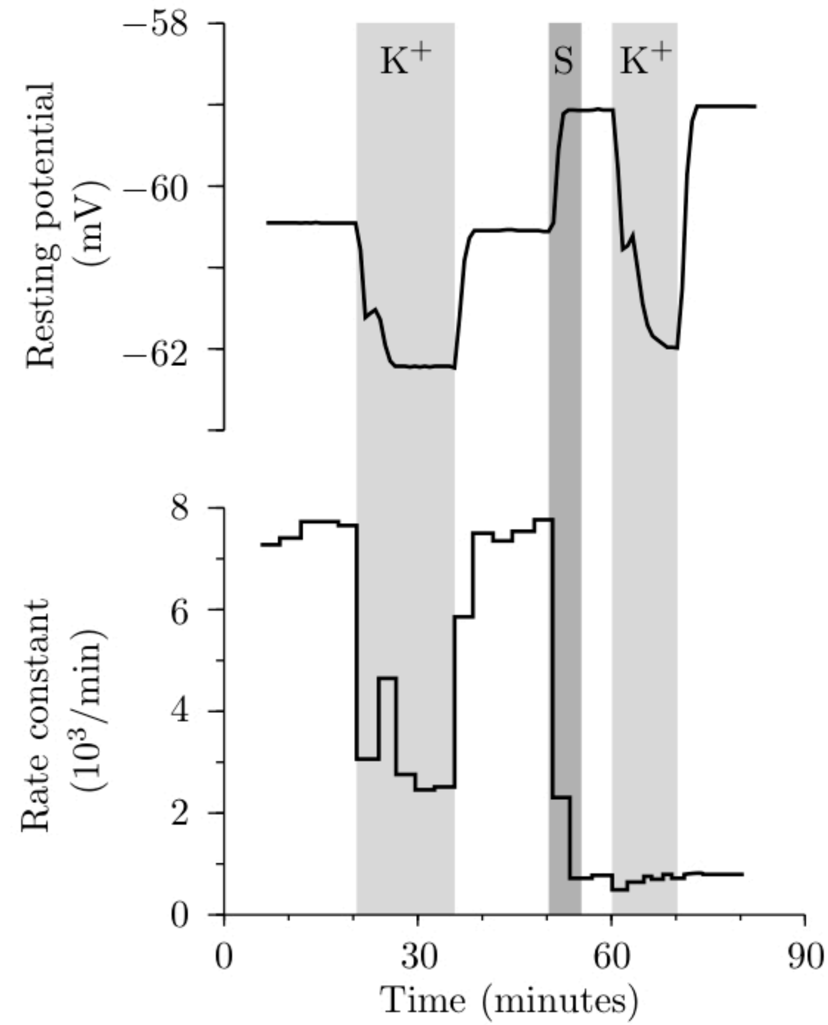


Figure 7.44

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

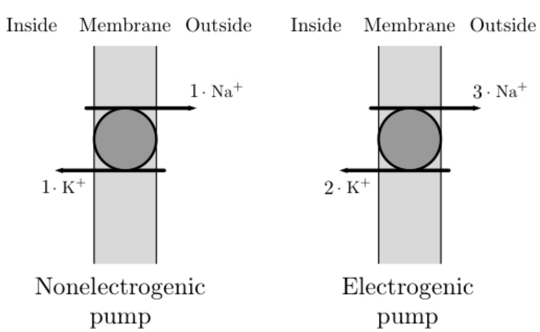


Figure 7.34

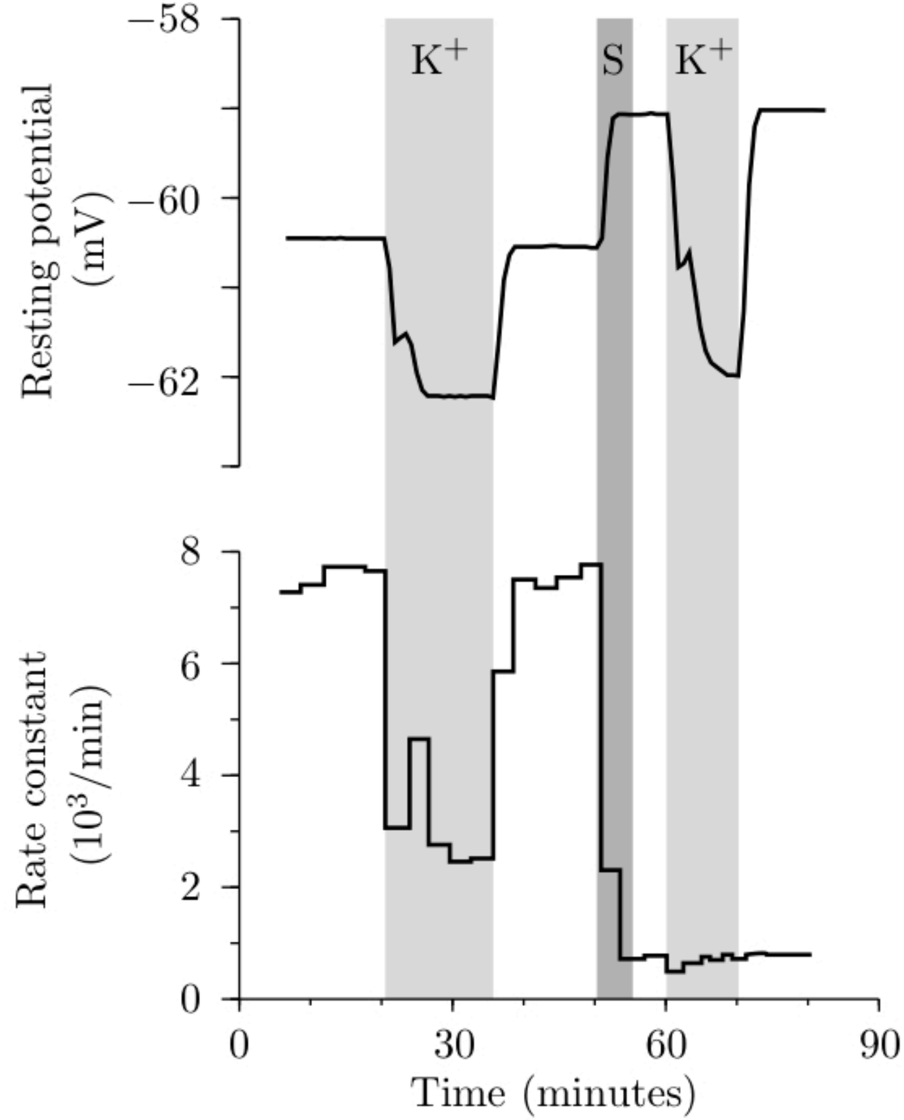


Figure 7.44

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)

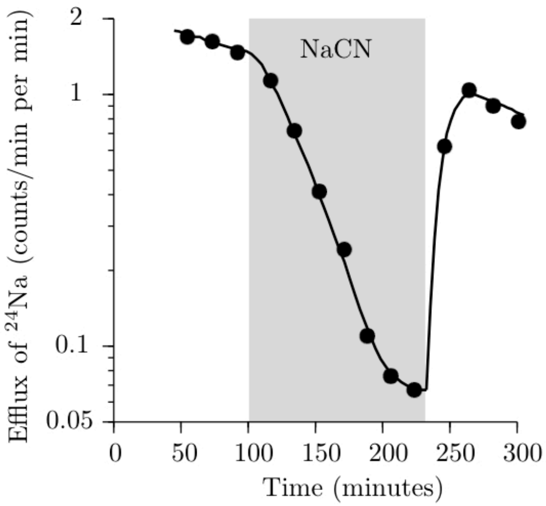


Figure 7.36

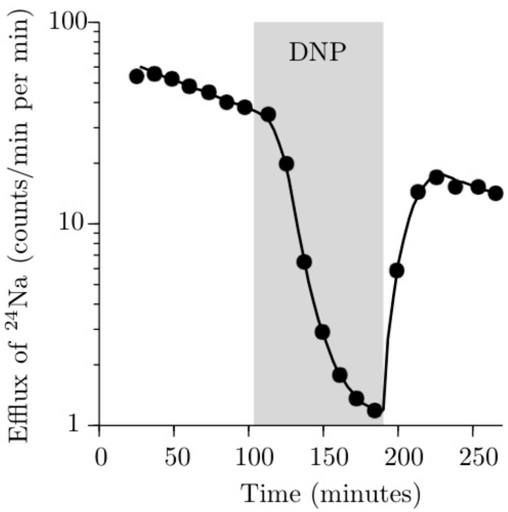


Figure 7.37

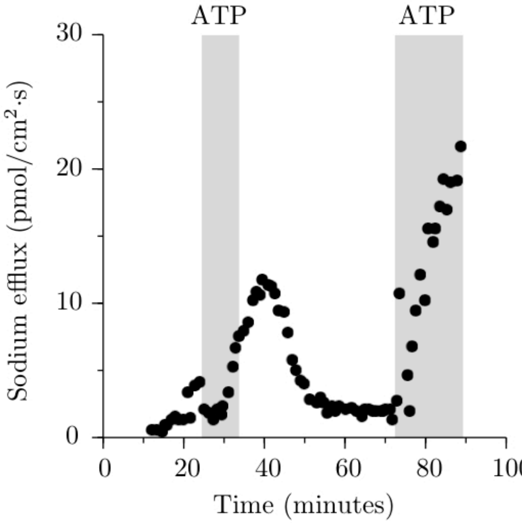


Figure 7.39

- 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)

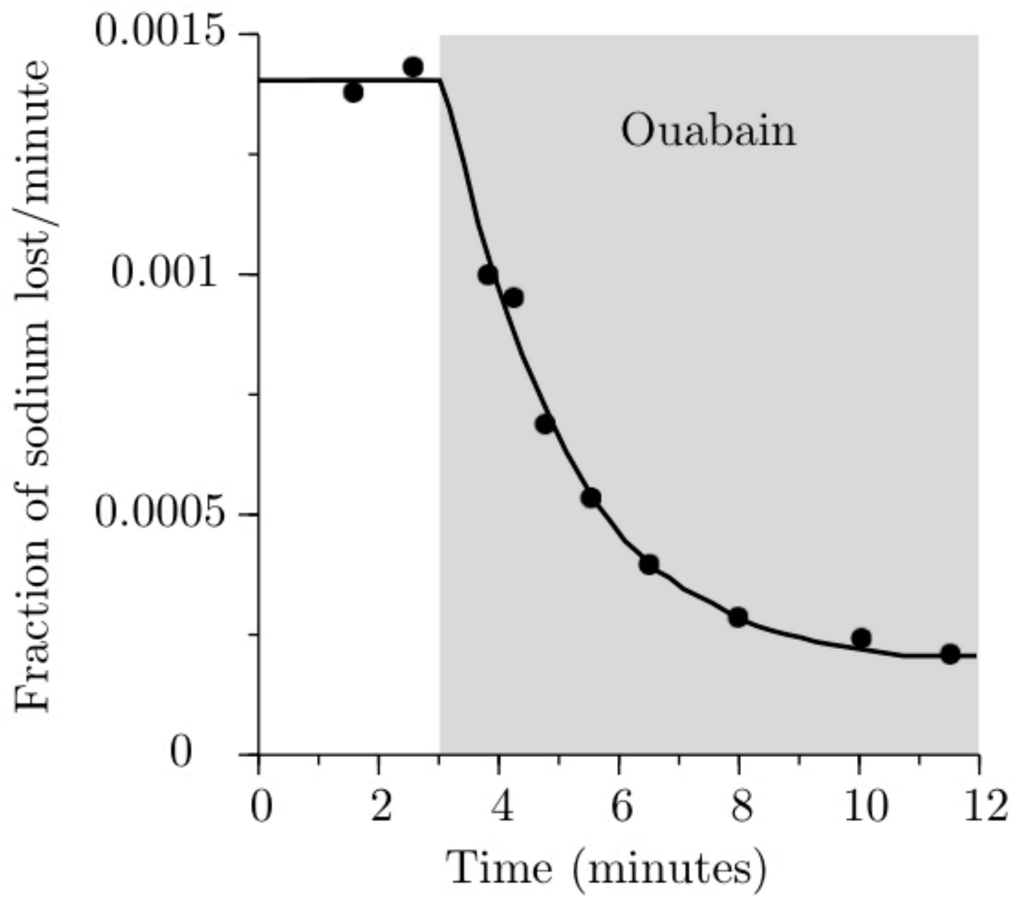


Figure 7.41

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

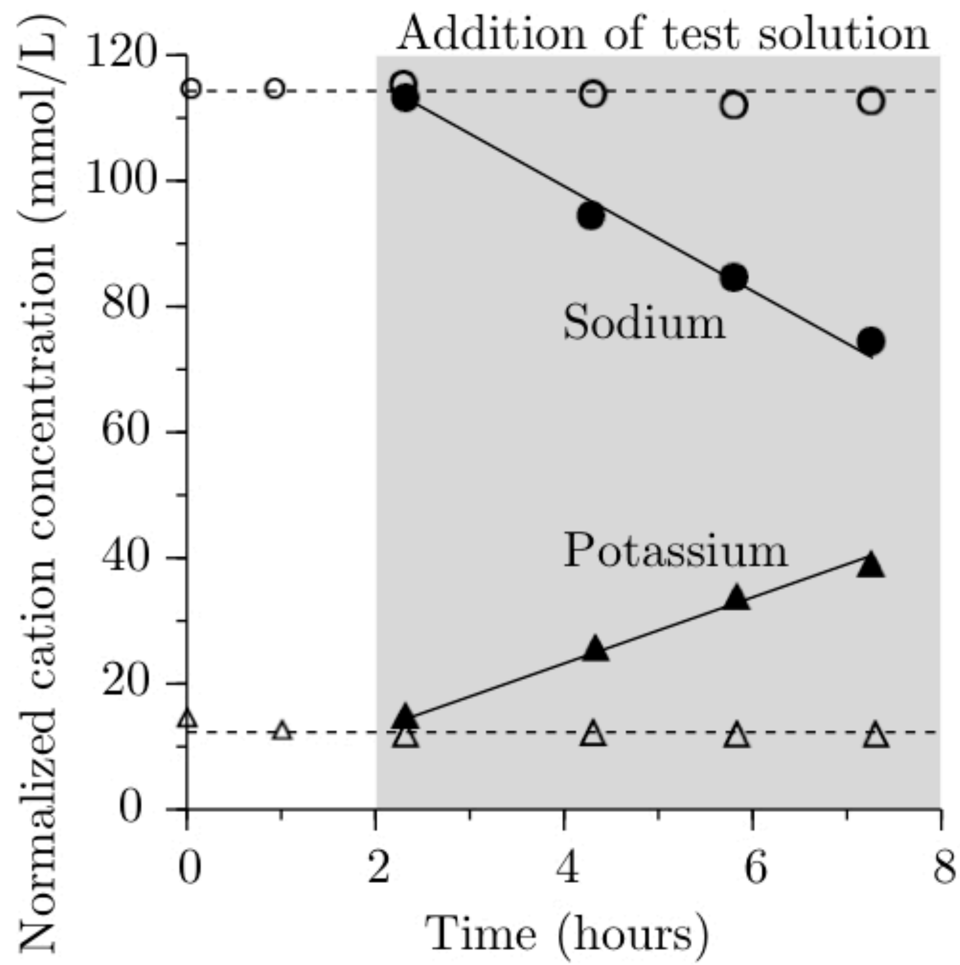
(though these drugs had long been used to treat heart failure, it wasn't until ~1950s that it was understood how)

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

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?

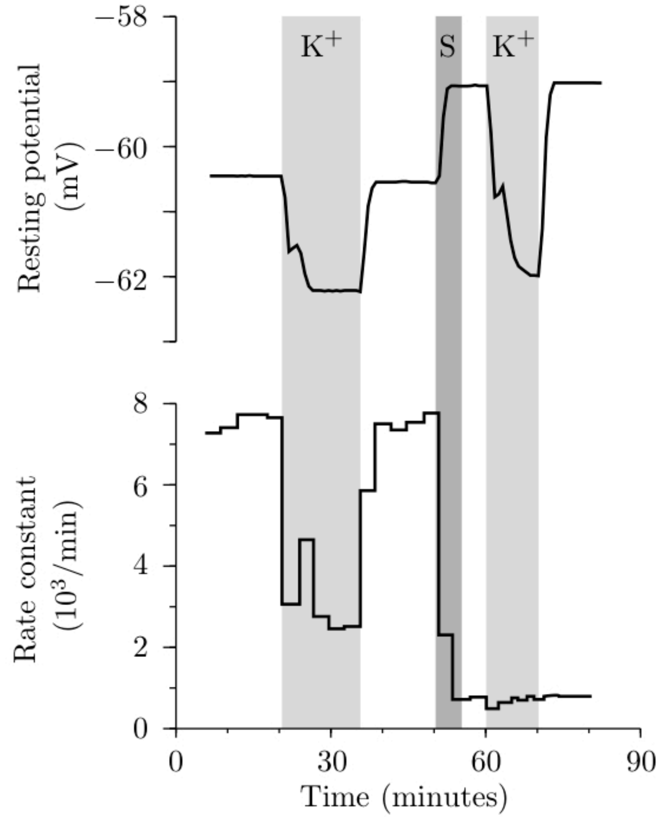


Figure 7.44

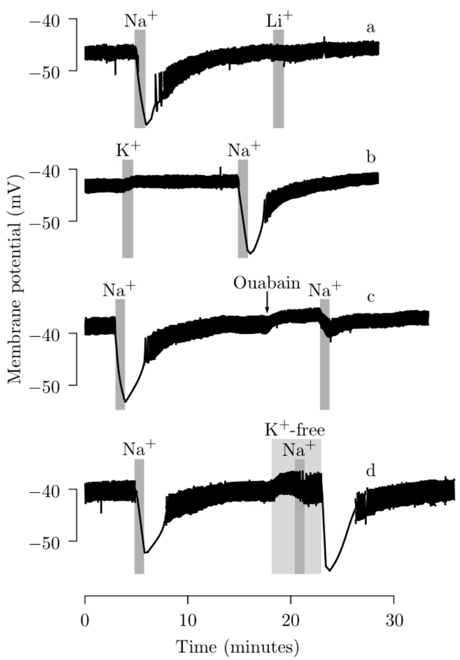


Figure 7.45

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

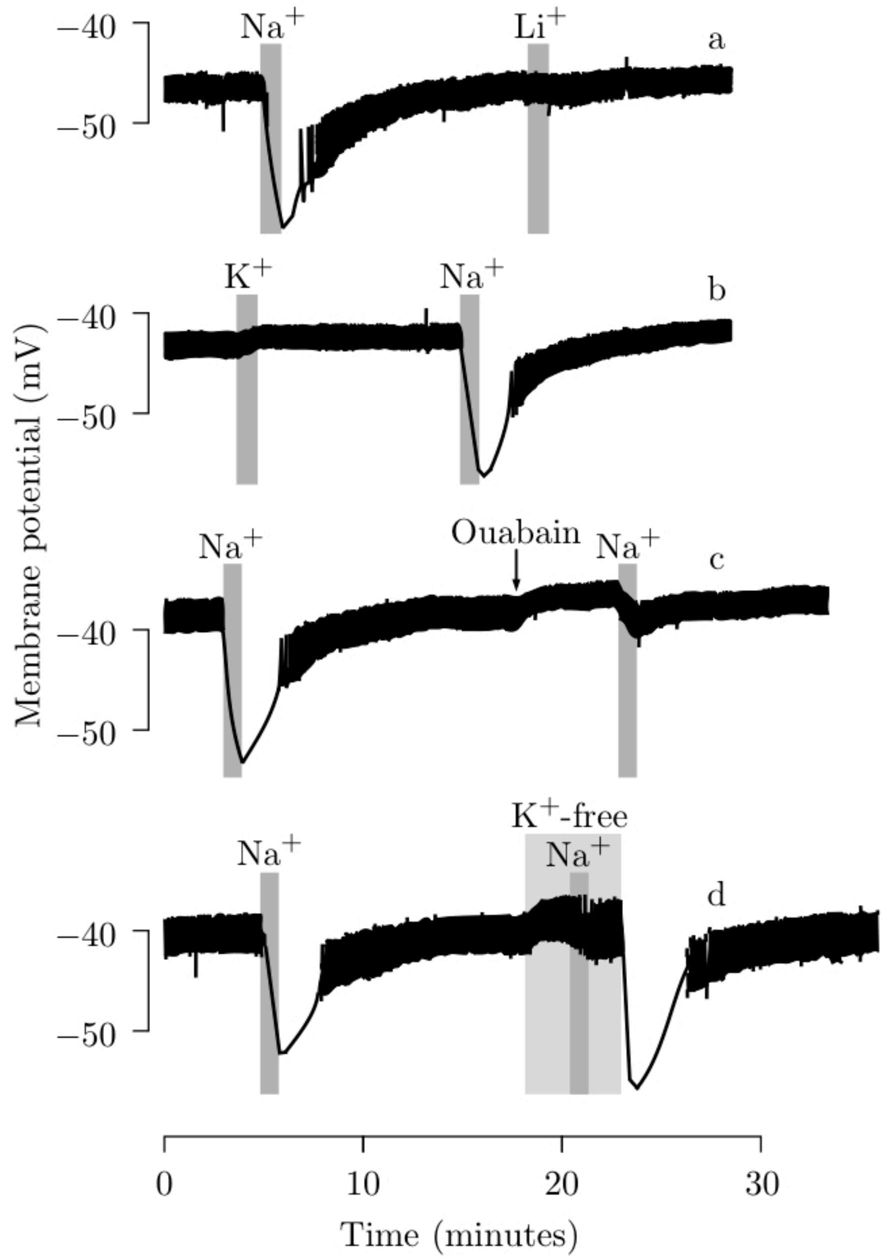


Figure 7.45

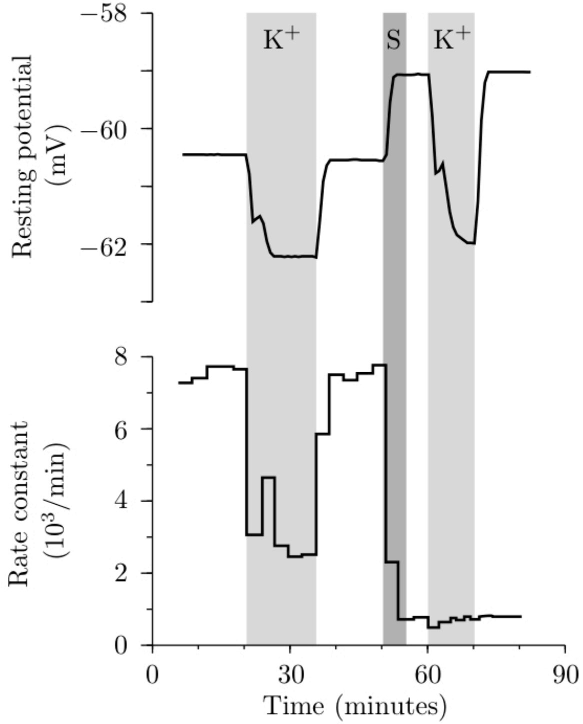


Figure 7.44

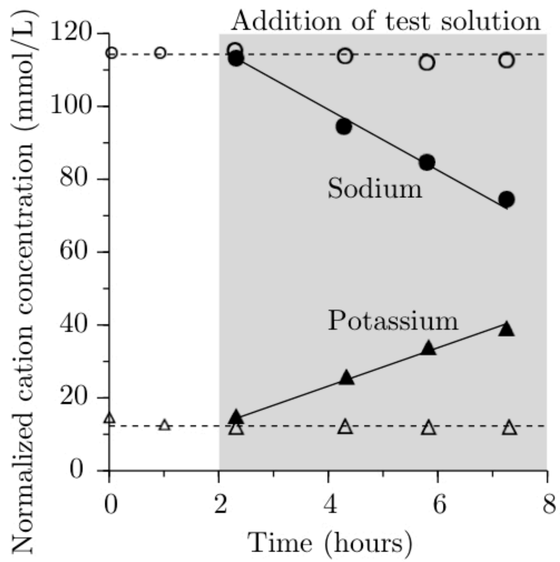


Figure 7.42

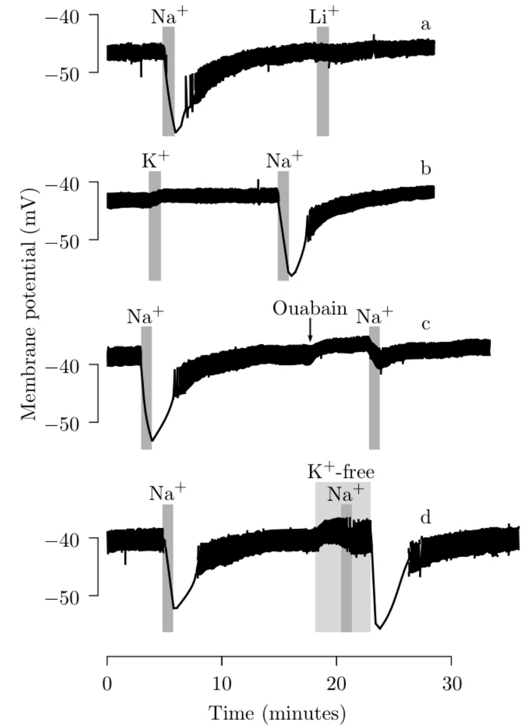


Figure 7.45

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

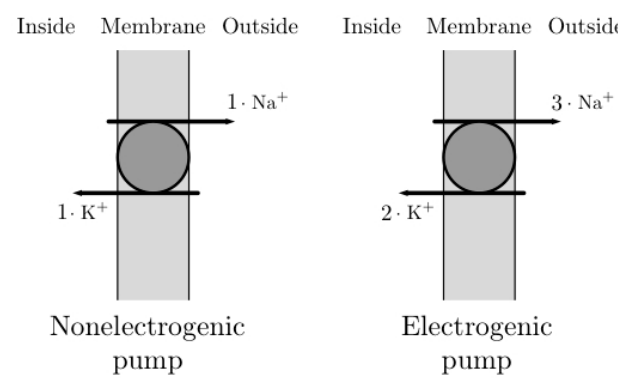


Figure 7.34

(Na⁺-K⁺)-ATPase

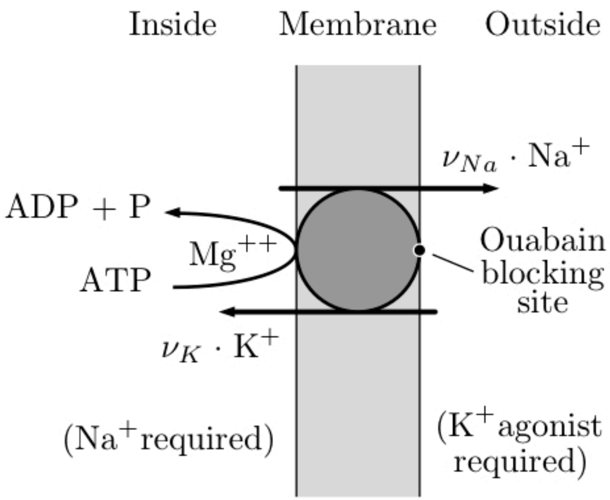


Figure 7.50

→ J.C. Skou (Nobel Prize in 1997)

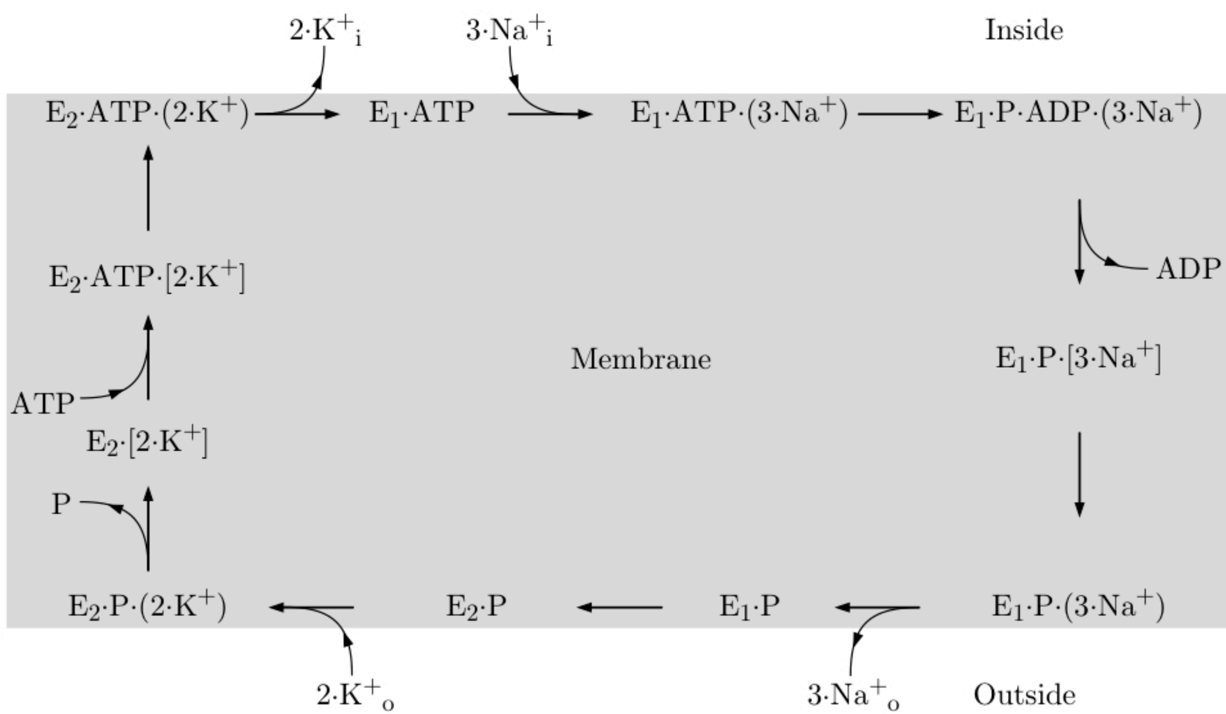
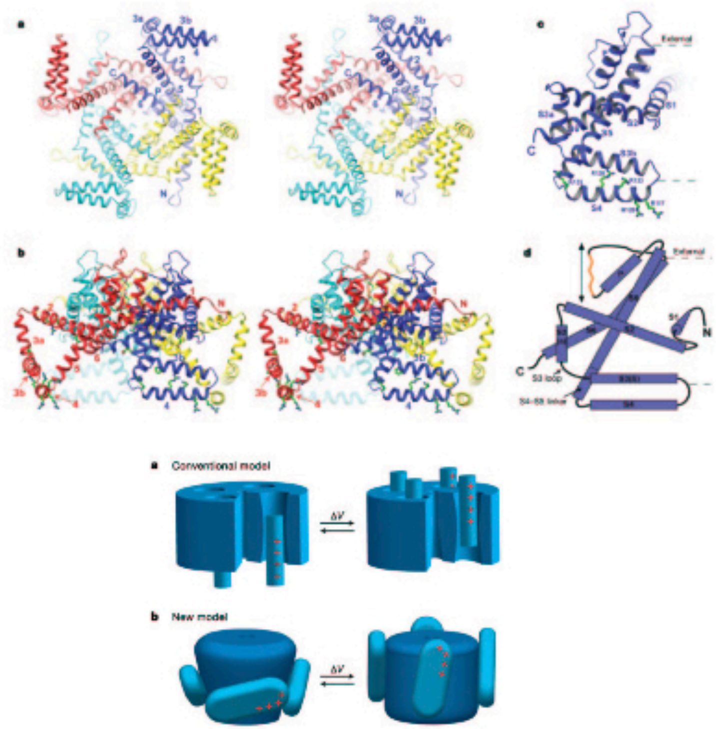


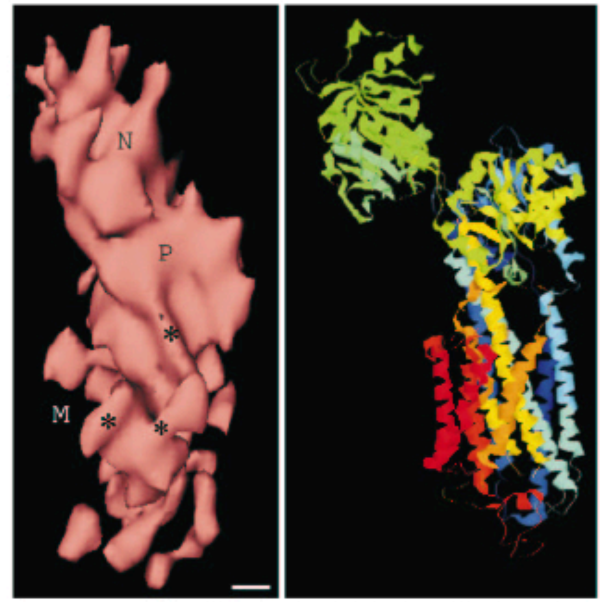
Figure 7.51

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]

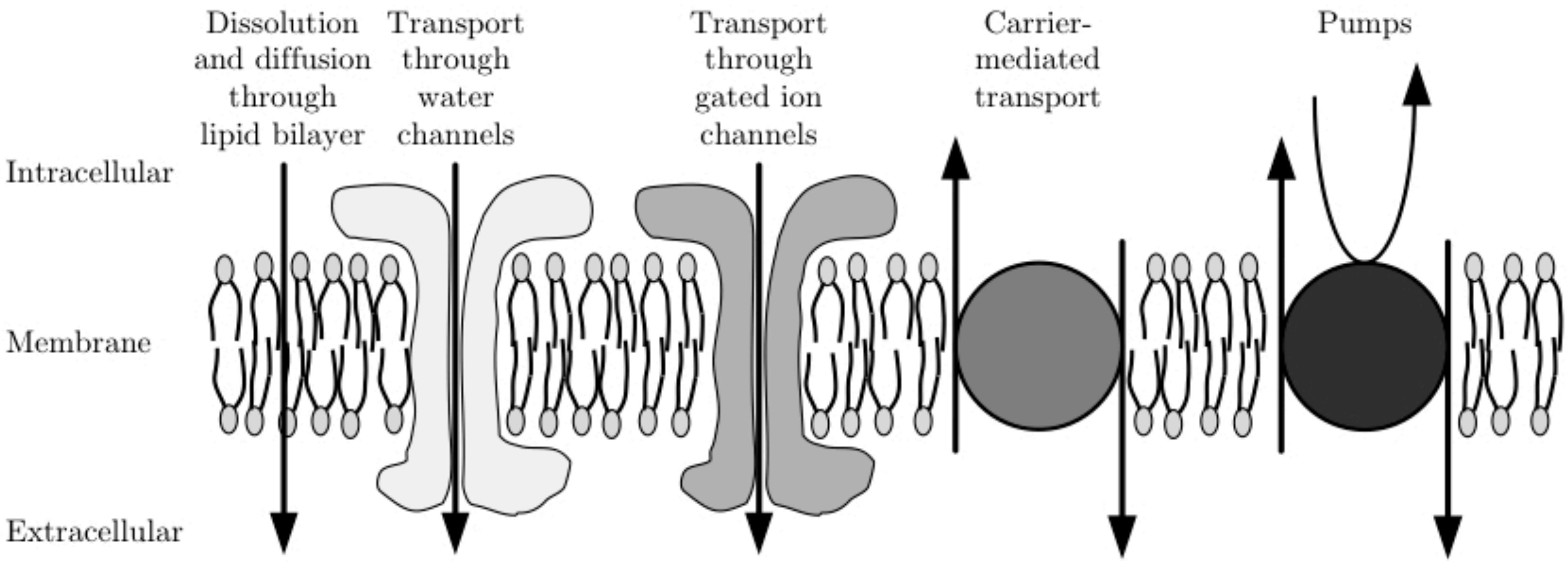

Dissolution and diffusion through lipid bilayer
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Figure 2.19

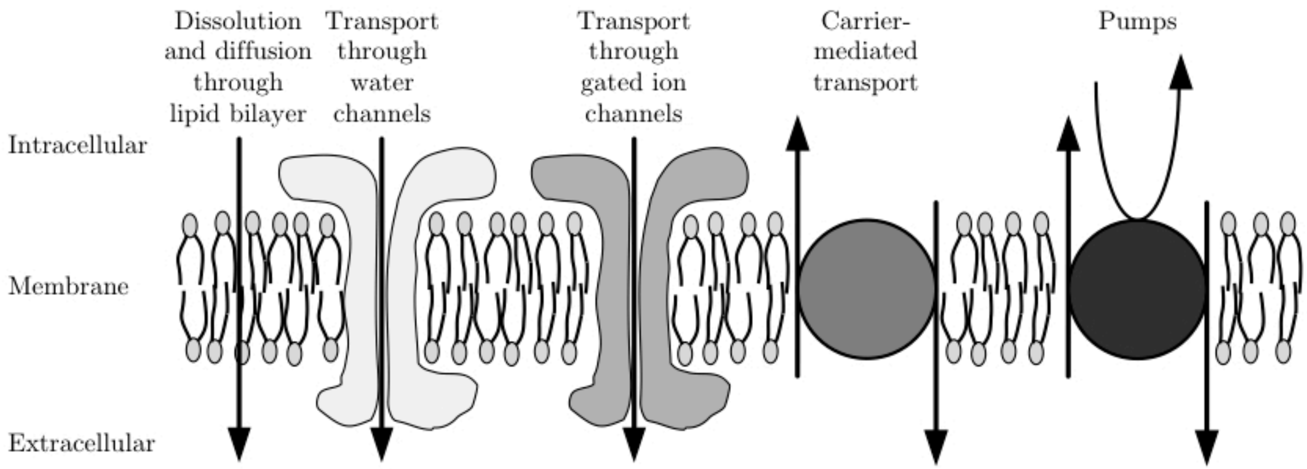

Figure 2.19

(Voltage-)gated ion channels

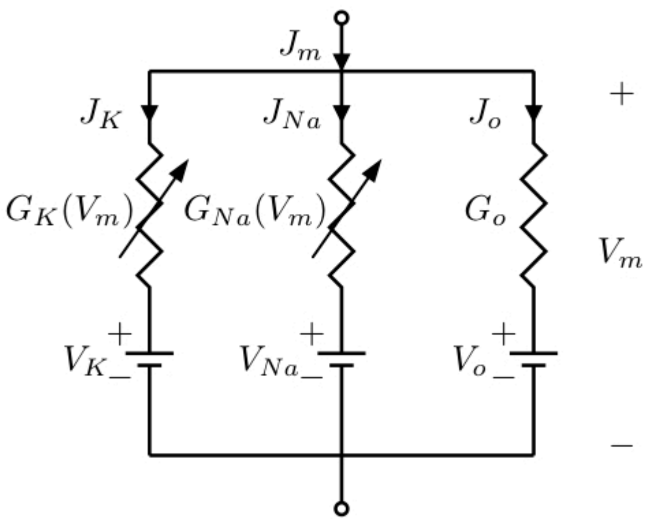

Figure 7.32

Pumps

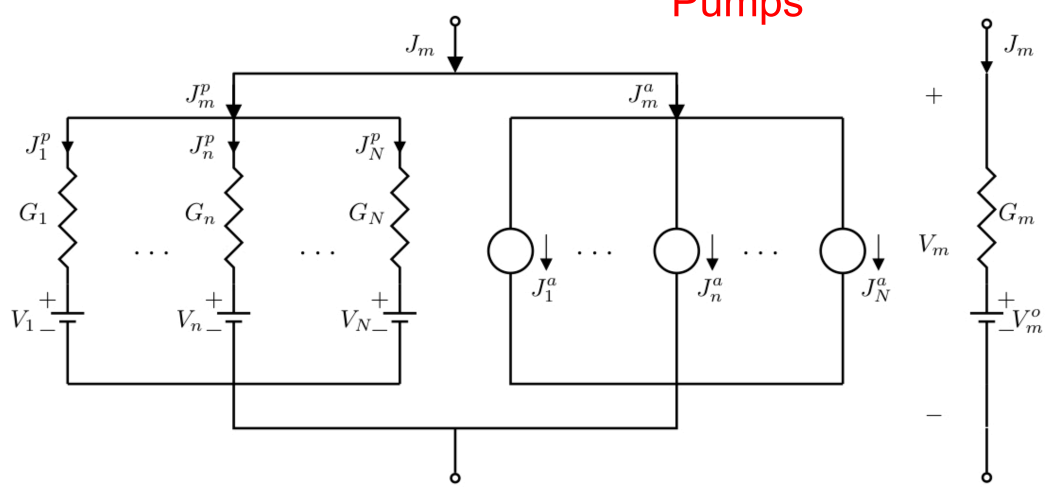

Figure 7.33

Looking Ahead: Hodgkin-Huxley network

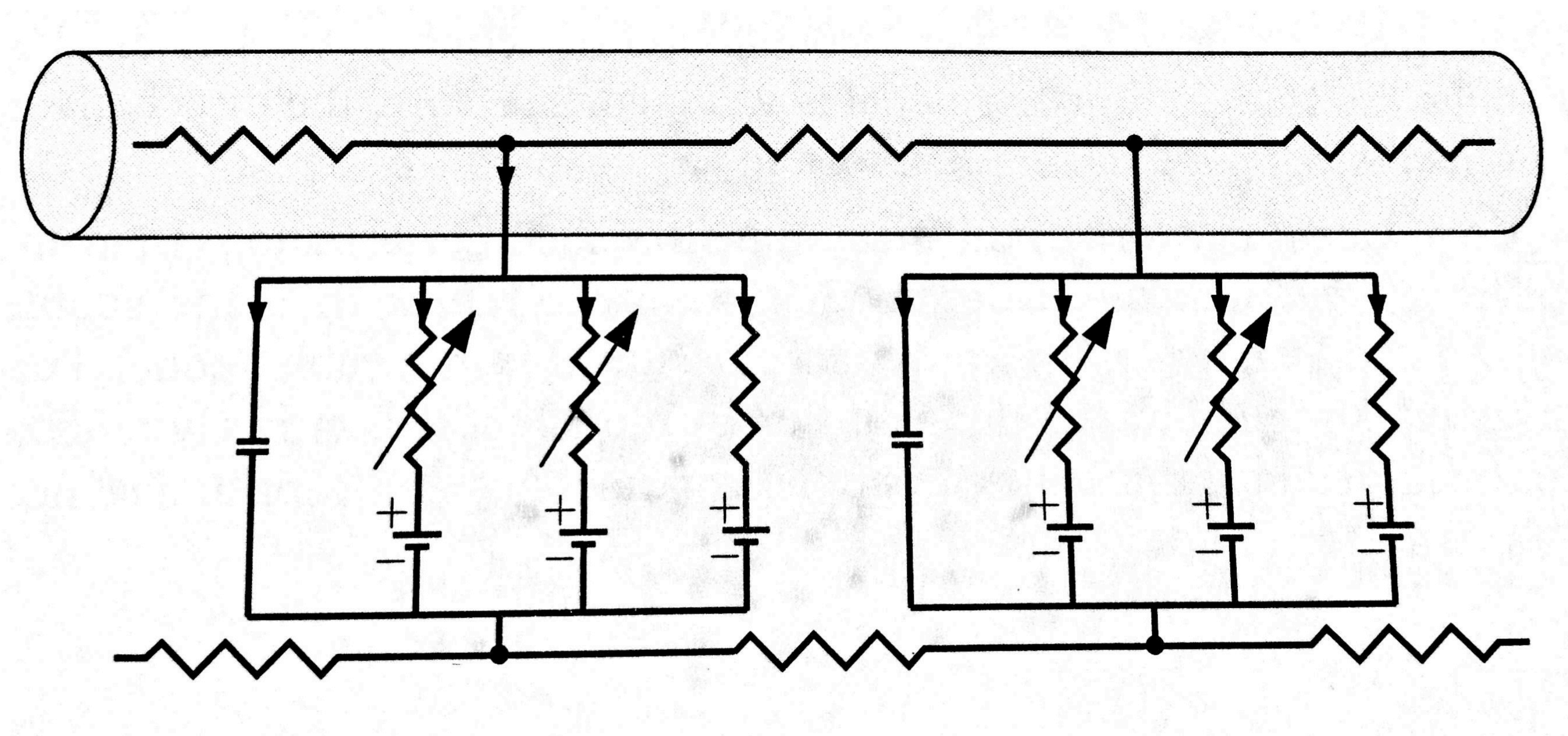

Fig.4.7 (vol.2)