tetrodotoxin has been shown to block a fraction of the resting sodium conductance, indicating a contribution by voltage-activated sodium channels.[9]

In central nervous system neurons, chloride channels may account for as much as 10 percent of the resting membrane conductance[13] and channels presumed to underlie this conductance have been described.[14]

ACTIVE TRANSPORT OF IONS

The Na–K Pump

The viability of nerve cells is maintained by the constant transport of sodium and potassium across the cell membranes against their electrochemical gradients. This perpetual task is carried out by the Na–K pump, the required energy being obtained from hydrolysis of adenosine triphosphate (ATP). Indeed, it has been shown that the phosphatase itself is an integral part of the ion transport system. The properties of the enzyme have been summarized succinctly in a review by Skou.[15] It consists of two molecular subunits: α, with an apparent molecular mass of about 100 kD, and β, about 38 kD. The active enzyme appears to exist in the membrane as a tetramer, $(\alpha\beta)_2$. The stoichiometry of the enzyme is as expected from the transport characteristics: An average of three sodium and two potassium ions are bound for each molecule of ATP hydrolyzed. The requirement for sodium is remarkably specific. It is the only substrate accepted for net outward transport; conversely it is the only monovalent cation *not* accepted for inward transport. Thus lithium, ammonium, rubidium, cesium, and thallium are all able to substitute for potassium in the external solution but not for sodium in the internal solution. The requirement for external potassium is not absolute. In its absence the pump will extrude sodium at about 10 percent of capacity in an "uncoupled" mode. The transport system is blocked specifically by the digitalis glycosides, particularly ouabain and strophanthidin.

Both the α and β subunits have been sequenced,[16,17] and various models have been proposed for their tertiary structure. The α subunit has six major hydrophobic regions capable of forming transmembrane helices; the β subunit has only one such region. Various schemes for the transport mechanism have been proposed. All involve alternate exposure of sodium and potassium binding sites (presumably within a channel-like structure) to the extracellular and intracellular solutions. The cyclic conformational changes are driven by phosphorylation and dephosphorylation of the protein and are accompanied by changes in binding affinity for the two ions. Thus sodium is bound during intracellular exposure of the sites and subsequently released to the extracel-

[13]Gold, M. R. and Martin, A. R. 1983. *J. Physiol.* 342: 99–117.
[14]Krouse, M. E., Schneider, G. T. and Gage, P. W. 1986. *Nature* 319: 58–60.
[15]Skou, J. C. 1988. *Methods Enzymol.* 156: 1–25.
[16]Kawakami et al. 1985. *Nature* 316: 733–736.
[17]Noguchi et al. 1986. *FEBS Letters* 196: 315–320.

lular solution; potassium is bound during extracellular exposure and released to the cytoplasm.

Experimental evidence that the pump is electrogenic

Transport of sodium and potassium was studied in squid axon by Hodgkin and Keynes and their colleagues[18,19] and in snail neurons by Thomas.[20,21] To examine the relations among internal sodium concentration, pump current, and membrane potential, Thomas used two intracellular pipettes to deposit ions in the cell, one filled with sodium acetate and the other with lithium acetate (Figure 7A). A third intracellular pipette was used as an electrode to record membrane potential. A fourth pipette was used as a current electrode for voltage clamp experiments (Chapter 4), and a fifth, made of sodium-sensitive glass, to monitor the intracellular sodium concentration. To inject sodium, the sodium-filled pipette was made positive with respect to the lithium pipette. Thus, current flow in the injection system was between the two pipettes, with none of the injected current flowing through the cell membrane. The result of such a sodium injection is shown in Figure 7B. After a brief injection the cell became hyperpolarized by about 20 mV and gradually recovered over several minutes. Injection of lithium (by making the lithium pipette positive) produced no hyperpolarization.

Several lines of evidence showed that the potential change after sodium injection was due to the action of a sodium pump and not to changes in membrane permeability. For example, the input resistance of the cell did not decrease, as might be expected if hyperpolarization were the result of an increased permeability to potassium or chloride. The hyperpolarization could, however, be greatly reduced or abolished by addition of the transport inhibitor ouabain to the bathing solution (Figure 7C), as would be expected if it were due to pump activity. Similarly, sodium injection had little effect on potential when potassium was absent from the external solution; reintroduction of potassium after injection, however, resulted in immediate hyperpolarization (Figure 7D).

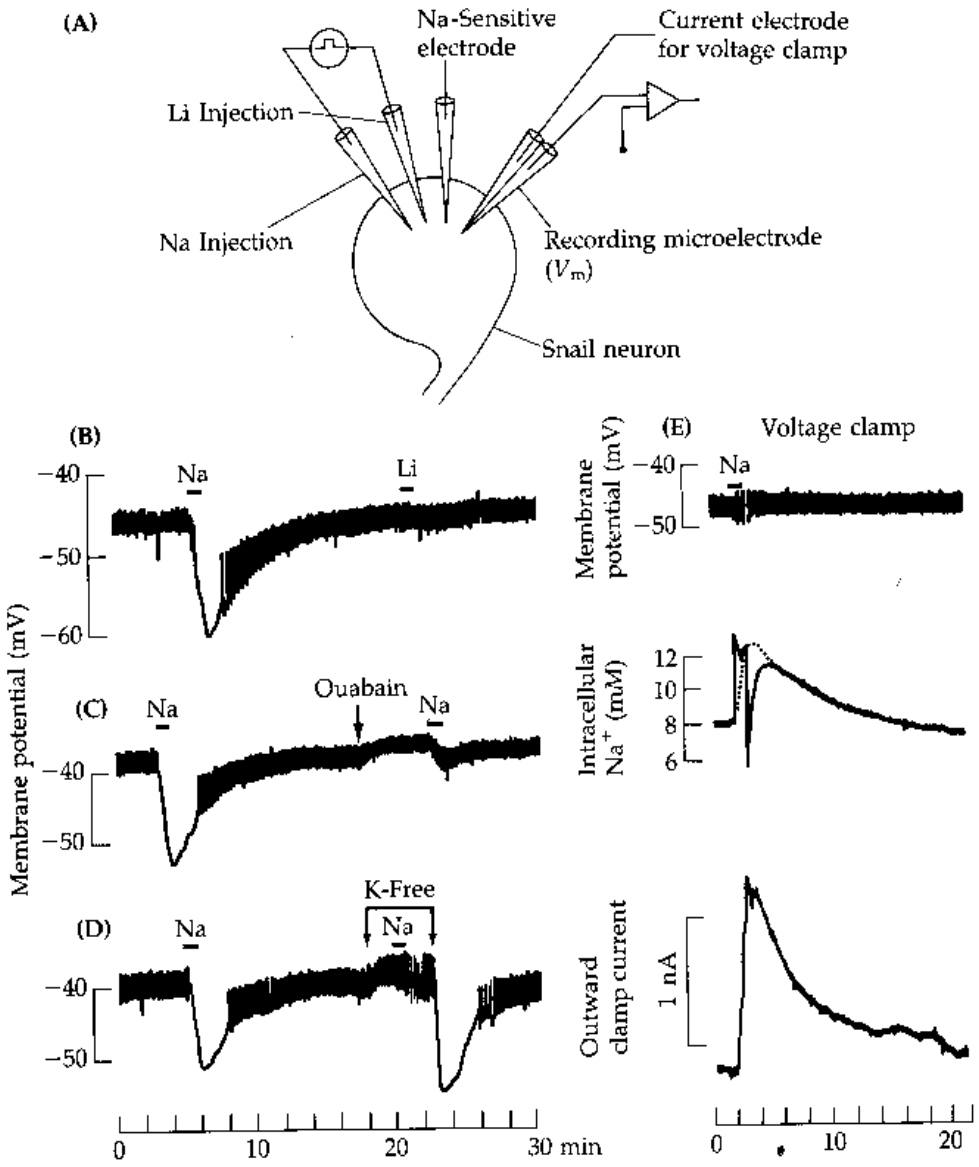
Quantitative estimates of the pump rate and the exchange ratio were obtained by voltage clamp experiments in which membrane current was measured while the membrane potential was being held constant (clamped). At the same time, intracellular sodium concentration was monitored. Sodium injection gave rise to an outward surge of current whose amplitude and duration followed the intracellular sodium concentration (Figure 7E). The total charge carried out of the cell, measured by integrating the total membrane current, was only about one-third of the charge injected in the form of sodium ions. This evidence was consistent with the idea that for every three sodium ions pumped out of the cell, two potassium ions were carried inward.

[18]Hodgkin, A. L. and Keynes, R. D. 1955. *J. Physiol.* 128: 28–60

[19]Baker, P. F. et al. 1969. *J. Physiol.* 200: 459–496.

[20]Thomas, R. C. 1969. *J. Physiol.* 201: 495–514.

[21]Thomas, R. C. 1972. *J.Physiol.* 220: 55–71.



7 EFFECT OF SODIUM INJECTION. Changes in intracellular sodium concentration, membrane potential, and membrane current following injection of sodium into snail neurons. (A) Sodium is injected by passing current between two electrodes filled with sodium acetate and lithium acetate (see text). A sodium-sensitive electrode measures $[Na]_i$; two other electrodes measure membrane potential and pass current through the cell membrane to obtain the voltage clamp records in (E). (B) Hyperpolarization of the membrane following intracellular injection of sodium. (The small rapid deflections are spontaneously occurring action potentials, reduced in size because of the poor frequency response of the pen recorder.) Injection of lithium does not produce hyperpolarization. (C) After application of ouabain (20 μg/ml), which blocks the sodium pump, hyperpolarization by sodium injection is greatly reduced. (D) Removal of potassium from the extracellular solution blocks the pump, so that sodium injection produces no hyperpolarization until potassium is restored. (E) Voltage clamp records. Sodium injection results in increased intracellular sodium concentration and in outward current across the cell membrane. The sharp deflections on the sodium concentration record are artifacts from the injection system. The time course of the concentration change is indicated by dashed lines. (After Thomas, 1969.)