titative discrepancies may be significant.

Those who hope for the best look forward to the use of the patch technique for the direct measurement of the properties of neurones too small to be measured by means of intracellular electrodes and for distinguishing between the effects of different extracellular agents (including drugs) on the permeability of the cell membrane. At least one experiment[10] suggests that the effects of local anaesthetics on the cell membrane may be to interrupt the flow of ions through a membrane channel, perhaps by physically blocking it, perhaps by some special interaction with the receptor. The application of the same technique to detached patches of cell membrane offers a way of exploring the intracellular influences on ion transport through neuronal membranes. □

Making potassium run uphill

THE mechanism that concentrates potassium ions within neurones (and other cells), the sodium–potassium pump, has recently been given a degree of notoriety because of the suggestion by Professor Efraim Racker's group at Cornell University[11] that phosphorylation of the enzyme responsible might be the crucial step in a cascade of biochemical reactions leading to malignancy. Although frequently overshadowed by the hunt for active receptors on neuronal cell surfaces, the characterization of the molecular components of the pump has made good progress in the past decade, but controversies and uncertainties persist.

Like the contents of other kinds of cells, the contents of neurones are in profound chemical disequilibrium with the extracellular space. It is therefore a little odd that while there is great interest centred on the search for neurotransmitters that will help to undermine this state of disequilibrium by making neuronal membranes more permeable to ions, the mechanism that maintains the disequilibrium appears to be regarded as a somewhat traditional branch of biochemistry — but is not well understood on that account.

Part of the trouble may be that the agent responsible has been recognized as a protein and as an enzyme bound in the cell membrane, but was originally known by the most undescriptive (and misleading) name in the biochemists' vocabulary — that of a K^+- and Na^+-dependent membrane-bound ATPase. Although more is now known of this pump, the true nature of this essential constituent of the cell membrane is far from clear.

Uphill work

Another reason why the sodium–potassium pumping mechanism receives less attention than it deserves is that it plays a crucial part not merely in glamorous organs such as the nervous system but also in muscles, conspicuously (in everyday clinical experience) in those of the heart, whose supply of potassium must be artificially made good following the use of glycosides such as digitalis in heart disease.

In all cells, the normal function of the pump is to extrude Na^+ and to take in K^+ ions. Even in resting cells, leakage of the ions in the direction of thermodynamic equilibrium is continuous, although not as great as when they are depolarized. Ion transport by the sodium–potassium pump must be sufficient, in the resting cell, to balance passive leakage, but must also be capable of restoring the loss of potassium, and of removing the unwanted increment of sodium, after a period of activity by a nerve (or muscle) cell.

The normal resting voltage of a neurone is a direct measure of the free energy involved in the process — the height of the hill up which water (represented by potassium) must be made to flow. Physical chemists will recognize that the voltage corresponding to the maintenance of K^+ concentrations inside and outside the membrane of, say, C_1 and C_2 is given by the Nernst equation as

$$RT/F \ln(C_2/C_1)$$

where R is the gas constant, F the Faraday constant and T the temperature. To account for the maintenance of multiple ion-gradients, the concentrations in this equation must be replaced by those of all the ion species weighted according to their permeabilities through the membrane. In the resting neurone, this entails a slight reduction of the voltage due to potassium disequilibrium that would otherwise obtain.

The pump

The essential ingredients of the Na^+/K^+ pump are two protein molecules, one a hydrophobic protein of about 120,000 daltons and the other a glycoprotein of 55,000 daltons (see Fig.5). The two components are closely associated, and it appears to be common ground that equal numbers of the two molecules are involved in transporting Na^+ and K^+, that the protein is embedded in the cell membrane and that its effectiveness in transporting sodium (normally outwards) and potassium (normally inwards) involves both phosphorylation (by ATP) and a conformational change of the enzyme.

There is, however, disagreement about whether the pump exists in membranes as a dimer of a linked pair of protein molecules (see refs 12, 13) and some doubt whether the customary view that the export of sodium is followed sequentially by the import of potassium must be replaced by the view that the two cations are transported more or less simultaneously. Participants at a conference at Yale University earlier in the summer appear to have been persuaded that simultaneous transport may now be more plausible.

Either way, the phosphorylation of the enzyme is accomplished by the transfer of one phosphate group from a molecule of ATP — a process for which Na^+ is necessary — and the subsequent dephosphorylation (catalysed by K^+). Transmembrane transport appears to involve the sequestering of ions within the structure of the protein and the kinetics and thermodynamics of the process appear to be consistent with the export of three Na^+ and the import of two K^+ ions for each cycle of phosphorylation and dephosphorylation.

With the help of sodium–potassium pumps in special systems, red cell ghosts for example, it has been possible to demonstrate[14] that the pump can be made to operate abnormally in suitable circumstances, pumping either Na^+ or K^+ alone, or even operating backwards (which merely confirms the inference from the Second Law of Thermodynamics that Maxwell's demon does not exist).

Several important issues remain unanswered. What, for example, is the significance of the close molecular homology around the site of phosphorylation (an aspartic acid residue) in the sodium–potassium pump and the analogous pump for Ca^{2+}? It would be no great surprise if these analogous enzymes had a common evolutionary origin. What are the mechanisms whereby the mechanism of the pump may be blocked by drugs, glycosides for example? This question is clinically significant, as is the open question whether the pump has some relevance to the use of lithium in the treatment of manic depressives. □

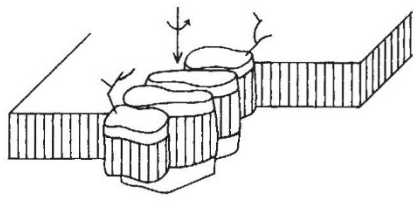

Fig.5 Drawing of the imagined molecular structure Na^+- and K^+-dependent ATPase. The subunits are drawn so that their volumes correspond to molecular weights of 120,000 and 55,000 using as a scale the width of the bilayer (4.0 nm).