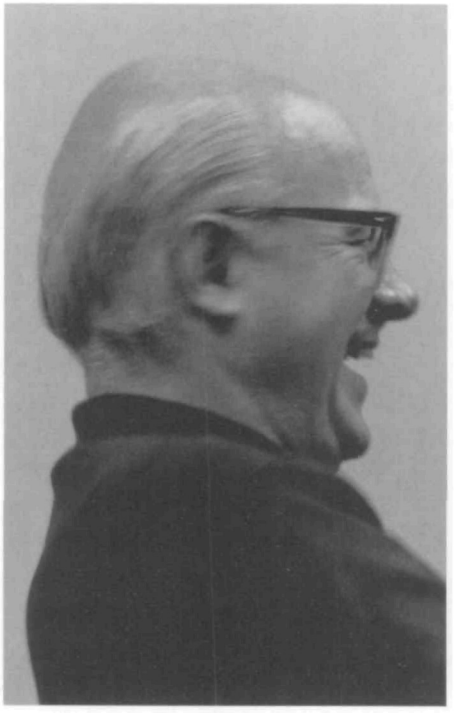
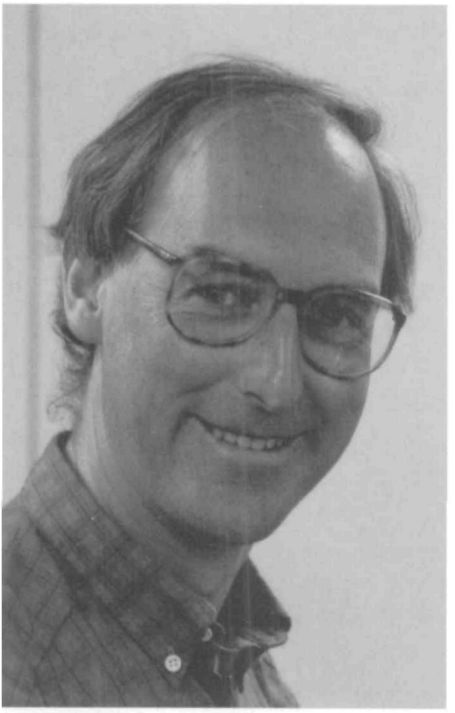
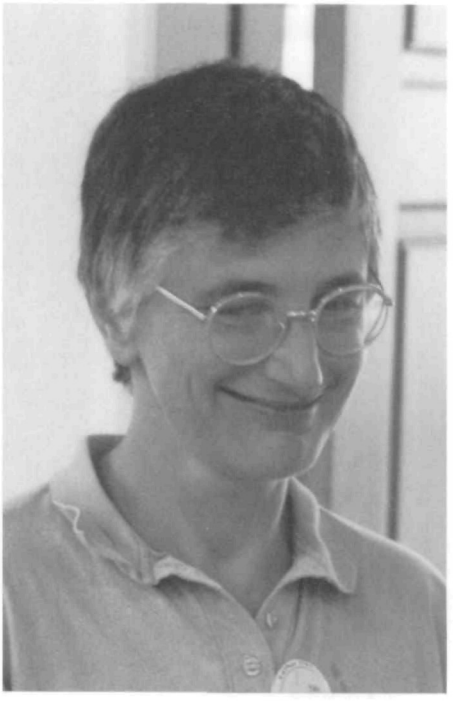


C. Slayman



B. Harvey



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SOME REMARKS ON THE INTEGRATED ACTIONS OF PUMPS, COTRANSPORTERS AND CHANNELS

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The biological significance of a particular molecular machine is defined only by its interactions with other molecular machines. While current research tends to emphasize direct interactions of macromolecules, indirect actions – often identified vaguely as 'physiological' functions or more specifically as transmitter- or messenger-mediated behavior – are clearly at least as important as direct interactions in the overall biological picture. What V-ATPases *can* accomplish becomes really interesting only when we know what they actually *do* accomplish; for example, in warehousing metabolic reserves, in compartmental buffering, in exporting adjuvant proteins and transmitter molecules and in processing nutrients. Wherever vacuolar-type ATPases appear, their action must be coordinated with actions of other transporters: i.e. with other pumps, co- or countertransporters, or *bona fide* channels; and with specific regulatory systems impinging on the same membranes.

Global variables which can modulate such interactions include membrane voltage, transmembrane chemical gradients, absolute solute composition (including pH) of cytoplasmic or extracellular fluids, redox potentials and, in some circumstances, luminous flux, temperature, etc. And much of transport biophysics has sprung from the fact that experimental values for such variables place essential thermodynamic constraints on the underlying reaction mechanisms.

To take a simple case in point, consider the question of how the free-living cells of many algae and fungi can concentrate nutrients from rarified environments to ratios of 10^5–10^6. One such nutrient is potassium, whose mode (or modes) of accumulative transport in non-animal cells, amazingly, still remains deeply in dispute; such cells clearly and categorically lack the P-ATPase-mediated exchange of K^+ for Na^+ which characterizes almost all animal cells (but which cannot sustain a concentration ratio much above 10^4). Now, however, because patch electrode technology has opened up the examination of ion pores – or *channels* – as molecular entities, it has become fashionable to ascribe 'almost all' of K^+ transport physiology in plants and fungi to the action of K^+ channels. But chemical accumulation of ions *via* a passive pathway, such as a channel, requires coupling to either electrical or hydrodynamic flow; and the latter must be zero in a static, turgid plant or fungal cell (and small in any cell that survives long). The energetic equivalent of a concentration ratio of 10^5–10^6 is near 30 kJ mol^{-1} or – for a univalent ion – about 300 mV. With a normal free energy of ATP hydrolysis less than 50 kJ mol^{-1}, any causally related ATP-driven charge pump could drive *no more than one ion per ATP split*

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(i.e. 1 mol of charge per mol of ATP). This constraint immediately rules out both V- and F-type ATPases as primary generators underlying putative channel-mediated K^+ accumulation, since these enzymes function, as far as is known, with stoichiometries of 2 H^+ (or perhaps more) per ATP split.

Animal physiologists and biochemists are often surprised to realize that plant and fungal cells can, and often do, sustain plasma membrane voltages approaching 300 mV. It is a necessary attribute of the free-living life style, and it has evolved with a P-type ATPase which indeed pumps a single ion (H^+) for each ATP molecule hydrolyzed. The observations on accumulative potassium transport in plant and fungal cells, however, are even more severe. It is quite easy under laboratory conditions to produce stable accumulation ratios which exceed those allowed by *measured* membrane voltages acting through any kind of passive pathway. The inference drawn from this was that, although the cooperative action of P-ATPases and K^+ channels may account for *some* K^+ accumulation, under extreme conditions more complex mechanisms *must* operate. In the case of the mycelial fungus, *Neurospora crassa*, and the giant pond alga, *Chara australis*, these mechanisms have been shown to be a K^+/H^+ symport and a K^+/Na^+ symport, respectively (Rodriguez-Navarro *et al.* 1986; Smith and Walker, 1989).

By a related argument, once gastric acid secretion had been shown to be the product of an ATPase (Ganser and Forte, 1973), it was clear that earlier 'proofs' of major intrinsic electrogenicity in the process must be spurious, since an ATP-driven pump that is known to create pH differences of ~7 units or more has very little energy left for net movement of charge. Overall electroneutrality of the gastric H^+-ATPase was quickly corroborated in ionophore experiments on vesicle-bound ATPase (Sachs *et al.* 1976), but almost a decade had passed before a convincing demonstration was assembled of the cooperating transport systems that gave the ensemble appearance of an electrogenic acid pump.

In fact, electrophysiological studies of the gastric P-ATPase attached to bilayer lipid membranes have recently shown the *partial reaction driven by ATP* to be electrogenic; i.e. charge displacement is an early step in proton pumping (van der Hijden *et al.* 1990). Actual release of the protons, however, requires entry of K^+ into the enzyme, which compensates the displaced charge and, in the process, is itself countertransported. Because the supply of protons, ultimately from the blood, is effectively infinite, while the supply of potassium ions from the gastric surface is finite, continuation of proton secretion requires K^+ to be recycled. This recycling is accomplished under normal circumstances by the opening of independent K^+ and Cl^- channels in the luminal membrane of gastric parietal cells, resulting in transport of salt down a chemical gradient and providing Cl^- for secretion with H^+ (Wolosin and Forte, 1981). The different processes are coordinated, at least in part, by the *mode* of gastric stimulation: insertion of intracellular tubulovesicles bearing the ATPase into apical canaliculi bearing the channels (Sachs *et al.* 1982).

As an introduction to the V-ATPases, Bill Harvey has already presented an excellent discussion of the way in which cooperating transport systems in the midgut of phytophagous insects can determine surprising *practical* functions for the ATPase. The original description of massive electrogenic K^+ transport *via* goblet cells of the *Cecropia* midgut epithelium (Harvey *et al.* 1967, 1968), plus later identification of both a

concentrated ATPase activity and a morphological feature – the so-called portasomes (Harvey *et al.* 1981) – which resemble the V(or F)-ATPases, evoked two different functional schemes: either this particular enzyme must differ from its likely congeners in actually pumping K^+ rather than H^+, or a *bona fide* H^+-ATPase must be coordinated with other transport systems to give the ensemble appearance of a K^+ pump. Only recently has the latter circumstance been clearly demonstrated (Wieczorek *et al.* 1989, 1991; Wieczorek, 1992; Harvey, 1992).

More generally, about half of this symposium has dealt with one aspect or another of the integrated functions of V-type ATPases, and more than half to come will consider such questions as well. In the present session, we shall begin – as a continuation of the previous session – with a report by Brian Harvey on V-ATPase-mediated proton pumping at the apical membrane of the frog skin epithelium and its relationship to the process of sodium reabsorption/salt conservation. From that, we shall shift to studies on single-celled eukaryotic organisms. Adam Bertl will describe his patch-clamp studies on vacuolar membranes of the yeast *Saccharomyces*, revealing a prominent vacuolar release pathway which probably functions in cytoplasmic calcium homeostasis and also in second-messenger routing for control of plasma-membrane channels. And Tilly Bakker-Grunwald will present her very exciting and novel experiments on the pathogenic primitive protozoan *Entamoeba histolytica*, which appears to have retained a functional proton-pumping V-ATPase in its plasma membrane and used it – as animal cells apparently must – to power sodium transport.

References

GANSER, A. L. AND FORTE, J. G. (1973). K^+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. *Biochim. biophys. Acta* **307**, 169–180.

HARVEY, W. R. (1992). Physiology of V-ATPases. *J. exp. Biol.* **172**, 1–17.

HARVEY, W. R., CIOFFI, M. AND WOLFERSBERGER, M. G. (1981). Portasomes as coupling factors in active ion transport and oxidative phosphorylation. *Am. Zool.* **21**, 775–791.

HARVEY, W. R., HASKELL, J. A. AND NEDERGAARD, S. (1968). Active transport by the cecropia midgut. III. Midgut potential generated directly by active K-transport. *J. exp. Biol.* **48**, 1–12.

HARVEY, W. R., HASKELL, J. A. AND ZERAHN, K. (1967). Active transport of potassium and oxygen consumption in the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **46**, 235–248.

RODRIGUEZ-NAVARRO, A., BLATT, M. R. AND SLAYMAN, C. L. (1986). A potassium–proton symport in *Neurospora crassa*. *J. gen. Physiol.* **87**, 649–674.

SACHS, G., CHANG, H., RABON, E., SCHACKMANN, R., LEWIN, M. AND SACCOMANI, G. (1976). A nonelectrogenic H^+ pump in plasma membranes of hog stomach. *J. biol. Chem.* **251**, 7690–7698.

SACHS, G., WALLMARK, B., SACCOMANI, G., RABON, E., STEWART, H. B., DIBONA, D. R. AND BERGLINDH, T. (1982). The ATP-dependent component of gastric acid secretion. *Curr. Topics Membr. Transport* **16**, 135–159.

SMITH, F. A. AND WALKER, N. A. (1989). Transport of potassium in *Chara australis*: I. A symport with sodium. *J. Membr. Biol.* **108**, 125–137.

VAN DER HIJDEN, H. T. W. M., GRELL, E., DE PONT, J. J. H. H. M. AND BAMBERG, E. (1990). Demonstration of the electrogenicity of proton translocation during the phosphorylation step in gastric H^+K^+-ATPase. *J. Membr. Biol.* **114**, 245–256.

WIECZOREK, H. (1992). The insect V-ATPase, a plasma membrane proton pump energizing secondary active transport: molecular analysis of electrogenic potassium transport in the tobacco hornworm midgut. *J. exp. Biol.* **172**, 335–343.

WIECZOREK, H., PUTZENIECHNER, M., ZEISKE, W. AND KLEIN, U. (1991). A vacuolar-type proton pump energizes K^+/H^+-antiport in an animal plasma membrane. *J. biol. Chem.* **266**, 15340–15342.

WIECZOREK, H., WEERTH, S., SCHINDLBECK, M. AND KLEIN, V. (1989). A vacuolar-type proton pump in a vesicle fraction enriched with potassium transporting plasma membranes from tobacco hornworm midgut. *J. biol. Chem.* **264**, 11143–11148.

WOLOSIN, J. M. AND FORTE, J. G. (1981). Functional differences between K^+-ATPase membranes isolated from resting or stimulated rabbit fundic mucosa. *FEBS Lett.* **125**, 208–212.