Shedding gloomy light into the black box of the Ussing chamber

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To the Editor: In a recent article on Ussing chambers (1), I noticed a reference to a theoretical paper of mine (2) that revisited and highlighted the deficiencies in the original (3) mathematics describing the movement of isotope through the three compartments of a typical chamber. The original mathematical description was deemed to produce a satisfactory and usable derived equation, familiar to Ussing chamber users as $J_{\text{net}} = J_{\text{ms}} - J_{\text{sm}}$, and was not reconsidered again for over forty years. $J_{\text{net}}$ is the arithmetical difference between $J_{\text{ms}}$ and $J_{\text{sm}}$. $J_{\text{ms}}$ is the measured flux of isotope moving from the mucosal to the serosal compartment. $J_{\text{sm}}$ is the measured flux of isotope moving from the serosal to the mucosal compartment. Both fluxes are assumed to be unidirectional (i.e., isotope moves in one direction only). On reexamination of the three-compartment description, further insights were reached, but, more importantly, the above equation was seen to be extremely unlikely to be valid. All mathematical treatments of the Ussing chamber are necessarily black box approaches, since the flow of isotope is described solely in terms of time-dependent differential equations governing movement between compartments. My 2005 paper was an attempt to shed some light into the black box because only by considering the internal detail of the model can one recognize the significance and test the validity, misleading or otherwise, of any inherent assumption. The guide to Ussing chambers (1) restates the central tenets of Ussing chamber work that net flux is the result of appropriately measured unidirectional fluxes, that the fluxes are independent, and that the fluxes can be seen to correlate with electrical events occurring in the tissue. These are all highly unlikely conclusions, as my reworking of the flux equations shows.

Since the 1964 treatment allows only unidirectional movement from tissue to end compartment, when the reverse process is modeled there is no permeability allowing it. The more general 2005 treatment allows flux from the end back to the tissue compartment and does not require the value attributed to this backflux to alternate between finite and zero, depending on the direction and hence the volition of the observer. The inclusion of backflux allows for the experimental fact that material added to a source compartment will appear in the end compartment, regardless of which source compartment is chosen, thereby overcoming the contradiction within the simpler model. In the general model, isotope appearance in a sink compartment is governed by all rate coefficients. The measured fluxes $J_{\text{ms}}$ and $J_{\text{sm}}$ are seen to be composites of unidirectional fluxes but are not themselves unidirectional. They are seen to be the result of isotope that entered the end compartment from the tissue minus isotope that entered and left that compartment during the same time interval. This in turn implies an interdependence not an independence to measured flux data.

What are the consequences of the general model, verifiable by examining past flux measurement data? The general model draws attention to a deficiency in the treatment of flux data when based on the simpler treatment; namely that the measured and assumedly unidirectional fluxes are not unidirectional and hence $J_{\text{net}}$ will not equal $J_{\text{ms}} - J_{\text{sm}}$. $J_{\text{net}}$ is not a fixed difference between two fixed arithmetical rates but will be time dependent, falling to zero as equilibrium is approached. The fluxes are interdependent, making it unlikely that the independence condition will be met even at the early stages of the transfer. When a very small fraction of the source isotope reaches the sink compartment, some of the isotopically labeled atoms will migrate back across into the intermediate compartment, even at very early times after the start of any flux measurement process. Increasing the sink volume also will not overcome this problem, nor will vigorous stirring since both tissue surfaces will have an unstirred layer that will prevent stirring forcing the local concentration down to zero.

The Solomon treatment makes the measured unidirectional fluxes independent of one another but only because the zero backflux assumption makes them so by definition, not because of experimental observation. In contrast, the general description shows that measured fluxes must be interdependent since the equations that describe flux contain all rate coefficients of the model as sums and products in the various asymptotes and constants that make up the model solution. This interdependence or reciprocity allows a choice to be made between the general model and the zero-backflux model, since flux reciprocity is predicted and can be shown to have occurred in past data. There is no requirement for reciprocity in the Solomon model. The general model is therefore the better model not just on logical grounds but also because it explains more features of Ussing chamber experiments. It demonstrates that a factor that changes measured $J_{\text{ms}}$ flux will also reciprocally alter the $J_{\text{sm}}$ flux. The general model therefore explains why omitting glucose or adding ouabain to the Ussing chamber will also change measured fluxes in a reciprocal manner leading to enhanced secretory fluxes; reduced absorption is a predictable consequence of using these agents but the apparently enhanced secretion is not. It is a consequence only of reduced absorption but manifests itself as apparently enhanced secretion of isotope.

This reciprocity principle is particularly important for the electrogenic chloride ion model as the basis for secretion, since increases in the measured $J_{\text{sm}}$ flux ion are taken as proof that some bacterial toxins increase enterocyte chloride secretion. The general model indicates that this cannot be categorical proof of chloride secretion since an increased $J_{\text{sm}}$ flux would also occur if the absorptive ($J_{\text{ms}}$) flux were inhibited. Data showing enhanced chloride ion appearance in the luminal compartment after enterotoxin treatment are therefore not incontrovertible proof of enhanced secretion but can equally well be explained by diminished absorption. This is a considerable difficulty for the theory of enhanced enterocyte chloride ion secretion as a basis for diarrheal disease. The general model shows that such flux measurements fail as a category of evidence for enterocyte secretion. The problem lies in the
interpretation of what change in the measured fluxes means. The general model shows that the maximum permissible inference is that enterotoxin has altered ion transport, not that it has necessarily caused enhanced secretion from the enterocyte. A further perceived utility of Ussing chamber measurements is that short-circuit current will equate with ion fluxes, leading to further insights when coupled with molecular biological techniques. This may be so, but the simple equating of fluxes with short-circuit current is also likely to lead to erroneous conclusions. A concern must be that disregarding what the general model mathematics indicates will continue to sanction the process of equating the unequatable and shed little light on intestinal physiology.

REFERENCES