Plant Physiol. (1969) 44, 1013–1015

Short Term Influx as a Measure of Influx Across the Plasmalemma

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Received February 13, 1969.

Abstract. **It is shown that the influx of tracer to a plant cell must be measured for less than one third of the half-time for exchange of cytoplasmic tracer, and any subsequent wash must be negligibly brief, if the initial influx measured over a finite period is to be a good estimate of the plasmalemma influx. Complications due to lack of knowledge of the cytoplasmic exchange rate constant and to extracellular contents make it difficult to make such an estimate from influx measurements alone. The use of influx measurements is further discussed.**

This note consists of a quantitative consideration of the idea that the influx of tracer to plant cells over a short period is a measure of the plasmalemma influx.

The simplest description of a plant cell is in terms of a small cytoplasmic compartment outside and in series with a large vacuolar compartment. At or near a steady state (*i.e.* when chemical fluxes and concentrations are effectively constant with time), the movement of a labeled isotope from one compartment to another will be proportional to the difference in specific activity. The movement of tracers will therefore follow first order kinetics. In this particular model, tracer movements will be described by relatively simple equations, which will relate tracer kinetic data to chemical fluxes and compartment contents (2, 6, 7, 9). This model has been shown to be a first approximation to the actual situation in the giant algal coenocyte, *Nitella translucens* (6, 7) and an attempt has been made to find if this is also true for excised carrot tissue (2). The following considerations are based on this simple model. The plasmalemma influx and efflux will be called M_{oc} and M_{co}; the tonoplast influx and efflux, M_{cv} and M_{vc}; and the cytoplasmic content, Q_c. Tracer fluxes will be called M^*_{oc}, *etc.*

The net tracer influx to the cell is initially equal to $M_{oc} \cdot s_o$. s_o will be taken as 1, and omitted from the following equations. Subsequently the specific activity in the cytoplasm will rise:

$$s_c = \frac{M_{oc}}{M_{co} + M_{cv}} \quad (1 - e^{-k_c \cdot t})$$

where k_c is the rate constant for exchange of tracer in the cytoplasm, and equals $\frac{M_{co} + M_{cv}}{Q_c}$. It should be noted that k_c describes the rate of change of tracer in the cytoplasm for a particular value of M_{co}, M_{cv} and Q_c, and therefore one of the conditions for the general applicability of the model is that the experimental system is at or near a steady state. k_c will be a constant for all Q_c only if M_{co} and M_{cv} are proportional to Q_c, which *a priori* may be considered unlikely.

As s_c rises the loss of tracer to the external solution will rise, and the net tracer influx to the cell at any instant will be

$$M^*_{oc} - M^*_{co} = M_{oc} - M_{co} \cdot s_c$$

$$= M_{oc} - M_{co} \cdot \frac{M_{oc}}{M_{co} + M_{cv}} \cdot (1 - e^{-k_c \cdot t}) \quad \text{(I)}$$

The instantaneous net tracer influx to the cell will fall from M_{oc} at $t=0$, to $\frac{M_{oc} \cdot M_{cv}}{M_{co}+M_{cv}}$ as t becomes large compared with $\frac{1}{k_c}$. This final influx can be regarded as a quasi-steady overall influx to the vacuole, since the cytoplasmic specific activity is now rising only very slowly due to the equally slow rise in the specific activity in the very large vacuole.

The question is, how long is the period at the beginning of an experiment when the measured influx to the cell is effectively equal to M_{oc}?

The measured tracer influx to the cell is the tracer taken up divided by the loading time. The net tracer entering the cell between 0 and t is found by integrating equation I. The average net tracer influx between 0 and t (the experimental measurement) will therefore be:

$$M_{oc} - \frac{M_{co} \cdot M_{oc}}{M_{co} + M_{cv}} \cdot \frac{1}{t} \cdot \int_0^t (1 - e^{-k_c \cdot t})\,dt$$

$$= M_{oc} - \frac{M_{co} \cdot M_{oc}}{M_{co} + M_{cv}} \cdot \left[1 - \frac{1}{k_c \cdot t}(1 - e^{-k_c \cdot t})\right] \quad \text{(II)}$$

The fractional approach to the final steady value of influx is independent of the values of the fluxes. For simplicity, the case when all the fluxes are equal will be considered. The same conclusions regarding the distinction between the initial and the final quasi-steady influx will apply whatever the relative values of the plasmalemma and the tonoplast fluxes. To retain generality, t can be expressed in cyto-

plasmic exchange half-times ($t = nt_{1/2c}$). $t_{1/2c} = \frac{0.693}{k_c}$, and therefore $k_c t = 0.693n$. Values of the average net tracer influx (equation II) were calculated for various values of n, and a curve is drawn through these points, plotted against n, in Fig. 1. It will be apparent that the experimental tracer influx falls off fairly rapidly, compared with the rate of cytoplasmic exchange, as the length of the loading period is increased. In order to estimate the difference between the initial and the quasi-steady influx to better than 90 % (the dashed line in Fig. 1) one must be able to measure influx over less than 0.31 cytoplasmic half-times, and this may be as short a period as 3 min (2).

In making an estimate of the initial flux to the cell one has to allow for the extracellular content, which will be a large fraction of the total taken up by a tissue in short periods. This may be measurable in a separate experiment, but then one is faced with the difficulty of measuring a small difference between 2 large and variable values.

If the cell is washed in inactive solution after the loading period to remove extracellular contents, activity will at the same time be lost from the cytoplasm to the external solution and to the vacuole. Equation II can be modified to take this into account. The lower curve in Fig. 1 shows the effect on the estimated influx of washing for 1 cytoplasmic half-time. In this case the difference between the initial influx and the quasi-steady influx will be underestimated by 50 % even after the shortest loading periods. After washing for about 5 half-times the estimated influx will be equal to the quasi-steady influx to the vacuole *however short the loading period*. The initial lag in the arrival of tracer in the vacuole during the early period of loading is exactly compensated for by the tracer transferred from the cytoplasm to the vacuole during a subsequent wash-out, leaving a total as though there had been a quasi-steady influx to the vacuole from the beginning of loading (2, 5, 6, 9). In the tissues examined so far, the time taken to wash out extracellular contents is such that an appreciable fraction of the cytoplasmic content would be exchanged at the same time (*e.g.* 2, 9).

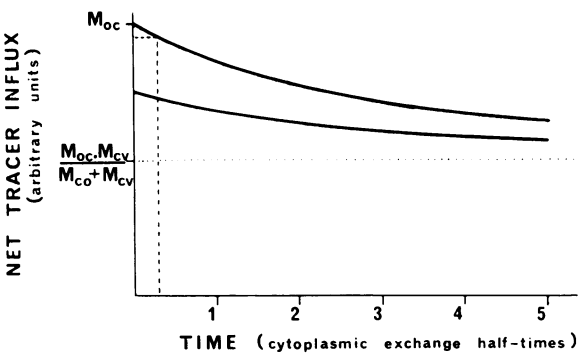

FIG. 1. The average net tracer influx to a plant cell between 0 and t as a function of t. The curves are constructed from the model discussed in the text. Upper curve—no wash after loading; lower curve—loading period followed by washing in inactive solution for 1 cytoplasmic exchange half-time. The horizontal dashed line lies at 90 % of the difference between M_{oc} and the final quasi-steady value $\frac{M_{oc} \cdot M_{cv}}{M_{co} + M_{cv}}$. The vertical dashed line indicates the corresponding loading period.

Thus, while in principle it would be possible to do influx experiments over a short enough period to distinguish between the initial and the quasi-steady influx, complications due to extracellular diffusion and cation exchange on the cell walls make the measurement frequently inaccurate or impossible. Further, there is a more basic difficulty in attempting to estimate M_{oc} from influx measurements alone, which would not be avoided even if extracellular contents were no complication. Before one can tell how short a loading time is necessary to obtain a good estimate of M_{oc}, and hence whether difficulties due to extracellular diffusion can be avoided, one has to know k_c. One could also attempt to obtain M_{oc} by extrapolation, but again one needs to know k_c, since the extrapolation is not linear but depends on k_c. However, influx has always to be measured as a small difference between 2 large and variable values, and such measurements are inherently not accurate enough to allow one to determine k_c generally.

It must be concluded that one cannot rely on influx measurements *alone* in establishing a value for the plasmalemma influx; and that under the conditions of most published experiments the influx measured is approximately equal to the quasi-steady influx to the vacuole, across both plasmalemma and tonoplast.

However, the overall influx may be very nearly equal to either the plasmalemma influx or the tonoplast influx under some conditions, and if this is known to be the case then influx measurements can be used alone in investigating individual transport processes.

The quasi-steady influx will be nearly equal to the plasmalemma influx if, under the conditions of the experiment, the majority of the tracer getting into the cytoplasm subsequently passes to the vacuole. This will be the case if the tonoplast influx is an order of magnitude larger than the plasmalemma influx [as in *Nitella translucens* (6)]; and will also be the case, even when the tonoplast and plasmalemma influxes are comparable in size, if the plasmalemma efflux is relatively small, *i.e.* when net influx is approximately equal to the plasmalemma influx. Under these conditions the quasi-steady influx will be only a little less than the initial influx to the cell. The quasi-steady influx will be nearly equal to the tonoplast influx if, under the conditions of the experiment, only a small fraction of the tracer getting into the cytoplasm passes into the vacuole,

i.e. the cytoplasmic specific activity is nearly equal to that in the external solution. This will be the case when the tonoplast influx is an order of magnitude smaller than the plasmalemma influx [as in *Nitellopsis obtusa* (8)], or even when they are comparable in size if there is a large net efflux. In this case the quasi-steady influx will be much less than the initial influx to the cell.

This approach is utilized frequently in investigations of certain giant algal coenocytes (in which the above considerations can be tested directly by physical separation of the cytoplasm and the vacuole) (*e.g.* 4, 6, 11), and has also been used in investigating higher plant cells (1, 3, 9, 12).

Acknowledgment

I am grateful to Professor M. G. Pitman for helpful comments on the first draft of this manuscript. The work was supported by a United States Atomic Energy Commission contract to Professor G. G. Laties, and a Fulbright travel grant to the author from the U. S.-U. K. Educational Commission.

Note Added in Proof

Welch and Epstein (1969, Plant Physiol. 44: 301–04) report measurements of influx to barley roots over a period of 1 min, which is short compared with any known cytoplasmic exchange half-time. These short loading periods were followed by a 30 min wash in inactive solution, which, as emphasized above, means that the flux measured will still approximate to the overall quasi-steady influx to the vacuole. The extent to which this is so will depend on how closely net chemical fluxes obey first order kinetics, and on whether plasmalemma efflux and tonoplast influx are equally sensitive to temperature changes, since the final wash of Welch and Epstein was at a lower temperature than that of the loading solution.

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