Mechanisms of Transfer between Environment and Cell

In a recent publication, ASCHHEIM[1] astonished himself by rediscovering the fact that adsorption of the Langmuir type is formally analogous to the conventional treatment of single-step enzyme reactions. He seems unaware that this was first pointed out by HALDANE[2], that a simple adsorption-carrier model was considered by REINER in 1939[3], that the analogy was proved and discussed by REINER in 1959[4] and pointed out in the same year by EDSALL and WYMAN[5]. ASCHHEIM also overlooks a large body of pertinent experimental work, such as has come from studies with bacteria[6,7].

ASCHHEIM's conclusion that active transport is due to adsorption on intracellular sites is a complete *non sequitur*. If the formal properties of two mechanisms are identical, it is obviously impossible to decide which mechanism holds merely by comparing data with the algebraic relations. On the same grounds, of course, the alternatives rejected by ASCHHEIM get no support from such a comparison.

This does not mean that no conclusions at all can be drawn from properly arranged kinetic data. We are dealing with a system of at least two phases, with perhaps a third if we count the cell surface. Ignoring for the moment a true enzymatic mechanism, we have three possibilities: (1) adsorption on the cell surface only; (2) adsorption inside the cell after entrance by diffusion; and (3) entrance into the cell by a carrier.

(1) Denoting the total adsorbent by A_t and extracellular concentration of the substance to be bound by S_e, the steady-state (and also maximum) value for material bound (by Langmuir adsorption) is $C_m = A_t S_e/(S_e + K)$, where $K = k_{-1}/k_1$ is the dissociation constant of the adsorption complex. The increase in bound material with time is determined by the differential equation $dC/dt = k_1 S_e(A_t - C) - k_{-1} C$. We can solve this and get $C = C_m\{1 - \exp[-(k_1 S_e + k_{-1})\, t]\}$. It is understood here that S_e is independent of time (maintained constant by some experimental device).

(2) If internal adsorption occurs after diffusion across the cell surface, we denote the internal free compound by S_i, the other components as before. The rate of penetration by diffusion across the surface[8] is $h_1 S_e - h_2 S_i$. In the complete steady state, we have therefore $C_m = A_t S_i/(S_i + K)$, and $S_i = HS_e$, where $H = h_1/h_2$ is the ratio of the permeability coefficients. Hence we can write $C_m = A_t S_e/(S_e + K/H)$. The total material taken up is $C_m + S_i$. If we assume that the carrier is almost always in the steady state, and follow the time course as S_i approaches its maximum value, we have $S_i = HS_e\,(1 - e^{-h_2 t})$, while C_m is as before.

(3) Let the total carrier concentration be A_t. The carrier complex C is formed and broken with rate constants k_1 and k_{-1}, and the complex releases free material intracellularly (S_i) at the rate $k_2 C$. The free S_i may also leak out of the cell at the rate hS_i. In the steady state, $C_m = A_t S_e/(S_e + K)$, and $S_i = K' C_m$, where $K' = k_2/h$. The total material taken up is $C_m + S_i = (K' + 1)\, C_m$. If we let carrier be always in the steady state, and follow the time development of free material S_i, we get $S_i = K' C_m(1 - e^{-ht})$, with C_m as before.

If we compare these results for the steady state, we see that surface adsorption still follows a Langmuir isotherm. On the other hand, internal adsorption after penetration by diffusion has an extra term that is linear in S_e. This term will be small if the penetration rate is small (small H); but the hyperbolic term will also decrease with H, unless K is very small or A_t very large. Thus one would expect, in experiments with increasing external concentrations, to find a concentration curve for uptake that starts like a hyperbola but, instead of levelling off completely, approaches a straight line with slope H. In the carrier case, the Langmuir isotherm form will again occur.

Further information, however, comes from time experiments. All cases approach maximum uptake more or less exponentially. But the curve for surface adsorption can be written as $\ln(1 - C/C_m) = -(k_1 S_e + k_{-1})\, t$ in order to get the value of the coefficient of t in the exponent; this coefficient is a measure of the rate at which the steady value is approached. The equation shows that this will be linear in S_e, not a constant. The time equation for intracellular adsorption is more complex, but the comparison is possible with a simple trick. Writing U for the total uptake,

$$U = (1 - e^{-h_2 t})\,\{H S_e + A_t H S_e/[K + H S_e(1 - e^{-h_2 t})]\}\,.$$

The slope of the U vs $\cdot\, t$ curve is

$$dU/dt = e^{-h_2 t}\{H S_e h_2 + K A_t H S_e h_2/[K + H S_e(1 - e^{-h_2 t})]^2\}\,.$$

In this expression, the term in square brackets will approach $(K + H S_e)^2$ with increasing time; thus $\ln(dU/dt)$ will approach const. $- h_2 t$. The corresponding function for the surface adsorption case, $\ln(dC/dt)$, will approach const. $-(k_1 S_e + k_{-1})t$. Thus, if we plot $\ln(dC/dt)$ or $\ln(dU/dt)$ respectively against time, the limiting slope will be dependent on S_e in the one case, independent in the other. As for the carrier case, it will have a unique property, since $U = C_m[K'(1 - e^{-ht}) + 1]$: the $U - t$ curve, carefully extrapolated back to $t = 0$, will not approach a zero value, but the finite value C_m. This intercept may of course be difficult to detect if A_t is very small while K' is very large. Even in this case, the discrimination is possible: we will have $dU/dt = K' C_m h e^{-ht}$, which will have a linear plot of $\ln(dU/dt)$ against t just like the surface adsorption case, but with a slope independent of S_e, whereas the corresponding plot for intracellular adsorption will be curved, and only approach a straight line for sufficiently large values of t.

Combinations of mechanisms might occur: e.g., a surface carrier mechanism followed by internal adsorption. There is a novel feature in the steady state. The maximum value for internally bound material will be

$$C_m = A_t B_t K' S_e/[K_a K_b + (K_b + K' A_t) S_e]\,,$$

where A_t is the amount of surface carrier and B_t that of internal adsorbent. Since these quantities, in an experiment with a mass of tissue or a cell suspension, would be proportional to the tissue mass, uptake will no longer be proportional to tissue mass as in the cases previously considered, but will vary with mass M as $a M^2/(b M + c)$.

Résumé. Un essai de choix entre plusieurs mécanismes théoriques du transport actif dans les cellules, dans des conditions d'équilibre de flux, n'apparaît pas satisfaisant à la lumière de cette étude. Des analyses cinétiques capables de constituer les séparations désirées entre ces mécanismes sont présentées.

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