BBA 75315

VESICULAR TRANSPORT ACROSS ENDOTHELIAL CELLS

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(Received March 12th, 1969)

SUMMARY

The transport of large molecules across endothelial cells by means of self diffusion of micropinocytotic vesicles is discussed, and it is shown that a consistent explanation of the observed rates of transport can be given if the mean time of attachment of the vesicles to the plasma membrane is of the order of a few seconds.

INTRODUCTION

It was first suggested by PALADE[1,2] that the transport of substances across cells might occur through the motion of micropinocytotic vesicles which took in material on one side of the cell and discharged it on the other. With such a mechanism the rate of transport of substances would be independent of molecular weight if it is assumed that vesicles are in communication with the fluids on either side of the cell long enough for their contents to equilibrate with these fluids. Experimental evidence discussed by RENKIN[3] shows that rates of transport across blood capillary endothelium decrease with increasing molecular weight but apparently become constant for weights in excess of about 60000. Thus the transport of large molecules may well occur by means of vesicles, although there must be some other mechanism allowing faster transport of smaller molecules. We confine our attention to the former process.

CASLEY-SMITH[4] produced evidence for the Brownian motion of such vesicles in living cells and SHEA AND KARNOVSKY[5] discussed this motion in a quantitative fashion to offer an explanation of a possible transport process. It is, however, simpler to consider the diffusion of the vesicles rather than their Brownian motion. A simple model will be set up and discussed in the light of CASLEY-SMITH's[6] recent observations on dimensions and number of vesicles in endothelial cells. The figures given in this reference have since been modified by its author (personal communication) to allow for tissue shrinkage in specimen preparation, and these later figures will be used in this paper.

VESICLE DIFFUSION

Consider a plane-parallel sided cell, or layer of cells, of infinite extent so that the problem is one-dimensional. There is a certain number of vesicles per unit volume, and, at any instant, a certain average number attached per unit area of the cell surfaces. On one side of the cell there is a medium containing a concentration c_1 of a substance

to be transported, and on the other side the concentration of the same substance is c_2 (Fig. 1).

We assume, and justify later, that the vesicles are attached to the plasma membranes long enough for their contents to become identical with the external media. Then vesicles of Type 1 containing Medium 1 are released from A (Fig. 1) into the space AB and some of them diffuse across to B, and similarly vesicles of Type 2 diffuse in the opposite direction. Suppose that in the cell close to A there are N_{10} vesicles of Type 1 and N_{20} of Type 2 per unit volume, so that $N_{10} + N_{20} = N_0$ is the total number of vesicles per unit volume at $x = 0$.

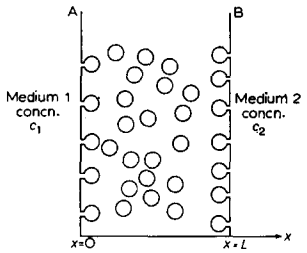

Fig. 1. Section of idealized cell with free and attached vesicles.

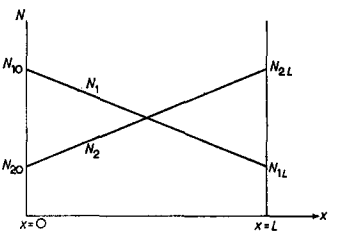

Fig. 2. Vesicle concentration distributions for a symmetrical system.

If n is the average number of vesicles attached, at any instant, per unit area of A, the number leaving per sec from unit area is

$$\nu = \frac{n}{\tau} \tag{1}$$

where τ is the mean time of attachment. Considering only a steady state situation the total number of vesicles, of both types, attaching to unit area of A per sec is also ν. The numbers of Types 1 and 2 attaching must be in the ratio of N_{10} to N_{20}. Thus if ν vesicles of Type 1 are released from A per sec, in the same time $\nu\ (N_{10}/N_0)$ of the same type attach. Hence the net number of Type 1 vesicles leaving unit area of A per sec is:

$$\nu - \nu \frac{N_{10}}{N_0} = \nu \frac{N_{20}}{N_0} \tag{2}$$

From the first diffusion equation (Fick's law) the number of particles of Type 1 crossing unit area per sec along OX $= -D\ (\partial N_1/\partial x)$ where N_1 is the number of Type 1 vesicles per unit volume at x, and D is the self-diffusion coefficient of the vesicles in the cytoplasm. For very low concentrations this is identical with the normal diffusion coefficient, a statement supported by the measurements of IRANI AND ADAMSON[7] on sucrose solutions. Thus, assuming that the vesicle concentration is low enough, we may consider that each kind of vesicle diffuses independently of the other, but with the same normal diffusion coefficient since they are distinguishable only by their contents.

Then for vesicles of Type 1 at $x = 0$

$$\nu \frac{N_{20}}{N_0} = -D \left(\frac{\partial N_1}{\partial x}\right)_{x=0} \tag{3}$$

The second diffusion equation

$$\frac{\partial N_1}{\partial t} = D \frac{\partial^2 N_1}{\partial x^2}$$

becomes, for a steady state condition when $\partial N_1/\partial t = 0$,

$$\frac{\partial^2 N_1}{\partial x^2} = 0$$

The solution of this subject to the boundary condition given by Eqn. 3 together with $N_1 = N_{10}$ at $x = 0$, is

$$N_1 = N_{10} - \frac{\nu}{D} \frac{N_{20}}{N_0} x \tag{4}$$

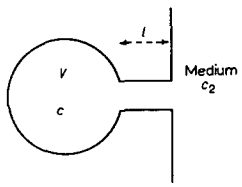

Fig. 3. Attached vesicle. The symbols are explained in the text.

Similarly, for vesicles of Type 2, the number attaching per sec per unit area at $x = 0$ is $\nu N_{20}/N_0$

$$\therefore \nu \frac{N_{20}}{N_0} = D \left(\frac{\partial N_2}{\partial x}\right)_{x=0}$$

and

$$N_2 = N_{20} + \frac{\nu}{D} \frac{N_{20}}{N_0} x \tag{5}$$

From Eqns. 4 and 5 for all x, $N_1 + N_2 = N_{10} + N_{20} = N_0$ so that the concentration of vesicles is constant throughout the volume and equal to N_0.

If we now assume that $\nu = n/\tau$ is the same at both surfaces the symmetrical distributions of Fig. 2 result with $N_{10} = N_{2L}$ and $N_{20} = N_{1L}$. Then from Eqn. 4 together with $N_{10} + N_{20} = N_0$

$$N_0 = N_{20} \left(2 + \frac{\nu L}{D N_0}\right) \tag{6}$$

The volume v of Medium 1 removed per sec per unit area from A and transported across the cell is, from Eqn. 2,

$$v = \nu \frac{N_{20}}{N_0} V$$

where V is the interior volume of a vesicle. Hence from Eqn. 6

$$v = \frac{\nu V}{2 + \frac{\nu L}{D N_0}} \tag{7}$$

or, using Eqn. 1

$$v = \frac{nV}{2\tau + \frac{nL}{DN_0}} \tag{8}$$

The mass of any particular component, of concentrations c_1 and c_2 on either side of the cell, transported per sec per unit area is

$$M = v(c_1 - c_2)$$

i. e.

$$M = \frac{nV\,(c_1 - c_2)}{2\tau + \frac{nL}{DN_0}} \tag{9}$$

Evidently if $2\tau >> nL/DN_0$ then $v = \frac{1}{2}\,(n/\tau)\,V$, a result which is readily obtained by assuming complete mixing of the vesicles in the cytoplasm, a condition approached as $D \to \infty$. The other extreme case occurs when $2\tau << nL/DN_0$, *i.e.* when the time of attachment is negligible and the rate of transport is governed entirely by the rate of diffusion of the vesicles. Under these conditions when D is small enough

$$v = D\frac{N_0}{L}V$$

THE MEAN TIME OF ATTACHMENT τ

Of the quantities appearing in Eqn. 8, n, N_0, L and V have been measured by CASLEY-SMITH[6] whose values for blood capillarly endothelium will be used in the following calculations. The normal diffusion coefficient (as distinct from the self-diffusion coefficient) can be calculated, provisionally, from a well known formula valid for very low concentrations of spherical particles:

$$D = \frac{kT}{6\pi\eta r}$$

where k is Boltzmann's constant, T is the absolute temperature, η is the viscosity of the solvent, and r is the particle radius.

The mean time of attachment τ has not yet been found either experimentally or by an independent theoretical derivation, and at present we can do no more than find its value using Eqn. 8 and the data for v given by RENKIN[3], together with CASLEY-SMITH's[6] modified measurements.

The experimental results from which v may be inferred are measurements of P, the volume transported per sec for a fixed weight of tissue not specifically stated. RENKIN[3] estimates this as about 50–100 g with a preference for the lower figure, and also gives some data from which the effective area across which transport occurred could be calculated. Assuming a weight of tissue of 50 g for which $P = 1.5 \cdot 10^{-4}$ cm$^3 \cdot$ sec^{-1} corresponding to particles of molecular weight in excess of 60000 which we assume to be transported by vesicles only, and allowing for a 30% obstruction of the diffusion cross-section by the cell nuclei, we find $v = 8.6 \cdot 10^{-4}\,\mu^3 \cdot \text{sec}^{-1} \cdot \mu^{-2}$. This figure might well be in error by a factor of 2 or 3.

In the calculation of D the value of η the viscosity of the cytoplasm presents some uncertainty. From values tabulated by SHEA AND KARNOVSKY[5] a reasonable choice seems to be $\eta = 0.5$ P. It appears that the viscosity may be higher near the cell boundaries, as also is the density of vesicles, but for the present we assume uniformity across the cell. With $\eta = 0.5$ P and CASLEY-SMITH's[6] modified mean maximum and minimum vesicle diameters of 79.5 and 61.1 nm. $D = 1.13 \cdot 10^{-9}$ or $1.48 \cdot 10^{-9}$ cm^2·sec^{-1}. In view of the doubt about the viscosity we take $D = 1.3 \cdot 10^{-9}$cm^2·sec^{-1}.

According to CASLEY-SMITH[6], after allowing for tissue shrinkage, $n = 125/\mu m^2$, $N_0 = 730/\mu m^3$, $L = 0.261$ μm, $V = 8.5 \cdot 10^4$ nm^3. From these figures $nL/DN_0 = 0.34$. Then from Eqn. 8 $\tau = 6.0$ sec.

Under these conditions $nL/DN_0 << 2\tau$ and the rate of transport is determined almost entirely by τ. However, apart from uncertainty about the cytoplasmic viscosity, it is certain that D is overestimated by the formula used because the vesicles occupy some 30% of the cytoplasmic volume[6] so that the concentration of diffusing particles is in fact very high. There appears to be no theory of self-diffusion in concentrated solutions but the experiments of IRANI AND ADAMSON[7] on sucrose solutions give a qualitative indication of the likely behaviour of vesicles in high concentrations. IRANI AND ADAMSON[7] show that as the concentration tends to zero the self-diffusion coefficient approaches the normal coefficient, but with increasing concentration both the normal and self-diffusion coefficients of sucrose decrease, the former by a factor of about 5 when the concentration reaches 3 M. The self-diffusion coefficient falls off to a less extent. It appears therefore that, even if the value of $\eta = 0.5$ P is realistic, D should be considerably less than the figure given above. Even so if one decreases D by a factor of 10, a plausible figure, τ is modified only from 6.0 to 4.5 sec; a factor of 30 reduces τ to 1.0 sec.

It remains to show that a time of attachment of this order is sufficient to allow the contents of the vesicles to equilibrate with the external media.

Consider a vesicle of interior volume V connected to the medium containing a component in concentration c_2 by a cylindrical neck of length l and cross-sectional area A (Fig. 3).

Suppose that at time t the concentration in the vesicle is c, and that initially at $t = 0$ it was c_1. For simplicity, since we are concerned only with orders of magnitude, assume a linear concentration gradient $(c-c_2)/l$ along the neck. The rate of flow of material along the neck $= \Delta\,[(c-c_2)/l]\,A$ where Δ is the diffusion coefficient for the dissolved substance. Since the rate of loss of material from the vesicle is $-\mathrm{d}(cV)/\mathrm{d}t$ it follows that

$$\frac{\mathrm{d}c}{\mathrm{d}t} + \frac{\Delta A}{Vl}(c - c_2) = 0$$

The solution of this, subject to the initial conditions, is

$$c - c_2 = (c_1 - c_2) \exp\left(-\frac{\Delta A}{Vl} t\right)$$

Thus in a time such that $(\Delta A/Vl)\, t \approx 10$ the vesicle contents have effectively equilibrated with the outside medium.

According to CASLEY-SMITH[6] $l = 30$ nm, and the mean inner diameter of the neck is 15·1–18·1 nm for blood capillary cells. For substances whose molecules could

pass freely along the neck $\Delta \approx 10^{-7}\text{cm}^2 \cdot \text{sec}^{1-}$, or greater, and with these figures $\Delta A/Vl \approx 10^3$.

Thus even for very large molecules in solution equilibrium will be established in about 10^{-2} sec.

DISCUSSION

It appears that the proposed mechanism of vesicle transport accounts satisfactorily for the transfer of large molecules across the endothelium if the average time of attachment of the vesicles to the plasma membrane is of the order of several seconds with an upper limit of about 6 sec. In making this latter estimate the greatest sources of error seem to be in the experimental determination of v, the volume transported, and in the estimated viscosity of the cytoplasm. Apart from the need for more accurate measurements of these quantities, it may be suggested that some experimental studies of self-diffusion in concentrated solutions of large particles (for example polystyrene latex particles) would be valuable. In the meantime the physical problem of the attachment of vesicles to, and their rate of detachment from, the plasma membrane awaits a solution.

Finally it may be remarked that discussion of the problem in terms of Brownian movement is also valid only for very low concentrations of vesicles, and for the real situation an account in terms of diffusion appears more promising if coupled with relevant experimental studies of diffusion constants.

ACKNOWLEDGEMENT

I am indebted to Dr. J. E. Casley-Smith for bringing this problem to my attention and allowing me to use his experimental results before publication.

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