BBA Report

BBA 71157

A simple method of determining relative permeabilities of liposomes to non-electrolytes

MARTYN W. HILL and B. ELEAZAR COHEN

A.R C. Institute of Animal Physiology, Babraham, Cambridge (Great Britain)

(Received September 11th, 1972)

SUMMARY

1. An osmotic method of measuring the permeability ω of liposomes is derived theoretically.

2. The equations obtained are tested experimentally and good agreement found.

3. It is therefore shown that the maximum slope of the absorbance time curve after the minimum volume is a good measure of the permeability under certain conditions.

The permeabilities of liposomes to a large range of non-electrolytes is much too large to be measured conveniently at equilibrium by isotopic techniques. It has been shown that a smectic mesophase of phospholipid (liposomes[1]) swell and shrink under the action of osmotically induced forces, and that the total volume occupied by the liposomes is proportional to the reciprocal of the optical extinction coefficient at 450 nm (ref. 2). This means that volume changes, induced osmotically, can be observed by light scattering at 450 nm and a measure of the permeability obtained.

De Gier *et al.*[3], using liposomes made up in 50 mM KCl, used the initial swelling rates as a measure of solute permeability when they were diluted into isotonic non-electrolyte outside. The slope obtained in this case will, however, depend on both σ, the reflection coefficient and ω, the permeability coefficient, for both the osmotic pressure and the permeability will determine the initial rates. There is no reason why σ and ω should have the same temperature dependence and hence the activation energies quoted will inevitably be some amalgam of that for σ and ω.

Various osmotic techniques have been used with both liposomes and natural cells to measure permeability and related parameters. Goldstein and Solomon[4], using red cells, measured σ, the reflection coefficient, by observing the concentration of permeant that balances the osmotic force of an impermeant at zero volume flow. Sha'afi *et al.*[5] use a

minimum volume technique with the aid of a computer to determine the permeability of red cells. Jacobs[6] used a similar technique on cells and presented the original analysis of the system. Jacobs' analysis was later shown to be incomplete in terms of the irreversible thermodynamics of Kedem and Katchalsky[7] and here we will use the latters' equations as a starting point of our analysis.

The following two equations are for the volume J_v and solute $\dot{n}_s$ fluxes, using their notation

$$J_v = \frac{dV_a}{dt} = Lp\,(\Delta p - \Delta\pi_i) - \sigma Lp\,(RT\,\Delta C_s + \phi\Delta\pi_i) \tag{1}$$

$$\dot{n}_s = \frac{dn_s}{dt} = J_v\,(1-\sigma)\,\bar{c}_s + \omega\,(RT\,\Delta C_s + \phi\Delta\pi_i) \tag{2}$$

where $\bar{c}_s = \dfrac{\Delta C_s}{\Delta \ln C_s}$; $\sigma = -\left(\dfrac{\Delta\pi_i}{\Delta\pi_s}\right)_{J_v=0}$

$$\omega = \left(\frac{J_s}{\Delta\pi_s}\right)_{J_v=0} \qquad Lp = \left(\frac{J_v}{\Delta\pi_i}\right)_{\Delta p=0}$$

In the present case we identify n_s as the number of permeable solute molecules inside the aqueous compartment of the liposome of sequestered volume V_a, and use the conditions, that $\Delta p = 0, \phi\Delta\pi_i << RT\Delta C_s$. If also we consider that $(n_s/V_a) << C_s$ where C_s is the outside concentration of the permeable species and C_i of the impermeable species, both being sensibly constant as V_a is much smaller than the outside volume, the first term in Eqn 2 can be neglected,

$$\text{then } J_v = -LpRT\,(\Delta C_i + \sigma\Delta C_s) \tag{3}$$

$$\text{and } \frac{dn_s}{dt} = A\omega RT\left(C_s - \frac{n_s}{V_a}\right) \tag{4}$$

where A is area of the liposomes.

The condition for maximum J_v or linear volume change is $d^2V_a/dt^2 = 0$, that is, differentiating Eqn 3

$$\frac{d\Delta C_i}{dt} = -\sigma\frac{d\Delta C_s}{dt}$$

or

$$\frac{dV_a}{dt} = \sigma\frac{dn_s}{dt}\left(\frac{n_i}{V_a} + \sigma\frac{n_s}{V_a}\right)^{-1} \tag{5}$$

We can arrange things such that the liposomes first shrink under an induced osmotic force, pass through a minimum volume and then swell as permeant on the outside diffuses in under a concentration gradient, followed by its concomitant water. The rate of swelling passes through a maximum, and then decreases as the system approaches its final equilibrium volume. The system will be never far from a stationary state after the minimum volume, that is

$$\left(\frac{n_i}{V}+\frac{\sigma n_s}{V}\right)\sim C_i+\sigma C_s$$

and using Eqns 4 and 5

$$\frac{dV_a}{dt}=A\omega RT\sigma\left(C_s-\frac{n_s}{V_a}\right)(C_i+\sigma C_s)^{-1} \tag{6}$$

$$\text{or}\ \frac{dV_a}{dt}=A\omega RT\left(1+\frac{C_i}{\sigma C_s}\right)^{-1}\left(1-\frac{n_s}{C_sV_a}\right) \tag{6a}$$

To test Eqn 6 it was rearranged so

$$1/dV_a/dt=\left(\frac{C_i}{C_s}.\frac{1}{A\omega\sigma RT}+\frac{1}{A\omega RT}\right)\left(1-\frac{n_s}{V_aC_s}\right)^{-1} \tag{6b}$$

and experimental values proportional to $1/dV_a/dt$ for different C_i/C_s were found, and plotted to test for linearity.

Egg phosphatidylcholine, phosphatidic acid (96:4) liposomes[1] were made up in 20 mM KCl and 0.18 ml of this rapidly added to 2.5 ml of various hypertonic solutions of KCl and non-electrolytes mixed in the following concentration ratios (mM): 0:100, 5:100, 10:100, 15:100, 20:100, 20:60 and 20:40. The final lipid concentration was 0.5 mM. The time courses of the optical changes were monitored graphically and by digital print out of the voltage analogue ψ of the absorbance at 450 nm at 1-s time intervals, or slower. A second difference of zero, between readings, was used as a criterion of linearity of the optical changes after the minimum volume, that is $d\psi/dt$ = constant and the slope taken in that range. dV_a/dt is proportional to $d\psi/dt$ as the changes in absorbance are small. Values of the inverse of the slope of the linear voltage–time curve were plotted against the ratio C_i/C_s. Figs 1a and 1b show examples of three non-electrolytes, urea, malonamide and erythritol, at 15 and 30 °C. The linearity of these plots indicate that the term n_s/V_aC_s in Eqn 6b is negligible. It also shows that, in this case, the intercept at $C_i/C_s = 0$ is proportional to ω, the permeability coefficient and the slope divided by the intercept is a measure of σ.

Table I gives the details of the results with permeabilities expressed relative to erythritol at both temperatures; separate batches of liposomes of different unknown areas were used for each temperature so no direct comparison of ω is possible. No other data are available for σ for liposomes but the values mirror the permeability in a meaningful way.

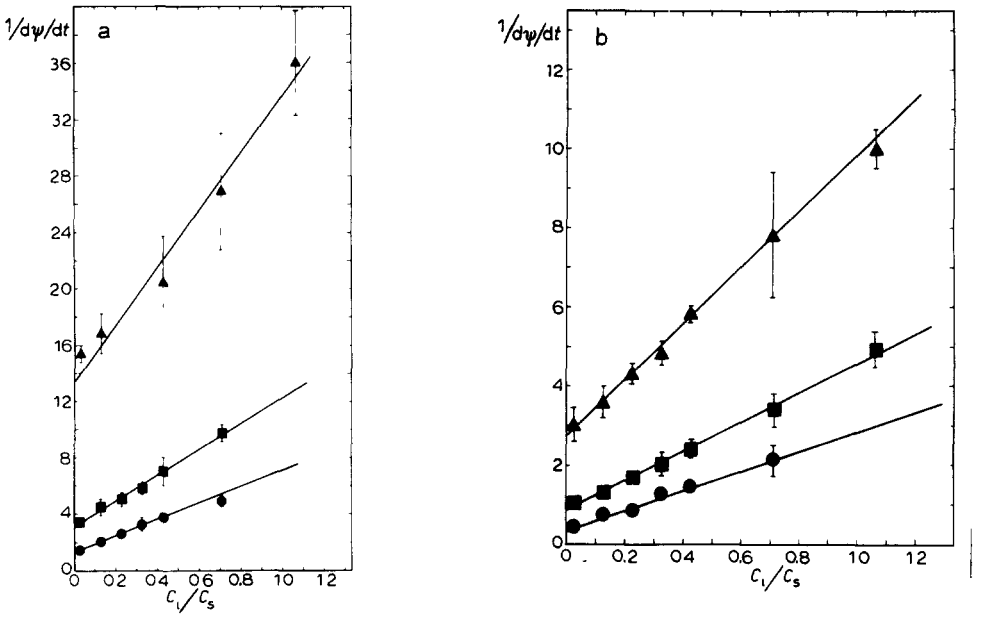

Fig. 1. The reciprocal of the maximum voltage–time slope after the minimum volume plotted as a function of the ratio of the outside concentration of impermeant to permeant. (a) at 15 °C and (b) at 30 °C. ▲——▲, erythritol; ■——■, malonamide; ●——●, urea. The points represent the average of four determinations, error bars denote range of uncertainty in measuring the slope. Note that the concentration of impermeant species C_i is equal to twice the KCl concentration.

TABLE I

REFLECTION COEFFICIENT σ AND RELATIVE PERMEABILITY TO ERYTHRITOL

Reflection coefficient σ and relative permeability to erythritol were calculated from Fig. 1 for experiments at 15 and 30 °C. Values are quoted ± their S.D.

Solute	*Liposomes Batch 1 (15 °C)*		*Liposomes Batch 2 (30 °C)*	
	ω/ω (erythritol)	*σ*	*ω/ω (erythritol)*	*σ*
Urea	9.5 ± 0.6	0.23 ± 0.04	7.6 ± 1.2	0.14 ± 0.03
Malonamide	4.0 ± 0.3	0.34 ± 0.06	3.2 ± 0.3	0.23 ± 0.04
Erythritol	1.0 ± 0.1	0.66 ± 0.2	1.0 ± 0.1	0.38 ± 0.06

We can see from Eqn 6a that if $n_s/V_a \ll C_s$, then dV_a/dt is linear. Also, if $n_s/V_a \ll C_s$ and $C_i/\sigma C_s \ll 1$ then,

$$\frac{dV_a}{dt} = A\omega RT$$

indicating that in this case the linear part of the volume changes after the minimum volume is a measure of the permeability coefficient ω.

Using small values of C_1/C_s, Cohen and Bangham[8] have used this method to determine the relative permeabilities of liposomes to a set of non-electrolytes whose olive oil/water partition coefficients range from $1 \cdot 10^{-2}$ to $1 \cdot 10^{-5}$. For solutes with higher partition coefficients, for example, the alcohols, the main condition of the analysis that $n_s/V << C_s$ holds during the time after the minimum volume will not necessarily be true and so there will not be a linear slope after the minimum volume.

B.E.C. acknowledges financial support from Universidad Central de Venezuela during the course of the present investigation.

REFERENCES

1 Bangham, A.D. (1968) *Prog. Biophys. Mol. Biol.* 18, 29–95
2 Bangham, A.D., de Gier, J. and Greville, G.D. (1967) *Chem Phys. Lipids* 1, 225–246
3 De Gier, J., Mandersloot, J.G., Hupkes, J.V., McElhaney, R.N. and Van Beek, W.P. (1971) *Biochim. Biophys. Acta* 233, 610–618
4 Goldstein, D.A. and Solomon, A.K. (1961) *J. Gen. Physiol.* 44, 1–17
5 Sha'afi, R.I., Rich, G.T., Mikulecky, D.C. and Solomon, A.K. (1970) *J. Gen Physiol.* 55, 427–450
6 Jacobs, M.H. (1933) *J. Cell. Comp. Physiol.* 2, 427–444
7 Kedem, O. and Katchalsky, A. (1958) *Biochim. Biophys. Acta* 27, 229–246
8 Cohen, B.E. and Bangham, A.D. (1972) *Nature* 236, 173–174