MATHEMATICAL DESCRIPTION OF NONEQUILIBRIUM PASSIVE TRANSPORT IN BIOSYSTEMS

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The time course of passive transport of low-molecular mass substances in a nonequilibrium system of alternating aqueous and lipidic phases was expressed as function of physico-chemical properties of the biosystem (phase volumes, interface area, diffusion layer thickness) and of the transported compounds (diffusion coefficients, partition coefficients).

A detailed study of transport of low-molecular mass substances in biosystems is important for the understanding of processes in living cells[1]. Informations about transfer and distribution of these substances are also of significance for the study of biological effects of xenobiotics. The time-dependent concentration of a xenobiotic in individual compartments of the biosystem is one of the factors determining the place, mode, and intensity of the effect. The transfer of xenobiotics to effective sites is mostly considered as passive transport through lipidic regions of membranes, which may be repeated several times[2-11]. Imperfect knowledge of the dependence of this process on the physico-chemical parameters of both the biosystem and transported substances is an obstacle in developing xenobiotics of optimum biological efficiency[4]. The known methods describing quantitative structure-activity relations[3-6] do not take the time effects into account. Introducing the time into the corresponding equations seems therefore desirable.

The aim of the present work was to derive mathematical equations making it possible to calculate the nonequilibrium concentration of a low-molecular mass substance distributed in any compartment of a biosystem by passive transport as function of the physico-chemical parameters of both the biosystem and transported substance.

Formulation of the Problem

In view of the passive transport of xenobiotics, a biosystem is composed of alternating aqueous and lipidic phases[2-4,7-11], whose interfaces are, owing to mutual interaction, more ordered than the bulks[12-14]. To describe the concentration distribution in such a system, it is necessary to use the second law of Fick for every phase with res-

pect to its inhomogeneity and to consider the different solubility of the considered substances in the aqueous and lipidic phases. An exact solution would be too complicated. Therefore, simplifying assumption must be introduced.

Diffusion proceeds much more rapidly in the bulk than in the diffusion layers. The diffusion coefficients of substances with a molar mass up to 500 g/mol are of the order of 10^{-9} m^2 s^{-1} in most liquids[15], and the "compartments" of real biosystems have an effective thickness (ratio of volume to area through which transport takes place) at most 10^{-6} m. Under these conditions, the bulk concentration attains equilibrium after less than 0·1 s, *i.e.* practically instantaneously.

Transfer through interface with diffusion layers is hence the only rate-determining step. It can be characterized by transport parameters l_1 and l_2 (from water to lipid and *vice versa*) if the concentration gradients in the diffusion layers are linear and the values of l_1 and l_2 (Eqs 2*a*,*b*) are independent of the concentration. The same assumptions have been used in the theory of passive transport [4,7-11].

Nonequilibrium passive transport in N-compartment system composed of equal aqueous and equal lipid phases (Fig. 1) can under the above assumptions be described by a system of linear differential equations of the first order, which can be written in the matrix form

$$-\dot{\mathbf{c}} = \mathbf{B}\mathbf{c}\,, \tag{1}$$

where $\mathbf{c}$ and $\dot{\mathbf{c}}$ are column vectors of concentrations and their time derivatives in the compartments. Matrix $\mathbf{B}$ (N × N) has elements $b_{i,i} = A_i l_x / V_i$ ($i = 1, 2, \ldots, N$, V_i is the volume of i-th phase, and A_i is the contact area between i-th and $i + 1$st compartment; $A_N = 0$; $x = 1$ for i odd, $x = 2$ for i even) and $b_{i+1,i} = -A_i l_x / V_{i+1}$ ($i = 1, 2, \ldots, N - 1$). The system (*1*) was solved by standard methods[16,17].

The transport parameters are given as[18,19]

$$l_1 = \alpha P/(\beta P + 1)\,, \tag{2a}$$

$$l_2 = \alpha/(\beta P + 1)\,, \tag{2b}$$

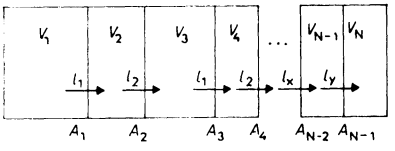


FIG. 1

Scheme of N-compartment system consisting of aqueous (odd indices) and lipid phases (even indices) of equal quality. V phase volume, A contact area, l_1 and l_2 transport parameters from water to lipid and *vice versa*, respectively ($x = 2$, $y = 1$ for N even; $x = 1$, $y = 2$ for N odd)

where $\alpha = D_L/h_L$, $\beta = D_L h_A/D_A h_L$, D is the diffusion coefficient in the aqueous and lipidic diffusion layers (subscript A and L) with an effective thickness h, and $P = l_1/l_2$ is the partition coefficient. In model systems organic solvent–water, α and β do not depend on the structure of transported substances[13,20–23] even in cases where these substances are not members of a homologue series and are partially or completely ionized or form ion pairs[14]. The quantities α and β thus become properties of the model system characterizing the thickness and properties of the diffusion layers on both sides of the interface. These constants together with the volumes of the phases and their contact areas represent the properties of the biosystem determining the time course of the distribution. Thus, the passive transport of substances in a biosystem is fully characterized by their partition coefficient.

RESULTS

Under the conditions considered, the passive transport is independent of the form of the interface, but depends on the "reciprocal thickness" $d_i = A_i/V_i$ of the compartments. If all the d_i values for a biosystem are different from one another, the dependence of the concentration c_i in i-th compartment on time can be obtained by solving the system of equation (*1*) (ref.[16]). For the aqueous phase (i odd) if at the beginning of the distribution, the substance considered is present only in the first compartment in a quantity of n mol:

$$c_i = (n/V_i)\, P^{-1} \prod_{j=1}^{i-1} d_j \Big(\sum_{m=1}^{y} a_1 e^{-b_1 t} + \sum_{m=1}^{x} a_2 e^{-b_2 t} \Big), \tag{3}$$

where

$$a_1 = P \prod_{n=1}^{x} (d_{2n} - P d_{2m-1})^{-1} \prod_{\substack{n=1 \\ n \neq m}}^{y} (d_{2n-1} - d_{2m-1})^{-1},$$

$$a_2 = \prod_{\substack{n=1 \\ n \neq m}}^{x} (d_{2n} - d_{2m})^{-1} \prod_{n=1}^{y} (d_{2n-1} - P^{-1} d_{2m})^{-1},$$

$$b_1 = l_1 d_{2m-1}, \quad b_2 = l_2 d_{2m}, \quad x = (i-1)/2, \quad y = (i+1)/2.$$

For lipid compartments (i even), we have

$$c_i = (n/V_i) \prod_{j=1}^{i-1} d_j \sum_{m=1}^{i/2} (a_1 e^{-b_1 t} + a_2 e^{-b_2 t}), \tag{4}$$

where a_1, a_2, b_1, and b_2 are the same as in Eq. (*3*) except that $x = y = i/2$.

Equations (*3*) and (*4*) cease to be valid if at least two of the exponents b_1 and b_2 are the same. Since, in general, $l_1 \neq l_2$, this case occurs when at least two aqueous or lipid phases have the same reciprocal thickness d. The lipidic region of biological membranes is formed by a double layer consisting mainly of phospholipids and cholesterol, so that the equality of the reciprocal thicknesses of the lipidic compartments may often be fulfilled.

In a special case, where for the aqueous phases $d_1 = d_3 = \ldots$ and for the lipid compartments $d_2 = d_4 = \ldots$, the solution[17] of the transport equations (*1*) has the following form, provided that the substance (n mol) is at time $t = 0$ present only in the first compartment. For aqueous compartments (i odd), with $x = (i - 1)/2$

$$c_i = (n/V_1)(Pd_1d_2)^x (d_2 - Pd_1)^{1-i} \left(e^{-l_1 d_1 t} \sum_{j=1}^{x+1} a_{2j-1,i} t^{j-1} + \right.$$

$$\left. + e^{-l_2 d_2 t} \sum_{j=1}^{x} a_{2j,i} t^{j-1}\right), \tag{5}$$

where the coefficients $a_{i,k}$ are given by the recurrent formulae

$$a_{1,i} = -a_{2,i}$$

$$a_{2j,i} = -\sum_{m=j}^{x} \left[a_{2m,i-1} \prod_{n=j}^{m-1} n(l_2d_2 - l_1d_1)^{-1}\right]$$

$$a_{2j-1,i} = (j-1)^{-1}(l_2d_2 - l_1d_1)\, a_{2j-3,i-1} \quad \text{for} \quad j > 1 .$$

For lipid compartments (i even)

$$c_i = (n/V_2)(Pd_1)^{i/2} d_2^{i/2-1} (d_2 - Pd_1)^{1-i} \left(e^{-l_1 d_1 t} \sum_{j=1}^{i/2} a_{2j-1,i} t^{j-1} + \right.$$

$$\left. + e^{-l_2 d_2 t} \sum_{j=1}^{i/2} a_{2j,i} t^{j-1}\right), \tag{6}$$

where

$$a_{2j-1,i} = \sum_{m=j}^{i/2} \left[a_{2m-1,i-1} \prod_{n=j}^{m-1} (-n)(l_2d_2 - l_1d_1)^{-1}\right]$$

$$a_{2,i} = -a_{1,i}$$

$$a_{2j,i} = (j-1)^{-1}(l_2d_2 - l_1d_1)\, a_{2j-2,i-1} \quad \text{for} \quad j > 1 .$$

Starting from the solution of Eqs (*1*) for the first aqueous phase

$$c_1 = (n/V_1)\, e^{-l_1 d_1 t}, \tag{7}$$

we can according to Eqs (5) and (6) determine in turn the concentration in any compartment as function of time. The terms $a_{i,k}$ for the first eight phases of an N-compartment model (N > 8) are given in Table I.

By convention, we set $\prod_{j=m}^{p} = 1$ and $\sum_{j=m}^{p} = 0$ for $p < m$. For the last compartment ($i = N$) we have $A_N = 0$ and the expression for c_N involves therefore only N − 1 exponential terms.

The expression (7) for the time dependence of the concentration in the first compartment is relatively simple. If l_1 is expressed from Eq. (2), by analysis of experimental dependences of c_1 on P for a real system the values of α and β for its first interface can be found. However, such an experiment has not been carried out as yet.

Basic informations about nonequilibrium passive transport can nevertheless be obtained from the quantities α and β measured on model interfaces. In Fig. 2 are shown the concentrations of a homologue series of N-(2-benziloyloxyethyl)-N,N-dimethylalkylammonium bromides in the first eight phases of an N-compartment system n-octanol/water after 1 h of distribution as functions of the partition coefficient P of these compounds, calculated from Eqs (5)–(7). The transport rate constants are functions of P according to Eqs (2*a*,*b*) and the constants are $\alpha = 0{\cdot}480$ V/A . . dm h^{-1}, $\beta = 0{\cdot}286$ (ref.[23]), $A = 0{\cdot}98$ dm^2, $V = 0{\cdot}5$ dm^3 (ref.[21]). In aqueous phases (odd numbers), the dependences are symmetrical, consisting of two linear branches with slopes $\pm(i - 1)/2$). For lipid compartments (even numbers), we obtain asym-

TABLE I

Coefficients a_{ij} in Eqs (5) and (6) for *j*-th phase of N-compartment system (N > 8); d_1 reciprocal thickness of aqueous phases, d_2 reciprocal thickness of lipid phases, l_1 and l_2 transport parameters in water–lipid direction and *vice versa*, respectively

	i							
j	1	2	3	4	5	6	7	8
			$l_2d_2 - l_1d_1$		$(l_2d_2 - l_1d_1)^2$		$(l_2d_2 - l_1d_1)^3$	
1	1	0	0	0	0	0	0	0
2	1	−1	0	0	0	0	0	0
3	− 1	1	1	0	0	0	0	0
4	− 2	2	1	1	0	0	0	0
5	3	−3	−2	−1	1/2	0	0	0
6	6	−6	−3	−3	1/2	−1/2	0	0
7	−10	10	6	4	−3/2	1/2	1/6	0
8	−20	20	10	10	−2	2	1/6	1/6

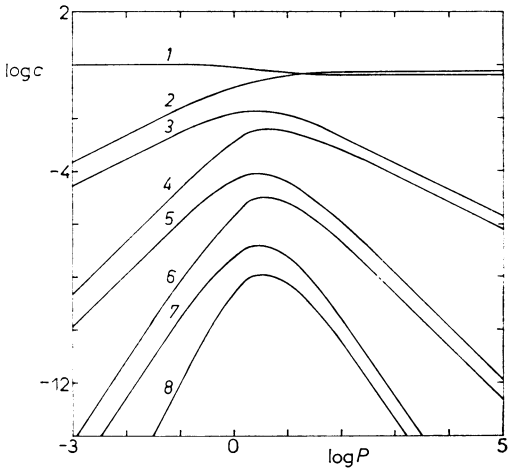
metric curves with integer slopes of their linear branches, $i/2$ and $(2 - i)/2$. Their form is independent of the time, they are only shifted with the time along the y axis ($\log c$). These findings are in full agreement with published data obtained by numerical methods under the same conditions[4,24-30].

The selectivity of the membrane system increases with the number of membranes, *i.e.* the substances must have a narrower lipophility interval to attain in a given compartment and time a concentration comparable with that of the "fastest" substance (with an optimum lipophility). According to Fig. 2, one tenth of the maximum concentration is attained by substances for which the values of $\log P$ are around 3·25, 2·55, 2·05, 1·70, 1·58, and 1·48 for the 3rd, 4th, 5th, 6th, 7th, and 8th compartment, respectively. Thus, the lamelar system lipid–water can at nonequilibrium conditions serve to separate substances according to their lipophility.

Curves analogous to those in Fig. 2 are obtained for the mentioned values of α and β in Eq. (*2*) also from Eqs (*3*) and (*4*) for systems with various phase thicknesses.

DISCUSSION

The above description of passive transport in a state far from equilibrium is especially suitable for xenobiotics, since it takes the accumulation of lipophilic substances in the membranes into account. In describing quantitative relations between physico-chemical properties and biological activity of substances, it is common practice to determine the biological activity after a constant time of action. The dependence of the concentration of a substance at a receptor on its partition coefficient in bilogarithmic coordinates is approximated by a parabola[3] or by bilinear curve[4,7,24,25]. Time is not involved in the equations, although its importance is obvious[4].



FIG, 2

Dependence of concentration c in phases 1—8 (curve numbers) for an N-compartment model (N > 8) on the partition coefficient P after 1 h of distribution. Curves calculated from Eqs (*5*), (*6*) and (*2*); $\alpha = 0{\cdot}245$ dm/h, $\beta = 0{\cdot}286$ (ref.[23]) $d_1 = d_2 = 1$ dm^{-1}.

Combination of Eqs (3)–(7) with the classical Hansch equation[3] makes it possible to describe the biological activity of a series of substances as function of their physico-chemical properties and time. The assumptions involved in the derivation of this equation and Eqs (3)–(7), *i.e.* negligible inactivation of the substances and their bondage to macromolecules except for the receptors, nonequilibrium distribution, instantaneous distribution in the bulks of the compartments, parameters in Eq. (2) independent of concentration, and biosystem composed of membranes of equal quality, must be satisfied.

The partition coefficient P in Eqs (3)–(7) refers to the membrane–water system. In practice, for the sake of simplicity of the determination, the reference system n-octanol–water is used. The validity of the Collander equation[31] is assumed for both systems. Accordingly, P in Eqs (3)–(7) must be replaced with aP^b, where a and b are constants.

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