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KINETICS OF UNIDIRECTIONAL TRANSPORT IN MULTIMEMBRANE SYSTEMS AS INFLUENCED BY BINDING TO MACROMOLECULES

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The distribution of externally added low-molecular-weight solutes in systems comprising alternating membraneous and aqueous compartments with binding capacity is described for special conditions of unidirectional transport and linear (i.e., far from saturation) binding. The kinetics of the process valid for a certain time period is expressed as the sum of exponential functions of time, their number being equal to the number of compartments investigated. The coefficients of the equation involve the rate and equilibrium parameters of the process as well as the volumes and the connecting areas of individual compartments. The degenerated case resulting from the cellular structure of biosystems is also considered. The description is shown to agree well with the results of drug potency testing, bioactivity being used to monitor concentration of an effector in the receptor region.

1. Introduction

The disposition of externally added low-molecular-weight substances in individual regions of biosystems is determined by the extent and relative rates of (a) transport through membranes, (b) binding to cell components and (c) elimination (i.e., metabolism, excretion, etc.). The last process is usually much slower than the former two steps and, hence, its influence can be neglected for certain time periods.

The problem of distribution of exogenous substances has so far been treated namely by drug designers, passive transport [1–5] and elimination [2,6,7], but in no case binding to cell components, being considered as the main processes responsible for the drug distribution. In those studies, transport has been treated as a reversible process and the resulting differential equations have been solved numerically. Unidirectional transport, however, could well represent the real situation for certain time intervals. The period of validity is proportional to the number of compartments (i.e., volumes with physicochemical properties different from their surroundings, e.g., cytoplasmic membrane, intracellular aqueous phases, endoplasmic reticulum). The non-covalent binding of solutes to cell components, preferably to proteins, is very fast [8] as compared with transport, and can be treated as instantaneous for simplicity. Under these conditions the differential equations describing transport kinetics combined with protein binding are completely integrable. The explicit solutions expressing the time course of the free solute concentration in any compartment of the biosystem, within certain time intervals, are presented here. The cellular structure of biosystems (i.e., periodic alteration of compartments with identical properties) is also taken into account.

2. Model and its description

Concerning the distribution of small molecules, the two-phase structure (alternating aqueous and

lipidic phases) of biosystems is of extraordinary importance. An exact mathematical description of the distribution in such a system requires: (a) the use of Fick's second law for each phase with respect to its inhomogeneity as the diffusion layers are more ordered than the bulk phases [9] (i.e., diffusivity is not constant within a phase); (b) taking into account the different solvation of substances in the aqueous and lipidic phases (i.e., concentration gradients at the interfaces are discontinuous). To simplify the description, the plausible assumption of practically instantaneous diffusion within the bulks of the compartments is introduced as the concentration of substances with a molar mass up to 500 g mol^{-1} (molar diffusivity of the order of 10^{-9} m^2 s^{-1} in most liquids [10]) equilibrates in the compartments of real biosystems (their ratio of volume to surface area being at most 10^{-6} m) within 0.1 s or less. Transport through interfaces with diffusion layers is then the only rate-limiting step and can be characterised by the rate parameters l_1 and l_2 (from water to membrane and vice versa).

Binding of drugs to macromolecules is often linear (i.e., far from saturation) [11] and practically instantaneous [8] under conditions of bioactivity testing. Then the concentration of the free solute in the i-th compartment (c_i) is described by:

$$c_i = c_{it}/(1 + K_i p_i) \quad (1)$$

where c_{it} is the total solute concentration in the i-th compartment, p_i the concentration of the binding sites providing that it is possible to group together all the classes of binding sites in the i-th compartment and to describe their binding abilities by the global association constant K_i.

The solute distribution in the N-compartment system composed of alternating aqueous and lipidic phases (fig. 1) is, under the above conditions, described by a set of first-order linear differential equations written in matrix notation as:

$$-\dot{\boldsymbol{c}} = \boldsymbol{B}\boldsymbol{c} \quad (2)$$

where $\boldsymbol{c}$ and $\dot{\boldsymbol{c}}$ are the N-dimensional column vectors of concentrations in the individual compartments and their time derivatives, respectively, the matrix $\boldsymbol{B}(N \times N)$ has elements: $b_{i,i} = A_i l_x / V_i(1 + K_i p_i)$ $(i = 1, 2, \ldots N)$; V_i is the volume of the i-th phase, A_i the contact area between the i-th and $(i+1)$-th compartment; $A_N = 0$; the value of x is specified in table 1 and $b_{i+1,i} = -A_i l_x / V_{i+1}$ $(1 + K_{i+1} p_{i+1})$ $(i = 1, 2, \ldots N-1)$. At time $t = 0$ the solute (n mol) is present only in the first compartment. The set of differential equations (eq. 2) was solved by standard methods [12,13].

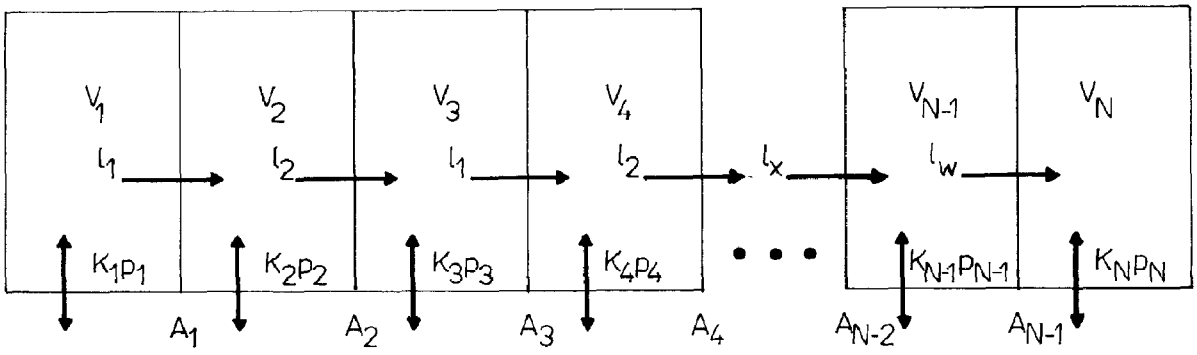

Fig. 1. Schematic outline of the N-compartment model system composed of alternating aqueous and lipidic phases (odd and even subscripts of V, respectively). V_i, volume of the i-th compartment; A_i, connecting area between the i-th and $(i+1)$-th compartment; l, transport rate parameters in direction water-lipid and vice versa (subscripts 1 and 2, respectively); K_i, association constant for binding to macromolecules (with concentration of binding sites p_i) in the i-th compartment.

3. Results and discussion

3.1. Systems with different compartments

Providing that the diagonal elements of matrix ***B*** (eq. 2) comprising the properties of individual compartments are different, the time course of the free solute concentration in the m-th compartment can be expressed as:

$$c_m = \left(n/\left(V_m(1+K_m p_m)\right)\right) \prod_{i=1}^{m-1} \lambda_i \times \sum_{j=1}^{m} \left[\exp(-\lambda_j t) / \prod_{\substack{n=1 \\ n \neq j}}^{m} (\lambda_n - \lambda_j) \right] \quad (3)$$

where

$$\lambda_i = l_x A_i / V_i (1 + K_i p_i) \quad (4)$$

The symbols and the values of x are specified in fig. 1 and table 1, respectively, n representing the molar amount of the substance.

3.2. Systems with cellular structure

The cellular structure of higher biosystems often causes invalidity of eq. 3 because of identical properties in certain compartments resulting in equality of the corresponding diagonal elements (eigenvalues) of matrix ***B*** (eq. 2). This phenomenon might also be observable, in addition to tissues of higher organisms, in eucaryotic microorganisms. Their cells are subdivided into a set of compartments by the endoplasmic reticulum and other intracellular membranes. In both the tissues and eucaryotic microorganisms we assume the periodical alteration of identical compartments starting with the M-th compartment. As the cellular substructures are usually surrounded by a membrane, M is an even number. Consequently, the diagonal elements (eigenvalues) of matrix ***B*** (eq. 2) can be written as:

$$\lambda_M = l_2 D_M = l_2 D_{M+2} = \ldots l_2 D_{N+1-w} \quad (5)$$

$$\lambda_{M+1} = l_1 D_{M+1} = l_1 D_{M+3} = \ldots l_1 D_{N+1-x} \quad (6)$$

where $D_i = A_i / V_i(1 + K_i p_i)$. The meaning of the symbols is as above; the values of w and x are given in table 1.

For biosystems having a cellular structure starting from the M-th compartment, the kinetics of distribution in the first $(M+1)$ compartments is given by eq. 3 but that for an $(M+m)$-th compartment $(m \geq 2)$ is described as:

$$c_{M+m} = E_{M+m} \left(\sum_{i=1}^{M+1} a_{i,M+m} e^{-\lambda_i t} + e^{-\lambda_M t} \sum_{i=1}^{y} a_{M+2i,M+m} t^i + e^{-\lambda_{M+1} t} \sum_{i=1}^{z} a_{M+2i+1,M+m} t^i \right) \quad (7)$$

where

$$E_{M+m} = (n/V_{M+2-x}) l_1^{(M+3)/2+z-w} l_2^{(M+3)/2+y-x} \times D_M^{y+1-x} D_{M+1}^{z+1-w} \prod_{i=1}^{M+1} D_i \quad (8)$$

and the coefficients a are given by the recurrent formulae

$$a_{i,M+m} = a_{i,M+m-1} / (\lambda_{M+2-x} - \lambda_i); \quad i = 1, 2, \ldots (M-1) \quad (9)$$

$$a_{M+2i+4-x,M+m} = a_{M+2i+2-x,M+m-1} / (i+1); \quad i = 0, 1, \ldots (y-1) \quad (10)$$

$$a_{M+m-1,M+m} = a_{M+m-1,M+m-1} / (\lambda_{M+2-x} - \lambda_{M+2-w}) \quad (11)$$

$$a_{M+2i+2-w,M+m} = \left(a_{M+2i+2-w,M+m-1} - (i+1) a_{M+2i+4-w,M+m}\right) / (\lambda_{M+2-w} - \lambda_{M+2-x}); \quad i = 0, 1, \ldots (z-1) \quad (12)$$

$$a_{M+2-x,M+m} = - \sum_{\substack{i=1 \\ i \neq M+2-x}}^{M+1} a_{i,M+m} \quad (13)$$

Table 1

The values of w, x, y, z

i, m	w	x	y	z
Odd	2	1	$(m-1)/2$	$(m-1)/2$
Even	1	2	$m/2$	$(m-2)/2$

Starting from the solution of eq. 2 for the first aqueous phase

$$c_1 = (n/V_1)e^{-l_1 D_1 t} \quad (14)$$

we can successively determine all the terms a needed for description of the time course of the free solute concentration in an arbitrary compartment. For $p < m$ we set $\prod_{l=m}^{p} = 1$ and $\sum_{l=m}^{p} = 0$.

3.3. Example

The data on xenobiotic influences on biosystems can be used for comparison of our results with biological reality, since biological activity monitors, under appropriate conditions, the drug concentration in the receptor compartment [14]. However, it is practically impossible to determine experimentally all the model parameters (l, Kp, A, V) for all the compartments of real biosystems. To cope with the problem, we use the dependence of both the rate parameters l and the association constant for protein binding K on the model partition coefficient P [11,15] as:

$$l_1 = aP/(bP + 1) \quad (15)$$

$$l_2 = a/(bP + 1) \quad (16)$$

$$K = dP^e \quad (17)$$

where constants a, b, d, e are dependent on the model system and experimental conditions used. Providing bioactivity within a series of drugs is tested under identical conditions (including a constant time of application) the partition coefficient P is the only variable as the values of a, b (eqs. 15 and 16), d, e (eq. 17), p (eqs. 1 and 2), A, V (eq. 2) are held constant under the circumstances. The plots of 'bioactivity vs. the partition coefficient P' are, in fact, quite common in studies of the relationships between physicochemical properties and bioactivity [2–7]. The corresponding dependences resulting from eq. 3 are given in figs. 2 and 3. Fig. 2 illustrates the effect of the time interval allowed for distribution on the shape of bioactivity vs. lipophilicity profile. The curves are at first bilinear, i.e., composed of two linear parts connected by a curved portion and later a minimum appears at log P about 0. In fig. 3 the bilinear profiles as influenced by variation in the constants d and e

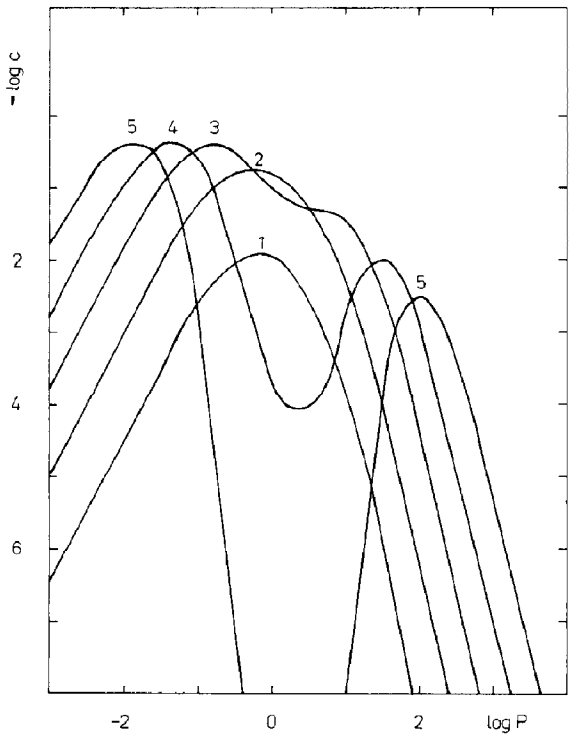

Fig. 2. Concentration-lipophilicity profiles for the fifth compartment after 3.2 (1), 10 (2), 32 (3), 100 (4) and 320 (5) h of distribution calculated according to eq. 3. Volumes of phases (in dm^3): 1, 0.5, 0.5, 0.3, 0.3 from the first to fifth compartment; all interfacial areas are 1 dm^2; $K_1 p_1 = K_3 p_3 = K_5 p_5 = P$; $K_2 p_2 = K_4 p_4 = 0$; transport rate parameters l_1 and l_2 obey eqs. 15, 16 with $a = 0.245$ dm h^{-1} and $b = 0.286$ [20].

from eq. 17 (B and A, respectively) are given. In the former case, mainly the changes in positions of curvatures without significant differences in the slopes of linear parts are observed whilst in the latter both features vary. The overall shape of the curves is virtually independent of the volumes of individual phases and their surface areas. The differences involve mainly vertical and horizontal shifts of either positions of the curvatures or the whole curves. Consequently, the dependences of similar shape also result from eqs. 7–13 for systems having cellular structure. As can be seen from figs. 2 and 3 four types of curves are generated by the equations presented: (1) composed of two linear parts connected by a curved portion

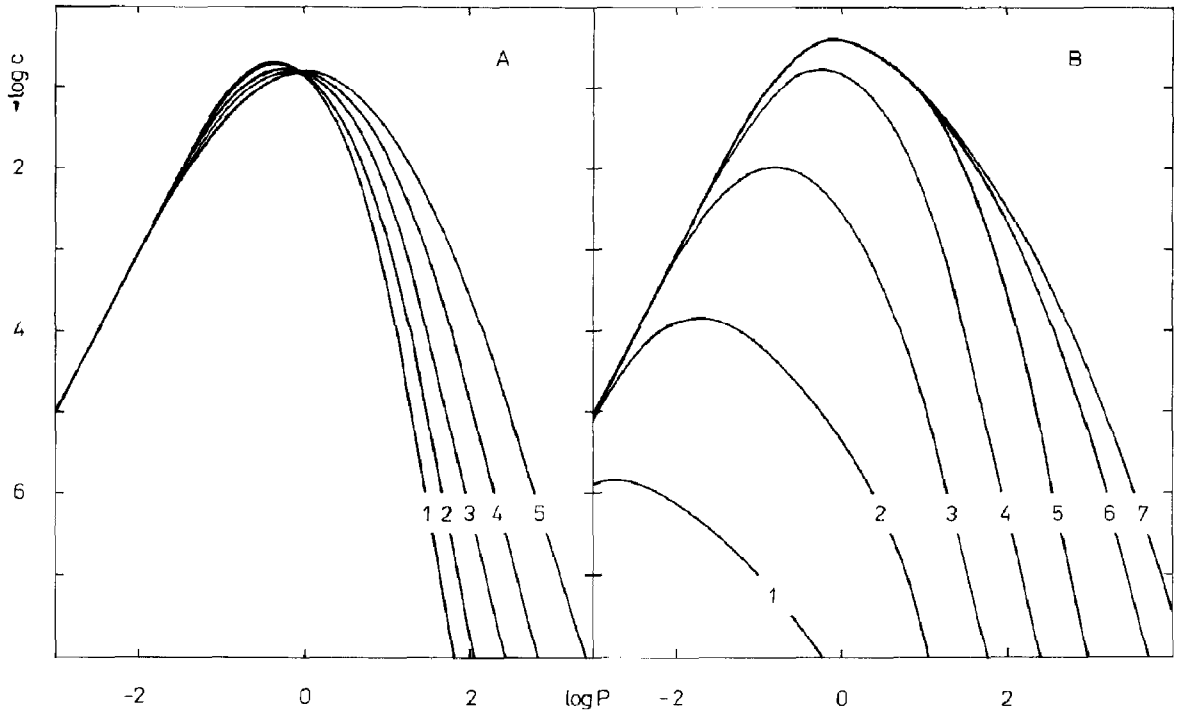

Fig. 3. Influence of e (A) and d (B) from eq. 17 on the concentration-lipophilicity profiles in the fifth compartment after 10 h of distribution. $K_2p_2 = K_4p_4 = 0$; $K_1p_1 = K_3p_3 = K_5p_5 = dP^e$. (A) $d = 1$, $e = 1.5$ (1), 1.25 (2), 1.00 (3), 0.75 (4) and 0.5 (5). (B) $e = 1$, $d = 1000$ (1), 100 (2), 10 (3), 1 (4), 0.1 (5), 0.01 (6) and 0.001 (7).

(fig. 2, curves 1, 2; fig. 3A) or (2) by a wider plateau (fig. 3B, curves 1–4); (3) comprising three linear parts joined by curved portions (fig. 3B; curves 5–7); (4) having two maxima separated by a minimum (fig. 2; curves 3–5).

The results of simulation can be compared with biological reality using data on biological activity in a congeneric series of drugs assessed under identical conditions which are limited to some 8–15 points as a rule. The original expressions (eqs. 3 and 7–13) are not suitable for this purpose as they: (1) comprise too many adjustable parameters with unknown but constant values (i.e., A, V, a, b, d, e); (2) adopt different forms for various compartments which are to be examined for all plausible possibilities as the receptor compartment is often unknown. Therefore, it seems advantageous to make use of an empirical equation we suggested previously [16]:

$$\log c = A \log P + \sum_{i=1}^{N} B_i \log(C_i'P + 1) + D' \quad (18)$$

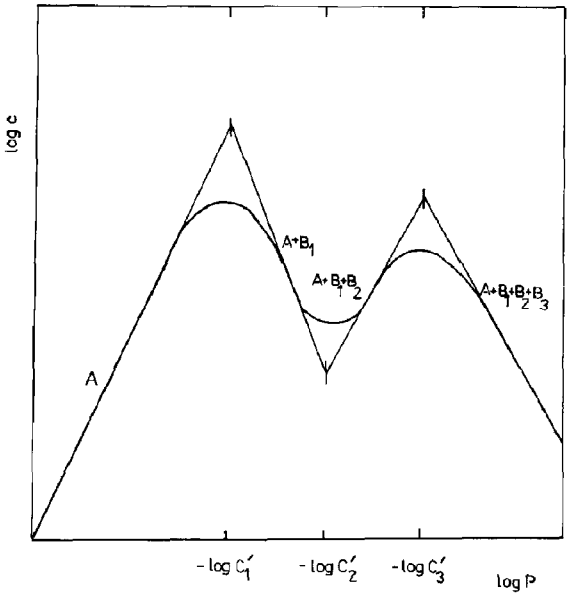

Fig. 4. The dependences of concentration c on the partition coefficient P described by eq. 18. Slopes in corresponding linear parts are given above the curves.

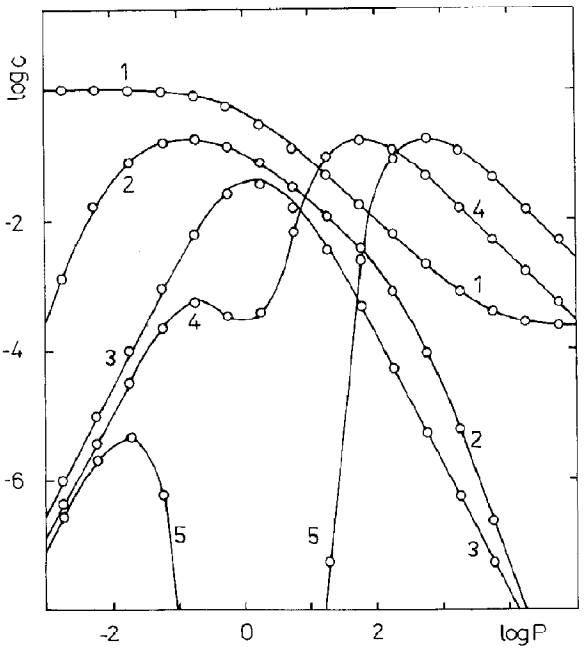

Fig. 5. Examples of fitting of the calculated concentration-lipophilicity profiles (points) by eq. 18 (lines). The equations and their statistics are given in table 2.

where c is concentration, P the partition coefficient, $N = 1$, 2 or 3, and A, B_i, C_i', D' adjustable parameters comprising all the invariable properties of the biological system investigated. Their relations to the shape of the corresponding curve are illustrated in fig. 4. The values of A and B_i determine the slopes in the linear parts, the magnitudes of C_i' being responsible for the positions of the curvatures. Examples of fitting are given in fig. 5, the optimized values of the adjustable parameters and statistics being summarized in table 2. As can be seen, a nearly perfect fit was obtained in all cases, indicating the ability of eq. 18 to describe the theoretical concentration vs. lipophilicity profiles. Its adaptation to biological conditions requires conversion of the model partition coefficient to the membrane/water partition coefficient P_M via the Collander equation [17]:

$$P_M = \text{const.}\ P^E \tag{19}$$

where E is constant for a congeneric series. Combination of eqs. 18 and 19 and appropriate rearrangement [14] provide the final expression for correlation of biological activity expressed as the isotoxic concentration c on the partition coefficient P:

$$\log(1/c) = A \log P^E + \sum_{i=1}^{N} B_i \log\left(C_i P^E + 1\right) + D \tag{20}$$

Eq. 20 represents theoretical descriptions (eqs. 3 and 7–13), its form being suitable for comparison with results of drug potency testing.

The hemolytic activity of α-monoglycerides [18,19] in aqueous and 2% ethanolic solution is plotted vs. lipophilicity in fig. 6. Micelle formation was suggested as a possible explanation for the decrease in activity of higher homologs in the aqueous medium as compared with that in the ethanolic solution [20]. In our context, however, it is rather the influence of the added ethanol on hydrophobic binding of the derivatives to proteins than a break-up of micelles which seems to be a

Table 2

The optimized values of adjustable parameters in eq. 18 describing the theoretical dependences of concentration on lipophilicity given in fig. 5

Statistical parameters: 16 points in all cases; the lowest $r = 0.999$ and $F = 304$, the greatest $s = 0.139$.

Curve	A	B_1	B_2	B_3	C_1'	C_2'	C_3'	D'
1	0 [a]	−1 [a]	1 [a]	0 [a]	1.075	2.279×10^{-4}	0 [a]	−0.021
2	3 [a]	−3 [a]	−1 [a]	−2 [a]	123.0	1.000	2.177×10^{-3}	5.644
3	2 [a]	−4 [a]	0 [a]	0 [a]	0.656	0 [a]	0 [a]	−0.528
4	2 [a]	−13.49	26.30	−15.83	2.309	5.893×10^{-1}	1.439×10^{-1}	−0.841
5	2 [a]	−125.3	249.0	−126.6	2.175	6.000×10^{-1}	1.612×10^{-1}	−0.808

[a] Nonoptimized value.

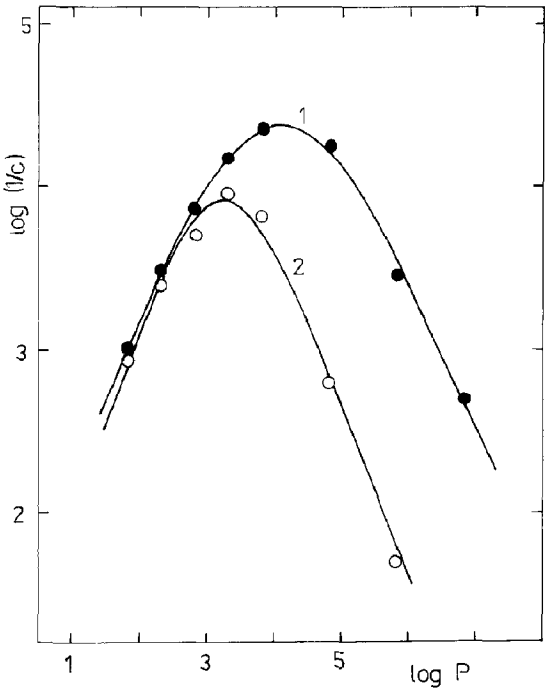

Fig. 6. Hemolytic activity of α-monoglycerides vs. lipophilicity [18,19] in aqueous (2) and 2% ethanolic solution (1). The curves correspond to eq. 20 with optimized values of adjustable parameters given together with statistics in table 3.

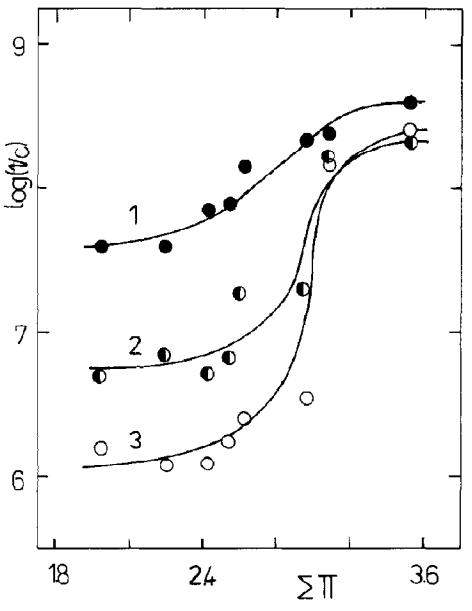

Fig. 7. Analgesic activity in rats of *N*-4-substituted-1-(2-arylethyl)-4-piperidinyl-*N*-phenylpropanamides at 2 (1), 4 (2) and 6 (3) h of application [21] vs. lipophilicity expressed as a sum of the *Π* constants of the varying substituents [22]. The lines correspond to eq. 20, the values of parameters and statistics being summarized in table 3.

more plausible explanation of the observation. Ethanol reduces the binding capacity of proteins which is equivalent to a decrease in constant d in eq. 17. The effect of the change in d on both the theoretical and experimental concentration vs. lipophilicity profiles is similar as follows from figs. 6 and 3B (e.g. curves 3 and 4), respectively, as well as from the fits of eq. 20 to the data given in table 3.

The dependences of analgesic activity in rats of *N*-4-substituted 1-(2-arylethyl)-4-piperidinyl-*N*-phenylpropanamides at various times of application [21] on the sum of the π constants representing additive increments of individual sub-

Table 3

The optimized values of adjustable parameters of eq. 20 describing the experimental concentration-lipophilicity profiles given in figs. 6 and 7 and the statistics

Fig.	Curve	A	B_1	B_2	C_1	C_2	D	E	n	r	s	F
6	1	1 [b]	−2 [b]	0 [b]	2.768×10^{-4}	0 [b]	1.457	0.864	8	0.998	0.056	68.1
6	2	1 [b]	−2 [b]	0 [b]	3.689×10^{-4}	0 [b]	1.079	1.054	7	0.997	0.096	30.4
7 [a]	1	0 [b]	1.784	−1.534	3.565×10^{-3}	6.274×10^{-4}	7.339	1.017	8	0.981	0.204	3.250
7 [a]	2	0 [b]	5.015	−4.888	6.257×10^{-4}	1.800×10^{-4}	6.745	1.125	8	0.931	0.691	0.818
7 [a]	3	0 [b]	9.526	−8.552	4.971×10^{-4}	2.131×10^{-4}	5.757	1.087	8	0.909	1.066	0.613

[a] $10^{\Sigma\Pi}$ was used instead of P.

[b] Nonoptimized value.

stituents to the 1-octanol/water partition coefficient [22] are given in fig. 7. Five derivatives with the substituents $R_1 = CH_3$ and $R_3 = H$ or $COCH_2CH_3$ (numbering as in the original article [21]) were excluded. The dependences measured at the second, fourth and sixth hour can be described by eq. 20, the values of optimized parameters and the statistics being summarized in table 3. A statistically significant conclusion can hardly be made on the basis of individual curves in fig. 7. Considering them as a set, however, quantitative similarity in the time development of theoretical and experimental curves (figs. 2 and 7, respectively) can be seen.

The observed quantitative agreement of the presented biological data with eqs. 3 and 7–13 might indicate their ability to describe the disposition of low-molecular-weight compounds in individual regions of biosystems for certain periods of application. In both the examples considered, obviously a nonequilibrium period of drug transport was involved as back-diffusion causes a specific 'angular' shape of the bioactivity-lipophilicity profiles [4,5].

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