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A THEORETICAL MODEL OF THE TEMPERATURE- AND PRESSURE-INDUCED PHASE TRANSITION OF PHOSPHOLIPID BILAYERS

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A statistical thermodynamic model of phospholipid bilayers is developed. In the model, a new concept of a closely packed system is applied, i.e., a system of hard cylinders of equal radii, the radius being a function of the average number of *gauche* rotations in a hydrocarbon chain. Using this concept of a closely packed system, reasonable values are obtained for the change in specific volume at the order-disorder transition of lecithin bilayers. In addition to interactions between the lipid matrix and water molecules, between the head groups themselves and between hydrocarbon chains, as well as the intramolecular energy associated with chain conformation, the Hamiltonian of the membrane also includes the energy of the pressure field. Thus, the phase transition of phospholipid membranes induced not only by temperature but also by hydrostatic pressure is described by this model simultaneously. In accordance with the experimental results, a linear relationship is obtained between the phase transition temperature and phase transition pressure. The other calculated phase transition properties of lecithin homologues, e.g., changes in enthalpy, surface area, thickness and *gauche* number per chain are in agreement with the available experimental data. The ratio of kink to interstitial conduction of bilayers is also estimated.

1. Introduction

According to the experiments, the order-disorder transition of phospholipid bilayers depends not only on the temperature but also on the pH and salt concentration [1] and on the hydrostatic pressure [2]. In several papers the temperature-induced phase transition of bilayers was explained by statistical mechanical models [3–9]. Only one phenomenological model has appeared [10] to explain the pressure-induced phase transition. The major purpose of our work is to present a statistical mechanical model for describing the phase transition of bilayers induced not only by temperature but also by hydrostatic pressure. Our model is similar in part to the model of Scott and Cheng [9] and of Jacobs et al. [7]. Using a new interpretation of the concept of close-packed area, our model contrasts with the model of Jacobs et al. [28], giving reasonable values for the change in specific volume at the order-disorder transition of lecithin bilayers. The following experimentally determined parameters of DMPC, DPPC and DSPC membranes are also well described by the model: transition temperature, transition pressure, enthalpy change, Clapeyron slope, surface area change and *gauche* number change per chain. Introducing a pressure energy term into the free energy function of phospholipid membranes, we conclude—in accordance with the experimental findings—that transition pressure is a linear function of the melting temperature.

Abbreviations: DPMC, DPPC, DPPC, dimyristoyl-, dipalmitoyl-, distearoylphosphatidylcholine, respectively.

2. Model

It is important to clarify the concept of close-packed area for a better understanding. In the case of membranes this has a special importance be-

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cause the effective cross-sectional area of lipid molecules increases with increasing energy content of the system, e.g., on heating of the membrane. Here the excitation of intramolecular degrees of freedom leads to a change in the effective cross-sectional area of the molecules. In the case of these systems one can use the following definition of close-packed area: it is the minimal surface area of the system for a given internal (or conformational) energy of the molecules. By this definition, the basic conformational characteristics of the molecules remain unchanged in the closely packed system. In our case, the membrane shows minimal surface area if the hydrocarbon chains contain only kinks. Implicitly, one can find a similar idea in the paper of Scott and Cheng [9], where their membrane model consists of a system of hard cylinders of varying radii corresponding to the different number of kinks or jogs in their chains.

In our model, which contrasts with Scott's model, the membrane is supposed to consist of hard cylinders of equal radii, the radius being a function of the average configurational energy per hydrocarbon chain. At the same time, as in Jacobs' model [7], we take into consideration the fact that *trans-gauche* isomerization may result in a conical shape of the molecules, demanding a larger area than for the cylindrical shape of molecules containing only kinks.

However, for the estimation of this repulsive or excluded volume interaction between molecules, we have to take into consideration the different definitions of close-packed area in Jacobs' model and ours.

2.1. The conformation-dependent close-packed area

The average number of *gauche* rotations per hydrocarbon chain is denoted by $\langle g \rangle$. In the formation of one kink the chain is shortened by one CH_2 unit of length ($h_0 = 1.27$ Å). Thus, the average length of a hydrocarbon chain in the closely packed system is:

$$\langle l \rangle_c = [n - \langle g \rangle / 2]\, h_0 \tag{1}$$

where n is the number of CH_2 groups per chain. The effective cross-sectional area of a hydrocarbon chain in a closely packed system ($\mathfrak{A}'_0$) can be defined according to Scott and Cheng (see eq. 5 in ref. 9) by the following formula:

$$A'_0 l_0 = \mathfrak{A}'_0 \langle l \rangle_c \tag{2}$$

where $l_0 (= nh_0)$ is the length of a hydrocarbon chain in the all-*trans* conformation and A'_0 the cross-sectional area of a hydrocarbon chain in the all-*trans* state. Finally, let us take into consideration that the cross-sectional area of a head group aligned parallel to the membrane surface is slightly larger than that of the pair of hydrocarbon chains in the all-*trans* state ($A_0 > 2A'_0$ [11]). However, the effective cross-sectional area of a pair of hydrocarbon chains ($2\mathfrak{A}'_0$) can be larger than that of the polar head, A_0, when the number of *gauche* states exceeds a nonzero threshold value. In this case, the close-packed area of the membrane is determined by the effective cross-sectional area of the hydrocarbon chains and not by the constant head group area. Thus, the conformation-dependent close-packed area of the membrane, $\mathfrak{A}_0$, is given by eq. 3:

$$\mathfrak{A}_0 = N \cdot \max \begin{cases} A_0 \\ 2\mathfrak{A}'_0 = 2A'_0 (1 - \langle g \rangle / 2n)^{-1} \end{cases} \tag{3}$$

where $A_0 = 40$ Å^2 [7] and $2A'_0 = 38.5$ Å^2 [12] for one phospholipid molecule, and N is the number of lipid molecules in one layer of a bilayer membrane.

2.2. The pressure energy

In the case of nonzero external isotropic (hydrostatic) pressure, we have to consider the pressure energy (H_{pres}) in addition to the Hamiltonian of a bilayer membrane, thus the partition function of one monolayer of the membrane (Q) will be [13]:

$$Q = \sum_{\substack{\text{all} \\ \text{conformations}}} \exp - (H_{monolayer} - H_{pres}) / kT \tag{4}$$

where the summation runs over all configurations of the monolayer. The pressure energy term which describes the work of the pressure field on one half of the membrane is:

$$H_{pres} = -pV = -p\mathfrak{A}_0 (a + 1) \langle l \rangle \tag{5}$$

where p is the pressure, V and A the actual volume

and area of one layer of the membrane, respectively, $\alpha[=(A-\mathcal{A}_0)/\mathcal{A}_0]$ the relative surface area and $\langle l\rangle$ denotes the effective vertical equilibrium length of a molecule, i.e., the monolayer thickness.

2.3. Partition function

The partition function of one monolayer of the membrane (Q), like the Jacobs' model [7], consists of three factors:

$$Q(A|N,T,p)=Q_{HD}\cdot Q_{CH}^{2N}\cdot Q_{LR}. \tag{6}$$

Q_{HD} is the partition function of a two-dimensional system of hard disks with cross-sectional area $\mathcal{A}_0/N$ [14–16]:

$$Q_{HD}=\left(\alpha^2\mathcal{A}_0/N\right)^N \exp N(0.06-0.12\alpha +0.382\alpha^2+0.243\alpha^3+\dots) \tag{7}$$

Here it is supposed tacitly that the partition function of cylinders, i.e., pairs of hydrocarbon chains containing kinks which move rapidly up and down, agrees approximately with the partition function of infinitely hard disks with cross-sectional areas $\mathcal{A}_0/N$. Q_{CH} is the partition function of a hydrocarbon chain:

$$Q_{CH}=1+\sum_{i=1}^{m}(1+2\exp-\epsilon/kT)^{m-i} \times 2\exp-[\epsilon+P_{HD}\Delta A]/kT \tag{8}$$

where P_{HD}, the pressure of hard disks with cross-sectional areas $\mathcal{A}_0/N$, is:

$$P_{HD}=(NkT/\mathcal{A}_0)(2/\alpha+1.0+0.67\alpha+1.5\alpha^2+\dots)/(1+\alpha) =(NkT/\mathcal{A}_0)f(\alpha). \tag{9}$$

where T is the absolute temperature, k Boltzmann's constant, $2N$ the number of hydrocarbon chains in one layer of a bilayer membrane, $m(=n-2)$ the number of carbon-carbon bonds which can exist in either the *gauche* or *trans* conformation, i the location of the first *gauche* bond in a chain, and ϵ the energy of a *gauche* bond relative to a *trans* bond ($\epsilon=500$ cal/mol $=2.1$ kJ/mol [7]). Eq. 8 corresponds formally to eq. 7 of Jacobs' model [7], however, there are two basic differences. We estimate the excluded volume interaction between the molecules by the term, $P_{HD}\Delta A$. This work must be done if the chain increases its effective cross-sectional area by ΔA relative to the minimal area $\mathcal{A}_0/2N$. This happens when *trans-gauche* isomerizations result in a conical shape of the lipid molecules, demanding a larger area than for the cylindrical shape of molecules containing only kinks. This increase in area must take place against an average pressure due to the presence of other chains. In this model we set this pressure equal to the hard disk pressure, P_{HD}, where the cross-sectional area of a disk is $\mathcal{A}_0/N$. Thus, we take into consideration the fact that a given chain feels the pressure of the surrounding molecules having generally larger effective cross-sectional areas than the molecules in the all-*trans* state. The second difference is in the formula for ΔA (cf. eq. 6 in ref. 7):

$$\Delta A=\gamma(m-i+1)\,\mathcal{A}_0/N \tag{10}$$

where γ is a dimensionless adjustable parameter and is expected to be independent of chain length. Here it is supposed—as in Jacobs' model—that the change in effective cross-sectional area is dependent on the distance of the first *gauche* bond from the head group and is proportional—in contrast to Jacobs' model—to the effective cross-sectional area of the hard cylinders, $\mathcal{A}_0/N$. By means of this definition, we can take into consideration that the greater the number of *gauche* states belonging to the bending segment, the larger the effective thickness and horizontal projection of the chain segment.

The third factor of the partition function, Q_{LR}, involves the long-range interactions between the lipid molecules and water, between the head groups themselves and between the hydrocarbon chains:

$$Q_{LR}=\exp-N(\mathcal{A}_0/A)\,\Delta H_s(2n+\delta)/kT \tag{11}$$

where ΔH_s is the heat of sublimation per CH_2 group of the long-chain hydrocarbon, its value being taken as -1840 cal/mol (-7.7 kJ/mol) of CH_2 groups [17] and δ characterizes the strength of the interactions between the head groups and between the lipid matrix and the water molecules. This formula is similar to the corresponding one in Jacobs' model (see eq. 4 in ref. 7). In our formula all three interactions are considered to be proportional to $\mathcal{A}_0/A$ in spite of the fact that the areas of

the polar heads are independent of the chain conformation ($A_0 = 40$ Å^2). However, the error arising from this inconsistency can be eliminated because the attractive interactions between polar heads are very small compared to the repulsive effect of water on the lipid matrix, i.e., the head-head interaction energy is less than -500 cal/mol [11] and the sum of the attractive head-head interaction and repulsive water-lipid interaction is less than 11776 cal/mol ($= \delta \Delta H_s$) [7].

We note also that a similar formula for the repulsive water-lipid interaction term can be found in the paper of Nagarajan and Ruckenstein (see eq. 12 in ref. 18) where the change in interfacial tension is proportional to $(\mathcal{A}_0/N) - A_0$.

As Q_{CH} is a geometric series it has a simpler form:

$$Q_{CH} = 1 + B\frac{(q^{m-1}-1)}{(q-1)} \tag{12}$$

where

$$B \equiv 2\exp-(\epsilon/kT + f(\alpha))$$
$$q \equiv (1+2\exp-\epsilon/kT)\exp-\gamma f(\alpha). \tag{13}$$

By means of Q_{CH}, formulae for the average *gauche* number per chain $\langle g \rangle$ and for the effective thickness of one layer of the bilayer membrane $\langle l \rangle$ can be derived (see the appendix). Thus

$$\langle g \rangle = 1 + [(B\,\partial Q_{CH}/\partial q) - 1]/Q_{CH} = \left[-kT\frac{\partial \ln Q_{CH}}{\partial \epsilon}\right] \tag{14}$$

and

$$\langle l \rangle = \tfrac{1}{2}(2m + 1 - \{-1 + q\,\partial Q_{CH}/\partial q\}/Q_{CH})\,h_0 + h \tag{15}$$

where h is the vertical length of the head group ($h = 8$ Å [19]). Fortunately, as Q_{CH} is independent of $\mathcal{A}_0$ and $\langle l \rangle$, by means of eqs. 3, 5, 14 and 15 explicit formulae can be obtained for $\mathcal{A}_0$ and $\langle l \rangle$ in closed forms. Substituting these formulae for $\mathcal{A}_0$ and $\langle l \rangle$ into eq. 6, the partition function of one layer of a bilayer membrane is also obtained in a closed form. By means of this partition function one can calculate a number of thermodynamic and geometrical properties of phospholipid bilayer membranes.

Table 1

Calculated properties of the temperature- and pressure-induced phase transition of phospholipid bilayers

lc, data of the liquid-crystalline phase; Δ, liquid-crystalline values minus the respective solid-phase values; P_m, transition pressure; T_m, transition temperature; H, enthalpy; l, average length of a lipid molecule; A, actual surface area per molecule; V, actual membrane volume per molecule; g, average *gauche* number per hydrocarbon chain; $\mathcal{A}_0$, closely packed surface area per molecule.

	P_m (MPa)	T_m (°C)	ΔH (kJ/mol)	l_{lc} (Å)	Δl (Å)	A_{lc} (Å^2)
DMPC	0.1	24.5	33	20.5	−3.9	51.6
($n = 14$, 678 g/mol)	0.1	23.3 [8]	28.2 [21]			
DPPC	0.1	41.7	46.1	21.5	−5.5	54
($n = 16$, 734 g/mol)	0.1	41.4 [8]	41.4 [21]	21.6 [20]	−3 [20]	56.3 *
	40	48.8		21.6	−5.45	53.42
	40	50 [2]				
DSPC	0.1	55.5	58.9	22.5	−7.1	55.7
($n = 18$, 790 g/ml)	0.1	54.3 [8]	46.1 [21]			

3. Results and discussion

The Gibbs free energy of the membrane can be calculated from:

$$G = -2kT \ln Q. \quad (16)$$

In fig. 1, the Gibbs free energy is plotted against the area per molecule, at different pressures, and at fixed temperature and vice versa.

These curves have two different minima and the deeper one determines the equilibrium state of the membrane. When the absolute minimum of the curve corresponds to the smaller area of about 40 Å^2 the membrane is in the gel or crystalline state and when it corresponds to the larger area of about 55 Å^2 it is in the liquid-crystalline state. The relative values of the minima and consequently the state of membrane can be altered by changing the external conditions. The increase in temperature triggers a gel to liquid-crystalline transition while the increase in isotropic pressure results in a transition in the opposite direction. Under certain external conditions, i.e., for given pairs of values of temperature and pressure, the two minima have the same values. These pairs of values are the transition temperature, T_m, and the transition pressure, P_m, and in these cases the two phases of the membrane corresponding to the two minima coexist. Fig. 2 shows the transition pressure as a function of the transition temperature for DPPC membranes. The solid line is the calculated curve

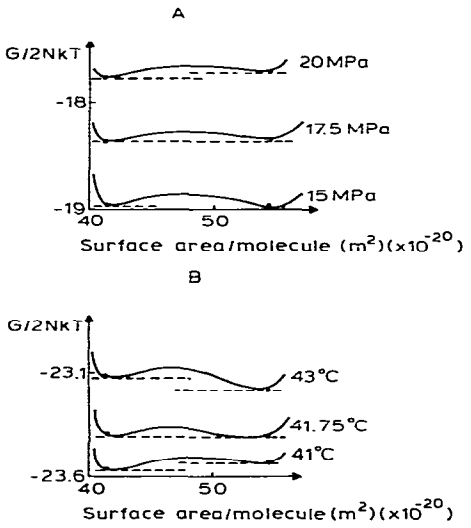

Fig. 1. Calculated Gibbs free energies in dimensionless units plotted against the surface area per molecule: (A) at different pressures and constant temperature (45.1°C). (B) at different temperatures and constant pressure (0.1 MPa) for DPPC membranes.

and the shaded area contains the measured values. In accordance with the experimental results [2], a linear relationship was obtained between the melting temperature and transition pressure. By means of the partition function a number of other thermodynamic and geometrical properties can be

ΔA (Å^2)	$\bar{a}_{\alpha(lc)}$ (Å^2)	$\Delta\bar{a}_0$ (Å^2)	V_{lc} (Å^3)	ΔV (Å^3)	dP_m/dT_m (MPa/°C)	g_{lc}	Δg
10	43.8	3.8	1060 1096 [22]	42.5 30.5 [22]	4.99 4.9 [27]	3.4 3.5 [23]	3.3 2.5 [23]
12.6	45.1	5.1	1160 1218 [22]	43.3 45.3 [22]	5.45 4.37 [27]	4.7 4.5 [23]	4.6 3.5 [23]
12.25	44.95	4.9	1154	41.35 45 [2]	5.62	4.6	4.5
14.5	46	6	1254 1347 [22]	37.1 59.3 [22]	6.18	6 6.5 [23]	5.8 4 [23]

* A_{lc} is calculated from V_{lc} and l_{lc} from $A_{lc} = V_{lc}/l_{lc}$.

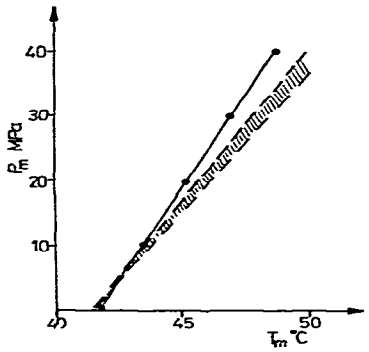

Fig. 2. Phase transition pressure as a function of phase transition temperature for DPPC membranes. Solid line, calculated curve; shaded area, experimental data [2].

calculated for phospholipid bilayers. Table 1 shows the calculated and measured phase transition properties of DMPC, DPPC and DSPC bilayers.

When calculating ΔV, ΔA, $\Delta \mathcal{A}_0$ and ΔH, the respective fluid-phase values minus solid-phase values were taken where the actual surface area (A), close-packed area ($\mathcal{A}_0$) and bilayer volume (V) were determined by eqs. 3, 5, 14 and 15.

The enthalpy changes for different phospholipids were calculated according to (see the appendix):

$$\Delta H = \Delta\left(2\epsilon\langle g\rangle + \Delta H_s[2n+\delta]\,(\alpha+1)^{-1} + pV/N\right)$$
$$= \Delta\left(-\frac{k}{N}\frac{\partial \ln Q}{\partial\, 1/T}\right) \qquad (17)$$

Besides the parameters in table 1 the average thickness of DPPC membranes in the liquid-crystalline state was calculated as a function of temperature and pressure. From the slopes of these curves, -1.3×10^{-3} °C^{-1} was obtained as the linear thermal expansion coefficient and 2×10^{9} N m^{-2} as Young's modulus. The experimental data obtained were -2×10^{-3} °C^{-1} [24] and 10^{9} N m^{-2} [25], respectively. The Clapeyron slopes were obtained from P_m versus T_m curves.

4. Conclusions

The model calculations presented in the previous section show that the values obtained especially for the liquid-crystalline state are comparable with the measured phase transition properties of phospholipid bilayers at different temperatures and pressures. However, the calculated changes in thermodynamic properties at the phase transition do not fit so well to the experimental data. This inconsistency arises from the structural differences in the gel phase of multilamellar systems (the experimental data in table 1 concern these systems) and that of single bilayers (the model concerns these systems) [26]. Taking into consideration that the close-packed area of a bilayer depends on the average conformation of hydrocarbon chains, rather good values are obtained for the volume changes.

We note here that the calculated volume changes are 3-times greater when we do not bear in mind the fact that the cross-sectional area of a head group is slightly larger than that of the pair of hydrocarbon chains in the all-*trans* state (i.e., when $\mathcal{A}_0 = 2NA'_0(1-\langle g\rangle/2n)^{-1}$ is applied) and the volume changes are negative when the close-packed surface area is considered to be constant (see fig. 8 in ref. 28). The calculated values of the enthalpy changes approach the experimental data better than those obtained from Jacobs' model [7] by 4 kJ/mol. The other important advantage of this model is that only the parameters of Jacobs' model are used, namely $\gamma = 0.47095$ and $\delta = -6.4$. (These values of γ and δ were fitted by Jacobs et al. to yield the correct transition temperatures of the lecithin homologues under atmospheric pressure.)

By means of this model the surface area increase, $A - A_0$, can be separated:

$$A - A_0 = (\mathcal{A}_0 - A_0) + (A - \mathcal{A}_0) \qquad (18)$$

where $\mathcal{A}_0 - A_0$ relates to kink formation in the hydrocarbon chains and $A - \mathcal{A}_0$ to the formation of other structural defects of hydrocarbon chains and to the long-range loosening of the lateral packing. According to our results for A and $\mathcal{A}_0$, and assuming a linear relationship between the lateral loosening and membrane permeability for small molecules, 36% of transport occurs through kinks and 64% through other structural defects in the liquid-crystalline state.

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Appendix

A.1. Average length of a lipid molecule

According to Jacobs et al. [8], the effective vertical equilibrium length of lipid molecules, if $\langle i \rangle$ is the average location of the first *gauche* bond, can be expressed by:

$$\langle l \rangle = \tfrac{1}{2}(m + \langle i \rangle + 1)\, h_0 + h \tag{19}$$

Considering that $\langle i \rangle = m + 1$, when the chains are in the all-*trans* state, the average of $m - \langle i \rangle$ is:

$$\langle m - i \rangle \equiv \langle j \rangle = \left[-1 + B \sum_{j=0}^{m-1} j q^j \right] Q_{CH}^{-1}$$
$$= \left[-1 + q(\partial Q_{CH}/\partial q) \right] Q_{CH}^{-1} \tag{20}$$

where

$$\partial Q_{CH}/\partial q = B\left[(m-2)\, q^{m-1} - (m-1)\, q^{m-2} + 1\right]/(q-1)^2 \tag{21}$$

and

$$Q_{CH} = 1 + B \sum_{j=0}^{m-1} q^j \tag{22}$$

Substituting eq. 20 into eq. 19 the average length will be:

$$\langle l \rangle = \tfrac{1}{2}\left(2m + 1 - \{-1 + q(\partial Q_{CH}/\partial q)\}\, Q_{CH}^{-1}\right) h_0 + h \tag{23}$$

A.2. Average gauche number per chain

As ϵ/kT is the value conjugated to the *gauche* number, the average *gauche* number per chain can be calculated in the following way [13]:

$$\langle g \rangle = -\frac{kT}{2N}\frac{\partial \ln Q}{\partial \epsilon} = -\frac{kT}{Q_{CH}}\frac{\partial Q_{CH}}{\partial \epsilon} = -\frac{kT}{Q_{CH}} \times \left[\frac{\partial B}{\partial \epsilon}\frac{Q_{CH} - 1}{B} + \frac{\partial q}{\partial \epsilon}\frac{\partial Q_{CH}}{\partial q} \right] \tag{24}$$

and from eq. 13 it follows that

$$\frac{\partial B}{\partial \epsilon} = \frac{\partial q}{\partial \epsilon} = \frac{-B}{kT}. \tag{25}$$

Thus, $\langle g \rangle$ is given by:

$$\langle g \rangle = 1 + \left[(\partial Q_{CH}/\partial q)\, B - 1\right]/Q_{CH} \tag{26}$$

A.3. Average enthalpy per molecule

Since $1/kT$ is the value conjugated to the enthalpy, the enthalpy per molecule is:

$$H = -\frac{k}{N}\frac{\partial \ln Q}{\partial (1/T)} = -\frac{k}{N} 2N \frac{\partial \ln Q_{CH}}{\partial (1/T)} + \Delta H_s (2n + \delta)(\alpha + 1)^{-1} + pV/N$$
$$= 2\epsilon \left\{ 1 + (B\, \partial Q_{CH}/\partial q - 1)\, Q_{CH}^{-1} \right\} + \Delta H_s (2n + \delta)(\alpha + 1)^{-1} + pV/N$$
$$= 2\epsilon \langle g \rangle + \Delta H_s (2n + \delta)(\alpha + 1)^{-1} + pV/N. \tag{27}$$

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