X-Ray Studies on Phospholipid Bilayers. VIII. Interactions with Chlorpromazine · HCl

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Chlorpromazine is a widely used phenothiazine tranquilizer known to alter the shape of normal erythrocytes and their osmotic fragility. In order to understand the nature of the interactions chlorpromazine · HCl (CPZ · HCl) was made to interact with phospholipid bilayers formed by dimyristoylphosphatidylethanolamine (DMPE) and dimyristoylphosphatidylcholine (DMPC). This study was carried out by X-ray diffraction on crystalline powders of various molar mixtures of CPZ · HCl with DMPE and DMPC, with and without water. It was found that CPZ · HCl significatively affects the bilayer structure of DMPC in the presence of water, but not that of DMPE.

Introduction

Phospholipids constitute a very important and interesting class of biological molecules. They exhibit an amphiphilic character as they are formed by long hydrocarbon chains and polar groups. One of the most important of their organized structures is the bilayer, which is found in the biological membranes. In the human erythrocytes the phospholipids are asymmetrically distributed: in the external monolayer are preferentially found diacylphosphatidylcholines (lecithins) and sphingomyelin while in the inner are located diacylphosphatidylethanolamines (cephalins) and phosphatidylserine [1]. Although the functional significance of this asymmetry has not been fully clarified, this laboratory has made some contributions to that end [2].

The structural studies of biological membranes are seriously hindered because of the large number of molecules present, their irregular distribution and state of fluidity. It is for this reason that phospholipid bilayers have been used as simpler models for studying the structural features of biomembranes [2–7]. An additional application of these models is their use to understand how chemical compounds of biological interest and therapeutical drugs affect the phospholipid bilayer of cellular membranes [8, 9].

Chlorpromazine is a tranquilizer derived from phenothiazine which is widely used to treat many psychotic disorders, particulary those which involve hyperactivity and anxious excitment [10]. It belongs to a group of drugs that, independently of their specific therapeutic activity, presents the common characteristic of being amphiphilic cationic, *i.e.*, are small molecules having an hydrophobic ring bound to a short chain with a charged amino group. However, chlorpromazine and several other related phenothiazines are know to alter the shape of human erythrocytes [11, 12] and change their osmotic fragility [13]. It has been suggested that the effect of the drug on the erythrocytes might arise from a different uptake of the drug by the two monolayers of the bilayer [14], by the formation of an intrabilayer nonbilayer phase [11] or that the drug may alter the protein-lipid interactions releasing the phosphatidylethanolamines from the inner monolayer [12]. On the other hand it has been reported that this type of drugs depresses the phase-transition temperature of liposomes prepared from different phospholipids [15, 16].

In this paper are presented the results obtained from the interaction of chlorpromazine · HCl (CPZ · HCl) with bilayers made of dimyristoylphosphatidylethanolamine (DMPE) and dimyristoylphosphatidylcholine (DMPC). These phospholipids – which have been extensively studied in our laboratory [2–9] – only differ in their terminal amino groups (NH_3^+ in DMPE and $N(CH_3)_3^+$ in DMPC). Their interactions were studied by X-ray powder diffraction, at room humidity and temperature, on dry and water containing samples with different molar proportions of the drug and each phospholipid.

Materials and Methods

Synthetic L-α-dimyristoylphosphatidylethanolamine (DMPE) from Calbiochem (Lot 405416),

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L-α-dimyristoylphosphatidylcholine (DMPC) from SIGMA (Lot 81 F – 8365) and chlorpromazine · HCl (CPZ · HCl), a gift from Silesia Labs, were used without further purification. Powder mixtures of CPZ · HCl with DMPE and DMPC were prepared in the molar ratios of 10:1, 5:1, 1:1 and 1:5. They were dissolved in chloroform UVASOL from Merck and left to dry very slowly and carefully in order to facilitate their possible interactions. The residues, in the form of crystalline powders, were introduced in low absorbing 0.5 mm diameter X-ray capillaries, sealed and mounted in 114.6 mm diameter Debye-Scherrer powder cameras. Other samples, prepared in the same way, were put into 1.5 mm diameter capillaries and then a 20% in weight or excess of liquid water was added. X-ray diffraction of these specimens were obtained in flat-plate cameras provided with 0.25 mm diameter glass collimators [7] until equilibrium was reached. The same procedure was followed with pure samples of each phospholipid and the drug. Period Ni-filtered CuKα radiation was used. The relative intensities of the reflections were measured from the films in a Joyce-Loebl MK III CS microdensitometer. All experiments were carried out at room humidity and temperature (50–70% r.h. and 19 °C ± 2 °C).

Results

Fig. 1 shows the microdensitograms of DMPE and CPZ · HCl pure and dry as well as of their mixtures in the molar ratios of 10:1, 5:1, 1:1 and 1:5, while their observed interplanar spacings and relative intensities are presented in Table I.

Fig. 2 and Table II show the same type of information for DMPC and CPZ · HCl. The analysis of both figures and tables indicates that all the reflections observed in the mixtures belong to either the corresponding phospholipid or to CPZ · HCl. In fact, their spacings are practically the same when pure or mix-

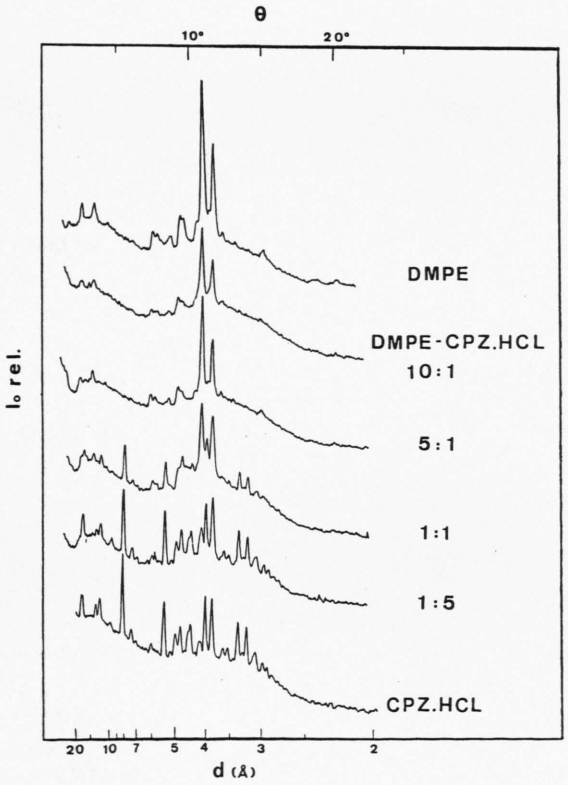

Fig. 1. Densitometer traces of X-ray powder diffraction patterns of dry DMPE, CPZ · HCl and molar mixtures.

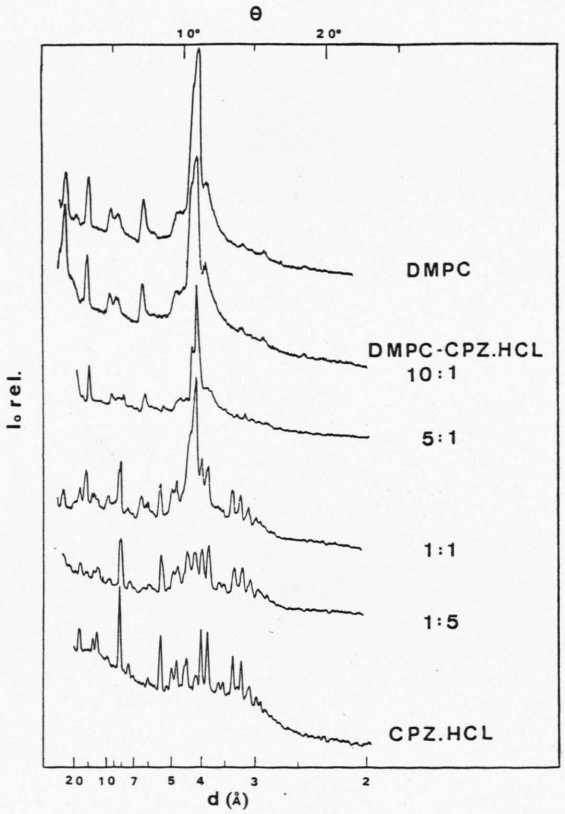

Fig. 2. Densitometer traces of X-ray powder diffraction patterns of dry DMPC, CPZ · HCl and molar mixtures.

Table I. Comparison of observed spacings (do) and relative intensities (Io) of dry powders of DMPE and CPZ · HCl and of their molar mixtures[a, b].

DMPE		DMPE : CPZ · HCl 10:1		5:1		1:1		1:5		CPZ · HCl	
do [Å]	Io	do [Å]	Io	do [Å]	Io	do [Å]	Io	do [Å]	Io	do [Å]	Io
51.4	[c]	51.4	[c]	51.4	[c]	51.4	[c]	51.4	[c]		
17.0	49	17.3	32	17.0	32	17.5	5	17.7	9		
		15.1	13	15.2	4	15.9	65	15.9	86	15.9	73
12.8	49	12.8	34	12.8	33	12.8	24	12.7	18		
								11.8	34	11.9	30
						11.2	24	11.1	59	11.1	69
10.2	10	9.94	6	10.2	3	10.3	6				
						9.41	3	9.41	15	9.37	20
						7.90	106	7.94	199	7.90	189
7.31	7	7.31	9	7.31	8						
						7.23	15	7.17	39	7.17	26
				6.74	2	6.84	5	6.81	11	6.81	16
				6.35	4	6.37	4	6.42	4	6.42	5
										6.13	5
5.94	68	5.99	32	5.94	43	5.91	29	5.91	25	5.87	27
5.72	43	5.75	20	5.74	23	5.72	24	5.72	16	5.68	9
5.52	13	5.50	2	5.47	10	5.50	2	5.50	5	5.50	3
5.28	9	5.31	2	5.25	11	5.30	66	5.28	166	5.28	191
5.11	35	5.14	12	5.10	24	5.07	4				
								5.01	5	5.01	10
4.79	84	4.82	54	4.79	70	4.81	51	4.84	45	4.82	63
4.66	75	4.68	25	4.65	49	4.66	50	4.65	63	4.65	76
						4.57	20	4.54	8	4.53	7
4.48	5			4.46	2	4.44	9	4.42	50	4.42	62
4.26	65	4.30	5	4.25	30	4.33	23	4.34	60	4.33	77
						4.23	2	4.23	4	4.22	8
4.06	598	4.09	338	4.05	297	4.07	279	4.09	93	4.09	37
3.93	59	3.90	2	3.93	5	3.96	42	3.96	127	3.95	143
3.83	310	3.84	166	3.83	152	3.83	180	3.81	158	3.80	162
3.65	19	3.65	13	3.65	18						
3.53	7			3.52	8	3.60	8	3.58	30	3.57	33
						3.50	13	3.49	28	3.48	30
3.41	10	3.42	5	3.40	10	3.40	2	3.39	3	3.37	8
						3.31	40	3.31	104	3.31	102
						3.24	3	3.24	6	3.24	7
3.20	7	3.20	6	3.18	24	3.18	40	3.17	68	3.17	90
						3.06	27	3.05	45	3.05	57

[a] The spacings and intensities were measured from diagrams obtained from Debye-Scherrer powder cameras, except those indicated as c.
[b] Several additional reflections below 3.0 Å were also observed.
[c] Very intense reflection measured from flat-plate diagrams. D = 14 cm.

ed, varying only their intensities as a function of their respective phase concentration. On the other hand, the reflections of both phospholipids, either pure or mixed with the drug, indicate that they present the bilayer structure, which have been described elsewhere [6, 7]. This proves, unambiguously, that the drug does not chemically interact with any of the phospholipids involved neither intercalates in their bilayers in the absence of water. Otherwise, different or at least additional reflections would have been observed. About the same results were obtained when water was added to the mixtures of DMPE with CPZ · HCl. Table III shows a comparison between spacings and intensities of the 1:1 mixture containing an excess of liquid water after eleven weeks with those of the pure lipid under the same conditions, while their X-ray diagrams are shown in Fig. 3. From them it can be seen that: a) most of

Table II. Comparison of observed spacings (do) and relative intensities (Io) of dry powders of DMPC and CPZ · HCl and of their molar mixtures[a, b].

DMPC		DMPC : CPZ · HCl								CPZ · HCl	
		10:1		5:1		1:1		1:5			
do [Å]	Io	do [Å]	Io	do [Å]	Io	do [Å]	Io	do [Å]	Io	do [Å]	Io
55.2	[c]	54.5	[c]	54.5	[c]	54.5	[c]	54.5	[c]		
27.4	262	26.9	271	27.6	18	27.4	78	27.3	43		
18.5	40	18.4	70	18.3	13	18.4	20	18.6	6		
15.6	10	15.6	11	15.9	7	15.9	29	16.1	53	15.9	88
13.7	270	13.7	238	13.7	64	13.7	143	13.8	19		
						12.0	12	12.1	22	11.9	36
				11.1	5	11.2	40	11.1	57	11.1	84
9.31	139	9.31	112	9.35	23	9.31	47	9.35	26	9.35	24
8.26	99	8.27	89	8.30	13	8.34	6				
				7.90	19	7.90	165	7.97	238	7.90	227
7.31	6	7.28	7								
				7.19	12	7.16	30	7.22	36	7.17	31
6.81	5	6.77	5	6.77	9	6.83	12	6.86	15	6.81	19
6.25	278	6.23	206	6.28	51	6.24	90	6.26	16		
										6.13	6
				5.84	8	5.87	23	5.92	31	5.87	32
										5.68	11
5.63	26	5.64	24	5.61	8	5.62	12				
										5.50	4
				5.28	17	5.29	177	5.31	164	5.28	229
5.17	12	5.14	16								
						5.02	8	5.07	7	5.01	12
4.76	96	4.74	60	4.79	13	4.81	102	4.86	80	4.82	76
				4.70	21	4.67	100	4.67	92	4.65	91
								4.51	9	4.53	8
				4.43	6	4.44	29			4.42	74
								4.37	186		
4.27	762	4.25	744	4.31	143	4.31	146			4.33	92
4.13	1015	4.11	840	4.14	298	4.12	272	4.16	235	4.22	10
										4.09	44
				3.95	17	3.94	27	3.97	210	3.95	172
3.88	89	3.87	82	3.89	20						
				3.80	10	3.81	64	3.83	208	3.80	194
3.68	42	3.67	10								
3.59	8			3.60	9	3.58	17	3.60	35	3.57	40
				3.47	7	3.49	20	3.50	32	3.48	36
3.36	8					3.40	6	3.42	4	3.37	10
				3.30	14	3.31	92	3.33	101	3.31	122
						3.24	8	3.25	9	3.24	8
3.18	38	3.17	29	3.17	11	3.17	81	3.19	108	3.17	108
3.05	16	3.05	10	3.04	10	3.06	24	3.07	28	3.05	68

[a] The spacings and intensities were measured from diagrams obtained from Debye-Scherrer powder cameras, except those indicated as c.

[b] Several additional reflections below 3.0 Å and one of 40.5 Å in the mixtures 10:1 and 1:5 were also observed.

[c] Very intense reflection measured from flat-plate diagrams. D = 14 cm.

DMPE spacings and intensities are similar when pure or mixed with the drug; b) despite the high solubility in water of CPZ · HCl some of its reflections are still present in its 1:1 mixture with DMPE, and c) pure and mixed DMPE with excess of water show similar spacings as the dry samples (Table I) although differences in intensities, due to incorporated water, can be observed.

The results observed in DMPC alone and in its mixture with the drug are very different if water is present. As it can be seen in Fig. 4, the X-ray diagrams of DMPC and its 1:1 mixture with CPZ –

Table III. Comparison of observed spacings (do) and relative intensities (Io) of DMPE and DMPE:CPZ·HCl 1:1 in powder with an excess of water[a, b].

DMPE		DMPE:CPZ·HCl 1:1	
do [Å]	Io	do [Å]	Io
51.5	[c]	51.5	[c]
25.8	15	25.5	13
16.9	28	17.0	28
12.8	59	12.7	47
–	–	11.3	4[d]
10.2	7	10.3	10
–	–	9.51	3[d]
–	–	7.97	6[d]
7.36	12	7.30	11
5.97	23	5.94	32
5.72	6	5.72	15
–	–	5.46	4[d]
5.14	25	5.13	29
4.80	50	4.78	38
4.64	6	4.63	39
4.22	1	4.22	18
4.06	182	4.05	261
3.83	88	3.81	72
3.65	8	3.65	16
–	–	3.50	8[d]
3.39	14	3.39	16[d]
–	–	3.29	4[d]
3.20	6	3.17	11[d]
–	–	3.07	12[d]

[a] The spacings and intensities were measured from flat-plate diagrams. D = 6 cm.
[b] Several additional reflections below 3.0 Å were also observed.
[c] Very intense reflection.
[d] Reflections that correspond to CPZ·HCl.

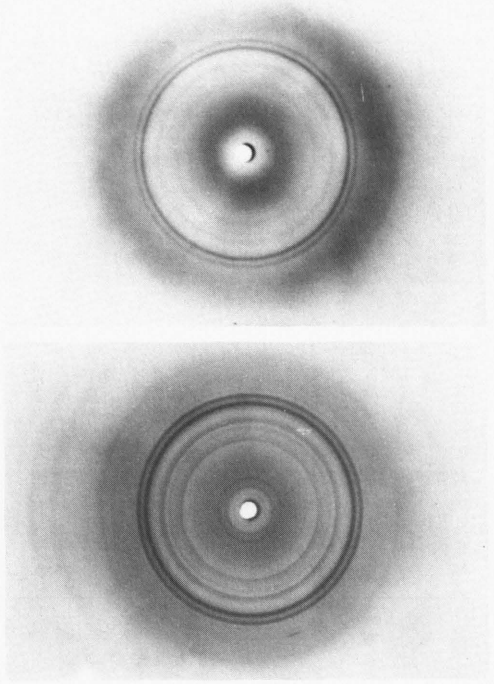

Fig. 3. X-ray powder diagrams of samples containing an excess of water. (a) DMPE; (b) DMPE:CPZ·HCl 1:1. Flat-plate camera, D = 6 cm.

both containing 20% by weight of water – show only a few reflections. However, while those of DMPC indicate an increase of the bilayer width from 55 Å when dry (Table II) to 58 Å when wet, that of the mixture contracts to 42 Å. Moreover, the latter diagram does not show the intense and well defined reflection of 4.2 Å exhibited by the X-ray pattern of DMPC. Instead, a rather broad and diffuse ring of 4.5 Å is now present. A further expansion of DMPC bilayer width to 63 Å is produced when in contact with an excess of water. On the other hand, its 1:1 mixture with the drug fails to show any reflection under this condition except the diffuse scattering produced by the liquid water (Fig. 5). These results

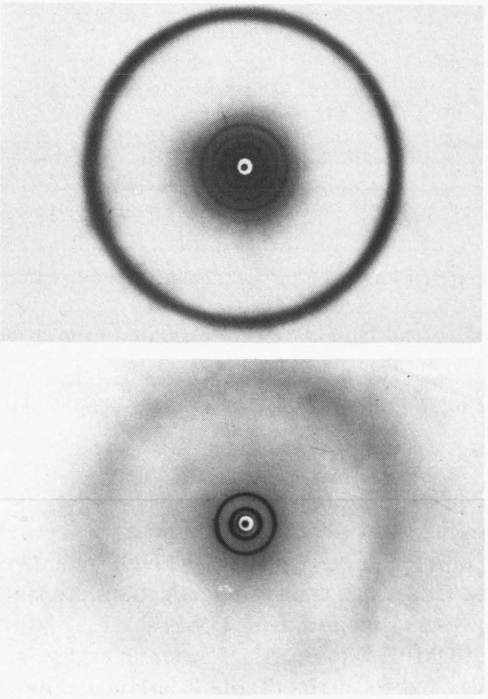

Fig. 4. X-ray powder diagrams of samples containing 20% by weight of water. (a) DMPC; (b) DMPC:CPZ·HCl 1:1. Flat-plate camera, D = 9 cm.

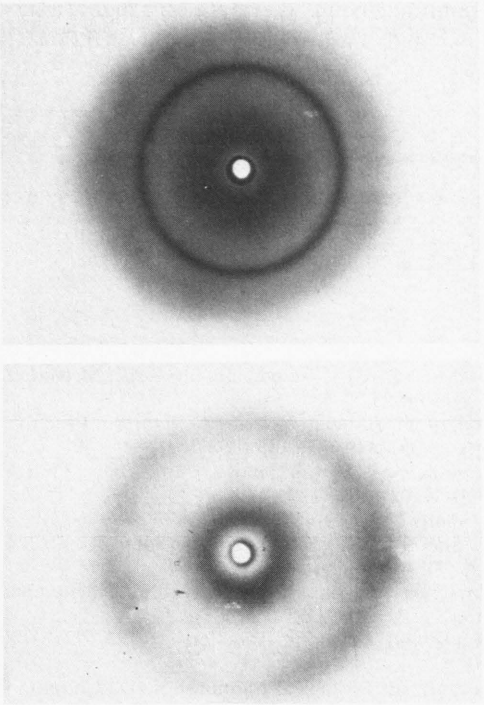

Fig. 5. X-ray powder diagrams of samples containing an excess of water. (a) DMPC; (b) DMPC:CPZ·HCl 1:1. Flat-plate camera, D = 6 cm.

clearly indicate that CPZ·HCl is able to disrupt the organized structure of DMPC bilayers when water is present.

Discussion

The phospholipids DMPE and DMPC have been extensively studied in our laboratory [2–9]. They form bilayers structurally related to those present in biological membranes. Therefore they constitute useful models for studying the way drugs and biologically relevant chemicals affect the structure of cellular membranes, particularly their lipidic part. That is the reason why CPZ·HCl, a widely used drug and known to alter the shape and other properties of erythrocytes, was made to interact with DMPE and DMPC bilayers.

The observed results clearly indicate that CPZ·HCl does not affect the bilayer structure of DMPE, even if there is an excess of water or, when dry, at a molar ratio as high as five times that of the lipid. This can be explained on the basis of the great stability of DMPE bilayers. In fact, the molecules pack very tightly due to the large number of hydrophobic and electrostatic interactions and the small size of their polar groups, remaining unchanged even when there is a large excess of water [7]. On the other hand, DMPC bilayers although less tightly packed than DMPE [6], neither is affected by CPZ·HCl in a chloroform solution or when dry. However, in the presence of water the structure of DMPC bilayers is deeply affected by CPZ·HCl. In fact, when the water concentration is as low as 20% by weight, the X-ray diagram of the 1:1 mixture shows only the first and second order reflections of a 42 Å bilayer repeat (Fig. 4b). Under the same conditions DMPC exhibits the six first orders of a bilayer repeat of 58 Å (Fig. 4a). Besides, the 4.2 Å reflection – which corresponds to the average separation of the extended and hexagonally packed hydrocarbon chains – is replaced in the mixture for a 4.5 Å diffuse ring, indicating a liquid – like conformation of the chains [17]. Now, when an excess of water is added, the mixture does not show any reflection due to the lipid or CPZ·HCl but the scattering due to the liquid water (Fig. 5b). Under these conditions DMPC shows about the same reflections as with 20% of water (Fig. 5a), but the bilayer separation increases to 63 Å. These results clearly indicate that CPZ·HCl is able to destroy the organized structure of DMPC bilayers when water is present. This effect might be explained, in the first place, by the fact that DMPC molecules are less tightly packed than those of DMPE, given the weaker electrostatic interactions and larger size of its head group. Therefore, as water is added it produces a gradual separation of DMPC bilayers [3]. When water reaches a concentration of 20% CPZ·HCl has been dissolved and the molecules might attach to the bilayer in such a way that the protonized amino groups can interact with the negatively charged phosphates and the aromatic rings can be directed towards the hydrophobic part of the phospholipid bilayer [15, 16]. This kind of intercalation of the drug might be responsible for the fluidity changes reported in liposomes [15, 16, 18].

The 13 Å decrease in DMPC bilayer width might be explained by the resulting liquid-like conformation of opposing hydrocarbon chains or, as it has been suggested, by their mutual interdigitation [19]. If the latter is true then the penetration would be rather deep, of about ten methylene groups (considering 2.5 Å the length of two CH_2 groups). In any

case, the addition of an excess of water would neutralize the weakened interbilayer electrostatic attractions inducing a larger separation and rotational disorder of the bilayers. This could explain the abscence of reflections from DMPC.

On the basis of these considerations it can be concluded the CPZ · HCl modifies the shape of erythrocytes because of its interaction with DMPC. This phospholipid is located in the external monolayer of the cell membrane being, therefore, in a highly aqueous media and easily accessible to CPZ · HCl molecules.

Acknowledgements

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