Structure of an adsorbed dimyristoylphosphatidylcholine bilayer measured with specular reflection of neutrons

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ABSTRACT Using specular reflection of neutrons, we investigate for the first time the structure of a single dimyristoylphosphatidylcholine bilayer adsorbed to a planar quartz surface in an aqueous environment. We demonstrate that the bilayer is strongly adsorbed to the quartz surface and is stable to phase state changes as well as exchange of the bulk aqueous phase. Our results show that the main phase transition is between the L_α phase and the metastable $L_{\beta'}^*$ phase, with formation of the $P_{\beta'}$ ripple phase prevented by lateral stress on the adsorbed bilayer. By performing contrast variation experiments, we are able to elucidate substantial detail in the interfacial structure. We measure a bilayer thickness of 43.0 ± 1.5 Å in the L_α phase (T = 31°C) and 46.0 ± 1.5 Å in the $L_{\beta'}^*$ phase (T = 20°C). The polar head group is 8.0 ± 1.5 Å thick in the L_α phase. The water layer between the quartz and bilayer is 30 ± 10 Å for the lipid in both the L_α and $L_{\beta'}^*$ phase. Our results agree well with those previously reported from experiments using lipid vesicles and monolayers, thus establishing the feasibility of our experimental method.

INTRODUCTION

To facilitate the investigation of biological membrane processes, there is a need for model lipid systems that closely parallel such membranes. In the past, investigators have often relied on lamellae, monolayers, or vesicles, but all of these systems have disadvantages in their ability to mimic a biological membrane. The obvious drawback of lamellae is that they consist of many bilayers and are complicated by interbilayer interaction. Monolayers are likewise an unsatisfactory substitute for the bilayer structure of biological membranes. It is possible to prepare single-bilayer vesicles, but they generally have high curvature and are difficult to characterize exactly. We propose that a better model system for structural studies is a single phospholipid bilayer adsorbed to a planar solid surface submerged in an aqueous phase. This system has the desired unilamellar structure and has the advantage of being geometrically well defined. Supported bilayers have been used as model systems in the past (1, 2), but there has been a need for a sensitive experimental probe to elucidate details of the adsorbed bilayer structure. In this communication, we demonstrate that specular reflection of neutrons fulfills this need.

Specular reflection of neutrons is a relatively new experimental technique for measuring interfacial structure, but it has already been shown to be applicable to both vapor/liquid (3–5) and liquid/solid interfaces (6). The intensity of neutrons specularly reflected from a surface yields information on the scattering-length-density profile normal to the surface, from which the structure at the interface can be deduced. Neutrons provide a distinct advantage over x-rays, in that they interact markedly differently with hydrogen and deuterium. Thus by selective deuteration of a lipid, one can probe different segments in a lipid bilayer.

We demonstrate in the following that our experimental method provides a sensitive measure of structural changes in a phospholipid bilayer across phase transitions. We are also able to measure the thickness of the water layer separating the adsorbed bilayer from the solid surface. Furthermore, extension of this work to study lipid/protein interaction is straightforward, as protein can readily be exposed to the adsorbed bilayer from the aqueous phase.

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MATERIALS AND METHODS

We performed the experiments at the D17 spectrometer at the Institut Laue-Langevin in Grenoble, France. We measured the intensity of specularly reflected neutrons of wavelengths 12 and 30 Å at grazing angles between 0.9 and 5.8 degrees, thus reaching momentum transfer normal to the surface up to 0.11 $Å^{-1}$. Fig. 1 is a schematic diagram of the sample cell and scattering geometry as seen from above. The sample cell consists of a 10 × 5 × 1-cm Suprasil quartz block (Hellma, UK) sealed with a Viton O-ring to a Teflon box. The quartz block was cleaned by sputtering with argon and rinsed with ultra-clean water (Elga UHQ) immediately before use. The lipid bilayer is adsorbed to the quartz block and the cell is filled with water. Neutrons pass through the length of the block, being reflected from the quartz/water interface.

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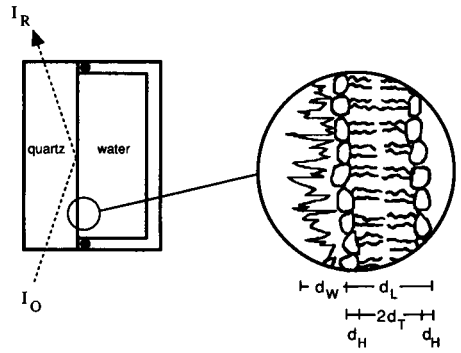

FIGURE 1 Schematic diagram of the sample cell as seen from above. The incident neutron beam, I_o, passes through the quartz block and is reflected at the quartz/water interface as I_R. The enlargement depicts the lipid bilayer adsorbed at the quartz surface. The thicknesses of the various layers are indicated: d_H is thickness of the polar head group, d_T is thickness of the hydrocarbon tail group, d_W is thickness of the water layer, and d_L is thickness of the entire lipid bilayer.

We purchased Lα-dimyristoylphosphatidylcholine (DMPC) and DMPC with perdeuterated hydrocarbon chains (DMPC-d_{54}) from Avanti Polar Lipids, Inc. (Birmingham, AL). We prepared a solution of small, unilamellar vesicles by sonication of 20 mg lipid in 2 ml water for ~25 min using a Branson tip sonicator operated in pulsed mode with 50% duty cycle at 30 W output. We diluted the resulting vesicle solution to a concentration of 0.2 mg DMPC/ml water and adjusted the temperature to 31°C before filling the sample cell, also held at 31°C. Upon filling the sample cell, the vesicles spontaneously collapse at the quartz surface and form a stable bilayer separated from the surface by a thin water layer (7). The bilayer formation is complete within several minutes.

We performed experiments at two different temperatures, $T = 31°C$ and $T = 20°C$, above and below the main phase-transition temperature for DMPC, $T_m = 24°C$. We studied the system under three contrasts: Case I, DMPC in a 19/31 wt/wt H_2O/D_2O mixture approximately contrast matched to quartz (CMQ water); Case II, DMPC in D_2O; and Case III, a 49/51 wt/wt DMPC/DMPC-d_{54} lipid mixture such that the hydrocarbon tails contrast matched the quartz in CMQ water. We measured the reflectivity for all three contrasts at $T = 31°C$, but studied only the first two cases at $T = 20°C$ due to beam time limitations. We analyzed the data as previously reported using the optical matrix method (8–10). The fits to the data are generally not unique, but by studying the sample under different contrasts, we are able to determine the correct fitting parameters because the same model for the interfacial structure must apply at all contrasts.

To establish reproducibility of the measurements and integrity of the bilayer, we conducted the following set of experiments. We first measured the reflectivity at $T = 31°C$ with the vesicle solution in CMQ water remaining in the sample cell. We then lowered the temperature to $T = 20°C$ and held the sample at this temperature for ~4 h. We raised the temperature back to 31°C and repeated the first measurement. We then replaced the vesicle solution with pure CMQ water and once again repeated the reflectivity measurements. The reflectivity data for a given Q in all three experiments (all at the same temperature) differ by at most ±8%. Hence, the bilayer remains tightly adsorbed to the quartz in spite of phase state changes and exchange of the bulk aqueous phase, and the presence of vesicles in the aqueous phase does not alter the reflectivity data.

RESULTS

The structure of a bilayer adsorbed at the quartz/water interface can be modeled as a series of discrete layers, each having a different thickness and scattering length density. Roughness parameters can be included to account for interfacial surfaces that are not perfectly smooth. Thus the parameters in the fitting procedure include the number of layers, the thickness and scattering length density of each layer, and the roughness at each interface. In fitting the data, we began by assuming the simplest possible model and increased the number of parameters until we obtained a satisfactory fit. We found that the data could be fitted well without including interfacial roughness, indicating that the effects of the adsorbed bilayer overshadow those of interfacial roughness. When we nevertheless include interfacial roughness of 17 Å, e.g., the roughness we measured for the clean quartz surface, the changes in the fitted parameters to produce the same quality fit remain within the range of experimental error. Therefore, in the following, we ignore interfacial roughness when fitting the reflectivity data.

We expect that the lipid bilayer is separated from the quartz surface by a thin water layer, and that the head groups are hydrated. An alternative model has been proposed (6) for a surfactant adsorbed to a quartz surface, in which the head groups are in direct contact with the quartz, and water in the head-group region is concentrated in patches between dehydrated head groups. While our reflectivity data can be fitted assuming this model, we reject this model for an adsorbed lipid bilayer on two grounds. First, because lipids adsorbed to a quartz surface are known to diffuse laterally (7), the head groups cannot be in direct contact with the quartz, but must be separated by a thin water layer as represented in Fig. 1. Second, the choline head group of DMPC is zwitterionic and thus provides a favorable environment for water molecules. Therefore, we do not expect patches of water between dehydrated head groups, but believe that there is substantial penetration of water into the head groups themselves.

Fig. 2 shows the theoretical scattering-length-density profiles for each of the three different contrasts studied. Table 1 gives the theoretical values for the scattering length densities. The difference between the scattering length densities of the head group and hydrocarbon tail group is relatively small, so we assume that both head and tail groups constitute a single uniform layer. In case I, where the water is contrast matched to quartz, the model consists simply of this single lipid layer. The aqueous phase for case II is pure D_2O, which gives a

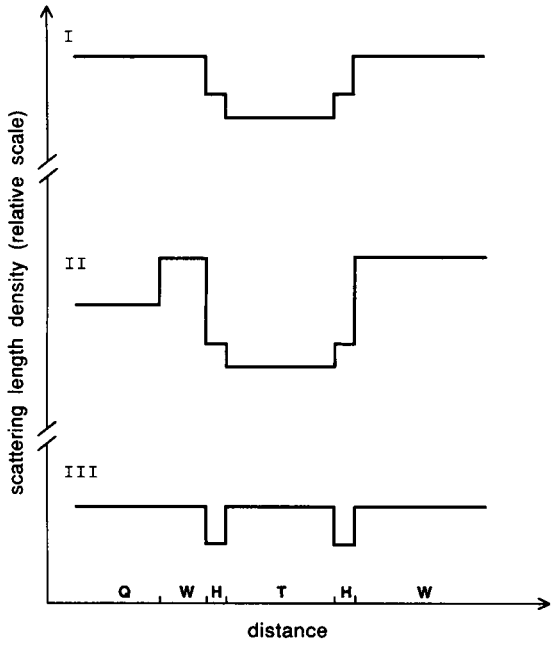

FIGURE 2 Theoretical scattering-length-density profiles for the three cases studied. Layers of differing scattering length density are indicated on the abscissa: Q, quartz block; W, water; H, polar head group; T, hydrocarbon tail groups. Case I corresponds to DMPC in an aqueous phase of water contrast matched to the quartz (CMQ water), while the aqueous phase is D_2O in case II. Case III portrays a mixture of DMPC/DMPC-d_{54} having hydrocarbon tails contrast matched to the quartz in an aqueous phase of CMQ water.

large contrast to lipid and quartz, and we need a two-layer model (consisting of a water layer and a lipid layer) to fit the data. Case III is an extension of case I, but here the hydrocarbon tail region is also contrast matched to quartz. Consequently, only the two head-group regions contribute to reflectivity, so we assume a three-layer model (head group 1, hydrocarbon tail

TABLE 1 **Theoretical scattering length densities calculated from the scattering length density of individual atoms (11)**

Material	Scattering length density
	10^{-6} Å^{-2}
DMPC	0.28
DMPC head group	1.20
DMPC tail group	−0.49
D_2O	6.35
quartz	3.41*
CMQ water	3.52

The specific volume of DMPC was taken as 0.97 (12).
*Measured value.

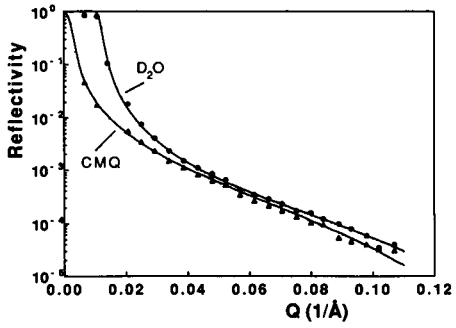

FIGURE 3 Reflectivity data as a function of momentum transfer Q for a bilayer of DMPC at T = 31°C. Data for two different cases are shown: Case I with CMQ water as the aqueous phase (▲), and case II with D_2O as the aqueous phase (●). The solid lines are best fits to the data, and the parameters used in the fitting routine are reported in Table 2.

region, and head group 2), in which the first and third layers are identical.

Best fits to the reflectivity data for DMPC at T = 31°C are shown in Fig. 3, where data are displayed for both CMQ water (case I) and D_2O (case II) aqueous phases. The reduction in reflectivity when using CMQ water as compared with D_2O is obvious and indicates the sensitivity of the measurements to contrast variation. Fig. 4 reveals the sensitivity of the fits to variation of the fitted parameters.

Table 2 summarizes the fitted parameters for all experiments. The fitted scattering length densities differ from the theoretical values in Table 1, suggesting that some mixing of the layers occurs. In case II, for instance, the fitted scattering length density ρ_w for the D_2O layer between the quartz and lipid is found to be $\sim 3.7 \times 10^{-6}$ Å^{-2} in contrast to the theoretical value for D_2O of 6.35×10^{-6} Å^{-2}. This indicates that the so-called water layer is not pure, but actually consists primarily of quartz, which has a scattering length density of 3.41×10^{-6} Å^{-2}. This is expected since quartz surfaces are not perfectly smooth and water fills the pits in the jagged surface. Including quartz roughness of 17 Å in the fitting routine as smearing of the scattering length density at the quartz/water interface does not alter the quality of fit or the fitted parameters within experimental error. Hence, the D_2O water layer is satisfactorily modeled as having sharp boundaries and with ρ_w intermediate between pure D_2O and quartz.

The thickness of the water layer can be determined from case II because the D_2O water phase provides a strong contrast to both quartz and lipid. We measure a thickness d_w of 30 ± 10 Å at both T = 31°C and T = 20°C. This corresponds well with recent data collected in

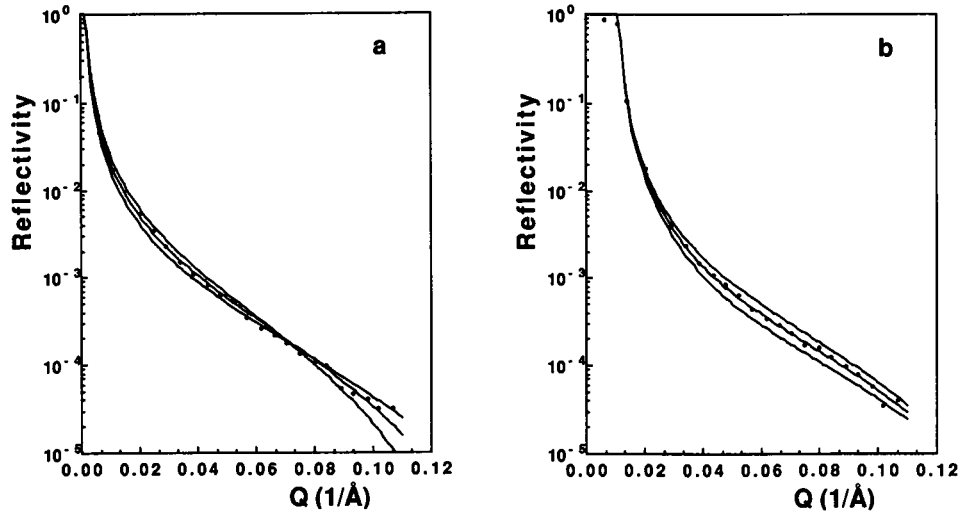

FIGURE 4 Reflectivity data for DMPC at T = 31°C showing the sensitivity of the fits to variation of the fitted parameters. (*a*) Case I, for which CMQ water is the aqueous phase. Fitted curves correspond to lipid thickness d_L = 38, 43 (best fit), and 48 Å with other fitting parameters held constant. (*b*) Case II, for which D_2O is the aqueous phase. Fitted curves correspond to ρ_L = 1.6, 2.0 (best fit), and 2.4 × 10^{-6} $Å^{-2}$ with other fitted parameters held constant. The values for the fitted parameters that we held constant are shown in Table 2.

a nuclear magnetic resonance study on single bilayers of DMPC adsorbed to micron-size glass spheres, in which a thickness of 17 ± 5 Å for the water layer was measured (13).

The fitted values for scattering length density of the lipid bilayer in Table 2 are higher than the theoretical values in Table 1, indicating that water penetrates the lipid layer. Consequently, ρ_L for cases I and II are different because the water that penetrates the lipid is an H_2O/D_2O mixture in case I, but pure D_2O in case II. Using data from case II where the contrast between the D_2O water phase and lipid is more pronounced, we calculate the volume fraction of water α in the lipid layer from

$$\rho_L^{fit} = \alpha\rho_{D_2O} + (1 - \alpha)\rho_L^{theory}. \quad (1)$$

TABLE 2 Experimental results for DMPC

Case	T	d_L	ρ_L	d_W	ρ_W	d_H	ρ_H	d_T	ρ_T
	°C								
I	31	43	0.7	—	—	—	—	—	—
II	31	43	2.0	30	3.7	—	—	—	—
III	31	43	—	—	—	8	1.6	13.5	3.5
I	20	46	0.7	—	—	—	—	—	—
II	20	46	2.1	30	3.7	—	—	—	—

Lipid dimensions d_L, d_H, and d_T are reported in Ångstroms with an error of ±1.5 Å, while the error in the water layer thickness d_W is ±10 Å. The scattering length density ρ is reported as 10^{-6} $Å^{-2}$ with an error of ±0.2 × 10^{-6} $Å^{-2}$. The three cases I, II, and III representing different constrasts are defined in the text. The dashes indicate that the quantity could not be determined under the contrast of that particular case.

This yields a water content of approximately α = 30% at both T = 31°C and T = 20°C, indicating that there is surprisingly no difference in the degree of head-group hydration across the main phase transition for an adsorbed bilayer. The data from case I support that the degree of hydration is the same in both phases, although the value of α calculated from case I is only 13%. We plan to perform additional experiments to verify our finding for degree of hydration, as well as to find the reason for the discrepancy in α between cases I and II.

The phase state for DMPC at T = 31°C is the lipid L_α phase, while between the pretransition temperature T = 12°C and the main transition T = 24°C, DMPC normally adopts a ripple structure designated as the $P_{\beta'}$ phase. The ripples have an amplitude of ~15 Å (14) and a wavelength of ~120 Å (15), so this structure would be manifested as a considerable increase in the lipid thickness and a significant smearing of the water/lipid interface as compared with that measured for the L_α phase. Our results shown in Table 2 give no indication for such a drastic structural change. The bilayer thickness d_L at T = 20°C increases by only 3 Å compared with the L_α phase, corresponding to changes normally observed for the transition from the L_α phase to the $L_{\beta'}$ phase (16). We conclude that the adsorbed bilayer does not form the ripple phase $P_{\beta'}$, but instead forms a phase analogous to the low-temperature $L_{\beta'}$ phase. Using micromechanical techniques, Evans et al. (17, 18) were the first to observe such a nonrippled phase at intermediate temperatures (denoted as $L_{\beta'}^*$), and they found that formation of the $L_{\beta'}^*$ phase is possible only when the bilayer is under

stress. In our experiments, the adsorbed bilayer is apparently under sufficient lateral stress to preclude formation of the ripple phase, and the main phase transition occurs between the $L_{\beta'}^*$ and the L_α phase. Our results support the conclusion of Evans et al. that the $L_{\beta'}^*$ phase has the same characteristics as the low-temperature $L_{\beta'}$ phase.

DISCUSSION

Our results provide, for the first time, detailed structural information on a single lipid bilayer adsorbed to a planar solid support. As our experimental approach is novel, we are limited to comparing our results with those obtained using other experimental techniques. The assumptions inherent in each technique vary and also different lipid systems are used, so it is not surprising that the reported results vary somewhat. The thickness $d_L = 43 \pm 1.5$ Å that we measure for DMPC in the L_α phase is in good agreement with the results obtained using unilamellar DMPC vesicles where d_L is reported as 44 Å (12) from small-angle x-ray scattering and 37.3 Å (19) and 41 Å (20) from small-angle neutron scattering. Our result is larger than all those measured for lamellae using x-ray diffraction where d_L is reported as 34.5 Å (21), 35.5 Å (14), and 36 Å (15).

The past small-angle scattering studies with vesicles did not include measurements for the $L_{\beta'}$ phase, so we are restricted to comparison with multilamellar systems measured with x-ray diffraction. We have already noted for the L_α phase that x-ray diffraction experiments yield a smaller thickness d_L as compared with both small-angle scattering results and our reflectivity results. Likewise, d_L measured with x-ray diffraction for DMPC in the $L_{\beta'}$ phase is also smaller than our result, as we measure $d_L = 46 \pm 1.5$ Å as compared with $d_L = 42.4$ Å (14) and 44 Å (15) reported from x-ray diffraction measurements on lamellae. We also wish to make comparisons with experiments on lamellae that employ neutrons, because as in our method, the measurements rely on differences in scattering length density in the sample. Therefore, we refer to Büldt et al. (22, 23), who used neutron diffraction to study the structure of Lα-dipalmitoylphosphatidylcholine (DPPC) lamellae in 25% water. To account for the two additional CH_2 groups in the hydrocarbon tail of DPPC, we subtract 5.1 Å (as per Eq. 2) from their result for bilayer thickness to arrive at a thickness $d_L = 43.7$ Å for DMPC in the $L_{\beta'}$ phase, smaller than our result of 46 ± 1.5 Å, but in agreement with x-ray diffraction results. This suggests that the discrepancy between our thickness measurements and those using lamellae may lie, at least in part, in the structure of the model lipid system itself, as our results agree more closely with those collected for unilamellar vesicles.

There have recently been studies of phospholipid monolayers at the air/water interface using x-ray (24) and neutron reflection (5). In those studies, the thickness of the polar head group was determined. Helm et al. (24) measured a thickness between 7.9 and 8.4 Å for the choline head group of DPPC in the L_α phase. This corresponds well to our measurement of 8 ± 1.5 Å for the choline head group of DMPC. Bayerl et al. (5) used neutron reflection to measure the dimensions of a 7:3 mixture of DMPC/Lα-dimyristoylphosphatidylglycerol (DMPG). They determined a head group thickness of ~8 Å, again in excellent agreement with our result. There is a major structural difference, however, between an adsorbed bilayer and a monolayer at the air/water interface, as Bayerl et al. found that the L_α to $L_{\beta'}$ phase transition is accompanied by a drastic change in head group hydration. We did not observe such changes in our bilayer system across the main phase transition.

It has been shown (16) that the thickness of the choline head group is the same within experimental error for both L_α and $L_{\beta'}$ phases, with the increase in thickness of the $L_{\beta'}$ phase being attributed to freezing of the hydrocarbon chains. Using a head group thickness of 8 Å (measured for the L_α phase), we determine a thickness d_T of ~15 Å for the hydrocarbon tails in the $L_{\beta'}^*$ phase. This can be used to calculate the chain tilt angle because the maximum length of a hydrocarbon tail consisting of n CH_2-groups is given by (25)

$$d_T^{max} = 1.5 + (n)(1.265 \text{ Å}), \quad (2)$$

which for DMPC is 16.7 Å. The chain tilt angle β is given by

$$\cos(\beta) = d_T^{fit}/d_T^{max}, \quad (3)$$

which yields a tilt angle of $26 \pm 7°$ from our measurements. This is in good agreement with Needham and Evans (18), who report a chain tilt angle of 24.2° for DMPC in the $L_{\beta'}^*$ phase at $T = 20°C$. Our result also agrees well with Janiak et al. (15), who measured a tilt angle of ~30° for DMPC at $T = 20°C$.

CONCLUSIONS

We have shown that the specular reflection of neutrons is a powerful technique for measuring the structure of a single phospholipid bilayer adsorbed to a planar solid surface. An adsorbed bilayer is a good model lipid system because it is geometrically well characterized, is stable to phase state changes and bulk water exchange, and it duplicates the unilamellar structure of a biological

membrane. Selective deuteration of the lipid and contrast variation experiments permit accurate determination of the adsorbed bilayer structure including total bilayer thickness, head group thickness, hydrocarbon tail thickness, chain tilt angle, and degree of lipid hydration. We are also able to measure the thickness of the water layer between the solid surface and the adsorbed bilayer. The very good agreement between our measurements and those of other investigators justifies our model for the interfacial structure as a series of discrete layers with sharp boundaries. The experimental technique has potential for examining structural changes induced by the coupling of peripheral proteins or amphiphilic peptides to the bilayer, and such investigations are presently in progress.

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