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Instability of Langmuir-Blodgett Layers of Barium Stearate, Cadmium Arachidate and Tripalmitin, Studied by Means of Electron Microscopy and Infrared Spectroscopy

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Abstract. Results of an investigation of the stability of n-layers of barium stearate, cadmium arachidate and tripalmitin by means of electron microscopy and attenuated total reflection infrared spectroscopy are reported. Odd and even numbered barium stearate n-layers with n = 1,2,3,4,5 are found to rearrange spontaneously from a regular film into ultrastructures of irregular, flat islands of varying thickness. The kinetics of the phase transformation of the first layer depends on the substrate, that of n-layers appears to be dependent on n, the temperature, and the surrounding medium. The kinetic behaviour of odd and even numbered layers is distinctly different. Similar studies on cadmium arachidate layers reveal much slower kinetics of the rearrangement process. In the case of tripalmitin n-layers it is shown that electron microscopy and infrared spectroscopy yield valuable complementary information about ultrastructure and molecular structure of the layers in correlation with the rearrangement process, which also occurs with this system. Consequences of the results of this paper for work published in various fields are briefly discussed.

Key words: Langmuir-Blodgett Layers — Instability — Crystallization — Electron Microscopy — Infrared Spectroscopy.

1. Introduction

The structure of molecular single and multilayers has been the subject of continuous studies since the discoveries made by Blodgett (1935). The same author emphasized that the deposition of the first layer on a solid is of a different character than that of subsequent layers (Blodgett and Langmuir, 1937). Based on electron diffraction patterns of single and multilayers of stearic acid and barium stearate Storks and Germer (1937) and Germer and Storks (1938)postulated that the first layer is closely but irregularly packed with the molecules oriented nearly perpendicular to the substrate surface. The upper layers were found to form a pseudohexagonal planar lattice. Only a few years after the discovery of the Langmuir-Blodgett layers, evidence was obtained indicating that multilayers do not necessarily form a "twodimensional single-crystal" as is often assumed.

As early as 1935 it was known that under certain conditions calcium stearate and barium stearate can overturn in the deposited layer (Blodgett, 1935) so that deposition of successive layers becomes disturbed or even impossible. In the latter case deposition only occurs when dipping the plate, but no substance is attached when it is withdrawn. Blodgett (1935) called these "non-alternating"

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layers x-films, and the "alternating" layers y-films. Langmuir (1938) discussed possible mechanisms of the so called "overturning" of longchain molecules in the layers.

X-ray investigations by Holley and Bernstein (1937) and Bernstein (1938) resulted in the very interesting fact that the longchain spacing of both x- and y-films is approximately the same, and furthermore that this distance is about 2 Å shorter, than that determined by interferometric measurements. Therefore the x-layers must have been completely rearranged after deposition.

More recently several authors have dealt with the phenomena of overturning (Ehlert, 1965; Charles, 1971; Honig, 1973 and Honig *et al.*, 1973). The first evidence for the conversion of Langmuir-Blodgett layers of longchain esters into microcrystals was given by Stenhagen (1938). The final proof of the structure of ethyl stearate was given by Knott *et al.* (1940). By x-ray investigations, these authors showed that freshly deposited multilayers show no microcrystalline structure, but that this appears after $^1/_2$ to 1 hr. The reciprocal lattice of "old" multilayers is furthermore identical with that of a single crystal of the ester, rotated about the long axis of the cell. These observations imply the instability of freshly deposited multilayers of ethyl stearate and their crystallization at random around the axis perpendicular to the substrate surface.

Epstein (1950) was the first to investigate Langmuir-Blodgett layers by means of electron microscopy. He interpreted the irregularities observed as due to micelle formation. Further electron microscopic investigations of monolayers have been carried out by Ries and Kimball (1955). They found that n-hexatriacontanoic acid transferred to collodion exists at low and intermediate film pressure as irregular islands and is continuous only at pressures just below the point of collapse. On the other hand Sheppard *et al.* (1965) reported that monolayers of n-parafinic acids, alcohols and amines are homogeneous when transferred from water to bare or Parlodion-coated glass slides. Stearic acid and barium stearate monolayers transfered between 5 dynes/cm and collapse pressure exhibited homogeneous texture (Sheppard *et al.*, 1964).

In a systematic study Spink (1967) investigated the influence of the substrate material on the structure of stearic acid deposited under various film pressures. From combined electron microscopic and autoradiographic density experiments, monomolecular films on mica were found to form monolayers of uniform thickness, whereas films on quartz or glass formed islands of irregular shape and thickness. No irregular film structure was found on collodion or Formvar substrate.

A pronounced correlation between the transfer ratio and the crystal plane of a silver substrate was found, though no clear differences in film structure were recognizable.

To our knowledge, only few kinetic studies of the rearrangement process of layers of regular thickness into layers of irregular thickness and island structure have been reported. A study in this direction was made by Smith (1967) who investigated the kinetic behaviour of erucic acid in the presence of copper in the subphase. Although the experiments of this author appear difficult to interpret, they do indicate that irreversible transformations of the films occured. As early as 1930 Zocher and Stiebel (1930) demonstrated in a careful study that fast compression of fatty acid films generally produces complicated irreversible coagulation phenomena leading to colloidal particles. It appears possible that Smith's experiments were subject to this phenomenon.

In this paper we report our results from a study of irreversible transformations of the ultrastructure of some lipid layers. Our work was initiated by the observation that tripalmitin layers transferred to germanium substrate undergo irreversible transformations clearly detectable by both internal reflection spectroscopy (ATR-IR) and electron microscopy (EM). Odd numbered layers of barium stearate on substrates of germanium, carbon film and glass, and even numbered layers on carbon film, were shown in the case of $n \geqq 2$ to transform spontaneously into layers composed of flat islands of irregular shape and thickness. The rearrangement progressed through a sequence of ultrastructural changes and lead to a crystalline phase independent of the substrate but kinetically dependent on layer number, surrounding medium (air, water) and temperature. Monolayers on carbon film also exhibited this rearrangement but monolayers deposited on the other substrates were found to be stable under the conditions applied in this study (cf. Table 1). In a comparative study of the kinetics of the rearrangement process of n-layers ($n = 1,3,5$) of barium stearate and cadmium arachidate on glass, the latter is found to transform at a much slower rate. In the case of the transformation process of tripalmitin layers ($n = 4$) deposited on hydrophobized glass, the EM observations are paralleled by synchronous measurements of the ATR-IR spectra. These give a detailed insight into the changes of the molecular conformation accompanying the rearrangement process. The stability of such n-layers will be discussed mainly with regard to the use of oriented layer assemblies as model membranes.

2. Experiments

2.1. Deposition of Monolayers on Solid Substrate

The transfer of a monolayer from an aqueous surface onto a solid substrate was performed analogous to the method worked out by Blodgett (1935). All substrates exhibited a hydrophilic surface when thoroughly cleaned by ultrasonic treatment with methanol and water followed by plasma cleaning[1]. (No cleaning was performed on carbon coated grids.) The first layer is attached when the plate is withdrawn from the subphase through the compressed film, the second layer by dipping the plate in and so on. Therefore the plate has an odd number of layers with a hydrophobic surface when in the air and an even number of layers when submersed in the aqueous subphase.

The film balance was equipped with a servomechanism, allowing the film pressure to be kept constant within 0.15 dynes/cm during the deposition of the film. The temperature of the bath was controlled to ± 0.2 °C, whereas the surrounding atmosphere was at room temperature (25° C). The transfer ratio observed during the formation of layers on glass or germanium plates was approximately unity, whereas the ratio for EM grids could not be checked. However, the layers were formed on EM grids in exactly the same manner as they were deposited on the germanium and glass plates.

All relevant information on the conditions for film preparation is collected in Table 1.

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[1] Harrick Sci. Corp., Croton Dam. Road, Box 867, Ossining, N.Y. 10562, U.S.A.

Table 1. Conditions of sample preparation and storage

Sample (Fig.)	Temperature of subphase[a]	Film pressure (dynes/cm)	Transfer speed[b] (mm/min)	Substrate	Storage Temp. [° C]	Storage Time [hours]	Remarks
1	25	30	2.2	Ge-ATR plate	25	24	
2	18	18	2.7	carbon film	25	0 and 24	EM: Samples frozen in Freon 22 to stop rearrangement process
3	18	18	2.7	carbon film	18	2 and 4	EM: Samples frozen in Freon 22 to stop rearrangement process
4	22.5	30	6.0	glass	25	BaSt 18 CdAr 23	
5	25	25	6.0	carbon film	30	0 and $3^1/_2$	EM: Samples frozen in Freon 22 to stop rearrangement process
6	16	18	2.2	glass hydrophobized with 1 layer BaSt	15 25	0 and 6 60	EM: Samples frozen in Freon 22 to stop rearrangement process
7	16	18	2.2	Ge-ATR plate hydrophobized with 1 layer Ba-Stearate	15	$1^1/_2$, 4 and 22	Internal reflection plate was kept at 15° C during the whole experiment

[a] Composition of subphase: $3 \cdot 10^{-5}$ mol/l $BaCl_2$, $4 \cdot 10^{-4}$ mol/l $KHCO_3$ for all samples except Cd-arachidate layers.
$2 \cdot 10^{-6}$ mol/l $CdCl_2$, $4 \cdot 10^{-4}$ mol/l $KHCO_3$ for Cd-arachidate layers, sample 4.

[b] The deposition onto the Ge-ATR plate took approximately 5 to 10 min per layer.

2.2. *Preparation for Electron Microscopy*

2.2.1. Replication

For EM investigation, the layers were replicated in a Balzers freeze-etch device BA 500 M[2]. Its vacuum chamber was equipped with a brass table cooled by liquid nitrogen. This table was used as a support for the samples during replication. The samples were put on the table at room temperature, then the bell jar was evacuated. During evacuation to a pressure of $2 \cdot 10^{-6}$ Torr the brass table was cooled to a temperature of —100° C. The oil diffusion pump was equipped with a nitrogen cooled baffle. Furthermore an additional cold finger at —170° C was positioned above the object to avoid contamination. Under these conditions no water condensation on the specimen occurs. The shadow-casting of the samples was done by evaporating 2 nm platinum at an angle of 35° to 40° with an electron beam gun (Moor, 1973). The replica thickness was measured with a quartz crystal film-thickness monitor. To reinforce the replica, an additional 20 nm of carbon were evaporated vertical to the object plane. The replicas were then floated onto a clean water surface. Formvar coated EM grids, which were reinforced by 10 nm of carbon, were used to pick up the replicas from the water surface. Cleaning of the replicas was found to be unnecessary.

2.2.2. Preparation of Samples for Kinetic Studies

To achieve rapid fixation of time dependent states of the layers, the samples were frozen in liquid Freon 22 and then transfered to liquid nitrogen. Since freezing should occur rapidly, the samples had to be small. Therefore the layers were deposited directly onto specially prepared EM grids of 2.3 mm diameter. Several rows of EM grids were arranged on a hydrophobized glass slide (10 min treatment with Rhodorsil 5% in chloroform, dried at 60° C and thoroughly cleaned by ultrasonic treatment with ethanol and water) and covered by a film of collodion. The edges of the slide were sealed by dipping in a 2% solution of collodion in butylacetate. Afterwards the whole slide was coated with 10 nm of carbon. Such slides were used for deposition of the lipid layers on the grids. In this way, simultaneous experiments could be made by freezing single grids of the same row at different times after layer deposition.

Under liquid nitrogen, the frozen samples were put on the table of a brass object holder. Still under nitrogen the holder was then closed with a cover with a conical edge to ensure a tight fit. Then it was screwed onto a precooled support in the freeze-etch apparatus (—170° C). The vacuum chamber was evacuated to below 10^{-5} Torr and the object holder warmed up to —90° C. After about 20 min, the outer surface of the closed object holder was free of ice. Then the cover was lifted, exposing the samples on the table. A cold finger was positioned above the samples to sublimate remnants of ice. The samples were then shadow-casted as described[3].

The preparation of even numbered layers required additional manipulations. Whereas in the production of odd numbered layers, the deposition of the last layer

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[2] (Balzers Union Aktiengesellschaft, Postfach 75, FL-9496 Balzers, Fürstentum Liechtenstein).

[3] A more detailed report of this technique and its use for preparation of biological objects will be published elsewhere.

should lead to a dry multilayer, the last layer of an even numbered system should be hydrophilic. In the latter case, the samples were kept under water as long as possible in a Teflon jar. To transfer the sample from the jar to the liquid Freon, the surface of the subphase was first cleaned by sweeping it several times with a Teflon bar. The samples were then taken out of the subphase, excess water was sucked off by filter paper, and the slide transfered, while still wet, into the liquid Freon. The duration of the sublimation of the ice from these samples had to be adapted individually according to the amount of ice covering the layers.

Photography. All the micrographs have been photographically reversed, so that the evaporated platinum appears white whereas the shadows of the structures appear black. To simplify the visual interpretation of the pictures, all the micrographs in the figures are arranged in such a way that the shadow-casting is directed from top left to bottom right.

2.3. Attenuated-Total-Reflection Infrared Spectroscopy

For a detailed discussion of ATR-IR-spectroscopy the reader is referred to Harrick (1967).

The spectra were scanned with a Perkin Elmer Mod. 325 infrared spectrometer, equipped with a KBr-grid polarizer and two internal reflection attachments (Wilks Mod. 9 and Mod. 50). Single pass germanium internal reflection plates (50 × 20 × 1 mm) were used, with an angle of incidence of 30 degrees. By this means one obtains about 85 internal reflections. The plates were thermostated to ± 0.2° C. The mean spectral slit width was 1 cm^{-1}.

2.4. Chemicals

The water used for the film balance was distilled three times: once with $KMnO_4$ followed by a double distillation in a quartz apparatus. Cadmium chloride (AnalaR) was obtained from the British Drug Houses Ltd., Laboratory Chemicals Division, Poole, England, $KHCO_3$ (pro analysis) from E. Merck, Darmstadt, Germany and barium chloride, stearic acid, arachidic acid and tripalmitin (all puriss.) from Fluka AG, Buchs (SG), Switzerland.

3. Results and Analysis of Data

The conditions applied for layer deposition are listed in Table 1.

3.1. Control of Effect of EM *Preparation*

In order to obtain a control for the effect of the shadowing process on the ultrastructure of the layers, ATR-IR spectra were taken on a number of test layers

(i) before and after evacuation
(ii) with and without cooling to —100° C
(iii) before and after shadowing with platinum and carbon.

Tests (i) and (ii) yielded no detectable effects, in particular no detectable loss of substance from the layers. Test (iii) with platinum shadowing could not be carried out, since Pt-shadowing rendered ATR-IR measurement impossible. Test samples with carbon shadowing showed that the condensation of the carbon film produced a slight acceleration of the rearrangement process of the layers.

3.2. Electron Microscopy

3.2.1. Odd Numbered Layers of Ba-Stearate on Germanium Plate (Fig. 1)

Barium stearate layers were transfered to a germanium internal reflection plate in order to be investigated by ATR-IR spectroscopy. Electron micrographs of these samples were produced to get an idea of the quality of both the surface of the germanium plate and the ultrastructure of the deposited layers.

The results are shown in Fig. 1. It should be mentioned that germanium after being polished optically plane still has a rather rough surface with many traces produced during the polishing process (Fig. 1a). In spite of this the transfer ratio is not significantly influenced. The first layer covers the plate homogeneously (Fig. 1b) and can scarcely be distinguished from the blank. Samples with 3 and 5 layers, however, showed distinct inhomogeneities such as patches (P, Fig. 1c) and islands of an unexpected thickness of up to 50 nm (I, Fig. 1d),

3.2.2. Kinetics of the Ultrastructural Rearrangement Process of Odd and Even Numbered Layers of Barium Stearate on Carbon Film

1. Odd Numbered Layers (Fig. 2). The first layer attaches homogeneously to the carbon substrate as was previously observed in the case of the germanium substrate. But in contrast to the latter, it shows continuous transformation into small aggregates (A) of about 100 nm diameter within 24 hrs (Fig. 2a). In samples with 3 layers (Fig. 2b) small aggregates and holes (H) could be seen immediately after sample preparation. In the course of time a continuous formation of flat islands with rounded contours could be observed. These formations reach measurements in the micrometer range.

Samples with 5 layers (Fig. 2c) showed the same features even more pronouncedly than those with 3 layers. In addition, after 24 hrs, the islands had assumed crystalline contours (C). It should be pointed out that the structural transformation of the first layer differs from that of subsequent layers. Fig. 2 shows, that the upper layers form large islands within 24 hrs. However, the characteristic ultrastructure of the first layer is always seen between the islands. Obviously the first layer on carbon film rearranges into small aggregates independent of the total number of layers.

2. Even Numbered Layers (Fig. 3). After 2 hrs of storage submerged in the subphase (cf. 2.2.2) 2 layers of barium stearate have transformed into islands resembling flat droplets, often connected by small bridges (Fig. 3a). Two hours later the contours of the islands show the beginning of crystallization (Fig. 3b). The same experiments performed with 4 layers demonstrate analogous behaviour (Fig. 3c, 3d). The thickness of the islands is about 30 nm, i.e. double that of samples 3a and 3b. It should be noted that the layers stored under water show very different kinetics of ultrastructural transformation than 3 layers exposed to the atmosphere (compare Fig. 2).

3.2.3. Comparison of Barium Stearate and Cadmium Arachidate Layers on Glass (Fig. 4)

1, 3, and 5 layers of both barium stearate (BaSt) and cadmium arachidate (CdAr) were transfered onto glass and stored for 18 hrs (BaSt) and 23 hrs (CdAr) at 25° C.

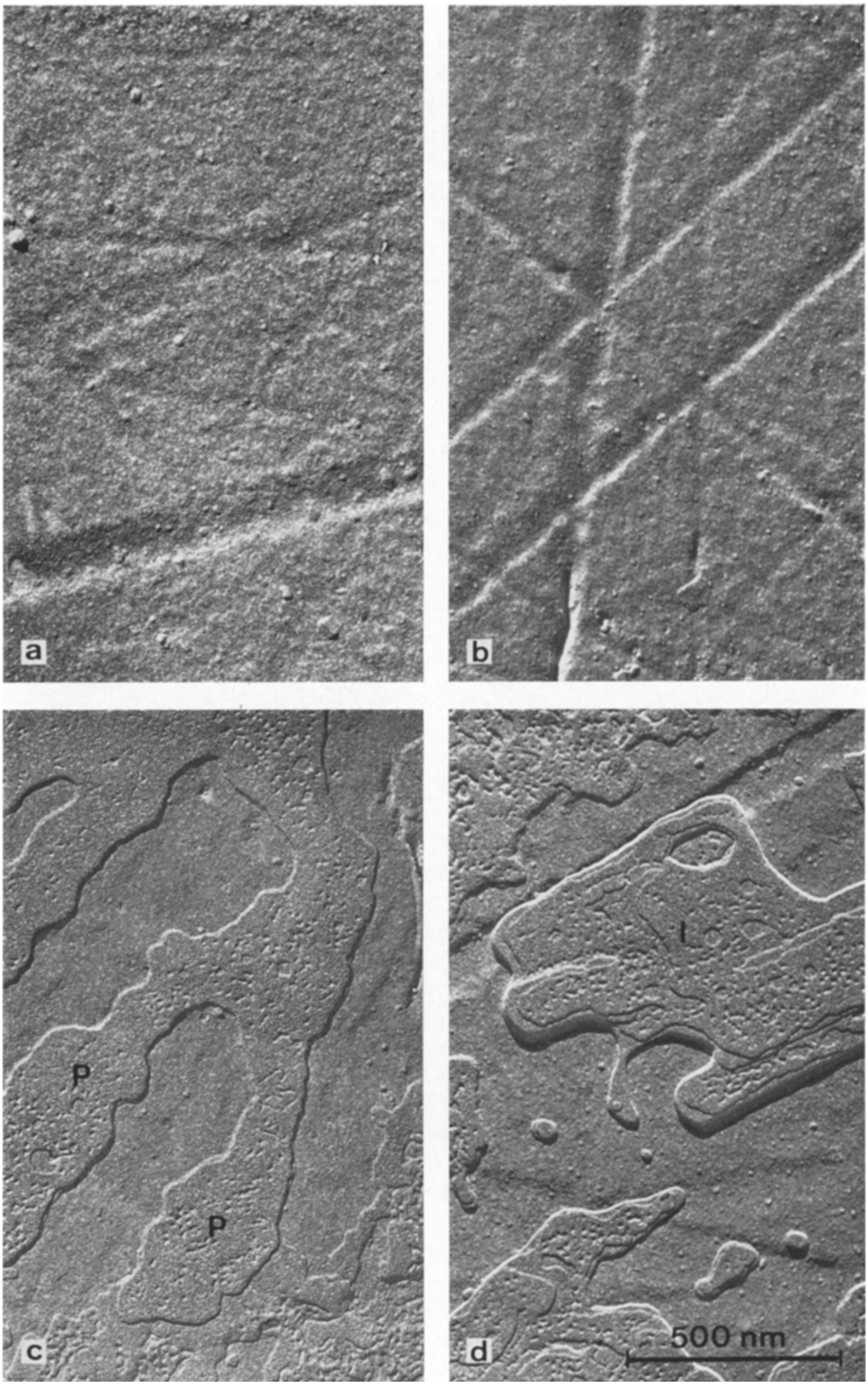

Fig. 1 (Legend see p. 90)

The first layer of both substances attached homogeneously and remained stable. The monolayers can scarcely be distinguished from a clean glass surface, analogous to monolayers of BaSt on germanium, cf Sect. 3.2.1.

Three layers of BaSt, however, show flat islands (I) and holes (H). Furthermore a large number of small pores (P) of about 5 nm diameter cover the surface homogeneously except for the areas within the holes (Fig. 4c). Similar pores could also be observed in some regions of the CdAr triple layers (Fig. 4d), but no island formation was evident.

5 layers of BaSt (Fig. 4e) show drastic reorganization, whereas the corresponding CdAr sample (Fig. 4f) only exhibits irregularities in the form of pores and small holes, comparable the BaSt triple layered sample.

Cadmium arachidate layers deposited at a surface pressure of 18 dynes/cm or 30 dynes/cm did not show any significant difference in their ultrastructural appearance.

3.2.4. Peculiar Observations in the Layer Rearrangement Process (Fig. 5)

In the course of the present study a large number of micrographs were inspected. Although the ultrastructures reported in sect. 3.2.1-3 were found to be typical, some phenomena were observed which should be included both for completeness and demonstration of extreme cases. An example is provided by the rearrangement process of barium stearate in 3 layers on carbon film substrate. Fig. 5a indicates a possible, though apparently not frequent, mechanism of the rearrangement process. Starting with a regular triple layer, the final ultrastructure is reached by the folding over and back again of the second and third layers, so forming 6 layers over the first which remains in place.

Fig. 5b shows a section of the same triple layer frozen $3^1/_2$ hrs after preparation. It shows a typical crystal of approximately 40 nm thickness with rather well developed crystal faces and kink type displacements. Each crystal is surrounded by an area depleted of material but still covered with small aggregates.

3.2.5. Kinetics of the Ultrastructural Rearrangement Process of Tripalmitin Layers (Fig. 6a)

Immediately after deposition the tripalmitin layers already show flat islands with irregular contours. However, there seem to be large areas with intact double layers, marked by T_2 in the micrographs. Furthermore 4 layered regions may be detected, marked by T_4. The first layer (BaSt), marked B, is thought to remain stable. It should be mentioned that during preparation the transfer ratio of the single layers dropped from $n \geqq 3$ to noticeably below 1. The decrease of the transfer ratio may be correlated with the existence of the T_4 region, the area of which, according to Fig. 6a, may be estimated to be smaller than the area of the T_2 layers. Hence the deterioration of the transfer ratio may result from the formation of incomplete third and fourth layers. Fig. 6b shows the state of the 4 layers after 6 h ageing at 15° C. The ultrastructure found immediately after deposition is still clearly recognizable between the crystals. Growth of flat crystals has already taken place to a considerable extent.

After 60 h ageing (Fig. 6c), the rearrangement into crystals is practically complete, and the original 4 layers of tripalmitin have almost totally disappeared.

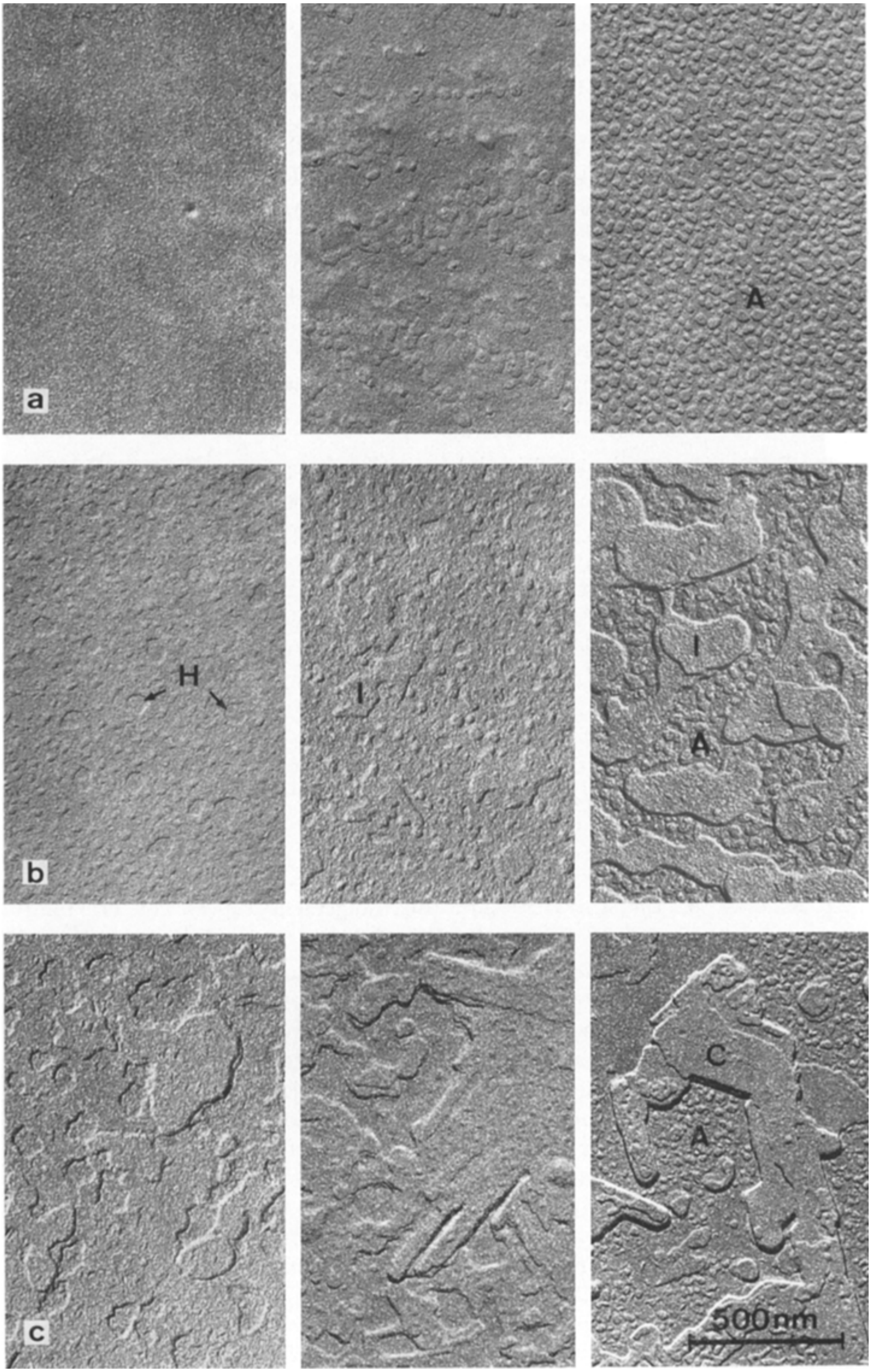

Fig. 2 (Legend see p. 90)

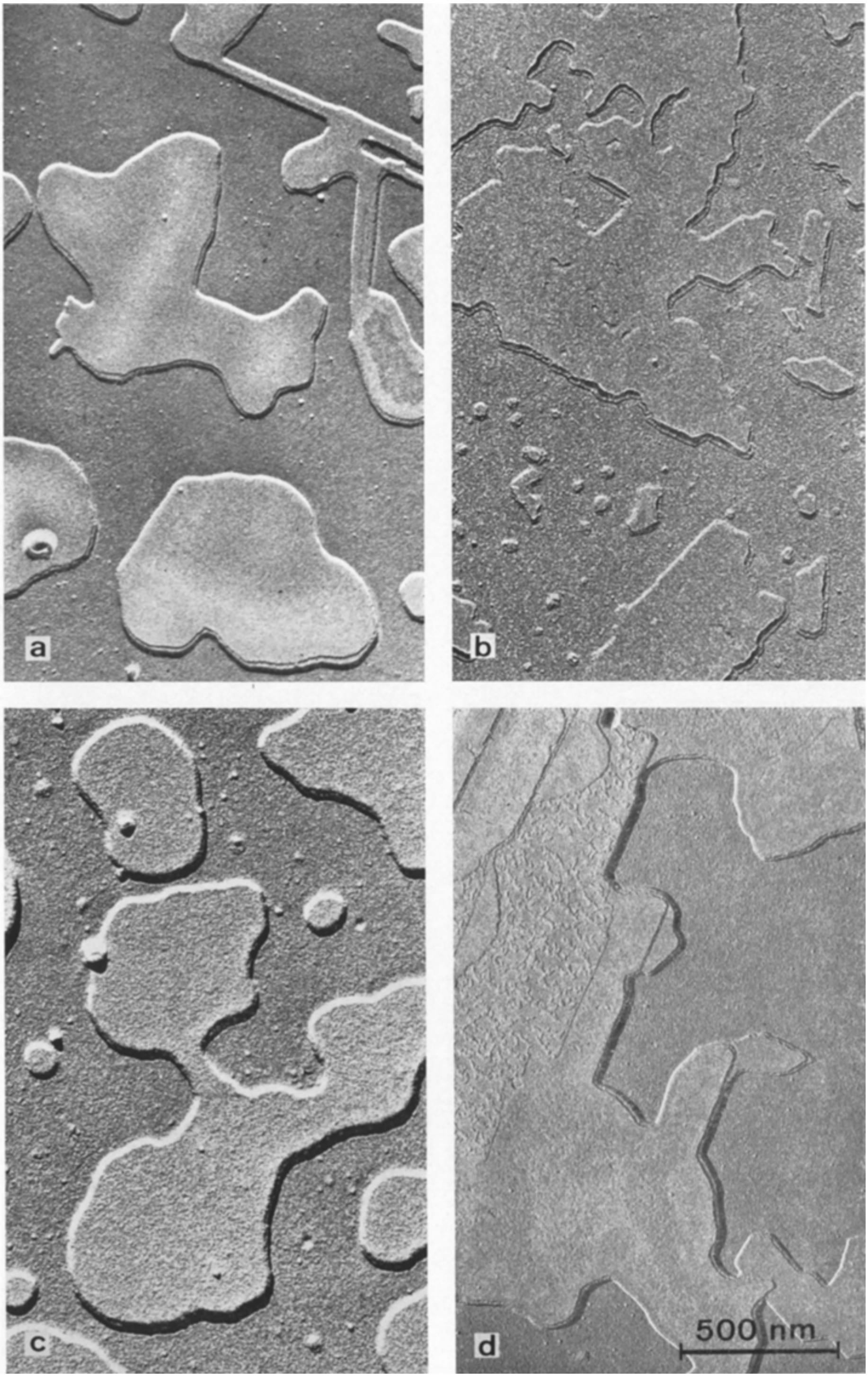

Fig. 3 (Legend see p. 90)

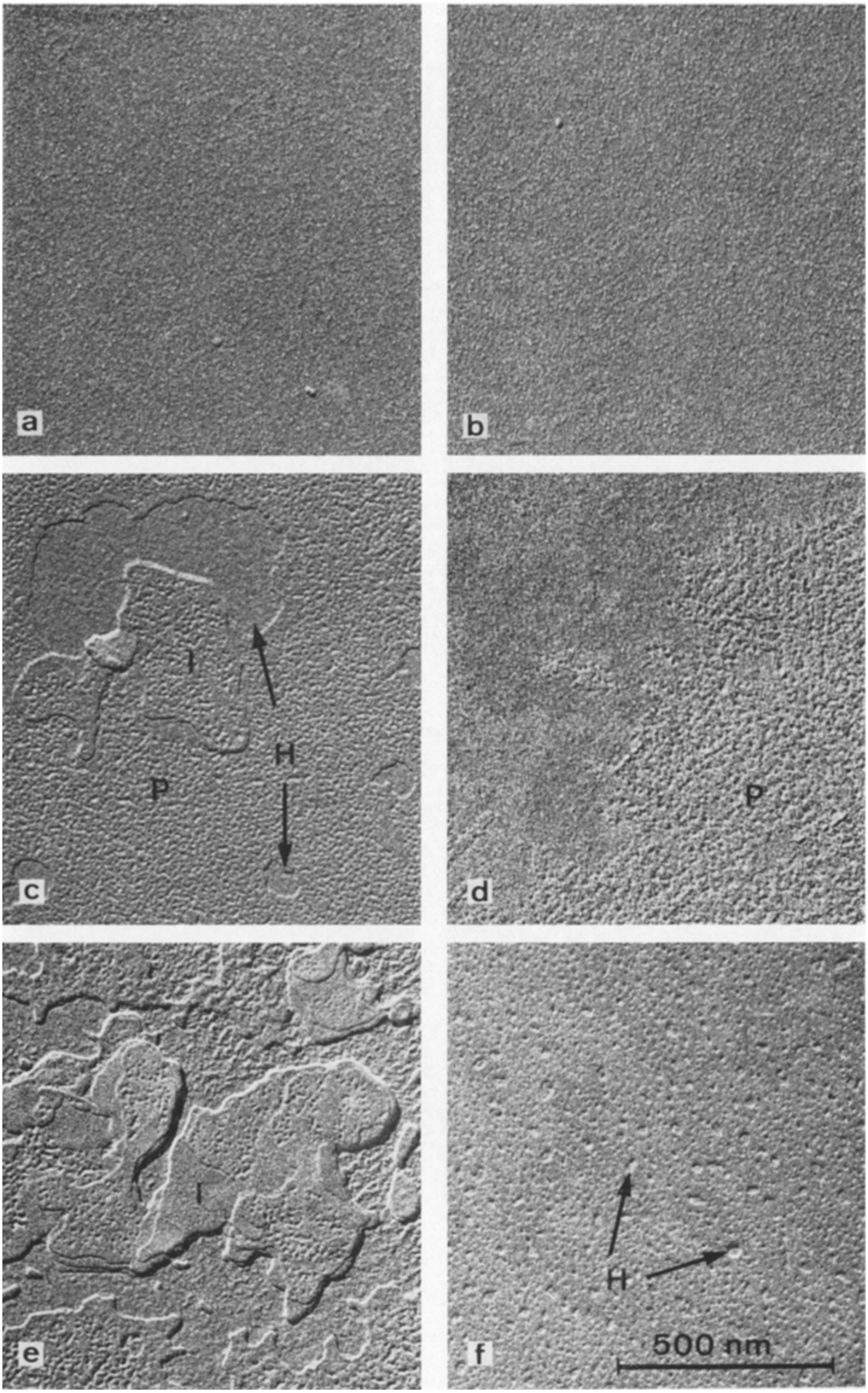

Fig. 4 (Legend see p. 90)

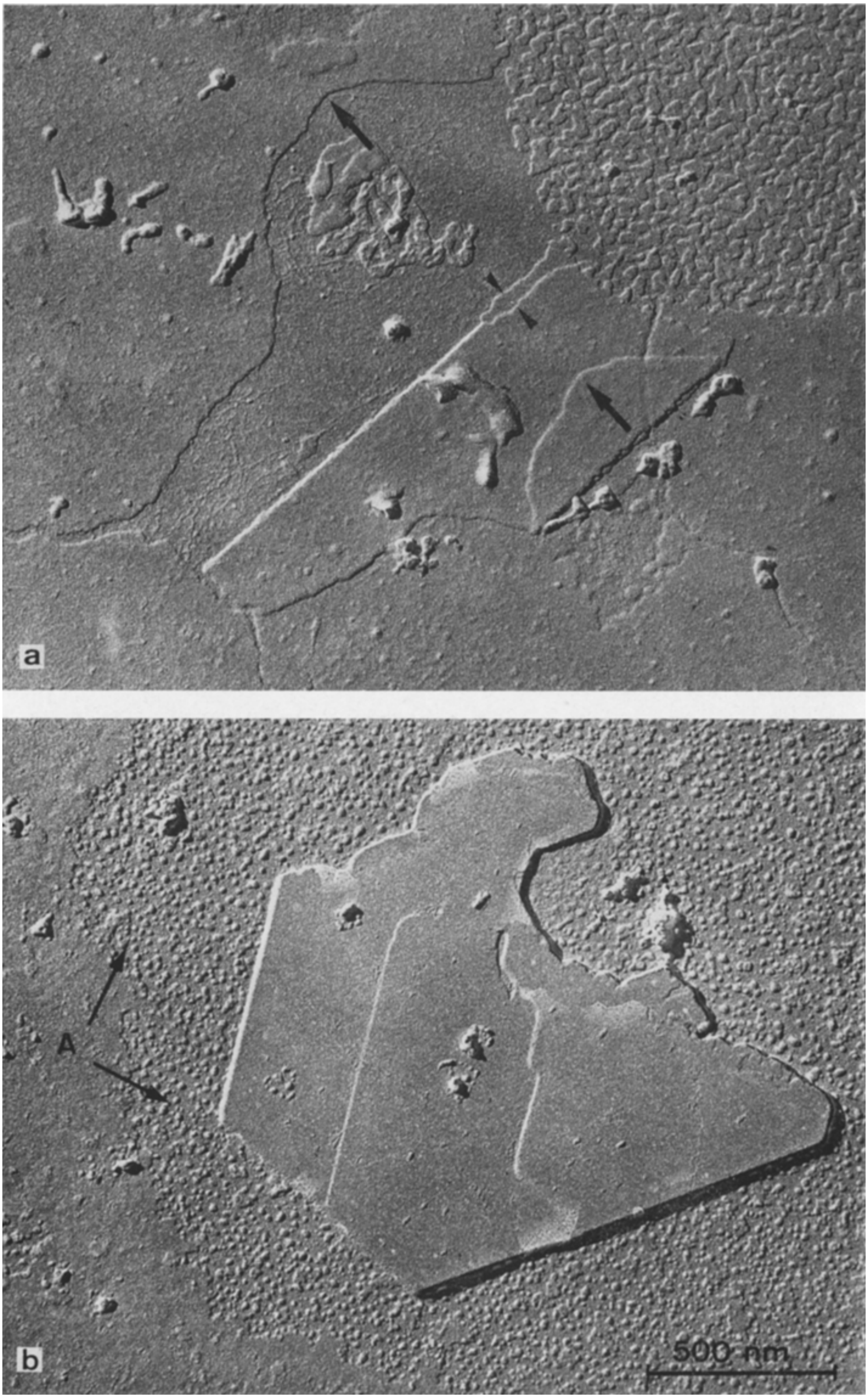

Fig. 5 (Legend see p. 90)

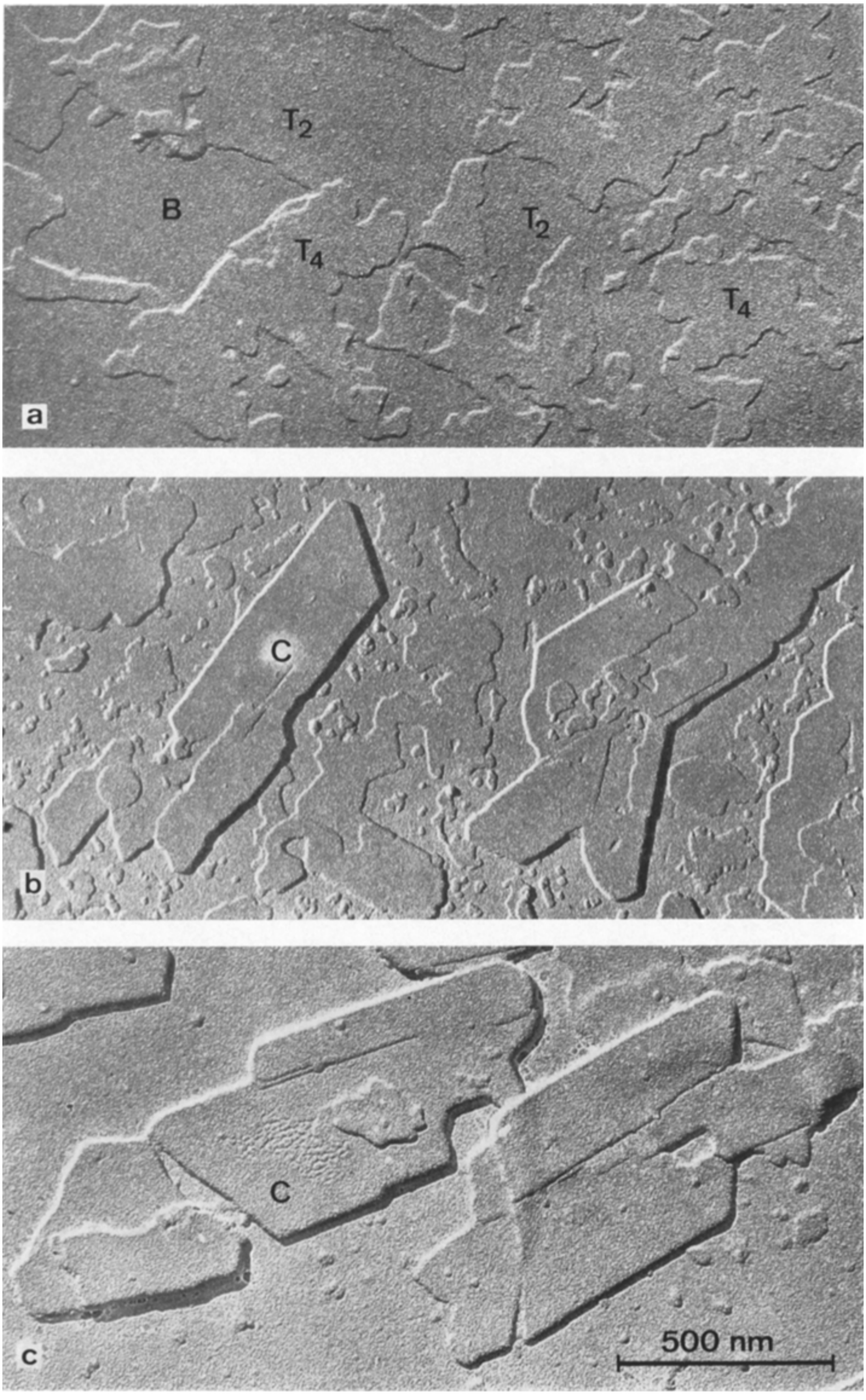

Fig. 6 (Legend see p. 90)

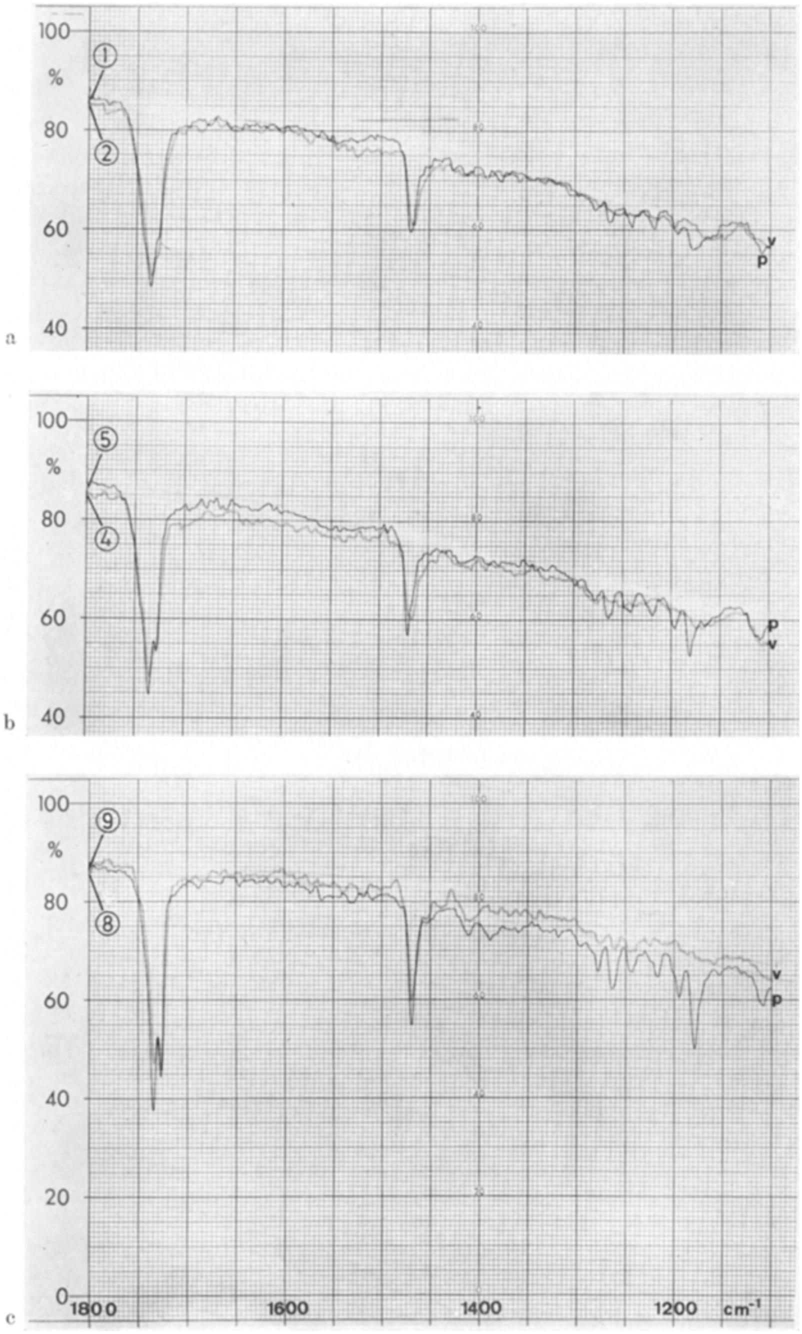

Fig. 7 (Legend see p. 90)

Probably the barium stearate monolayer is still intact, though its existence cannot be detected directly.

3.3. *ATR-IR Spectra*

3.3.1. ATR-IR spectra taken of 8 layers of tripalmitin on germanium substrate (ATR-plate) are shown in Fig. 7. The layers were prepared under the same conditions as those for the EM studies of this system, cf 3.2.5. and Table 1, the substrate being germanium instead of glass. To improve the signal/noise ratio in this critical measurement 8 layers instead of 4 layers were used. It is believed that ATR-IR experiments with 8 layers yield results that are relevant also for 4 layers in respect to the qualitative aspects of the rearrangement process, which is considerably faster for 8 layers. No qualitative differences from 4 layers have so far been detected. For completeness ATR-IR spectra were also taken on 4 layers but will not be reproduced here. More details on the kinetics of tripalmitin layer rearrangement will be published later. The spectra shown in Fig. 7 are typical for

Fig. 1a—d. Odd numbered layers of barium stearate on germanium substrate (platinum replica). a Ge substrate surface before layer deposition, showing scratches due to polishing process. b monolayer on substrate, c 3 layers, d 5 layers. I: islands (up to 50 nm thickness) P: patches. For conditions of sample preparation and age of layers cf Table 1. × 60000

Fig. 2a—c. Kinetics of rearrangement process of odd numbered layers of barium stearate on carbon film. a monolayer, b 3 layers, c 5 layers from left to right 0, 4 and 24 hrs after preparation. A: aggregates, I: islands, H: holes. C: crystals. For conditions of sample preparation cf Table 1. × 44000

Fig. 3a—d. Kinetics of rearrangement process of even numbered layers of barium stearate on carbon film. a, b 2 layers, 2 and 4 hrs after preparation. c[1], d 4 layers, 2 and 4 hrs after preparation. For conditions of sample preparation cf Table 1. × 44000

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[1] The grainy appearance of this micrograph is due to incomplete sublimation of the ice.

Fig. 4a—f. Comparison of barium stearate (left) and cadmium arachidate (right) layers on glass substrate. a, b monolayers, c, d 3 layers, e, f 5 layers 18 hrs and 23 hrs, respectively, after preparation. H: holes, I: islands, P: pores. For conditions of sample preparation cf Table 1. × 60000

Fig. 5a—b. 3 layers of barium stearate on carbon film. a region with double folding of the upper two-layers, micrograph taken of layers frozen immediately after preparation. b crystal formed by rearrangement of 3 layers after $3^1/_2$ hrs. A: aggregates. For conditions of sample preparation cf Table 1. × 52000

Fig. 6a—c. Kinetics of ultrastructural rearrangement process of tripalmitin in 4 layers on glass substrate hydrophobized with 1 layer of barium stearate. a immediately after deposition at 15° C. b 6 hrs after deposition at 15° C. c 60 hrs after deposition, stored at 25° C. T_2: 2 layered areas, T_4: 4 layered areas, B: BaSt first layer. For conditions of sample preparation cf Table 1. × 60000

Fig. 7a—c. ATR-IR-Spectra of tripalmitin in 8 layers deposited on Ge substrate hydrophobized with a barium stearate monolayer. a Approx. 1 hr 30 min after deposition, p: parallel and v: perpendicular polarization. b Approx. 4 hrs after deposition, p: parallel and v: perpendicular polarization. c Approx. 22 hrs after deposition, p: parallel and v: perpendicular polarization

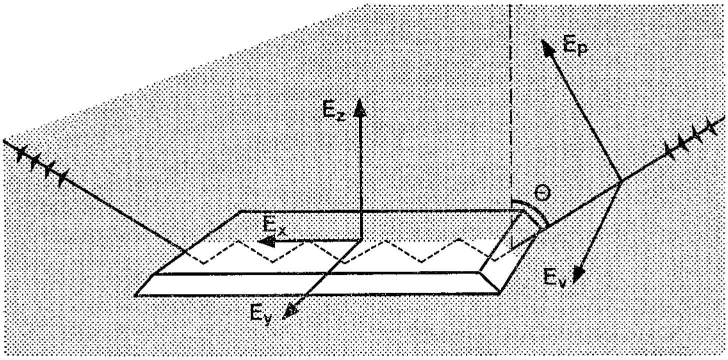

Fig. 8. ATR set up. θ: angle of incidence. E_p, E_v: parallel and perpendicular polarized components of the electric field of incident light. E_x, E_y, E_z: electric field components with respect to the coordinate system corresponding to the internal reflection plate ($E_p \rightarrow E_x, E_z$, $E_v \rightarrow E_y$)

the state of tripalmitin layers a short time after deposition on the reflection plate. They demonstrate the fact that Langmuir-Blodgett layers of tripalmitin are not stable under the conditions given in Table 1. The spectroscopic behaviour of the multilayers agrees well with the findings made by means of electron microscopy.

3.3.2. Interpretation of Spectra

Interpretation of the spectra will be made along the lines followed by Fringeli *et al.* (1972). For convenience the notation of polarizations commonly used in ATR-IR spectroscopy is repeated in Fig. 8.

(i) Carbonyl Stretching Vibrations near 1743, 1736 and 1728 cm^{-1} [ν(C = O)]. This band appears quite broad and only weakly structured in the spectra taken $1^1/_2$ hrs after deposition (Fig. 7a). In spectra taken immediately after deposition the weak shoulders at 1743 and 1728 cm^{-1} are lacking. The 3 bands are attributed to the ν (C = O) stretchings of the 3 ester groups of tripalmitin. All components of this band are clearly polarized and thereby indicate an ordering of the molecules in the layer after ageing for 90 min. In the freshly prepared layers no polarization could be detected. Very probably this means that fresh layers feature neither ordering of the molecules in the layers nor freezing of particular conformations of the single molecules.

Spectra of 8 layered samples aged approx. 4 hrs (Fig. 7b) at T = 15° C, show clearly narrower ν (C = O) bands at the same frequency. The high frequency component has considerably lower intensity and the 1736/1728 cm^{-1} components are clearly resolved. Polarization of the components is noticeably changed by the ageing process, this change may result from the formation of particular conformations near the polar part of the tripalmitin molecules. Furthermore the narrowing of the absorption band is correlated with the rearrangement of the ultrastructure of the layers into crystalline domains.

The spectra of 8 layers aged for $22^1/_2$ hrs at 15° C (Fig. 7c) feature further narrowing of the ν (C = O) bands. The 1743 cm^{-1} component has disappeared. This fact may be explained by the assumption that the tripalmitin molecules have reached the conformation characteristic for the crystal (Fringeli *et al.*, 1972). This also is borne out by the polarization of the 1736 and 1728 cm^{-1} bands and is in agreement with the EM results and the behaviour of other relevant IR bands.

(ii) Methylene Bending Bands near 1470 cm^{-1} [$\delta(CH_2)$]. The behaviour of the δ (CH_2) bands during the ultrastructural rearrangement process may be understood in close analogy with the ν (C = O) bands. Measurement of the polarization of the CH_2-bending absorption bands near 1470 cm^{-1} proved to be quite laborious. As a consequence, the parallel and perpendicular polarized spectra could not be taken at the same time. At short times the band consists of two components at 1470 and 1466 cm^{-1}, which are essentially unpolarized at 2 hrs 12 min and at 1 hr 34 min, respectively. After 4 hrs ageing the doublet 1470/1466 cm^{-1} shows the following behaviour (cf. Fig. 7b):

(a) The 1466 cm^{-1} component has decreased in intensity.

(b) The polarization of the 1466 cm^{-1} band is considerably more pronounced as may be seen from its y-component.

After 23 hrs of ageing the 1470 cm^{-1} band is clearly dominant in both y-polarization (23 hrs 14 min) and xz-polarization (22 hrs 36 min).

The 1470 cm^{-1} band corresponds to the δ (CH_2) band observed on crystalline tripalmitin. The nature of the 1466 cm^{-1} band is not fully understood at the present time. However, according to the facts mentioned before, it may be reliably related to the molecular order in the Langmuir-Blodgett layers. In relation to the most striking feature of this band, namely its transient y-polarization, it should be mentioned that in these experiments the ATR-plate was withdrawn from the subphase in the y-direction (cf. Fig. 8). The polarization indicates a certain amount of anisotropy which arises in the course of the rearrangement process, but no physical model explaining the phenomenon has so far been found.

3.3.3. Sequences of Wagging Vibrations [$\gamma_w(CH_2)$] 1310 to 1190 cm^{-1}

As has been discussed in an earlier paper, (Fringeli *et al.*, 1972), the wagging sequences may be observed only if the CH_2-chains of tripalmitin are in the all-trans (zig-zag) conformation. Even very slight deviations from this conformation cause the wagging sequence to vanish. In the case of crystalline tripalmitin as used in this work, the wagging sequence is nearly fully xz-polarized. In Fig. 7a wagging bands are faintly detectable with xz-component (1 hr 40 min), but no y-component (2 hrs 25 min) is observed.

At later times the intensity of the full wagging sequence increases concomitantly with the rearrangement of the ultrastructure (cf Fig. 6). The polarization remains unaltered. The following conclusions may be drawn from the behaviour of the wagging bands.

(i) In Langmuir-Blodgett layers deposited at 18 dynes/cm the CH_2-chains are not in all-trans conformation, but must contain at least one, possibly several gauche defects. This has been confirmed by direct measurement of the ATR-IR spectra at very short ageing times (10 min).

(ii) No direct evidence is provided by the ATR-measurements with respect to the non all-trans conformations of the CH_2-chains in the Langmuir-Blodgett layers. Therefore no information can be given for the number of all-trans defects in this state. However, as may be inferred from the transfer ratio at 18 dynes/cm, the average orientation of CH_2-chains is perpendicular to the substrate plane.

(iii) The rearrangement process of the ultrastructure involves the formation of the all-trans conformation of the CH_2-chains of the tripalmitin molecules. Simultaneously the chains must orient themselves perpendicularly to the substrate plane.

3.3.4. Vibrations of the -CH_2OCOCH_2-groups (1180 cm^{-1})

This band is characteristic for a planar conformation of the -CH_2OCOCH_2-group of tripalmitin molecules. As may be seen from Fig. 7a, this band at 1180 cm^{-1} is rather weak after 2 hrs ageing time at 15° C. Polarization measurements yielded no intensity for the y-component (2 hrs 27 min) but significant intensity for the xz-component (1 hr 49 min). With prolonged duration of the rearrangement process (Fig. 7b, c), the band increases in intensity but remains nearly fully xz-polarized. Interpretation of this observation is as follows:

(i) The pronounced xz-polarization proves that the oscillating dipole moment is oriented perpendicularly to the substrate plane. Therefore the -CH_2OCOCH_2-group has nearly planar conformation, in close analogy to the findings in the case of crystallized tripalmitin, (Fringeli *et al.*, 1972).

(ii) The relatively large half-width of this band complex at short ageing times may be associated with the existence of a diversity of conformations. This behaviour is closely analogous to the behaviour of the ν (C = O) band, cf Sect. 3.3.1.

(iii) There is an obvious analogy between the time behaviour of the CH_2-wagging and the -CH_2OCOCH_2-bands. Both clearly indicate the formation of crystalline tripalmitin.

4. Remarks and Conclusions

4.1. Complexity of the Phenomena

First some remarks must be made regarding the complexity of the phenomena investigated in this paper:

(i) The problem of significant sampling of EM micrographs must be mentioned. This problem has to be dealt with by inspection of a large number of micrographs and visual selection of a significant sample.

(ii) A further problem is the uniformity of the BaSt samples used in this work. No attempt has been made to analyse the deposited layers with respect to their BaSt-stearic acid composition. Variation of contrast in apparently homogeneous regions may originate from the inhomogeneous distribution of BaSt and stearic acid in the sample (cf. Fig. 3a and 3d).

(iii) Whereas EM of shadow-casted specimens yields information about the local ultrastructure down to 3 nm lateral resolution, the ATR-IR measurement provides an average over the full sample layer spread over the ATR plate. This should be kept in mind when correlating results from the two methods.

(iv) Finally it should be stated that the rearrangement process starts with the deposition of the second layer. Therefore exact definition of the age of an n-layer (n $\geq$ 2) becomes difficult. In information on ageing times given in this paper the end of the deposition process of the n-layer is assumed to be zero hour.

4.2. Relation to Other Work

In view of the relevance of reliable information about ultrastructure and molecular structure of oriented layers it seems appropriate to correlate the results obtained in this work with results reported by other workers.

(i) In a paper by U. P. Fringeli *et al.* (1972) ATR spectra of tripalmitin layers were used to obtain information about the conformation of the tripalmitin molecules building up the layers. Regarding the results of that investigation now in the light of the findings of this work, one should state that the former very probably relate to CH_2-chain conformations in the aged, i.e. rearranged layers and not to the Langmuir-Blodgett layers.

(ii) In a series of papers by H. Kuhn and co-workers energy transfer between dye molecular layers spaced by CdAr-layers has been studied as a function of distance. The reader is referred to Kuhn *et al.* (1972) for detailed information. The conclusions presented in that work depended heavily on the assumption of regular layers, and some tests were provided to support this assumption. It had also been reported by Kuhn *et al.* (1972), that the distance-intensity relations of the Förster-Galerkin theory of inductive energy transfer were less accurately fullfilled in layer assemblies built with barium stearate and tripalmitin than in cadmium arachidate layers.

The findings of Kuhn's group with respect to the stability and uniformity of monolayers are confirmed in this work. Furthermore, agreement exists with respect to the stability of cadmium arachidate layers, which we find rearrange much more slowly than barium stearate and tripalmitin layers. However even cadmium arachidate layers exhibit a considerable density of defects as is demonstrated by Fig. 4, which clearly shows pores and holes in the 3 and 5 layered samples. It appears rather surprising that these defects do not lead to greater deviations from the Förster relation than were actually found by Kuhn *et al.* (1972). The deviations found for barium stearate and tripalmitin layer assemblies may now, in the light of our findings, be interpreted as caused not by diffusion but by the formation of the insular ultrastructure of the barium stearate and tripalmitin layers.

(iii) In an interesting paper, Honig (1973) recently discussed possible mechanisms for formation of defects in films (x-type-films) and also presented a stability theory for such systems.

He also showed that the formation of x-type films of arachidic acid occurs mainly when immersed in the subphase and becomes noticeable from n-layers with $n \geqq 6$ on (Honig *et al.*, 1973). Though arachidic acid has not been studied in this work, it seems quite probable that defects in the films on the ultrastructural level are produced not only in contact with the subphase, but also spontaneously, leading to the rearrangement process reported in this paper.

4.3. Concluding Remarks

First, it may be stated that the rearrangement process described in this paper definitely bears a resemblance to phase transitions and the melting process of triplamitin layers observed in earlier work, (Fringeli *et al.*, 1972). Though no EM study of the melting process is available at this time, the time behaviour of characteristic bands of the ATR-IR spectra is strictly analogous, though in reverse order of sequence to that of the rearrangement process. Second, the transport process involved in the rearrangement should be mentioned. No definite information about this important step in the formation of ultrastructures of flat islands of irregular thickness from a film of regular thickness seems to be available. Most probably the driving force of the whole process is the decrease in free surface energy connected with the formation of crystalline islands.

A further remark about the structure of the tripalmitin molecules in the Langmuir-Blodgett layers should be made. Regarding the properties of monolayers and multilayers immediately after deposition as revealed in the present work, one is inclined to consider the layer state (multilayer state at time $t = 0$) as very similar to the liquid crystal state in the smectic phase. However the n-layer state appears to be intrinsically instable and in fact rearranges spontaneously into the irregularly structured forms documented in the micrographs Fig. 1 to 6.

Finally, the results of this work should make clear that studies on lipid n-layers require careful examination of their ultrastructure, especially if such systems are used as models in optical and E.S.R. spectroscopy, as well as in high resolution electron microscopy. Furthermore, for theoretical investigations, the diversity of ultrastructures occuring during the rearrangement process should be considered.

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