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ELECTRICAL CAPACITY OF BLACK LIPID FILMS AND OF LIPID BILAYERS MADE FROM MONOLAYERS

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SUMMARY

Planar bilayer membranes were formed from monolayers of a series of monounsaturated monoglycerides and lecithins. The hydrocarbon thickness of these membranes, as calculated from the electrical capacity, increases with the length of the fatty acid chain. The specific capacity of monoolein bilayers was found to be 0.745 $\mu F/cm^2$ which is nearly twice that of a monoolein black film made in the presence of decane, but is close to that obtained after freezing out the solvent from the black film. The hydrocarbon thickness of the bilayer, as calculated with a dielectric constant of 2.1, is considerably less than twice the length of the extended hydrocarbon chain of the monoglyceride.

The specific capacity (C_m) of bilayers made from monoolein monolayers showed a negligible voltage dependence, whereas the C_m increased significantly at a voltage of 150 mV in the case of Mueller-Rudin-type monoolein films with *n*-decane as a solvent.

INTRODUCTION

Artificial lipid bilayer membranes have been used in the past as models for biological membranes. These model membranes have been made mainly using the technique developed by Mueller et al. [1] which allows the formation of optically black films of macroscopic area from a large variety of lipids. This method is limited by the fact that the membranes usually retain some of the solvent used for their formation and have a thickness greater than the thickness of the bilayer portion of a biological membrane [2–10]. Furthermore, asymmetric membranes cannot be made by this technique. These limitations are not present in an alternative method which was first described by Langmuir and Waugh [26] and recently revised and improved [11–14]. With this method, two lipid monolayers are joined with their hydrocarbon tail to form a bilayer across a hole in a supporting sheet. In this way, lipid bilayer membranes can be obtained which are virtually solvent free. An important structural parameter of these membranes is the thickness of the hydrophobic core which may be evaluated by the electrical capacity of the membrane. With bilayers made from monolayers of

a number of lipids such as egg lecithin, bovine cardiolipid, glyceroldioleate or phosphatidylserine, Montal and Mueller observed values of specific capacity between 0.9 and 1.0 $\mu F/cm^2$ which is close to the specific capacity of many biological membranes [12]. The small membrane area used in these experiments (less than 0.1 mm^2), however, makes it difficult to ascertain whether the membrane is really flat and has an area equal to the area of the aperture on which the membrane is formed. For this reason we have re-examined the problem of membrane thickness and have carried out capacitance measurements with bilayer membranes made from a series of monoglycerides and lecithins of varying chain-length both by the monolayer technique and by the original technique of Mueller and Rudin.

MATERIALS AND METHODS

The monoglycerides with C_{14} to C_{24} *cis*-mono-unsaturated fatty acid chains (monomyristolein, monopalmitolein, monoolein, monoeicosenoin, monoerucin, mononervonin) were obtained from Nu Chek Prep, Elysian, Minn., U.S.A. All samples consisted mainly of the α isomer with small amounts of the β isomer; they were used without further purification. The C_{18}, C_{22}, C_{24} *cis*-mono-unsaturated lecithins (dioleoyl-, dierucoyl- and dinervonoyllecithin) were synthetized by K. Janko [24]. The longer-chain *n*-alkanes were from Fluka or Merck (for gas chromatography); hexane and pentane were Uvasol grade and KCl analytical grade, and were purchased from Merck.

Bilayer membranes from monolayers were formed as described by Montal and Mueller [12]. Each of the two Teflon troughs had a volume of about 2 cm^3 and an air water interface of about 1 cm^2. The whole assembly was mounted in a thermostatted metal block; if not otherwise indicated, the temperature was 25°C. A thin plastic septum which was clamped between the troughs served as a support for the membrane. These Teflon sheets had a thickness of 12.5 μm and were obtained from Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A. (Membrane Kit No. 5937 for oxygen electrodes). A hole 0.2–0.3 mm in diameter was melted into the septum by means of a heated platinum wire. The holes obtained in this way were not always circular; their area was carefully measured using a microscope with a micrometer grating in the ocular. The error of the area measurements was estimated to be about 5 %. Prior to the formation of the monolayers, the water surface in the troughs was cleaned by aspiration, and the water levels ajusted to just below the hole in the septum by means of two syringes. The lipid was then added to the water surface as a 0.1–1 % (w/v) solution in *n*-hexane or *n*-pentane. The amount of the lipid (5–10 μl of the solution on each trough) was in large excess compared with the amount needed to form a monolayer. After the solvent was evaporated (or nearly so) the water level on one side was ajusted with the syringe to just above the hole. Then the level on the other side was slowly and carefully raised. The formation of the membrane was checked by continuously recording the electrical capacity.

For the measurement of the electrical capacity two different methods were used. In most experiments with bilayers made from monolayers, rectangular voltage pulses of 10 or 20 mV (if not otherwise indicated) were applied through Ag/AgCl electrodes to the membrane from a Philips PM 5770 pulse generator. The decay of the capacitive current was measured as a voltage drop across the input resistance of

the 5A22N differential amplifier of a Tectronix 5115N storage oscilloscope. Depending on the required time resolution and current sensitivity, an input resistance between 10 KΩ and 1 MΩ was chosen. The capacity C was obtained from the exponential decay time τ of the current according to the relation $C = \tau J_0/V$, where J_0 is the current extrapolated to zero time and V the applied voltage. In this way the input capacity of the differential amplifier canceled out. The membrane capacity C_m was obtained by subtracting the capacity of the septum from the total capacity C. This correction was usually less than 10 %; from the uncertainty in the area of the wetted portion of the septum the error introduced by this correction into the calculation of C_m was estimated to be about 2 %. In most experiments with ordinary black films the capacity was measured with a Wayne Kerr B 221 bridge which was operated with an external sine-wave generator (General Radio 1210-C) together with a null detector (General Radio 1232-A). The frequency of the a.c. signal applied to the membrane was 2 KHz and the amplitude 20 mV peak-to-peak. Both methods were checked using an equivalent circuit simulating the membrane/solution system; the results agreed within 1–2 %. Black lipid films were formed in the usual way [15] from a 0.5–2 % (w/v) monoglyceride solution in a hydrocarbon solvent. As the monoglycerides with longer hydrocarbon chains (20 : 1, 22 : 1, or 24 : 1) were not soluble in such a high concentration in the *n*-alkanes, the mixture was briefly heated to 40 °C to obtain a clear solution. The specific membrane capacity C_m was found to be largely independent of the concentration of the solution. For instance, the values observed with monoolein/hexadecane membrane differed by less than 3 % when the monoolein concentration was varied between 0.1 and 10 % (w/v). The diameter of the hole in the teflon wall on which the membrane was formed was either 1 or 2 mm. The actual area of the black film was measured in each experiment with an eyepiece micrometer with an estimated error of less than 5 %. The membrane cell was equipped with Ag/AgCl electrodes and was mounted in a thermostated metal block. If not otherwise indicated, the capacity was measured 5 min after the membrane had turned completely black. Within the limits of error, the specific capacity was independent of the KCl concentration in the aqueous phases between 10 and 100 mM.

RESULTS

(*a*) *Bilayers made from monolayers*

When the monoglyceride was added as a *n*-hexane solution to both water surfaces and a long time (several minutes) was allowed for the solvent to evaporate, no membranes could be obtained on a carefully cleaned plastic septum. With shorter waiting times, or when a small amount of *n*-hexane was added to the solvent-free monolayers, bilayers could be formed. As the formation of a membrane takes only a few seconds, many membranes could be obtained within minutes by manipulating the syringes and the capacity of each membrane could be measured. During successive trials the capacity of the bilayer increased with increasing waiting time and appeared to reach a limiting value. Soon after this stage, further attempts to form a membrane were no longer successful.

Alternatively, if a membrane was formed soon after the addition of the lipid/hexane solution to the water surface, the capacity of this membrane slowly

increased and approached a stationary value that agreed within the limits of error with the final capacity reached by a succession of separate membranes. Such an experiment is represented in Fig. 1. If it is assumed that the membrane area remains constant during the experiment (see below) a likely interpretation of Fig. 1 would be that the bilayer slowly loses hexane to the aqueous phase until a final thickness is reached. In rare cases, membranes of unusually low capacity were obtained which presumably formed from a collapsed portion of the monolayer.

When the surroundings of the hole in the plastic septum were pretreated with a dilute solution of vaseline (a mixture of long-chain hydrocarbons) in a volatile solvent, membranes could be formed even after the evaporation of the hexane from the monolayer. The same result was often obtained when the septum was in contact with the lipid on the water surface for a prolonged time prior to membrane formation. In both cases the membrane had a capacity that was often lower by 5–10 % than the limiting capacity of bilayers formed on a cleaned septum. Possibly, by the pretreatment, a torus of vaseline or lipid is formed on the rim of the hole in the septum, which facilitates the formation of the film.

Membranes with the limiting capacity which were formed from *n*-hexane solutions on a cleaned septum lasted up to several minutes. Much longer lifetimes (up to several hours) were observed with membranes made on a vaseline- or lipid-pretreated septum or with hexadecane-stabilized films (see below). Despite the present technical limitation that vaseline or small amounts of a solvent are required in order to get long-lasting membranes, the method of bilayer formation from monolayers offers advantages over the conventional technique of membrane formation.

Critical for the quantitative interpretation of the experimental data is the question whether the membrane is flat during the capacity measurement and has an area equal to the geometrical area of the hole in the septum. It could be argued that the membrane is bulged when it is formed and that therefore an area larger than that of the hole has to be used in the calculation of the specific capacity. In order to study this question in more detail, the membrane capacity was measured as a func-

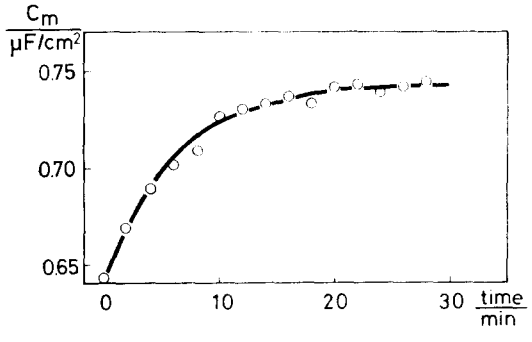

Fig. 1. Time dependence of the specific capacity C_m of a membrane formed immediately after the addition of a solution of monoolein in *n*-hexane to the water surface. The aqueous phase contained 10 mM KCl and was partially saturated with *n*-hexane (25 °C). C_m has been calculated under the assumption that the membrane area remains constant during the experiment and that it is equal to the area of the hole ($8 \cdot 10^{-4}$ cm²).

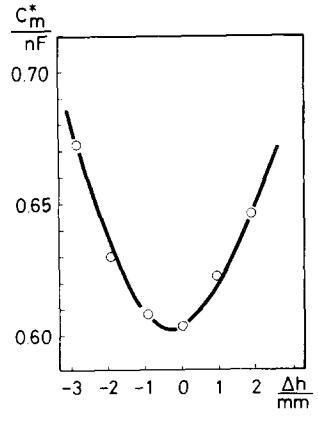

Fig. 2. Membrane capacity C_m* as a function of the height difference Δh of the water levels on both sides of the membrane. The membrane was formed from a monolayer of monoolein (solvent: *n*-hexane) and was in the final state. By manipulating one of the syringes, an initial height difference of $\Delta h = -2.6$ mm was created. Δh was then reduced stepwise to zero and increased to positive values. From C_m* ($\Delta h = 0$) and from the area of the hole ($8.2 \cdot 10^{-4}$ cm^2) the specific membrane capacity is calculated to be $C_m = 0.735\,\mu$F/cm^2.

tion of a deliberately created difference Δh in the water levels on both sides of the septum (Fig. 2). After a change in Δh which was produced by manipulating one of the syringes, the membrane capacity C_m^+ reached a new value in one to several seconds. As seen from Fig. 2, C_m^+ has a minimum at $\Delta h = 0$ (the initial state after the formation of the membrane) and increases for both positive and negative values of Δh. In principle, an increase of C_m^+ with Δh could result either from a decrease in the thickness or from an increase in the area of the membrane. The first possibility, however, is very unlikely in view of the high value of Young's modulus of a lipid bilayer membrane [5, 31]. The experiment therefore suggests that the newly formed membrane is flat, but may be bulged under the influence of a hydrostatic pressure difference ΔP. The additional lipid needed for the increase in membrane area is presumably supplied from the monolayers adsorbed to the septum. Similar results were also obtained with membranes from monoglyceride/*n*-hexadecane monolayers (see below). If r is the radius of curvature of the bulged membrane, then the interfacial tension γ of the membrane is given by $\gamma = r\Delta P/4$. The values of γ calculated from this relation are 1.2 dyne/cm for monoolein membranes stabilized with *n*-hexane, and 1.4–2.9 dyne/cm for *n*-hexadecane-stabilized monoolein membranes, depending on the composition. These values lie in range of γ reported from other bilayer membranes [16, 17]. The limiting values of the specific capacity C_m of membranes obtained from a series of monoglycerides are represented in Fig. 3 (see also Table I). Fig. 3 also gives the hydrocarbon thickness d of the membrane which is calculated from the relation $C_m = \varepsilon_0\varepsilon/d$, where $\varepsilon_0 = 8.85 \cdot 10^{-12}$F/m is the permittivity of free space and ε the dielectric constant of the membrane. In all calculations, a value of $\varepsilon = 2.1$, corresponding to the average dielectric constant of a long chain hydrocarbon has been used [7]. As the polar layers of the membrane can be represented as a very large capacitor in series with a smaller capacitor (the

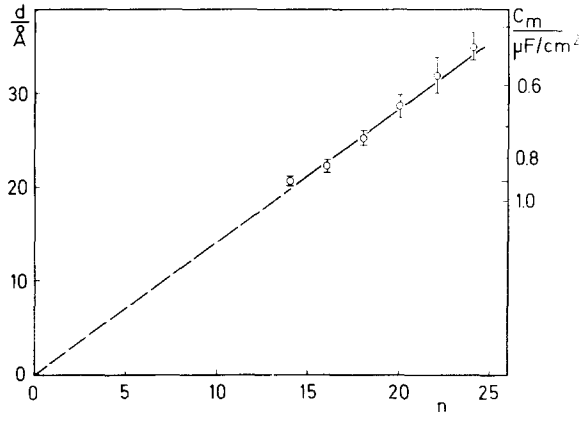

Fig. 3. Specific capacity C_m and hydrocarbon thickness d of bilayer membranes made from monoglyceride monolayers (compare Table I). n is the number of carbon atoms in the mono-unsaturated fatty acid chain of the monoglyceride. The monolayer was formed from an n-hexane solution of the monoglyceride on an aqueous phase containing 10 mM KCl (25 °C). The bars represent the standard deviation of the thickness.

TABLE I

Specific capacity C_m and hydrocarbon thickness d of bilayer membranes made from monoglyceride and lecithin monolayers. The monolayer was formed from an n-hexane solution of the lipid on an aqueous phase containing 10 mM KCl (25 °C). The value of d has been calculated with a dielectric constant of $\varepsilon = 2.1$. n is the number of carbon atoms in the mono-unsaturated fatty acid of the lipid.

n	C_m (μF/cm^2)	d (Å)	Number of membranes
Monoglycerides			
14	0.912 ± 0.020	20.4 ± 0.5	4
16	0.847 ± 0.026	21.9 ± 0.7	34
18	0.745 ± 0.024	25.0 ± 0.8	33
20	0.657 ± 0.026	28.3 ± 1.2	27
22	0.590 ± 0.035	31.5 ± 1.9	27
24	0.538 ± 0.024	34.6 ± 1.5	23
Lecithins			
18	0.721 ± 0.021	25.8 ± 0.8	13
22	0.569 ± 0.023	32.7 ± 1.3	30
24	0.481 ± 0.027	38.6 ± 2.2	5

hydrophobic core of the membrane), d is very nearly equal to the thickness of the hydrocarbon layer of the membrane.

It is seen from Fig. 3 and Table I that the thickness of bilayers made from monoglyceride monolayers is proportional to the number n of carbon atoms in the fatty acid chain, the increment per CH_2 group being about 1.4 Å.

Table I also contains the capacities and thicknesses of membranes made from three different lecithins. The capacity value of 0.721 μF/cm^2 for the di-(18 : 1)-lecithin may be compared with $C_m = 0.76\ \mu$F/cm^2 reported by Fettiplace [32] for bilayers

made from egg lecithin monolayers (egg lecithin mainly contains C_{16} and C_{18} fatty acid chains [33]). It is seen from Table I that the capacity of a lecithin membrane does not differ significantly from the capacity of a monoglyceride membrane of the same chain length. The values for bilayers from dioleoyllecithin and from dierucoyllecithin should be compared with the values of black films with *n*-decane as solvent obtained immediately after blackening (0.36 and 0.32 μF/cm^2, respectively [28]). In contrast to the bilayers made from monolayers, the specific capacity of black films from lecithin dispersed in *n*-decane increases by about 20 % for 3 h after blackening (Pohl, G., unpublished results). It should also be mentioned that Requena and Haydon obtained a value of 0.390 ± 0.005 μF/cm^2 for the specific capacity of thin dioleoyllecithin/decane films from the voltage dependence of the film tension [29, 30].

Bilayer membranes were also formed from mixtures of monoolein and *n*-hexadecane in varying ratios (Fig. 4). The monoolein/hexadecane mixture was added to the aqueous surface as an *n*-pentane solution and the pentane was allowed to evaporate prior to membrane formation. It is seen from Fig. 4 that the specific membrane capacity at low mol fractions (x) of the monoolein in the mixture is rather small, around 0.57 μF/cm^2, but increases with increasing x. Extrapolation to $x = 1$ would give a value in the vicinity of $C_m = 0.75$ μF/cm^2 which agrees well with the limiting value of 0.745 μF/cm^2 for monoolein bilayers made from *n*-hexane solutions (Table I). Similar results were obtained with tetradecane as the solvent. A possible explanation for the low and composition-independent C_m value near $x = 0$ could be that in this case the membrane is bordered by a liquid torus around the rim of the hole and has the properties of a black film (of the Mueller-Rudin type). This interpretation is supported by the close agreement between the capacity value of $C_m = 0.57$ μF/cm^2 near $x = 0$ and the value obtained with black films made of monoolein in *n*-hexadecane (Table II). Additional experiments with bilayers made from mixed monolayers of *n*-hexadecane and various monoglycerides at low mole fraction x also yielded capacity values that agreed within the limits of error with the capacity

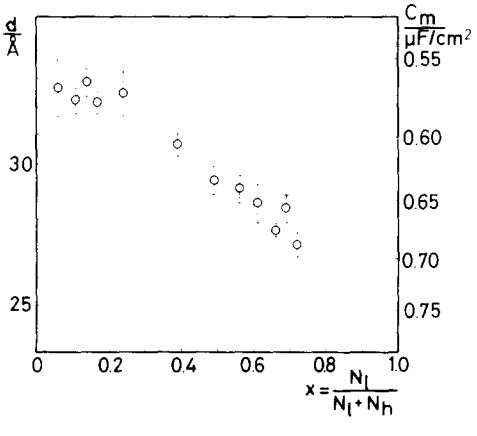

Fig. 4. Specific capacity C_m and hydrocarbon thickness d of bilayer membranes made from mixtures of monoolein and *n*-hexadecane. The mixture has been added to the water surface as a dilute solution in *n*-pentane. N_l and N_h are the number of mol of lipid and hexadecane, respectively. Each point is the average of 10–16 membranes. The bars denote the standard deviation of C_m. (10 mM KCl, 25 °C).

TABLE II

Specific capacity C_m and hydrocarbon thickness d of black films made by the Mueller-Rudin technique from different mono-unsaturated monoglycerides. n is the number of carbon atoms in the fatty acid chain of the monoglyceride. The value of d has been calculated with $\varepsilon = 2.1$. In each case the capacitance has been measured 5 min after the membranes has turned completely black. The aqueous phase contained 100 mM KCl, the temperature was 25 °C. The film-forming solution contained between 0.5 and 2 % (w/v) of the monoglyceride in either n-decane or n-hexadecane. Each C_m value is the average of 10–20 membranes; the error limits are given as standard deviations.

	n-Hexadecane		n-Decane	
n	C_m (μF/cm^2)	d (Å)	C_m (μF/cm^2)	d (Å)
14	0.772 ± 0.019	24.1 ± 0.6	0.485 ± 0.030	38.3 ± 2.5
16	0.661 ± 0.020	28.1 ± 0.9	0.445 ± 0.025	41.8 ± 2.5
18	0.585 ± 0.018	31.8 ± 1.0	0.390 ± 0.018	47.7 ± 2.3
20	0.498 ± 0.015	37.3 ± 1.2	0.345 ± 0.015	53.9 ± 2.5
22	0.383 ± 0.025	48.5 ± 3.4	0.318 ± 0.013	58.4 ± 2.5
24	0.271 ± 0.022	68.6 ± 4.0	0.275 ± 0.015	67.6 ± 3.9

given in Table II for black lipid films made from the same lipid in n-hexadecane.

The compressibility of lipid bilayers can be studied by measuring the voltage dependence of the capacitance C_m [6, 9, 15, 18–23]. In the case of monoolein bilayers made from monolayers with n-hexane as a solvent it was found that C_m remained constant at 0.74 μF/cm^2 within the limits of error (± 3 %) up to $V = 300$ mV. This is in sharp contrast to the behaviour of monoolein membranes that have been made by the Mueller-Rudin technique (see below).

(*b*) *Black lipid films made by the Mueller-Rudin technique*

The specific capacity of black lipid films made by the Mueller-Rudin technique

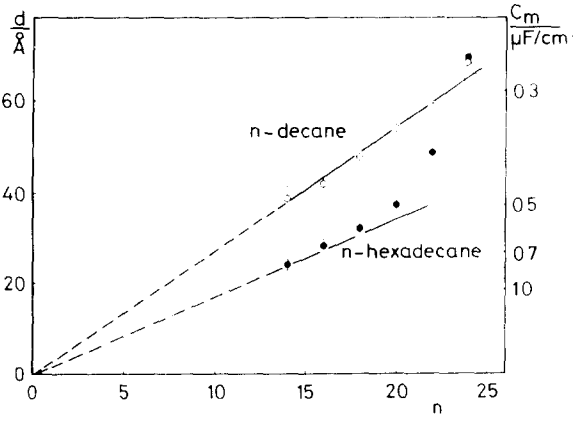

Fig. 5. Specific capacity C_m and hydrocarbon thickness d of black films made by the Mueller-Rudin technique from different mono-unsaturated monoglycerides (compare Table II). Each C_m value is the average from 10–20 membranes, the bars denoting the standard deviation. ○, n-decane solution; ●, n-hexadecane solution.

from various monoglycerides in either *n*-decane or *n*-hexadecane was measured. The results are summarized in Table II and Fig. 5. With *n*-decane as a solvent, the hydrocarbon thickness d increases nearly linearly with the number n of carbon atoms, in the fatty acid chain the increment per CH_2 group being about 2.9 Å. With *n*-hexadecane as a solvent, d is proportional to n only for low n (increment $\sim$1,9 Å per CH_2 group) and then increases faster than n.

The membrane capacity C_m and thickness d as a function of the chain length of the *n*-alkane solvent are listed in Table III for for monoolein membranes. As already described by Fettiplace et al. [7] and White [17], C_m increases with increasing chain length of the *n*-alkane. Our values agree with those reported by Fettiplace et al. [7], whereas White [17] obtained capacities that were higher by 1–7 %.

We were able to confirm the observation of White [10, 17] that by lowering the temperature below 16 °C the liquid torus of a monoglyceride/*n*-hexadecane membrane can be frozen and that this process is accompanied by a large increase in the specific membrane capacity C_m. With monoolein membranes, C_m increased from 0.585 μF/cm^2 at 25 °C to 0.68 μF/cm^2 at 14 °C, and the corresponding values for mononervonin (C_{24}) membranes were 0.271 and 0.45 μF/cm^2.

We have also studied the voltage-dependence of the capacitance of black lipid films made by the Mueller-Rudin technique. The wellknown phenomenon that the specific capacity increases with increasing voltage has been interpreted previously by the assumption that solvent trapped in the film is squeezed out into microlenses under the influence of the compressive forces generated by the electric field. The time evolution of the capacity change was studied in the following way. Rectangular voltage pulses of variable length were applied to the film and the charging current during the rising and the falling phase of the pulse was recorded with a storage oscilloscope as described in the Experimental section. As the charging time was always negligible compared with the pulse duration, the capacity change ΔC_m that occurred after time t was obtained by comparing the current records at the rising and falling phase of the pulse of length t. The results obtained in this way which may be compared with findings of Requena et al. [31] are shown in Fig. 6.

TABLE III

Specific capacity C_m and hydrocarbon thickness d of black lipid films made by the Mueller-Rudin technique from monoolein in different *n*-alkanes. v is the number of carbon atoms of the alkane. d was calculated with $\varepsilon = 2.1$. The lipid in the film-forming solution was 0.5–1 % (w/v). The aqueous phase contained 100 mM KCl, the temperature was 30 °C in the case of *n*-octadecane and 25 °C in all other cases. Each point was obtained from 12–20 different membranes.

v	C_m (μF/cm^2)	d (Å)	C_m (μF/cm^2)		
			Ref. 7	Ref. 6	Ref. 17
6	0.380±0.012	48.9±1.5	—		—
8	0.394±0.013	47.2±1.6	—		—
10	0.390±0.018	47.7±2.3	0.383		0.406
12	0.416±0.016	44.7±1.8	—	0.413	0.432
14	0.469±0.018	39.6±1.6	0.465		0.475
16	0.585±0.018	31.8±1.0	0.580		0.625
18	0.705±0.032	26.4±3.0	—		—

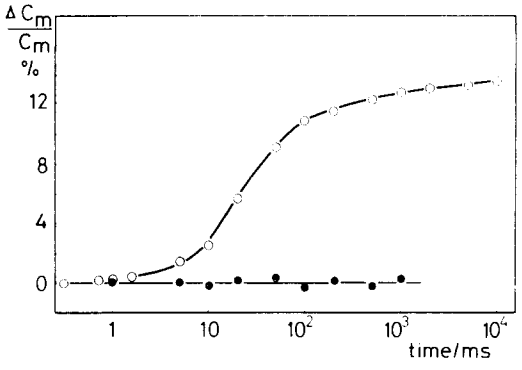

Fig. 6. Relative increase of the membrane capacity C_m after the application of a voltage V_n = 150 mV as a function of time t. 25 °C, 0.1 M KCl. ●, bilayer membrane made from monoolein monolayers with *n*-hexane as a spreading solvent. All points have been obtained from the same membrane; ○, black lipid film made by the Mueller-Rudin technique from a 1 % (w/v) solution of monoolein in *n*-decane. The area of the black film was 1 mm² and the area of the torus surrounding the film was about 1 % of the film area. All points have been obtained from the same film. When the torus area was deliberately made large (> 10 % of the film area), ΔC_m showed the normal time course up to about 1000 ms, but thereafter continued to increase up to considerably higher values than observed normally.

The capacity rise occurred in a broad time range and could not be described by a single exponential function. Most of the change took place within 200 ms. It was found that at any time t the capacity could be represented by a quadratic function of the applied voltage V:

$$\Delta C_m/C_m = \alpha V^2$$

At t = 50 ms, α = 3.8 V^{-2} was found, and at t = 500 ms (near saturation), α = 5.4 V^{-2}. Similar results were also obtained with monoolein/*n*-hexane films. In contrast, bilayer membranes made from monoolein monolayers (with *n*-hexane as a spreading solvent) did not show any change in C_m following a voltage jump over a time range up to at least 1 s (Fig. 6).

DISCUSSION

In this study we have shown that bilayers can be formed at 25 °C from monoglyceride monolayers which have a larger capacity and a smaller thickness than black lipid films made from the same lipid in an *n*-alkane solvent. The difference in thickness of the membranes made by the two techniques could be reduced by selecting the proper solvent necessary to form the black film. For monoolein bilayers made from monolayers we observed a specific capacity C_m of 0.745 μF/cm². The highest capacity values so far reported for monoolein films made by the Mueller-Rudin technique were obtained with *n*-hexadecane as a solvent and ranged between 0.580 and 0.625 μF/cm² at an ionic strength of 0.1 M (refs 7, 17 and this paper). In this study we were also able to obtain monoolein films with *n*-octadecane as a solvent at 30 °C which had a specific capacity C_m of 0.705 μF/cm². In a recent paper, White [10] reported that monoolein/hexadecane films undergo a transition to a higher

specific capacity upon cooling below the freezing point of *n*-hecadecane. He interpreted this finding as a freezing out of the hexadecane from the film into solid microlenses leaving the rest of the bilayer largely free of solvent. Our value of $C_m = 0.745$ $\mu F/cm^2$ is close to the C_m value observed by White at 10 °C, below the freezing point of the solvent. Although the specific capacity of bilayers from monoolein monolayers at 10 °C is not known, the difference of their C_m values at 10 and 25 °C should be quite small, so that both the monoolein bilayers from monolayers and the monoolein films after the freezing out of the solvent are likely to have about the same composition and structure.

Although we were not able to form bilayers from monolayers in the complete absence of the spreading solvent (*n*-hexane), the solvent content of these films is likely to be very small for the following reason. Fettiplace et al. [7] have presented arguments that the area A per lipid molecule in a black film is very nearly the same as the area in a bulk hydrocarbon/water interface with which the film is in equilibrium. They have determined in this way values of A for monoolein ranging from 36.5 to 39.5 $Å^2$ per molecule. If v is the molar volume of the hydrocarbon chain of monoolein, the hydrocarbon thickness d of a solvent-free bilayer may be calculated as $d = 2v/A$. Taking 1-heptadecene as representative for the hydrocarbon chain of oleic acid [7], a volume of $v = 502$ $Å^3$ per chain at 25 °C is calculated from the data of White [10]. With an average value of $A = 38$ $Å^2$ per molecule this gives $d = 26.4$ Å. This value is close to the hydrocarbon thickness calculated from the specific capacity of the bilayer formed from a monoolein monolayer ($d = 25.0$ Å, Table I) assuming a dielectric constant of $\varepsilon = 2.1$ [7].

The calculated hydrocarbon thickness of the monoolein bilayer, $d = 25.0$ Å, is considerably less than twice the length l of a fully extented oleic acid chain ($2l = 40$ Å). Furthermore, it is seen from Fig. 3 that the increment of membrane thickness per CH_2 group of the fatty acid chain is about 1.4 Å, whereas twice the length increment per CH_2 group of a straight alkane chain is 2×1.25 Å $= 2.5$ Å. These findings seem to indicate that the hydrocarbon chains in the membrane are in a more or less coiled state. It is difficult, however, to exclude the possibility, that the value of the dielectric constant of the hydrocarbon layer of the membrane is different from the bulk value $\varepsilon = 2.1$ used for the calculation of d [25]. Strictly speaking, ε is a function of position in the membrane [20], and the average value of ε may vary to some extent with the chain length of the lipid, Furthermore, Griffith et al. [27] obtained some evidence that water may be able to penetrate into the membrane thus altering the polarity of the lipid core [12].

In the case of black films made by the Mueller-Rudin technique, an average increment d of 2.9 Å per CH_2 group is observed over the whole series of monoglycerides with *n*-decane as a solvent (Fig. 5), whereas with *n*-hexadecane the increment increases with the chain length of the fatty acid. As values of A (the area per lipid molecule in the bilayer) are not available for any other monoglyceride besides monoolein, the solvent content of these films cannot be exactly calculated but qualitatively it seems that there is a tendency for a higher solvent content with increasing *n*.

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