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THE COMPOSITION OF BLACK LIPID MEMBRANES FORMED FROM EGG-YOLK LECITHIN, CHOLESTEROL AND *n*-DECANE

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Summary

1. The ratio of lecithin to *n*-decane molecules in bilayer membranes was found to be approximately one hundred-times greater than in the original membrane mixture.

2. If the membrane is prepainted, it is important both to prepaint and to dry the aperture under N_2 in order to keep the lipid composition of the bilayer membranes constant.

Formation of bimolecular lipid membranes of the Mueller—Rudin type involves the spontaneous thinning of a thick membrane (approx. 10 000 Å thick)to an extremely thin membrane (approx. 70 Å thick) surrounded by a thick torus (vol. torus/vol. bilayer approx. 10^5). There is no reason to assume that the composition of the thin bilayer region will be the same as that of the original solution applied to the polymer support. If a proper understanding of the molecular organisation and mechanism of membrane processes is to be achieved, it is important to ascertain the composition of this bilayer region. Cook et al. [1] estimated the composition of membranes indirectly by studying the physical properties of membrane mixtures at bulk oil—water interfaces. However, this method is not applicable to complex systems. Henn and Thompson [2] fixed radioactively labelled membranes with $La(NO_3)_3$ and $KMnO_4$ and subsequently analysed their composition. One of the limitations of this method is that fixing membranes with salts alien to the environment in which they are usually studied may alter their composition.

An elegant experiment was developed by Pagano et al. [3] in which drops of mercury are passed through black lipid membranes. A portion of the

black lipid membrane envelopes the mercury drop on its passage through the membrane but the membrane is not ruptured. By radioactively labelling the membrane, it is possible to determine its composition. This technique was adopted, with modifications, using membranes formed from egg-yolk lecithin, cholesterol and *n*-decane. By using [^{32}P]lecithin with either *n*-[1-^{14}C]decane or [26-^{14}C]cholesterol (Radiochemical Centre, Amersham) it was possible to determine the ratios of lecithin to *n*-decane and lecithin to cholesterol in the bilayer as compared to that in the bulk phase. The ^{32}P-labelled lecithin was obtained by injecting a chicken with ^{32}P-labelled sodium phosphate (1 mCi, Radiochemical Centre, Amersham, 0.5 ml) in the interperitoneal space and collecting eggs subsequently laid. The lecithin was extracted from the eggs according to a modified method of Dawson [4] and Wren [5,6].

The membranes were prepared on an aperture (1 mm diameter) in a Teflon disc supported horizontally in a Teflon cell. The bathing solutions were electrically insulated from each other, thus enabling the measurement of membrane resistance and capacitance. The bathing solution was isotonic saline buffered at pH 7.4 (Tris—HCl, 0.01 M). The membrane mixture consisted of an equimolar ratio of lecithin and cholesterol, and molar ratio of hydrocarbon to lecithin of approx. 150:1. The Teflon was prepainted with membrane mixture (3 μl) and allowed to dry at 37 °C.

Mercury was dropped through the membranes and the membrane-coated mercury drops were collected using a technique very similar to that reported by Pagano et al. [3]. Recordings of the potential difference (V_m) across the membrane indicated a rapid decrease as the mercury drop passed through, with a gradual recovery (< 5 s) to the initial value V_m. Interference patterns were sometimes observed subsequent to the passage of drops through membranes; further drops were not passed until the membranes were completely black again. The capacitance/area readings before and after experiments were found to be constant. By taking a known quantity of a radioactive sample and counting it under standardised conditions, it was possible to convert the disintegrations per minute into the number of molecules present in the sample. Although, in a few cases, the activity recorded for ^{32}P β-radiation is much higher than that of ^{14}C radiation thus there is no possibility of detecting ^{14}C radiation in the ^{32}P-channel of the scintillation counter.

Initially the composition of membranes containing [^{14}C]decane and [^{32}P]lecithin was studied. Once the Teflon had been prepainted, successive membrane mixtures were made with the same membrane mixture on the same Teflon aperture. The results in Table I show a decrease in the ratio of lecithin molecules in the bilayer during the time of the experiment (3—4 h). The same decrease was observed when the ratio of lecithin to cholesterol molecules was determined (Table II). The lipid content of the initial membrane is very different from those made subsequently. By comparing the ratios of lecithin to *n*-decane and lecithin to cholesterol obtained for the first membranes made in the two experiments with the ratios for the last

TABLE I

DETERMINATION OF THE RATIO OF *n*-DECANE/LECITHIN MOLECULES

Once the Teflon had been prepainted, successive membranes were made with the same membrane mixture on the same Teflon aperture. The samples were counted for 20 min and an average of four readings taken; percentage error of counts, $^{14}C \approx 2\%$, $^{32}P \approx 5\%$.

dpm		No. of molecules in the bilayer sampled		Ratio of *n*-decane to lecithin
n-[^{14}C]Decane	[^{32}P]Lecithin	*n*-[^{14}C]Decane ($\times 10^{-14}$)	[^{32}P]Lecithin ($\times 10^{-15}$)	
451	43	40.7	77.1	1:19
433	6	38.6	10.7	1:3.9
840	13	74.8	22.8	1:3.0
424	3	37.6	6.2	1:1.6
793	3	77.3	5.3	1:0.7

TABLE II

DETERMINATION OF THE RATIO OF CHOLESTEROL TO LECITHIN MOLECULES

Conditions and errors as stated in Table I.

dpm		No. of molecules in bilayer sampled		Ratio of cholesterol to lecithin
[^{14}C]Cholesterol	[^{32}P]Lecithin	*n*-[^{14}C]Decane ($\times 10^{-15}$)	[^{32}P]Lecithin ($\times 10^{-16}$)	
39 939	470	6.9	77.2	1:112
33 072	120	5.7	11.7	1:21
38 787	69	6.7	2.8	1:4
33 113	58	5.7	0.8	1:1.4

membranes in the same experiment, the extent of the changes in the membrane lipid content becomes apparent:

	Table I (lecithin/decane)	Table II (lecithin/cholesterol)	Combined Results (lecithin/decane/cholesterol)
First membrane	19 :1	112 :1	19 :1:0.17
Last membrane	0.7:1	1.4:1	0.7:1:0.5

Initially there is relatively high concentration of lecithin in the bilayer, but over the period of the experiment (3—4 h) the lecithin contribution decreases. Eventually lecithin and cholesterol are incorporated into the membranes to about the same extent. This change in composition could be due to lipid oxidation as a result of the membrane mixture being spread on a surface exposed to air during prepainting. Oxidation of the lipid could result in unidentified oxidation products so that lecithin or its oxidation products are incorporated into the bilayer much more readily than cholesterol or its oxidation products. To verify this, it was decided to prepaint and to dry the Teflon support in a stream of nitrogen. As indicated in Table III, the ratio of cholesterol to lecithin remained relatively constant when compared to the results of Table II. The experiment with [^{14}C]decane and [^{32}P]lecithin was therefore repeated, prepainting under nitrogen. The results, depicted in Table IV, show that a much more constant ratio for lecithin/decane was obtained.

TABLE III

Comparison of the ratio of cholesterol to lecithin molecules in bilayer sampled with that of the membrane mixture when the Teflon support was prepainted and dried under N_2. Two different sets of experiments were performed; the lecithin/cholesterol ratio in the first experiment was 6.6 ± 4.8 (mean ± S.D.) and for the second experiment 2.3 ± 1.2 (mean ± S.D.). The membrane mixture samples were taken before and after the sampling experiments were carried out. Samples were counted for 20 min and an average of 10 readings taken, percentage error for counts $^{14}C \approx 1\%$, $^{32}P \approx 3\%$. The membrane mixture was sampled before being used for membrane formation and again after the last membrane was formed. The results indicate that there is a slight tendancy for lecithin to plate out on the glass surface of the containing vessel during the time course of the experiment.

dpm		No. of molecules in bilayer sampled		Ratio of cholesterol to lecithin	Capacitance/area ($\mu F \cdot cm^{-2}$)
[^{14}C]Cholesterol	[^{32}P]Lecithin	[^{14}C]Cholesterol ($\times 10^{-14}$)	[^{32}P]Lecithin ($\times 10^{-14}$)		
4093	3	7.0	53.4	1:7.6	0.33 ± 0.03
2663	0.5	4.6	5.9	1:1.3	0.29 ± 0.03
2099	3	3.6	50.4	1:14	0.30 ± 0.03
3566	3	6.1	50.4	1:8	0.37 ± 0.03
7025	1	12.0	23.7	1:2	0.33 ± 0.03
Membrane mixture					
before 108,825	27	—	—	1:2.5	—
after 140,770	12	—	—	1:0.8	—
4071	0.5	7.0	8.9	1:1.3	0.28 ± 0.03
6352	1	10.9	17.8	1:1.6	0.29 ± 0.03
845	0.5	1.5	5.9	1:4	0.30 ± 0.03
Membrane mixture					
before 100,585	17	—	—	1:1.8	—
after 58,288	6	—	—	1:1.1	—

TABLE IV

THE RATIO OF *n*-DECANE TO LECITHIN MOLECULES IN BILAYER SAMPLED WHEN TEFLON PREPAINTED AND DRIED UNDER N_2

Lecithin/*n*-decane ratio was 1.2 ±0.4 (mean ±S.D.). Conditions as stated in Table III.

dpm		No. of molecules in bilayer sampled		Ratio of *n*-decane to lecithin	Capacitance/area ($\mu F \cdot cm^{-2}$)
[^{14}C]Decane	[^{32}P]Lecithin	n-[^{14}C]Decane ($\times 10^{-15}$)	[^{32}P]Lecithin ($\times 10^{-15}$)		
637	4	5.4	7.4	1:1.4	0.33 ± 0.03
736	5	6.3	8.3	1:1.3	0.27 ± 0.03
1910	3.5	16.0	6.2	1:0.4	0.27 ± 0.03
355	2.5	2.9	4.5	1:1.5	0.28 ± 0.03
316	1	2.7	2.1	1:0.8	0.34 ± 0.03
248	2	2.1	3.9	1:1.8	0.27 ± 0.03
Membrane mixture					
before 93,543	9	—	—	$1:2.0 \cdot 10^{-2}$	—
after 103,345	16	—	—	$1:3.2 \cdot 10^{-2}$	—

The results show that, where lipids susceptible to oxidation are being used, the widely adopted technique of prepainting the membrane aperture should only be employed if the procedure is carried out under nitrogen. Clearly, if dried in air, variable membrane compositions result, until three to four membranes have been made. The existance of a variable composition should be minimised in all black lipid membrane experiments.

The ratio of lecithin/*n*-decane in the bilayer region is about 100 × as great as in the bulk membrane mixture. The ratio of lecithin/cholesterol is approximately the same in the bilayer region as in the membrane mixture. Pagano et al. [3], using glyceryl monooleate at 25 °C suggested, that there were three different types of membranes containing varying amounts of hydrocarbon. The membranes containing the greatest amount of hydrocarbon were infrequently found (2 cases in 23) but the other two types occurred almost equally. One type contained the same number of hydrocarbon/lipid molecules in the bilayer (10 experiments in 23); the other had approximately 10 × as many hydrocarbon/lipid molecules (11 experiments in 23) [3]. The latter was attributed to microscopic lenses, or to the possibility of a hydrocarbon layer interspaced between two monolayers of lipid and hydrocarbon. No membranes were encountered in this series of experiments, where the hydrocarbon content was in excess of the lecithin content. Fettiplace et al. [7], using a two component system of lecithin and *n*-decane at 20 °C, reported a ratio of approx. 1:1, and a slightly lower value was obtained with a glyceryl monooleate/*n*-decane system. The detection of microlenses would be difficult using this method.

The difference in molecular geometry between glyceryl monooleate and lecithin might account for the greater hydrocarbon content of the former membranes; lecithin has two long hydrocarbon chains together with a charged polar group, whereas glyceryl monooleate possesses only one hydrocarbon chain and no charged polar group. It may thus be possible to eliminate the formation of microscopic lenses by choosing lipids of an appropriate molecular geometry. If black lipid membranes are being used as models of biological membranes, it is important that the solvent content is minimised. A better understanding of the molecular parameters (geometry and organisation) necessary for low solvent incorporation in membranes will only be achieved by a systematic study of the composition of black lipid membranes containing different classes of lipids.

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