LIPIDS AND MEMBRANES

J.A. LUCY

Department of Biochemistry and Chemistry,
Royal Free Hospital School of Medicine,
8 Hunter Street, London, WC1N 1BP, England

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1. Introduction

Today, as in 1964, those who are concerned with membranes are broadly divided into two main groups: investigators who work on lipids and others who study proteins. Whether one pursues mainly this aspect or that is often more the result of individual interest and background rather than the outcome of a decision that one particular aspect of membranes is more important than another. Hence although the present article is about lipids and membranes, it is hoped that the reader – if he is a protein biochemist – will neither take it amiss that membrane proteins are scarcely mentioned, nor conclude that workers on lipids believe that the diverse properties of biological membranes can be satisfactorily explained without reference to their protein components.

It is nevertheless apparent that studies of lipid systems per se made during the last ten years have contributed to current ideas on the structure and function of biological membranes. For example, Van Deenen and his colleagues at Utrecht have obtained much information from investigations on synthetic phospholipids, in particular from investigations on the properties of phospholipids at the air–water interface [1]. The behaviour of lipid bilayer membranes, first studied by Mueller et al. [2], and now intensively investigated, has shown that many of the physical properties of natural membranes can be imitated by bilayers of phospholipid [3]. More recently a new method [4] of forming bilayers from lipid monolayers has made studies of the effects of asymmetry on the behaviour of lipid bilayers possible: this technique is likely to be of great value in relation to the asymmetric properties of natural membranes that are discussed below. The liposome model, introduced by Bangham at Cambridge and widely applied in numerous laboratories elsewhere [5], has not only provided much information on the permeability properties of lipid membranes vis-a-vis their natural counterparts, but is being developed as a potential clinical tool in the treatment of inborn errors of metabolism [6]. Finally, physical studies of membrane lipids by X-ray diffraction [7], and of phospholipids and their interactions with cholesterol made by thermal techniques, nuclear magnetic resonance, electron spin resonance and infra-red spectra [8], and by using fluorescent probes [9], have all contributed important insights on the roles of lipids in membranes.

This article is primarily concerned with some salient features of lipids in biological membranes, both when membranes behave as lipoprotein barriers and compartmentalize the cell and its component organelles, and also when these same membranes lose their identity and integrity by fusing together thereby allowing the contents of previously separated entities to unite.

2. Lipids in membrane composition, permeability, structure and function

Endeavours to relate membrane function to structure require a quantitative knowledge of the

lipids present in membranes, and the past ten years have seen the emergence of a few generalizations that can be made regarding membrane composition. Regarding the proportion of lipid to protein, it is found that membranes that behave mainly as barriers like the myelin sheath and erythrocyte membranes contain relatively high proportions of lipid. By contrast inner mitochondrial membranes, which are primarily 'functional' rather than 'barrier' membranes, contain more protein than lipid. Secondly, the quantity of cholesterol seems also to be related to whether or not the membrane behaves as a boundary: plasma membranes and myelin being high and inner mitochondrial membranes low in cholesterol. The phospholipids of plasma membranes are usually more saturated than those of subcellular organelles, – retinal rod outer segment membranes being highly unsaturated [10]. Plasma membranes are enriched in sphingomyelin. On passing from the endoplasmic reticulum via Golgi membranes to the plasma membrane in rat liver, a progressive change in composition has been observed with phosphatidylcholine decreasing and sphingomyelin increasing [11]. In general, the membranes of the Golgi complex (smooth microsomes), zymogen granules and the plasma membrane are relatively rich in sphingomyelin and cholesterol [12]. Interestingly, the specific phospholipid distribution that is characteristic of the subcellular particles of normal liver has been found not to occur in organelles isolated from hepatoma cells [13].

Lysophospholipids are reported to comprise a significant percentage of the total phospholipids of some subcellular organelles, e.g. 5–6% lysophosphatidylcholine in mitochondria, microsomes and nuclei of hepatoma cells [13] and up to 24% in the chromaffin granules from tumours of human chromaffin tissue [14]. It is essential, however, to distinguish between genuinely endogenous lysolecithin and that formed during subcellular fractionation procedures as a result of enzymic hydrolysis of membrane phospholipids. The latter is especially a problem in the analysis of the phospholipids of pancreatic tissue [12,15], where artefactual levels of up to 20% of lysolecithin have been observed.

It will probably be many years yet before we understand the precise function of each class of phospholipids found in biological membranes. Usually it is assumed that the different phospholipids all fulfil some kind of structural role but this need not necessarily be so. The number of enzymes showing various degrees of phospholipid-dependence for activity is growing apace. In a recent review article [16] some 26 enzymes have been listed that essentially fulfil the two criteria for lipid-dependence: these are loss of activity following the removal of lipid, and reactivation on adding lipid to the inactive enzyme. Conceivably then some phospholipids, phosphatidylserine for example, may be important not so much as structural elements but as modifiers of membrane-bound enzymes. This is a controversial field however and views differ, for instance concerning the precise phospholipid-dependence of the $(Na^+\text{-}K^+)$-ATPase activity of membranes. The complications that are possible in such a system are well illustrated by the following scheme, recently published by Goldman and Albers [17], which may indicate some preferential involvement of different phospholipids in the various partial reactions of $(Na^+\text{-}K^+)$-ATPase activity.

$$E_1 + ATP \underset{Mg^{2+}}{\overset{Na^+}{\rightleftharpoons}} E_1P + ADP \quad \text{(phosphatidylserine)}$$

$$E_1P \xrightarrow{Mg^{2+}} E_2P \quad \text{(phosphatidylethanolamine, phosphatidylcholine?)}$$

$$E_2P + H_2O \xrightarrow{K^+} E_2 + P_1 \quad \text{(phosphatidylserine)}$$

$$E_2 \xrightarrow{-Mg^{2+}} E_1 \quad \text{(phosphatidylethanolamine)}$$

In addition to variations in polar group, phospholipids in natural membranes exhibit varying degrees of unsaturation and hydrocarbon chain length. Human erythrocyte membranes contain acyl chains ranging from C_{12} to C_{22}, and with up to six double bonds. Saturated chains are found at the 1-position and unsaturated chains at the 2-position in most membrane phospholipids; the compositions of lysolecithin molecules formed from membrane lecithin by the attack of phos-

pholipases will therefore depend critically on whether the enzymes are of the A_1 or A_2 type. Studies of phospholipids at the air–water interface have shown that, as compared with a fully-saturated phospholipid, a molecule in which one of the chains is unsaturated occupies a relatively large surface area as a result of the difficulty of closely packing molecules possessing unsaturated linkages [1]. The closeness of packing of membrane phospholipid molecules influences the permeability by simple diffusion through the membrane of ions, e.g. K^+, and small polar molecules, e.g. glycerol, and it is found that liposomes show increasing permeability to these species with increasing unsaturation of their component phospholipids [1,18,19]. Similar considerations apply to phospholipids containing acyl chains of medium length (e.g. C_{10} and C_{14}): these phospholipids also show a relatively gaseous-type behaviour in monomolecular films [1].

One of the features of membranes that has attracted continuous attention over the years is the effect of cholesterol on the properties of membranes. Studies of behaviour at the air–water interface showed that mixing cholesterol with an expanded monolayer of phospholipid molecules resulted in a 'condensing effect', with the molecules in the mixed film occupying less space than would be anticipated from a knowledge of the areas occupied by the individual molecules [20]. The relatively high permeability to polar molecules and ions of liposomes prepared from unsaturated phospholipids is also reduced by the presence of cholesterol molecules in the liposome membrane [19]. Recently many observations have been reported that have clarified difficulties experienced in interpreting the interactions between cholesterol and phospholipids in terms of the chemical structure of phospholipid molecules. It is now apparent that the effects of cholesterol on membranes depend on whether the membrane phospholipids are above or below their transition temperatures. Below the transition temperature the hydrocarbon chains of the phospholipids will be relatively rigid or gel-like: with such arrays of molecules, cholesterol has a disruptive influence that allows increased movement of the acyl chains. With phospholipids that are above their transition temperature, the steroid nucleus prevents flexing of the hydrocarbon chains thus reducing fluidity. Cholesterol may possibly have a dual role in different regions of the same membrane, preventing the formation of crystalline, gel-like areas in some regions while simultaneously inhibiting the motion of relatively unsaturated phospholipids in more fluid, liquid crystalline regions [21].

It might perhaps be suggested that one of the major developments in membrane research since 1964 is the emergence of the concept of fluidity in the hydrocarbon moieties of the lipid components of membranes, coupled with contemporary views on the mobility of the protein and glycoprotein components [22], as illustrated for example by the movement of surface antigens [23]. Finally, among the most recent of the ideas concerning the organisation of membrane lipids in relation to membrane function is that of asymmetry. Biological membranes are asymmetric with regard to the distribution of their proteins and glycoproteins. In addition, a considerable body of evidence that is based on the use of specific reagents for individual phospholipids [24] and on the use of purified enzymes that are presumed to act only on the exterior of the cell when haemolysis is absent [25], indicates that individual species of phospholipid are differentially distributed in the outer and inner halves of membranes. The outer part of the erythrocyte plasma membrane seems to consist predominantly of choline-containing phospholipids (lecithin and sphingomyelin) almost to the exclusion of other phospholipids. Conversely there appear to be few reactive phosphatidylserine or phosphatidylethanolamine residues on the outer surface [24]. Cholesterol may be predominantly in the outer half of the bilayer at least in myelin [26]. Specific allocations of different species of membrane lipid to one side or to the other of membranes would seem to take us one further step forward in understanding why membranes contain so many different kinds of lipid molecules.

3. Lipids in membrane instability: the fusion of membranes

While the formation of living creatures may initially have been dependent on the barrier properties of phospholipid membranes, which sequestered small droplets of biochemical soup away from the primeval sea, the development of complex life forms would seem to have relied no less on the ability of phospholipid membranes to fuse. Without membrane fusion there would be no fertilization, cell division, cellular differentiation, lysosomal digestion, or secretion of proteins, to name a few of the many phenomena for which membrane fusion is obligatory.

Attention is frequently drawn to the stability of the phospholipid bilayer. An overview of cellular function indicates, however, that an equally important property of biological membranes is their incipient instability. To quote from Lewis Thomas, 'I think that nature does not see the sharpness of the differences among organelles and cells and organs and organisms and populations as we must see them' [27]. If it is accepted that the phospholipid bilayer provides the basic structural framework for the majority of biological membranes, what then are the factors responsible for decreasing the thermodynamic stability of the bilayer in a controlled manner but to such an extent that primary lysosomes fuse with phagocytic vacuoles, and myoblasts fuse into long, multinucleated myotubes?

One possible mechanism by which this may occur involves the transient presence in membranes of lysolecithin, produced and subsequently destroyed by membrane-bound enzymes. This suggestion is indirectly supported by the relatively high concentration of lysolecithin in chromaffin granules which release catecholamines to the cell exterior by exocytosis [14], and by the ability of lysolecithin to induce cell fusion in a variety of systems [28–30]. However, it has recently been reported that lysolecithin inhibits the fusion of myoblasts in vitro; subsequent removal of lysolecithin allowed cell fusion to proceed [31]. Searches for the involvement of lysolecithin in virus-induced cell fusion have also yielded negative results [32,33]. These observations do not necessarily preclude this lysophospholipid from being involved in other instances of membrane fusion in biology and pathology although it seems possible, in view of the rapidity and severity of the damage to membrane structures induced by lysolecithin itself, that other lysophospholipids may be more suitable candidates. In this connection it is interesting to note that lysophosphatidylethanolamine produces the most remarkable shape changes in rat erythrocytes, and it has been proposed that the sequence of membrane transformations produced by lysophosphatides in vitro can satisfactorily explain most of the age-dependent changes found in vivo in red blood cells [34].

De Boer and Loyter [35] have reported the formation of polynucleate avian erythrocytes on treatment with a mixture of polylysine and phospholipase C, while Toister and Loyter [36] have found that fusion of avian erythrocytes can also be induced by Ca^{2+} at pH 10.5. It has also been reported that phospholipid vesicles, notably phosphatidylserine, fuse mammalian cells cultured in vitro [37].

Studies in this laboratory have shown that more than thirty different fat-soluble substances induce chicken erythrocytes to fuse into multinucleated cells, (fig. 1), [38]. Some of these lipids, e.g. glyceryl monooleate [39], oleylamine [40], and retinol [41] are also effective in the interspecific fusion of fibroblasts. The majority of these fusogenic molecules are unsaturated, or medium-chain-length (C_{10}–C_{14}) saturated, carboxylic acids – or esters of these two classes of fatty acids. C_6–C_9, C_{15}, C_{16} and C_{18} saturated acids did not induce fusion within 4 hr in the test system investigated. In related experiments the addition of the fusogenic, capric, oleic or linoleic acids, but not fusogenically inactive palmitic or stearic acids, to lecithin liposomes was found to give rise to new structures composed of undulating lamellae, (see ref. [42], fig. 2). Fusion of chicken erythrocytes and the modification of the structure of aqueous dispersions of lecithin in negatively-stained preparations are therefore two further features held in common by medium-chain-length and unsaturated carboxylic acids,

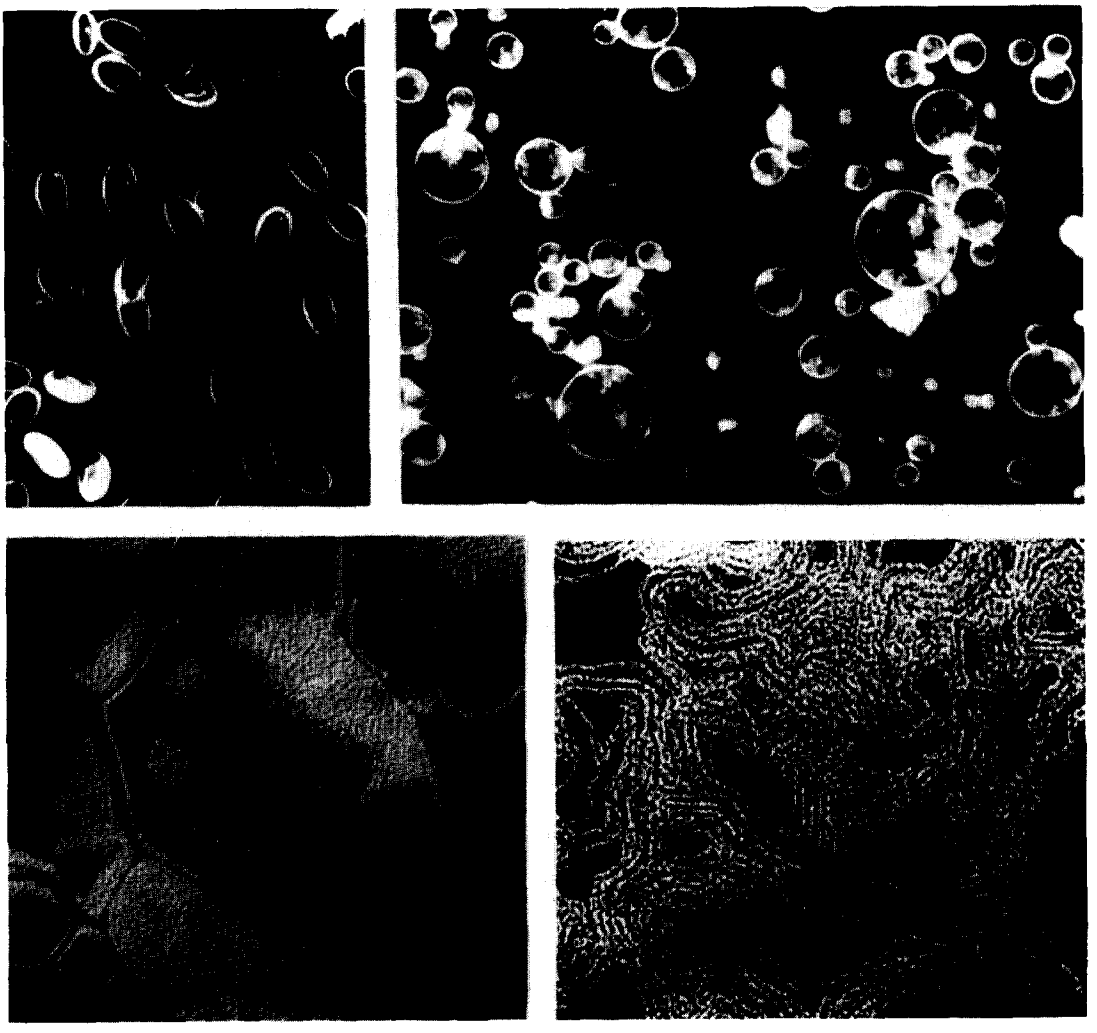

Fig. 1. (a) and (b): Light micrographs of hen erythrocytes, dark field, × 400, prepared by Mr. Q.F. Ahkong; (a) Untreated, washed cells (approx. 3 × 10^8 cells/ml) suspended at 37°C in Eagle's medium containing 80 mg/ml Dextran 60C [37]. (b) Cells prepared as in (a) and then treated at 37°C for 30 min with glycerylmonooleate (100 μg/ml), followed by 2 hr at 37°C with bovine serum albumin (6 mg/ml). Incubation with protein allows cells to complete the fusion process but traps glyceryl monooleate thus limiting both the extent of fusion and lysis. (c) and (d): Electron micrographs of negatively-stained lipids reproduced with permission from Howell et al. [42], × 200000: (c) Egg lecithin sonicated in potassium phosphotungstate at pH 5.6; (d) Egg lecithin treated with oleic acid.

additional to their parallel behaviour at the air–water interface and similar permeability properties in liposome membranes. The active fatty acids have relatively low melting points. Insertion of such molecules into membranes will presumably increase the proportion of hydrocarbon chains in a relatively liquid state. As it has recently been observed that hen erythrocytes are also fused into multinucleated cells by the application of heat alone in the absence of exogenous lipids [43], it would seem that an increase in the fluidity of the hydrocarbon interior of membranes may provide a second possible mechanism by which their stability may be reduced sufficiently to permit fusion to proceed. This idea is supported by vesicles of phospholipid that are

below their transition temperature producing less cell fusion than similar numbers of vesicles containing lipids at or above their transition temperature [37].

Some of the low-melting lipids discussed above may exert fusogenic properties in vivo but there is currently no evidence that any of them is actually involved either directly or indirectly in membrane fusion occurring under normal or pathological conditions, except that retinol is well-known to labilize lysosomes and to be active in facilitating the extracellular secretion of lysosomal enzymes [44].

It is interesting to note in conclusion that there would seem to be a paradox relating to the membranes of secretory organelles, e.g. those of zymogen granules. These membranes contain high proportions of cholesterol and sphingomyelin. They therefore resemble plasma membranes and may be considered to be more likely to fuse with them [45]. The incorporation of cholesterol into dipalmitoylphosphatidylglycerol-dipalmitoylphosphatidylcholine vesicles has, however, been reported to reduce their ability to fuse cells [37]. Furthermore the permeability of these membranes is probably also minimal as a result of their cholesterol content, despite the fact that the cell swelling which always precedes cell fusion when chicken erythrocytes are treated with fusogenic lipids indicates that increased membrane permeability may be an essential component of the fusion process [38].

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