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SYMPOSIUM ON 'NUTRITIONAL ASPECTS OF MEMBRANE STRUCTURE AND FUNCTION'

Some principles of membrane structure

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Our current view of the biological membrane is summarized in the fluid-mosaic model which pictures membrane proteins as floating in a sea of phospholipids, the latter being organized as a bilayer (Singer & Nicolson, 1971). The phospholipids, with their hydrophobic fatty acyl chains, provide the basic permeability barrier for the membrane and at the same time provide a structural support for the various membrane proteins. Despite the apparent simplicity of their role, the phospholipid composition of the membranes is highly complex, from which one of two opposite conclusions can be drawn. One is that the composition of the membrane is finely balanced to produce a fully functional membrane and that each species of phospholipid is present for a distinct purpose. Alternatively, it can be argued equally well that the complexity of lipid composition reflects the relative unimportance of the lipid composition for membrane function, so that many chemically distinct lipid species can be used to produce a membrane which is functionally competent. Clearly these two different views will produce different interpretations of any dietary effects on lipid composition and membrane function.

Because of the sheer complexity of most biological membranes, both of the lipid and the protein components, answers about the basic properties of membranes are most likely to come from studies of highly simplified model systems. Fortunately we have a good model for the phospholipid bilayer portion of the membrane in the liposome that can be made by mixing phospholipids with water. Depending on the details of preparation, these 'liposomes' consist either of vesicles surrounded by a single lipid bilayer, or of a number of concentric bilayers. We are not as fortunate when it comes to simplified membrane-protein systems, as relatively few membrane proteins have been purified in amounts sufficient for detailed biochemical study. Here I will concentrate on one of the best understood of these proteins, the [Ca^{2+}, Mg^{2+}]ATPase that can be purified from muscle sarcoplasmic

reticulum, usually from rabbit. It is not yet clear to what extent conclusions drawn from study of this particular protein can be extended to other membrane proteins but, by analogy with previous studies of water-soluble proteins, it seems likely that many of the basic features of membrane proteins (including the way in which they interact with phospholipids) will be common to all such proteins.

Simple lipid bilayers

One of the most basic properties of the lipid bilayer is its fluidity. Phospholipids can exist in two distinct physical states, a gel phase (equivalent to a solid) and a liquid crystalline phase (a liquid-like state). In the gel phase the phospholipids are tightly packed with the fatty acyl chains in a fully extended, all-*trans* form. The system is highly ordered and rigid, with properties like those of a solid. In marked contrast, in the liquid crystalline phase, there is considerable freedom of motion for the phospholipids with rotation about C–C bonds of the fatty acyl chains and lateral diffusion of phospholipids within the plane of the membrane. In the liquid crystalline phase the viscosity of the fatty acyl chains is comparable to that of a light machine oil so that movement of non-polar molecules through the bilayer will be relatively free, whereas in the gel phase, motion is restricted and permeability will be very low for all molecules. Since one role of the membrane is as a permeability barrier, it might be thought that its lipids would be in the gel phase. This is not so because of the conflicting requirements of the various roles of the membrane. First, in the gel phase, the membrane is 'brittle' so that the cell becomes osmotically fragile (Melchior, 1982). Second, the other major component of the membrane, the proteins, generally require a fluid environment in which to function (see p. 150).

The temperature of the transition between the gel and liquid crystalline states depends on the class of phospholipid (phosphatidylcholine, phosphatidylethanolamine, etc.), the length of the fatty acyl chains and the degree of unsaturation (Table 1).

As illustrated in Table 1, phospholipid structure can be manipulated in a variety of ways to ensure that lipids are in the liquid–crystalline phase at physiological temperatures. First, phosphatidylcholines have lower transition temperatures than phosphatidylethanolamines. Second, short fatty acyl chains result in low transition temperatures, although phospholipids containing fatty acid chains shorter than myristic acid (C_{14}) do not form stable bilayers. Third, a more effective strategy favouring the liquid crystalline phase is the introduction of unsaturation into the fatty acyl chains, most effectively by a *cis*-double bond at the centre of the chain (as in oleic acid); *cis*-double bonds at other positions or *trans*-double bonds have less effect. These effects can be understood in terms of the difficulty of packing unsaturated chains in the close-packed gel phase. Phospholipids containing one saturated and one unsaturated chain have a phase-transition temperature between the phase-transition temperatures for the respective phospholipids with two identical fatty acyl chains, so that, for example, the phase-transition temperature for 1-stearoyl-2-oleoyl phosphatidylcholine is 0° (Table 1). It is important to

Table 1. *Transition temperatures for phosphatidylcholines and phosphatidylethanolamines**

Fatty acid†	Transition temperature (°) Phosphatidylcholine	Phosphatidylethanolamine
14:0/14:0	24	48
16:0/16:0	42	60
18:0/18:0	54	
22:0/22:0	75	
18:1Δ9-*cis*/18:1Δ9-*cis*	−20	−16
18:1Δ9-*trans*/18:1Δ9-*trans*	10	35
18:0/18:1Δ6-*cis*	30	
18:0/18:1Δ9-*cis*	0	
18:0/18:1Δ12-*cis*	12	
18:0/18:1Δ16-*cis*	43	

*From Lee (1983).

†The first and second fatty acids are attached to the 1- and 2-positions of the phospholipids respectively.

realize that the mixed chain lipid is very different from a 1:1 mixture of distearoylphosphatidylcholine and dioleoylphosphatidylcholine: such a mixture at physiological temperatures would contain a mixture of lipids in the gel phase (mostly distearoylphosphatidylcholine) and in the liquid crystalline phase (mostly dioleoylphosphatidylcholine) (Lee, 1983). Most membranes contain roughly equal quantities of saturated and unsaturated fatty acids, so that, if these were randomly incorporated into phospholipids, phospholipids with two saturated chains, one saturated and one unsaturated chain and two unsaturated chains in a molar ratio of 1:2:1 would be obtained. That this is not observed, but rather that the majority of the phospholipids contain one unsaturated and one unsaturated chain, is due partly to the presence of phospholipase A_2 (*EC* 3.1.1.4) with its high specificity for saturated chains at the 1-position and saturated chains at the 2-position (Van den Bosch, 1980), and partly to the specificities of choline phosphotransferase (*EC* 2.7.8.2) and ethanolamine phosphotransferase (*EC* 2.7.8.1) (Holub, 1978). Effects of multiple unsaturation have not been studied in much detail but it has been shown that introduction of the first double bond has the greatest effect with the second having less effect and further double bonds in fact tending to cause a slight increase in transition temperature (Coolbear *et al.* 1983).

The permeability of lipid bilayers to neutral molecules has been shown to be dependent both on lipid phase and on lipid structure. Permeability is very low for gel-phase lipid. In the liquid crystalline phase, permeability increases with decreasing chain length and increasing unsaturation (De Gier *et al.* 1968; Demel *et al.* 1968). For some bilayers, addition of cholesterol reduces permeability whereas for others it has no effect (Demel *et al.* 1972).

The [Ca^{2+}, Mg^{2+}]*ATPase*

The [Ca^{2+}, Mg^{2+}]ATPase can be purified from rabbit muscle sarcoplasmic reticulum and reconstituted into lipid bilayers of defined composition so that effects of changing phospholipid structure and membrane fluidity on enzyme activity can be tested directly. It is found that the activity of the ATPase is markedly dependent on the chemical structure of the surrounding phospholipid, both of the phospholipid head group and of the fatty acyl chain (Table 2 and Fig. 1). The optimal lipid head group for activity is phosphatidylcholine with phosphatidylethanolamine and phosphatidylserine supporting lower activity. Activity is also chain-length dependent, the optimal chain length being approximately C_{18}, with chains shorter than C_{16} or longer than C_{22} supporting significantly lower activities. Finally, the values in Table 2 illustrate the importance of the liquid crystalline phase for activity; whereas the ATPase is active in bilayers of dipalmitoylphosphatidylcholine at 45° in the liquid crystalline phase, the ATPase exhibits no activity at 25° when the lipid will be in the gel phase (Warren *et al.* 1974).

These effects of phospholipid on activity seem not to follow from interactions at a small number of essential sites on the ATPase but rather from non-specific interactions at the whole of the lipid–protein interface of the ATPase. Thus, as illustrated in Fig. 1, changes in activity occur gradually with changing lipid composition of the membrane, and very major changes in the phospholipid composition of the membrane are required to bring about significant changes in activity. It has also been shown that binding constants of phospholipids to the ATPase are almost independent of structure, so that the composition of the lipid mixture around the ATPase in the membrane (the annular lipids) will parallel the bulk composition of the membrane (East & Lee, 1982). The ATPase will not therefore be buffered from any effects of changing lipid composition.

From time-to-time it has been suggested that, beyond the requirement for lipid to be in the liquid crystalline phase, the exact viscosity or fluidity of the membrane is also important. The concept of 'homoviscous adaptation' postulates that

Table 2. *Relative activities of the* [Ca^{2+}, Mg^{2+}]*ATPase reconstituted into phospholipid bilayers*•

Phospholipid	Percentage activity†
Dioleoylphosphatidylcholine	150
Dioleoylphosphatidylethanolamine	75
Dioleoylphosphatidylserine	50
Dipalmitoylphosphatidylcholine (45°)	90
Dipalmitoylphosphatidylcholine (25°)	0
Dimyristoleoylphosphatidylcholine	50

•From Bennett *et al.* (1978), East & Lee (1982).
†Expressed relative to that of the ATPase in the native sarcoplasmic reticulum membrane.

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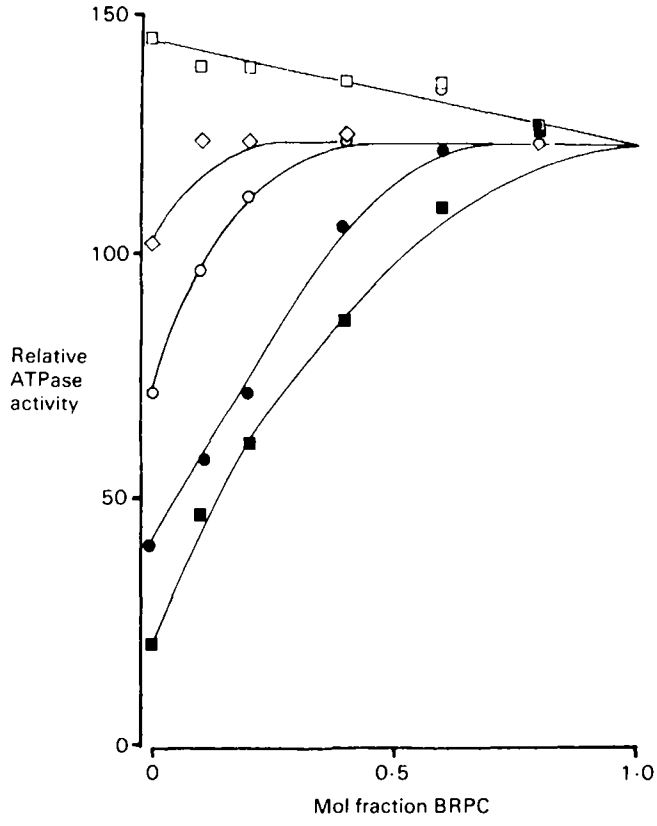

Fig. 1. Effect on ATPase activity of reconstitution with mixtures of di (9,10-dibromostearoyl) phosphatidylcholine (BRPC) and dioleoylphosphatidylcholine (□), brain phosphatidylserine (○), egg phosphatidylethanolamine (◇), dipalmitoyl phosphatidylcholine (●) and dimyristoyl phosphatidylcholine (■). Activities measured at 37° and expressed relative to that of the original unsubstituted ATPase.

organisms alter the lipid compositions of their membranes in order to maintain a constant viscosity independent of environmental conditions (Sinensky, 1974). An organism can increase the fluidity of its membranes by decreasing the average chain length, or increasing the average unsaturation of its phospholipid acyl chains or by decreasing the cholesterol content of its membranes. Membrane fluidity can be decreased by the opposite changes. It is thus held that the particular fatty acyl chain distribution found for the phospholipids in a membrane is that required to provide the appropriate fluidity at the environmental temperature. The importance of fluidity for the [Ca^{2+}, Mg^{2+}]ATPase can be tested by reconstituting the ATPase into bilayers of a series of phospholipids differing in chain unsaturation, and searching for a correlation between ATPase activity and a measure of lipid fluidity such as the electron spin resonance order parameter of a spin-labelled fatty acid incorporated into the bilayer. As shown in Fig. 2, there is no correlation between fluidity and activity. Particularly notable is the lack of effect of cholesterol on the activity of the ATPase reconstituted with dioleoylphosphatidylcholine: cholesterol

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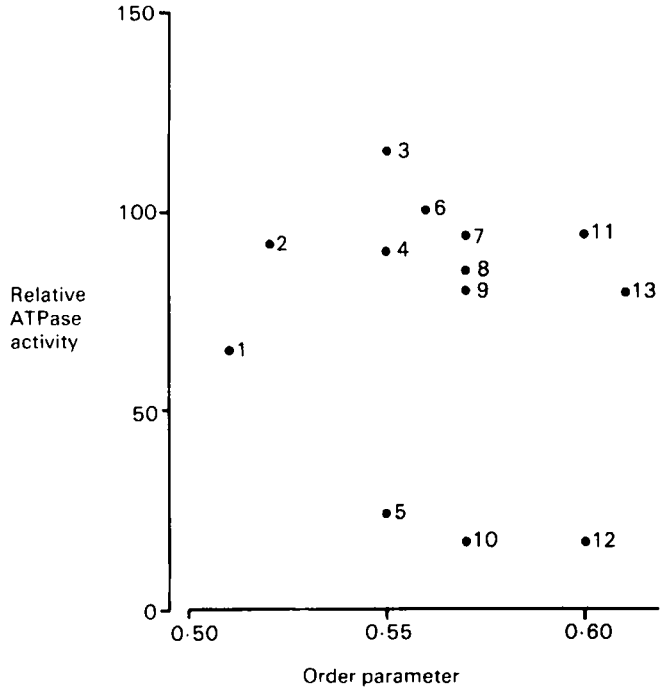

Fig. 2. Relative ATPase activities for the ATPase reconstituted with various phospholipids as a function of the electron spin resonance order parameter for those phospholipids. Full details are given in East *et al.* (1984) but noteworthy are 5, dimyristoleoylphosphatidylcholine; 6, dioleoylphosphatidylcholine; 10, dimyristoyl phosphatidylcholine and 13, dioleoylphosphatidylcholine + cholesterol (1:1, molar ratio).

is known to cause a marked decrease in the fluidity of phospholipids in the liquid–crystalline phase (Simmonds *et al.* 1984). It is clear that the actual chemical structures of the lipids in the membrane are more important than some generalized property of the membrane such as fluidity.

Lipid composition of the sarcoplasmic reticulum

We can now see whether the general features of membrane structure described previously provide an understanding of the lipid composition of muscle sarcoplasmic reticulum and any changes with diet. Table 3 lists the relative contents of the major fatty acids in the phosphatidylcholine and phosphatidylethanolamine fractions of rabbit muscle sarcoplasmic reticulum. The chain lengths observed in the membrane (C_{16}–C_{20}) correspond to those that support high activities for the [Ca^{2+}, Mg^{2+}]ATPase (Table 2).

The content of saturated relative to unsaturated fatty acyl chains is approximately 40:60, with the saturated chains being concentrated at the 1-position of the phospholipids, and the unsaturated at the 2-position; the major monounsaturated fatty acid is oleic acid. These properties ensure that the lipids will be in the required liquid crystalline phase at physiological temperatures. The

Table 3. *Fatty acid content of phospholipids of rabbit muscle sarcoplasmic reticulum*•

	Composition (mol %)					
	Phosphatidylcholine			Phosphatidylethanolamine		
Fatty acid	Total	Position 1	Position 2	Total	Position 1	Position 2
14:0	0·2	0·4	0·6	–	–	–
16:0	40	74	5·5	26	49	5
18:0	4	8	0·3	14	26	2
18:1	17	8	26	14	5	21
18:2	25	8	41	6	2	11
20:4	8	–	15	16	–	27

•Summarized from Marai & Kuksis (1973*a*).

membrane contains relatively little cholesterol. Although the lipid composition is distinct from that in other tissues such as liver, there is no evidence that the particular composition is in some way specially suited for the function of muscle sarcoplasmic reticulum. Thus the fatty acid composition of sarcoplasmic reticulum from rabbit, rat, chicken and human muscle all show some differences (Marai & Kuksis, 1973*b*) and that from lobster is distinctly different with a much greater proportion of unsaturated fatty acids (Madeira & Antunes-Madeira, 1976).

Changes in phospholipid composition have been observed during development. In chicken, there is a marked decrease in palmitate and increase in linoleate (18:2) with development (Boland *et al.* 1974), but with relatively little change in the amounts of the various phospholipid classes. In contrast, in rabbit sarcoplasmic reticulum there is a large increase in the proportion of phosphatidylcholine in the membrane together with an increase in fatty acid unsaturation (Sarzala *et al.* 1975).

Table 4. *Effects of diet on fatty acid composition (mg/g) of phosphatidylcholines from turkey breast*•

	Diet		
Fatty acid	Standard	+ Beef fat	+ Anchovy oil
16:0	330	330	330
18:0	110	120	110
18:1	220	240	150
18:2	250	230	90
20:4	20	30	30
20:5	3	4	110
22:6	2	6	70
Total saturated	440	460	470

•From Neudoerffer & Lea (1967).

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Unfortunately, there are few reported studies of dietary manipulation of the phospholipids of sarcoplasmic reticulum. However, the results in Table 4 show that enriching the diet in saturated fatty acids with beef fat has no significant effect on phospholipid composition, and the total content of saturated fatty acids remains slightly less that 50%. Addition of highly unsaturated fatty acids to the diet, however, causes a marked change in the unsaturated fatty acid content of the membrane, but with little change in the saturated fatty acids; in particular the content of the polyunsaturated fatty acids 20:5 and 22:6 is increased at the expense of 18:1 and 18:2. This pattern of change is fairly typical for other membranes where the saturated fatty acid content cannot be raised much above 50%, but where classes of unsaturated fatty acids can be interchanged.

Discussion

It is clear that membrane phospholipids need to be considered in relation to the rest of metabolism and not in terms of their role in the membrane alone. Thus phospholipids of the plasma membrane are important in cell stimulation when, for example, phosphatidylethanolamine is methylated to phosphatidylcholine (Hirata & Axelrod, 1980) and the turnover of phosphatidylinositol is increased (Michell *et al.* 1981). The membrane phospholipids also act as a reservoir for arachidonic acid, the major precursor of prostaglandins and other eicosanoids.

Studies with model systems suggest that the requirements for a functional membrane system are quite simple. First, only phospholipids containing fatty acyl chains longer than C_{12} form stable bilayers. However, the activity of at least one membrane protein (the $[Ca^{2+}, Mg^{2+}]$ATPase) has been shown to be sensitive to the chain length of the surrounding phospholipids, with chains shorter than C_{16} supporting low activity. If this is a general phenomenon, then it would explain why fatty acids shorter than C_{16} are found in membranes only in trace amounts. Second, it seems that lipids must be in the liquid–crystalline phase to support enzyme activity, again at least for the $[Ca^{2+}, Mg^{2+}]$ATPase. This requires phospholipids to contain either two unsaturated fatty acyl chains or one saturated and one unsaturated. Phospholipids with two unsaturated chains tend to form highly permeable bilayers, an unsatisfactory characteristic. Further, presumably more metabolic energy is expended in the production of di-unsaturated phospholipids. Naturally occurring phospholipids therefore generally contain one saturated and one unsaturated fatty acyl chain. Mammalian plasma membranes also contain large amounts of cholesterol; although this is not necessary for membrane formation (bacterial membranes for example generally contain no sterol) it would serve to reduce the permeability of the membrane, without necessarily reducing the activities of membrane proteins (Simmonds *et al.* 1982, 1984).

Within the necessary constraints outlined previously, the phospholipid composition of a membrane can be changed by dietary means. In particular, it is possible to alter the relative proportions of the various unsaturated fatty acids within the membrane, although the relative amounts of saturated and unsaturated

fatty acids cannot be changed much from 1:1. The relative constancy of the ratio, saturated: unsaturated fatty acid is ensured by the specificity of phospholipase A_2 and probably also by the specificity of choline phosphotransferase and ethanolamine phosphotransferase (Holub, 1978). Possible dietary changes in membrane lipids are also limited by processing of ingested fatty acids during the synthesis of plasma lipoproteins. The very marked differential rates of absorption of cholesterol and plant sterols such as β-sitosterol (which differs from cholesterol only in the presence of an ethyl group on the side chain) is also noticeable and seems to be due to differences in the rate of esterification before incorporation into chylomicrons (Grundy, 1983). These natural protective mechanisms are largely absent for the isomeric fatty acids (*trans*-isomers and unusual *cis*-isomers) found in hydrogenated vegetable and fish oils, which can be incorporated into phospholipids to produce species with significantly altered properties (Table 1). Fortunately, in man, relatively low amounts of these isomeric acids are found in membranes (Ohlrogge *et al.* 1981).

Although it has been suggested that a finely balanced lipid composition is necessary in the membrane to maintain the appropriate membrane viscosity, this now seems rather unlikely. Although the argument is rather circular, presumably if the exact composition and viscosity of the membrane were important then there would be mechanisms to prevent changes in these factors with changing diet; in fact, quite large changes can often be produced by dietary means (Table 4). Even in tissues such as brain and retina, which show exceptional resistance to loss of essential fatty acids by dietary deprivation, no particular role for the essential fatty acids in membrane structure has been demonstrated. Thus severe essential fatty acid deficiency can be induced in rats with corresponding changes in the lipid composition of peripheral nerve myelin, but no functional or structural changes were observed in the nerve (Yao *et al.* 1980). Again, when rhodopsin is incorporated into bilayers of egg-yolk phosphatidylcholine, the kinetics of the light-induced transitions are similar to those in rod outer segments despite the very different fatty acid composition: very slow rates were observed in bilayers of dimyristoylphosphatidylcholine (O'Brien *et al.* 1977), but this can be attributed to the short fatty acyl chains.

It seems that transport proteins at least are rather insensitive to membrane viscosity, the actual phospholipid structure being more important (Fig. 2). Other membrane events might, of course, be more sensitive to viscosity. Thus, for example, in the mobile-receptor hypothesis, the β-receptor with its bound hormone is believed to diffuse laterally and collide with mobile adenyl cyclase molecules (adenylate cyclase; *EC* 4.6.1.1). These collisions, in the presence of GTP, lead to activation of adenyl cyclase. It might be thought that such collisional coupling would be very sensitive to membrane viscosity. However, lateral diffusion of the β-receptor has been determined to be very slow, presumably limited by interprotein aggregation, or anchoring to cytoskeletal structures: lipid viscosity is then unlikely to have much effect (Axelrod, 1983). Indeed it now seems as if protein diffusion, rather than being something to be achieved by the cell is

something largely to be overcome: diffusion of membrane proteins is a simple consequence of the liquid crystalline lipid environment in which they reside.

REFERENCES

Axelrod, D. (1983). *Journal of Membrane Biology* **75**, 1–10.

Bennett, J. P., Smith, G. A., Houslay, M. D., Hesketh, T. R., Metcalfe, J. C. & Warren, G. B. (1978). *Biochimica et Biophysica Acta* **513**, 310–320.

Boland, R., Martonosi, A. & Tillack, T. W. (1974). *Journal of Biological Chemistry* **249**, 612–623.

Coolbear, K. P., Berde, C. B. & Keough, K. M. W. (1983). *Biochemistry* **22**, 1466–1473.

De Gier, J., Mandersloot, J. G. & Van Deenen, L. L. M. (1968). *Biochimica et Biophysica Acta* **150**, 666–675.

Demel, R. A., Geurts van Kessel, W. S. M. & Van Deenen, L. L. M. (1972). *Biochimica et Biophysica Acta* **266**, 26–40.

Demel, R. A., Kinsky, S. C., Kinsky, C. B. & Van Deenen, L. L. M. (1968). *Biochimica et Biophysica Acta* **150**, 655–665.

East, J. M., Jones, O. T., Simmonds, A. C. & Lee, A. G. (1984). *Journal of Biological Chemistry* **259**, 8070–8071.

East, J. M. & Lee, A. G. (1982). *Biochemistry* **21**, 4144–4151.

Grundy, S. M. (1983). *Annual Review of Nutrition* **3**, 71–96.

Hirata, F. & Axelrod, J. (1980). *Science* **209**, 1082–1090.

Holub, B. J. (1978). *Journal of Biological Chemistry* **253**, 691–696.

Lee, A. G. (1983). In *Membrane Fluidity in Biology* vol. 2 pp. 43–88 [R. C. Aloia, editor]. New York: Academic Press.

Madeira, V. M. C. & Antunes-Madeira, M. C. (1976). *Canadian Journal of Biochemistry* **54**, 516–520.

Marai, L. & Kuksis, A. (1973*a*). *Canadian Journal of Biochemistry* **51**, 1248–1261.

Marai, L. & Kuksis, A. (1973*b*). *Canadian Journal of Biochemistry* **51**, 1365–1379.

Melchior, D. C. (1982). *Current Topics in Membranes and Transport* **17**, 263–316.

Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. & Creba, J. A. (1981). *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences* **296**, 123–137.

Neudoerffer, T. S. & Lea, C. H. (1967). *British Journal of Nutrition* **21**, 691–714.

O'Brien, D. F., Costa, L. F. & Cott, R. A. (1977). *Biochemistry* **16**, 1295–1303.

Ohlrogge, J. B., Emken, E. A., Gulley, R. M. (1981). *Journal of Lipid Research* **22**, 955–960.

Sarzala, M. G., Pilarska, M., Zubrzycka, E. & Michalak, M. (1975). *European Journal of Biochemistry* **57**, 25–34.

Simmonds, A. C., East, J. M., Jones, O. T., Rooney, E. K., McWhirter, J. & Lee, A. G. (1982). *Biochimica et Biophysica Acta* **693**, 398–406.

Simmonds, A. C., Rooney, E. K. & Lee, A. G. (1984). *Biochemistry* **23**, 1432–1441.

Sinensky, M. (1974). *Proceedings of the National Academy of Sciences, USA* **71**, 522–525.

Singer, S. J. & Nicholson, G. L. (1971). *Science* **175**, 720–731.

Van den Bosch, H. (1980). *Biochimica et Biophysica Acta* **604**, 191–246.

Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G. & Metcalfe, J. C. (1974). *Biochemistry* **13**, 5501–5507.

Yao, J. K., Holman, R. T., Lubozynski, M. F. & Dyck, P. J. (1980). *Archives of Biochemistry and Biophysics* **204**, 175–180.

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