Biomembranes

Structural and Functional Aspects

Edited by
Meir Shinitzky

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Preface

This majority of physiological functions correspond to an integrated series of molecular events which are in part associated with membranes. The membranal elements in such processes are either receptors, enzymes or transport channels, and their function is by and large controlled by the membrane dynamics. Volume 2 of this series deals with membrane functional units which take part in fundamental physiological processes. This volume is introduced by a chapter on lipid composition which bridges between the dynamic aspects presented in Volume 1 and the basic functional aspects presented in the following chapters. The material in Volumes 1 and 2 was carefully selected to provide a comprehensive ground for understanding complex physiological processes like signal transduction which is the subject of the forthcoming Volume 3.

Rehovot, October 1993 Meir Shinitzky, *Editor*

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CHAPTER 1

Membrane Lipids and Aging

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Abbreviations

CE	—	cholesterol esters
Cer-P	—	phosphoceramide
Cer-P-Eth	—	ceramide phosphorylethanolamine
Chol	—	cholesterol
CL	—	cardiolipin
COH	—	cholesterol 7α hydroxylase
CPK	—	creatine phosphokinase
D	—	coefficient of lateral mobility of the mobile fluorophores
DAG	—	diacylglycerol
DHAP	—	dihydroxyacetone phosphate
DPH	—	1,6-diphenyl-1,3,5-hexatriene
FA	—	fatty acid
FRAP	—	fluorescence recovery after photobleaching
GSH	—	reduced glutathione
G3P	—	glycerol-3-phosphate
HDL	—	high density lipoprotein
HMG-CoA	—	3-hydroxy-3-methylglutaryl-coenzyme A
H_2O_2	—	hydrogen peroxide
LDL	—	low density lipoprotein
LPC	—	lysophosphatidylcholine
LPE	—	lysophosphatidylethanolamine
MDA	—	malondialdehyde
NBD-PE	—	N-4-nitrobenzo-2-oxa-1,3 diazolyl phosphatidyl-ethanolamine
PA	—	phosphatidic acid
PC	—	phosphatidylcholine
PE	—	phosphatidylethanolamine
P-ethanolamine	—	phosphorylethanolamine
PG	—	phosphatidylglycerol
P_i	—	inorganic phosphate

PI	—	phosphatidylinositol
PKC	—	protein kinase C
PL	—	phospholipid
PM	—	plasma membrane
PS	—	phosphatidylserine
R	—	fraction of fluorophores that are mobile on the experimental time scale
RBC	—	red blood cells
SOD	—	superoxide dismutase
SPM	—	sphingomyelin
SUV	—	small unilamellar vesicles
TBA	—	thiobarbituric acid
TBARS	—	thiobarbituric acid reactive substances
TAG	—	triacylglycerols
TMR-S Con-A	—	tetramethyl rhodamine succinyl concanavalin A
TPA	—	transparinaric acid
VLDL	—	very low density lipoprotein

Introduction*

The growing awareness of the active participation of lipids in signal transduction and protein function, in addition to their structural role, lends increasing support to the idea that changes in membrane lipid composition can have significant implications, particularly in the aging process. An understanding of the relationship between lipid composition and membrane function requires consideration of (1) the intermediary metabolism of lipids, (2) lipid-lipid and lipid-protein interactions affecting membrane organization and dynamics and the functional consequences of such interactions, (3) the specific effects of lipids on proteins, and (4) chemical modifications of proteins by lipids.

Despite the apparent enormous diversity of lipid composition in eucaryotic cells, the cells' lipid composition is organized at many levels. Membrane lipid composition is unique for each cell type. Organs and tissues have characteristic lipid compositions [352–354,465]. The various subcellular organelle membranes of eucaryotic cells maintain unique lipid compositions. There is a gradient of cholesterol and

*The references in this section are only representative.

sphingomyelines in normal cells — their highest mole fraction is present in the plasma membrane while the membranes of the nucleus and mitochondria are almost free of these two lipids. This occurs even in cells which are poor in sphingomyelin such as rat hepatocytes (Fig. 1). There are claims that 90% of the cholesterol in fibroblasts is actually located in the cell plasma membranes and that the majority of the remaining cholesterol is derived from internalized plasma membrane [229].

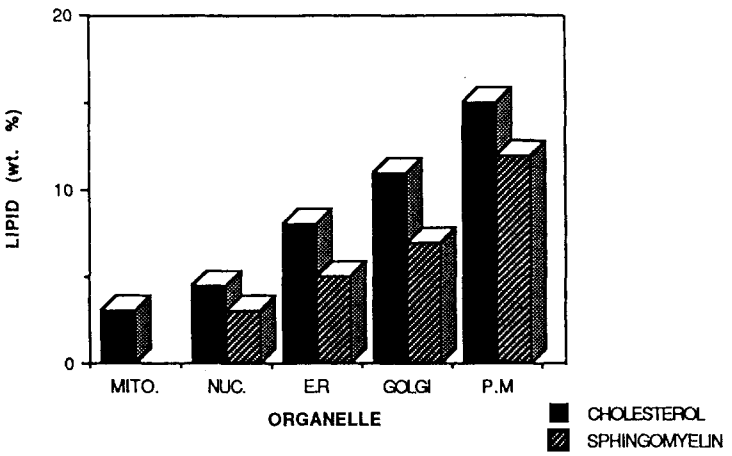

Fig. 1. The ratio of sphingomyelin and cholesterol levels in membranes of rat hepatocyte organelles. From [307].

Cardiolipin (CL) seems to be located almost exclusively in the mitochondrial membrane. Phosphatidylethanolamine (PE) is also highly enriched in this organelle. An additional level of organization is the asymmetric distribution of lipids between the two membrane leaflets. The outer leaflet of plasma membranes is enriched with choline phospholipids and is poor in amino phospholipids. The amino phospholipids are more abundant in the inner leaflet. (For reviews see [15,112,455, 487].) The unique composition of organelle membranes and the asymmetric distribution of their lipids is retained and is therefore an integral part of cellular lipid homeostasis.

It is well known that the lipid composition of cell membranes is altered during normal physiological processes such as aging (for a review see [15,383,385]), cell development [201,223,383] and in the course of various pathological processes described elsewhere in this chapter. The major compositional change that seems to take place in many but not all aging systems is an increase in cholesterol to phospho-

lipid ratio (review in [383,385]). Another often cited change is an increase in the ratio of saturated to unsaturated lipid acyl chains [383, 385]. The ratio of sphingomyelin (SPM) to phosphatidylcholine (PC) is a third important age-related variable [15,383]. This ratio increases in a number of tissues and cells during aging. SPM is highly saturated and may be responsible for a substantial part of the increased levels of saturated acyl chains observed in these systems [15]. Each of these changes leads to membrane rigidification and an increase of its order (see section on Physical Aspects of Age-Related Alterations in Membrane Lipid Composition). The reduction in the level of polyunsaturated acyl chains in the form of phospholipids may in part be related to their peroxidation (see section on Aging Theories Related to Lipids and Lipid Peroxidation). Peroxidation may lead to the crosslinking of various membrane components and may contribute to an increase in membrane order and rigidity [207]. The increase in order and rigidity is referred to as a decrease in membrane fluidity [383,452].

Decreased membrane fluidity seems to affect many membrane-associated activities. Among these are the activity of various enzymes [90,99,141,151,171,262,299,360,365,383,476], modulation of ion pumps [476], the accessibility of receptors and their interaction with the membrane bilayer [39,383], signal transduction [6,178,184,240,279, 281,383], membrane permeability [54,128,248,328,343,460,495,496], electrical conductivity [343], membrane potential [432], transport of small molecules [84, 127, 173, 174, 237, 270, 283, 343, 380], cell fusion [84], and agglutination [381,382]. Membrane fluidity has been shown to affect protein conformation and state of aggregation [338,476], the availability of protein substrates [63,386], as well as cell shape and flexibility [1,101].

The impact of severe alteration of lipid composition on cells is exemplified by the lipidoses. In these lipid storage diseases, certain lipids accumulate in specific tissues and seem to be responsible for the symptoms that are associated with these disease states [23,100,220,293, 413,427]. The maintenance of specific lipid composition and organization must then be important to proper cell function.

Recent studies indicate that the relationship between cell membrane structure and the function of proteins may be much more intimate than previously realized. Lipid regulation of membrane functions may be mediated via the effects of specific lipids on defined membrane activities; lipids such as glycosylphosphatidylinositol [119], fatty acids [259] and prenyl moeities [138] covalently bind to protein and thereby affect

their association with membranes. Additionally, there are general effects which are mediated through membrane organization and dynamics [262, 383]. Alterations in lipid composition such as those that take place in aging will then have broad implications for the maintenance of vital cell support systems.

Review of Experimental Data

Membrane lipid composition and their changes with age

The major age-dependent alterations of membrane lipid composition observed in mammals seem to take place in tissues in which the cells have a long life span and a slow turnover of phospholipids [51,352,353]. Much data have been accumulated on age-dependent alterations in membrane lipid composition of organs and tissues of mammals [199, 348,352–354; Table I). In most cases the results are descriptive and do not deal with the causes for the compositional changes. There are many difficulties in evaluating aging processes, in general, and the involvement of membrane lipid composition, in particular. Complications arise from the need to differentiate between the processes of maturation and aging, to establish and define primary events, and to distinguish between primary and secondary events including disease, diet and climate-related factors.

At the cellular level one must consider differences in the lipid turnover of each specific cell type. In addition, differences in the mitotic capabilities of adult stage cells may complicate the assignment of an accurate "age." Mitotic capabilities have been categorized by Goldstein and Shmookler-Reis [140] as (1) continuous mitotic: cells which are capable of continuous division throughout life. These include hematopoietic, epidermal, gastrointestinal and spermatogenic systems; (2) intermittent mitotic: cells which turnover slowly and upon induction start to divide and proliferate. This group includes cells such as hepatocytes and connective tissue fibroblasts; and (3) nonmitotic: cells which completely lose their mitotic capability. Examples are skeletal and cardiac muscle cells and neurons.

The vascular system

During human aging there is a striking increase in the relative content of sphingomyelines and cholesterol in the membranes of cells comprising the aorta and arteries (Fig. 2) [107,108]. This suggests positive

coupling between the levels of these two lipids [353,354]. A linear correlation between the logarithm of age (in years) and total phospholipids [354] could be accounted for mostly by a large increase in sphingomyelin (Fig. 3). A similar correlation was described by Smith and Cantab [398] and Kummerow [222]. It is worth noting that during aging SPM can reach 55% of the total phospholipid, whereas in the advanced aortic lesion of atherosclerosis it can be as high as 70–80%. The age-related increase is not limited to the endothelial cells since the fibrous plaques of the intima are also enriched with sphingomyelin and cholesterol [222,398,399]. Sphingomyelin accumulation was, in addition, observed in the agranular endoplasmic reticulum, the plasmalemma of smooth muscle cells, the principal cells of the intima and the inner wall of the blood vessels [305,325,326].

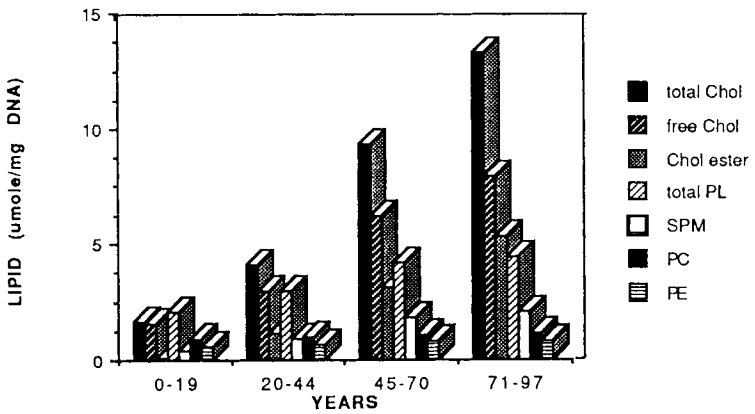

Fig. 2. Age-dependent changes of phospholipid composition in human aorta. Data from [107].

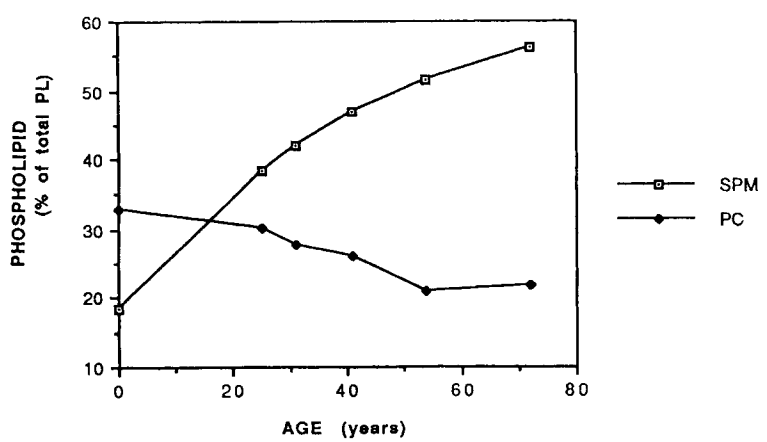

Fig. 3. Age-dependent changes of sphingomyelin and phosphatidylcholine in human aorta. Data from [354].

TABLE I

Changes in lipid composition with aging

System	Age	Lipid component		Ref.
A. Tissues and Cells				
Human aorta, arteries	1–84 y	Chol	(+)	[353]
		SPM	(+)	
Human aorta	25–72 y	Chol	(+)	[354]
		SPM	(+)	
		PC	(−)	
		PE	(−)	
		PS	(−)	
		PG	(−)	
		PI	(−)	
Human aortic intima and media	0–79 y	Chol	(+)	[107]
		CE	(+)	
		SPM	(+)	
		PL	(+)	
		PC	(0)	
		Pe	(0)	
Human aortic intima and media	26–60 y, 6 month–40 y	Chol	(+)	[398, 399]
		CE	(+)	
		SPM	(+)	
		PC	(−)	
Human coronary arteries	Young vs. old	SPM	(+)	[222]
		PC	(−)	
		PE	(−)	
		LPC	(+)	
Rhesus monkey aortic intima and media	0–24 week vs. >40 week	SPM	(+)	[325, 326]
		PC	(−)	
		PE	(−)	
Human brain and nervous system	0–100 y	Chol	(+)	[353, 355]
		SPM	(+)	
		PC	(−)	
		PE	(−)	
		Galactosyl ceramide	(+)	
		Sulphatide	(+)	
Human brain analyzed by region	3–92 y	Chol	(var)	[406]
		PL	(0)	
		Dolichol	(+)	
		Ubiquinone	(−)	

TABLE I, continued

System	Age	Lipid component		Ref.
Rat myelin		Chol	(+)	[463]
Rat brain	2–30 months vs. 24–28 months	Chol	(+)	[384]
Rat brain		PL saturation	(+)	[179]
Rat cerebral cortex	2–24 months	Chol/PL	(−)	[251]
Mouse cerebral cortex	6–28 months	PI	(−)	[426]
		PS	(0)	
Human lens	0–80 y	SPM	(+)	[43]
		PC	(−)	
		PE	(−)	
		PS	(−)	
		PI	(0)	
		LPC	(0)	
		LPE	(+)	
Human lymphocytes	10–80 y	Chol	(+)	[55, 340, 341]
Human platelets	17–86 y	Chol	(+)	[71]
Human RBC	8–95 y	Chol	(+)	[7]
		PL	(0)	
Human	39–81 y	Chol		[199]
lung			(+)	
heart			(0+)	
spleen			(0+)	
liver			(+)	
kidney			(0+)	
pancreas			(0)	
adrenal			(+)	
Rat	3 vs. 10 months	Chol		
lung			(0+)	
heart			(+)	
spleen			(0+)	
liver			(+)	
kidney			(0+)	
Human skin, leg stratum cornea	1–50 y	CE	(−)	[358]
		PL	(0)	
		TG	(−)	
Rat skeletal muscle	10 vs. 25 months	Chol/PL	(+)	[466]

TABLE I, continued

System	Age	Lipid component		Ref.
Rat diaphragm muscle sacrolemmal membrane fraction	Mature vs. aged	Chol PL Chol PL	(+) (0) (0) (0)	[401]
(B) Subcellular organelles				
Mouse cortical synaptosomes	4–28 months	Chol PL PE/PC PC sat.	(+) (+) (−) (+)	[471]
Mouse cortical synaptosomes	2–24 months	PC	(−)	[432]
Mouse lymphocyte plasma membrane	2–30 months	Chol PL	(+) (−)	[340]
Rat liver plasma membrane	3–24 months	PC Prot/PL Chol/PL Prot/chol	(−) (+) (+) (0)	[415]
Rat liver plasma membrane	3–16 months	PL Chol Chol/PL SPM PC PE SPM/PC	(+) f. (+) m. (−) (+) (−) (−) (−) f. (+) m. (−)	[314]
Rat liver plasma membrane	1–20 months	Total PL PC/PL SPM/PL Chol/PL	(+) (+) (+) (+)	[315]
Monkey liver microsomes	16–27 y 1–25 y	Chol PL	(+) (0)	[250]
Rat liver microsomes	3–27 months	Chol Chol/PL SPM PE PS+PI FA sat.	(0) (+) (0) (0) (0) (0)	[367]

TABLE I, continued

System	Age	Lipid component		Ref.
Mouse		SPM/PC	(+)	[156]
liver microsomes		PE/PC	(+)	
		PL sat.	(−)	
Rat	4 vs. 33 months	Chol	(+)	[241]
heart mitochondria		SPM	(+)	
		PC	(0)	
		PI	(0)	
		PG	(−)	
		PL sat.	(+)	
Rat	5 vs. 28 months	Chol	(+)	[304]
liver mitochondria		PL	(−)	
		Chol/PL	(+)	
		CL	(−)	
Rabbit	6 weeks vs. >1 y	PL	(0)	[206]
jejunum brush border		PC	(−)	
membrane		SPM	(−)	
		PE/PL	(+)	
		PS/PL	(+)	
		PI/PL	(−)	
		CE	(−)	
ileal brush border		PL	(+)	
membrane		Chol	(+)	
		PS	(+)	
		SPM/PL	(+)	
		Chol/PL	(−)	
		PC/PL	(−)	
		PI/PL	(−)	
Rat	10–30 months	PL/prot	(+)	[327]
brush border		Chol/PL m.	(+)	
membranes		f.	(0)	
		FA sat. index	(0)	
Rat	6 vs. 24 months	Chol	(+)	[237]
kidney cortex		SPM	(+)	

sat, saturation; m, male; f, female; (+), significant increase; (−), significant decrease; (0), no change; (0+), small increase; var, variable.

The major source of SPM influx into the aortic walls is from serum lipoproteins [379]. Rabbits fed cholesterol-rich diets show an exponential increase in the rate of entry of serum SPM into the aortic wall which suggests that coupling exists between the level of sphingomyelin and the

level of cholesterol in biological membranes [379]. Rat aortic smooth muscle cells in culture take up "remnants" of very low density lipoproteins (VLDL) [33]. These remnants, like LDL, are enriched in SPM and cholesterol when compared to other lipoproteins [18,96]. Such coupled relationships have been reported for various membranes both *in vitro* in tissue culture and *in vivo* under various physiological conditions (for more details see [307] and relevant parts of the sections on Cholesterol, Cholesterol/Phospholipid Ratio and Specific Effects on Membrane Proteins).

Recent observations on the prelesional event in atherogenesis indicate a progressive accumulation of extracellular densely packed uni- or multilamellar vesicles within the aortic intima. Analysis by various electron microscopy techniques indicate that the vesicles appear in cell-free subendothelial spaces and are protein poor. Interaction with filipin indicates that these vesicles are enriched with unesterified cholesterol [387]. This may lead to the loss of integrity of the endothelial cell barrier — the first phase in the atherogenic process [164]. There are indications that cholesterol itself, and especially oxidized cholesterol, is toxic to the arterial endothelium [143]. (See also the section on Aging Theories Related to Lipids and Lipid Peroxidation.)

Recently major studies on humans such as the Framingham and Helsinki heart studies indicate that with age, human arteries become more susceptible to atherogenic changes at any given plasma cholesterol level [59,60]; see the section on Cholesterol. There are, however, studies indicating that aging may lead to arterial changes which diminish the degree to which the rate of atherosclerosis is modulated by plasma cholesterol level [142].

Heart and blood cells

Human and mouse lymphocytes show an age-dependent increase in cholesterol content which seems to affect immune competence [55,330,340–342]. The physiological effect of increased cholesterol levels is mediated by a consequent increase in membrane microviscosity (decrease in membrane fluidity). This may be one of the causes of diminished immune responses in aging. This hypothesis is supported by the findings of Shinitzky and coworkers that reducing the cholesterol to phospholipid mole ratio in membranes of lymphocytes from old rodents or humans restored their responsiveness. This was achieved *in vitro* by incubation of the lymphocytes with egg phosphatidylcholine small

unilamellar vesicles [342] and *in vivo* by feeding old rodents or humans diets enriched with lipid emulsion composed of egg triglycerides:egg phosphatidylcholine:egg phosphatidylethanolamine 7:2:1 (mole ratio) (referred to as AL-7:2:1) [6,330]. This work suggests that intervention and manipulation of the age-related alterations in membrane lipid composition are feasible.

Araki and Rifkind [7] studied age-related changes in erythrocyte lipid composition of healthy adult human males between the ages of 18–95 years. They reported that the major age-dependent change is an increase in cholesterol level with no change in total phospholipid content. They correlated the increased in cholesterol level with an increase in osmotic fragility. The authors suggest that the increase in cholesterol content may be a result of a change in the distribution of cholesterol between various plasma components. Brosche and Platt [47] found an increase of cholesterol to a maximum in the sixth decade of life and a decline thereafter in comparing human erythrocyte membrane ghosts of healthy elderly subjects (four age groups from 70–90 years). Abe et al. [1] found an increase in red blood cell membrane viscosity with age, reduced deformability and increased osmotic fragility in aging rats. Yechiel and Barenholz [unpublished] observed an age-dependent increase in SPM/PC and cholesterol/phospholipids mole ratios parallel to an increase in osmotic fragility in rat erythrocytes. Similar changes in the lipid composition of rat heart during the course of aging were also detected [24]. For a review of RBC membrane changes during aging, see [24a].

Yechiel and Barenholz [479] found that in the heart and erythrocytes of 18-month-old rats the membrane lipid composition can be reversed to that of young rats by intravenous administration of small unilamellar liposomes of egg PC, referred to as lipid replacement therapy. Egg PC (0.5–2.0 g/kg) causes a reduction in the level of both SPM and cholesterol and increases the level of PC. Preliminary results also suggest that such manipulation affects biochemical and physiological functions. Erythrocytes of old rats after lipid replacement therapy show osmotic fragility which is similar to those of young cells. There is an increase in the level of the enzyme creatine phosphokinase (CPK) during rat aging in plasma and in heart tissue. Lipid replacement therapy reverses these changes in the activity of CPK [478]. The effects of PC liposome administration on membrane lipid composition of various tissues and in plasma is beyond the scope of this paper, and readers are referred to reviews by Williams et al. [468] and Williams and Tall [467].

The nervous system and the eye

In the nervous system of humans, the rate of formation of new membranes — and therefore the total amount of membrane lipids in this system — is greater than the loss by cell death up to the fourth decade of life. During this decade the rate of loss begins to exceed the rate of formation of new membranes [352]. In human brain as well as in other mammals, SPM and galactosyl ceramide gradually "replace" PC. PE is "replaced" by sulphatide. It is of interest that in the invertebrate, which does not have SPM, the level of other sphingolipids such as ceramide phosphorylethanolamine or ceramide phosphoethylamine increases with age [352].

Similar but more striking increases in SPM and cholesterol levels were also found for the hard tissues of the eyes such as the cornea, the lens and the chorda, but not in the retina. In humans, the age-dependent changes in lipid composition of the eye are very dramatic. The SPM content in the human lens rises to the extent that SPM becomes the dominant phospholipid, reaching a level of 70% of the total phospholipids. The PC level is reduced in parallel, falling to a level as low as 5.0% [43,44,118]. Senile cataract, one of the frequent abnormalities occurring in the eye upon aging, is another example of an increase in SPM and concomitant reduction of PC and PE levels. The SPM isolated from the eye of cataract patients is more saturated than that isolated from normal patients [292]. The increase in SPM level is again paralleled by increased cholesterol content [78]. In the cornea (Fig. 4) [45], SPM increases with age to become the major lipid. The central part and the outer annular part of the cornea have different compositions. In *arcus senilis*, in which the cornea has an opaque ring at the periphery, the lipid composition of the *arcus* seems "aged" compared with the "younger" central part.

Maneta-Peyret et al. [251] found no striking change in the lipid composition of 2- vs. 24-month-old rats when rat cerebral cortex was examined. The cholesterol to phospholipid ratio (Chol/PL) decreased with small increases in cardiolipin, hydroxysulfatides, and phosphatidylinositol (PI). Except for a small increase in oleic acid, there were no significant changes in phospholipid acyl chain composition. They examined changes in fatty acid distribution using anti-fatty acid (oleic) antibodies and found age-related modifications in different layers of the cortex. Sun et al. [426] noted a decrease in phosphatidylserine (PS) in the cerebral cortex of 6- vs. 28-month-old mice with no other significant

changes in PL content. Miyamoto et al. [272] found an increased molar ratio of Chol to PL when comparing cerebral cortex of 2-, 12- and 24-month-old rats. They correlated an increase in membrane viscosity with a loss of cortical α_1-adrenergic receptor density. For a general review on the nervous system and aging, see [369,400].

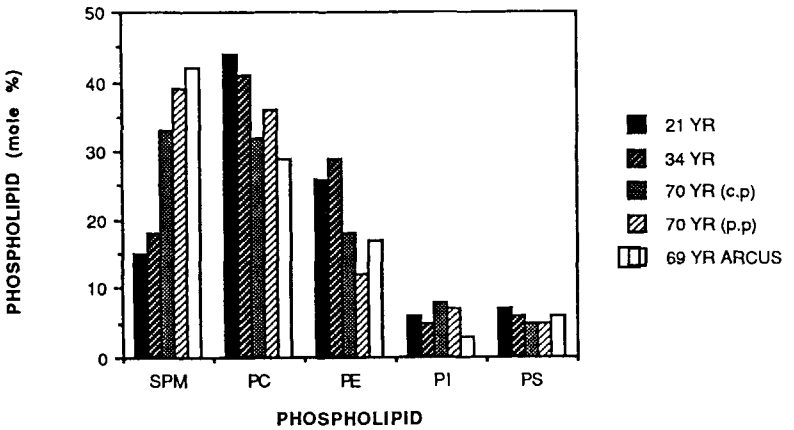

Fig. 4. The phospholipid composition of human cornea as a function of age. Data from [45]. c.p. =central part, p.p. =peripheral part, *arcus*=opaque ring of *arcus senilis*.

In vivo *lipid compositional changes in isolated membranes*

Stommel, Berlet and Debuch [423] analyzed purified myelin from young (below 50 years) vs. old (above 50 years) human brains. They did not find an increase in the Chol/PL ratio; they did find a significant decrease in PS content. Differences in acyl chain composition were detected only in sphingolipids. In addition they detected a general increase in hydroxy fatty acid residues.

Wood [471] examined changes in the lipid composition of cortical synaptosomes from mice aged 4, 16 and 28 months. With age, cholesterol levels increased, phospholipid level increased marginally and, therefore, the Chol/PC ratio increased. The major phospholipid compositional change was a decrease in PE/PC and increased saturation in PC. They compared these changes with the responses of young and old mice to ethanol treatment, treatment which is expected to increase fluidity. Membranes of older mice were less responsive to ethanol, exhibiting smaller changes in fluidity than young membranes. In addition, the concentration of ethanol needed to inhibit α-aminobutyric acid (GABA) release by 50% was twofold higher in membranes of old mice when compared with young mice [425].

Recent studies by Wood et al. [472] describe an age-related increase in dolichol in synaptic membranes obtained from mice. It was found that in older animals, the dolichol isoprenologue chain length shifted toward shorter chains. Wood proposed that dolichol may regulate the fluidity of synaptic plasma membrane in old animals to maintain optimal fluidity. This may be an additional mechanism of homeoviscous adaptation [388]. An increase in dolichols may also act at the biochemical level by contributing to the elevation of enzymatic protein glycosylation which has been reported in diabetes [22]. During aging, enzymatic glycosylation may be at least as relevant as nonenzymatic glycosylation [233]. The fact that the level of both cholesterol and dolichols increase concomitantly suggests that this increase may be related to an increase in the activity of the enzyme HMG-CoA reductase, the first specific step in the biosynthesis of isoprenoid compounds in animals (see Fig. 8; and [138]).

Tanaka and Ando [432] analyzed age-dependent alteration in phospholipid composition of mouse nerve terminals (synaptosomes) between the age of 6–27 months. The major change they found was a reduction in PC level. The authors related this to the fact that the resting membrane potential becomes less negative with advancing age, which may be explained by the decrease in the activity of Na^+,K^+ ATPase. Reduction in PC level without compensation by other lipids will result in a decrease in total phospholipid content in the synaptosomes, which indeed was found, and to an increase in cholesterol to phospholipid and sphingomyelin to PC mole ratios. Viani et al. [458] also found decreased membrane PLs, no change in cholesterol level and therefore increased Chol/PL with age in synaptosomes of rats of 1, 12 and 24 months old. Fluidity was decreased and membrane bound Na^+,K^+ ATPase activity was reduced.

Spinedi et al. [415] analyzed the liver plasma membranes of Wistar rats aged 3 and 24 months and found positive correlations between protein/PL and Chol/PL ratios. The protein/Chol ratio remained unaffected. Among the major PL classes, they found that only PC content decreased with age. Using diphenylhexatriene (DPH) as a probe, Nokubo [290] detected a progressive increase in liver plasma membrane microviscosity (the reciprocal of fluidity) in male rats after 2 months, whereas in female rats, microviscosity began to increase only after 24 months. The activities of the enzymes Mg^{++}-ATPase and Na^+, K^+ ATPase did not change significantly, whereas 5' nucleotidase activity at pH 9.1 decreased in males.

Schmucker et al. [367] analyzed the lipid content of liver microsomes of young (3–4 months), mature (12–16 months) and senescent (25–27 months) male Fisher rats. Cholesterol content did not change appreciably, but there was a significant increase in the Chol/PL ratio between 16 and 27 months, increasing from 0.49–0.65. No net change in the acyl chain saturation index was found. Hawcroft, Jones and Martin [156], examining mouse liver microsomes, found declining mixed function oxidase activity paralleling a decrease in the ratio of PC to PE and SPM and a reduction in saturation of PL acyl chains. Maloney et al. [250] studied the effects of aging on the liver microsome mixed function oxidase system of monkeys aged 1–25 y. In their lipid analysis they found minor changes in total phospholipid content and no significant changes in the different PL classes. Microsomal cholesterol increased between 16–25 y, resulting in a rise in the Chol/PL ratio. Electron spin resonance spectroscopy indicated a decrease in the fluidity of the lipid domain. They found no significant changes in the concentration of microsomal protein or cytochromes P-450 content. At 19 y, NADPH cytochrome C reductase activity was increased.

Lewin and Timiras [241] isolated mitochondrial membranes from the myocardium of 4- and 30-month-old male Long Evans rats and observed increased cholesterol content and increased proportion of SPM and phosphatidylglycerol (PG). They also analyzed variability in acyl chain composition and found increased levels of saturation for most PL.

Pratz and Corman [327] studied enzyme activities, protein electrophoretic patterns and the lipid composition of kidney brush border membranes in 10-, 20- and 30-month-old male and female Wistar rats. Membrane protein patterns remained essentially the same. The Km of three hydrolases — maltase, L-aminopeptidase and alkaline phosphatase — were not affected; the Vmax of maltase and alkaline phosphatase decreased in 10- vs. 30-month-old rats. The Vmax of L-aminopeptidase was not affected. The PL/protein ratio was constant between 10 and 20 months and then increased from 20–30 months for both males and females. In males, the cholesterol levels increased faster than the phospholipid level, and therefore the Chol/PL ratio was greater at 30 months than at 10 months. In females this ratio remained unchanged. The acyl chain composition stayed the same in males and decreased in females by 10% in the proportion of arachidonic acid. In a second study on isolated brush border vesicles of 10- and 24-month-old rats, they found at a 20% rise in total Chol/mg protein with age correlated with a

decrease in salt permeability but not the osmotic water permeability of these membranes [328].

Brush border membranes of renal cortical tissue of 6- vs. 24-month-old rats were analyzed by Levi et al. [237]. They attempted to correlate decrements in renal tubular function particularly related to inorganic phosphate (P_i) transport with changes in lipid composition and fluidity. They reported an increase in Chol and SPM concomitant with a decrease in membrane fluidity based on fluorescence anisotropy of DPH. Low phosphorus diets in younger adults resulted in lower levels of Chol and SPM and increased fluidity. Aged rats did not show significant changes in lipid composition as a result of the low phosphorus diet, suggesting that this may be related to the impairment of function.

Keelan et al. [206] analyzed the lipid content of brush border membranes of rabbit intestine. They compared mature vs. weanling animals and found that the ratio of total PL to total cholesterol was not affected. The total amount of PL increased. There was a decrease in the percent of PC per total lipid and an increase in the percent of SPM and PS. Cholesterol and cholesterol esters also increased. In the jejunum, decreased amount and proportion of the choline phospholipids PC and SPM and increased proportions of the amino phospholipids PS and PE were detected. In ileal brush border membranes, they found a relative increase in PS and SPM and decrease in PC and PI.

Zlatanov et al. [490] studied age-related alteration in lipid composition of testicular plasma membrane in Wistar rats. They found that cholesterol to protein ratio increased 2.5-fold from 1–20 months, phospholipid to protein ratio increased 1.5-fold from 1–7 months followed by a late decrease in this ratio. This resulted in an increase in the Chol/PL mole ratio from 0.31 at the first month to 1.1 at the 20th month. These compositional changes were consistent with age-related changes found in the lateral mobility of the lipid probe 5-(N-octadecanoyl)amino fluorescein (AF18) (see the section on Bulk Effects: Membrane Organization and Dynamics).

Studies of cells in culture

The complexity of the *in vivo* systems and the long period of time required to accumulate data during *in vivo* studies (i.e., 3 y for rats) have encouraged the use of cell cultures as model systems for aging studies. How well they reflect the *in vivo* situation is still an unresolved issue (for a review, see [160]). The primary support for model systems

is the accumulating evidence that the proliferative capacity of cells *in vitro* decreases as a function of donor age [160]. Studies of epidermal cells [335], human liver cells [235], articular chondrocytes [3] and human arterial smooth muscle cells [34] all indicate decreased ability to replicate as donor age increases. Schneider and Mitsui [368] compared cultures of human upper arm skin fibroblasts from young and old donors and found that replicative capacity, migration of cells, cell number at stationary phases and *in vitro* life span decreased with increasing donor age. Evans and Georgescu [113] found that the proliferative capacity of articular cartilage derived cells from various mammalian species was directly related to the life spans of the donor.

The work of Hayflick and Moorhead [161] formed the basis for the use of cell cultures as models for aging. They demonstrated that the growth curve of primary cell cultures can be described by three phases, of which the third phase is considered analogous to *in vivo* aging. The cells have a limited life span defined by a limited number of passages which is typical of each cell type. Phase III begins when the number of cells start to decrease after the cells have reached a finite number of doublings. There is increasing evidence today suggesting that cultures of normal cells which progress from phase II to phase III may undergo changes similar to *in vivo* cells when aging. It is well documented that cell death in phase III is due to an inherent property and not due to factors such as medium components or culture conditions (for a review, see [157,160,291]).

Rigorous studies of aging cells in culture should include assessment of DNA and RNA content, synthesis and turnover; protein content and turnover; enzyme activities; kinetics of the cell cycle; cell structure and morphology (see [158,318]). When considering *in vitro* model systems, one must be aware that: (1) not all cell types have continuous mitotic capability; (2) there may be nonsynchrony of cell division in culture; and (3) viral infection and chemically induced transformation may induce changes in some cells in a given cell population which are irrelevant to *in vivo* aging. Such processes may explain the observed heterogeneity in cell ability to divide and to senesce [348]. Therefore, a procedure such as the "thymidine labeling index" proposed by Cristofalo and Sharf [82] is required by which one can assess the percent of dividing cells in each population. Despite the controversial aspects of correlating *in vivo* aging with cellular senescence, cells in culture may serve as a means for studying the contribution of membrane lipid composition to structure-function relationships. At least some of the age-dependent changes in lipid composition do occur in cells in culture.

One of the main advantages of the use of cells in culture is that their lipid composition can be manipulated either biochemically by affecting lipid metabolism or physically by the use of lipid donors or acceptors. These include modified or unmodified lipoproproteins, liposomes of defined lipid composition or lipid emulsions [6,302,479,480,484]. Manipulating cell lipid composition by the use of lipid donors or acceptors occurs via two mechanisms. The first involves a process of exchange and/or transfer with a donor of defined lipid composition [6, 301,480,481,483,484]. This approach was used by Pagano and coworkers to study the intracellular movement of various fluorescent lipid analogues in cells [300]. The advantage of this process is that it does not involve immediate metabolic transformation of most lipids. The lipids are introduced to the cell into the external leaflet of the cell plasma membrane and move to other cellular membranes via various modes of intracellular lipid traffic [300,455]. The second mechanism is based on endocytosis in which the donor particles (liposomes or defined lipoproteins) are taken up by the cells into lysosomes and/or endosomes. This method has a major drawback in that the lipids may be modified in the lysosomes.

Heart cells

Rogers [346] compared phospholipids and their acyl chain compositions in adult and newborn rat hearts as well as in cultured neonatal rat heart cells. In adults, the proportion of linoleic acid was higher and palmitic acid was lower when compared to newborn or cultured cells. In heart cells after 3 d in culture, the proportion of arachidonic acid resembled that in newborn and adult and showed a gradual and significant decline with culture age. The gradual shift in acyl chain composition as the cells aged in culture was attributed to an outgrowth of mesenchymal cells characterized by a low relative proportion of arachidonic acid while the number of myocytes remain unaltered. They found an absence of significant change in PL composition of heart cells for up to 3 weeks in culture, except SPM content which was higher in 3, 7, and 21 d cultures than in both newborn or adult heart.

The effect of changes in membrane composition was studied by Barenholz and co-workers [479,480,483,484] with of three cell culture systems: (1) monolayers of myocytes prepared from newborn rat hearts, (2) monolayers of fibroblasts from newborn rat hearts, and (3) cultured heart cell reaggregates prepared from rats of various ages.