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APPENDIX I

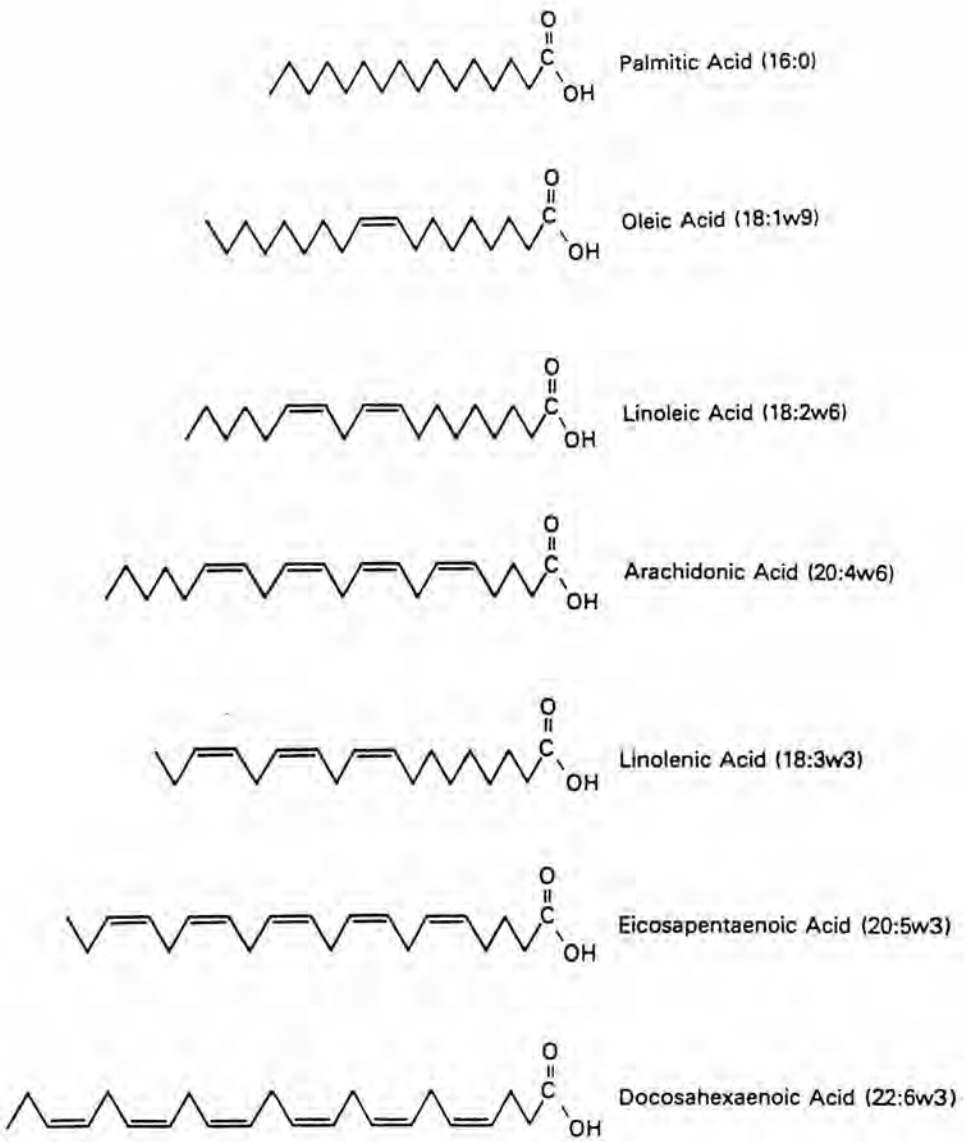

Figure 21 Structural formulas for omega-6 (linoleic acid, 18:2ω6) and omega-3 (alpha-linoleic acid, 18:3ω3) fatty acids. The first number (before the colon) gives the number of carbon atoms in the molecule and the second gives the number of double bonds. ω6 and ω3 indicate position of the first double bond in a given fatty acid molecule.

Table 33 Fatty acid composition of food fats (g fatty acid per 100 g total fatty acids)

	Caprylic $C_{8:0}$	Capric $C_{10:0}$	Lauric $C_{12:0}$	Myristic $C_{14:0}$	Pentadecylic $C_{15:0}$	Palmic $C_{16:0}$	Palmitoleic $C_{16:1}$	Hexadecadienoic $C_{16:2}$	Margaric $C_{17:0}$	Stearic $C_{18:0}$	Oleic $C_{18:1}$	Linoleic $C_{18:2}$	Linolenic $C_{18:3}$*	Stearidonic $C_{18:4}$	Arachidic $C_{20:0}$	Eicosenoic $C_{20:1}$	Eicosadienoic $C_{20:2}$	Arachidonic $C_{20:4}$	Timnodonic $C_{20:5}$	Behenic $C_{22:0}$	Erucic[6] Cetoleic[6] $C_{22:1}$	Docosatetraenoic $C_{22:4}$	Docosapentaenoic $C_{22:5}$	Clupanodonic $C_{22:6}$	Lignoceric $C_{24:0}$	Selacholeic $C_{24:1}$	References
Plant lipids																												
Almond oil	0	0	0	trace	–	7.0	0.6	–	0.1	1.2	67.3	23.7	0.2	–	0	–	–	–	–	0	0	–	–	–	0	–	21
Cocoa butter	–	0	trace	0.1	–	27.6	0.4	–	trace	33.9	34.0	3.1	trace	–	0.9	–	0	–	–	0	0	–	–	–	–	–	21
Coconut oil	10.1	6.6	45.8	18.4	–	8.0	trace	–	–	2.4	6.0	1.7	0	–	0	–	–	–	–	0	0	–	–	–	–	–	21
Corn oil	0	0	0	trace	–	11.5	0.1	–	trace	2.0	28.3	56.3	1.0	–	0.6	–	–	–	–	0	0	–	–	–	–	–	21
Cottonseed oil	0	trace	0.1	0.6	–	22.6	0.5	–	trace	2.6	18.1	52.3	1.7	–	0.5	–	–	–	–	0.2	0.7	–	–	–	–	–	21
Grape-seed oil	–	0	trace	0.04	–	6.7	0.1	–	–	3.1	12.1	77.3	0.7	–	0	–	0	–	–	0	–	–	–	–	–	–	21
Linseed oil	–	0	trace	trace	–	6.0	0.1	–	trace	5.1	21.1	14.9	53.0	–	trace	–	–	–	–	trace	0	–	–	–	–	–	21
Margarine**	2.2–5.0	1.9–3.7	14.2–29.3	6.1–11.4	–	9.4–22.1	–	–	–	3.3–8.0	14.3–38.1	10.3–29.3	0–2.5	–	–	–	–	–	–	–	–	–	–	–	–	–	22
Mustard-seed oil	0	0	trace	0.05	–	2.9	0.2	–	–	1.0	19.4	9.7	21.4	–	0.7	–	0.4	–	–	0.4	44.4	–	–	–	0.3	2.7	21
Olive oil, European[1]	0	0	0	trace	–	11.8	0.9	–	trace	2.8	74.5	8.7	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	24
–, Tunisian	–	0	0	0	–	16.8	1.9	–	trace	2.5	58.9	18.8	0.9	–	–	–	–	–	–	–	–	–	–	–	–	–	21
Palm-kernel oil	4.9	3.9	48.7	16.0	–	7.6	0	–	–	1.9	14.2	2.6	0	–	–	–	–	–	–	–	–	–	–	–	–	–	21
Palm oil	–	–	trace	1.5	–	45.1	trace	–	trace	4.8	36.8	10.2	0.5	–	0.5	–	0	–	–	trace	0.2	–	–	–	–	–	21
Peanut oil	–	0	trace	trace	–	10.6	0.1	–	0.1	3.5	49.5	28.8	1.3	–	1.8	–	–	–	–	3.1	0.1	–	–	–	1.6	–	21
Rapeseed oil	0	0	0	0.05	–	3.6	0.2	–	trace	1.4	15.5	13.9	19.0	–	0.8	7.5	0.5	–	–	0.5	45.5†	–	–	–	0.5	–	21
Safflower oil	0	0	trace	0.1	–	7.5	0.1	–	trace	2.6	12.7	75.5	0.8	–	0.7	–	–	–	–	0.2	0.1	–	–	–	–	–	21
Sesame oil	–	0	trace	trace	–	8.9	0.3	–	trace	6.0	40.7	41.7	1.7	–	0.8	–	–	–	–	0	0	–	–	–	–	–	21
Soybean oil	0	0	0	0.1	–	10.7	0.1	–	0.1	3.8	23.0	52.4	8.9	–	0.6	–	–	–	–	0.4	trace	–	–	–	–	–	21
Sunflower-seed oil	–	0	trace	0.1	–	6.6	0.1	–	trace	4.3	22.4	65.2	0.3	–	0.4	–	–	–	–	0.7	0	–	–	–	–	–	21
Walnut oil	–	0	0	0.02	–	7.0	0.1	–	trace	2.1	18.7	59.8	13.3	–	trace	–	–	–	–	0	0	–	–	–	0	–	21
Animal lipids																												
Cow's milk	1.0	2.5	2.9	10.1	–	24.7	3.3	–	–	9.5	22.5	4.0	2.8	–	trace	–	–	–	–	–	–	–	–	–	–	–	26
Eggs	–	–	–	0.6	–	25.5	4.5	–	–	9.9	45.4	13.0	1.7	–	–	–	–	–	–	–	–	–	–	–	–	–	23
Fish																												
Cod	–	–	–	1.8	–	0.5	33.4	2.4	0.9	4.0	11.8	1.2	0.8	1.2	–	1.6	–	3.2	12.4	–	0.7	–	0.6	21.9	–	–	25
Cod-liver oil	–	–	–	2.8	–	0.4	10.7	6.9	1.2	3.7	23.9	1.5	0.9	2.6	–	8.8	0.5	1.0	8.0	–	5.3	0.3	1.3	14.3	–	0.5	25
	–	–	–	3.7	0.1	0.2	12.8	11.8	0.9	3.2	25.7	1.0	0.4	1.6	trace	6.7	0.2	0.2	12.7	trace	3.8	0.2	1.5	6.9	trace	1.0	26
Eel	–	–	–	5.9	1.6	0.6	14.0	12.4	0.7	1.4	27.8	1.0	trace	–	–	28.3	–	0.5	0.8	trace	trace	trace	0.7	0.5	–	trace	24
Herring	–	–	–	7.6	–	0.4	18.3	8.3	0.5	2.2	16.9	1.6	0.6	2.8	–	9.4	–	0.4	8.6	–	11.6	trace	1.3	7.6	–	0.9	23
Mackerel	–	–	–	4.9	–	0.5	28.2	5.3	1.0	3.9	19.3	1.1	1.3	3.4	–	3.1	–	3.9	7.1	–	2.8	–	1.2	10.8	–	0.8	25
Salmon	–	–	–	15.1	–	trace	15.3	5.6	1.5	3.8	27.2	1.5	0.9	–	trace	8.0	–	0.5	3.4	trace	10.5	–	0.7	2.5	–	trace	24
Trout	–	–	–	2.1	–	0.8	11.9	8.2	1.5	4.1	19.8	4.6	5.2	1.5	–	3.0	0.6	2.2	5.0	–	1.3	0.6	2.6	19.6	–	0.7	25
Tunny	–	–	–	4.5	–	0.6	22.1	2.8	0.8	6.1	21.7	2.1	1.2	0.5	–	2.0	–	3.0	13.2	–	trace	–	1.5	17.3	–	–	27
Beef	–	–	–	3.2	0.6	26.9	6.3	–	1.2	13.0	42.0	2.0	1.3	–	–	trace	–	1.0	trace	–	–	–	trace	–	–	–	8
Chicken	–	–	–	1.3	trace	26.7	7.2	–	trace	7.1	39.8	13.5	0.7	–	–	0.6	–	0.7		–	–	–	–	1.0	–	–	8
Duck	–	–	–	0.6	trace	22.8	4.4	–	trace	5.5	52.8	12.1	0.6	–	–	trace	–	trace	trace	–	–	–	trace	–	–	–	8
Lamb	–	–	–	5.4	0.6	24.2	1.3	–	1.0	20.9	38.2	2.5	2.5	–	–	trace	–	0	trace	–	–	–	trace	–	–	–	8
Pork	–	–	–	1.6	trace	27.1	3.4	–	trace	13.8	43.8	7.4	0.9	–	–	0.7	–	trace	trace	–	–	–	trace	–	–	–	8
Turkey	–	–	–	1.0	trace	25.0	5.0	–	0.5	10.0	21.5	20.0	1.0	–	–	0.4	–	5.0	1.5	–	–	–	2.0	5.0	–	–	8

* In plant lipids $C_{18:3} + C_{20:1}$. [6] Erucic acid in plant lipids, cetoleic acid in fish. ** Values based on 9 Swiss samples; for American brands, see WEIHRAUCH et al.
[1] Spain, France, Italy. † New strains contain less erucic acid.

Table 34 Content of ω3 Fatty Acids and Other Fat Components in Selected Fish

Fish	Fatty acids							Cholesterol
	Total fat	Total saturated	Total mono-unsaturated	Total poly-unsaturated	18:3	20:5	22:6	
Anchovy, European	4.8	1.3	1.2	1.6	—	0.5	0.9	—
Bass, striped	2.3	0.5	0.7	0.8	Tr	0.2	0.6	80
Bluefish	6.5	1.4	2.9	1.6	—	0.4	0.8	59
Carp	5.6	1.1	2.3	1.4	0.3	0.2	0.1	67
Catfish, brown bullhead	2.7	0.6	1.0	0.8	0.1	0.2	0.2	75
Catfish, channel	4.3	1.0	1.6	1.0	Tr	0.1	0.2	58
Cod, Atlantic	0.7	0.1	0.1	0.3	Tr	0.1	0.2	43
Croaker, Atlantic	3.2	1.1	1.2	0.5	Tr	0.1	0.1	61
Flounder, unspecified	1.0	0.2	0.3	0.3	Tr	0.1	0.1	46
Grouper, red	0.8	0.2	0.1	0.2	—	Tr	0.2	—
Haddock	0.7	0.1	0.1	0.2	Tr	0.1	0.1	63
Halibut, Greenland	13.8	2.4	8.4	1.4	Tr	0.5	0.4	46
Halibut, Pacific	2.3	0.3	0.8	0.7	0.1	0.1	0.3	32
Herring, Pacific	13.9	3.3	6.9	2.4	0.1	1.0	0.7	77
Herring, Round	4.4	1.3	0.8	1.5	0.1	0.4	0.8	28
Mackerel, king	13.0	2.5	5.9	3.2	—	1.0	1.2	53
Mullet, striped	3.7	1.2	1.1	1.1	0.1	0.3	0.2	49
Ocean perch	1.6	0.3	0.6	0.5	Tr	0.1	0.1	42
Plaice, European	1.5	0.3	0.5	0.4	Tr	0.1	0.1	70
Pollock	1.0	0.1	0.1	0.5	—	0.1	0.4	71
Pompano, Florida	9.5	3.5	2.6	1.1	—	0.2	0.4	50
Salmon, Chinook	10.4	2.5	4.5	2.1	0.1	0.8	0.6	—
Salmon, pink	3.4	0.6	0.9	1.4	Tr	0.4	0.6	—
Snapper, red	1.2	0.2	0.2	0.4	Tr	Tr	0.2	—
Sole, European	1.2	0.3	0.4	0.2	Tr	Tr	0.1	50
Swordfish	2.1	0.6	0.8	0.2	—	0.1	0.1	39
Trout, rainbow	3.4	0.6	1.0	1.2	0.1	0.1	0.4	57
Tuna, albacore	4.9	1.2	1.2	1.8	0.2	0.3	1.0	54
Tuna, unspecified	2.5	0.9	0.6	0.5	—	0.1	0.4	—

[a] Values are given as g/100 g edible portion, raw except for cholesterol, which is given as mg. Dash (—) denotes lack of reliable data for nutrient known to be present. Tr, trace (less than 0.05 g/100 g of food).

Adapted from the U.S. Department of Agriculture Provisional Table on the Content of Omega-3 Fatty Acids and Other Fat Components in Seafoods, as presented by Simopoulos et al.

Table 35 Effects of ω3 Fatty Acids on Factors Involved in the Pathophysiology of Atherosclerosis and Inflammation

Factor	Function	Effect of ω3 fatty acid
Arachidonic acid	Eicosanoid precursor; aggregates platelets; stimulates white blood cells	↓
Thromboxane	Platelet aggregation; vasoconstriction; increase of intracellular Ca++	↓
Prostacyclin ($PGI_{2/3}$)	Prevent platelet aggregation; vasodilation; increase cAMP	↑
Leukotriene (LTB_4)	Neutrophil chemoattractant increase of intracellular Ca++	↓
Tissue plasminogen activator	Increase endogenous fibrinolysis	↑
Fibrinogen	Blood clotting factor	↓
Red cell deformability	Decreases tendency to thrombosis and improves oxygen delivery to tissues	↑
Platelet activating factor (PAF)	Activates platelets and white blood cells	↓
Platelet-derived growth factor (PDGF)	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen-free radicals	Cellular damage; enhance LDL uptake via scavenger pathway; stimulate arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulate eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil O_2 free radical formation; stimulate lymphocyte proliferation; stimulate PAF; express intercellular adhesion molecule-1 on endothelial cells; inhibit plasminogen activator, thus, procoagulants	↓
Endothelial-derived relaxation factor (EDRF)	Reduces arterial vasoconstrictor response	↑
VLDL	Related to LDL and HDL level	↓
HDL	Decreases the risk for coronary heart disease	↑
Lp(a)	Lipoprotein (a) is a genetically determined protein that has atherogenic and thrombogenic properties	↓
Triglycerides and chylomicrons	Contribute to postprandial lipemia	↓

Adapted from Weber, P.C. and Leaf, A., *World Rev. Nutr. Diet*, 66, 218, 1991.

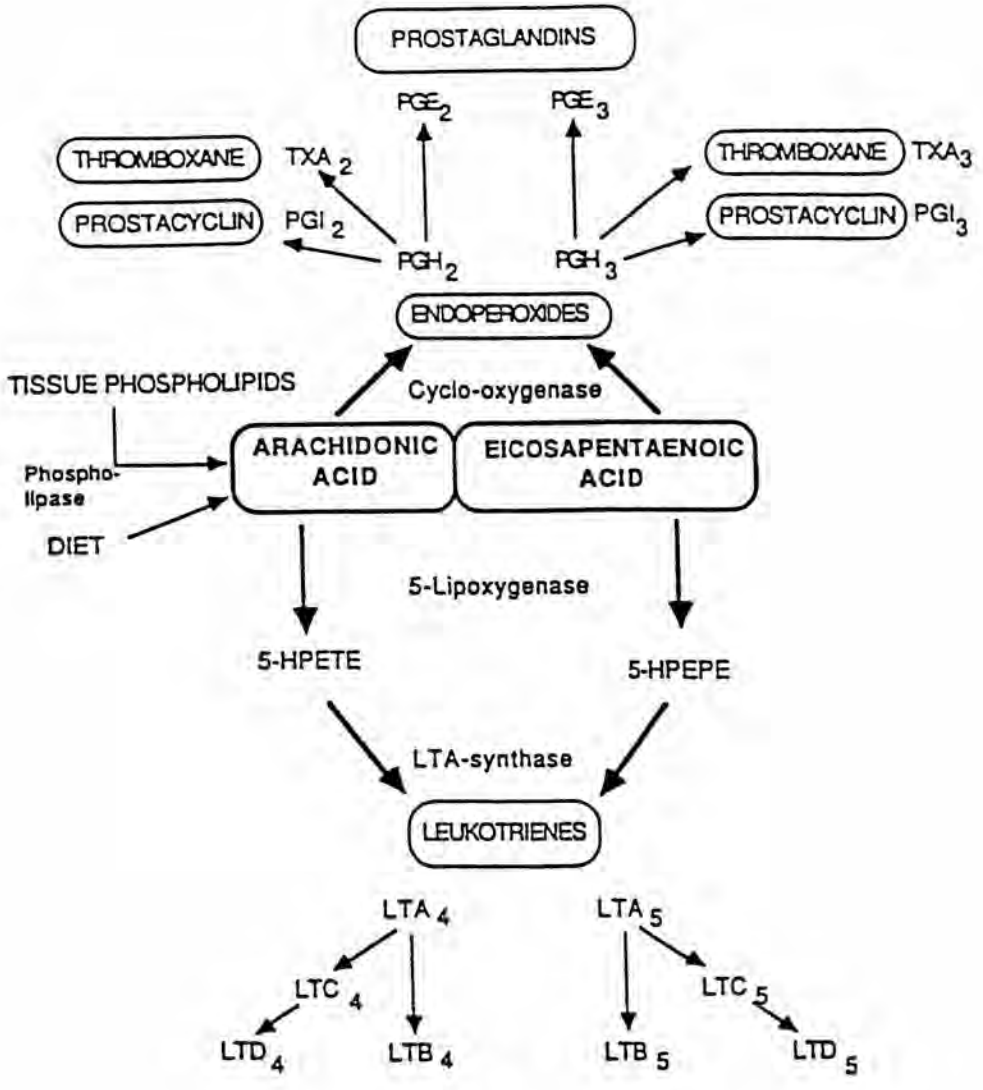

Figure 22 Oxidative metabolism of arachidonic acid and eicosapentanoic acid by the cyclooxygenase and 5-lipoxygenase pathways. 5-HPETE denotes 5-hydroperoxyeicosatetranoic acid and 5-HPETE denotes 5-hydroxyeicosapentanoic acid.

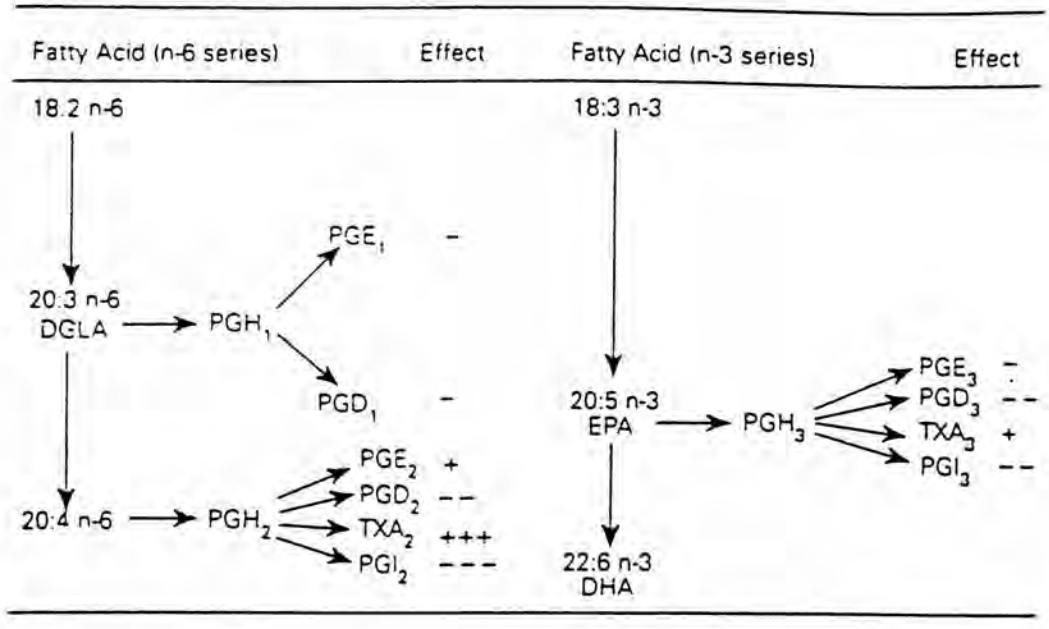

Figure 23 Effect of different prostaglandins on platelet aggregation.

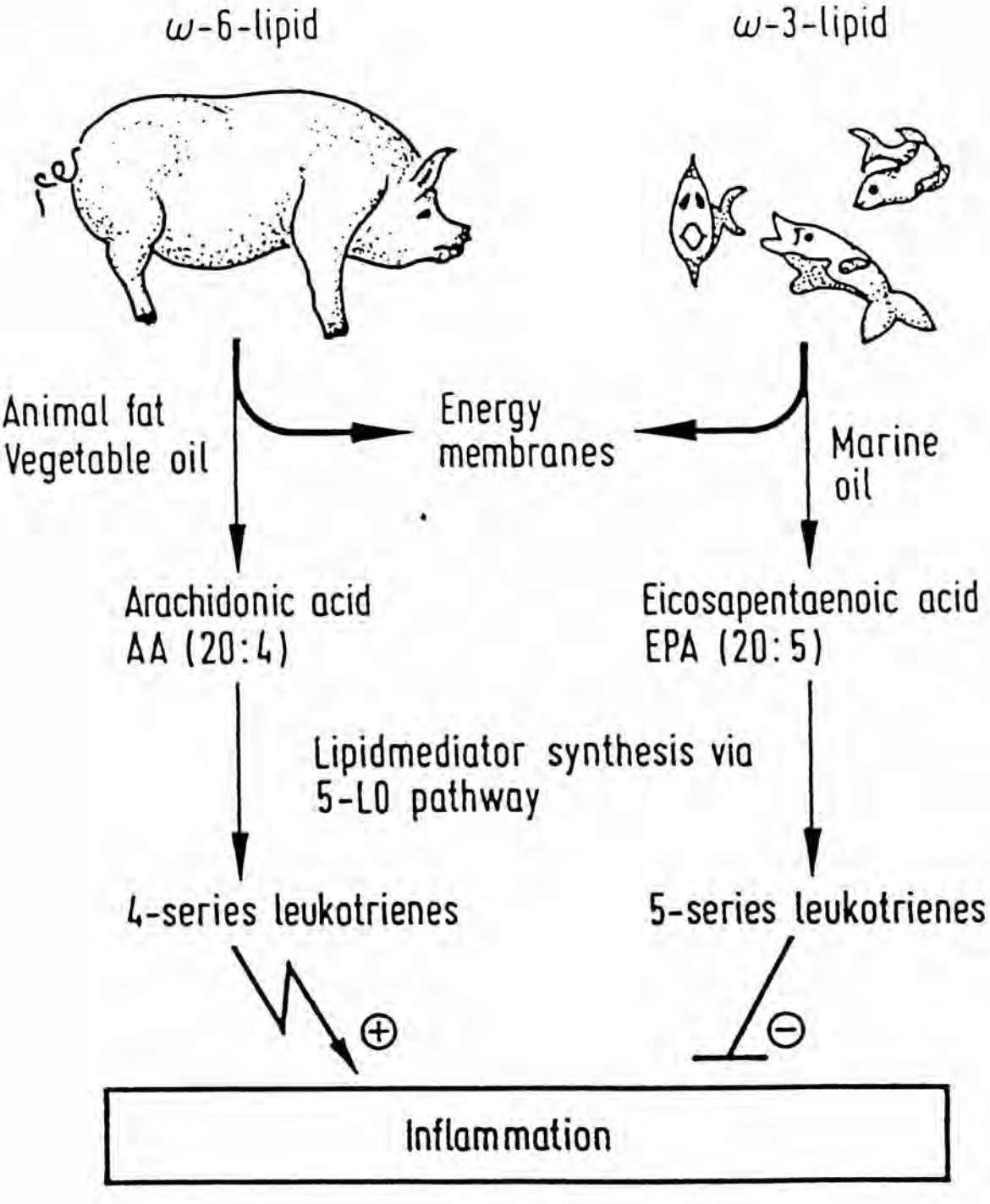

Figure 24 Dietary sources of lipid mediator precursors.

APPENDIX II

mentioned in BNFTF

8

FUNCTIONS OF UNSATURATED FATTY ACIDS

The major roles of lipids can be described conveniently as:

- energy storage;
- energy-providing;
- structural;
- metabolic.

Individual lipids may have different roles to play at different times or sometimes they play different roles at the same time. This chapter will focus mainly on the structural roles of lipids since unsaturated fatty acids have unique roles to play here.

8.1 ENERGY STORAGE ROLE

8.1.1 Adipose tissue

8.1.1.1 Fatty acid composition of storage lipids

Adipose tissue represents the largest reservoir of storage lipids. Storage lipids (triacylglycerols) tend to contain more saturated and monounsaturated fatty acids than structural lipids (phospholipids), although the composition of human storage triacylglycerols can be influenced by the composition of the diet. Fatty acids are mobilised from adipose tissue (lipolysis) to meet energy demands at times when dietary energy is limiting. The release of stored fatty acids is regulated by the amounts and types of different dietary components and by hormones, whose secretion may also be regulated in part by the diet (see Chapter 7).

Storage lipids may be derived directly from the fat in the diet or they may be synthesised in the liver, mammary gland or adipose tissue from dietary carbohydrates (see Chapter 7). The capacity of these tissues to synthesise fatty acids is geared to the needs of the body and is under dietary and hormonal control. The range of fatty acids that can be made is limited, usually to palmitic, stearic and oleic acids. Human adipose tissue has the enzymic machinery for fatty acid synthesis, but activities are very low. When there is little fat in the diet, the fatty acid pattern of adipose tissue is mainly dependent on the liver's biosynthetic activity and is characteristic of the species. The introduction of fat into the diet can suppress the synthetic activity of the tissues and mechanisms operate to transport dietary fatty acids into the storage lipids so that the fat composition is more characteristic of the diet.

Because of the relatively high proportion of energy derived from fat in most industrialised countries, the diet is much the most important determinant of the composition of the fatty acids in adipose tissues.

8.1.1.2 Effects of dietary unsaturated fatty acids on storage lipids

Giving vegetable oils rich in linoleic acid (18:2 n-6) to animals and man causes an enrichment of this fatty acid in plasma and storage triacylglycerols (Field and Clandinin, 1984; Becker, 1989). The low levels of arachidonic acid (20:4 n-6) in these lipids may also be elevated, though not markedly, after giving linoleic acid due to biosynthesis in the liver. Alpha linolenic acid (18:3 n-3) is markedly elevated in the plasma triacylglycerols of rats given linseed oil in the diet and there is some deposition in adipose tissue. Likewise, EPA and DHA are enriched in the storage lipids of rats given certain fish oils containing these acids, but less so than 18:2 n-6 in rats fed vegetable oils (Jandacek *et al.*, 1991). Very recently, accumulation of EPA and DHA has been measured in the adipose tissue triacylglycerols of human beings consuming fish oils in the diet (Lin and Conner, 1990).

If rats are given cod liver oil, which is rich in the long chain monounsaturated fatty acids gadoleic (20:1 n-9) and cetoleic (22:1 n-11), their cardiac triacylglycerols (but not phosphoglycerides) are enriched in these fatty acids. Giving diets containing a high proportion of the monounsaturated acid erucic acid (22:1 n-9) (present in older varieties of rapeseed oil) can also result in the transitory

accumulation of triacylglycerols rich in erucic acid in the cardiac muscle of a variety of experimental animals (Norum *et al.*, 1989).

There are numerous examples of dietary hydrogenated oils, which contain different amounts of fatty acids (mainly monounsaturates) with *trans*-unsaturation, causing marked elevations of *trans* fatty acids in triacylglycerols of plasma, adipose tissue and milk (British Nutrition Foundation, 1987). These substitutions occur mainly in positions 1 and 3 of triacylglycerols. High dietary inclusions of C18 *trans*-unsaturated fatty acids may also cause increases in the *trans*-monounsaturated fatty acid content of tissue phosphoglycerides. This occurs mainly at position 1 (British Nutrition Foundation, 1987).

Starvation of rats whose diets originally contained vegetable oils rich in linoleic acid results in preferential mobilisation of 16:0 and 18:1 and a selective retention of linoleic acid and arachidonic acid. Starvation of rats whose diets originally contained fish oils rich in n-3 fatty acids results in the selective retention of DHA in triacylglycerols but a selective removal of EPA (Cunnane, 1989).

8.1.1.3 Analysis of adipose tissue as an indicator of dietary fatty acids

The lipids in adipose tissue are slowly, but continuously, being replaced. The continual breakdown and resynthesis of lipids is called lipid turnover. Lipid replacement may occur

(i) by complete synthesis of the lipid from its simplest precursors;
(ii) by replacement of parts of the molecules;
(iii) by replacement of whole lipid molecules that have been transported from another site and that have gone through the lipolysis and re-esterification cycle.

Turnover allows a finer degree of metabolic control in a dynamic system than would be possible in a more static system. Although in some tissues lipids turnover very rapidly, in adipose tissue turnover is so slow that the fatty acid composition of adipose tissue gives a reasonable reflection of the habitual fat consumption over a long period of time.

The biopsy technique can be used to obtain small pieces of adipose tissue for fatty acid analysis. It does not give a direct measure of the dietary content of all types of fatty acid because the fatty acids are not necessarily deposited in direct proportion to their content in the diet. It is, however, most useful for the essential fatty acid, linoleic acid. Different amounts of linoleic acid can be fed in diets and corresponding amounts can be found in adipose tissue.

The technique has also been useful for monitoring the dietary intake of *trans* fatty acids. It is less useful for some of the longer chain polyunsaturated fatty acids, e.g. arachidonic acid, which tend to accumulate in adipose tissue to a lesser extent than would be predicted from their content in the diet. This is probably because they are preferentially esterified into the structural phospholipids, leaving less to be stored in the fat tissue.

8.1.2 Storage lipids in other tissues

The triacylglycerols in the fat globules of milk can be regarded as an energy store for the newborn baby.

Other tissues, such as the liver and heart of mammals, can accumulate fat in the form of small globules but only for short-term use. The extensive accumulation of fat in mammalian liver and heart is a pathological condition. However, some species of fish, particularly gadoids, normally store fat in the liver rather than subcutaneously or around the internal organs.

8.2 ENERGY-PROVIDING ROLE

Fatty acids are mobilised from adipose tissue to meet the demands for energy at times when dietary energy is limiting, for example in starvation or in strenuous exercise. The release of stored energy is regulated by the amounts and types of different dietary components and by hormones, whose secretion may also be regulated in part by diet. Thus, the post-prandial elevation of the hormone insulin in the blood is one of the factors that ensures that fatty acids from circulating lipoproteins are shunted into adipose tissue via the action of lipoprotein lipase, whilst the hormone-sensitive lipase that breaks down the stored fat is inhibited. During fasting, the relative deficiency of insulin and sufficiency of glucagon allows the hormone-sensitive lipase to release fatty acids from the stored fat. They are then mobilised into the bloodstream and carried in a complex with albumin to other tissues where they can be broken down by the process of beta-oxidation (described in Chapter 7) to yield energy that drives metabolic reactions.

Different types of fatty acids may be oxidised to different extents. There is some evidence that the essential fatty acids may be conserved for their more vital membrane functions at the expense of the non-essential fatty acids.

8.3 STRUCTURAL ROLES IN MEMBRANES

8.3.1 Membrane structure

Current theories of membrane structure envisage that most of the phospholipid is present as a bimolecular sheet with the fatty acid chains in the interior of the bilayer (the 'fluid mosaic' model of Singer and Nicholson, 1972). Membrane proteins are located at the internal or external face of the membrane, or projecting through from one side to the other (Figure 8.1).

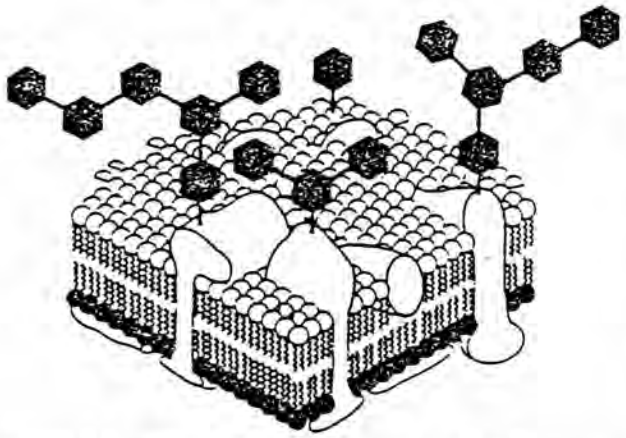

Figure 8.1 Structure of cellular membrane: the Singer–Nicholson fluid-mosaic proposal for membrane structure. Carbohydrate groups on lipids and proteins face the extracellular space.

There may be polar interactions between the phospholipid headgroups and ionic groups on the proteins as well as hydrophobic interactions between the fatty acid chains and hydrophobic amino acid sequences. Lipid molecules are quite mobile along the plane of the membrane but there is limited movement across the membrane. The patterns of lipid molecules on each side of some membranes which exhibit 'membrane asymmetry' are quite different.

The lipid provides a flexible structure in which are located the proteins that control many metabolic activities within the membrane. The proteins in question may be enzymes, transporters or receptors for substances such as hormones, antigens or cell-growth factors. The physical properties of the membrane are strongly influenced by the lipid composition. They are important because they are regulated in the face of environmental changes (diet, temperature, etc) by subtle differences in the proportions of amphiphilic lipids and sterols and changes in the fatty acid composition of the lipids.

An important feature of the physical properties of membranes is the degree of freedom for molecules to move about in the membrane, generally described as membrane fluidity. Fluidity is, in part, related to the packing of lipids in the bilayer. Among the most important factors that affect this packing are:

(i) the nature of the fatty acid chains;
(ii) the amount of choiesterol (in animal membranes);
(iii) interactions, both polar and non-polar, between lipids and proteins.

8.3.2 Role of unsaturated fatty acids in membrane structure

Unsaturated fatty acids are found in membranes mainly esterified in phosphoglycerides.

The presence of unsaturation in the chains of fatty acids affects their shape and their ability to pack together. Saturated chains can be constrained to pack together in crystalline arrays that give low fluidity. With the introduction of one double bond, a bend in the molecule is formed and the space occupied by the fatty chain is much increased. The chains, therefore pack less well together and the fluidity increases. There is not a linear relationship, however, between the degree of unsaturation and the fluidity. After the introduction of two or three double bonds, the fluidity begins to level off and even decrease again. This is because the highly polyunsaturated fatty acids begin to adopt a helical configuration that allows closer packing again (Stubbs and Smith, 1990).

Computer modelling studies have confirmed that the minimal energy conformation of glyceride-linked DHA can either be an 'angle-iron' shape or, more likely, a helix (Applegate and Glomset, 1986).

Consequences of helicity in PUFA molecules are:

(i) The molecules are shortened: e.g. DHA (22:6 n-3) is significantly shorter than arachidonic acid (20:4 n-6) which is shorter than stearic acid (18:0).
(ii) The molecules pack together better than kinked chains so that they form more compact structures.

Substitution of *trans*-monounsaturated for *cis*-monounsaturated fatty acids may lead to a decrease in the fluidity of the lipid bilayer because the *trans*-unsaturated acids can pack together more like saturated acids. However, when dietary *trans*-monounsaturated fatty acids are incorporated into membranes, there is little influence on overall fluidity because the *trans*-unsaturated acids replace saturated fatty acids at position 1 of phosphoglycerides rather than replacing *cis*-unsaturated fatty acids at position 2.

The stability of mammalian membranes is crucially dependent upon the types of unsaturated fatty acids that are incorporated into the phosphoglycerides of the bilayer. Stability is highly dependent on the presence of the essential fatty acids and the longer chain, more highly polyunsaturated fatty acids derived from them. Thus, in the condition of essential fatty acid deficiency (see Chapter 7) essential fatty acids of the n-6 family are replaced by non-essential fatty acids of the n-9 family. The resulting membrane structure is more permeable to water and the efficiency of diverse metabolic processes that occur in the membrane matrix is much reduced.

The contribution of lipids containing specific combinations of fatty acids to the structure of particular membranes is discussed in Section 8.3.6.

8.3.3 Membrane traffic

In general, different cellular membranes have different phosphoglyceride compositions and, in a given membrane such as the plasma membrane which surrounds the cell, phosphoglycerides are sited asymmetrically in the two bilayers. The phosphoglycerides, PtdIns and PtdSer are located predominantly on the cytoplasmic face, whereas PtdCho is found predominantly on the serosal face. Precisely how this is achieved is not known in detail, although two mechanisms may be involved (Van Meer, 1989):

(i) Transfer of the phosphoglycerides from the endoplasmic reticulum where they are synthesised by means of phospholipid binding proteins is especially well characterised for the PtdCho transfer proteins. These are involved particularly in the transfer of PtdCho from the endoplasmic reticulum to the outer mitochondrial membrane.
(ii) Transfer of phospholipids to their final membrane destinations can also take place as vesicles, often generated on intracellular membranes known as the Golgi apparatus. The preferential location of both sphingomyelin and cholesterol in the plasma membrane rather than in internal cell membranes is due to concentration and selection of these lipids in Golgi vesicles.

The molecular selection mechanisms underlying the sorting of phospholipids for their final destinations in the cell are currently unknown. In membrane trafficking, lipids may well be selected in association with specific membrane proteins (Lisanti and Rodriguez-Boudan, 1990).

8.3.4 Establishment of the fatty acid composition of membrane lipids

8.3.4.1 Biosynthetic mechanisms

Individual phosphoglycerides in membranes are characterised by specific combinations of fatty acids in positions 1 and 2 of the molecules. It is not known to what extent these characteristic fatty acid compositions are determined by acylation reactions within the endoplasmic reticulum, or by subsequent selection of particular phosphoglyceride molecular species in the various transport processes described in Section 8.3.3. Selectivity can, in principle, occur at any one of a number of stages in lipid biosynthesis (see Chapter 7) (Gurr and Harwood, 1991):

(i) cellular uptake of free fatty acids (involving fatty acid binding proteins);
(ii) activation of free fatty acids to CoA derivatives;
(iii) chain elongation and/or shortening and further desaturation reactions;
(iv) acylation reactions during biosynthesis of phosphatidic acid followed by selection of diacylphosphoglyceride species;
(v) acyl transfer reactions with preformed phosphoglycerides;
(vi) membrane sorting and trafficking processes (Section 8.3.3).

Many of these processes exhibit species and tissue specificity.

8.3.4.2 Influence of dietary fat on membrane lipid composition

While the composition of membrane lipids is less susceptible to dietary influence than that of storage lipids (Section 8.1.1), modification of the fatty acid composition by dietary fatty acids is well established and, in recent years, has been particularly well studied for PUFA of the n-3 and n-6 series.

The observed changes are not always readily interpretable in terms of tissue lipid biochemistry. For example, when rats are given a diet in which linoleic acid predominates, arachidonic acid (20:4 n-6) is the major PUFA in the liver phosphoglycerides PtdCho and PtdEtn, while 22-carbon PUFA of the n-6 family are present in very low concentrations. In rats given diets containing mainly alpha linolenic acid (18:3 n-3), the major fatty acids in these liver phosphoglycerides are EPA (20:5 n-3) and DHA (22:6 n-3). When the diet contains appreciable amounts of both linoleic acid and alpha linolenic acid, the major PUFA of liver phosphoglycerides are arachidonic acid and DHA. These results do

not directly reflect the activities of the fatty acid desaturases and elongases in rat liver microsomes (Sprecher, 1989, 1991) (see Chapter 7).

Nevertheless, if the conversions of linoleic acid and alpha linolenic acid into their higher PUFA metabolites are studied *in vivo* in the livers of rats that are essential fatty acid-deficient (Chapter 7) with dietary levels of linoleic acid and alpha linolenic acid that are below minimum dietary requirements, and taking into account the inhibitory effects of the n-3 series upon the n-6 series, then interpretable dose–response relationships exist (Lands, 1991). This underlines the importance of working within the responsive range of essential fatty acids in nutritional metabolic research and offers hope that situations involving more complex dietary mixtures of fatty acids, and tissues other than liver, may ultimately be understood in quantitative terms.

Similar effects in enriching membrane phospholipids with the long chain metabolites of linoleic acid and alpha linolenic acid administered in the diet occur in tissues other than the liver, e.g. platelets and neutrophils. However, the proportion of C20 and C22 PUFA ultimately present in tissue membrane lipids can vary widely: e.g. the major phosphoglycerides of both platelets and neutrophils contain much more C20 than C22 PUFA from either C18 dietary precursor (Sprecher, 1989). Since these tissues, as well as heart muscle cells, are not active in PUFA biosynthesis, the observed effects are a combination of liver biosynthetic activities and selective uptake and esterification reactions in the tissues in question.

Extreme examples of tissues with highly selective uptake and esterification mechanisms are brain and retina (see Chapter 9). Postnatal photoreceptor cells, and probably brain cells, appear to derive most of their high concentrations of DHA from the liver or the diet rather than by their own synthetic activity. However, the retina has both extracellular and intracellular fatty acid binding proteins with a high affinity for DHA. Moreover, DHA is activated to its CoA ester by a high affinity acyl-CoA synthetase in retina (Bazan, 1989). Brain also has a PtdEtn fatty acyltransferase selective for DHA (Masuzawa *et al.*, 1989). Processes such as these are fundamental in concentrating DHA in the brain, retina and testis.

Increases in the n-3 PUFA content of the phospholipids PtdCho, PtdEtn and PtdSer have been consistently observed when animals and human beings are given diets supplemented with fish oils rich in EPA and DHA. The composition of PtdIns, which is dominated by a molecular species containing stearic acid (18:0) at position 1 and arachidonic acid (20:4 n-6) at position 2, remains relatively unchanged by such diets. The physiological consequences of such changes in composition, especially in platelets and neutrophils, will be described in later sections.

In experiments where n-3 PUFA supplements are provided as triacylglycerols, ethyl esters or as the free fatty acids, the synthesis of fatty acids by the liver *de novo* is largely suppressed. The liver may, however, be involved in the modification of the dietary fatty acids by chain elongation in the case of EPA or retroconversion in the case of DHA. Therefore the spectrum of fatty acids delivered to cells is not necessarily the same as that administered in the diet. Additional factors in determining the ultimate fatty acid composition of the cellular phosphoglycerides of the target cells include:

(i) the capacity to oxidise the fatty acids, whether completely by beta-oxidation or partially by retroconversion;
(ii) the potential for selective introduction of fatty acids at any of the stages in phosphoglyceride biosynthesis (Section 8.3.4.1);
(iii) the dietary dose and its rate of absorption;
(iv) the developmental and physiological state of the animal.

It is not, at present, possible to predict outcomes with confidence, but dietary fish oils can be said to raise levels of long chain n-3 PUFA substantially in the major phosphoglycerides in most body tissues.

8.3.5 Specific fatty acid combinations in membrane lipids

8.3.5.1 Polyunsaturated fatty acids

The impressive advances in molecular biology in the last few decades have inevitably focused much more attention on the protein than the lipid components of membranes. Membrane proteins can now be purified with relative ease; many of their genes, especially those for receptor proteins and associated signal transduction processes, have been cloned. Lipids, with one or two notable exceptions, have been generally relegated to a rather non-specific, somewhat inconsequential background role in membrane structure and function. However, even very slight changes in phosphoglyceride fatty acids can cause marked changes in membrane properties. This is especially true for the PUFA in the phosphoglycerides. For example, the substitution of the Mead fatty acid (20:3 n-9) for arachidonic acid (20:4 n-6) generates a membrane that is less compact and more permeable to ions (Evans and Tinoco, 1978). Similarly, the substitution of arachidonic acid (20:4 n-6) by EPA (20:5 n-

3) has marked effects on eicosanoid production in the membranes (see Section 8.5).

This has implications for tissues rich in n-3 PUFA including retina (see Section 8.3.6.5), brain and testis. Mammalian brain, particularly the 'cephalin' fraction (i.e. mainly PtdEtn), is also a rich source of DHA. Moreover the PtdEtn fraction of brain is rich in 'plasmalogens', i.e. phosphoglycerides containing a vinyl ether-linked chain at position 1 instead of an ester-linked chain. Ethanolamine plasmalogens from mammalian, including human, brain have 16:0, 18:0 and 18:1 substituents in approximately equal abundance at position 1 with DHA, EPA and arachidonic acid in approximately equal abundance at position 2. Diacyl PtdEtns are much richer in 18:0 as well as in DHA suggesting that 18:0/22:6 n-3 is a major molecular species in this phosphoglyceride. The content of PUFA, especially DHA, decreases in both lipids with increasing age.

The preceding sections have established that fatty acids are incorporated into individual membrane lipid classes with a high degree of specificity and into specific positions in the individual phosphoglycerides, consistent with their possessing highly specific functions. The highly characteristic distribution of 18:0 and 20:4 n-6 on positions 1 and 2 of PtdIns has been mentioned. To understand fully the functions of fatty acids in membranes requires a consideration of specific molecular species of not only the more common phospholipids, PtdCho, PtdSer, PtdEtn and Ptd Ins, but also the generally less studied classes such as cardiolipin, sphingomyelin, cerebrosides, gangliosides and sulphatides.

8.3.5.2 Monounsaturated fatty acids

This marked molecular specificity in membrane lipids is seen with fatty acids other than PUFA. Very long chain monounsaturated fatty acids such as erucic acid (22:1 n-9) and nervonic acid (24:1 n-9) are not readily incorporated into phosphoglycerides and indeed are relatively rare in natural phosphoglycerides. However, in rats fed diets rich in erucic acid (22:1 n-9), this fatty acid is preferentially incorporated into cardiolipin which itself is preferentially located in mitochondria (Blomstrand and Svensson, 1974). The same occurs with partially hydrogenated fish oil, though only the *cis* and not the *trans* isomers are incorporated into cardiolipin from the oil (Blomstrand and Svensson, 1983). Nervonic acid (24:1 n-9) is so named because of its relative abundance in brain associated traditionally with sphingomyelin. In the human brain, it accounts for some 20–25% of the total fatty acids.

8.3.6 Role of specific lipids in bilayer structures

Two general structural roles for lipids can be considered in biomembranes:

(i) that which relates to the bulk phase of the membrane or the bilayer *per se*;
(ii) that which relates to the direct interaction of lipids with proteins (see Section 8.3.7).

It is known that different and specific lipid compositions are used to produce membranes with different bulk structural properties. Some examples follow.

8.3.6.1 The role of membrane lipids in maintaining fluidity

The retailoring of individual phospholipids by acyl exchange reactions is of major significance in maintaining the fluidity of the membrane (Lynch and Thompson, 1984). For example, acyl exchange between 18:0/18:0 and 18:1/18:1 to generate 18:0/18:1 is a very efficient means of 'fluidising' membranes. Other processes, including altering phospholipid head groups, altering the overall fatty acid composition of the membrane, and altering the cholesterol content, are also important.

Of special significance is the relatively recent acceptance that the abundance of C20 and C22 PUFA, especially n-3 PUFA, in membranes can no longer be accounted for simply in terms of membrane fluidity (see Section 8.3.2). This is because increased unsaturation above a certain number of double bonds, exceeded in C20 and C22 PUFA, does not necessarily translate into increased 'fluidity', either in the sense of the potential of the molecules to undergo segmental, rotational or lateral motions, or in the sense of their ability to form ordered lipid domains as reflected by their phase transition temperatures (Dratz and Deese, 1986).

8.3.6.2 The apical membrane of epithelial cells

It is important that the apical membrane of epithelial cells has a low permeability to ions and water. Hydrogen bond interactions between the carbohydrate groups of glycolipids located on the outer (serosal) side of the bilayer can generate a high viscosity membrane with the required characteristics (Van Meer, 1988).

8.3.6.3 The erythrocyte membrane

The red blood cell is a dramatic example where specific fatty acids can alter cell shape. Specific phosphatidyl choline (PtdCho) molecules can be

transferred experimentally into the erythrocyte membrane using a phospholipid exchange protein (Jos *et al.*, 1985). The incorporation of 16:0/18:1 PtdCho into the membrane generates 'normal' (biconcave) cells, 16:0/16:0 PtdCho generates convex cells and the incorporation of 18:2/18:2 PtdCho generates concave cells.

8.3.6.4 Myelin

With its highly specific lipid composition, myelin is a classical example of an ion-impermeable, insulating cell membrane whose unique structure and properties are probably determined largely by specific interactions between membrane proteins and phospholipids and the cytoskeleton of the cell.

8.3.6.5 Rods

The bilayer regions of the outer segments of rods have a very high content of di22:6 n-3 (DHA) phosphoglycerides (Tinoco, 1982). This clearly represents a structural specialisation to generate a highly structured but fluid membrane, possibly with an enhanced elasticity. The membrane may then be able to accommodate the high frequency of conformational changes undergone by the high density of rhodopsin molecules contained within it.

A recent striking discovery is the presence in retinal lipids of very long chain PUFA (VLCPUFA) with even-numbered chain lengths from 22 to 36 carbon atoms (Aveldano, 1987). Phosphoglycerides containing VLCPUFA in rod outer segments seem to be closely associated with the dominant membrane protein, rhodopsin.

8.3.7 Lipid – protein interactions

The second general role of lipids in biomembranes relates to their direct molecular interactions with proteins at the lipid–protein interface. The fact that highly specific detergents are required to crystallise a membrane protein such as bacteriorhodopsin (Roth *et al.*, 1989) implies that equally specific interactions between phospholipids and the protein occur in the native membrane. Phosphoglycerides containing long chain PUFA may fulfil this role in mammalian rod outer segments. Interactions of this type, though currently undefined, could be of obvious importance not only in stabilising the hydrophobic helix within the membrane but possibly also in facilitating the folding of the protein into its active conformation within the membrane and in its subsequent function (Dratz and Deese, 1986; Neuringer and Conner, 1989).

Such interactive roles of specific lipids with proteins in facilitating and participating in conformational changes necessary for membrane protein function are well reflected in the many examples of the activities of purified and partially purified membrane proteins being influenced by their lipid environment, other than simply in terms of bulk membrane 'fluidity'. Thus the molecular specificity of lipids in biomembranes is intimately related to both the structural and functional specificities of biomembranes. This applies both to roles of specific lipids in determining particular bulk structural properties of membranes, and to roles of specific lipids in interacting with proteins to determine particular metabolic functions of membranes. Thus the importance of interactions between proteins and other molecules in their immediate environment applies as much to the organic (membrane) phase of the cell as it does to the aqueous (cytosoluble) phase. However, the organic phase is particularly challenging chemically and this has undoubtedly delayed full appreciation of the specific interactions that can occur within cell membranes. Some examples of specific lipid-protein interactions follow.

8.3.7.1 Transporter proteins

The catalytic parameters of the erythrocyte glucose transporter (V_{max}, K_m, turnover number) are markedly influenced by the nature of the lipids associated with it, such as head group composition, and the degree of unsaturation and chain length of constituent fatty acids (Carruthers and Melchior, 1986).

8.3.7.2 Enzymes

The activities of Ca/Mg ATPase from sarcoplasmic reticulum (fundamental in muscle contraction), adenyl cyclase (fundamental in agonist-induced formation of cAMP) and 5-nucleotidase (fundamental in generating the extracellular agonist adenosine) are all markedly influenced by the levels of n-6 and n-3 PUFA in the membrane lipids. Thus in animals fed diets enhanced in fish oils rich in EPA (20:5 n-3) and DHA (22:6 n-3), the activity of Ca/Mg ATPase is decreased, adenyl cyclase is increased and 5-nucleotidase is increased (Kinsella, 1990). These diets result in enhanced levels of 22:6 n-3 in phosphoglycerides in all of the cell membranes in question.

8.3.7.3 'Policeman' role

Phosphatidyl inositol (PtdIns) has a well-defined role in locating, and probably directing, various enzymes to the external surface of the lipid bilayer, where they act extracellularly (Cow *et al.*, 1986). Such enzymes include acetylcholinesterase and 5-nucleotidase, and in all cases the enzymes are anchored in the bilayer externally to the cell

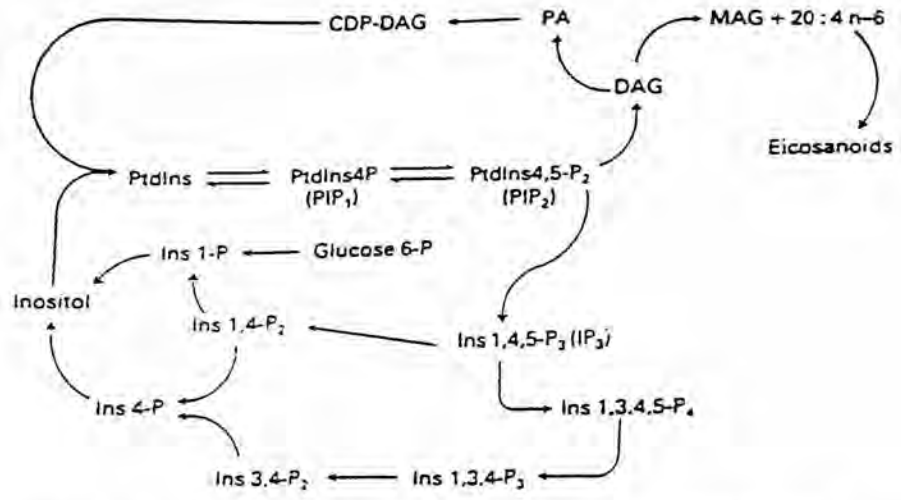

Figure 8.2 The inositol–lipid cycle. CDP-DAG = CDP-diacylglycerol; PA = phosphatidic acid; MAG = monoacylglycerol; PIP_1 = phosphatidyl inositol 4 phosphate; PIP_2 = phosphatidyl inositol 4,5 biphosphate. IP_3 = inositol 1,4,5 triphosphate; PtdIns = phosphatidyl inositol; Ins = inositol; DAG = diacylglycerol.

through being linked to PtdIns via glycosyl substituents on the proteins.

8.4 ROLE OF UNSATURATED FATTY ACIDS IN MEMBRANE METABOLIC CONTROL VIA THE INOSITOL LIPID CYCLE

8.4.1 Mechanism

A major example of membrane lipid metabolism of central importance in cellular physiology is the inositol lipid cycle. This cycle is now known to underlie the responses of very many cells to a range of hormones, neurotransmitters and cell growth factors (see Figure 8.2).

To initiate the cycle, an extracellular agonist (e.g. acetyl choline or an alpha adrenergic agonist) interacts with a specific cell receptor. The receptor/agonist complex then interacts with a specific G-protein system containing alpha, beta and gamma subunits to generate GTPase activity. This results in activation of a specific phospholipase C (PLC). PLC then cleaves PtdIns 4,5 bisphosphate (PIP_2) to generate two important, short lived second messengers: inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 is converted through the sequential action of a series of phospho-monoesterases to yield inositol. DAG is converted sequentially to diacylglycerophosphate and then to cytidyl-diacylglycerophosphate (CDAGP). CDAGP is coupled with inositol to yield cytidyl-monophosphate (CMP) and PtdIns. PtdIns is then phosphorylated sequentially by two ATP kinases to yield, first, PtdIns 4 phosphate (PIP_1) and then, finally, the starting molecule PIP_2 is regenerated. In this way, a whole series of hormones and neurotransmitters can generate two discrete intracellular messengers, IP_3 and DAG.

A different mechanism for activating the cycle appears to hold for cell growth factors since these extracellular agonists interact with receptors that frequently possess tyrosine kinase activity (Boyer *et al.*, 1989). At least one substrate of the latter is a PLC isoenzyme that is activated by phosphorylation to cleave PIP_2. The net effect is the same, i.e. the production of IP_3 and DAG. At least five immunologically distinct PLCs are now known from mammalian tissues and at least four of these are apparently represented by separate gene products.

8.4.2 Role of the second messengers (IP_3 and DAG)

The inositol lipid cycle generates the two important second messengers, IP_3 and DAG.

IP_3 activates the release of intracellular stores of calcium ions from the endoplasmic reticulum and probably also facilitates the entry of calcium ions into the cell (see British Nutrition Foundation, 1989).

The role of DAG as a second messenger is less well defined. Together with calcium ions and PtdSer, it activates a protein kinase C that phosphorylates numerous intracellular proteins with, so far, largely undefined functions. There is evidence, however, that one of the systems activated through phosphorylation is a membrane transport system that can cause alkalinisation of the cell interior.

The elevation of both intracellular calcium and

alkalinisation are essential for cell division. These metabolic controls are therefore very important in processes involving the rapid division of cells in the immune response and in tumour growth.

8.4.3 Importance of the inositol lipid cycle in nutrition

The inositol lipid cycle, which is central to the control of cell metabolism throughout the body and whose regulation underlies very many fundamental physiological control processes, not least those relating to cell division and growth, is intimately involved in lipid nutrition and metabolism in at least three ways:

(i) The specific PLC that hydrolyses PIP_2 is a plasma membrane bound enzyme. As with other membrane enzyme systems, including the G proteins and adenyl cyclases, it can in principle be influenced by the nature of the lipid phase in which it operates.

(ii) The dominant species of PtdIns in the plasma membrane has the fatty acid composition 18:0/20:4 n-6. Many isoenzymes of PLC exist, often with high tissue specificity (e.g. in the retina). There is also evidence that the molecular species composition of PtdIns may be tissue-specific within a given species and also species-specific for given tissues. The significance of this, particularly how it relates to the EFA intake of the species and how it may influence the action of PLC on PtdIns, especially via isoenzymes, is currently unknown.

(iii) The possibility of variations in the fatty acid composition and molecular speciation of PtdIns raises some questions. Are there consequences for the activation of protein kinase C by DAG since the latter presumably reflects the molecular species composition of its precursor PtdIns? In particular, can the content of C20 and C22 n-3 and n-6 PUFA be altered in PtdIns and its derived DAG by dietary means? Similar considerations apply to the fatty acid and molecular species composition of the PtdSer necessary for the co-activation of protein kinase C with DAG and calcium ions; since the activation process appears to occur in association with the cell membranes. This complex area is not understood but the potential influence of dietary EFA on the biochemistry of the phosphatidyl inositol cycle clearly represents an area of high priority for future studies.

It has been reported that eicosapentaenoic acid (EPA), added as the free fatty acid to the medium used to culture bovine aorta endothelial cells, inhibits the production of platelet-derived growth factor (PDGF) by the cells (Fox and Dicorleto, 1988). Several lines of evidence suggest that oxidative damage to the EPA is necessary for the observed inhibitory effect. Whether this is principally a physiological or a pathological effect is not known but this finding alone justifies a very high priority for this topic in future work on EFA nutrition. Some of these considerations will be amplified in following chapters dealing with thrombogenesis (Chapter 13), tumour formation (Chapter 16) and the immune response (Chapter 19).

8.5 ROLE OF UNSATURATED FATTY ACIDS IN MEMBRANE METABOLIC CONTROL VIA EICOSANOID FORMATION

The second major area where unsaturated fatty acids can exert membrane metabolic control is via their critical role as precursors of the eicosanoids (Johnson *et al.*, 1983). These are a complex group of highly biologically active, often short-lived compounds with 20 carbon atoms produced by cells to act in their immediate environment.

8.5.1 Discovery of eicosanoids

While research was being undertaken to determine which fatty acids have EFA activity and how this was related to chemical structure, it was discovered that the human uterus, on contact with fresh human semen, was provoked into either strong contraction or relaxation (Kurzrok and Lieb, 1930). Von Euler (1934) then showed that a fatty acid fraction in lipid extracts from sheep seminal plasma caused marked stimulation of smooth muscle. The active factor was named prostaglandin and was shown to exhibit a variety of physiological and pharmacological properties at extremely low concentrations. The chemical structures of the prostaglandins suggested that the substances might originate from arachidonic acid and this was subsequently proved in a series of very elegant biochemical experiments using radioactively labelled arachidonic acid.

8.5.2 Types of eicosanoids (see Figure 8.3)

Eicosanoids are hydroxylated derivatives of C20 PUFA that can be categorised into four groups:

(i) cyclic products, formed by an initial peroxida-

tion reaction, that are known collectively as the prostanoids and include prostaglandins, prostacyclins and thromboxanes (see Section 8.5.3);

(ii) linear products, initiated by the 5-hydroperoxidation of PUFA by a 5-lipoxygenase, known collectively as leukotrienes (LT) (see Section 8.5.4);

(iii) other lipoxygenase products of PUFA such as those formed by the action of 12-lipoxygenase on arachidonic acid or EPA;

(iv) mono-oxygenase products of PUFA formed by the action of cytochrome P_{450}: e.g. the hydroxylation of arachidonic acid at the methyl carbon atom or the adjacent carbon atom.

8.5.3 Prostanoids

Prostanoids are a group of metabolically related compounds of great physiological importance which are formed in every tissue. Because of their extreme potency and their short circulation time, they are considered local hormones. They all contain 20 carbon atoms, arranged in a (bi)cyclic structure, carrying two side chains, one of which is terminated by a carboxyl group (COOH). The other side chain contains a hydroxyl group (OH) at carbon number 15 and is terminated by a methyl group (CH_3). Consequently prostanoids can be considered cyclic hydroxy fatty acids. Depending on the configuration of the cyclic part of their molecule, prostanoids are divided into prostaglandins (PGs, containing a ring of five carbon atoms), thromboxanes (TXs, containing a ring of six carbon atoms) or prostacyclins (containing a ring of five carbon atoms).

8.5.3.1 Functions

The C20 polyunsaturated fatty acids can generate a whole range of related compounds but with subtle differences in structure, which exert a range of profound physiological activities at very low concentrations. The prostaglandins have the ability to contract smooth muscle, to inhibit or stimulate the adhesion of blood platelets and to cause constriction or dilation of blood vessels with related influence on blood pressure (see Chapter 14).

In addition to the prostaglandins themselves, the prostanoids include the metabolites of prostaglandin H. These include the prostacyclins and thromboxanes. These have essentially opposing physiological effects. Prostacyclins, formed in the wall of blood vessels, are among the most powerful known inhibitors of platelet aggregation. They relax the arterial walls and promote a lowering of blood pressure. Thromboxanes, found in platelets, stimulate the platelets to aggregate (an important mechanism in wound healing), contract the arterial wall and promote an increase in blood pressure. The balance between these activities is important in maintaining normal vascular function (see Chapter 13).

All prostanoids are produced locally near to their sites of action, are released in minute quantities, act rapidly, and are quickly destroyed by a battery of degradative enzymes. The breakdown products are excreted in urine and their measurement has been employed to estimate their daily production by the body.

8.5.3.2 Biosynthesis of prostanoids (Figure 8.4)

The first step in the biosynthesis of prostanoids is the release of the essential fatty acid (EFA) as the free fatty acid from the membrane phospholipid in which it is stored. This release is catalysed by phospholipase A_2. The EFA substrate then binds to the enzyme cyclo-oxygenase. This multi-functional enzyme is a single polypeptide with a mass of 70,000 Daltons containing two haem groups per molecule. It has two activities:

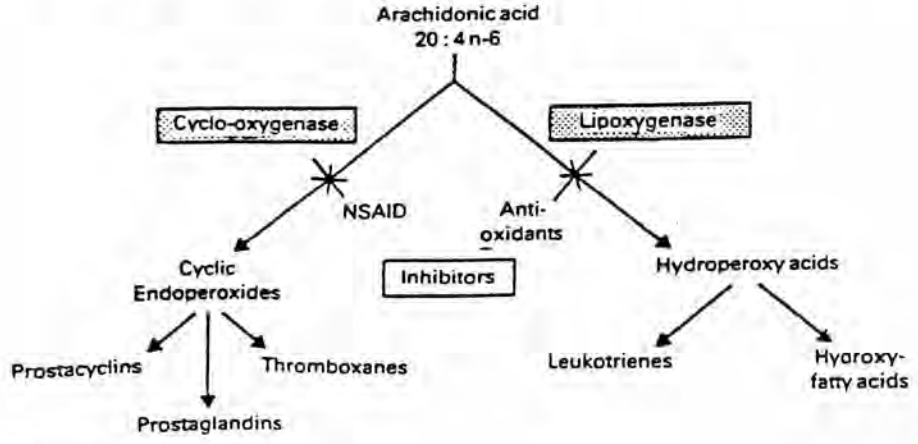

Figure 8.3 Types of eicosanoid.

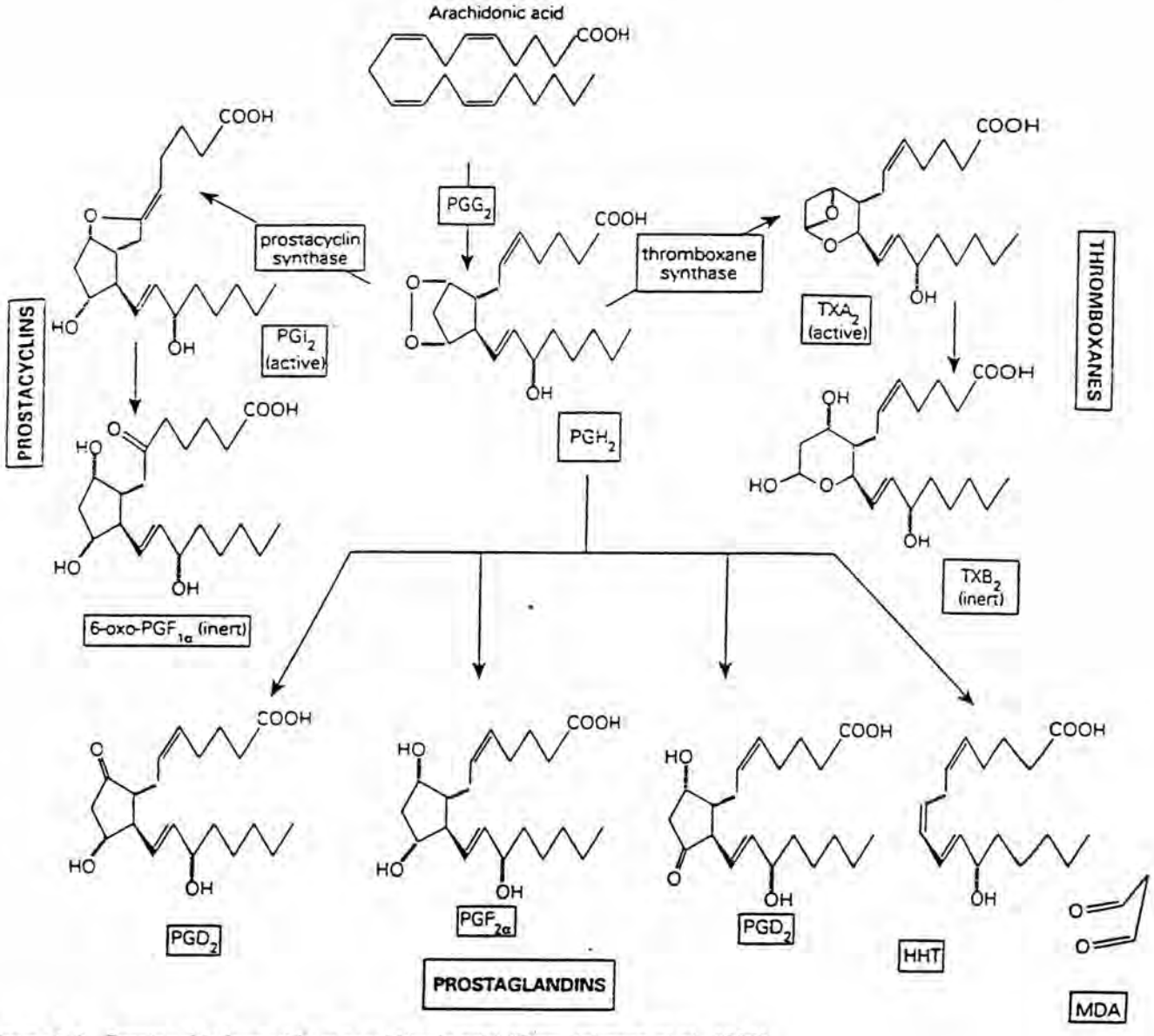

Figure 8.4 Prostanoids formed from arachidonic acid. (From Johnson *et al.*, 1983)

(i) oxygen atoms are inserted in the folded fatty acid chain to yield a cyclic endoperoxide. This intermediate is prostaglandin G (PGG) (Figure 8.5).

(ii) catalytic activity reduces the peroxide function of PGG to an hydroxy group and thus forms prostaglandin H (PGH).

Prostaglandin H (PGH) is the key intermediate for conversion into a wide range of other prostaglandins and eicosanoids. Figure 8.4 illustrates the range that can be produced if arachidonic acid is the substrate for cyclo-oxygenase. The balance of activities of the enzymes that catalyse these reactions determines the pattern of eicosanoids formed in any given tissue. Diet is one of the factors affecting these patterns as discussed in Section 8.5.5.

PGG and PGH are unstable precursors of all other members of the prostanoid family. Thus platelets and many other tissues contain thromboxane synthetase that catalyses conversion of the endoperoxide intermediates to thromboxane A_2 (TXA_2) which is inherently unstable and decays to the inert TXB_2. In other tissues, prostacyclin synthetase forms PGI_2 from the unstable endoperoxides. PGI_2 is also unstable, though less so than TXA_2, and decays to an inert form. Other tissues may contain enzymes that isomerise the endoperoxides to PGE, PGF and PGD, although these reactions may also occur by non-enzymic rearrangements.

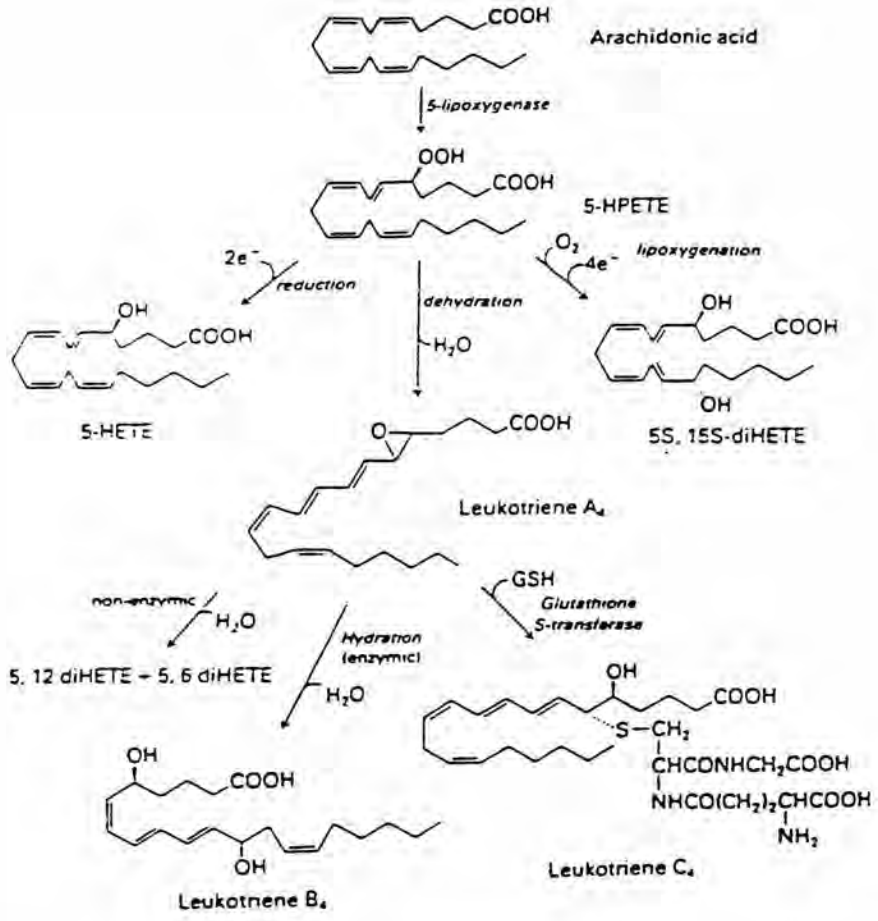

Figure 8.5 Biosynthesis of leukotrienes. (From Gurr and Harwood, 1991)

8.3.4 Leukotrienes

8.3.4.1 Functions of leukotrienes

An alternative route for the conversion of C20 unsaturated fatty acids into biologically active oxygenated products is by 5-lipoxygenase peroxidation. The end products of peroxidation are called leukotrienes, the name derived from the cells (leukocytes) in which they were originally recognised.

Leukotrienes have a range of potent biological activities including the contraction of respiratory, vascular and intestinal smooth muscles. Some are chemotactic agents for cells of the immune system (see Chapter 19).

8.3.4.2 Biosynthesis of leukotrienes

In the linear pathway (Figure 8.5), the enzyme 5-lipoxygenase catalyses the oxygen-dependent hydroperoxidation of arachidonic acid (20:4 n-6) to yield 5-hydroperoxyeicosatetraenoic acid (5-HPETE) or the unstable intermediate, 5-epoxy derivative leukotriene A_4 (LTA_4).

LTA_4 can be further metabolised in two ways (Figure 8.5):

(i) LTA_4 can be converted by an epoxide hydrolase to yield LTB_4 which is a powerful chemoattractant produced by leukocytes to attract additional white cells to sites of tissue damage.

(ii) LTA_4 can react with glutathione S-transferase to form the sulphidopeptidyl leukotrienes known as LTC_4, LTD_4 and LTE_4. These are known collectively as the 'slow reacting substances A' (SRS-A).

The human 5-lipoxygenase has been purified and its gene has been cloned. The enzyme is unstable, has a mass of 78,000 Daltons and requires ATP and calcium ions, as well as other undefined protein and membrane fractions, for optimal activity. The 5-lipoxygenase activating protein (FLAP) has been identified in polymorphonuclear leukocytes, and has a mass of 18,000 Daltons. Its function appears to be to facilitate the transfer of lipoxygenase from the cytosol to the plasma membrane. Inhibitors of FLAP block the biosynthesis of 5-lipoxygenase.

8.5.5 Effect of dietary unsaturated fatty acids on eicosanoid formation

8.5.5.1 Prostanoids

Arachidonic acid gives rise to the 2-series prostaglandins as shown in Figure 8.4, but essential fatty acids of both n-6 and n-3 families can give rise to similar products differing in numbers and patterns of double bonds (see Figure 8.6). Dihomogamma linolenic acid (DGLA) (20:3 n-6) generates the 1-series PG, and eicosapentaenoic acid (EPA) (20:5 n-3) generates the 3-series PG (see Figure 8.7). Competitive effects occur between these precursor fatty acids in the cyclo-oxygenase reaction. Thus, when membrane phospholipids have elevated levels of n-3 PUFA after dietary administration of fish oils, formation of 2-series PG from arachidonic acid is depressed and formation of the 3-series derivatives is elevated. In general, the 3-series derivatives are less pharmacologically active than the 2-series.

It was once thought that the main criterion of essentiality in a fatty acid was its ability to be converted into a physiologically active eicosanoid. There are now known to be exceptions to this rule. Although it is difficult entirely to separate the membrane and eicosanoid functions of EFA, certain EFA may contribute to the integrity of membranes in ways that have little to do with their conversion into eicosanoids (see Sections 8.3.6 and 8.3.7).

In addition to the prostaglandins themselves, there are also prostacyclins and thromboxanes which are metabolites of prostaglandin G. Again, although arachidonic acid generates the 2-series prostacyclins and thromboxanes, DGLA and EPA generate the 1- and 3-series of these compounds.

8.5.5.2 Leukotrienes

Arachidonic acid (20:4 n-6) is converted into the 4-series LT. Similarly dihomogamma linolenic acid (20:3 n-6) is converted into the 3-series LT and eicosapentanoic acid (20:5 n-3) is converted into the 5-series LT. As in the case of cyclo-oxygenase, competititve effects occur between these precursor fatty acids in the 5-lipoxygenase reaction and, in particular, EPA (20:5 n-3) and DHA (22:6 n-3) competitively inhibit the formation of the 4-series

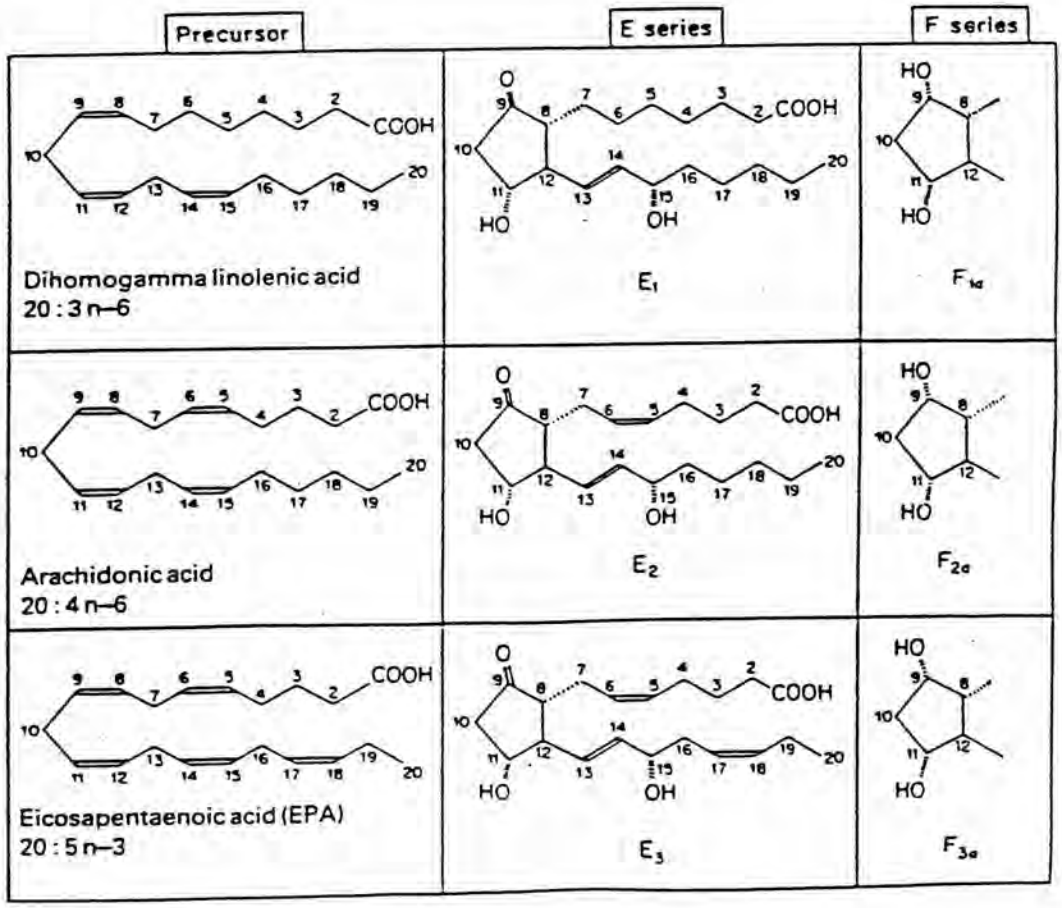

Figure 8.6 Different unsaturated fatty acids precursors produce different prostaglandins. (From Gurr and Harwood, 1991)

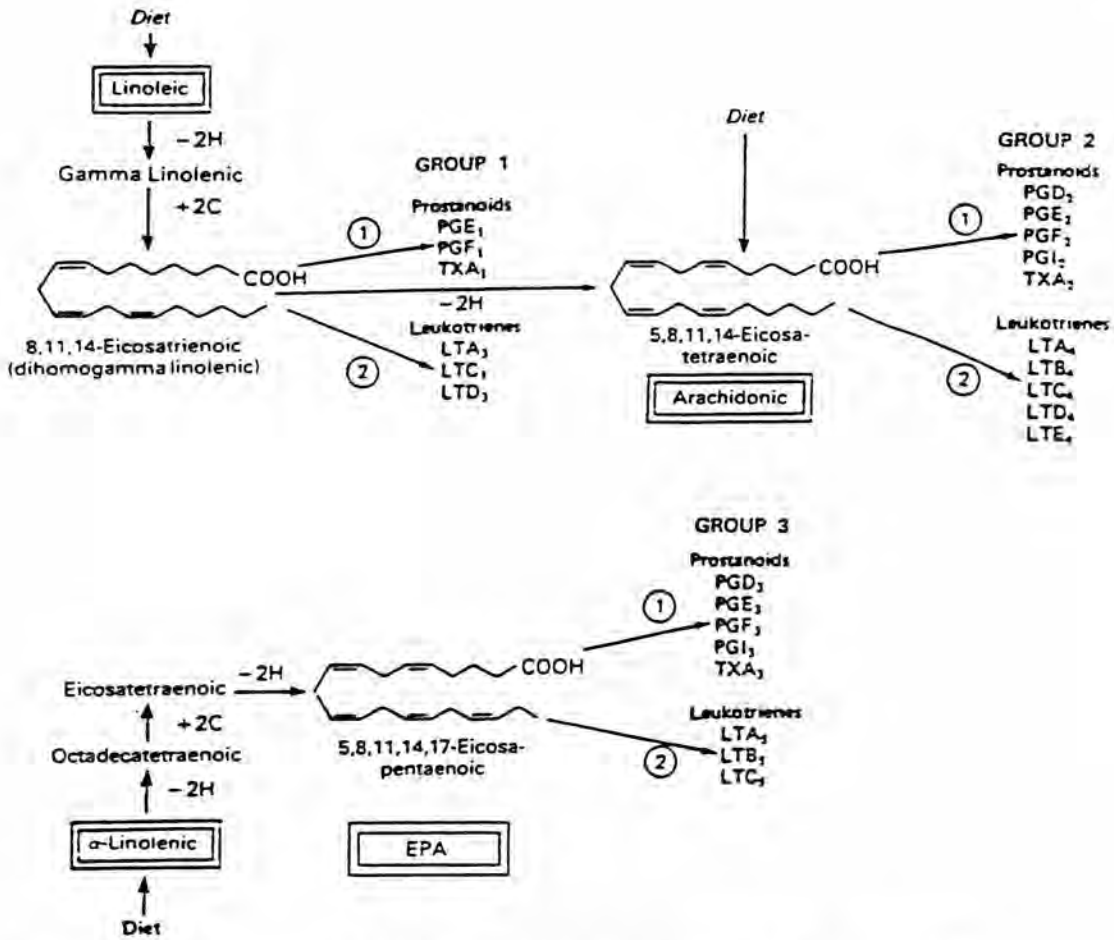

Figure 8.7 The three groups of eicosanoids and their biosynthetic origin. (From Murray *et al.*, 1988)

LT from arachidonic acid (20:4 n-6). Thus, elevation of membrane phospholipid levels of n-3 PUFA by feeding fish oils to animals depresses the formation of 4-series LT and increases the formation of 5-series products. The latter are generally less active pharmacologically than the 4-series.

8.5.5.3 Overall effect and dependence of eicosanoid formation on fatty acid substrate

Figure 8.7 is a summary of the biosynthetic origins of the eicosanoids. It shows how the range of eicosanoids that can be produced depends on which fatty acid provides the substrate for either cyclo-oxygenase (to produce prostaglandins, prostacyclins and thromboxanes) or lipoxygenase (to produce the leukotrienes).

8.5.6 Control of eicosanoid formation

8.5.6.1 Control via phospholipase

Formation of eicosanoids requires the hydrolysis of plasma membrane phospholipids by phospholipase A_2 to generate the free PUFA that are the substrates for the cyclo-oxygenase and the lipoxygenases. The phospholipases in question are activated by a range of natural tissue-specific agonists interacting with specific cell membrane receptors: e.g. the thrombin-induced formation of prostaglandins by platelets (see Chapter 13). The mechanism of activation of the phospholipase A_2 involves specific G protein systems similar to those already described for activation of phospholipase C in the inositol lipid cycle. Many compounds which inhibit phospholipase A_2 block the production of eicosanoids in isolated cells and tissues, although these compounds tend to be non-selective in their actions.

Glucocorticoids appear to inhibit eicosanoid production by controlling phospholipase A_2 activity and this effect is caused by the generation of protein(s) collectively termed lipocortins. Some of these proteins have been cloned and at least one has eicosanoid inhibitory and anti-inflammatory activity.

8.5.6.2 Control via intracellular calcium

Elevation of intracellular calcium also induces

prostaglandin production, probably through activation of phospholipase A_2. However, controversy still exists over the precise substrate for the phospholipases which produce PUFA for eicosanoid production. PtdCho, PtdEtn, PtdIns and phosphatidic acid have all been implicated as substrates in various tissues. Hydrolase activity may also liberate PUFA from diacylglycerols.

8.5.6.3 Interactions with inositol lipid cycle

Complex interactions between the inositol lipid cycle and the eicosanoids can occur under physiological conditions. Eicosanoids are among the natural activators of the cycle. Such complexity is well illustrated by considering the mode of action of the leukotriene LTD_4 in mammalian cell systems (Crook *et al.*, 1989).

LTD_4 interacts with a specific cell receptor which, through a G protein system, activates a phospholipase C to hydrolyse PIP_2 to IP_3 and DAG. DAG, together with the calcium ions released through the action of IP_3, activates a protein kinase C. This in turn activates a DNA isomerase by phosphorylation. The active gene produces mRNA which is translated into a specific protein that activates a phospholipase A. This then acts on plasma membrane phospholipids to release arachidonic acid. Cyclo-oxygenase and 5-lipoxygenase can then act in the usual way to generate more prostaglandins and leukotrienes. Thus, the original LTD_4 signal is successfully amplified.

8.5.6.4 Implications for dietary manipulation

These highly efficient cascade reactions illustrate the great complexity of the processes which can amplify the original signal. The processes are highly cell-specific and typical of the biochemical controls underlying phenomena such as blood clotting and tissue inflammation that will be dealt with in Chapters 13 and 19. Their complexity should not obscure the basic fact that both the production and mode of action of the eicosanoids are fundamental membrane phenomena. They can, in principle, be influenced by perturbating membrane structure more or less specifically. However, because of the very direct link between eicosanoids and the essential fatty acids, eicosanoid metabolism is particularly amenable to dietary manipulation.

APPENDIX III

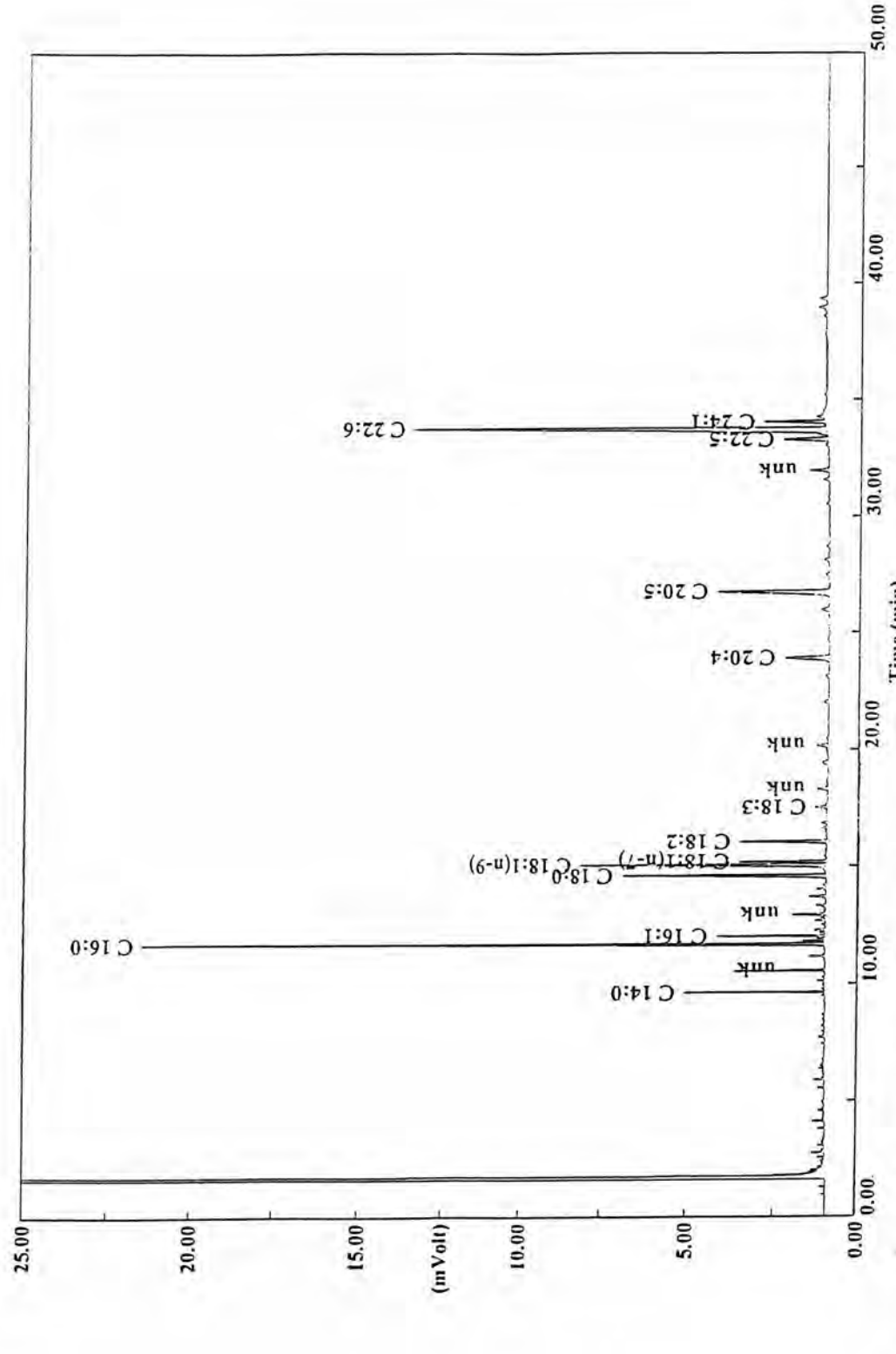

Figure 25 Gas-liquid chromatogram of fatty acid of total lecithin derived from grade A Danish fish meal .

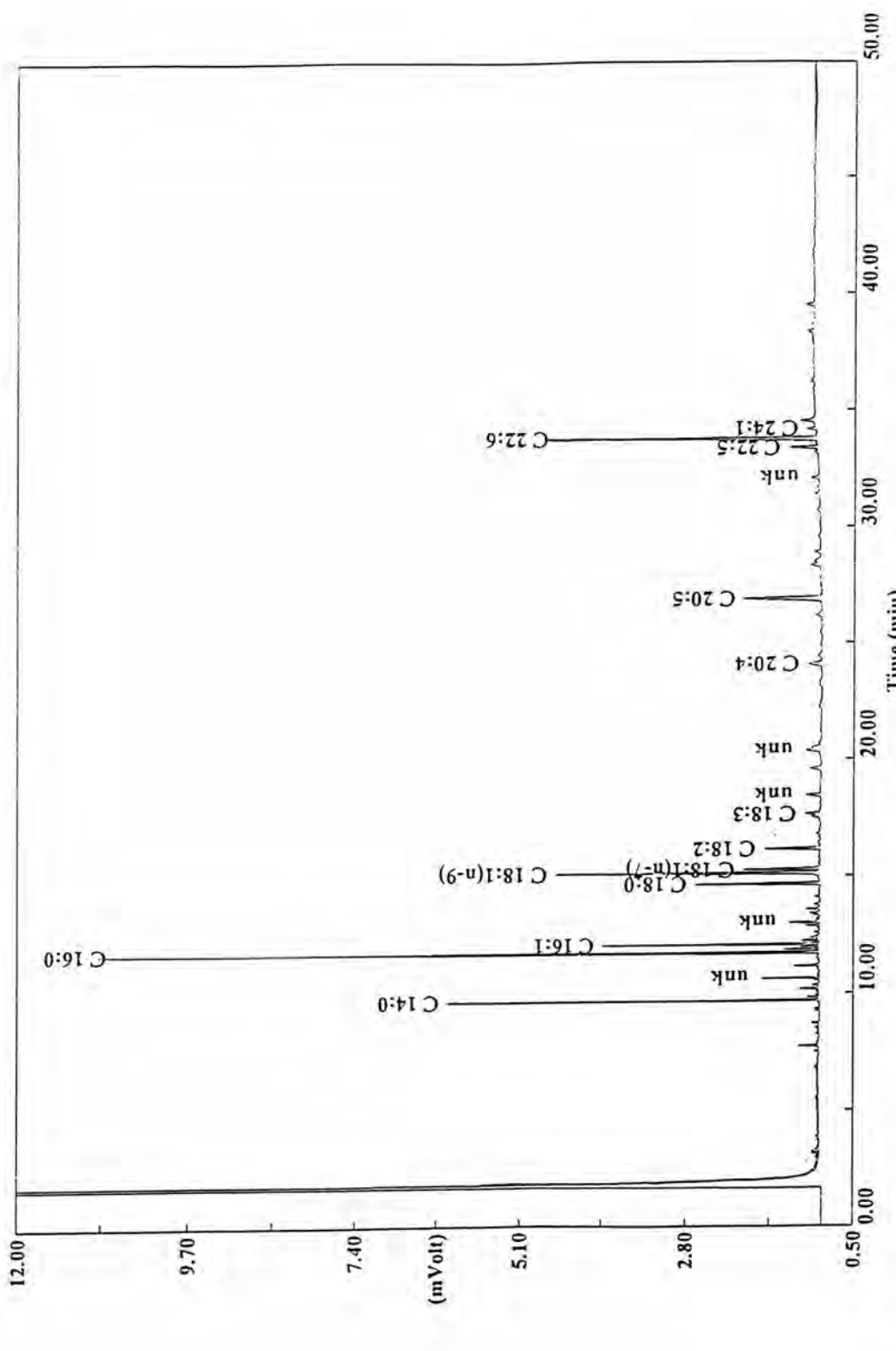

Figure 26 Gas-liquid chromatogram of fatty acid of triglycerides fraction of lecithin derived from grade A Danish fish meal

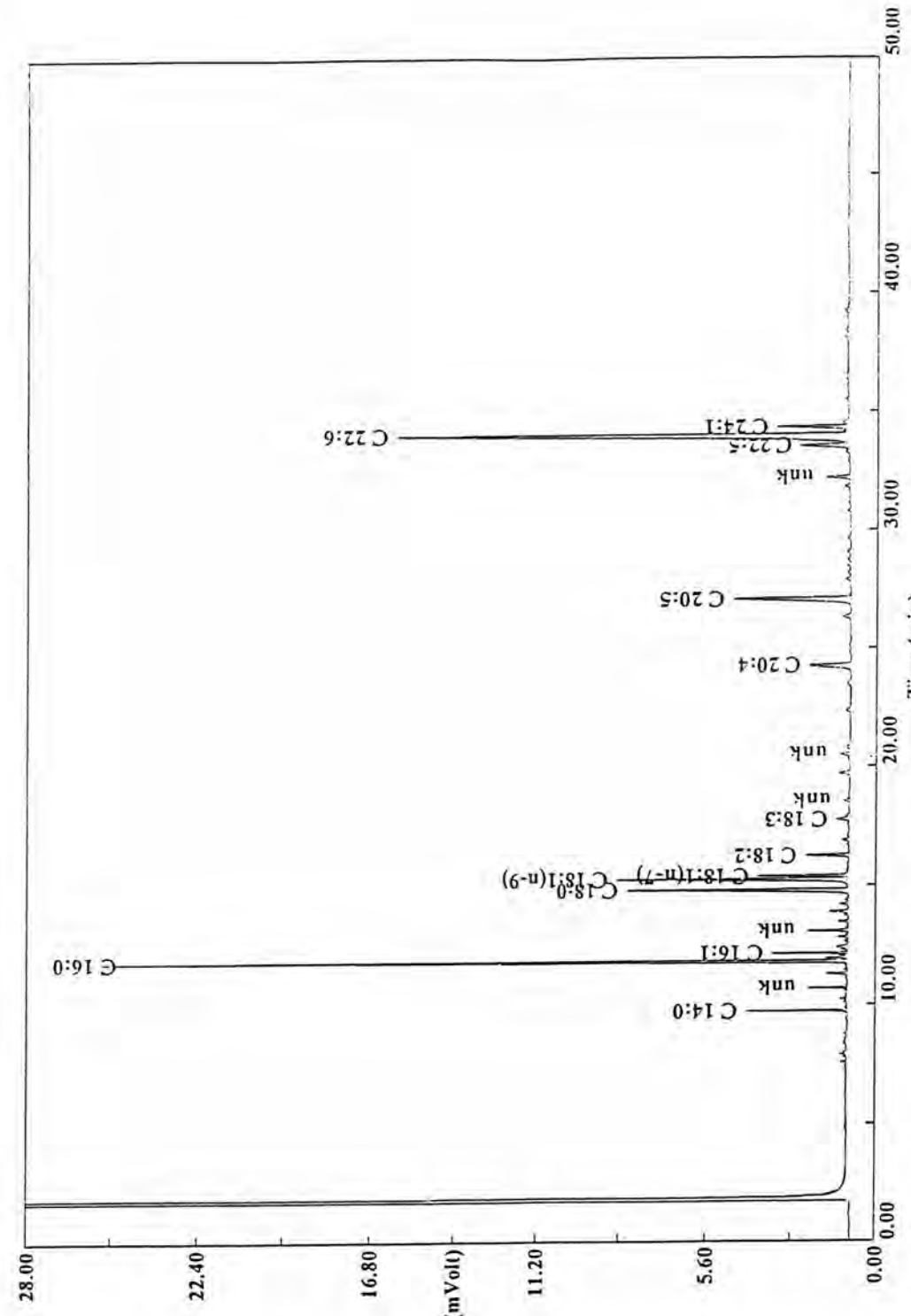

Figure 27 Gas-liquid chromatogram of fatty acid of phospholipids fraction of lecithin derived from grade A Danish fish meal.

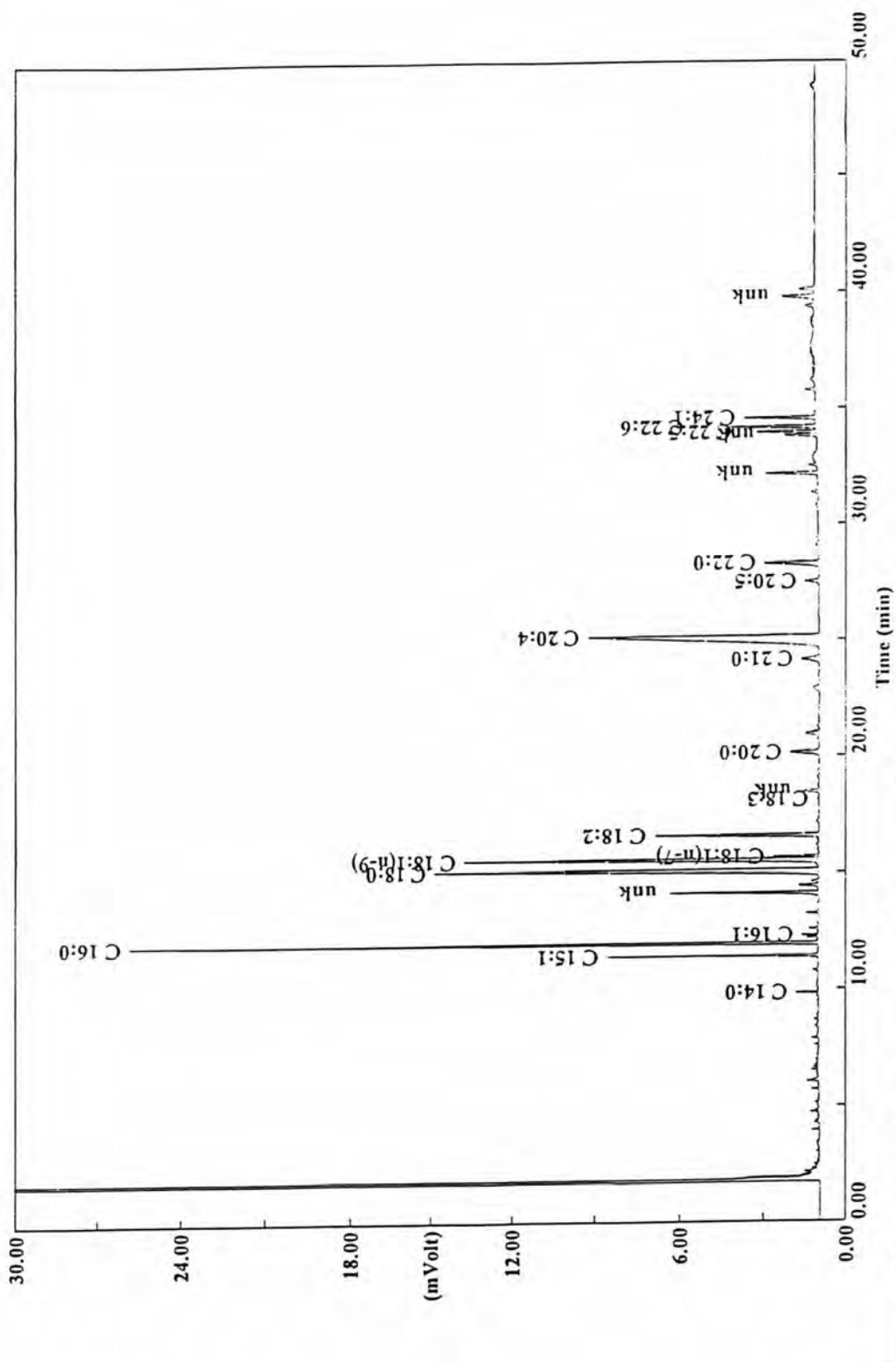

Figure 28 Gas-liquid chromatogram of fatty acid of platelets after the incubation with liposome-free NSS for 1 h at 22°C.

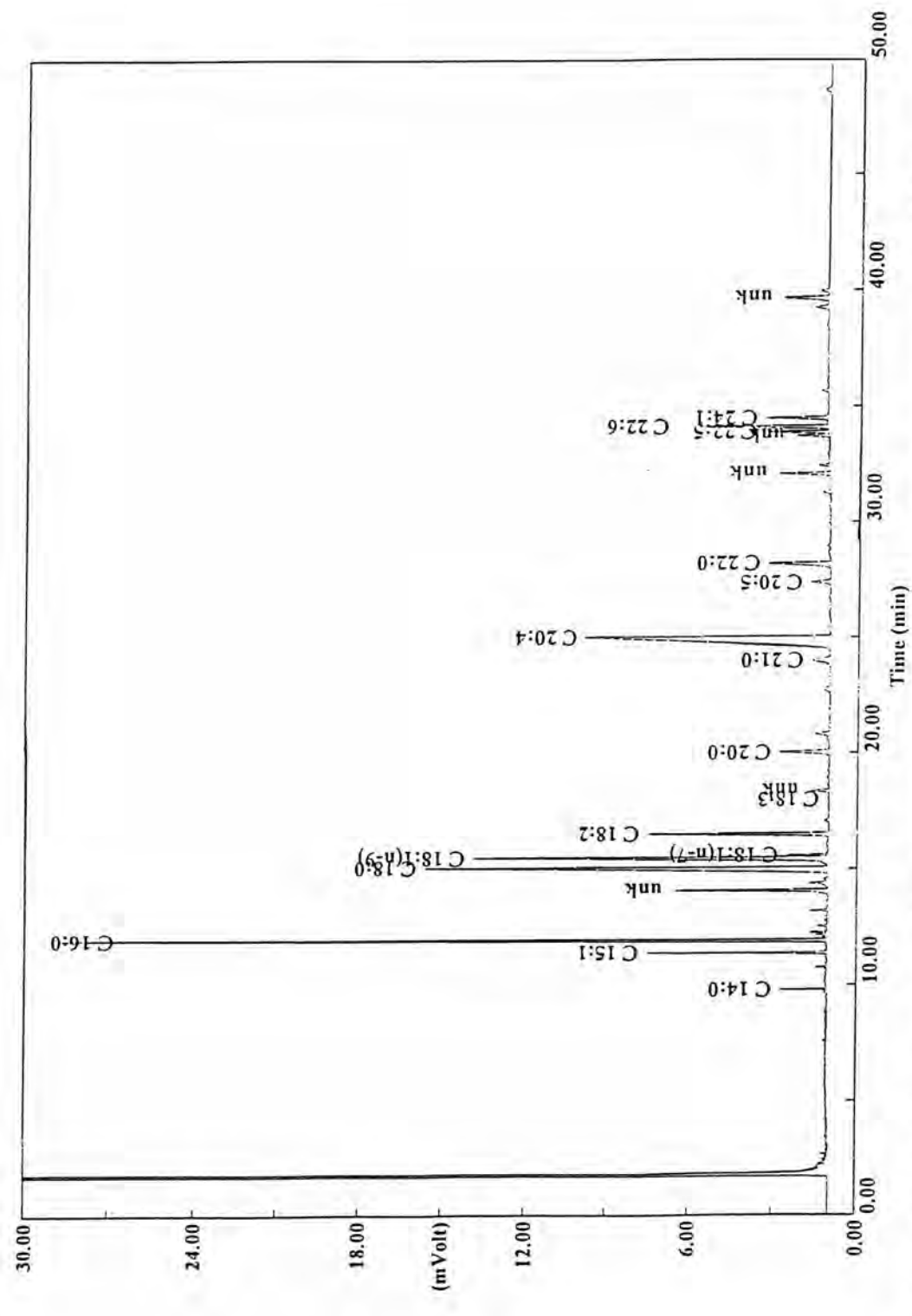

Figure 29 Gas-liquid chromatogram of fatty acid of platelets with plasma after the incubation with FM-LRFE for 1h at 22°C in the presence of lecithin at the concentration of 600 mg/dl.

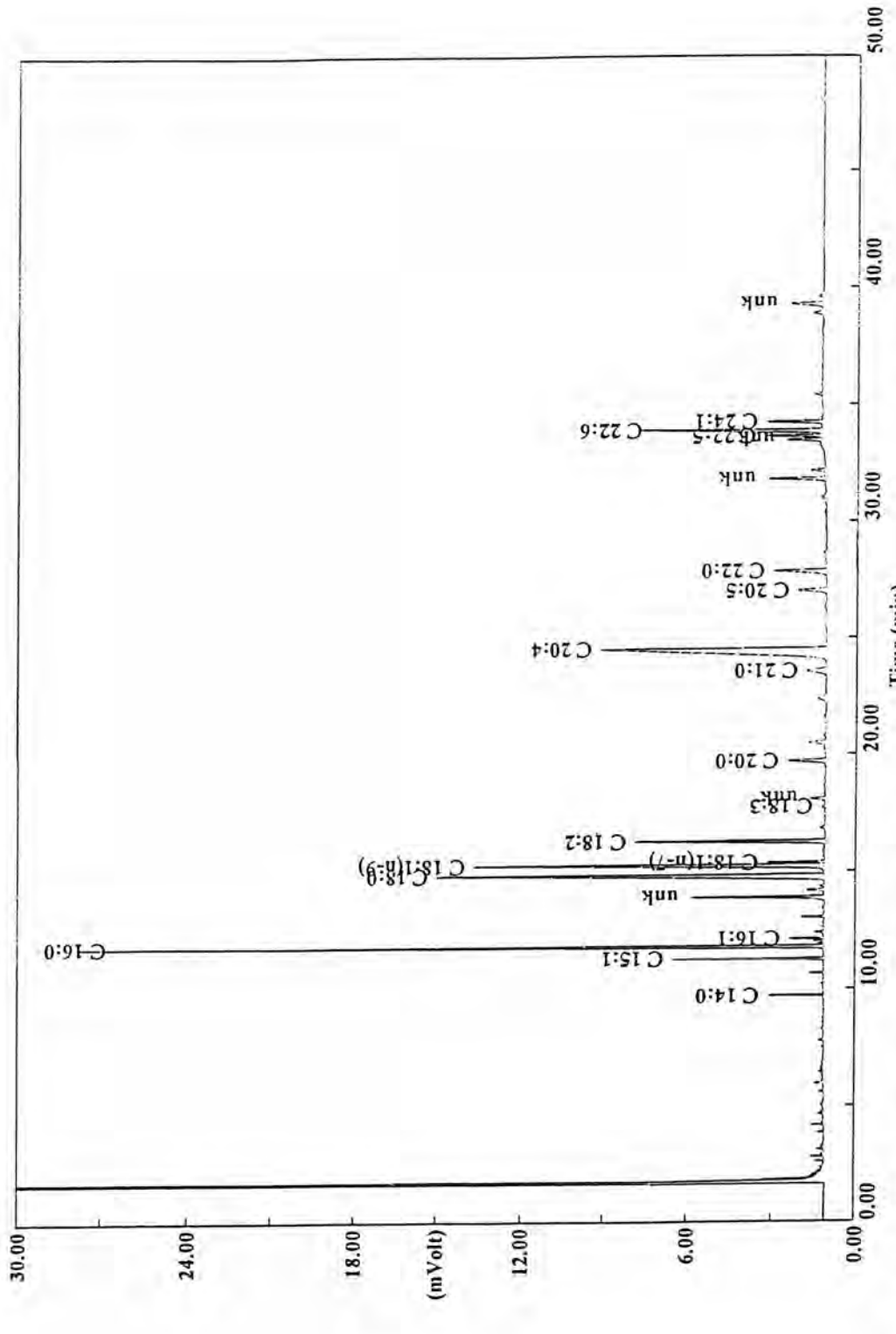

Figure 30 Gas-liquid chromatogram of fatty acid of platelets without plasma after the incubation with FM-LRFE for 1h at 22°C in the presence of lecithin at the concentration of 600 mg/dl.

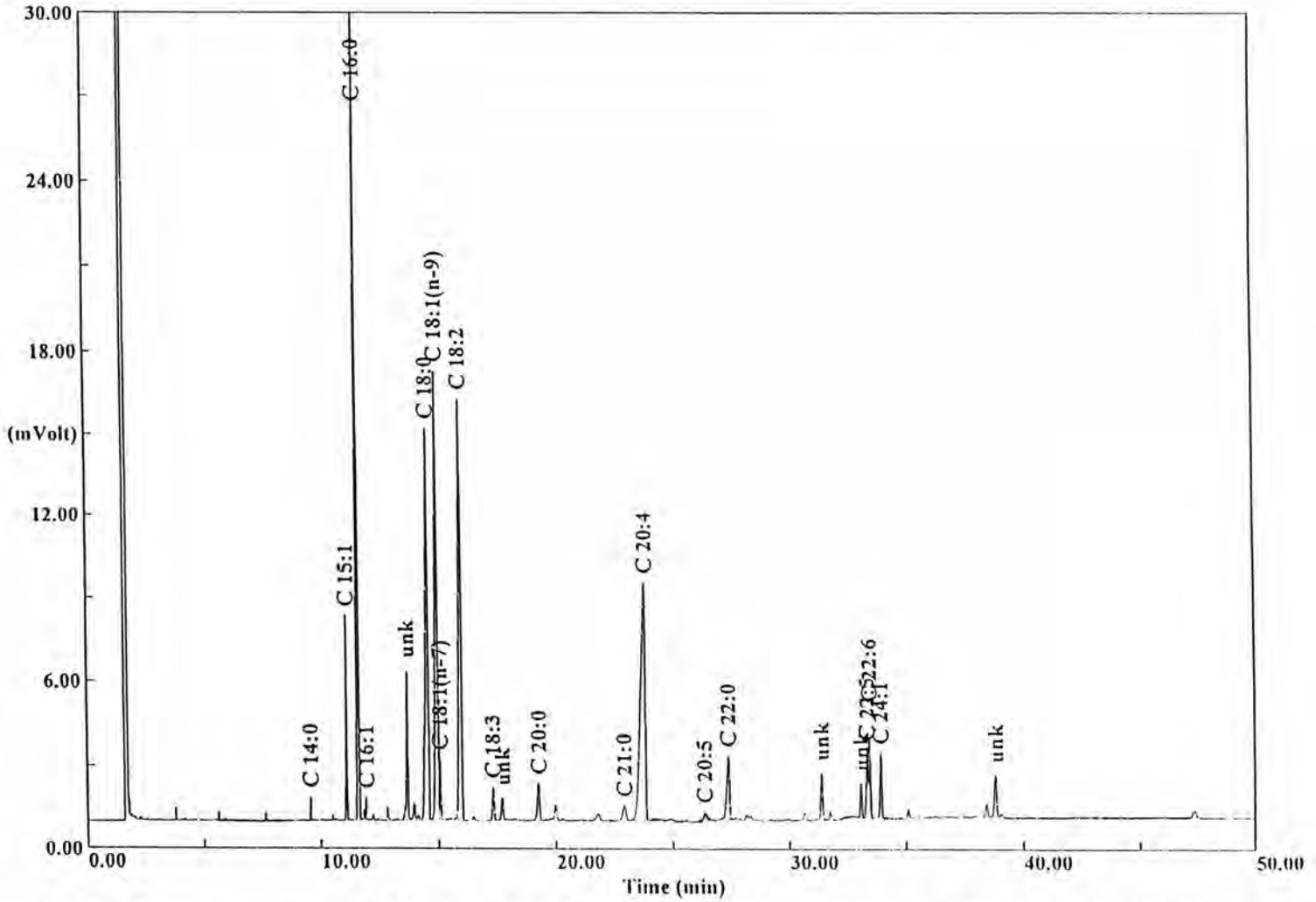

Figure 31 Gas-liquid chromatogram of fatty acid of platelets without plasma after the incubation with SY-LRFE for 1h at 22°C in the presence of lecithin at the concentration of 600 mg/dl.

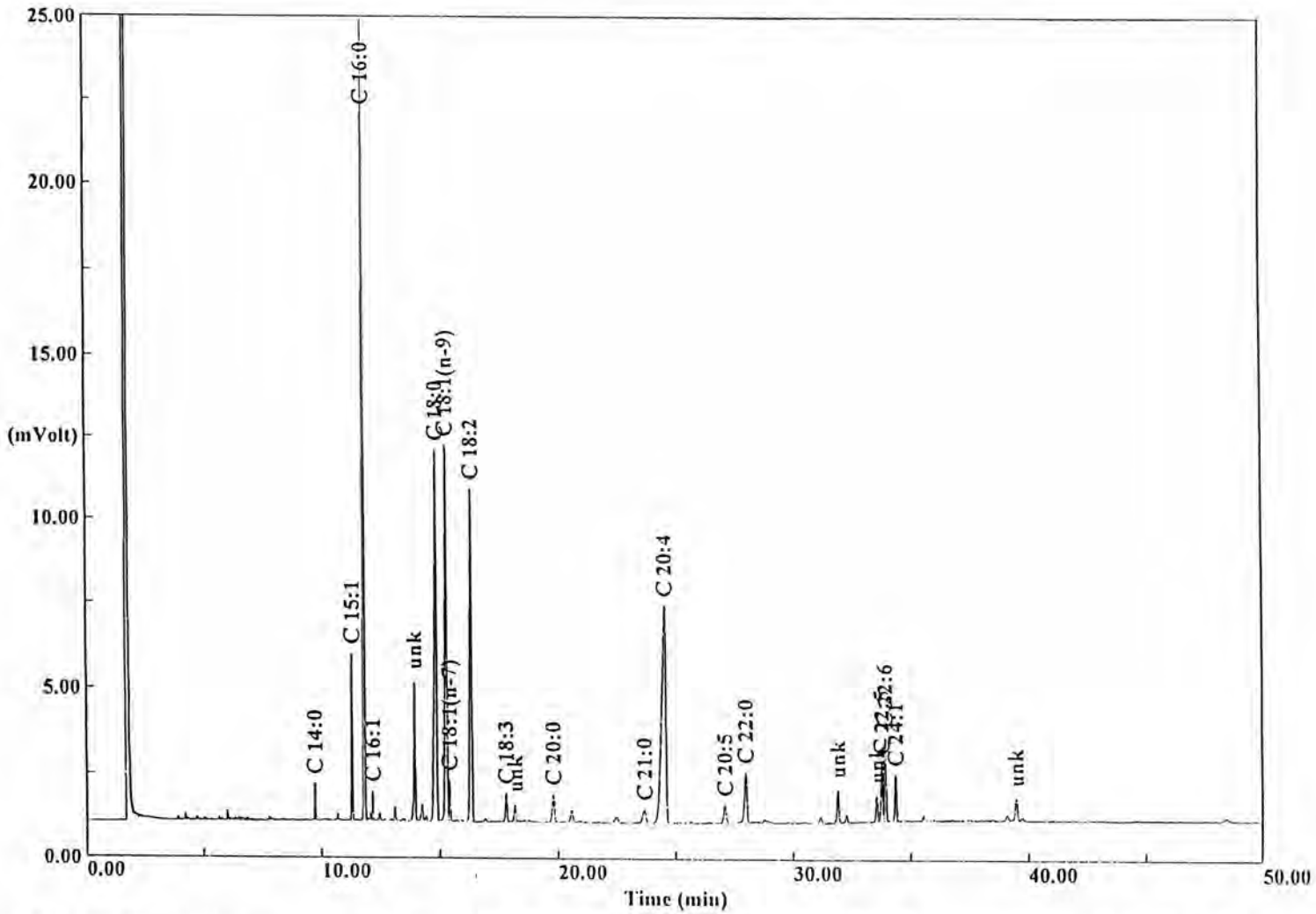

Figure 32 Gas-liquid chromatogram of fatty acid of platelets without plasma after the incubation with SL-FOFE for 1h at 22°C in the presence of lecithin at the concentration of 600 mg/dl.

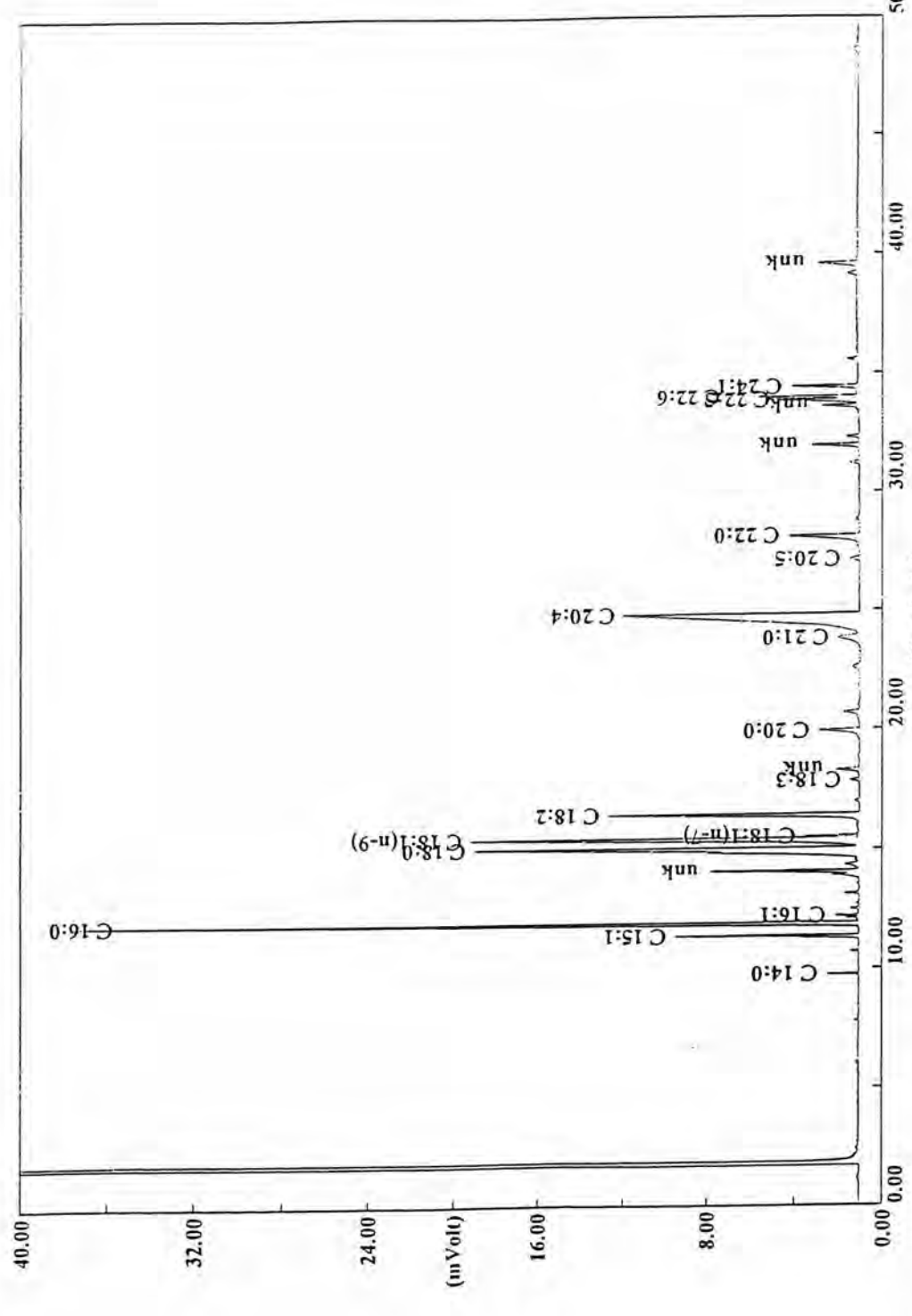

Figure 33 Gas-liquid chromatogram of fatty acid of platelets without plasma after the incubation with 20%Lipofundin for 1h at 22°C in the presence of lecithin at the concentration of 600 mg/dl.

BIOGRAPHY

Miss Supantitra Chanprasert was born on November 2, 1971 in Samutsakhon,Thailand. She graduated with Bachelor degree of Science in Medical Technology from Faculty of Medicine, Chulalongkorn University in 1995. She has studied for Master degree in Biotechnology Programme at Chulalongkorn University since 1995.