Lipid Biochemistry

An introduction

Lipid Biochemistry

An introduction

Fourth edition

M.I. Gurr
Nutrition Consultant and Visiting Professor
University of Reading and
Oxford Polytechnic

J.L. Harwood
Professor of Biochemistry
University of Wales
Cardiff

CHAPMAN & HALL
London · New York · Tokyo · Melbourne · Madras

UK	Chapman and Hall, 2–6 Boundary Row, London SE1 8HN
USA	Chapman and Hall, 29 West 35th Street, New York NY10001
JAPAN	Chapman and Hall Japan, Thomson Publishing Japan, Hirakawacho Nemoto Building, 7F, 1-7-11 Hirakawa-cho, Chiyoda-ku, Tokyo 102
AUSTRALIA	Chapman and Hall Australia, Thomas Nelson Australia, 102 Dodds Street, South Melbourne, Victoria 3205
INDIA	Chapman and Hall India, R. Seshadri, 32 Second Main Road, CIT East, Madras 600 035

First edition 1971
Second edition 1975
Third edition 1980
Fourth edition 1991

Typeset in 10/12pt Times by EJS Chemical Composition, Midsomer Norton, Bath, Avon
Printed in Great Britain by St Edmundsbury Press, Bury St Edmunds, Suffolk

ISBN 0 412 26610 5 (HB)
0 412 26620 2 (PB)

British Library Cataloguing in Publication Data

Gurr, M. I. (Michael Ian)
Lipid biochemistry.–4th ed.
1. Organisms. Lipids
I. Title II. Harwood, J.L.
574.19247

ISBN 0–412–26620–2

Library of Congress Cataloging-in-Publication Data

Available

Contents

1 The nature of lipids and their place in living things

1.1 DEFINITIONS

The word 'lipid' (in older literature spelled also as lipide or lipoid) is used by chemists to denote a chemically heterogeneous group of substances having in common the property of insolubility in water, bu solubility in non-polar solvents such as chloroform, hydrocarbons or alcohols. It is necessary to use this definition based on physical properties since there may be little or no chemical relationship between the numerous different compounds now classified as lipids, many of which are described in this book. It is not always possible to discern a clear distinction between the terms fat and lipid. The term fat is more familiar to the layman and brings to mind substances that are clearly fatty in nature, greasy in texture and immiscible with water. Familiar examples are butter and the fatty parts of meats. Fats are thought of as solid in texture as distinct from oils which are liquid at ambient temperatures. Chemically, however, there is little distinction between a fat and an oil, since the substances that the layman thinks of as edible fats and oils are composed predominantly of esters of glycerol with fatty acids. These are called triacylglycerols and are chemically quite distinct from the oils used in the petroleum industry, which are generally hydrocarbons. Lipid to the chemist embraces the wider range of fatty substances that are described in this book.

1.2 STRUCTURAL CHEMISTRY AND NOMENCLATURE

Lipids occur throughout the living world in microorganisms, higher plants and animals. In this book, they will be described mainly in terms of their

functions although from time to time it will be convenient, even necessary, to deal with lipid classes based on their chemical structures and properties.

The naming of lipids often poses problems. When the subject was in its infancy, research workers would give names to substances that they had newly discovered. Often, these substances would turn out to be impure mixtures and as the chemical structures of individual lipids became established, rather more systematic naming systems came into being. Later, these were formalized further under naming conventions laid down by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB). Thus, triacylglycerol is now preferred to triglyceride but the latter is still frequently used especially by clinical workers and you will need to learn both (Chapter 4). Likewise, outdated names for phospholipids: Lecithin (for phosphatidylcholine (Chapter 6) and Cephalin (for phosphatidylethanolamine and phosphatidylserine) will be avoided in this book but you should be aware of their existence. Further reference to lipid naming and structures will be given in Chapter 6.

The very complex naming of the fatty acids is discussed in detail in Chapter 3. Their main structural features are their chain lengths, the presence of unsaturation (double bonds) and of substituent groups. In regard to chain length, it is cumbersome to have to say every time: 'a chain length of ten carbon atoms' and we shall, therefore, refer to a '10C fatty acid'. If we wish to refer to a specific carbon atom in a chain, we shall write, for example: 'the substituent at C10'. The numbering of fatty acid carbon atoms is done from the carboxyl end of the chain with the carboxyl carbon as C1. An important aspect of unsaturated fatty acids is the opportunity for isomerism, which may be either positional or geometric. Positional isomers occur when double bonds are located at different positions in the carbon chain. Thus, for example, a 16C monounsaturated fatty acid may have positional isomeric forms with double bonds at C7 and C9, sometimes written $\Delta 7$ and $\Delta 9$. (The position of unsaturation is numbered with reference to the first of the pair of carbon atoms between which the double bond occurs.) Geometric isomerism refers to the possibility that the configuration at the double bond can be *cis* or *trans*. (Although the convention *Z/E* is now preferred by chemists instead of *cis/trans*, we shall use the more traditional and more common *cis/trans* nomenclature throughout this book.) In the *cis* form, the two hydrogen substituents are on the same side of the molecule, while in the *trans* form they are on opposite sides (Figure 1.1).

Another important feature of biological molecules is their stereochemistry. In lipids based on glycerol, for example, there is an inherent asymmetry at the central carbon atom of glycerol. Thus, chemical synthesis of phosphoglycerides yields an equal mixture of two stereoisomeric forms

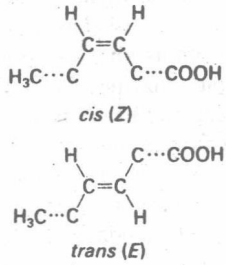

Figure 1.1 Geometrical isomerism in unsaturated fatty acids.

whereas almost all naturally occurring phosphoglycerides have a single stereochemical configuration, much in the same way as most natural amino acids are of the L (or *S*) series. In the past, naturally occurring compounds were designated L-α- and represented by the Fisher projection (Figure 1.2). The glycerol derivative was put into the same category as that glyceraldehyde into which it would be transformed by oxidation, without any alteration or removal of substituents. Phosphatidylcholine was therefore named: L-α-phosphatidylcholine. The IUPAC-IUB convention has now abolished the DL (or even the more recent *RS*) terminology and has provided rules for the unambiguous numbering of the glycerol carbon atoms. Under this system, phosphatidylcholine becomes, 1,2-diacyl-*sn*-glycero-3-phosphorylcholine or more shortly, 3-*sn*-phosphatidylcholine. The letters *sn* stand for stereochemical numbering and indicate that this system is being used. The stereochemical numbering system is too cumbersome to use routinely in a book of this type and, therefore, we shall normally use the terms 'phosphatidylcholine' etc. but introduce the more precise name when necessary.

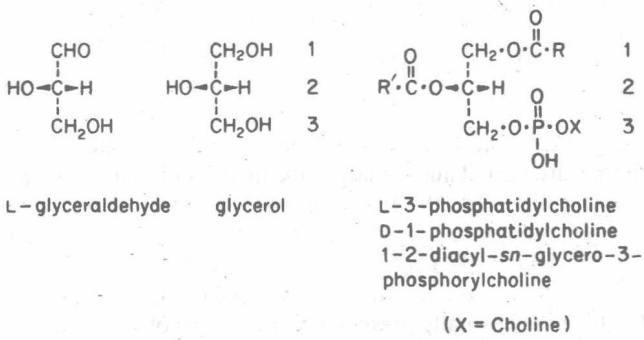

Figure 1.2 The stereochemical numbering of acylglycerols.

Another field in which nomenclature has grown up haphazardly is that of the enzymes of lipid metabolism. This has now been formalized to some extent under the Enzyme Commission (EC) nomenclature. The system is incomplete and not all lipid enzymes have EC names and numbers. Moreover, the system is very cumbersome for routine use and we have decided not to use it here. You will find a reference to this nomenclature in the reading list should you wish to learn about it. Enzymes that catalyse the biosynthesis of certain molecules are sometimes called synthetases or alternatively synthases. We shall standardize on the term **synthetase** in this book.

1.3 FUNCTIONS OF LIPIDS

The major roles of lipids can be described conveniently as structural, storage and metabolic, although individual lipids may have several different roles at different times or even at one and the same time.

1.3.1 Structural lipids: these are important at surfaces and in membranes – barriers between one environment and another

Lipids play an important part in biological structures whose purpose is to provide barriers that protect organisms against their environment. The simplest type of barrier is simply a layer of lipids on the surface of the skin or fur of animals, the surface of leaves in plants, or associated with the walls of microorganisms.

The importance of lipids in such barriers lies in their ability to exclude water and other molecules. The characteristic physical feature of lipids, namely their water insolubility, derives from the chemical structure of part of the lipid molecule which is described as hydrophobic (Greek: water-hating). In lipids that are esters of fatty acids, the hydrophobic moiety is the hydrocarbon chain of the fatty acid. Some idea of the varied chemistry of the fatty acids can be obtained by reading Chapter 3. The nature of the fatty acid chain plays a major role in determining the physical properties of those lipids of which they are part. Thus the larger the number of double bonds (higher degree of unsaturation), the lower the melting point of the acyl chains. Within the groups of saturated fatty acids, the melting point is also lowered as the chain length decreases, or if the chain is branched. Similar hydrocarbon chains with hydrophobic properties are also seen in fatty alcohols which are normally present as components of wax esters (Chapter 4). The aliphatic hydrocarbon chain is not the only hydrophobic structure

found in nature. The sterol ring system is widespread and the most abundant sterol in the animal kingdom is cholesterol. Other sterols, such as β-sitosterol are major constituents of plants. Because they contain an alcohol function, they may also form esters with fatty acids (sterol esters) which are amongst the most hydrophobic of all body lipids (Chapter 6).

Lipids also form an integral part of biological membranes. All living cells are surrounded by a membrane that provides a barrier between the cell and its environment. They also occur within the cell, providing a structure in which many metabolic reactions take place. In mammals, the lipids involved in membrane structures are mainly the glycerophospholipids and unesterified (free) cholesterol, while in plants, the glycosylglycerides are predominant, especially in the chloroplasts and β-sitosterol is the most abundant sterol rather than cholesterol. The chemistry of these structural lipids and their role in membrane architecture are described in Chapter 6, while their biochemistry forms the basis for Chapter 7.

The importance of these compounds lies in their possession of chemical groupings that associate with water (hydrophilic groups) in juxtaposition with hydrophobic moieties. These sorts of lipids are often called polar lipids, or more technically amphiphilic (Greek: liking both) and this amphiphilic nature is of immense importance in respect of their properties in membranes and in foods. (In contrast, hydrophobic fats, without polar groups, such as triacylglycerols, wax esters and sterols are often called neutral, apolar or non-polar lipids, but these are imprecise terms and best avoided.)

Phosphoglycerides are amphiphilic lipids in which the polar moiety is the phosphate group plus an organic base, such as choline. In glycosylglycerides the polar group is a sugar. Brain and nervous tissue are particularly rich in glycolipids based on the alcohol, sphingosine, as distinct from glycerol.

Current theories of biological membrane structure envisage that most of the lipid is present as a bimolecular sheet with the fatty acid chains in the interior of the bilayer. Membrane proteins are located at intervals at the internal or external face of the membrane or projecting through from one side to the other (Chapter 6). There may be polar interactions between the phospholipid headgroups and ionic groups on the proteins as well as hydrophobic interactions between 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. Indeed the composition of lipid molecules on each side of some membranes is quite different, a phenomenon called **membrane asymmetry**.

The physical properties of the membrane, which are strongly influenced by the lipid composition, seem to be important in so far as they are regulated in the face of environmental changes (diet, temperature, etc.) by subtle changes in the proportions of amphiphilic lipids, sterols and fatty acids. Such changes in physical properties may modulate the activities of membrane

proteins, such as enzymes, transporters of small molecules across the membrane or **receptors** for substances such as hormones, antigens or nutrients.

1.3.2 Storage lipids: the high-energy density of triacylglycerols makes them ideal as long-term fuel stores

Fatty acids in the form of simple glycerides constitute an important source of fuel in mammals and in many plants. The triacylglycerols are by far the most important storage form (Chapter 4).

Whereas structural lipids have a fairly conservative fatty acid composition, with a high proportion of unsaturated acids, the fatty acid composition of storage lipids is more variable, reflecting to a large extent the composition of the diet in simple stomached animals, such as man. In general, storage lipids in mammals tend to have a preponderance of saturated and monounsaturated fatty acids. In the plant kingdom, the storage lipids of seed oils have a wide variety of fatty acids. In a particular plant family, one fatty acid tends to predominate in the seed oil and this is frequently of unusual structure. The biggest reservoir of fatty acids to supply the long term needs of human beings for energy is the adipose tissue. Fatty acids are mobilized from this 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. Other tissues, such as the liver of mammals, can accommodate fat in the form of small globules but only in the short term. The excessive accumulation of fat in mammalian liver is a pathological condition. However, many species of fish normally store fat in the liver or the flesh rather than in the adipose tissue.

Milk fat can also be regarded as a kind of energy store, for the benefit of the new-born, and like adipose tissue fat, is composed mainly of triacylglycerols. Egg yolk lipids likewise provide a store of fuel and nutrients for the developing embryo.

Higher plants also make use of various kinds of lipids as storage fuels. Many seeds store triacylglycerols to provide energy for the germination process. These normally occupy the embryo of the seed, although in some cases like the avocado, the lipid is contained in the mesocarp of the fruit. Some fruits, like the jojoba, use wax esters as the fuel storage form.

In animals, storage fat may be derived directly from the fat in the diet or it may be synthesized in the adipose tissue, mammary gland or liver from simple sugars. The capacity of these tissues to synthesize fatty acids is geared to the animal's needs and is under strict dietary and hormonal control. When

there is little fat in the diet, the fatty acid pattern of a storage tissue is dependent on its own biosynthetic activity or the fatty acids supplied to it by the liver. The introduction of fat into the diet suppresses to varying extents the synthetic activity of the tissues and mechanisms operate to transport dietary fat into the storage fat so that the fat composition is more characteristic of the diet.

Plants of course must synthesize all their storage fat from simple precursors. This occurs at a certain stage after flowering and the resulting seed oil is frequently characterized by a very specific pattern of fatty acids in which one, often of unusual structure, predominates.

An important aspect of lipids in a living organism is their dynamic state. In all parts of the organism, lipids are continually being broken down or removed from the tissue and replaced. This continual breakdown and resynthesis is called turnover. Lipid replacement may occur by complete synthesis of the lipid from its simplest precursors, by replacement of parts of the molecules or by replacement of whole lipid molecules that have been transported from another site. Turnover allows a finer degree of metabolic control than would be possible in a more static system.

The chemistry and biochemistry of these storage lipids is discussed entirely within Chapter 4. Chapter 5 deals mainly with the dietary lipids of mammals and their assimilation by the body. The emphasis is on human nutrition.

As the foods we eat originate from living things, so, therefore our dietary lipids are derived from the structural and storage lipids of plants and animals. These lipids are digested in the alimentary tract by hydrolytic enzymes. The digestion products are taken up into the intestinal absorbing cells and resynthesized into complex lipids. Lipids absorbed and resynthesized in this way are transported to sites of storage or metabolism depending on the animal's current energy needs. The route of transport is the bloodstream, which is essentially an aqueous fluid in which small molecules are dissolved and macromolecules and cells are suspended. This poses a problem when fatty substances, insoluble in water, have to be accommodated. The solution to the problem is the conjugation of lipids with proteins to form a wide range of transport particles called lipoproteins.

Lipids transported as lipoproteins are taken up into tissues where they may be stored as energy reserves in the adipose tissue, incorporated into the structural lipids of membranes or oxidized to supply energy, depending on the nutritional and physiological state of the organism at the time. Much of the lipid in the body can be synthesized by body tissues. However, some fatty acids (the essential fatty acids) and the fat-soluble vitamins have to be supplied in the diet because, although vital for the animal, they cannot be synthesized endogenously.

Deficiencies in the diet or genetically determined errors in metabolism

give rise to disease states. Lipids may be important in the aetiology of these diseases or play a part in therapy. These aspects of health and disease are addressed in Chapter 5.

1.3.3 Lipids in metabolic control: storage and barrier functions make use of the bulk properties of lipids. At the level of individual molecules, lipids participate as chemical messengers and are involved in the control of metabolism

Lipids not only contribute to the structure of cells and provide an energy store, they also participate in the transmission of chemical messages in living organisms.

In Chapters 3, 5 and 7, while discussing the biosynthesis and breakdown of specific types of lipids and the way in which their own metabolism is controlled, we allude to their specialized physiological roles. Thus, in Chapter 3, we discuss the conversion of specific polyunsaturated fatty acids into a whole range of oxygenated fatty acids, the eicosanoids, which have a variety of potent physiological effects at extremely low concentrations. These effects, which include stimulation of muscle contraction and stimulation or inhibition of platelet aggregation, are indeed so powerful that the eicosanoids need to be produced at or near the site of their action and quickly destroyed. They are, in effect, local hormones. The way in which our understanding of how nutrition influences their production and the way in which they act is explored further in Chapter 5, while their role in the regulation of the immune system is discussed in Chapter 8.

The formation of eicosanoids is an example of tightly controlled enzymic peroxidation of lipids. Peroxidation reactions can also occur chemically and unless controlled by the presence of natural antioxidant systems can give rise to massive cell damage, disintegration and disease. The chemistry involved is discussed in Chapter 3, while the pathological implications are described in Chapter 8.

A variety of lipid molecules take part in diverse aspects of metabolism and its control. Polyunsaturated fatty acids and their metabolites have been discussed above. Others are the fat-soluble vitamins, retinol (vitamin A) and tocopherol (vitamin E) (Chapters 5 and 8). Sterols, such as cholesterol, regulate membrane function and act as precursors for a range of molecules with diverse metabolic activities: cholecalciferol (vitamin D), which is metabolized further to hydroxylated derivatives that regulate calcium metabolism and other aspects of cellular function (Chapters 5 and 7); bile acids, which are involved in lipid absorption (Chapters 4 and 7) and steroid hormones (Chapter 7).

Perhaps the greatest interest in recent years has been the development of

ideas about the role of the inositol phospholipids whose breakdown products act as **second messengers** in cell signalling. This story begins in Chapter 7 in relation to the metabolism of the inositol phospholipids and continues in Chapter 8 with a discussion of their role in cells. Indeed, we have collected together in Chapter 8 a diversity of material which has a common theme, namely, specific physiological functions of lipids. In addition to those described above, the chapter covers adaptive changes in membranes, the use of lipid vesicles for clinical purposes such as drug delivery, the roles of lipids particularly the sphingolipids in membrane receptors. It also discusses the involvement of lipids in skin diseases, in the so-called lipid storage diseases and their role in pulmonary surfactant.

This book is about the chemical nature of the many types of water-insoluble substances known as lipids: how they are synthesized and broken down; how they are incorporated into and released from living cells and tissues; how they contribute to the structural elements of cells (Chapters 3–7). It is also concerned with the normal biological control of these metabolic processes and the implications for the organisms when the normal metabolic control mechanisms are faulty or when environmental conditions create a stress that requires a process of adaptation. It is about the roles of lipids in biological structures, the means by which they provide metabolic energy and about their important functions in the foods we eat. Although it is probably true to say that most students of biochemistry are interested in the metabolism of man and other mammals, it should not be forgotten that, because of the vast quantities of plants and microorganisms distributed over the earth's surface, the plant kingdom accounts for much the largest proportion of the world's lipids. Consequently, this book will still deal very extensively with plant lipids even though there has been increased emphasis on human lipid biochemistry compared with previous editions.

REFERENCES

Farquar, J.W., Insull, W., Rosen, P., Stoffel, W. and Ahrens, E.H. (1959) Nomenclature of fatty acids. *Nutrition Reviews*, **17**, Suppl.

Gunstone, F.D. (1967) *An Introduction to the Chemistry and Biochemistry of Fatty Acids and their Glycerides*, Chapman and Hall, London.

IUPAC–IUB Commission on Biochemical Nomenclature (1978) *Biochemical Journal*, **169**, 11–14.

Nomenclature Committee of the International Union of Biochemistry (1984) *Enzyme Nomenclature*, Academic Press, London.

2 *Isolation, separation and detection of lipids*

Since lipids are characterized generally by their ability to dissolve in water-immiscible organic solvents, advantage is taken of this property at many stages of analysis.

2.1 EXTRACTION OF LIPIDS FROM NATURAL SAMPLES

Extraction of lipids from natural samples, like that of many biological molecules, is best accomplished as soon as possible to minimize the degradative changes which would otherwise take place. When storage has to take place it is best done at as low a temperature as possible (say −20°C or less) and under an inert nitrogen atmosphere to minimize oxidation of groups such as double bonds. If great care is not taken during the extraction process, many lipids will be partly or completely lost. For example, the polyphosphoinositides (section 8.7, 8.8) are extremely labile due to the very active degradative enzymes present in many animal tissues. Similar degradative enzymes can pose particular problems in plant tissues since they are active at very low temperatures (certainly at −20°C) and also retain activity (or may even be activated) in organic solvents. Such enzymes are obviously best inactivated as quickly as possible by, for example, brief exposure of the tissue to steam or boiling water or by a prior extraction with hot isopropanol – all measures which inactivate the lipases.

The actual extraction method depends on the type of tissue and also the lipids it is desired to analyse. However, few lipids can be extracted by a single solvent and binary mixtures are usually used. One of the components should have some water solubility and hydrogen bonding ability because

lipid–protein complexes such as those encountered in membranes have to be split.

A very common method is that of Bligh and Dyer. These workers used a mixture of chloroform and methanol in a ratio with tissue water (1 : 2 : 0.4) to form a one-phase system. Homogenization of tissues in this mixture efficiently extracts most lipids. More chloroform and methanol are then added to give two phases, the upper (aqueous) one containing non-lipid impurities; the lower (chloroform) phase can then be removed, washed with fresh upper phase and finally evaporated to dryness. Residual water can be removed with anhydrous sodium sulphate or by filtration through Sephadex columns. In some cases it may be desirable to use salt or dilute acid solutions in the upper phase to prevent losses of polar lipids. Finally after removing solvent by vacuum evaporation, the crude lipid residue should be protected from oxidation by inert nitrogen gas. In fact, before storage for any length of time, lipids are best redissolved in a small amount of solvent containing an antioxidant such as 2,6-di-*tert*-butyl-*p*-cresol(BHT) before storage at −20°C or less in the dark under nitrogen.

2.2 LIKELY COMPONENTS OF THE CRUDE LIPID EXTRACT

Since the initial extraction has been based on solubility properties, the crude lipid extract will contain any molecule which dissolves preferentially in the organic solvents used. Thus, significant quantities of non-lipids, e.g. hydrophobic proteins, may be present at this stage. The mixture of lipids (Table 2.1) will depend on the nature of the sample extracted.

Table 2.1 Major components of typical lipid extracts from different tissues

Erythrocytes	*Liver*	*Leaves*	*Cyanobacteria*	*Gram⁻Bacteria*
P. Lipid	P. Lipid	P. Lipid	P. Lipid	P. Lipid
Sphingolipid	Sphingolipid	—	—	—
—	—	Glycosyl-glycerides	Glycosyl-glycerides	—
Sterols	Sterols	—	—	—
—	TAG	—	—	—
		Others*‡	Others*	Others†

TAG = triacylglycerols, P. Lipid = phosphoglycerides; * pigments; † lipopolysaccharide; ‡ waxes, cutin.

2.3 GENERAL FEATURES OF LIPIDS IMPORTANT FOR THEIR ANALYSIS

Most of the lipids cited as major components in different tissues contain esterified fatty acids. These are termed acyl lipids. This is important during analysis for several reasons:

1. the nature of the fatty acids determines much of the physical and biological properties of the lipid and, therefore, it is of importance to analyse their properties;
2. the type of fatty acid influences the stability of the sample;
3. because mixtures of fatty acids are found in any given lipid class, the latter contains a number of molecular species.

Total fatty acid analysis is usually performed by forming volatile derivatives (such as methyl esters) for gas chromatography. Special techniques have to be used sometimes to avoid destroying unusual functional groups such as cyclopropene rings. Moreover, the danger of auto-oxidation means that analysis of samples containing polyunsaturated fatty acids has to be especially careful. For complete identification of individual acyl groups, degradation or derivatization usually has to be employed – for example, when double bond positions have to be assigned.

In order to determine the positional distribution of acyl groups on, say, the glycerol backbone, enzymic cleavage is usually employed. For example, phospholipases are available which have a specificity for either the *sn*-1 or the *sn*-2 position. Use of such enzymes will release fatty acids from one position and these can be separated from the partly deacylated product. Analysis of fatty acids and deacylated lipid will then reveal which fatty acids are present at each glycerol carbon.

Molecular species of lipids can be separated on the basis of size and/or unsaturation. In the past it has often been necessary to block or remove the polar part of the lipid making analyses time-consuming. For example, because the charge on the head-group of phosphatidylcholine is large in relation to differences in acyl unsaturation, it was usually necessary to degrade such phosphoglycerides to diacylglycerols before analysis by chromatography. Modern methods of HPLC have, however, rendered such methods unnecessary provided that adequate methods of detection are available.

Single lipid classes can be separated from each other by methods which make use of differences in their size and charge. They can often be provisionally identified by co-chromatography with authentic standards in various systems. Important constituent groups will be revealed by spectroscopic techniques or with specific colour reagents. However, unambiguous identification may require that the various products of