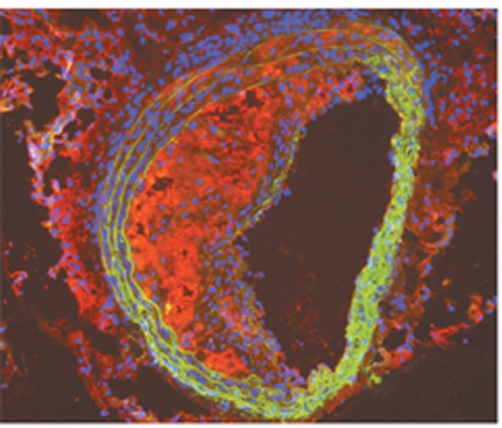
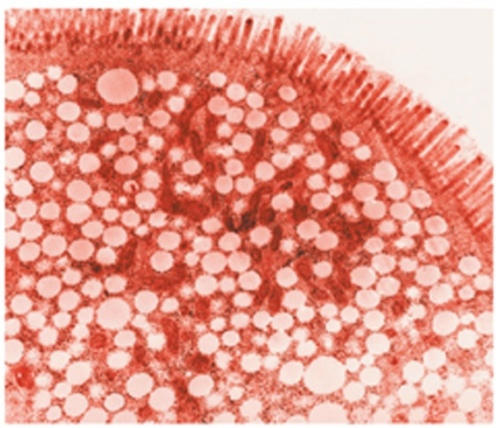
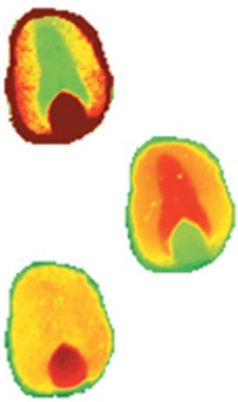
SIXTH EDITION

LIPIDS

BIOCHEMISTRY, BIOTECHNOLOGY AND HEALTH







Michael I. Gurr, John L. Harwood,
Keith N. Frayn, Denis J. Murphy
and Robert H. Michell

WILEY Blackwell

Lipids

Lipids: Biochemistry, Biotechnology and Health

SIXTH EDITION

(formerly Lipid Biochemistry: An Introduction, Editions 1–5)

BY

Michael I. Gurr

John L. Harwood

Keith N. Frayn

Denis J. Murphy

Robert H. Michell

WILEY Blackwell

Registered office: John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

Library of Congress Cataloging-in-Publication Data

Names: Gurr, M. I. (Michael Ian), author. | Harwood, John L., author. | Frayn K. N. (Keith N.), author. | Murphy, Denis J., author | Michell, R. H., author. Lipid biochemistry. Preceded by (work):
Title: Lipids:Biochemistry, Biotechnology and Health / by Michael I. Gurr , John L. Harwood, Keith N. Frayn, Denis J. Murphy, and Robert H. Michell.
Description: 6th edition. | Chichester, West Sussex ; Hoboken, NJ : John Wiley & Sons Inc., 2016. | Preceded by Lipid biochemistry / by Michael I. Gurr, John L. Harwood, and Keith N. Frayn. 5th ed. 2002. | Includes bibliographical references and index.
Identifiers: LCCN 2016000533 (print) | LCCN 2016002203 (ebook) | ISBN 9781118501139 (pbk.) | ISBN 9781118501085 (Adobe PDF) | ISBN 9781118501108 (ePub)
Subjects: | MESH: Lipids
Classification: LCC QP751 (print) | LCC QP751 (ebook) | NLM QU 85 | DDC 572/.57–dc23
LC record available at http://lccn.loc.gov/2016000533

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Cover images: Left panel: An artery partially occluded by an atherosclerotic plaque (Section 10.5.1). The red stain is for macrophages that are present in the plaque and become foam cells. The green stain is for smooth muscle cells in the arterial wall and capping the plaque. Photo courtesy of Thomas S. Davies and Susan Chazi, Cardiff University, UK from work funded by the British Heart Foundation.
Middle panel: An enterocyte from human jejunum displaying multiple lipid droplets a few hours after consuming a fatty meal (Section 7.1.3). The figure also shows mitochondria (dark) and the microvilli (brush border). Electron micrograph courtesy of Dr M Denise Robertson, University of Surrey, UK from work funded by the Biotechnology and Biological Sciences Research Council (BBSRC). Reproduced, with permission from BMJ Publishing Group Ltd, from MD Robertson, M Parkes, BF Warren et al. (2003) Mobilization of enterocyte fat stores by oral glucose in man. Gut 6: 833–8.
Right panel: Distribution of different molecular species of phosphatidylcholine within developing oilseed rape embryos as revealed by MALDI-MS imaging (Section 9.3.1). Red shows high concentrations and green low. Photo courtesy of Helen Woodfield and Drew Sturtevent from work funded by the BBSRC in Prof. Kent Chapman's laboratory at the University of North Texas, USA.
Background: Gettyimages/manuela schewe-behnisch / eyeem

Set in 8.5/12pt, MeridienLTStd-Roman by Thomson Digital, Noida, India

1 2016

Contents

Preface

Our main aims in writing this book have been, as ever, to aid students and other researchers in learning about lipids, to help staff in teaching the subject and to encourage research in this field. Since the publication of the Fifth Edition in 2002, there have been huge advances in our knowledge of the many aspects of lipids, especially in molecular biology. Far more is now known about the genes coding for proteins involved in lipid metabolism and already techniques of biotechnology are making use of this knowledge to produce specialized lipids on an industrial scale. The new knowledge has also had a far-reaching influence on medicine by revealing the role of lipids in disease processes to a much greater extent than hitherto and allowing for advances in diagnosis and disease prevention or treatment. We have endeavoured to reflect as many of these advances as possible in this new edition. Although modern textbooks of general biochemistry or biology now cover lipids to a greater extent than when our first edition was published in 1971, a book devoted entirely to lipids is able to go into far more detail on all these diverse aspects of the subject and to discuss exciting new developments with greater authority. It should be emphasized here that we have referred to a wide range of organisms – including archaea, bacteria, fungi, algae, 'higher' plants and many types of animals and not restricted ourselves to mammalian lipids.

Because of this research activity, we have rewritten large parts of the book and have given it a new title that reflects the fact that it is increasingly difficult to identify old boundaries between subjects such as biochemistry, physiology and medicine. This runs in parallel with changes in university structure: away from narrow 'departments' of 'biochemistry', zoology', 'botany' and the like, towards integrated 'schools' of biological sciences or similar structures. The increasing diversity of the subject requires greater specialist expertise than is possible with one or two authors. Accordingly, we have brought two new colleagues on board and one of the original authors has been given the role of coordinating editor to assure, as far as possible, consistency of style, so that we could avoid identifying authors with chapters. The authors have consulted widely among colleagues working in lipids and related fields to ensure that each chapter is as authoritative as possible. We are grateful for their help, which is recorded in the acknowledgements section. As a result, advances in such topics as enzymes of lipid metabolism, lipids in cell signalling, lipids in health and disease, molecular genetics and biotechnology have been strengthened.

The need to include new material has had to be balanced against the need to keep the book to a moderate size, with a price within most students' budgets. Some things had to go! As in the Fifth Edition, we decided to restrict some material of historical interest. Nevertheless, we thought that the inclusion of many short references to historical developments should remain, to add interest and to put certain aspects of lipidology in context. We have also removed some of the material that dealt with analytical procedures so that we could focus more on metabolic, physiological, clinical and biotechnological aspects. Chapter 1 now summarizes lipid analytical methods, with ample references to more specialist literature but has a section on lipidomics to highlight modern approaches to lipid profiling in biological fluids and tissues. This introductory chapter also contains a guide to finding your way around the book, which we hope students will find useful. We shall appreciate comments and suggestions so that future editions can be further improved.

MI Gurr
JL Harwood
KN Frayn
DJ Murphy
RH Michell

Acknowledgements

Over the years, we have received invaluable assistance from many colleagues in the compilation of this book and our thanks have been recorded in the previous five editions. Their contributions are still significant in this new edition and we are also grateful to the following for helping us with new material.

In Chapter 1, Jules Griffin provided valuable assistance with the lipidomics section. The substantial section on fatty acid biosynthesis has been brought up to date with help from Stuart Smith and his colleague Marc Leibundgut, whose huge expertise has been much appreciated. Many other aspects of Chapter 3 have benefited from the help of John Cronan Jr., Michael Schweizer, Marc Leibundgut and Ivo Fuessner. Bill Christie's wide knowledge of lipid chemistry, nomenclature and analysis has been invaluable throughout the book. Deficiencies in our knowledge of fat-soluble vitamins have been rectified by David Bender (Chapter 6); recent advances in comparative aspects of lipid metabolism by Caroline Pond (Chapter 7); lipids in immunity by Parveen Yaqoob and Philip Calder (Chapter 10); lung surfactant by Fred Possmayer (Chapters 4 & 10) and lipoproteins in human metabolism and clinical practice by Fredrik Karpe and Sophie Bridges (Chapters 7 & 10). Gary Brown and Patrick Schrauwen helped with information on inborn errors of lipid metabolism; Jenny Collins with cancer and lipid metabolism; and Sara Suliman with understanding lipodystrophies (Chapter 10).

Our thanks are due to the Wiley-Blackwell team for guiding us through the intricacies of the publication process. Particular mention should be made of Nigel Balmforth, who has been associated with *Lipid Biochemistry* from its early days with Chapman & Hall, then Blackwell and finally Wiley. Finally, after the enormous amount of work that goes into writing a book of this complexity, the authors conclude that all 'i's and 't's must have been dotted and crossed. It takes an expert, conscientious and helpful copy-editor to put a stop to this complacency and create a much better product. Martin Noble has done just that. Thank you all.

About the authors

Michael I. Gurr was Visiting Professor in Human Nutrition at Reading and Oxford Brookes Universities, UK.

John L. Harwood is Professor of Biochemistry in the School of Biosciences, Cardiff University, UK.

Keith N. Frayn is Emeritus Professor of Human Metabolism at the University of Oxford, UK.

Denis J. Murphy is Professor of Biotechnology in the School of Applied Sciences, University of South Wales, UK.

Robert H. Michell is Emeritus Professor of Biochemistry in the School of Biosciences, University of Birmingham, UK.

About the companion website

www.wiley.com/go/gurr/lipids

The website includes:

- Powerpoint slides of all the figures from the book, to download
- Pdfs of all tables and boxes from the book, to download
- Updates to Further Reading and additional figures to download

CHAPTER 1

Lipids: definitions, naming, methods and a guide to the contents of this book

1.1 Introduction

Lipids occur throughout the living world in microorganisms, fungi, higher plants and animals. They occur in all cell types and contribute to cellular structure, provide energy stores and participate in many biological processes, ranging from transcription of genes to regulation of vital metabolic pathways and physiological responses. In this book, they will be described mainly in terms of their functions, although on occasion it will be convenient, even necessary, to deal with lipid classes based on their chemical structures and properties. In the concluding section of this chapter, we provide a 'roadmap' to help students find their way around the book, so as to make best use of it.

1.2 Definitions

Lipids are defined on the basis of their solubility properties, not primarily their chemical structure.

The word 'lipid' is used by chemists to denote a chemically heterogeneous group of substances having in common the property of insolubility in water, but solubility in nonaqueous solvents such as chloroform, hydrocarbons or alcohols. The class of natural substances called 'lipids' thus contrasts with proteins, carbohydrates and nucleic acids, which are chemically well defined.

The terms 'fat' and 'lipid' are often used interchangeably. The term fat is more familiar to the layman for 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 generally solid in texture, as distinct from oils which are liquid at ambient temperatures. Natural fats and oils are composed predominantly of esters of the three-carbon alcohol *glycerol* with *fatty acids*, often referred to as 'acyl lipids' (or more generally, 'complex lipids'). These are called triacylglycerols (TAG, see Section 2.2: often called 'triglycerides' in older literature) and are chemically quite distinct from the oils used in the petroleum industry, which are generally hydrocarbons. Alternatively, in many glycerol-based lipids, one of the glycerol hydroxyl groups is esterified with phosphorus and other groups (phospholipids, see Sections 2.3.2.1 & 2.3.2.2) or sugars (glycolipids, see Section 2.3.2.3). Yet other lipids are based on sphingosine (an 18-carbon amino-alcohol with an unsaturated carbon chain, or its derivatives) rather than glycerol, many of which also contain sugars (see Section 2.3.3), while others (isoprenoids, steroids and hopanoids, see Section 2.3.4) are based on the five-carbon hydrocarbon isoprene.

Chapter 2 deals mainly with lipid structures, Chapters 3 and 4 with biochemistry and Chapter 5 with lipids in cellular membranes. Aspects of the biology and health implications of these lipids are discussed in parts of Chapters 6–10 and their biotechnology in Chapter 11. The term 'lipid' to the chemist thus embraces a huge and chemically diverse range of fatty substances, which are described in this book.

1.3 Structural chemistry and nomenclature

1.3.1 Nomenclature, general

Naming systems are complex and have to be learned. The naming of lipids often poses problems. When the subject was in its infancy, research workers gave names to substances that they had newly discovered. Often, these

Lipids: Biochemistry, Biotechnology and Health, Sixth Edition. Michael I. Gurr, John L. Harwood, Keith N. Frayn, Denis J. Murphy and Robert H. Michell.

substances would turn out to be impure mixtures but as the chemical structures of individual lipids became established, rather more systematic naming systems came into being and are still evolving. Later, these were further formalized under naming conventions laid down by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB). Thus, the term 'triacylglycerols' (TAGs – see Index – the main constituents of most fats and oils) is now preferred to 'triglyceride' but, as the latter is still frequently used especially by nutritionists and clinicians, you will need to learn both. Likewise, outdated names for phospholipids (major components of many biomembranes), for example 'lecithin', for phosphatidylcholine (PtdCho) and 'cephalin', for an ill-defined mixture of phosphatidylethanolamine (PtdEtn) and phosphatidylserine (PtdSer) will be mostly avoided in this book, but you should be aware of their existence in older literature. Further reference to lipid naming and structures will be given in appropriate chapters. A routine system for abbreviation of these cumbersome phospholipid names is given below.

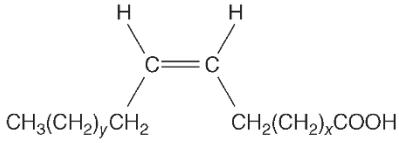
1.3.2 Nomenclature, fatty acids

The very complex naming of the fatty acids (FAs) is discussed in more detail in Chapter 2, where their structures are described. Giving the full names and numbering of FAs (and complex lipids) at each mention can be extremely cumbersome. Therefore a 'shorthand' system has been devised and used extensively in this book and will be described fully in Section 2.1, Box 2.1. This describes the official system for naming and numbering FAs according to the IUPAC/IUB, which we shall use routinely. An old system used Greek letters to identify carbon atoms in relation to the carboxyl carbon as C1. Thus, C2 was the α-carbon, C3 the β-carbon and so on, ending with the ω-carbon as the last in the chain, furthest from the carboxyl carbon. Remnants of this system still survive and will be noted as they arise. Thus, we shall use '3-hydroxybutyrate', *not* 'β-hydroxybutyrate' etc.

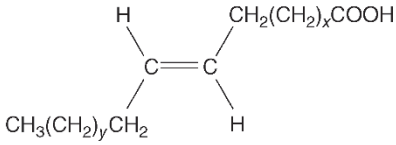
While on the subject of chain length, it is common to classify FAs into groups according to their range of chain lengths. There is no standard definition of these groups but we shall use the following definitions in this book: short-chain fatty acids, 2C–10C; medium-chain, 12C–14C; long-chain, 16C–18C; very long-chain >18C. Alternative definitions may be used by other authors.

1.3.3 Isomerism in unsaturated fatty acids

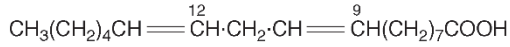
An important aspect of unsaturated fatty acids (UFA) is the opportunity for isomerism, which may be either positional or geometric. Positional isomers occur when double bonds are located at different positions in the carbon chain. Thus, for example, a 16C monounsaturated (sometimes called monoenoic, see below) fatty acid (MUFA) may have positional isomeric forms with double bonds at C7-8 or C9-10, sometimes written Δ7 or Δ9 (see Box 2.1). (The position of unsaturation is numbered with reference to the first of the pair of carbon atoms between which the double bond occurs, counting from the carboxyl carbon.) Two positional isomers of an 18C diunsaturated acid are illustrated in Fig. 1.1(c,d).



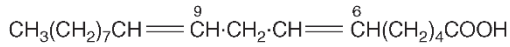
(a) *cis* (Z)



(b) *trans* (E)



(c) *cis*, *cis* –9, 12–octadecadienoic acid



(d) *cis*, *cis* –6, 9–octadecadienoic acid

Fig. 1.1 Isomerism in fatty acids. (a) *cis*-double bond; (b) a *trans*-double bond; (c) *c,c*-9,12-18:2; (d) *c,c*-6,9-18:2.

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 (Fig. 1.1a,b). *Cis* and *trans* will be routinely abbreviated to *c,t* (see Box 2.1).

1.3.4 Alternative names

Students also need to be aware that the term 'ene' indicates the presence of a double bond in a FA. Consequently, mono-, di-, tri-, poly- (etc.) unsaturated FAs may also be referred to as mono-, di-, tri- or poly- (etc.) enoic FAs (or sometimes mono-, di-, tri- or poly-enes). Although we have normally used 'unsaturated' in this book, we may not have been entirely consistent and '-enoic' may sometimes be encountered! Furthermore it is important to note that some terms are used in the popular literature that might be regarded as too unspecific in the research literature. Thus shorthand terms such as 'saturates', 'monounsaturates', 'polyunsaturates' etc. will be avoided in much of this text but, because some chapters deal with matters of more interest to the general public, such as health (Chapter 10) and food science or biotechnology (Chapter 11), we have introduced them where appropriate, for example when discussing such issues as food labelling.

1.3.5 Stereochemistry

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, 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. Students interested in the details of the stereochemistry of glycerol derivatives should consult previous editions of this book (see Gurr *et al.* (1971, 1975, 1980, 1991, 2002) and other references in **Further reading)**. 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, the phosphoglyceride, phosphatidylcholine, becomes 1,2-diacyl-*sn*-glycero-3-phosphorylcholine or, more shortly, 3-*sn*-phosphatidylcholine (PtdCho; Fig. 1.2). The letters *sn* denote *'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. or their relevant abbreviations, but introduce the more precise name when necessary.

1.3.6 Abbreviation of complex lipid names and other biochemical terms

Students will appreciate that the official names of complex lipids (and many other biochemicals) are cumbersome and research workers have evolved different systems for abbreviating them. In this latest edition we have incorporated all abbreviations into the index. At the first mention of each term in the text, we shall give the full authorized name followed by the abbreviation in parentheses. This will be repeated *at the first mention in each subsequent chapter*. Students should be aware that, unlike the IUB/IUPAC naming system, which is now generally accepted and expected to be used, the abbreviation system is still very much a matter of personal choice. Therefore students may expect to find alternative phospholipid abbreviations in some publications, for example PC, PE, PS and PI for

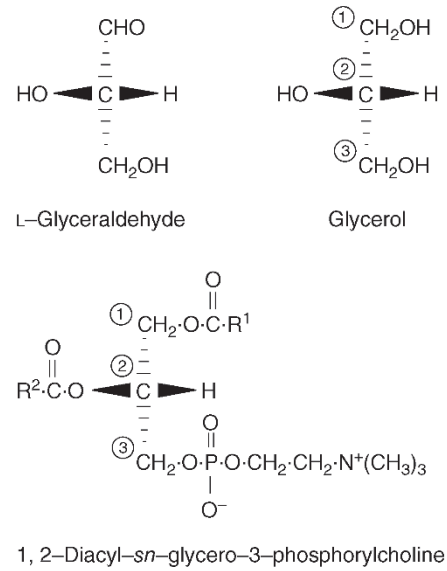


Fig. 1.2 The stereochemical numbering of lipids derived from glycerol.

phosphatidylcholine, -ethanolamine, -serine and –inositol, instead of the PtdCho, PtdEtn, PtdSer and PtdIns used here. With very few exceptions we have not defined abbreviations for well-known substances in the general biochemical literature, such as ATP, ADP, NAD(H), NADP(H), FMN, FAD etc.

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 **Further reading** should you wish to learn about it.

Since the last edition was published in 2002, there have been huge advances in molecular biology and, in particular, in identifying the genes for an ever-increasing number of proteins. Where appropriate, we have referred to a protein involved in human lipid metabolism, of which the gene has been identified and have placed the gene name in parentheses after it (protein name in Roman, gene name in Italic script).

1.4 Lipidomics

1.4.1 Introduction

Since the last edition of this book in 2002, there have been very considerable advances in analysing and identifying natural lipids. Much modern research in this field is concerned with the profiling of lipid molecular species in cells, tissues and biofluids. This has come to be known as 'lipidomics', similar to the terms 'genomics' for profiling the gene complement of a cell or 'proteomics' for its proteins.

Some older methods of lipid analysis, presented in previous editions, will be described only briefly here and the student is referred to **Further reading** for books, reviews and original papers for more detail. Before describing the modern approach to lipidomics, we describe briefly the steps needed to prepare lipids for analysis and the various analytical methods, many of which are still widely used.

1.4.2 Extraction of lipids from natural samples

This is normally accomplished by disrupting the tissue sample in the presence of organic solvents. Binary mixtures are frequently used, for example chloroform and methanol. One component should have some water miscibility and hydrogen-bonding ability in order to split lipid-protein complexes in the sample, such as those encountered in membranes (Chapter 5). Precautions are needed to avoid oxidation of, for example, UFAs. Control of temperature is important, as well as steps to inhibit breakdown of lipids by lipases (see Sections 4.2 & 4.6). The extract is finally 'cleaned up' by removing water and associated water-soluble substances (see **Further reading**).

1.4.3 Chromatographic methods for separating lipids

Once a sample has been prepared for analysis, chromatography can be used to separate its many lipid constituents. A chromatograph comprises two immiscible phases: one is kept stationary by being held on a microporous support; the other (moving phase) percolates continuously through the stationary phase. The stationary phase may be located in a long narrow bore column of metal, glass or plastic (column chromatography), coated onto a glass plate or plastic strip (thin layer chromatography, TLC, see Fig. 1.3) or it may simply be a sheet of absorbent paper (paper chromatography).

The principle of chromatography is that when a lipid sample (often comprising a very large number of molecular species) is applied to a particular location on the stationary phase (the origin) and the moving phase percolates through, the different components of the mixture partition differently between the two phases according to their differing chemical and physical properties. Some will tend to be retained more by the stationary phase, while others tend to move more with the moving phase. Thus, the components will move apart as the moving phase washes through the system (see Christie, 1997; Christie & Han 2010; and Hammond 1993 in **Further reading** for more details of the theory of chromatography).

Many types of adsorbent solid can be used as the stationary phase (e.g. silica, alumina). The moving phase may be a liquid (liquid chromatography, LC) or a gas (gas chromatography, GC – the original term gas-liquid chromatography, GLC, is now less used). Particularly good separations may now be achieved by GC (see Fig. 1.4) with very long thin columns packed with an inert support for the stationary phase or in which the stationary phase is coated on the wall of the column. This is useful for volatile compounds or those that can be converted

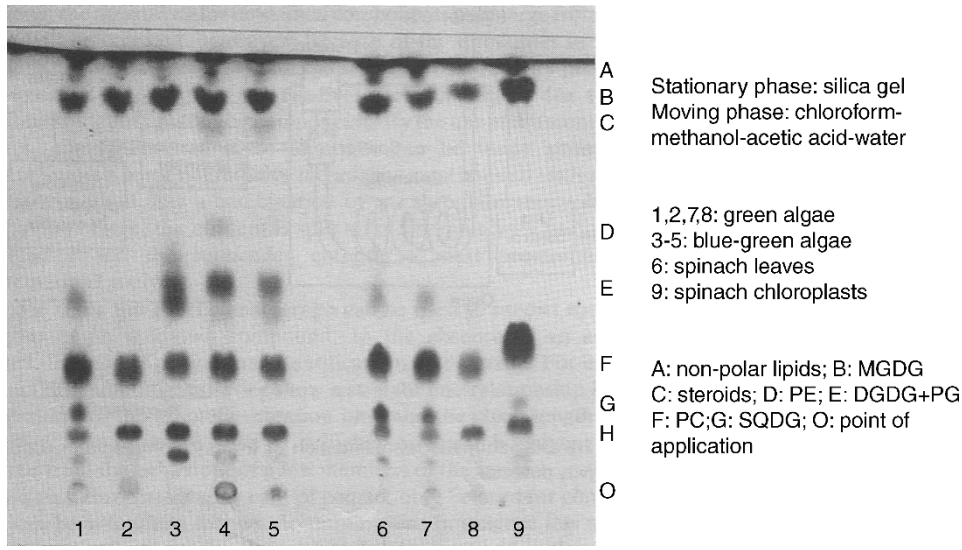


Fig. 1.3 Separation of lipid classes by thin-layer chromatography (TLC).

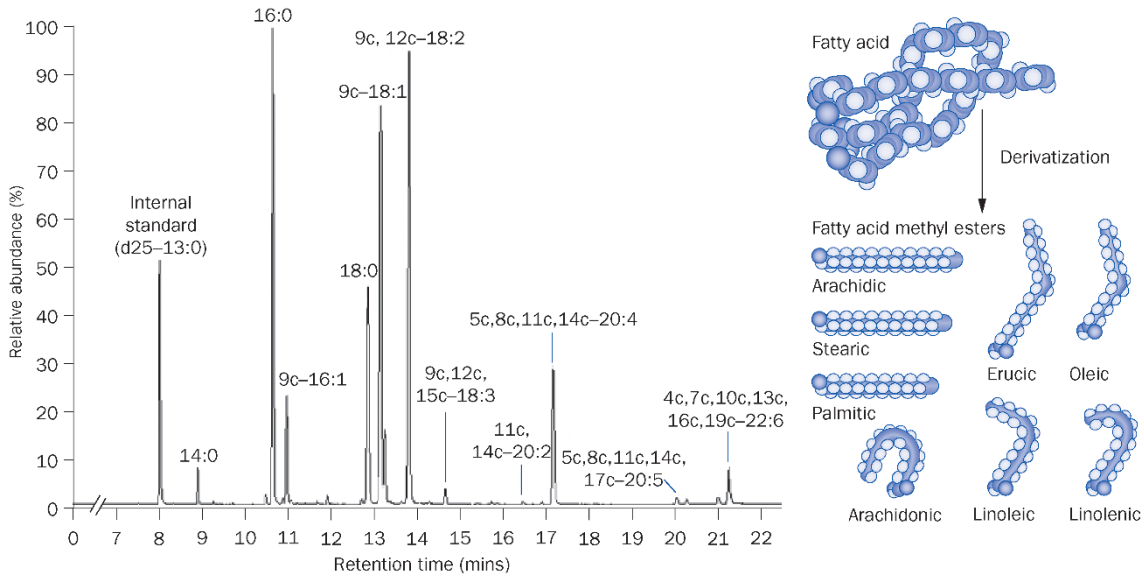


Fig. 1.4 Separation of fatty acid methyl esters by gas chromatography (GC). The figure shows the FA composition of a lipid extract of heart tissue as measured by GC on a capillary column. To the right of the chromatogram is depicted the conversion of a complex lipid into FA methyl esters in preparation for chromatography. The peaks on the chromatogram are labelled with shorthand abbreviations for FAs (see Box 2.1 for details). Detection is by a flame ionization detector. From JL Griffin, H Atherton, J Shockcor & L Atzori (2011) Metabolomics as a tool for cardiac research. *Na Rev Cardiol* **8**: 630–43; p. 634, Fig. 3a. Reproduced with permission of Nature Publishing Group.

into more volatile ones, such as the methyl esters of FAs (see Sections 2.1.8.1 & 11.2.4.2 for further details of the preparation of FA methyl esters). For less volatile complex lipids, LC in thin columns through which the moving phase is passed under pressure can produce superior separations: this is called high performance liquid chromatography (HPLC).

Once the components have been separated, they can be collected as they emerge from the column for further identification and analysis (see Section 1.4.4). Compounds separated on plates or strips can be eluted from the stationary phase by solvents or analysed in situ by various means. (Further information on methods of detection can be found in Christie & Han (2010) and Kates (2010) in **Further reading**.)

The power of modern lipidomics has been made possible by the combination of GC or LC with improved methods of mass spectrometry (MS) to provide detailed and sophisticated analyses of complex natural lipid mixtures and this is the subject of the next section.

1.4.4 Modern lipidomics employs a combination of liquid chromatography or gas chromatography with mass spectrometry to yield detailed profiles of natural lipids – the 'lipidome'

While individual FAs can be readily measured by gas chromatography-mass spectrometry (GC-MS), the commonest method to perform this analysis relies on cleaving FAs from the head groups that they are associated with and converting them into methyl esters by transesterification. This process is used to make the FAs volatile at the temperature used by GC-MS, but during this process information is lost, particularly about which lipid species are enriched in a given FA.

An alternative is to use LC-MS. In this approach, lipid extracts from biofluids and tissues can be analysed directly. The lipids are dissolved in an organic solvent and injected directly onto the HPLC column. Columns can contain a variety of chemicals immobilized to form a surface (stationary phase) that the analytes interact with. For the analysis of lipids, columns containing long chains of alkyl groups are most commonly used, in particular 8C and 18C columns, which have side-chain lengths of 8 and 18 carbons, respectively. The most commonly used HPLC method is referred to as 'reverse phase', whereby lipids are initially loaded onto a HPLC column and then the HPLC solvent is varied from something that is predominantly aqueous to a solvent that is predominantly organic, across what is termed a gradient. The solvents are referred to as the mobile phases. During this process, lipids are initially adsorbed on to the stationary phase, until their solubility increases to the point that they begin to dissolve in the mobile phase. In this manner, polar and nonpolar lipids can readily be separated and typically, in a lipid extract, lipid molecular species would elute in the order of nonesterified fatty acids (NEFAs), phospholipids, cholesteryl esters and TAGs. The chromatography serves two important purposes. Firstly, it reduces the complexity of the subsequent mass spectra generated by the mass spectrometer, making metabolite identification more convenient. Secondly, some metabolites can ionize more readily than others and this can produce an effect called 'ion suppression' where one metabolite ionizes more easily and reduces the energy available for the ionization of other species. As a result, the mass spectrometer may detect only the metabolite that ionizes readily and miss the other metabolites that do not readily form ions.

LC-MS is most commonly used with 'electrospray ionization' where the analytes are introduced to the mass spectrometer in the form of a spray of solvent. They are accelerated over an electric field across the capillary that introduces them into the mass spectrometer and the nebulization of the spray is often assisted by the flow of an inert gas. The inert gas causes the solvent to evaporate (desolvate), producing a fine spray of droplets. As the solvent evaporates, charges build up in the droplets until they explode into smaller droplets, finally producing an ion that is introduced into the mass spectrometer. While this may sound relatively destructive, this form of ionization is relatively 'soft', ensuring that the molecule itself or an adduct (a combination of the molecule and another charged species such as H^+, Na^+, K^+ or other ions present in the solvent) is formed. The ions are then detected by the mass spectrometer (Fig. 1.5).

While there are numerous designs of mass spectrometer, two common methods are often used in lipidomics. In high resolution MS, the mass accuracy achievable is so great that chemical formulae can be determined with reasonable precision. This is because only carbon-12 has a mass of exactly 12 atomic mass units, while other nuclides all have masses that slightly differ from a whole number. These mass deficits can be used to predict what