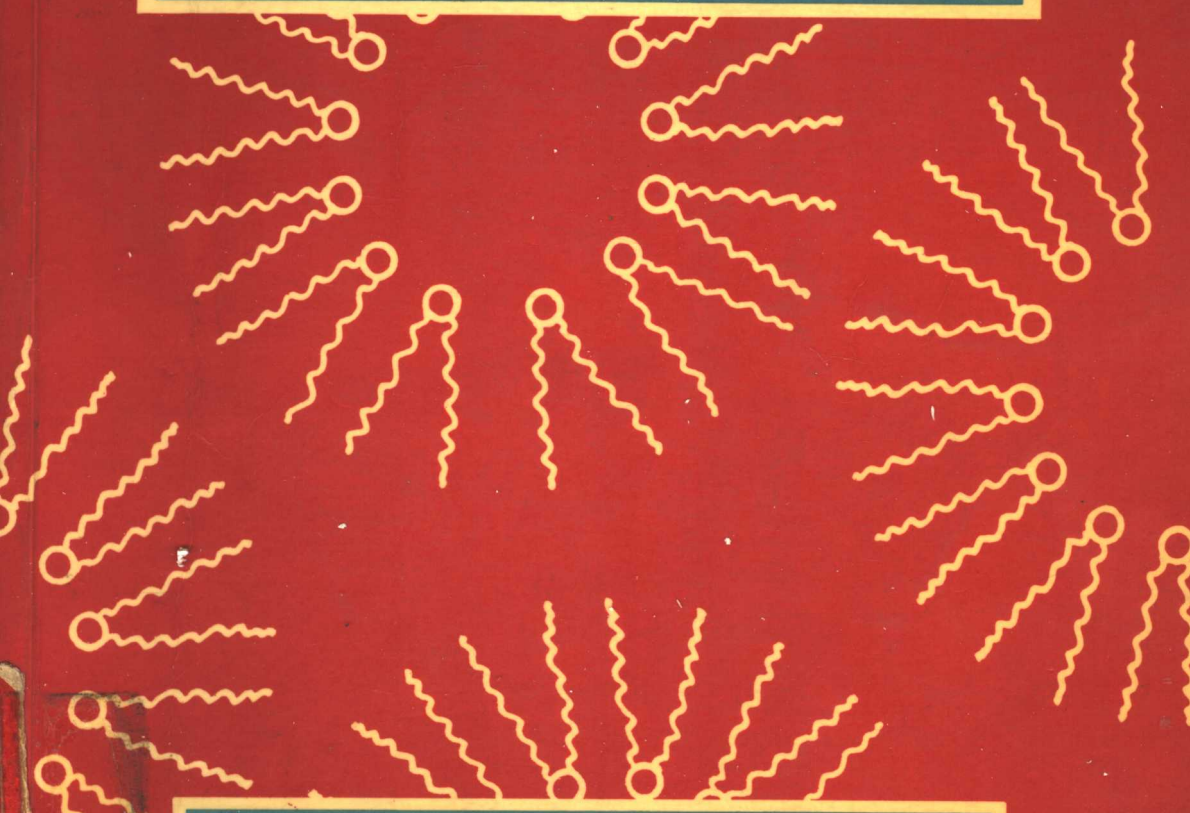
LIPID BIOCHEMISTRY

an introduction

M.I. GURR and A.T. JAMES

Third Edition



Science Paperbacks

Lipid Biochemistry:
An Introduction

M. I. GURR, B.Sc., Ph.D.
National Institute for Research in Dairying
Shinfield, Reading

A. T. JAMES, B.Sc., Ph.D.
Unilever Research Laboratory
Sharnbrook, Bedford

THIRD EDITION

1980
LONDON NEW YORK
CHAPMAN AND HALL
150TH ANNIVERSARY

Preface

In the preface to the Second edition, we made a prediction that many exciting developments would take place in the coming years that would change the face of a new edition. This has indeed been the case and the current edition reflects these new advances. Our picture of the structure of the fatty acid synthetase has changed dramatically, bringing a new concept in enzymology – the multicatalytic polypeptide chain. This new knowledge owes much to the exploitation of genetic mutants, the use of which is undoubtedly going to extend into many other areas of lipid biochemistry.

An understanding of the control of lipid metabolism has also advanced considerably during the last decade and we have tried to reflect that here, although it will be some years before a truly integrated picture can be obtained. For this reason we have continued to deal with the control of particular aspects of lipid metabolism – fatty acids, triacylglycerols, lipoproteins – in the specific chapters but we can foresee the time when a chapter on the overall integration of lipid metabolism will be appropriate and feasible. As a particular example, the exciting new concepts of the control of cholesterol metabolism in specific tissues *via* the interaction of low density lipoproteins with cell surface receptors have been described in Chapter 6.

Also included in this third edition are new concepts of the central role of the cyclic endoperoxide in prostaglandin metabolism and the newly discovered metabolites, prostacylins and thromboxanes, though the control of their production and their role *in vivo* remain speculative.

The final chapter has been renamed and largely rewritten. Part of the original material dealing with the control of triacylglycerol storage has been removed to a more appropriate section in Chapter 3, and replaced by an extended section on lipid metabolism in disease, and the new concepts of the role of fatty acids in immunity.

An important objective has been to maintain the compactness and conciseness of former editions and so as to accommodate new material we have discarded some sections that were either too speculative or too detailed. We hope that most of the errors that have been pointed out to us in the Second edition have been corrected without the introduction of too many new ones!

We are indebted to our many friends and colleagues throughout the world for their helpful criticisms, suggestions and comments. Our aims remain the same: firstly, to aid students in learning about lipids and staff in teaching a subject they may personally perhaps find dull; secondly, to influence students towards research in this area.

Finally, one of the greatest influences on our work and on lipid research in general has been Professor Konrad E. Bloch. In this, the year of his retirement, we are honoured to dedicate this book to him.

January 1980
Shinfield, Reading
Sharnbrook, Bedford

M.I.G.
A.T.J.

Acknowledgements

It is a pleasure to record our thanks to the many people who have helped in the preparation of this book. Our colleagues, Drs E. F. Annison, R. Bickerstaffe, H. van den Bosch, D. N. Brindley, R. H. Coleman, R. R. Dils, P. F. Dodds, G. A. Garton, G. M. Gray, J. L. Harwood, J. N. Hawthorne, C. H. S. Hitchcock, D. Howling, G. H. Hübscher, D. Husbands, R. Jeffcoat, V. Kamat, K. J. Kingsbury, R. B. Leslie, R. H. Michell, L. J. Morris, B. W. Nichols, J. McL. Philp, and H. S. A. Sherratt and Messrs D. Brett, S. Hall, P. Harris and M. P. Robinson are thanked for reading and commenting on different parts of the manuscript: their discussion has been invaluable, Miss C. Inchley and Mrs P. Benham are thanked for many arduous hours of typing and for deciphering our abysmal handwriting; Mrs E. A. Gurr and Mrs J. Geens for drawing some of the figures; Messrs D. Chance and H. Deakin and Miss L. Needham for preparing many photographs and diagrams. Permission to reproduce certain photographs and diagrams has kindly been given by Drs B. W. Nichols (Figs 1.2 and 1.3); Mr P. Harris (Fig. 1.9); Dr R. Jeffcoat and The Biochemical Society (Figs 2.1 and 2.7); Dr W. Stoffel and *Hoppe Seyler's Zeitschrift für Physiologische Chemie* (Fig. 2.17); Mr J. Edwards-Webb (Fig. 2.21); The Elsevier Publishing Co. (Fig 2.22); Dr L. J. Morris and John Wiley & Sons Ltd (Figs 2.23 and 3.6); Dr L. J. Morris and The Elsevier Publishing Co. (Fig. 2.24); Mr R. J. Taylor (Fig. 3.4); Mr R. Kenworthy (Fig. 3.12); Professor J. Senior and *The Journal of Lipid Research* (Figs 3.13 and 3.15); Mr C. Smith and Mr R. Kenworthy (Fig. 3.14); Dr V. Luzzati and *The Journal of Cell Biology* (Fig. 6.1), Dr R. B. Leslie (Fig. 6.4); Drs A. A. Benson, R. M. C. Dawson, D. E. Green, The American Oil Chemists Society, The National Academy of Sciences of the U.S.A, and Academic Press Inc. (Fig. 6.5a); Mr M. Stubbs (Fig. 6.6a); Dr D. E. Green and *The Journal of Cell Biology* (Fig. 6.6b); and Dr A. F. Henson (Fig. 6.6c); Professor O. Westphal and *Angewandte Chemie* (Fig. 6.7a); Dr L. Rothfield and the *Journal of Biological Chemistry* (Fig. 6.7b); Dr J. L. Strominger and The National Academy of Sciences of the U.S.A. (Fig. 6.8); Dr K. J. Kingsbury and St. Mary's Hospital (Fig. 7.2).

Contents

1 Lipids: what they are and how the biochemist deals with them

1.1 GENERAL INTRODUCTION

The word 'lipid' (in several different spellings) has long been used to denote a chemically heterogeneous group of substances, having in common the property of insolubility in water, but solubility in non-polar solvents such as chloroform, hydrocarbons or alcohols. Adequate coverage of the whole spectrum of such fat-soluble substances is beyond both the scope of so short a treatise and the capabilities of the authors. We shall therefore narrow our definition to include only those compounds which are esters of long chain fatty acids. Therefore large groups of biochemically interesting lipids such as the steroids and terpenes will not be covered, although our definition necessitates inclusion of, for example, the sterol esters.

Because, in our definition, the unifying feature is the long chain fatty acid, we will start by elucidating the various classes of naturally occurring long chain fatty acids (Chapter 2). The organic and physical chemistry of these compounds has been described in detail in a companion volume – F. D. Gunstone's 'An Introduction to the Chemistry and Biochemistry of Fatty Acids and their Glycerides' and chemical and structural aspects will be dealt with only in so far as it is necessary for an understanding of their biochemistry – anabolism and catabolism – which makes up the major part of Chapter 2. The tremendous upsurge of interest in lipids over the past two decades has been due mainly to the development of powerful new techniques for the separation, analysis and identification of these compounds whose immiscibility with water and rather similar physical properties had hitherto led to rather slow progress. We shall therefore end each chapter which concentrates on a distinct class of lipids with a section devoted to the most useful analytical methods, but devote the greater part of this present chapter to general chromatographic techniques.

From there, it is a natural step to describe the different classes of lipids derived from fatty acids: the so-called neutral lipids (non-polar esters of fatty acids with the alcohols, glycerol, cholesterol, vitamin A and other long chain alcohols, Chapter 3); the phospholipids (mixed-acid esters of fatty acids and phosphoric acid with glycerol or sphingosine, Chapter 4) and the glycolipids (a heterogeneous class in which each member contains a sugar moiety, Chapter 5).

Lipids rarely exist in an organism in the 'free' state but are more usually combined with proteins or carbohydrates as lipoproteins or lipopolysaccharides. These form the subject matter for Chapter 6. Lipids are important constituents of the food we eat: as providers of energy, as vitamins and for their contribution to the texture, flavour and palatability of food. These aspects are described in Chapter 7. Finally, we have included sections dealing with various diseases in which lipid metabolism is deranged – lipidoses, hyperlipoproteinaemias, heart disease, diabetes – in the appropriate chapters.

1.2 NOMENCLATURE AND STEREOCHEMISTRY

In any area of scientific investigation, the naming of compounds tends to develop rather haphazardly, each new substance being named according to the whim of its discoverer. Hence in fatty acid chemistry, many compounds have been named from the source of their extraction, for example, palmitic acid from palm oil. This results in a highly confusing and arbitrary system of jargon which puts great strain on the memory. Later there follows a period of rationalization when a logical system of chemical naming is substituted for the old trivial names. These chemical names often have the disadvantage of being rather long and cumbersome, and furthermore, if the naming committee is not truly international, more than one 'rational' system may develop side by side. As the authors have been involved in lipid research for a number of years, the use of trivial names has become second nature, and as they are much less cumbersome to write, we have tended to use them in this book. Therefore, when a certain compound is first introduced, its chemical name will be stated in parentheses after the trivial name; subsequently, the trivial name only will be used. Furthermore, the very convenient shorthand nomenclature for fatty acids, which has become very popular in the field of gas chromatography, will be used wherever possible (see Chapter 2, p. 18).

When a field has developed rapidly, substances which were originally thought to be pure, are later found to be mixtures of two or more rather similar components. Therefore a name which was once intended to denote a single compound eventually has to include a whole class of related substances. This difficulty arises in the case of the phospholipids where the term *Cephalin* includes both phosphatidyl ethanolamine, phosphatidyl serine and some other lipids. Confusion has arisen because some authors have used the term to mean simply phosphatidyl ethanolamine. The terms *Cephalin* and *Lecithin* (phosphatidyl choline) will be avoided in this book (except at the first mention of the compound), and the names phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl choline used consistently.

An important feature of the glycerophospholipids is their asymmetry at the central carbon atom of the glycerol. Thus, all naturally occurring phospholipids have the same stereochemical configuration, much in the same way as most natural amino acids are of the '*L*' series. Unfortunately, there has been much disagreement about the convention for naming the stereoisomers. In the past, the members of the natural series have been referred to as *L*-α-compounds and represented by the Fischer projection. In this case the glycerol derivative is put into the same category with that glyceraldehyde into which it would be transformed by oxidation, without any alteration or removal of substituents. As the glycerol hydroxyls

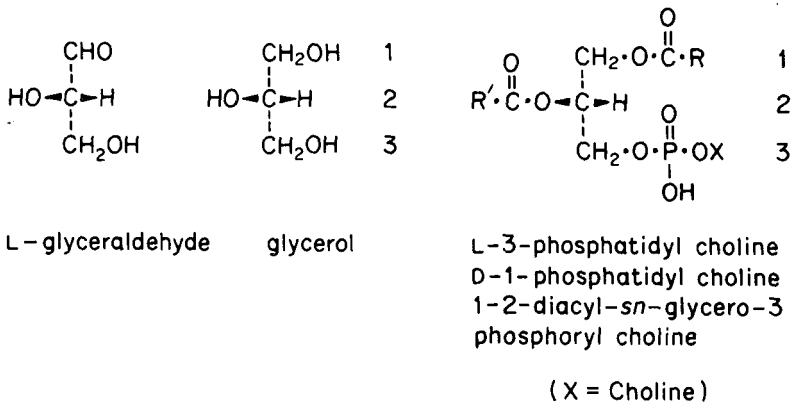


Fig. 1.1 The stereochemistry of phosphoglycerides

can be distinguished from each other, it is not clear from this nomenclature whether 'α' refers to the 1- or the 3-hydroxyl of glycerol; for this reason, *L*-3-phosphatidyl choline, for example, would be preferred (see Fig. 1.1). Others have used the equivalent alternative, *D*-1-phosphatidyl choline because it more closely followed the I.U.P.A.C.* convention that a compound be put into that optical series which gives its substituent the lower number. Another reason is that the natural diacylglycerols derived from phospholipids have almost universally been called *D*-αβ-diacylglycerols.

Recently, an international committee (the I.U.P.A.C.–I.U.B.† Commission on Biochemical Nomenclature) has recommended the abolition of the *DL* terminology and provided rules for the unambiguous numbering of the glycerol carbon atoms. Under this system phosphatidyl cholines become 1,2-diacyl-*sn*-glycero-3-phosphoryl cholines, or the shorter generic term would be: 3-*sn*-phosphatidyl choline. The letters *sn* stand for *stereochemical numbering* and indicate that this system is being used. The stereochemical numbering system is too cumbersome to use with regularity in a book of this type and therefore, normally we shall use the terms 'phosphatidyl choline' etc., but introduce the more precise name when necessary. A brief, but clear introduction to the subject of phospholipid stereochemistry can be found in reference 2.

While on the subject of stereochemistry, it is probably worth while to point out that the asymmetry of phospholipids leads to optical activity. Measurement of the *specific rotation* of a phospholipid is more useful to the organic chemist who is preparing stereochemically pure synthetic lipids than to the biochemist. What has often not been generally realized is the fact that many triacylglycerols also possess small but measurable rotations and this has provided a useful tool in the study of triacylglycerol structure.

*I.U.P.A.C.: International Union of Pure and Applied Chemistry.
†I.U.B.: International Union of Biochemistry.

Another field in which the nomenclature has grown up haphazardly and has recently been revised, is that of the enzymes of lipid metabolism. Quite often the trivial name of an enzyme gives no indication of the reaction catalysed, or there may be confusion between different names for the same enzyme. Nevertheless the Enzyme Commission (EC) nomenclature is very cumbersome for routine use and whenever the trivial name is sufficiently descriptive we will use it in this book but state the EC name at the first mention. Where confusion exists (as in the phospholipases, see Chapter 4) or the trivial name is not descriptive, then we shall use the EC name.

Biochemical knowledge has expanded so rapidly in recent years and the subject has become so diverse that it is becoming extremely difficult for a research worker to maintain an interest in all areas of the subject. The result is an increased tendency toward specialization. The student cannot afford to narrow his view to this extent and it is important to avoid regarding any one particular branch of biochemistry such as 'lipids' or 'sugars' as isolated disciplines, bearing no relationship with each other. Of course, all metabolic pathways are interrelated and in this book, we have tried to indicate the way in which reactions involving lipids are intimately connected with the whole functioning of an organism.

Until quite recently, most of the work on lipid metabolism had been done using a limited number of animal species – the rat has been a favourite source of material. Lipid metabolism of bacteria is now in vogue, but higher plants have had very little attention. We have tried not to narrow our attention to a few species but to choose examples from as wide a range as possible from both plant and animal kingdoms.

At the beginning, we stated that this is an introductory book for the student, not a research treatise. For this reason, we have preferred not to quote references in the text for each statement we have made, in the manner of a research paper or a review. At the end of each chapter will be a limited number of references to reviews which cover the appropriate topics in more detail and from which references to original and more specialized papers can be obtained. We also hope that the reader will select sections from different chapters to read in conjunction with each other, rather than reading the book in a 'cover to cover' manner. In a book of this size there is insufficient space for repetition and we hope that the system of cross referencing will be found adequate. As an example of what we mean, we might take the case of *sphingosine*. Although this base is an important constituent of some phospholipids, it will only be briefly mentioned in Chapter 4 but treated in more detail in Chapter 5.

1.3 ANALYTICAL TECHNIQUES

Natural lipids are complex mixtures of chemical species and of permutations of types of fatty acids esterified in each lipid class. This complexity hindered progress in lipid biochemistry because the normal chemical purification procedures of distillation, crystallisation, solvent extraction etc. were inefficient. Liberation, in the sense of ability to study single species, came only with the advent of chromatographic techniques, mainly in the years 1940 to 1960.

First catch your lipid

Before the different lipids can be resolved they must first be separated from all other

chemical types present. To do this, advantage is taken of their low water solubility and preference for water immiscible organic solvents, conferred by the long fatty side chains. Since membrane and plasma lipids are normally associated with proteins, solvents having some water solubility and hydrogen bonding ability are necessary to split the lipid-protein complex and even in some cases denature the protein (see Chapter 6). The most common extraction solvent is a mixture of chloroform and methanol (2:1 by volume). The tissue is best first broken up by use of an homogenizer or an ultrasonic probe, then extracted with the solvent and the organic layer removed. Addition of an equal volume of water will then cause a phase separation and the chloroform layer (containing the lipids) can be separated, washed with water, and dried over an absorbent such as anhydrous sodium sulphate or magnesium perchlorate. After filtering, the solvent can be vacuum evaporated to yield a crude lipid residue that should be protected from oxidation by a blanket of inert gas.

In plant tissue there is a danger of activating the lipases on extraction and so splitting many of the lipids to give free fatty acids. This is best prevented by a prior extraction with *iso*-propanol which inactivates the lipases.

Crude lipid separations can be done by selective extraction.

For some experiments it is sufficient to have a crude resolution of phospholipid and neutral lipid, and this can be achieved by extracting the dry lipid with cold dry acetone, when neutral lipids dissolve and most of the phospholipids remain behind. There is, however, still some carry-over of polar lipid into the neutral lipid.

Where only the mixed fatty acids are required the whole tissue or lipid extract can be hydrolysed by dilute aqueous or methanolic potassium hydroxide. The alkaline solution if extracted with light petroleum will yield a non-saponifiable fraction (e.g. sterols) and by extraction with ether after acidification the fatty acids themselves are obtained.

Exploitation of chromatography.

Unambiguous separation of lipid classes, molecular species of each class and individual fatty acids can be obtained only by chromatographic techniques. One of the great advantages of chromatography lies in its ability to handle very small amounts of material; indeed the limit is set only by the sensitivity of their detection. Fractional distillation on the other hand has to have enough material to wet the column packing and is difficult with less than about five grams of substance unless carrier distillation is used. Furthermore the high efficiency of a chromatogram enables molecules to be separated that possess only small differences in structure. The mechanism will be made clear later.

The principles of chromatography are based on distribution between two phases, one moving, the other stationary.

A chromatogram (so-named by its Polish inventor, Tswett, because he used the technique to separate plant pigments) consists of two immiscible phases (see Table 1.1). One phase is kept stationary either by being held on an inert microporous support or being itself a microporous or particulate adsorbent solid: the other phase is percolated continuously through the stationary phase. The phase pairs that can be used are as

follows (the mobile or moving phase being given first), together with their inventors and developers.

1. Liquid–solid (Tswett, 1911, Poland; Kuhn and Lederer, 1931, Germany)
2. Liquid–liquid (Martin and Synge, 1941, U.K.)
3. Liquid–charged gel (Moore and Stein, 1946, U.S.A.; Partridge, U.K.)
4. Liquid–uncharged gel (Boldingh, 1953, The Netherlands; Porath, 1959, Sweden; Determan, 1966, Germany)
5. Gas–solid (Claesson, 1946, Sweden; Phillips, 1949, U.K.; Turkeltaub, 1950, U.S.S.R.)
6. Gas–liquid (James and Martin, 1952, U.K.)

The liquid systems are capable of being used for all substances irrespective of molecular weight, whereas gas–liquid and gas–solid chromatograms can be used only for substances of molecular weight of up to around 700–800.

If we take any single substance and mix it with any of these phase pairs, it will distribute itself between the two phases, the ratio of the concentrations in the two phases (at equilibrium) being known as the partition coefficient.

The partition coefficient is a physical constant dependent on the nature and magnitude of solute-solvent interactions in the two phases. Let us consider two substances A and B and imagine that at equilibrium, substance A distributes itself between the two phases so that 90% is in the stationary phase and 10% in the moving phase. Substance B, however, distributes so that 10% is in the stationary phase and 90% in the moving phase. Then a mixture of A and B dissolved in a small volume of moving phase and applied to the chromatogram will begin to separate when the moving phase is added and washes them through the system. They will distribute themselves independently of one another: B will move as a zone at 9/10ths of the velocity of the moving phase and A at 1/10th of the velocity. Clearly the two substances will rapidly move apart, the original rectangular profile of the zones changing to the shape (ideally) of a Gaussian error curve because of diffusion. The substances can either be visualized *directly on the system* by colour sprays or can be *eluted* as pure components.

The two phases can be arranged in a variety of ways.

There are basically only two types of chromatogram geometry. (1) *The column* consists of a metal, glass or even plastic tube with a ratio of length to diameter of at least 10:1 and packed with either an adsorbent solid (silica gel, alumina etc.) or an inert solid, such as kieselguhr, of large surface area, that can hold by surface tension, a liquid as one member of the phase pair. Gas chromatograms can afford a much greater ratio of length to diameter because of their inherent lower resistance to flow than can liquid columns. The surface of kieselguhr normally wets with the more aqueous of a phase but this can be reversed by making its surface water-repellent by chemical treatment so that it will hold the least polar of the liquids, hence the term *reversed phase chromatography*. This technique was introduced by Howard and Martin in the U.K. specifically to separate long chain fatty acids, since their partition coefficients were so much in favour of the least polar of a phase pair, that if this were the moving phase, they moved too quickly through the column and hence showed inadequate resolution.

(2) *The plate or strip* consists of the stationary phase support arranged as a flat surface. Mixtures can be spotted and dried on the surface and when the bottom of the plate is immersed in the moving phase in a closed vessel, capillarity ensures that the liquid will move through the porous material (paper, or a porous solid held to a glass or metal surface). The fact that both phases exist as relatively thin films means that the solutes have only short distances to move as they pass from phase to phase. Very refined separations can thus be obtained rapidly. The thin layer chromatogram is particularly useful for lipid separations. The plates, which are usually of glass, are prepared in the following manner:

The plate, carefully cleaned so as to be grease-free, is laid on a horizontal surface and is coated evenly with an aqueous slurry of a suitable powdered adsorbent, usually silica gel. The adsorbent sometimes has a binder, such as calcium sulphate, added so as to increase the mechanical strength of the layer. The plate is then heated in an oven at a fixed temperature and for a fixed time so as to 'activate' the adsorbent. 'Activation' is the process of removing water from the gel: the lower the water content the higher the adsorption. The plates can then be stored either in airtight containers or at a fixed relative humidity.

In all types of chromatogram, complexing agents can be added to one or other phase to change the distribution coefficient in favour of that phase by complexing with specific chemical types e.g. borate ions for complexing with *cis*-hydroxyls, silver ions for complexing with double bonds (see Chapter 2, Figs. 2.23, 2.24).

In general, compounds are not eluted from flat plate chromatograms: instead, the development (i.e. the movement of the liquid phase) is stopped when the front has reached the end of the strip, the strip is dried to remove solvent and the position of the zones revealed by spraying. The sprays can be of a destructive type such as dilute sulphuric acid followed by heating (this produces black spots by carbonisation where there is an organic material) (Fig. 1.2) or a non-destructive type such as dichloro- or dibromo-fluoresceins that show a changed fluorescence where there is a zone. In the latter case the compounds can be recovered by scraping the adsorbent from the plate where the spray indicates a zone to be present, followed by extraction with a suitable solvent. Under standard conditions it will be found that a given substance will move relative to a standard substance to a constant ratio or *relative* R_F (See Fig. 1.2). This *relative* R_F is a useful confirmation of structure of an unknown substance but it is unwise to use it as an absolute indicator.

Only infrequently can every component of a complex mixture be resolved with one solvent system. However, by using two-dimensional development, i.e. first by one solvent system in one direction, then after drying the plate by running a different solvent at right angles, refined separations can be achieved. This is demonstrated in Fig. 1.3 for separation of plant lipids. The volatile solvent (containing ammonia) should be run first as it is difficult to remove the last traces of acetic acid. This lessens the effectiveness of the basic solvent and results in poor separations.

Where the zones are radioactive they can be detected by passing the plate under a windowless proportional counter masked by a thin slit. A typical trace is shown in Fig. 1.4. The pure substances can then be isolated by extraction after removing the appropriate area of adsorbent from the plate.

Table 1.1 Types of chromatogram used for lipid separations

Phase pair	*Geometry*	*Stationary phase*	*Moving phase*	*Separation*
Liquid/liquid	Column	Hydrocarbon	Aqueous-organic solvents	Long chain fatty acids
	Column	Aqueous buffer	Ether	Short chain fatty acids
	Thin layer or paper strip	Hydrocarbon or silicone	Aqueous acetone	Long chain fatty acids Neutral lipids
Liquid/uncharged gel	Column	Swollen gel (lipophilic sephadex)	Aqueous organic solvents	Neutral lipids
Liquid/ionized gel	Column	Swollen gel (diethylamino-cellulose)	Chloroform, chloroform–methanol, chloroform–methanol–ammonia	Separation of neutral lipids from basic and acidic charged lipids (phospholipids/sulpholipids)
Liquid/solid	Column	Silica gel or modified silica gel (e.g. silver nitrate-impregnated)	Range of solvents	All types of lipids
	Paper strip	Silica gel or modified silica gel (e.g. silver nitrate-impregnated)	Range of solvents	All types of lipids
	Thin layer	Silica gel or modified silica gel (e.g. silver nitrate-impregnated)	Range of solvents	All types of lipids
Gas/liquid	Column	High molecular weight hydrocarbons, silicone greases, polyesters	Permanent gas	Fatty acids of all chain lengths, glycerides of all types

The quantitation of compounds on thin layer plates has been extremely difficult to achieve in a one-step process. Densitometry, for example, is potentially useful but it is difficult to achieve a linear response and the results are unreliable. The problem has been partly solved by performing the chromatography not on a *plate* but on a silica rod. The rods are very heat resistant and can be passed through a flame to oxidize the organic compounds to CO_2 and water. The effluent gases can be detected quantitatively by passing them through a flame-ionization detector such as is used in gas–liquid chromatography (see Section 1.3.1). The resultant recording is shown in Fig. 1.5. This method still needs further development to be reliable enough for routine work but has great potential.

1.3.1 Gas–liquid chromatography

In this technique the moving phase is a permanent gas and the columns are either packed with *Celite*, on whose surface is the

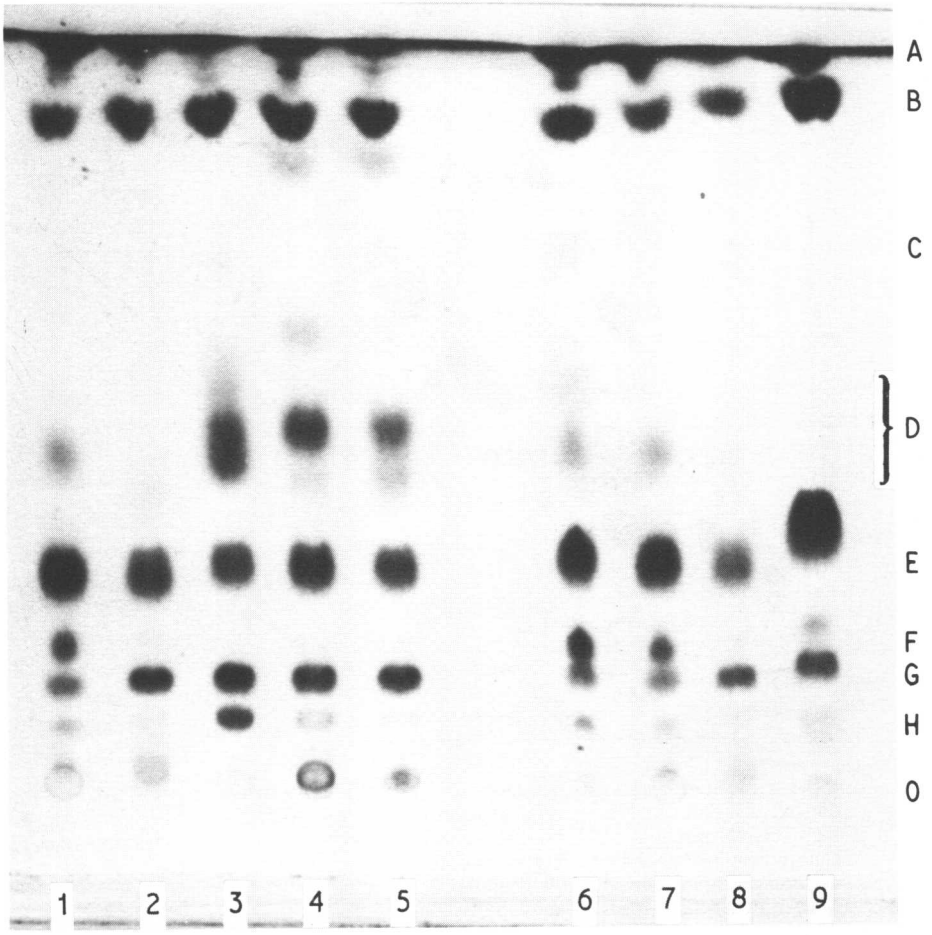


Fig. 1.2 Thin layer chromatogram of plant lipids.
Developed in chloroform-methanol-acetic acid-water (85:15:10:4, by vol.), on silica gel G. Compounds detected by spraying with 50% sulphuric acid and charring. The source of the lipids is as follows:

1. *Chlorella vulgaris*. 2. *Anacystis nidulans*. 3–5. Nitrogen-fixing blue-green algae. 6. Spinach leaves. 7. *Chlorella vulgaris*. 8. *Anacystis nidulans*. 9. Spinach chloroplasts. Identification of the lipids: A. Neutral lipids. B. Monogalactosyl diacylglycerol. C. Sterol glycoside. D. In 1, 6 and 7, Phosphatidyl ethanolamine. In 3–5, A mixture of glycosides of a fatty alcohol. E. Digalactosyl diacylglycerol + phosphatidyl glycerol. F. Phosphatidyl choline. G. Sulphoquinovosyl diacylglycerol. H. In 1, 6 and 7, Phosphatidyl inositol. In remainder: unknown. O. Origin of application.
The R_f of, for example, spot B relative to spot C is the ratio of the distances of the centres of spots B and C from the origin O. This depends only on the solvent, the nature of B and C, and the temperature.

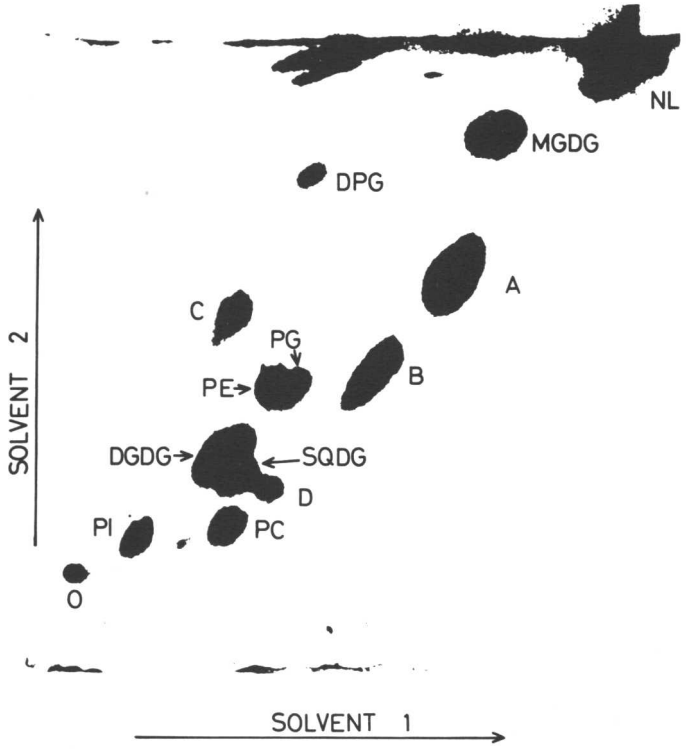


Fig. 1.3 Separation of a complex mixture of plant lipids by two-dimensional thin layer chromatography. Solvent 1: Chloroform-methanol-7N Ammonia, (65:25:4, by vol.). Solvent 2: Chloroform-methanol-acetic acid-water (170:15:15:2, by vol.). O: origin of application; PI: phosphatidyl inositol; PC: phosphatidyl choline; DGDG: digalactosyl diacylglycerol; SQDG: sulphoquinovosyl diacylglycerol; PE: phosphatidyl ethanolamine; PG: phosphatidyl glycerol; DPG: diphosphatidyl glycerol; MGDG: monogalactosyl diacylglycerol; NL: neutral lipids; A, B, C, and D: unknown. Compounds detected by spraying with 50% H_2SO_4 and charring.