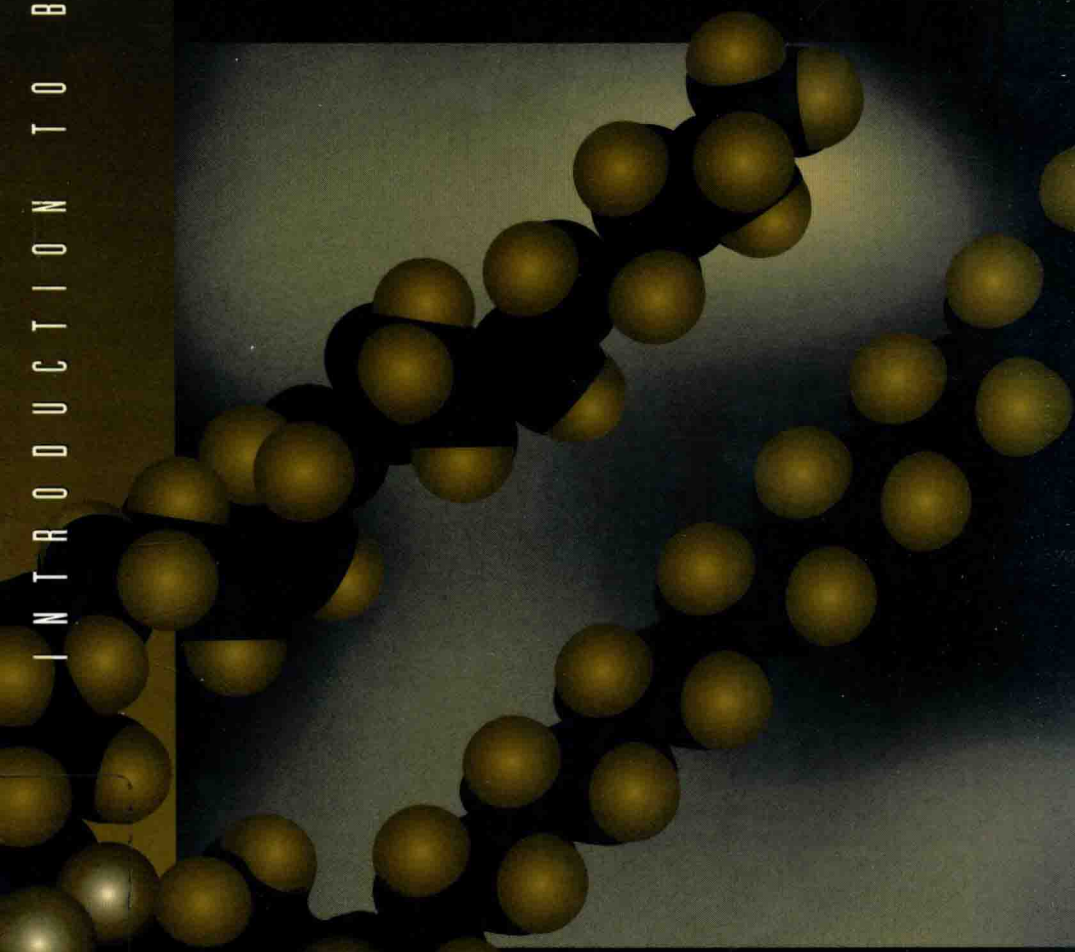
INTRODUCTION TO BIOTECHNIQUES

Lipid Analysis

F.W. HEMMING and J.N. HAWTHORNE



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LIPID ANALYSIS

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Abbreviations

ACDP	Advisory Committee on Dangerous Pathogens
COSHH	Control of Substances Hazardous to Health
CM	chylomicrons
DEAE	diethylaminoethyl
ECD	electron capture detector
ELISA	enzyme-linked immunosorbent assay
FID	flame ionization detector
GC	gas chromatography
GC–MS	gas chromatography–mass spectrometry
GLC	gas–liquid chromatography
GM	Geiger–Müller
HDL	high density lipoproteins
HETP	height equivalent to a theoretical plate
HIV	human immunodeficiency virus
HMIP	HM Inspectorate of Pollution
HPAEC	high-performance anionic exchange chromatography
HPLC	high-performance liquid chromatography
i.d.	internal diameter
IDL	intermediate density lipoproteins
IR	infrared
IRMA	immunoradiometric assay
LC	liquid chromatography
LDL	low density lipoproteins
LSC	liquid scintillation counting
MAb	monoclonal antibodies
MS	mass spectometry
NMR	nuclear magnetic resonance
PBS	phosphate-buffered saline
PG	prostaglandin
PI	phosphatidylinositol
PM	photomultiplier
POPOP	1,4-bis-2-(5-phenyloxazolyl)-benzene
PPO	2,5-diphenyloxazole
RIA	radioimmunoassay
RPO	Radiation Protection Officers
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SI	Système International
TLC	thin-layer chromatography
VLDL	very low density lipoproteins

Preface

In following our research interests in lipid biochemistry, we have been impressed not only by the wide range of lipid-soluble compounds found in living systems but also by the great variety of biological phenomena in which lipid-soluble compounds play an important role.

At its simplest, this is seen in the passive, protective hydrophobic barrier of waxes found in many insects and plant leaves or in the energy store of triacylglycerols found in many eukaryotic cells. All biological membranes are composed primarily of phospholipids which may fulfill several functions. They provide a permeability barrier, so important in compartmentation of eukaryotic cells, but also provide a lipid environment within which many proteins involved in cell- (and organelle-) surface phenomena function. The exciting discovery of the second messenger activity of inositol phosphates and of diacylglycerol drew attention to the critical part played by phosphatidylinositol in the transduction of messages across the plasma membrane of animal cells. A different activity is shown by the glycolipids of the plasma membrane in the form of a key function in cell recognition phenomena important, for example, in embryonic development and in malignancy.

Inside the cell, several lipids are anchored in membranes by polyisoprenoid chains in a manner appropriate to their role in electron transport (e.g. the quinones) or in protein glycosylation (the dolichols). However, not all biologically active lipids are membrane components and some, such as steroid hormones, prostaglandins and pheromones, can in trace quantities stimulate large biochemical and physiological changes.

This book deals with the basic aspects of the analytical techniques needed to investigate the complexity of lipids in living cells. On the one hand, it is intended to provide sufficient information to understand the experimental basis of and to assess observations already published. On the other hand, it aims to equip the lipid researcher with a background to techniques appropriate for any experimental studies planned.

We have deliberately not provided detailed protocols. These can be ephemeral and are probably best prepared by the individual based on the primary scientific literature or books on specific methodologies and bearing in mind local circumstances.

It is anticipated that the book will be of most use to advanced undergraduates or junior research workers setting out to get to grips with the analysis of a lipid. It is hoped that the arrangement of chapters will be 'user friendly', particularly for those with special interests in the analysis of one specific group of lipids or in the applications of one particular technique.

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1 Introduction

Recognition of the diversity and biological importance of lipids or of lipid modifications of other compounds (e.g. of proteins) continues to increase. In addition, variations in concentration of some lipids of animal tissues have important consequences for the health of the animal. This book aims to explain and discuss the principles of the methods used currently in the analysis of lipids to assist those wishing to understand or plan experiments in this area.

The term lipid is used broadly to describe compounds of biological origin that will partition into an organic solvent that is immiscible with water. Analysis is assumed to involve recognition, isolation and quantitative assay of a compound or group of compounds. Much of this book, therefore, consists of a discussion of the most suitable methods used in elucidating these aspects of analysis for each of the major groups of lipids.

Lipid-soluble compounds are grouped on a chemical basis. This has led to chapters on hydrocarbons, alcohols (plus phenols, quinols, quinones and aldehydes), fatty acids, esters, phospholipids and glycolipids. The final chapter deals with lipoproteins which contain several of these groups of compounds and present special analytical challenges. Since the analysis of several of these compounds involves the application of common techniques, it has proved convenient to precede the compound-based chapters with one dealing in general terms with the techniques used in lipid analysis. The successful analyst will ensure not only that he/she thoroughly understands the basis, and strengths and weaknesses of the methodology described in Chapter 2, but also is well versed with regard to the manufacturer's instructions for any equipment that is to be used.

Armed with this information, it is wise to prepare an analytical protocol and to attempt to anticipate particular needs or potential problems that the protocol may present. This book deliberately avoids providing protocols but recommends that these be written by the analyst with the aid of a detailed techniques text or original paper describing the analysis and taking account of the local situation.

Chapters 3–9 summarize much of the general information pertinent to preparing an appropriate protocol for individual compounds or groups of compounds.

Each of Chapters 3–9 opens with a brief account of the biological significance of the group of lipids under consideration. This biological context helps in assessment of the types of analytical questions that may need to be answered.

Analytical methods involve the application of physical and chemical techniques to complex mixtures. Clearly, the analyst will be best able to develop new methods, to optimize existing methods or simply to apply protocols derived from others if he/she has at least a basic appreciation of the chemical structures and properties of the compounds to be analyzed. For this reason, each of Chapters 3–9 includes a section dealing with this aspect. In describing the structures, internationally agreed nomenclature based on the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) and of the International Union of Biochemistry (IUB) has been used.

This section is followed by essentially qualitative aspects of analysis (detection, separation, purification) before discussing quantitation of particular lipids. This is not to relegate quantitative data but simply recognizes that some aspects of qualitative analysis may be essential features of quantitation. In fact, it is important to appreciate that most analyses should be aimed at providing good quantitative data. In order to be widely interpretable, this data should be expressed in SI units (recommended by the Système International d'Unités) with sufficient information to allow judgement of the reliability and reproducibility of the results. To this end, the protocol used in the determination should be sufficiently clear to allow someone else to repeat the work. Random experimental errors should be assessed and reported by determining the mean and standard deviation of a reasonable number of replicable determinations. Systematic errors cannot be treated in this statistical way. However, attempts should be made to assess them, for example, by adding known amounts of internal standards during the analysis and setting up appropriate control experiments. If systematic errors cannot be eliminated they should be quantitated and taken into account. For example, if an internal standard is routinely being determined at 75% of the quantity added, it is reasonable to report the content of that analyte as 133% (100/75) of that actually measured.

The analyst will also need to be aware of the sensitivity and specificity of an assay. It is reassuring to learn that analytical data reported are

well within the sensitivity of the method used and that they are unlikely to be compromised by the presence of other compounds. In attempting to maximize one of these aspects, it is possible that sacrifices will have to be made with regard to the other.

All quantitative analytical methods should be supported by good calibration data, preferably calibration curves covering the range of analyte concentrations being reported. Having taken this and other precautions emphasized above, it is incumbent upon the analyst to judge realistically the precision of the determination being reported. Stating data to the fifth figure (e.g. 15.473 mg g^{-1} should be avoided if the assay has a precision of ±1%. A more realistic statement would be 15.5 mg g^{-1}.

Finally, when reporting the results of lipid analysis, presentation of data and conclusions are all important. Currently available software for computers and wordprocessors assists greatly in this. Graphs, pie charts and histograms often get the essential message of comparative assays across to others more readily than do tables of numbers, especially if used as a visual aid to an oral report. Flow sheets may be a more accessible presentation of experimental protocol than text. It is essential for the analyst to consider the potential audience or readership of his/her analytical report and select an appropriate form of presentation.