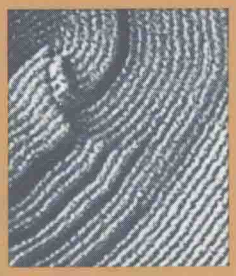

Biochemistry of Lipids and Membranes

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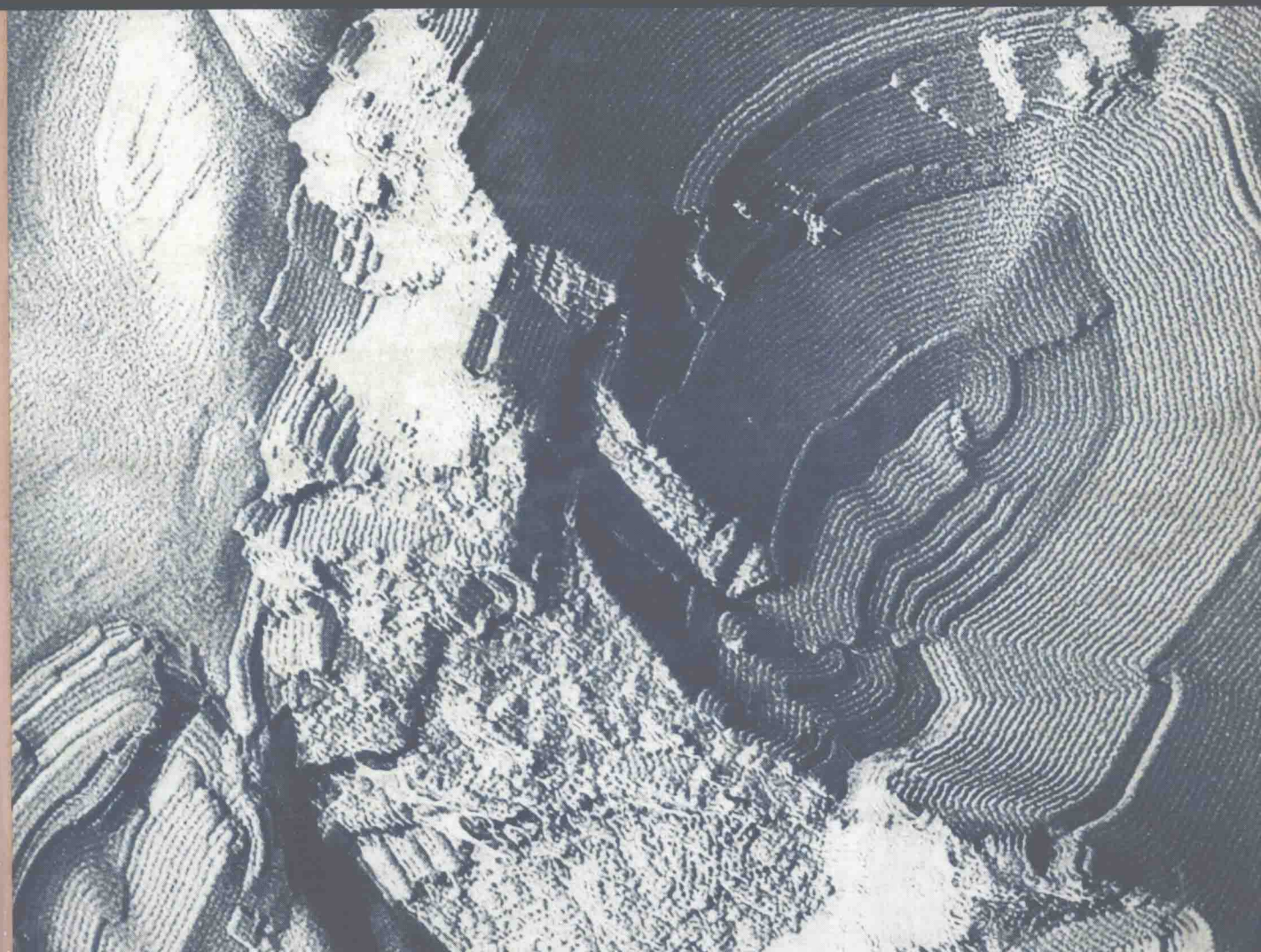

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For Konrad and Lore Bloch,
with thanks from several generations of lipid biochemists

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Preface

This book has two major objectives. One is to provide an advanced textbook in the field of lipid and membrane biochemistry. The second is to provide a clear summary of the field for scientists engaged in research in the area of lipids and membranes and related fields.

Biochemistry has matured to the point that advanced textbooks in the various subcategories are required. This book should satisfy that need for the field of lipid and membrane biochemistry. The chapters are written for students who have taken an introductory course in biochemistry. We assume the students are familiar with the basic principles and concepts of biochemistry and have a general background in lipid and membrane biochemistry.

The second objective relates to the need for a general reference and review book for scientists in the lipid and membrane field. Such a book does not presently exist. Certainly there are many excellent reviews available of the various topics covered by this book, and these reviews are cited in the appropriate chapters. The availability of a current, readable, and critical summary of the biochemistry of lipids and membranes should fill an important gap in scientists' libraries. The literature in the field is vast, and there are usually many constraints on researchers' time. This book should allow these scientists to become more familiar with other areas of lipid metabolism related to their research interests. Finally, this book should help clinical researchers keep abreast of developments in basic science that are important for subsequent clinical advances.

The first chapter was written by Konrad Bloch, who for 50 years has made very important contributions to the lipid and membrane field. His chapter differs from the other chapters in that he summarizes the advances of his major research topics during the past 10

years—the evolutionary and structural functions of cholesterol in membranes. It is a lively and thought-provoking contribution.

The second chapter, Physical Properties and Functional Roles of Lipids in Membranes, introduces advanced information on membrane lipids. A major theme of the chapter reminds us that membrane lipids do more than separate aqueous compartments in cells. The following chapters provide current information on the biochemistry of fatty acids, phospholipids, triacylglycerols, sphingolipids, eicosanoids, cholesterol, and lipoproteins. The book concludes with two chapters that summarize the rapidly developing areas of assembly of lipids and proteins into membranes.

The book does not attempt to cover the general area of structure and function of biological membranes, since that subject has already been covered in a large number of excellent books. Second, the addition of such material would greatly increase the length, and therefore the cost, of this book.

The naming of lipids and enzymes in the book generally adheres to the rules of IUPAC-IUB. For further information on the nomenclature of lipids, please see *Biochemical Journal* 171 (1978): 21–35. A new edition of *Enzyme Nomenclature* has recently been published by Academic Press.

The editors and contributors assume full responsibility for the content of the various chapters. We would be pleased to receive comments and suggestions about this book.

Finally, the editors and contributors are indebted to the many other people who have made this book possible. In particular, we extend our thanks to Judith Smith, Teresa Vollmer, Theresa Fillwoch, Tommyz Campbell, Dawn Oare, Patricia Knight, and Perry d'Obrenan. We also thank the following colleagues who have read parts of the book and made useful suggestions: Dave Severson, Subhash Basu, Carlos Hirschberg, Matt Spence, and Günter Blobel.

Dennis and Jean Vance
Vancouver, Canada
July 1984

Contents

CHAPTER 1

Cholesterol: Evolution of Structure and Function

Konrad Bloch

NATURAL OCCURRENCE OF STEROLS

Biochemical unity has been a dominant concept for several decades. Nucleic acids, proteins, carbohydrates, and phospholipids of the same general structure are shared by all forms of life. The genetic code is universal. Darwinian evolution, the common descent of organisms, is manifest at the chemical level. Superimposed on unity, biochemical diversity is phenotypically expressed, at least in part, by organic molecules that are not ubiquitous: they are found in or needed by some cells but not others. Hormones, pigments, sterols, and many other substances concerned with specialized function belong to this category. Cholesterol and the structurally related sterols of fungi and plants are, as far as we know, not universal. We can therefore state with certainty that the sterol structure is not essential for the life process per se. In a more speculative vein, we can say that sterols arrived late in the evolution of organisms. The appearance of oxygen in the biosphere was essential for the biosynthetic pathway of sterols to develop.

Sterols are common in eucaryotic cells but rare in procaryotes. Vertebrates without exception synthesize cholesterol; in no instance is the pathway deleted or incomplete. Most invertebrates, lacking the enzymatic machinery for sterol synthesis, rely on an outside sterol supply. This generalization, valid until recently, may need to be qualified. Drosophila cell lines appear to be viable and exist without measurable endogenous or exogenous sterol (Silberkang et al. 1983). Yeasts and fungi, again with some apparent exceptions, harbor

side-chain-alkylated sterols. While in photosynthetic organisms sitosterol and stigmasterol (see Figure 1.2 for structure) are the most abundant and widely distributed sterols, cholesterol and ergosterol are by no means absent. The classical distinction between animal and plant sterols no longer corresponds to reality.

The occasional presence of sterols in procaryotes is of unknown functional significance. Substantial sterol synthesis (4-methyl sterols) is known with certainty to occur in *Methylococcus capsulatus*, an aerobic methanotroph (Bird et al. 1971). In another well-documented case, the isolation of Δ^8-cholestenol from a myxobacterium has recently been reported (Kohl, Gloe, and Reichenbach 1983). Claims for the presence of sterol traces in other procaryotes need to be substantiated. At any rate, for the vast majority of bacteria and blue-green algae, sterol is not a required molecule. Notable and special cases are the grossly heterotrophic sterol-requiring *Mycoplasma* species, which normally parasitize animal or plant tissues (Edward and Fitzgerald 1951). In a few instances squalene-derived molecules other than those containing the typical tetracyclic steriod nucleus appear to be functionally equivalent to sterol, for example, pentacyclic triterpenes (see the following discussion).

METABOLIC AND PRECURSOR FUNCTIONS OF THE STEROL MOLECULE

Perhaps not surprisingly, the more advanced the organism, the more diverse the role of the sterol molecule. That cholesterol is the essential precursor for bile acids, corticoids, sex hormones, and vitamin D–derived hormones is well established for all vertebrates. These transformations (Figure 1.1) involve partial shortening or complete elimination of the isooctyl side chain as well as a wide variety of ring hydroxylations. Oxygen is an essential reagent for both side-chain and nuclear modifications catalyzed by highly specific mixed-function oxygenases, which often, but not invariably, involve cytochrome P_{450}. It is important to note that in all these transformations but one (vitamin D) the C_{19} carbocyclic ring system remains intact. Changes of the ring conformation from all trans (planar) to A/B cis occur only in the formation of ecdysone, an insect hormone, and in bile acid formation. The position of oxygen functions and the length of the truncated side chain determine hormone specificity.

In invertebrates, with their primitive endocrine system, transformations of diet-derived sterol are apparently restricted to hydroxylations and transformation from A/B trans to cis of the otherwise intact C_{27} sterol structure (ecdysone, see Figure 1.1). Side-chain shortening does not seem to occur. However, as an interesting example of environmental adaptation, certain insects have evolved a mechanism for converting nonanimal sterols to nutritionally competent cholesterol derivatives by removing C-24 alkyl groups from the

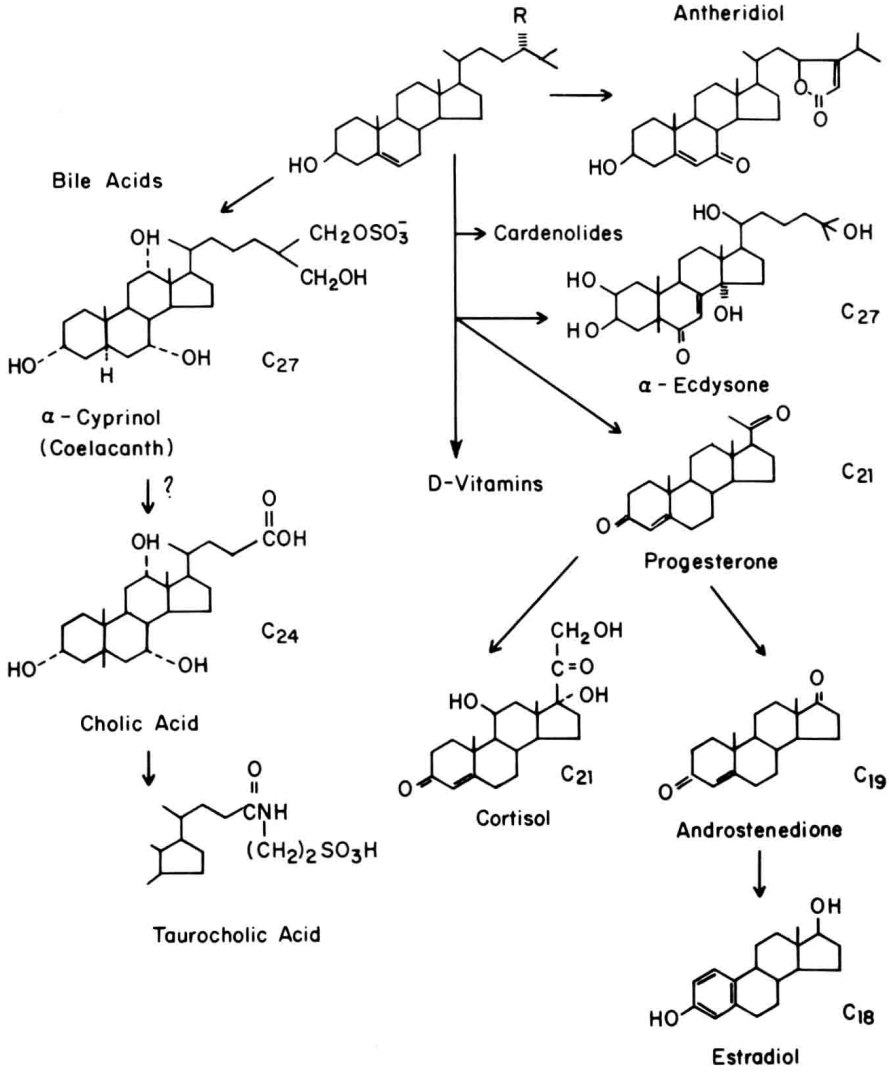

Figure 1.1. Functional evolution of the sterol molecule.

side chain. Thus the omnivorous cockroach dealkylates ergosterol or C_{29} plant sterols to 22-dehydrocholesterol, while *Dermestes vulpinus*, an obligate carnivore, lacks—because it does not need—the requisite dealkylating enzymes (Clark and Bloch 1959).

An extensive examination of marine invertebrates (sponges, gorgonians) has uncovered a bewildering variety of side-chain-modified sterols. Structures bearing additional alkyl or cyclopropane groups at six of the eight isooctyl sterol side-chain positions have been identified (Djerassi et al. 1979). It has been suggested, and to some extent documented, that the phospholipids of such marine organisms

contain unique structural features complementary to the side-chain-alkylated sterols.

In lieu of the mammalian bile acids of the cholic acid type, some crustaceans and perhaps also other invertebrates, elaborate aliphatic aminosulfonates as intestinal emulsifiers, presumably for aiding triacylglycerol absorption (van den Oord, Danielsson, and Ryhage 1965).

Metabolites of ergosterol, the prototypical sterol of yeast and fungi, have not been found or adequately characterized. We may conclude that in unicellular eucaryotes only unmodified sterol molecules play an essential or beneficial role.

The C_{24} side-chain-alkylated plant sterols sitosterol and stigmasterol do not appear to undergo functionally essential conversions involving the loss of ring skeletal carbon atoms. Surprisingly, however, numerous plant families produce ecdysone, either identical with the cholesterol-derived invertebrate molting hormones or variants thereof, in quantities exceeding those found in insects by up to five orders of magnitude or more. Plant-feeding insects therefore have the choice of deriving these hormones directly from their diet or by converting the sterols they ingest. The cardioactive digitalis glycosides (cardenolides and bufalins) formed from sterols in *Digitalis* and *Strophanthus* species are probably secondary metabolites, whose physiological function in the organism of origin is unknown.

Physiologically useful modifications of the sterol structure have not been described in the few bacterial sterol producers or sterol auxotrophs. Information on the role of sterols in procaryotes exists only for the wall-less mycoplasmas (see the following discussion).

STEROL PATTERNS

In some animal tissues (liver and brain) cholesterol comprises more than 95% of the sterol fraction. Cholesterol precursors (lanosterol and partially dealkylated lanosterol derivatives) account for the remainder. In some cells, for example, lymphocytes, the concentration of these intermediates may be substantial (Burns et al. 1982). Sterol absorption from the mammalian gastrointestinal tract appears to be specific for cholesterol and its precursors, regardless of diet. Fungal and plant sterols are effectively excluded except in rare hereditary disorders. This remarkable discrimination, which accounts for the homogeneity of tissue sterols, appears to be especially pronounced for the absorption process mediated by the brush border membranes. However, animal cells in culture, for instance, Chinese hamster ovary cells, readily take up plant sterols from the medium and may indeed metabolize them. By contrast marine invertebrates, whether or not they are sterol auxotrophs, appear to discriminate much less against dietary sterols, with the result that their tissue sterol compositions show varying degrees of complexity.

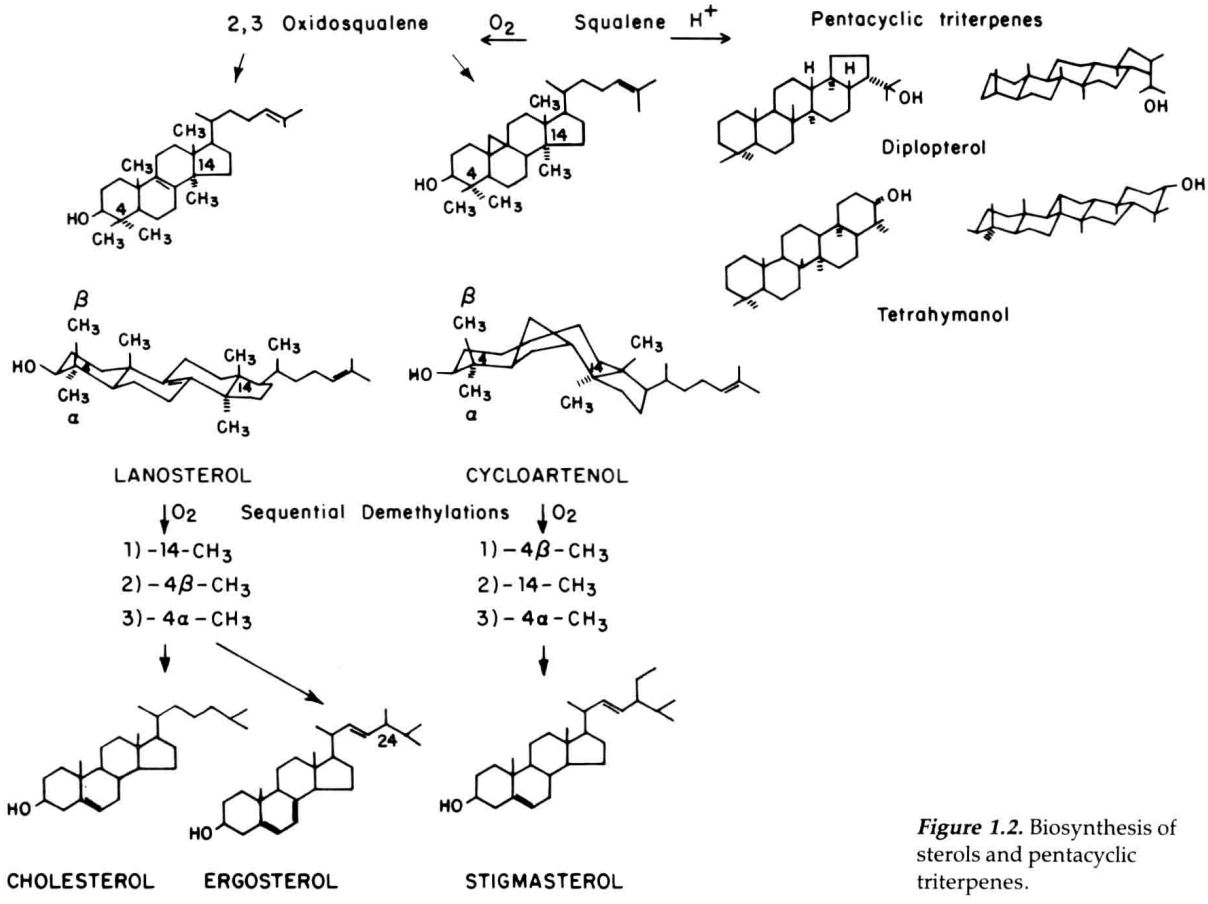

Figure 1.2. Biosynthesis of sterols and pentacyclic triterpenes.

Bewildering sterol mixtures are found in most lower and higher plants. While the C_{24}-ethyl sterols predominate, especially in plant leaves, sterols conventionally regarded as either typical for animals (cholesterol) or yeasts and fungi (ergosterol) (see Figure 1.2 for structure) are often present in substantial amounts. Especially striking is the fact that about a dozen species of red algae contain cholesterol exclusively. The argument made for invertebrates that the complexity of the sterol mixtures is attributable to indiscriminate absorption is not likely to hold in the case of plants. Since side-chain-alkylated sterols exhibit membrane properties quite different from those shown by cholesterol, the possibility that plants produce sterols for diverse functions deserves to be explored.

The amounts of sterol found in various animal tissues greatly exceed their bodily needs for the production of bile acids, steroid hormones, and vitamin D, probably by several orders of magnitude. According to conventional wisdom, the bulk of the tissue sterol is a