CHOLESTEROL

Chemistry, Biochemistry, and Pathology

Edited by

ROBERT P. COOK

University of St Andrews,
Queen's College,
Dundee, Scotland

Library of Congress Catalog Card Number: 57-14411

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE

In these days of specialization the singling out of a particular topic, or in this case of a single chemical entity, for detailed consideration is no novelty. The potential buyer will ask, does cholesterol justify a complete volume? The editor, in answering yes, can point out the universal distribution of cholesterol in all animal tissues and, it would appear, in all parts of their cells. But this is no novelty, water and numerous other chemical compounds are also universal components of cells. Cholesterol, however, has an additional interest; it has been implicated as a pathogen *sui generis*. It is a favored candidate in the etiology of atherosclerosis, that major affection of our arteries. It has also been suggested as one of the factors concerned in the causation of cancer and it is certainly found in a large number of human gallstones.

Since it is present in all cells and yet a possible cause of disease, we may well ask in the words of the title of one of the lectures given by the late Harry Deuel: "Cholesterol—Friend or Foe?"

The compound is, above all, an interesting chemical species. Why has Nature singled out this particular shape of molecule for cholesterol and the related steroid hormones whose actions are a constant source of wonder? Its biosynthesis also poses the problems of how and why a compound is cyclized instead of, as it were, remaining straight.

From the chemical point of view it is used *inter alia* as a model for studying stereochemical problems and as the chemist remarks it will be a "happy hunting ground" for years to come. This attitude has much to commend it—we study a subject because we are interested in it and if this leads to useful practical applications in the field of medicine then our time has been well spent.

We have tried to make the book of use to scientific workers, not only those dealing directly with cholesterol but to all concerned with the wider aspects of biology, chemistry, and medicine. For example, the metabolic transformations of this compound (and of other sterols) by a great variety of organisms are therefore discussed, and wherever possible this information is given in a tabular or diagrammatic form for ease of reference. To emphasize the practical intention of the book we have included an appendix of commonly used laboratory methods.

There will be found, undoubtedly, overlaps and omissions. To have been encyclopedic would have meant a multivolume treatise and the value of such a work is debatable.

The general plan in each chapter has been to cover representative work and to include references, particularly those of a review nature, from which the reader, if he be so minded, may accumulate even more knowledge on the specialized aspects of the subject.

The references are listed at the end of each chapter with the names of the first cited authors arranged in alphabetical order. A chronological order is followed when several publications under the same name are quoted. In Chapter 1 the full titles of all the journal articles are given because of their historical interest. In other chapter titles of articles (or an indication of their subject) are given only when they are of a review nature, such references being marked with an asterisk. The abbreviations used for journals are in general conformity with the "List of Periodicals Abstracted by Chemical Abstracts, 1956," published by the American Chemical Society, Ohio State University, Columbus 10, Ohio.

The contributors are all experienced in their particular fields of study and have cooperated willingly in this project. To them I owe my sincere thanks and any success the book may have is due to their help.

The editor would like to thank the Staff of Academic Press for their kind assistance in the various stages of publication. They have made it possible to include much recent work. The book may therefore be regarded as reasonably "up to date" to the end of 1957.

ROBERT COOK

Dundee, Scotland
January, 1958

LIST OF CONTRIBUTORS

DAVID ADLERSBERG, *The Mount Sinai Hospital, New York, New York*

WERNER BERGMANN, *Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut*

PETER BLADON, *Department of Chemistry, The Royal College of Science and Technology, Glasgow, Scotland*

GEOFFREY H. BOURNE, *Department of Anatomy, Emory University, Emory University, Georgia*

GEORGE S. BOYD, *Department of Biochemistry, University of Edinburgh, Edinburgh, Scotland*

ROBERT P. COOK, *University of St. Andrews, Queen's College, Dundee, Scotland*

HENRIK DAM, *Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark*

R. GORDON GOULD, *University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico*

OSCAR HECHTER, *The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts*

MARJORIE G. HORNING, *Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Maryland*

MICHAEL F. OLIVER, *Department of Cardiology, Royal Infirmary, Edinburgh, Scotland*

IRVINE H. PAGE, *Research Division, Cleveland Clinic Foundation and Frank E. Bunts Educational Institute, Cleveland, Ohio*

JAMES B. M. RATTRAY, *Department of Biochemistry, Queen's University, Kingston, Ontario, Canada*

HARRY SOBOTKA, *The Mount Sinai Hospital, New York, New York*

THRESSA C. STADTMAN, *Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Maryland*

CONTENTS

CHAPTER 1

HISTORICAL INTRODUCTION

Henrik Dam

"Weite Welt und breites Leben,
Langer Jahre redlich Streben,
Stets geforscht und stets gegründet,
Nie geschlossen, oft gründet
Ältestes bewahrt mit Treue,
Freundlich aufgefasstes Neue.
Heitern Sinn und reine Zwecke,
Nun, man kommt wohl eine Strecke."

GOETHE

I. The Discovery and Occurrence of Cholesterol

The compound now known as cholesterol was described for the first time in the latter half of the 18th century. De Fourcroy (1789) mentions that more than 20 years earlier Poulletier de la Salle had obtained from the alcohol-soluble part of human gallstones "une substance feuilleté, lamelleuse, brillante, assez semblable à l'acide boracique." Fourcroy prepared a larger quantity of the substance, which he believed was the same as "blanc de baleine" i.e. spermaceti. He also believed that the crystalline substance from gallstones was related to what he called "adipocire" ("fatty wax"), a matter obtained by treating grave wax with acids.* During the time between Poulletier's observation and Fourcroy's publication other workers, e.g. Conradi (1775; see also Gren, 1789), had

* Grave wax is a substance formed in cemeteries, particularly in moist places, during decomposition of the bodies.

extracted the substance from gallstones and made observations regarding its solubility.

Chevreul (1815) showed that the substance remained unchanged after boiling with potassium hydroxide, and thereby as well as by its melting point differed from spermaceti and "adipocire." In 1816 Chevreul introduced the designation *cholesterine* from Greek: chole, bile; and steros, solid.* Cholesterine was found by Chevreul (1824) in human and animal bile, by Lecanu (1838) in human blood, and by Couerbe (1834) in brain. Lecanu is credited by Gobley (1846) with its discovery in hens' eggs. Gobley's analyses of egg yolk are models of clarity. It was thereafter gradually recognized as a normal constituent of all animal cells and several secretions, as well as a component of certain pathological deposits. Vogel (1843) found it in atheromatous arteries, and Müller (1838) in the type of tumors which he called cholesteatomes.

Berthelot (1859) showed that "cholestérine" was an alcohol and prepared esters of it. Cholesterol oleate and palmitate were isolated from serum by Hürthle (1896). The palmitate and stearate were found in normal adrenals in 1909 by Rosenheim and Tebb. Windaus (1910b) showed that the cholesterol desposit in atheromatous aorta is present chiefly as esters, his work on this problem being taken up on Aschoff's suggestion.

II. Early Chemistry

Reinitzer (1888) published the correct summation formula of cholesterol, and Diels and Abderhalden (1904) showed that the alcohol group is secondary in that they converted cholesterol into cholestenone. The presence of a double bond was shown in 1868 by Wislicenus and Moldenhauer.

The elucidation of the constitution is mainly due to the arduous work of Windaus and his associates. Aided by parallel studies by Wieland on the chemically related bile acids and earlier studies of Mauthner and Suida on derivatives of cholesterol, Windaus (1919) arrived at a tentative formula which was changed in 1932 to the one now accepted (see Chapter 2).

The new formula was based on X-ray studies by Bernal (1932a) and by the finding of chrysene by catalytic dehydrogenation (Diels and Gädke, 1927), thoroughly discussed by Rosenheim and King (1932a, b) and by Bernal (1932b). Windaus (1932) and Wieland and Dane (1932)

* See Frontispiece; the term cholesterol was introduced in English and French literature in the beginning of the 20th century, cholesterin is still used in the German literature.

decided on the final formulation. Windaus (1919, 1932) has given reviews of the various stages of this research.

III. Related Compounds

A. Animal Sterols

Coprosterol (Greek: copros, dung)* the saturated derivative to which cholesterol is usually transformed in the large intestine, was apparently first observed by Marcet (1857, 1860) as crystals separating by cooling of an alcoholic extract of human feces treated with lime. The pictures he gave of the crystals and the description of their solubility and melting point (given as 92–96° C.) seem to indicate the identity with coprosterol. Marcet believed that the substance which he called "excretine" contained sulfur. A substance "stercorine" from feces mentioned by Flint (1862) may have been impure coprosterol (melting point given as 96.8° F.).

Coprostanol was independently discovered and exactly described by Bondzynski (1896) who gave it the designation "Koprosterin." Bondzynski and Humnicki (1896) examined it further and correctly attributed its formation to bacterial reduction of cholesterol, although the imitation of the process by bacteria *in vitro* without the presence of intestinal content or feces was not carried out until later (cf. Snog-Kjaer *et al.*, 1955, 1956).

The chemical relationship between coprostanol and cholesterol was elucidated by Windaus and Uibrig (1915) and Windaus (1916). They carried out the first transformation of cholesterol into coprostanol by chemical means *in vitro* and cleared up the difference between coprostanol and the isomeric compound obtained by catalytic hydrogenation of cholesterol: *dihydrocholesterol*† (then called β-cholestanol, now *cholestanol*) as being due to differences in the steric arrangement of a hydrogen atom.

Windaus and Neukirchen (1919) converted the hydrocarbon corresponding to coprostanol into cholanic acid and thus definitely proved the relationship between coprostanol and the bile acids.

Other animal sterols but containing 30 carbon atoms, namely, *lanosterol* and *agnosterol* from wool fat, were found by Windaus and Tschesche (1930).

Δ^7-Cholestenol was found by Fieser (1951) in commercial cholesterol and called by him "lathosterol" (Greek: latho, undetected). It was also found by Baumann and associates (e.g. Miller and Baumann, 1952) in

* Named now *coprostanol*: systematic nomenclature 5β-cholestan-3β-ol (see Chapter 2).

† Cholestanol (dihydrocholesterol) is in systematic nomenclature 5α-cholestan-3β-ol (see Chapter 2).

rodent skin as a sterol which gives a rapid reaction ("fast acting") with the Liebermann-Burchard reagent. Later it was shown to be widely distributed.

B. Plant Sterols

Sterols were found in plants, namely, in peas by Beneke (1862) and differentiated from cholesterol by Hesse (1878) who introduced the name *phytosterin* for a sterol isolated from Calabar beans. Thoms (1897) proposed to use the term phytosterol for all plant sterols.

Sitosterol (Greek: sitos, grain) from cereal germs was isolated by Burián (1897), *stigmasterol* from Calabar beans (*Phytostigma venenosum*, wherefrom the name was derived) by Windaus and Hauth (1906). Anderson and Shriner (1926) showed the existence of several isomeric forms of sitosterol.

Ergosterol was found in ergot by Tanret (1889) and called by him *ergostérine*. Ergosterol was prepared in impure form from yeast by Gérard (1895), and in pure form by Smedley-MacLean and Thomas (1920).

C. Relation to Vitamin D*

"Activation of cholesterol" by irradiation to antirachitic substances was described in 1925 by Hess *et al.* and by Steenbock and Black, and in 1926 by Rosenheim and Webster. The work of Rosenheim and Webster (1926, 1927a, b), Heilbron *et al.* (1926, 1927), Pohl (1927), and Windaus and Hess (1927) proved that the phenomenon was due to the activation of a contaminant of ordinary cholesterol with a substance closely related to ergosterol, which latter substance became active on irradiation.

7-Dehydrocholesterol was synthesized from cholesterol in 1935 by Windaus *et al.* The following year Boer and associates (1936) isolated this substance from samples of cholesterol and proved it to be the provitamin accompanying cholesterol.

Bills and McDonald (1926) activated cholesterol to an antirachitic substance without irradiation, namely, by heating it in an organic solvent with floridin. The vitamin obtained by this procedure was later identified by Raoul and co-workers (1954).

D. Relation to Steroid Hormones

The structural relation of cholesterol to certain *hormones* was disclosed when ovarian, testicular, and adrenal cortical hormones were isolated and their chemistry studied in the thirties of this century.

* For a more detailed account of the early history of the relation between cholesterol and the D vitamins reference is made to the review article by Bills (1935).

For the comprehensive literature concerning the relationship of cholesterol to hormones the reader is referred to Chapter 8 of this book and to monographs, such as that of Fieser and Fieser (1949). Here it may suffice to mention that Ruzicka and co-workers (1934) prepared androsterone from epicholestanol (cholestan-3α-ol) and that Fernholz (1934) converted stigmasterol into progesterone.

IV. Analytical Methods

Among the methods for detection and estimation of cholesterol the qualitative estimation was published in 1872.

The *Salkowski* color reaction with chloroform and sulfuric acid for qualitative estimation was published in 1872.

The *Liebermann-Burchard* reaction evolved in two stages. First, Liebermann (1885) described the color changes taking place when sulfuric acid is added to a concentrated solution of cholesterol in acetic anhydride (color shift through red to blue). Thereafter Burchard (1889) applied the reaction to a solution of cholesterol in chloroform or certain other water-free solvents and noted that a green color developed, the red and blue stages being bypassed when the solution of cholesterol is dilute.

The more sensitive reaction with acetyl chloride and zinc chloride in glacial acetic acid was described 1909 by Tschugaeff and Gasteff.

Windaus (1909, 1910a) introduced the *gravimetric digitonin method* which was a revolutionizing step not only in the quantitative determination but also in the isolation of sterols. Windaus' discovery was based on the observation by Ransom (1901) that cholesterol neutralizes the hemolyzing effect of saponin. Windaus, who was then working in the Institute of Chemistry, University of Freiburg, Germany, tested several saponins for their ability to precipitate cholesterol and found digitonin to be particularly suited for the purpose. The circumstance that the head of the Institute was the well-known investigator of saponins, Kiliani, probably favored this highly important study. Windaus also found that saponins react only with free sterols, not with sterol esters. Later (1916), he showed that the epi (3α-ol) forms of sterols are not precipitable with digitonin.

A micromethod combining digitonin precipitation with the Liebermann-Burchard reaction was developed by Schoenheimer and Sperry (1934) and revised by Brun (1939) and Sperry and Webb (1950).

Schoenheimer (1930) modified the original digitonin method for the purpose of determining saturated sterols in the presence of unsaturated, by adding bromine to the double bond of the unsaturated sterol. The bromine addition product did not precipitate with digitonin.

Windaus found that it is possible to extract the sterol from the digitonin addition compound by prolonged treatment with boiling xylene.

Another method, namely solution of the digitonide in pyridine in which it is dissociated, and subsequent precipitation of the digitonin with ether was introduced by Schoenheimer and Dam (1933).

V. Metabolism

Synthesis. Dezani (1913) and Dezani and Cattoretti (1914) were the first to show that cholesterol is synthesized in rats reared on a cholesterol-free diet. Their findings were confirmed for the same and other species, e.g. with infants by Gamble and Blackfan (1920), with human adults by Gardner and Fox (1921), with young dogs by Beumer and Lehmann (1923), with rats by Channon (1925) and Randles and Knudson (1925), with chicks by Dam (1929), and with laying hens by Schoenheimer (1929b).

The fact that cholesterol is an essential nutrient for certain insects was first established by Hobson (1935) in studies with blowflies.

Breakdown. The fact that the animal organism can also catabolize cholesterol was shown by Dam (1931) with chicks during the first two weeks after hatching, by Page and Menschick (1932) with rabbits fed cholesterol, and by Schoenheimer and Breusch (1933) with mice. Cook (1938) showed that feeding of cholesterol to rats resulted in an increase of acids in the feces in amount equal to the cholesterol broken down.

State and concentration in blood. Originally the interest centered mostly around the concentration of cholesterol and its esters in blood and particularly in plasma or serum. This was studied in relation to meals of varying composition with respect to fat and cholesterol and in relation to menstrual cycle, pregnancy, and to diseases such as atherosclerosis, lipidoses, xanthoma, and diseases of the liver and thyroid.

Of the enormous number of older studies on this subject mention may be made to those of Gardner and co-workers, e.g. Gardner and Gainsborough (1928), Bloor and Knudson (1917), Okey and Boyden (1927), and by Thannhauser and his colleagues. Thannhauser and Schaber (1926) studied particularly the so-called "Ester-sturz," namely, the pronounced fall in the ratio of ester to total cholesterol found in cases of liver disease.

More recently the interest, mostly in relation to atherosclerosis, has focused on the presence of cholesterol in lipoproteins and chylomicra. Of the rich literature on this subject the works of Gofman and his colleagues (e.g. Gofman *et al.*, 1950) shall be mentioned. However, the total cholesterol concentration in blood plasma is still receiving atten-

tion. particularly through the work of Keys and associates (e.g. Keys and Keys, 1954). This controversial subject is discussed in other chapters.

Absorption. The absorption of cholesterol from the intestine was demonstrated by Pribram (1906). J. H. Mueller (1915) showed that (in dogs) absorbed cholesterol is transported via the thoracic duct, and in 1916 he reported that pancreatic juice and bile further the absorption. Russian workers had already shown that cholesterol feeding in rabbits causes deposition of the substance in the arterial wall (Anitschkow and Chalatow, 1913). The same was shown later for other species, e.g. for chicks (Dauber and Katz, 1942). The importance of fat for the ready absorption of cholesterol was pointed out by Cook (1936).

Schoenheimer (1929a) demonstrated the marked difference in absorbability between cholesterol which is easily absorbed and plant sterols which are much more difficultly absorbed from the intestine of rabbits. Von Behring and Schoenheimer (1930) showed that saturated sterols (stanols) such as cholestanol and coprostanol behave like plant sterols in this respect. That rats may absorb some cholestanol when fed this substance was shown by Dam and Brun (1935).

Peterson (1951) first reported that plant sterols interfere with the absorption of cholesterol in chickens. A similar effect exerted by cholestanol was found by Siperstein *et al.* (1953), and had been observed in man by Dam (1934).

The study of the influence of various sterols on blood and tissue cholesterol, and on atherosclerosis, led to further information of their absorbability and convertibility to cholesterol; for example Cook and associates (1954) showed that rabbits absorb Δ^7-cholestenol (lathosterol), 7-dehydrocholesterol, and also cholestanol, and that all of these are potentially atherogenic. Nichols *et al.* (1955) found that prolonged feeding of cholestanol to chickens resulted in deposition of the substance in the vascular wall.

Excretion. Since Chevreul (1824) found cholesterol in normal bile it has been known that bile is one of the excretion paths. In 1926 Sperry showed with bile fistula dogs that a considerable amount of cholesterol is excreted through the intestinal wall.

Conversion to other sterols. Conversion of a small part of the body's cholesterol into cholestanol was reported by Schoenheimer and coworkers (1930), who found that a small amount of cholestanol usually accompanies cholesterol and is excreted into the intestine. A considerable amount of cholestanol was found in the sterile content of an intestinal loop of 14 years duration in a surgical patient by Boehm (1911).

Schoenheimer and associates suggested that the formation of cholestanol from cholesterol in the body was accompanied by desaturation of other cholesterol molecules.

The formation of 7-dehydrocholesterol from cholesterol in the intestinal wall of guinea pigs was later demonstrated by Glover *et al.* (1952).

With the *era of isotopes* an unforeseen development in the study of cholesterol metabolism began. Rittenberg and Schoenheimer (1937) undertook studies of cholesterol formation using deuterium oxide and suggested that the formation of cholesterol in the animal body involved the coupling of a number of small molecules.

After the tragic death of Schoenheimer in 1941, Bloch and Rittenberg (1942) began their now classical work on the formation of cholesterol from deuterium containing acetic acid which was followed by a large number of studies on similar lines by these and other investigators, especially after carbon-labeled acetate was introduced (Rittenberg and Bloch, 1945).

The detailed discussion of these studies belongs under special chapters of this book, but it shall be mentioned here that the new technique made it possible to demonstrate synthesis of cholesterol in individual tissues *in vitro*, such as liver, intestine, and arteries. Further, it was shown that feeding of cholesterol depresses cholesterol synthesis in, for example, the liver (see Gould *et al.*, 1953; Tomkins *et al.*, 1953), and the relation of squalene to cholesterol synthesis was attacked (Tomkins *et al.*, 1953).

Through the work of Wüersch and associates (1952) (the side chain), and Cornforth *et al.* (1953) (the ring structure) it became possible to map the cholesterol molecule according to which of the carbon atoms originate from the CH_3 and which from the COOH group of acetic acid. The complexity of the conversion is shown by the presence of "high counting companions" by Schwenk and his associates (e.g. Schwenk *et al.*, 1955).

Other workers began the study of the conversion of cholesterol into bile acids and hormones. Thus, Bloch *et al.* (1943) showed that deuterium-labeled cholesterol is converted into cholic acid, and other workers, especially Bergström (1952), have greatly extended the research on this subject.

Conversion to pregnandiol and probably to progesterone was reported by Bloch (1945). The formation of adrenal cortical hormones from cholesterol was demonstrated by Zaffaroni and associates (1951).

The conversion of cholesterol into coprostanol has also been taken up using the isotope technique. By suitable labeling of cholesterol, Rosenfeld and co-workers (1954) found that cholesterol can be con-