# Advances in Lipid Research

Volume 8

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# PREFACE

The breadth and diversity of lipid chemistry and biochemistry are well depicted in this volume of *Advances in Lipid Research*—the scope of topics in the seven chapters ranges from plants to animals.

The first chapter covers an area of great medical and pharmacological importance, namely, cholesterol turnover in man. With the current emphasis on cholesterol and its relation to heart disease, an insight into the metabolic behavior of cholesterol becomes paramount. Various aspects of the methodology in this field and suggestions concerning the possible mode of action of hypocholesteremic regimens are discussed. Another topic pertinent to the field of heart disease research is that of arterial metabolism. No picture of the development of heart disease can be complete without some understanding of the metabolic contribution of the arterial tissue. The second chapter is a detailed discussion of arterial composition and metabolism, especially as it pertains to esterified fatty acids and cholesterol. In the past two decades there has been much discussion of the essential fatty acids and their role in mammalian development, health, and disease. This area is thoroughly reviewed in the third chapter. The fourth contribution to this volume covers a topic of great biological importance, membranes. The participation of lipids in membrane structure is explored in this chapter. The last three chapters in this book deal with individual areas of interest in the lipid field. The fifth chapter presents an excellent review of the chemistry and biochemistry of the phospholipids and glycolipids which occur in the plant kingdom. Rumen metabolism is a subject not often associated with lipid metabolism; but as can be seen from the sixth contribution to this volume, it offers much of interest. The final chapter is a discussion of the surface chemistry of lipids. This discipline is germane to a number of important interests in lipid chemistry, among them the mechanics of pulmonary function and an understanding of membrane characteristics.

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# Cholesterol Turnover in Man

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## I. Introduction

In this review the major emphasis has been placed on cholesterol transport in man, but since this cannot be isolated from the much larger literature concerned with other species, the findings in animals, especially primates, has been brought into the discussion when relevant. Reviews of aspects of cholesterol metabolism that deal more comprehensively with species other than man include those by Danielsson (1963), Goodman (1965), Chevallier (1967), Treadwell and Vahouny (1968).

Cholesterol is an integral part of cell structure, is synthesized in most tissues, and, with the possible exception of mature neural tissue, participates in the constant renewal, exchange, and degradation of molecules which constitute cholesterol turnover. The magnitude of this turnover and the factors that regulate it differ among different tissues; some of these factors are unique for a specific tissue yet are not identical in the same tissue in all mammalian species. The heterogeneous nature of cholesterol metabolism emphasizes the oversimplification that is inherent in attempts to measure and quantitate cholesterol turnover in an entire organism. Nevertheless, because of the considerable endeavor and progress that has been made in this direction in recent years, this review will be devoted primarily to choles-

terol turnover in the living human. The complexities of cholesterol metabolism in individual tissues have been viewed as part of the whole.

Major emphasis has been placed on the measurement of cholesterol turnover, which reflects the balance between input of cholesterol from endogenous synthesis in tissues and from dietary cholesterol, and loss of cholesterol, which occurs mainly in the feces in the form of cholesterol, bile acids, and their degradation products.

Facets that deserve separate consideration, but only to the extent that they contribute to cholesterol turnover, include mechanisms of cholesterol absorption, the regulation of cholesterol synthesis, transport of esterified cholesterol in plasma lipoproteins, the turnover of bile acids, and the influence of diet.

## II. Measurement of Cholesterol Turnover

Ideal studies of cholesterol turnover have provided values for cholesterol absorption, cholesterol synthesis, and cholesterol degradation. Additional information which has been derived from studies of this sort relates to the flux of cholesterol between compartments within the body and the amounts of cholesterol that constitute these compartments or pools. Because of the association between atherosclerosis and the plasma cholesterol concentration, many of these studies have been directed to the elucidation of factors that determine this level.

Grundy and Ahrens (1969) have recently reviewed the major techniques used to measure cholesterol turnover and have compared their relative merits. In man, the methods have been confined either to the measurements of isotopic dilution as reflected by changes in the plasma or the feces following the administration of radioactive cholesterol, or by the direct quantitation of the excretion products in the feces. The direct measurement of tissue cholesterol turnover has been carried out in animals, but has been attempted in man only after death.

Studies of cholesterol turnover are lengthy because turnover is slow. Moreover, ideally the methods demand steady state conditions including constant sterol intake and unchanging body weight. Methods that require the collection of feces pose the additional problems of total collection of feces and corrections for losses of neutral steroids due to degradation of the sterol molecule. Techniques that merely require measurement of plasma cholesterol specific activity are

simpler to perform, but they do not answer the questions that can be solved by direct quantitation of fecal steroids or by a combination of the two methods.

The different techniques and the information that they have provided are discussed below.

## A. Plasma Specific Activity-Time Curve Analysis

The single intravenous injection of labeled cholesterol or precursor followed by analysis of the entire plasma cholesterol specific radioactivity time curve is undoubtedly the simplest of the turnover techniques. It is only recently, however, that a valid analysis of the plasma cholesterol specific activity curve has been described (Goodman and Noble, 1968). Furthermore, it has some limitations, since direct measurement of synthesis and absorption of cholesterol cannot be made. It does, however, provide information, albeit by indirect mathematical analysis, that cannot be derived otherwise (Goodman and Noble, 1968; Nestel *et al.*, 1969).

Early studies with labeled acetate were not continued for long enough to delineate the full plasma cholesterol specific activity-time curve. The emphasis was on the initial portion of the curve, with attempts to resolve this into a number of rate constants. Hellman *et al.* (1954), who administered labeled acetate to 7 subjects and followed the disappearance of plasma cholesterol radioactivity for up to 42 days, attempted to resolve the specific activity curve into 3 rate constants. The first, which was based on 2 or 3 observations along the steepest part of the curve, appeared to have a half-time of 0.20–0.47 days, which they interpreted as the appearance of newly synthesized cholesterol from the liver; the other two rate constants were of the order of 1.6–4.0 days and 15.5–24.8 days, respectively; although they have no precise physiological meaning, the middle rate constants probably reflect equilibration between plasma free cholesterol, erythrocyte cholesterol, plasma esterified cholesterol, and cholesterol in some tissues, such as intestine, which comprise pool A of Goodman and Noble (1968). Gould *et al.* (1955), who also injected radioacetate into human subjects, concluded that hepatic and plasma free cholesterol equilibrated with a half-time of about 1 hour; this conclusion was based on the finding that peak plasma free cholesterol specific activity was reached 3 hours after the injection of acetate and on the reasonable assumption that the plasma cholesterol originated almost entirely within the liver. Equilibration between plasma free cholesterol and

the cholesterol within erythrocytes occurred in 8-12 hours, which was only a little longer than they had previously reported in dogs (Eckles *et al.*, 1955). In common with all later studies, they observed that plasma free and esterified cholesterol reached isotopic equilibrium between the second and fourth days.

Although Gould *et al.* (1955) were probably correct in believing that cholesterol in liver and plasma equilibrate rapidly, the only direct comparisons of hepatic and plasma cholesterol specific activities are not in entire agreement. Chobanian and Hollander (1962) reported a much higher free cholesterol specific activity in the liver than in the plasma of a man who died 14 hours after an injection of radiocholesterol. This may have been due to the extreme illness of the patient because Nestel and Couzens (1966a) found similar specific activities in the plasma and in pieces of liver obtained at surgery 18 hours after injection of the label.

These early studies, some of which were carried out in patients with biliary fistula, also established the rapidity with which biliary cholesterol and bile acids equilibrate with plasma cholesterol (Rosenfeld and Hellman, 1959). Since these observations form the basis of the other isotopic balance techniques, they are discussed later.

The plasma cholesterol specific activity curve declines more slowly after the first few days, and reaches an exponential rate of disappearance after about the third week. The rate constant of this final portion of the curve varies considerably in different studies. Kurland *et al.* (1961), Chobanian *et al.* (1962), and Lewis and Myant (1967) reported half-times of over 50 days whereas others, such as Nestel *et al.* (1965a), Goodman and Noble (1968), Grundy and Ahrens (1969), and Nestel *et al.* (1969), found this to be frequently of the order of 30-40 days. This is of some importance because the analysis of the curve involves the extrapolation of the final exponential back to zero time. Whether the slower half-times represent an additional pool with a very slow turnover or an artifact brought about by the very low concentrations of radioactivity remaining in the plasma at this time, remains to be resolved.

The interpretation of this final portion of the specific activity curve has also undergone changes. In earlier studies it was considered to represent the equilibration and turnover of the entire miscible pool of cholesterol. This appeared to include cholesterol in most tissues, that in the nervous system and that sequestered in areas such as atheromatous plaques clearly falling outside this pool. This concept was derived from the excellent study of Chobanian and Hollander (1962), who measured the specific activity in many tissues as their patients