Refer to: Grundy SM: Cholesterol metabolism in man (Medical Progress). West J Med 128:13-25, Jan 1978

Medical Progress

Cholesterol Metabolism in Man

SCOTT M. GRUNDY, MD, PhD, *San Diego*

Recent investigations on cholesterol metabolism in man have led to new insights into diseases associated with abnormal accumulations of cholesterol in plasma (hypercholesterolemia), arterial tissues (atherosclerosis) and biliary tract (gallstones). Regulation of cholesterol synthesis under the influence of dietary and plasma cholesterol, may play a crucial role in determining biliary and tissue concentrations of this sterol. Plasma concentrations, on the other hand, appear to be controlled by complex mechanisms for secretion, transformation and removal of plasma lipoproteins. The recent identification of specific cellular receptors for uptake of plasma lipoproteins represents a significant advance for the understanding of regulation of both plasma and tissue concentrations, and possibly of the basic mechanisms underlying accumulation of cholesterol in atherosclerotic plaques.

ALTHOUGH CHOLESTEROL is a precursor for life-sustaining steroid hormones and a vital substance for cellular integrity, its insolubility in aqueous solutions creates numerous problems for its transport and excretion. Because of its low solubility, special mechanisms are required for transport of cholesterol from one site to another in the body. These include lipoproteins for plasma transport, binding proteins for intracellular transfers, and micellar solutions for biliary excretion and intestinal absorption. Despite the efficiency of these systems, precipitation of cholesterol cannot always be prevented. Therefore, deposition commonly occurs in the arterial wall as an initiating factor for atherosclerosis, and precipitation in bile leads to cholesterol gallstones. In this review basic pathways of cholesterol metabolism in man will be discussed, and in light of recent and important observations in this field speculation on pathogenesis of disorders of cholesterol metabolism—atherosclerosis and gallstones—will be presented.

From the Department of Medicine, University of California, San Diego, School of Medicine, and Veterans Administration Hospital, San Diego.

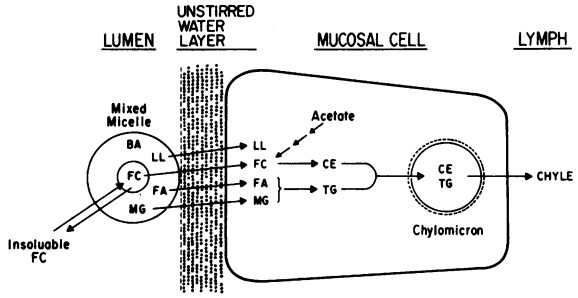
Reprint requests to: Scott M. Grundy, MD, PhD, Dept. of Medicine, Veterans Administration Hospital, 3350 La Jolla Village Drive, San Diego, CA 92161.

Cholesterol Absorption

Cholesterol enters the intestinal tract from two major sources—the diet and bile. A third possible source is secretion by intestinal mucosa. Intake of cholesterol in the American diet, which is derived entirely from animal foods (meat, eggs, milk, cheese and the like) averages 500 to 750 mg per day, but an even greater source of intestinal cholesterol is the bile. In people of normal weight biliary outputs of cholesterol range from 750 to 1,250 mg per day.[1,2] Amounts secreted by intestinal mucosa are much smaller, but have not been measured accurately. Although cholesterol in bile is unesterified, a small fraction from the diet may be in the form of esters in which cholesterol is linked with a fatty acid; however, any cholesterol ester entering the intestine is hydrolyzed rapidly by pancreatic cholesterol esterase.[3]

ABBREVIATIONS USED IN TEXT

CoA = coenzyme A
EHC = enterohepatic circulation
FFA = free fatty acids
HDL = high density lipoproteins
HMG CoA = β-hydroxyl-β-methylglutarate
IDL = intermediate density lipoproteins
LCAT = lecithin-cholesterol acyltransferase
LDL = low density lipoproteins
VLDL = very low density lipoproteins



BA = bile acids
CE = cholesterol ester
FA = fatty acids
FC = free (unesterified) cholesterol
LL = lysolecithin
MG = monoglycerides
TG = triglycerides

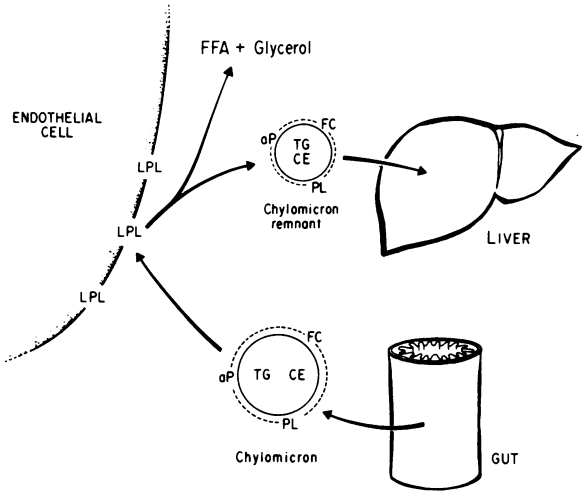
Figure 1.—Mechanisms of cholesterol absorption.

Mechanisms for cholesterol absorption are shown schematically in Figure 1. Before absorption, cholesterol must be solubilized by mixed micelles containing conjugated bile acids and hydrolytic products of triglycerides and lecithin—fatty acids, monoglycerides and lysolecithin.[4,5] Free (unesterified) cholesterol is dissolved in the hydrophobic center of the micelle. Bile acids are crucial detergents of the micelle; they are, in fact, obligatory for cholesterol absorption and when they are diverted from the intestine, as in total biliary obstruction, no cholesterol is absorbed. Even when bile acids are present, however, cholesterol absorption is incomplete; most reports indicate that only 30 to 60 percent of intestinal cholesterol enters body pools.[7-9] Since cholesterol must be in a soluble state before it can be absorbed, the primary factor limiting absorption, in most cases, is the amount of cholesterol that can be solubilized by micelles. Complete absorption of cholesterol is not possible because—in contrast to glucose and amino acids, for example—cholesterol in intestinal contents is never completely dissolved. Amounts of intestinal cholesterol that can be absorbed depend on intraluminal availability of bile acids and hydrolytic products of dietary fats, all of which can expand mixed micelles and promote absorption.[10,11] Maximum absorption of cholesterol occurs in the upper intestine where micelles are swollen by fatty acids and monoglycerides; absorption is less in the lower intestine, possibly due to disruption of micelles by fat absorption.[12]

In addition to solubilizing cholesterol, micelles promote its absorption by facilitating transport across the unstirred layer of water adjacent to the surface of the luminal cell.[13,14] This transport occurs by simple diffusion. Movement through the unstirred layer, and not penetration of the microvillus membrane, appears to be rate-limiting for cholesterol absorption. The micelle as a whole does not penetrate the cell membrane, and passage of cholesterol through structure lipid of the membrane occurs by monomolecular passive diffusion. Whether micelles specifically facilitate cholesterol uptake into the mucosal cell has not been determined, but the requirement of bile acids for cholesterol absorption suggests such a role.

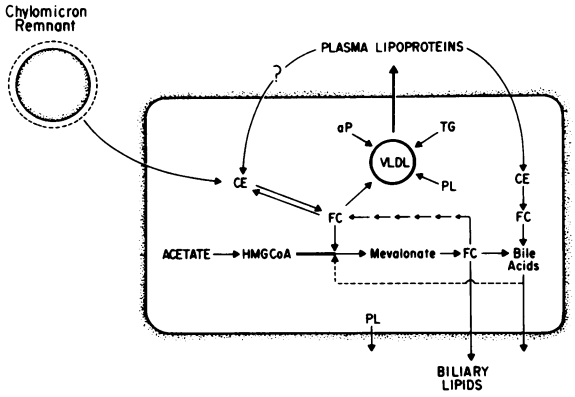
Another group of dietary constituents affecting cholesterol absorption are the plant sterols. These sterols, of which β-sitosterol is the most common, differ from cholesterol only slightly in chemical structure. They interfere with cholesterol absorption,[15] but for unexplained reasons are themselves absorbed in only trace amounts.[16] The plant sterols may decrease cholesterol absorption by more than one mechanism. Therefore, they may, to take three possibilities, displace cholesterol from mixed micelles, compete for its uptake by the mucosal cell membrane and(or) inhibit its esterification in the mucosa, thereby preventing its incorporation into chylomicrons. Normally, the diet contains relatively small amounts of plant sterols (200 to 300 mg per day), and in these amounts they probably retard absorption of cholesterol only minimally. When administered in relatively large amounts (5 to 15 grams per day), however, these sterols inhibit cholesterol absorption amounts completely,[17] and by this mechanism, reduce concentrations of plasma cholesterol.

In addition to lumenal uptake, mucosal cells have the capacity to synthesize cholesterol and in certain experimental animals, such as the rat and baboon, the intestine appears to contribute a significant fraction of the total body production of cholesterol.[18,20] Also, studies have shown that the human intestine can synthesize cholesterol,[21-23] but the level of this synthesis or fraction of total body synthesis is unknown. Amounts of cholesterol produced by the intestine may be regulated



aP = apoproteins
CE = cholesterol ester
FC = free (unesterified) cholesterol
FFA = free fatty acids
LPL = lipoprotein lipase
PL = phospholipids
TG = triglycerides

Figure 2.—Metabolism of chylomicrons.



aP = apoproteins
CE = cholesterol ester
FC = free (unesterified) cholesterol
HMG CoA = β-hydroxyl-β-methyl glutarate
PL = phospholipids
VLDL = very low density lipoproteins

Figure 3.—Hepatic metabolism of cholesterol.

by bile acids, for in the presence of bile acids mucosal synthesis is inhibited[24] and with bile diversion production is enhanced.[21] Presumably, newly-synthesized cholesterol mixes with cholesterol derived from intestinal lumen, and both are absorbed together by the same mechanism.

Before incorporation into chylomicrons most of the cholesterol in the mucosa becomes esterified. The chylomicron particle possesses a lipid-rich core consisting mainly of triglycerides, but also small amounts of cholesterol ester; in addition, it has a surface coat containing protein (including specific apoproteins), phospholipids and free cholesterol. Metabolism of chylomicrons is outlined in Figure 2. They are secreted into chyle and enter the blood stream through the thoracic duct. As they pass into capillary beds these fat particles come into contact with an enzyme, lipoprotein lipase, located on the surface of endothelial cells. This enzyme hydrolyzes triglycerides, with release of free fatty acids and glycerol. And after most of the triglycerides are removed from chylomicrons the remaining particles—called chylomicron remnants—are released into the plasma compartment. These remnants, which probably contain all absorbed cholesterol, are taken up by the liver.[25-27] Newly absorbed cholesterol, therefore, is transported almost exclusively to the liver.

Hepatic Metabolism

Cholesterol in the liver can be derived from three sources: (1) from newly-absorbed cholesterol (delivered by chylomicrons), (2) from peripheral tissues (delivered by plasma liproproteins) and (3) from cholesterol synthesized within the liver cell (Figure 3). Cholesterol that is taken up by the liver, from chylomicrons and other liproproteins, appears to be largely in the form of cholesterol esters[25-28] which can be either stored as esters or hydrolyzed to free cholesterol.

The liver is probably a major source of newly-synthesized cholesterol. While estimates have never been made of daily hepatic production of cholesterol in man, studies in primates suggest that at least half of whole body synthesis occurs in the liver.[23] In persons of normal weight, total body synthesis is in the range of 9 to 13 mg per kg of body weight per day, or for a normal 70-kg man, about 650 to 900 mg per day.[29] If data from nonhuman primates can be extrapolated to man, the human liver would make approximately 325 to 450 mg per day. The precusor for sterol synthesis is acetyl coenzyme A (CoA) and at least 21 steps are in the synthetic pathway for cholesterol.[30,31] Initial steps in cholesterol synthesis include the sequential formation of acetoacetyl CoA, β-hydroxyl-β-methyl glutarate (HMG CoA), and mevalonic acid. The conversion of HMG CoA to mevalonic acid appears to be the rate determining reaction of cholesterol formation. Through a series of condensation reactions, mevalonic acid is transformed into the long-chain hydrocarbon, squalene, which in another sequence of several reactions is cyclized and transformed into cholesterol.

In some way not understood completely cholesterol exerts a feedback inhibition on its own synthesis in the liver.[32-35] The site of feedback inhibition is primarily at the conversion of HMG

CoA to mevalonic acid which is mediated by the enzyme HMG CoA reductase. The activity of this enzyme seems to depend largely on amounts of cholesterol absorbed by the intestine and transported to the liver. The mechanisms by which this inhibition is mediated have not been determined fully. Both allosteric inhibition of the enzyme and reduction of enzyme synthesis are probably involved. Also, the nature of the active feedback agent is in dispute; while it has been generally thought that cholesterol itself is the major inhibitory factor, oxygenated products of cholesterol (for example, 25-hydroxy-cholesterol and 7-keto-cholesterol) have also been found to actively inhibit HMG CoA reductase.[36-40] Whether these latter products are important physiologically remains to be determined.

Although there is a general agreement that cholesterol derived from chylomicron remnants inhibits hepatic synthesis, the feedback potential for cholesterol from other sources is yet to be determined. It is widely assumed, but difficult to prove, that newly-synthesized cholesterol is locally active in feedback regulation; likewise, the activity of cholesterol delivered by plasma lipoproteins other than chylomicrons is uncertain. For example, in familial hypercholesterolemia total-body synthesis of cholesterol is not suppressed contrary to what might be expected.[8,15,29,41] This discrepancy again implies that chylomicron cholesterol is more potent than cholesterol from other lipoproteins in feedback regulation. Furthermore, even within different lipoprotein species cholesterol that is delivered to the liver may vary in potency for feedback inhibition as suggested by Nervi and Dietschy.[42]

Rates of hepatic cholesterogenesis may depend on several factors besides feedback regulation by cholesterol itself. One of these factors may be bile acids. The role of bile acids in regulation of hepatic cholesterol synthesis, however, is controversial and probably complex. The bile acids may influence cholesterol synthesis in at least three ways. First, since bile acids affect cholesterol absorption, an increase of bile acids in the intestine should promote absorption, which in turn will suppress cholesterol synthesis;[35,43,44] a deficiency of bile acids, on the other hand, has the opposite effect, causing increased synthesis. Second, bile acids suppress their own synthesis from cholesterol,[22,45] and should thereby increase hepatic concentrations of cholesterol; this mechanism should reduce synthesis through feedback inhibition by cholesterol itself. Third, bile acids may directly interfere with some step in the biosynthesis of cholesterol,[46,47] although this mechanism is doubted by some investigators. Therefore, actions of bile acids on regulation of cholesterol synthesis are complicated and have not been resolved completely, but they cannot be explained by a single mechanism.

Finally, synthesis of cholesterol in the liver may depend to some extent on food intake. Production rates during feeding considerably exceed those of fasting which may reflect, in part, availability of dietary substrate.[48-50] Furthermore, chronically high intakes of calories are associated with overproduction of cholesterol,[2,51-54] while restriction of calories reduces synthesis.[2] Whether the saturation of dietary fat, independent of total calories, influences cholesterol synthesis is unresolved; in some studies exchange of polyunsaturated fats for saturated fats has been reported to enhance production rates,[41,55-57] but in others, no differences in cholesterol synthesis has been found by exchange of the two fats.[58,59]

As shown in Figure 3, hepatic cholesterol can have three fates: it can be converted partly to bile acids, it can be secreted into bile as cholesterol itself and it can be secreted into plasma with lipoproteins. Transformation of cholesterol to bile acids occurs only in the liver. Normally about a third of the daily production of cholesterol—that is, about 200 to 300 mg per day—is converted into bile acids. The first step in this conversion—the formation of 7α-hydroxycholesterol—is the rate limiting step for production of both primary bile acids: cholic acid and chenodeoxycholic acid.[60-62]

After conjugation with taurine or glycerol, the primary bile acids are secreted into bile and pass through the biliary tract into the duodenum. They are reabsorbed almost completely in the small intestine. While it is generally held that reabsorption occurs mainly in the ileum,[63] recent studies by Hardison, von Bergmann and Grundy[64] have shown that a significant fraction of intestinal bile acids can be absorbed in the upper small bowel. Upon reabsorption, bile acids return via the portal vein to the liver where they are rapidly extracted and resecreted into bile, to complete the enterohepatic circulation (EHC). Because of the high efficiency of reabsorption (about 98 percent),[29] a pool of bile acids which is usually 2 to 3 grams in normal subjects is built in the EHC.

At each turn of the EHC a small fraction of

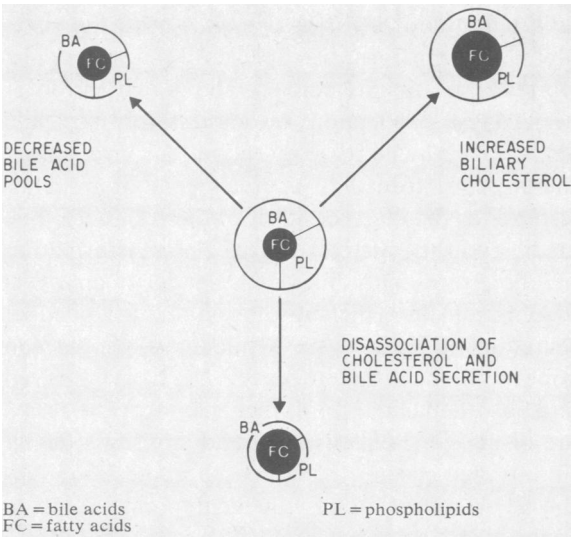


Figure 4.—Causes of supersaturated (lithogenic) bile.

bile acids passes into the large intestine, where, upon contact with colonic microorganisms, hydroxy-groups on their steroid nucleus are altered, producing "secondary" bile acids. Although cholic acid is converted largely to deoxycholic acid, and chenodeoxycholic acid to lithocholic acid, numerous other alterations are possible, and stools may contain a host of secondary bile acids.[15] Appreciable quantities of deoxycholic acid are absorbed in the colon so that it constitutes a significant portion (about a third) of bile acids in the EHC. Lithocholic acid, in contrast, is less well-absorbed, and it normally does not exceed 3 percent of biliary bile acids; most lithocholate in the bile of man is present as its sulfate ester,[65,66] due to its sulfation by the liver.

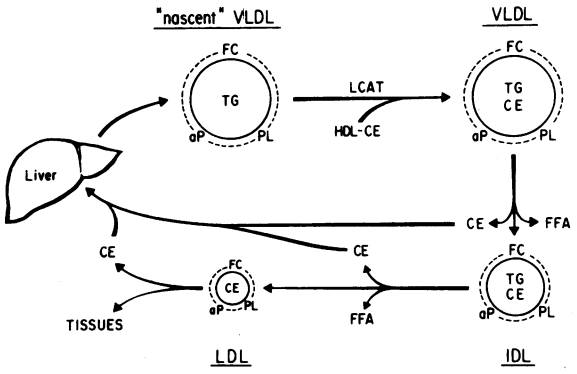
The second fate of hepatic cholesterol is direct secretion into bile. In the steady state, when extrahepatic pools of cholesterol are constant, biliary cholesterol is composed of newly-synthesized cholesterol plus that recycled from the intestine. In adults of normal weight, daily hepatic secretion of cholesterol ranges from 800 to 1,200 mg.[1] As mentioned above, biliary cholesterol enters the intestine along with dietary cholesterol, where 30 to 60 percent is reabsorbed. Unabsorbed cholesterol passes into the colon, is transformed partially into two other neutral steroids—coprostanol and coprostanone[67]—and is excreted with these latter two steroids without reabsorption from the colon.

Metabolism of biliary cholesterol has clinical significance because of the problem of gallstones, which very frequently contain large amounts of cholesterol. The cholesterol in bile is solubilized normally by mixed micelles containing bile acids and phospholipids, but if amounts of cholesterol exceed the solubilizing capacity of these two polar lipids, it is likely to crystallize and initiate stone formation.[68,69] Bile containing excess cholesterol, relative to bile acids and phospholipids, is called supersaturated or "lithogenic" bile. This abnormality may arise in at least three ways (Figure 4). First, there may be a deficiency of bile acids in the EHC (that is, low bile acid pools).[29,70,71] Second, there may be an excess secretion of biliary cholesterol.[2,29,72] Third, in the fasting state secretion of cholesterol may be disproportionately high or dissociated relative to outputs of the solubilizing lipids.[73] In addition, a combination of these abnormalities may coexist in the same patient.

A reduction in the pool size of bile acids has been found in many patients with cholesterol stones; the mechanisms for this abnormality have not been defined with certainty, but a defective regulation of bile acid synthesis appears to be the most likely cause.[29] When patients have an abnormal regulation of synthesis, a decline in pool size and enterohepatic cycling of bile acids resulting from usual daily losses in feces does not release adequately the feedback inhibition on bile acid synthesis, which are required to reexpand the pool to normal levels. A new steady state is therefore established in which bile acid pools are reduced; and although patients with this disorder make and excrete normal amounts of bile acids, they are unable to respond with transitory increments in synthesis that are needed to maintain the bile acid pool in the normal range.

Another cause of supersaturated bile is excessive secretion of cholesterol into bile,[2,29,72] which is usually linked to overproduction of cholesterol within the whole body. Increased cholesterol synthesis is most commonly caused by the high caloric intake associated with obesity. Therefore, the long-recognized association between obesity and gallstones can be explained by enhanced whole-body synthesis of cholesterol.[2] Yet, biliary cholesterol can be increased without obesity,[72] and more studies are needed to determine frequency and importance of this mechanism for production of supersaturated bile in nonobese subjects with gallstones.

Finally, supersaturated bile may arise from abnormal dissociation of hepatic secretion of cholesterol from outputs of its solubilizing lipids



aP = apoproteins
CE = cholesterol ester
FC = free (unesterified) cholesterol
FFA = free fatty acids
HDL = high density lipoproteins
IDL = intermediate density lipoproteins
LCAT = lecithin-cholesterol acyltransferase
LDL = low density lipoproteins
PL = phospholipids
TG = triglycerides
VLDL = very low density lipoproteins

Figure 5.—Plasma transport of cholesterol.

(bile acids and phospholipids).[73,74] According to current theory hepatic cholesterol destined for bile is dissolved first by phospholipids as occurs in many biological membranes, and thereafter, these cholesterol-phospholipid complexes are solubilized by bile acids to form mixed micelles. This mechanism, whereby cholesterol is intimately associated with phospholipids, can explain the finding in several animal species (dogs, rats and monkeys) that cholesterol and phospholipids fall in parallel with declining outputs of bile acids. However, a similar tight coupling of cholesterol and phospholipids does not always occur in man as it does in animals; and when bile acid outputs decline during the fasting state, due to storage of bile acids in the gallbladder, bile saturation with cholesterol can increase to a pronounced degree. This suggests that additional cholesterol which is unassociated with phospholipids can be secreted as well. Cholesterol secretion in man, therefore, appears to occur in a two-step process: first, a portion of biliary cholesterol is presolubilized by phospholipids before dissolution by bile acids, and second, additional cholesterol may be taken up by mixed micelles. In fact secretion of supersaturated bile during fasting almost certainly requires the presence of greater quantities of cholesterol than can be dissolved initially by phospholipids.

Secretion of biliary cholesterol in man, therefore, appears to be more complex than for many animals. This complexity is probably the result of the necessity to dispose of relatively more cholesterol. In comparison with certain animals, such as the dog and rat, man does not have the same ability for transforming cholesterol into bile acids; while these animals convert about two thirds of each day's production of cholesterol into bile acids, man converts only about a third. Man, therefore, is forced to excrete most of his cholesterol as cholesterol itself rather than as bile acids, and this relative "defect" in transformation of cholesterol into bile acids leads to an overloading of the efficient phospholipid-dependent secretion of cholesterol. While most people can cope with the usual loads of newly synthesized and absorbed cholesterol, their secretory processes are of marginal effectiveness and any slight deviation, either because of increased cholesterol synthesis or reduction of bile acid pools, leads to supersaturation and frequently to cholesterol gallstones.

Plasma Transport

A portion of cholesterol produced by the liver is transported to peripheral tissues for utilization. Since cholesterol is completely insoluble in aqueous solution, it cannot circulate freely in plasma and must be brought into solution by more complex mechanisms—specifically by liproproteins. Plasma lipoproteins are complex particles containing well-defined apoproteins, which are proteins that can bind lipids and facilitate their transport from the liver cell to plasma and within the plasma compartment. Basic pathways of lipoprotein metabolism are shown in Figure 5.

The major lipoprotein secreted into plasma is a relatively large particle (300 to 450 A) with a high lipid content that imparts rapid floatation in the ultracentrifuge; hence, the designation "very low density lipoproteins" (VLDL). These particles, which enter plasma in a "nascent" form,[75,76] possess a membranous coat consisting of specific apoproteins, phospholipids and free cholesterol, as well as a nonpolar core containing mostly triglycerides and possibly small amounts of cholesterol ester. When VLDL enters plasma, transformations in its structure begin to occur. The particle first acquires cholesterol ester in its core lipid by two possible mechanisms:[77,78] (1) high density lipoproteins (HDL), which are discussed below, may transfer cholesterol ester directly to VLDL and (2) free cholesterol on the VLDL surface coat can be esterified by the enzyme, lecithin-cholesterol acyltransferase (LCAT).

Degradation of VLDL begins through lipolysis of triglycerides which occurs at the surface of

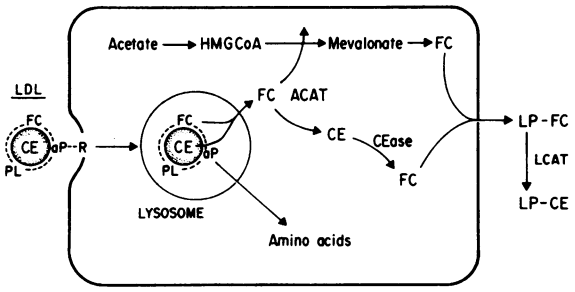
endothelial cells of capillaries; this reaction is catalyzed by the enzyme: lipoprotein lipase. As hydrolysis proceeds free fatty acids (FFA) and glycerol are released, size of VLDL is reduced and density of particles increases so as to yield a product designated intermediate density lipoprotein (IDL). Analogous to the remnant of chylomicron degradation, IDL probably can be taken up *in toto* by the liver of many animal species;[79] in normal man, however, IDL is degraded further to low density lipoprotein (LDL).[80,81] Precise mechanisms for conversion of IDL to LDL are not well understood, but most involve several steps: one is removal of certain apoproteins of the IDL coat; another is elimination of most remaining triglycerides; and finally, a portion of cholesterol ester appears to be removed. The site of removal of excess lipid and protein components has not been determined with certainty, but the liver may play a role.

The LDL particle size is in the range of 175 to 225 A; this lipoprotein contains mostly cholesterol ester in its core lipid with a single protein, apoprotein B, in its membranous coat. The fate of LDL has become a subject of great interest. Until recently, it was assumed that removal and degradation of LDL occurred in the liver;[82,83] this pathway has been brought into question, however, by Sniderman and associates.[84] These workers showed that in pigs LDL degrades normally even after hepatectomy; they suggested, therefore, that other tissues besides the liver may remove LDL, and this suggestion has been amply confirmed as will be discussed in the subsequent section.

Despite peripheral uptake and degradation of LDL, the liver may still dispose of a portion of the cholesterol on circulating lipoproteins. Thus, as VLDL and IDL are degraded some cholesterol ester is released and may go to the liver; also, recent studies by Sniderman[85] in humans have shown that the cholesterol ester content of LDL is decreased with passage across the splanchnic bed, which suggests that LDL cholesterol ester can be removed partly by the liver. If confirmed, this mechanism could be an important means for transport of cholesterol from lipoproteins or tissues to the liver.

Tissue Metabolism

Through recognition of extrahepatic degradation of LDL Sniderman and co-workers[84] focused attention on other sites of LDL uptake, and almost simultaneously Goldstein and Brown[86] observed



aP = apoproteins
ACAT = acyl CoA cholesterol acyltransferase
CE = cholesterol ester
CEase = cholesterol esterase
FC = free (unesterified) cholesterol
HMG CoA = β-hydroxyl-β-methyl glutarate
LCAT = lecithin-cholesterol acyltransferase
LDL = low density lipoproteins
LP = lipoproteins
PL = phospholipids
R = specific receptor

Figure 6.—Cholesterol metabolism in peripheral tissues.

degradation of LDL by cultured human fibroblasts. These initial studies were followed by intense investigation of metabolism of LDL and cholesterol in cultured cells. Important studies in this area have been carried out by Goldstein and Brown,[86,89] Steinberg, Weinstein, Carew and associates,[90,91] Stein, Stein and co-workers,[91-93] Avigan,[94] Fogelman, Edwards, Popjak and co-workers,[95-99] Ross[100] and Bierman and associates.[101] The following is a brief summary of the results of these investigations.

Basic steps in uptake and degradation of LDL by peripheral cells are shown in Figure 6. First, it should be emphasized that cholesterol is a major component of all mammalian plasma membranes, and one of the functions of plasma lipoproteins may be to provide membrane cholesterol for growth and survival of cells. The steps shown in Figure 6 thus represent essential mechanisms for intracellular cholesterol homeostasis; they have been shown to apply to cells from a variety of tissues including fibroblasts, smooth muscle cells, endothelial cells, polymorphonuclear leukocytes and lymphocytes.

Uptake of LDL by cells appears to be mediated by LDL-binding receptors on the surface of cells. The extent of binding is a function of the number of receptors, which is regulated in turn by cells' need for cholesterol. If a cell contains an abundance of cholesterol, due to excess LDL in the medium, the production of receptors is reduced, thereby decreasing cellular uptake of cholesterol; in reverse, depletion of cellular cholesterol increases the number of receptors, and uptake is facilitated. The physiochemical nature of the binding between LDL and its receptors has not

been determined; however, there appear to be at least two kinds of receptors: high-affinity and low-affinity receptors. But when LDL binds to high-affinity receptors, it enters the cell by absorptive endocytosis and is incorporated into endocytotic vesicles (endosomes) that fuse with lysosomes. Within the lysosome, the lipoprotein is dismantled: apoprotein B of the LDL coat is degraded to amino acids, and cholesterol esters, containing mostly polyunsaturated fatty acids (linoleic acid), are hydrolyzed by lysosomal acid lipase. The resulting unesterified cholesterol is discharged into the cell cytoplasm, where it can be incorporated into cell membranes, reesterified for storage within the cell, or excreted out of the cell. The esterification reaction, occurring through action of acyl CoA: cholesterol acyltransferase (ACAT), usually attaches a saturated fatty acid (palmititic acid) or monounsaturated fatty acid (oleic acid) to the cholesterol molecule; esterification is apparently stimulated by excess cholesterol to prevent abnormal accumulation of free (unesterified) cholesterol in membranes. Another mechanism for keeping intracellular cholesterol at an optimum level is by inhibition of cholesterol synthesis; inhibition of HMG CoA reductase, the key enzyme regulating cholesterol synthesis, appears to be mediated by an increase in intracellular unesterified cholesterol.

Besides specific receptor uptake of LDL, many cells appear to possess other mechanisms for degrading this lipoprotein. Brown and Goldstein[89] have proposed that LDL can also be incorporated into cells by bulk phase phagocytosis. By this mechanism, the fate of LDL cholesterol appears to be entirely different from that derived by specific receptor uptake. After phagocytosis, cholesterol esters are hydrolyzed, but free cholesterol acquired in this way seemingly does not inhibit HMG CoA reductase or stimulate esterification; instead it is returned into the surrounding medium without affecting the overall economy of cholesterol in the cell.

Discovery of pathways described above were made simultaneously with investigations on the metabolic defect in familial hypercholesterolemia. In this disease, plasma levels of LDL are notably elevated—an abnormality that appears to be due in large part to defective uptake of LDL by tissues. Three related, but different mechanisms have been implicated as causes of severe hypercholesterolemia; these defects have been revealed by studies in patients with related forms of homozygous familial hypercholesterolemia. These studies have been carried out in cultured skin fibroblasts, smooth muscle cells, and lymphocytes from affected patients. In some patients LDL receptors are totally absent; in others they are present but defective; and in the third group, receptors appear normal but cells are unable to internalize LDL. In all three types, LDL uptake is blocked and severe hypercholesterolemia develops. When LDL concentrations become extremely high in these patients an alternate pathway, which has been designated the scavenger path, apparently comes into play. This removal pathway, which maintains constant albeit very high levels of LDL, is probably distinct from the specific pathways and probably belongs to the reticuloendothelial (RE) system. A less severe form of hypercholesterolemia occurs in patients who are heterozygous for familial hypercholesterolemia; these patients possess specific receptors, but in reduced numbers; and while plasma LDL is elevated, their LDL concentrations are only about half those with the homozygous form.

Another defect in cellular cholesterol metabolism in familial hypercholesterolemia has been identified by Fogelman and associates.[96,97] These workers studied activation of cholesterol synthesis from acetate in leukocytes incubated in media containing lipid-free serum, and in comparison of leukocytes of normal subjects and patients with heterozygous familial hypercholesterolemia they found that the latter responded with greater activation of cholesterol synthesis than did normals. The leukocytes from hypercholesterolemic subjects were also found to release more newly synthesized cholesterol into the media than normal, which led these workers to suggest that cells from hypercholesterolemic patients may release cholesterol from cells too rapidly; whether this defect is related to a deficiency of LDL receptors on cells has not been determined.

Reverse Cholesterol Transport

As cellular membranes become saturated with cholesterol through uptake of LDL and internal biosynthesis there is need for removal of unused sterol into extracellular fluid for return to the liver. Mechanisms for the "reverse cholesterol transport" have not been determined with certainty, but several possible carriers have been suggested. Glomset[102] has proposed that HDL may be the agent of transport—a hypothesis that is supported by studies in cell culture by Stein and

associates[92,93] If this mechanism pertains, unesterified membrane cholesterol incorporated into HDL may be either transferred to VLDL through the action of LCAT or returned directly to the liver. Other possible carriers of cholesterol include LDL[85] or another plasma protein not previously recognized to transport lipids.[103] Since 1 to 1.5 grams of LDL-cholesterol are probably taken up by peripheral cells each day, this amount of cholesterol plus that synthesized in extrahepatic tissues must find its way back to the liver in some manner.

Hypercholesterolemia and Atherosclerosis

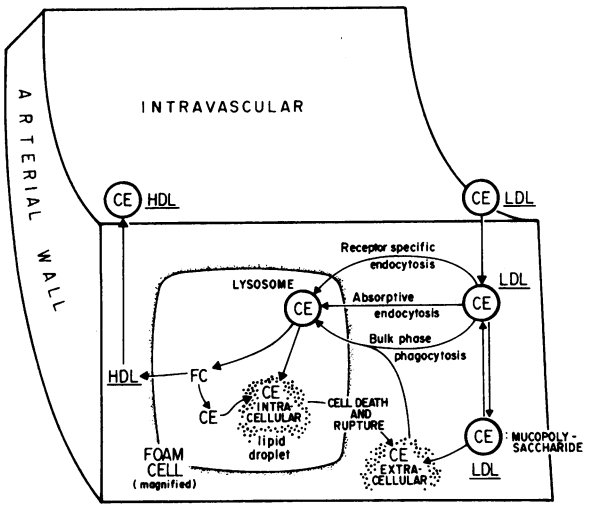
Deposition of cholesterol and its esters in the arterial wall is a major factor in development of atherosclerosis. A portion of cholesterol in atherosclerotic plaques may be derived by local synthesis, but evidence of several kinds indicates that plasma cholesterol is a significant, and probably a major, source. There is, in fact, abundant evidence for a positive correlation between levels of plasma cholesterol and rates of atherogenesis, as shown in a variety of ways for both man and experimental animals. For example, feeding of cholesterol to many species of animals causes hypercholesterolemia and atherosclerosis, and the extent of atherosclerosis usually parallels the rise in plasma cholesterol.[104] Studies in different human populations also have shown that those with high concentrations of plasma cholesterol have more atherosclerotic disease than those with low cholesterol;[105] and within a given population, as in the United States, atherosclerotic disease occurs more commonly in people with the higher levels of plasma cholesterol.[106-108] Furthermore, in familial hyperlipidemias, especially when concentrations of LDL-cholesterol are elevated to a pronounced degree, the incidence of atherosclerotic complications is extremely high.[109-111] Therefore, the relationship between elevated plasma cholesterol and atherosclerosis seems well established.

Because of this relationship, the definition of hypercholesterolemia becomes an important issue. Abnormal concentrations could be defined as those in the upper 5 to 10 percent of a given population. While this method is used commonly, it does not take in account the relationship between cholesterol levels and atherosclerosis. Another approach is to relate plasma cholesterol concentrations to risk for atherosclerosis which should have a more practical or clinical significance. Therefore, a definition of hypercholesterolemia in terms of ideal levels rather than "normal" concentrations would seem more logical and realistic.

According to a normal distribution curve for plasma cholesterol in adult Americans, concentrations greater than 275 mg per 100 ml are usually above the 90th percentile.[112] The Framingham study,[107,112] nevertheless, showed that even below this level cholesterol concentrations are correlated with the prevalence of clinical events related to atherosclerosis. In this particular study, for example, men with plasma cholesterol less than 190 mg per 100 ml were at only half the risk for coronary heart disease as those with levels of 190 to 250 mg per 100 ml. It is therefore reasonable to conclude that those with concentrations in the latter range are not entirely normal, and any definition or classification of hypercholesterolemia should reflect the graded correlation between cholesterol levels and atherosclerosis. One way to modify the term hypercholesterolemia might be according to degrees of severity, that is, mild, moderate, and severe; this categorization will be used in this discussion and should provide perspective for both diagnosis and treatment of hypercholesterolemia in clinical practice.

With these criteria, mild hypercholesterolemia could be defined roughly as cholesterol concentrations of 225 to 275 mg per 100 ml. Causes of mild hypercholesterolemia have not been examined sufficiently, but both environmental and genetic factors probably play important roles. The former include diet (especially diets rich in saturated fats and cholesterol), obesity and possibly lack of exercise. Genetic factors almost certainly can be implicated as well and while the role of heredity has been studied intensively in more severe forms of hypercholesterolemia,[113-115] less attention has been paid to genetic factors in milder elevations of cholesterol. More studies are needed therefore to dissect the relative importance of hereditary and environment in this latter category.

The importance of mild hypercholesterolemia is emphasized by several epidemiologic studies which show that in most patients with atherosclerotic disease there are only mildly elevated cholesterol concentrations.[107,112] Therefore, when consideration is given to the relation between hypercholesterolemia and atherosclerosis, the large number of people with mild hypercholesterolemia should not be overlooked. Actually, however, in mild hypercholesterolemia excess cholesterol may reside in any of several lipopro-



CE = cholesterol ester
HDL = high density lipoproteins
LDL = low density lipoproteins

Figure 7.—Mechanisms of cholesterol deposition in atherosclerosis.

tein fractions and the risk for atherosclerosis may be determined, in part at least, by which lipoprotein fraction carries the excess cholesterol. Since LDL is the most "atherogenic" of all lipoproteins, an increase of LDL-cholesterol probably confers the greatest risk. In patients with associated hypertriglyceridemia, the increment in cholesterol may be largely in VLDL and the role of VLDL in atherogenesis is controversial.[116-119] When VLDL alone is increased with low concentrations of total cholesterol (that is, less than 200 mg per ml), atherosclerosis may not be accelerated.[113,118] In other words, if cholesterol levels are in the ideal range, hypertriglyceridemia per se may not confer increased risk. Finally, in some patients an increase in cholesterol may be contained in HDL. Several recent studies have suggested that people with increased HDL-cholesterol, instead of being at increased risk, may be protected from atherosclerosis,[118,120] possibly through the action of HDL to mobilize cholesterol from the arterial wall.[92,93,102]

Concentrations of plasma cholesterol greater than 275 mg per 100 ml are abnormal by almost any criteria. With *moderate hypercholesterolemia* (plasma cholesterol of approximately 275 to 350 mg per 100 ml) dietary factors may still contribute significantly to the elevation, for many such patients are "dietary responsive" and removal of saturated fats and cholesterol from the diet will produce appreciable reduction in their plasma cholesterol. Hereditary factors, however, are also important in most patients with moderate hypercholesterolemia; at least two "monogenic" disorders, familial combined hyperlipidemia[114,115] and familial hypercholesterolemia,[113-115] as well as a polygenic form of hypercholesterolemia,[114,115] can increase plasma levels to this range.

By present criteria, sustained cholesterol levels greater than 350 mg per ml may be called *severe hypercholesterolemia.* When serum cholesterol is notably increased, regardless of what lipoprotein fraction it is found in, atherogenesis is greatly accelerated. The causes of severe hypercholesterolemia are largely genetic, and dietary factors are usually of lesser importance. The most common cause is familial hypercholesterolemia, but less commonly this degree of elevated cholesterol can occur in familial combined hypercholesterolemia or polygenic hypercholesterolemia.[114,115]

Mechanisms by which plasma LDL-cholesterol may accumulate in atherosclerotic plaques is shown in Figure 7. These mechanisms, which are based on current concepts of LDL metabolism, are largely speculative and further investigation will be required to determine the relative importance of each pathway as well as to uncover new ones. In the diagram, sites of cholesterol accumulations are depicted as cholesterol esters, but at each site there is also an excess of free cholesterol.

Lipoproteins, especially LDL, presumably enter the subintimal space by diffusion through the intima or from the vasovasorum. Subintimal cells, such as smooth muscle cells or tissue macrophages, may then internalize LDL through several mechanisms. As with other cells in peripheral tissues, LDL can be incorporated into lysosomes of arterial cells following interaction with specific receptors on the cell surface (receptor-specific endocytosis)—this kind of LDL uptake has been shown for both smooth muscle cells and macrophages studied *in vitro.* Also, nonspecific uptake might occur by either absorptive endocytosis or bulkphase phagocytosis. When excess cholesterol ester accumulates in these cells, by increased uptake of LDL or decreased removal of the ester, they are transformed in ways not well-understood into "foam" cells which have become engorged with numerous lipid droplets. In early atherosclerotic lesions, called fatty streaks, most cholesterol is intracellular and esterified, and its esters contain high proportions of oleic acid. Since cholesterol esters made within cells usually contain mostly monounsaturated fatty acids, this pattern of esterification in fatty streaks suggests that most

of the excess cholesterol esters in foam cells is derived from free cholesterol which had been released by hydrolysis of LDL-cholesterol esters in lysosomes. In other words, the foam cell esters are probably formed by the same pathway described above for normal cells. An alternate mechanism of foam-cell formation could be engorgement and rupture of lysosomes before ester hydrolysis, but if this occurred fatty acids of cholesterol esters should be largely polyunsaturated (for example, linoleic acid) as are present in plasma lipoproteins.

As atherosclerotic lesions develop and mature, large amounts of cholesterol begin to accumulate outside of cells. These depositions contain a higher proportion of unesterified cholesterol than those intracellularly, and they also have a greater portion of polyunsaturated cholesterol esters. The latter observation suggests that deposition of esters from plasma cholesterol occurs directly without previous hydrolysis in lysosomes. Such an accumulation could take place in two ways: first, LDL could be internalized into cells and then be released before hydrolysis, or second, LDL-cholesterol could be deposited extracellularly without cellular uptake. The latter mechanism could be facilitated by interaction of LDL with tissue mucopolysaccharides for it is well-known that certain mucoploysaccharides can precipitate LDL. The possibility that tissue mucopolysaccharides play an important role in precipitation of LDL within the atherosclerotic plaque makes an attractive hypothesis.

The extent of accumulation of cholesterol in atherosclerotic lesions is determined, of course, by the balance between influx and removal of cholesterol, but regulation of neither process is well-understood. Current evidence suggests that hyperlipoproteinemia and hypertension promote influx of lipids into the arterial wall, and by this mechanism accelerate atherosclerosis. Processes for removal of free and esterified cholesterol from the wall remain to be determined. However, HDL could be one factor in removal of cholesterol from the arterial wall because, as mentioned above, patients with unusually high levels of plasma HDL seem relatively resistant to clinical sequelae of atherosclerosis. Findings in studies in tissue culture are in accord with this proposal; HDL has been shown to inhibit uptake of LDL into cells,[120] and it may serve as a receptor for cholesterol released from cells.[102] An important question is, of course, whether human atherosclerotic plaques can be reversed. By reversal of mechanisms for atherogenesis (such as through decreased LDL and increased HDL levels), regression is theoretically possible and has been suggested by results of studies in animals[121] and preliminary results in man,[122,123] but remains to be established with certainty.

REFERENCES

1. Grundy SM, Metzger AL: A physiological method for estimation of hepatic secretion of biliary lipids. Gastroenterology 62:1200-1217, Jun 1972

2. Bennion LJ, Grundy SM: Effects of obesity and caloric intake on biliary lipid metabolism in man. J Clin Invest 56:996-1011, Oct 1975

3. Treadwell CR, Vahouney GV: Cholesterol absorption, *In* Code CF (Ed): Handbook of Physiology, Section 6. Alimentary Canal, Vol 3. Washington, DC, American Physiological Society, 1968, p 1407

4. Hofmann AF, Borgström B: Physico-chemical state of lipids in intestinal contents during their digestion and absorption. Fed Proc 21:43-50, Jan 1962

5. Hofmann AF, Borgström B: The intraluminal phase of fat digestion in man—The lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. J Clin Invest 43:247-257, Feb 1964

6. Siperstein MD, Chaikoff IL, Reinhardt WO: C^{14}-cholesterol—V. Obligatory function of bile in intestinal absorption of cholesterol. J Biol Chem 198:111-114, Sep 1952

7. Kaplan JA, Cox GE, Taylor CB: Cholesterol metabolism in man: Studies on absorption. Arch Path 76:359-368, Oct 1963

8. Quintao E, Grundy SM, Ahrens EH: Effects of dietary cholesterol on the regulation of total body cholesterol in man. J Lipid Res 12:233-247, Mar 1971

9. Grundy SM, Mok HYI: Determination of cholesterol absorption in man by intestinal perfusion. J Lipid Res 18:263-271, Mar 1977

10. Pihl A: The effect of dietary fat on the intestinal cholesterol and on the cholesterol metabolism in the liver of rats. Acta Physiol Scand 34:183-196, Feb 1955

11. Swell L, Flick DF, Field H Jr, et al: Role of fat and fatty acid in absorption of dietary cholesterol. Am J Physiol 180:124-128, May 1955

12. Westergaard H, Dietschy JM: The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. J Clin Invest 58:97-108, Jul 1976

13. Simmonds WJ: Uptake of fatty acid and monoglyceride, *In* Rommel K, Goebell H, Böhmer R (Eds): Lipid Absorption: Biochemical and Clinical Aspects. Lancaster, England, MTP Press Ltd, 1976, pp 51-64

14. Simmonds WJ, Hofmann AF, Theodor E: Absorption of cholesterol from a micellar solution: Intestinal perfusion studies in man. J Clin Invest 46:874-890, May 1967

15. Grundy SM, Ahrens EH Jr, Davignon J: The interaction of cholesterol absorption and cholesterol synthesis in man. J Lipid Res 10:304-315, 1965

16. Salen G, Ahrens EH Jr, Grundy SM: Metabolism of β-sitosterol in man. J Clin Invest 49:952-967, May 1970

17. Grundy SM, Mok HYI: Effects of low dose phytosterols on cholesterol absorption in man, *In* Greten H (Ed): Lipoprotein Metabolism. Berlin-Heideberg-New York, Springer-Verlag, 1976, pp 112-118

18. Dietschy JM, Siperstein MD: Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. J Lipid Res 8:97-104, Mar 1967

19. Dietschy JM, Wilson JD: Cholesterol synthesis in the squirrel monkey: Relative rates of synthesis in various tissues and mechanisms of control. J Clin Invest 47:166-174, Jan 1968

20. Dietschy JM, Weis HJ: Cholesterol synthesis by the gastrointestinal tract. Am J Clin Nutr 24:70-76, Jan 1971

21. Grundy SM, Ahrens EH Jr, Salen G: Interruption of the enterohepatic circulation of bile acids in man: Comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. J Lab Clin Med 78:94-121, Jul 1971

22. Dietschy JM, Wilson JD: Regulation of cholesterol metabolism. N Engl J Med 282:1128-1138, May 1970

23. Dietschy JM, Gamel WG: Cholesterol synthesis in the intestine of man: Regional differences and control mechanisms. J Clin Invest 50:872-880, Apr 1971

24. Dietschy JM: The role of bile salts in controlling the rate of intestinal cholesterogenesis. J Clin Invest 47:286-300, Feb 1968

25. Goodman, DS: The metabolism of chylomicron cholesterol ester in the rat. J Clin Invest 41:1886-1896, Jun 1962

26. Nestel PJ, Havel RJ, Bezman A: Metabolism of constituent lipids of dog chylomicrons. J Clin Invest 42:1313-1321, Apr 1963

27. Bergman EN, Havel RJ, Wolfe BM, et al: Quantitative studies of the metabolism of chylomicron triglycerides and cholesterol by liver and extrahepatic tissues of sheep and dogs. J Clin Invest 50:1831-1839, Sep 1971

28. Faergeman O, Havel RJ: Metabolism of cholesteryl esters of rat very low density lipoproteins. J Clin Invest 55:1210-1218, Jun 1975

29. Grundy SM, Metzger AL, Adler RD: Mechanisms of lithogenic bile formation in American Indian women with cholesterol gallstones. J Clin Invest 51:3026-3043, Dec 1972

30. Bloch K: The biological synthesis of cholesterol. Science 150:19-28, Oct 1965

31. Frantz ID Jr, Scroepfer GJ Jr: Sterol biosynthesis. Ann Rev Biochem 36:691-726, 1967

32. Gould RG: Lipid metabolism and atherosclerosis. Am J Med 11:209-227, Aug 1951

33. Tompkins GM, Sheppard H, Chaikoff IL: Cholesterol synthesis by liver—III. Its regulation by ingested cholesterol. J Biol Chem 201:137-141, Mar 1953

34. Gould RG, Taylor CB, Hagerman JS, et al: Cholesterol metabolism—I. Effect of dietary cholesterol on the synthesis of cholesterol in dog tissue in vitro. J Biol Chem 201:519-528, Apr 1953

35. Siperstein MD, Guest MJ: Studies on the site of the feedback control of cholesterol synthesis. J Clin Invest 39:642-652, Apr 1960

36. Kandutsch AA, Chen HW: Inhibition of sterol synthesis in cultured mouse cells by 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol. J Biol Chem 248:8408-8417, Dec 1973

37. Kandutsch AA, Chen HW: Inhibition of sterol synthesis in cultured mouse cells by cholesterol derivatives oxygenated in the side chain. J Biol Chem 249:6057-6061, Oct 1974

38. Kandutsch AA: Consequences of blocked sterol synthesis in cultured cells—DNA synthesis and membrane composition. J Biol Chem 252:409-415, Jan 1977

39. Brown MS, Dana SE, Goldstein JL: Regulation of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase activity in cultured human fibroblasts: Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. J Biol Chem 249:789-796, Feb 1974

40. Brown MS, Faust JR, Goldstein JL: Role of the low density lipoprotein receptor in regulating the content of free and esterified cholesterol in human fibroblasts. J Clin Invest 55:783-793, Apr 1975

41. Grundy SM: Effects of polyunsaturated fats on lipid metabolism in patients with hypertriglyceridemia. J Clin Invest 55:269-282, Feb 1975

42. Nervi FO, Dietschy JM: Inhibition of hepatic cholesterogenesis by different lipoproteins. Circulation (Supplement III) 50:46, Oct 1974

43. Frantz ID Jr, Schneider HS, Hinkelman BT: Suppression of hepatic cholesterol synthesis in the rat by cholesterol feeding. J Biol Chem 206:465-469, Jan 1954

44. Bhattathiry EPM, Siperstein MD: Feedback control of cholesterol synthesis in man. J Clin Invest 42:1613-1618, Oct 1963

45. Bergström S, Danielsson, H: On the regulation of bile acid formation in the rat liver. Acta Physiol Scand 43:1-7, Feb 1958

46. Fimognari GN, Rodwell VN: Cholesterol biosynthesis: Mevalonate synthesis inhibited by bile salts. Science 147:1038, Feb 1965

47. Back P, Hamprecht B, Lynen F: Regulation of cholesterol biosynthesis in rat liver: Diurnal changes of activity and influence of bile acids. Arch Biochem Biophys 133:11-21, Aug 1969

48. Shefer S, Hauser S, Bekersky I, et al: Biochemical site of regulation of bile acid biosynthesis in the rat. J Lipid Res 11:404-411, Sep 1970

49. Edwards PA, Muroya H, Gould RG: *In vivo* demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. J Lipid Res 13:396-401, May 1972

50. Dietschy JM, Brown MS: Effect of alterations of the specific activity of the intracellular acetyl CoA pool on apparent rates of hepatic cholesterogenesis. J Lipid Res 15:508-516, Sep 1974

51. Nestel PJ, Monger EA: Turnover of plasma esterified cholesterol in normocholesterolemic and hypercholesterolemic subjects and its relation to body build. J Clin Invest 46:967-974, Jun 1967

52. Nestel PJ, Whyte HM, Goodman DS: Distribution and turnover of cholesterol in humans. J Clin Invest 48:982-991, Jun 1969

53. Miettinen, TA: Cholesterol production in obesity. Circulation 44:482-850, Nov 1971

54. Nestel PJ, Schreibman PH, Ahrens EH Jr: Cholesterol metabolism in human obesity. J Clin Invest 52:2389-2397, Oct 1973

55. Grundy SM, Ahrens EH Jr: An evaluation of the relative merits of two methods for measuring the balance of sterols in man: Isotopic balance versus chromatographic analysis. J Clin Invest 45:1503-1515, Sep 1966

56. Good PDS, Shioda R, Kinsell LW: Dietary regulation of cholesterol metabolism. Lancet 2:604-607, Sep 1966

57. Nestel PJ, Havenstein N, Whyte HM, et al: Lowering of plasma cholesterol and enhanced sterol excretion with the consumption of polyunsaturated ruminant fats. N Engl J Med 288: 379-382 Feb 1973

58. Avigan J, Steinberg D: Sterol and bile acid excretion in man and the effects on dietary fat. J Clin Invest 44:1845-1856, Nov 1965

59. Grundy SM, Ahrens EH Jr: The effects of unsaturated dietary fats on absorption, excretion, synthesis, and distribution of cholesterol in man. J Clin Invest 49:1135-1152, Jun 1970

60. Danielsson H, Einarsson K, Johansson G: Effect of biliary drainage on individual reaction in the conversion of cholesterol to taurocholic acid. Eur J Biochem 2:44-49, Mar 1967

61. Shefer S, Hauser S, Bekersky I, et al: Feedback regulation of bile acid biosynthesis in the rat. J Lipid Res 10:646-655, Nov 1969

62. Shefer S, Hauser S, Lapar V, et al: Diurnal variation of HMG CoA reductase activity in rat intestine. J Lipid Res 13: 571-573, Sep 1972

63. Tyor MP, Grundy SM: Bile acid and cholesterol homeostasis, chap 52, *In* Bockus HL (Ed): Gastroenterology, Vol II. Philadelphia, WB Saunders Co, 1976, pp 109-120

64. Hardison WG, Grundy SM, von Bergmann K: Small bowel absorption of endogenous conjugated bile acid in man. Gastroenterology 71:911, Nov 1976 (abstr.) A-18/911

65. Cowen AE, Korman MG, Hofmann AF, et al: Metabolism of lithocholate in healthy man. Gastroenterology 69:59-66, Jul 1975

66. Cowen A, Korman MG, Hofmann AF, et al: Metabolism of lithocholate in healthy man. Gastroenterology 69:67-76, Jul 1975

67. Miettinen TA, Ahrens EH Jr, Grundy SM: Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. J Lipid Res 6:441-424, Jul 1965

68. Admirand WH, Small DM: The physiochemical chemical basis of cholesterol gallstone formation in man. J Clin Invest 47: 1043-1052, May 1968

69. Holzbach RT, Marsh M, Olszewski M, et al: Cholesterol solubility in bile—Evidence that supersaturated bile is frequent in healthy man. J Clin Invest 52:1467-1479, 1973

70. Vlahcevic ZR, Bell CC Jr, Buhac I, et al: Diminished bile acid pool size in patients with gallstones. Gastroenterology 59: 165-173, 1970

71. Swell LC, Bell CC Jr, Vlahcevic ZR: Relationship of bile acid pool size to biliary lipid excretion and the formation of lithogenic bile in man. Gastroenterology 61:716-722, 1971

72. Grundy SM, Duane WC, Adler RD, et al: Biliary lipid outputs in young women with cholesterol gallstones. Metabolism 23:67-73, Jan 1974

73. Metzger AL, Adler RA, Heymsfield S, et al: Diurnal variation in biliary lipid composition: Possible role in cholesterol gallstone formation. N Engl J Med 288:333-336, Feb 1973

74. Mok HYI, von Bergmann K, Grundy SM: Graded withdrawal of the bile acid pool on bile lipid secretion in man. Gastroenterology 69:848, Sep 1975

75. Hamilton RL: Synthesis and secretion of plasma lipoproteins—Pharmacological control of lipid metabolism. Adv Exp Med Biol 26:7-24, 1972

76. Hamilton RL, Kayden HJ: The liver and the formation of normal and abnormal plasma lipoproteins. *In* Becker FF (Ed): The Liver: Normal and Abnormal Functions. New York, Marcel Dekker, Inc., Part A, 1974, pp 531-572

77. Glomset JA, Nichols AV, Norum KR, et al: Plasma lipoproteins in familial lecithin-cholesterol acyltransferase: Further studies of very low and low density lipoprotein abnormalities. J Clin Invest 2:1078-1092, May 1973

78. Glomset JA, Norum KR, Nichols AV, et al: Plasma lipoprotein metabolism in familial lecithin-cholesterol acyltransferase deficiency. Scand J Clin Lab Invest 33 (Suppl 137):165-172, 1974

79. Faergeman O, Tsunako S, Kane JP, et al: Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. J Clin Invest 56:1396-1403, Dec 1975

80. Bilheimer DW, Eisenberg S, Levy RI: The metabolism of very low density lipoprotein proteins—I. Preliminary *in vitro* and *in vivo* observations. Biochim Biophys Acta 260:212-221, Feb 1972

81. Eisenberg S, Bilheimer DW, Levy RI, et al: On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein. Biochim Biophys Acta 326:361-377, 1973

82. Hotta S, Chaikoff IL: The role of the liver in turnover of plasma cholesterol. Arch Biochem Biophys 56:28-37, May 1955

83. Hay RV, Pottenger LA, Reingold AL, et al: Degradation of I^{125}-labelled serum low density lipoprotein in normal and estrogen-treated male rats. Biochem Biophys Res Commun 44: 1471-1477, Sep 1971

84. Sniderman AD, Carew TE, Chandler JG, et al: Paradoxical increase in rate of catabolism of low-density lipoproteins after hepatectomy. Science 183:526-528, Feb 1974

85. Sniderman A, Teng B, Marpole D, et al: Low density lipoprotein, the liver and cholesterol transport. Clin Res 23:333A, Apr 1975

86. Goldstein JL, Brown MS: Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. Proc Natl Acad Sci USA 70:2804-2808, Oct 1973

87. Goldstein JL, Brown MS: Bending and degradation of low density lipoproteins by cultured human fibroblasts: Comparison

of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. J Biol Chem 249:5153-5162, Aug 1974

88. Brown MS, Faust JR, Goldstein JL: Role of the low density lipoprotein receptor in regulating the content of free and esterified cholesterol in human fibroblasts. J Biol Chem 55:783-793, Apr 1975

89. Brown MS, Goldstein JL: Familial hypercholesterolemia: A genetic defect in the low density lipoprotein receptor. N Engl J Med 294:1386-1390, Jun 1976

90. Weinstein DB, Carew TE, Steinberg D: Uptake and degradation of low density lipoprotein by swine arterial smooth muscle cells with inhibition of cholesterol biosynthesis. Biochimica et Biophysica Acta 424:404-421, Mar 1976

91. Stein O, Weinstein DB, Stein Y, et al: Binding, internalization and degradation of low density lipoprotein by normal human fibroblasts and by fibroblasts from a case of homozygous familial hypercholesterolemia. Proc Natl Acad Sci USA 73:14-18, Jan 1976

92. Stein O, Stein Y: The removal of cholesterol from Landschütz ascites cells by high density apolipoprotein. Biochim Biophys Acta 326:232-244, Nov 1973

93. Stein Y, Glangeaud MC, Fainaru M, et al: The removal of cholesterol from aortic smooth muscle cells in culture and Landschütz ascites cells by fractions of human high density apolipoprotein. Biochim Biophys Acta 380:106-118, Jan 1975

94. Williams CD, Avigan J: In vitro effects of serum proteins and lipids on lipid synthesis in human skin fibroblasts and leukocytes grown in culture. Biochim Biophys Acta 260:413-423, Mar 1972

95. Fogelman AM, Edmond J, Polito A, et al: Control of lipid metabolism in human leukocytes. J Biol Chem 248:6928-6929, Oct 1973

96. Fogelman AM, Edmond J, Seager J, et al: Abnormal induction of 3-hydroxy-3 methylglutaryl coenzyme A reductase in leukocytes from subjects with heterozygous familial hypercholesterolemia. J Biol Chem 250:2045-2055, Mar 1975

97. Fogelman AM, Seager J, Edwards PS, et al: Mechanism of induction of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase in human leukocytes. J Biol Chem 252:644-651, 1977

98. Edwards PA, Popjak G, Fogelman EJ: Control of 3-hydroxy-3-methylglutaryl coenzyme A reductase by endogenously synthesized sterols *in vitro* and *in vivo*. J Biol Chem 252:1057-1063, Feb 1977

99. Edwards PA, Fogelman AM, Popjak G: A direct relationship between the amount of sterol lost from rat hepatocytes and the increase in activity of HMG-CoA reductase. Biochem Biophys Res Comm 68:64-69, 1976

100. Ross R: The smooth muscle cell—II. Growth of smooth muscle in culture and formation of elastic fibers. J Cell Biol 50:172-186, Jul 1971

101. Bierman EL, Stein O, Stein Y: Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. Circ Res 35:136-150, Jul 1974

102. Glomset JA: The plasma lecithin:cholesterol acyltransferase reaction. J Lipid Res 9:155-167, Mar 1968

103. Carew TE, Koschinsky T, Hayes SB, et al: A mechanism by which high-density lipoproteins may slow the atherogenic process. Lancet 1:1315-1318, Jun 1976

104. Katz LN, Stamler J: Experimental Atherosclerosis. Publication No. 124, American Lecture Series Monograph, *In*, Bannerstone Division of American Lectures in Metabolism. Springfield, Ill, Charles C Thomas, 1953

105. Keys A: Diet and the epidemiology of coronary heart disease. J Am Med Assoc 164:1912-1919, Aug 1957

106. Gofman JW, et al: Report of a cooperative study of lipoproteins and atherosclerosis: Evaluation of serum lipoprotein and cholesterol measurements as predictions of clinical complications of atherosclerosis. Circulation 14:691-741, Oct 1956

107. Kannel WB, Dawber TR, Friedman GD, et al: Risk factors in coronary heart disease—An evaluation of several serum lipids as predictors of coronary heart disease—The Framingham Study. Ann Internal Med 61:888-899, Nov 1964

108. Dayton S, Chapman JM, Pearce MS, et al: Cholesterol, atherosclerosis, ischemic heart disease, and stroke. Ann Intern Med 72:97-109, Jan 1970

109. Adlersberg D: Hypercholesterolemia with predisposition to atherosclerosis. Am J Med 11:600-614, Nov 1951

110. Epstein FH, Block WD, Hand EA, et al: Familial hypercholesterolemia, xanthomatosis and coronary heart disease. 26: 39-53, Jan 1959

111. Khachadurian AK: The inheritance of essential familial hypercholesterolemia, Am J Med 37:402-407, Sep 1964

112. Truett J, Cornfeld J, Kannel W: A multivariate analysis of the risk of coronary heart disease in Framingham. J Chron Dis 20:511-524, Dec 1967

113. Fredrickson DS, Levy RI, Lees RS: Fat transport in lipoproteins: An integrated approach to mechanisms and disorders. N Engl J Med 276:34-44, 94-103, 148-156, 215-225, 273-281, Jan 1967

114. Goldstein JR, Schrott HG, Hazzard WR, et al: Hyperlipidemia in coronary heart disease—I. Lipid levels in 500 survivors of myocardial infarction. J Clin Invest 52:1533-1543, Jul 1973

115. Goldstein JR, Schrott HG, Hazzard WR, et al: Hyperlipidemia in coronary heart disease—II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest 52:1544-1568, Jul 1973

116. Carlson LA, Ericsson M: Quantitative and qualitative serum lipoprotein analysis—Part I. Studies in healthy men and women. Atherosclerosis 21:417-433, May 1975

117. Carlson LA, Ericksson M: Quantitative and qualitative serum lipoprotein analysis—Part 2. Studies in male survivors of myocardial infarction. Atherosclerosis 21:435-450, May 1975

118. Rhoads GG, Gulbrandsen CL, Kagan A: Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. N Engl J Med 294:293-298, Feb 1976

119. Brunzell JD, Schrott HG, Motulsky AG, et al: Myocardial infarction in familial forms of hypertriglyceridemia. Metabolism 25:313-320, Mar 1976

120. Steinberg D, Miller NE, Weinstein DB: Uptake of high density and low density lipoproteins by cultured human fibroblasts: Relation to endocytosis. Circulation (Suppl. II) 45:54, Nov 1976

121. Armstrong ML, Warner ED, Connor WE: Regression of coronary atheromatosis in rhesus monkeys. Circ Res 27:59-67, Jul 1970

122. Basta LL, Williams C, Kioschos JM, et al: Regression of atherosclerotic stenosing lesions of the renal arteries and spontaneous cure of systemic hypertension through control of hyperlipidemia. Am J Med 61:420-423, Sep 1976

123. Rarndt, R, Blankenhorn DH, Craeford DW, et al: Regression and progression of early femoral atherosclerosis in treated hyperlipoproteinemic patients. Ann Intern Med 86:139-146, Feb 1977