review

The hepatobiliary axis and lipoprotein metabolism: effects of bile acid sequestrants and ileal bypass surgery

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Abstract Cholesterol excretion from the body is achieved almost exclusively via the hepatobiliary axis. Disruption of the integrity of this pathway by interruption of the enterohepatic circulation produces profound changes in cholesterol metabolism that affect every body tissue. This is particularly evident in the liver and gut which are the major sources of this sterol in the plasma. Elevated plasma cholesterol levels have been implicated in the pathogenesis of atherosclerosis and, in consequence, strenuous efforts have been made to find appropriate hypocholesterolemic therapy to reduce this risk. Medical or surgical interruption of the enterohepatic circulation is, to date, the most successful means of lowering plasma cholesterol, and in this review we examine the ramifications of such therapy on lipid and lipoprotein metabolism in the liver, gut, and plasma.—**Packard, C. J., and J. Shepherd.** The hepatobiliary axis and lipoprotein metabolism: effects of bile acid sequestrants and ileal bypass surgery. *J. Lipid Res.* 1982. **23:** 1081–1098.

INTRODUCTION

The liver and gut play a central role in human cholesterol metabolism, being involved jointly in its dietary absorption and its de novo synthesis (1). Moreover, they possess the unique ability to package and secrete the sterol into the circulation as a component of plasma lipoproteins (2), thus making it available to other tissues that use it for structural and metabolic purposes in preference to making it for themselves (3, 4). As a result, the liver and gut are together responsible for up to 75% of corporeal cholesterol production (5).

Low density lipoprotein (LDL)[1] is the major vehicle of cholesterol transport in human plasma (2, 6). It carries the sterol in two forms which occupy different sites in the particle; free cholesterol is located primarily in the phospholipid-protein matrix of the surface coat while esterified cholesterol is a component of the hydrophobic core (7, 8). LDL cholesterol is made accessible to parenchymal cells through the agency of a high affinity cell membrane receptor which, by binding the lipoprotein, initiates a sequence of events culminating in degradation of the particle and release of its contained cholesterol into the cell. This pathway is autoregulated in that assimilation of sufficient sterol to satisfy cellular requirements suppresses synthesis of the LDL receptor. Concurrently, endogenous cholesterol production is inhibited by down-regulation of the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase); and by balancing the rates of cholesterol synthesis and receptor-mediated lipoprotein assimilation, the cell is able to regulate its sterol content (3, 4, 9–12).

In addition to its involvement in the above physiological processes, cholesterol appears to play an important part in the pathogenesis of atherosclerosis and of its major clinical manifestation, ischemic heart disease. This lipid is found in the extracellular matrix of normal aorta (13); in diseased vessels (14) it appears in abundance in fatty streaks, intermediate lesions, or advanced plaques (where crystalline cholesterol deposits are char-

Abbreviations: VLDL, very low density lipoproteins d 0.95–1.006 g/ml; LDL, low density lipoproteins d 1.006–1.063 g/ml; HDL, high density lipoproteins d 1.063–1.21 g/ml; HMG CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; ACAT, acyl coenzyme A:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; 7α-hydroxylase, cholesterol 7α-hydroxylase; FH, familial hypercholesterolemia.

[1] Elaboration of the structure, function, and metabolism of plasma lipoproteins is beyond the scope of the present discussion and the reader is referred to the following excellent reviews of this subject, references 2, 7, 10, and 68.

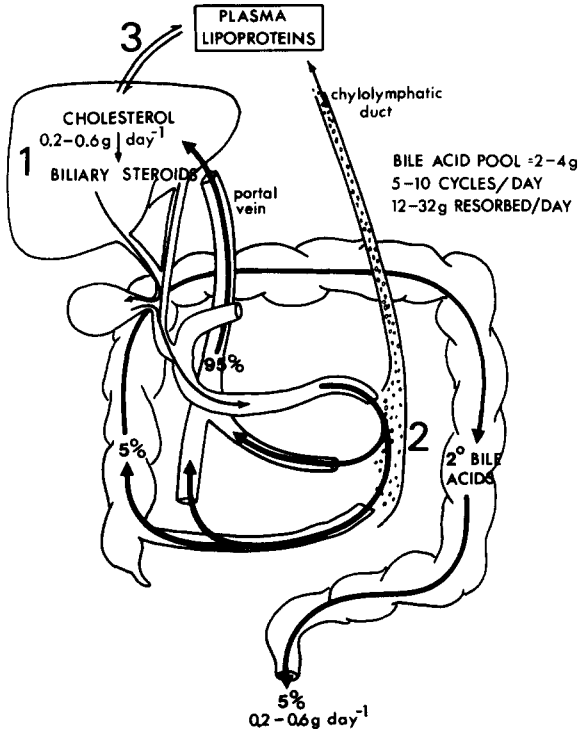

Fig. 1. Cholesterol, metabolism and the hepatobiliary axis. 1) Cholesterol, bile acid and lipoprotein metabolism in the liver; 2) cholesterol, bile acid and lipoprotein metabolism in the gut; 3) plasma lipoprotein composition and metabolism.

acteristic). Stenosis of the vessel with subsequent ischaemia and ultimate infarction of the tissue it supplies is often associated with the presence of these lesions. Consequently a great deal of effort has been directed towards discovering means whereby subendothelial cholesterol accumulation can be retarded and lipid present in existing deposits can be mobilized and transported to the liver for excretion. Since the circulating lipoproteins appear to be a major source of this excess sterol (15, 16), attempts have been made to lower plasma cholesterol levels by a variety of means including dietary manipulation (17, 18), pharmacologic agents (19–22) and surgical procedures (23, 24). The detailed mechanisms of action of these treatments are still unknown and none has yet been shown to make a significant impact on ischemic heart disease mortality.

Cholesterol excretion from the body is achieved almost exclusively via the hepatobiliary tree by elimination of the sterol itself or of its major metabolites, the bile acids (**Fig. 1**). Only a small component of the sterol loss occurs by epithelial sloughing. Under normal conditions most of the bile acids entering the intestinal lumen are reabsorbed by an active transport mechanism in the terminal ileum. Thus, although the bile acid pool (normally 2–4 g) circulates 5–10 times per day, the daily fecal loss of bile acids is only 0.3 to 0.6 g which is replaced by synthesis from cholesterol (25, 26). Interruption of this enterohepatic circulation of bile acids by high molecular weight sequestrant resins[2] (27, 28) or by ileal bypass surgery (29) increases the fecal loss of bile acids (27, 30, 31) and leads to augmented rates of bile acid synthesis, changes in hepatic cholesterol metabolism (1, 25, 26, 32, 33), and a drop in plasma lipoprotein cholesterol levels (27, 29). In this review we examine the impact of interruption of the enterohepatic circulation on cholesterol, bile acid, and lipoprotein metabolism under the following headings: I. Cholesterol, bile acids, and lipoprotein synthesis in the liver; II. Cholesterol, bile acid, and lipoprotein metabolism in the gut; III. Plasma lipoprotein composition and metabolism; and IV. Clinical implications in relation to the atherosclerotic process.

I. CHOLESTEROL, BILE ACID, AND LIPOPROTEIN SYNTHESIS IN THE LIVER

The liver, among its many other functions, occupies a key position in cholesterol metabolism. It exports this lipid both to the systemic circulation in the form of lipoproteins and to the intestinal lumen as biliary cholesterol and bile acids. In order to do this efficiently the organ must regulate synthesis of the lipoprotein lipids and apoproteins on the one hand and the various biliary products on the other. The control of these hepatic pathways of cholesterol metabolism is complex and interrelated and it is not surprising that perturbation of the system produces wide-ranging effects.

Normal hepatic cholesterol metabolism

The liver acquires cholesterol by direct synthesis and also by assimilation of the sterol from the circulating lipoproteins. Studies using animal models have shown that a number of lipoproteins, including partially degraded triglyceride-rich particles (chylomicrons and very low density lipoprotein remnants (34–36), and low density (37, 38) and high density lipoproteins (39) can deliver cholesterol to the liver. However, the relative contribution that each makes to this process is not yet known and undoubtedly varies in different animal species. When faced with a situation in which insufficient exogenous sterol is available, the hepatocyte (3, 40), in common with other cells (4), will synthesize cholesterol from acetyl coenzyme A. The rate-limiting step in this synthetic pathway is catalyzed by HMG CoA reductase,

[2] Reference to the bile acid sequestrants encompasses studies with both cholestyramine (the copolymer of styrene and divinylbenzene) and colestipol (the polymerization product of tetraethylenepentamine and epichlorohydrin). These resins appear to differ only in their binding capacity per gram weight (167).

located in the microsomal fraction of the cell (1). Regulation of this important enzyme is complex as is evident from recent reviews of the topic (11, 41, 42). The intensively studied rat liver reductase exhibits circadian rhythm (43), is influenced by hormonal factors (42), is stimulated by dietary lipid (33), and is depressed by fasting and cholesterol feeding (1, 42, 43). However, it is not clear how these effects are achieved. One important component is feedback inhibition by cholesterol and another isoprenoid structure, possibly isopentenyl adenine, which may regulate enzyme turnover (11). Additionally, the observation that the enzyme can be activated in vitro by dephosphorylation led to the suggestion that this may control its function in vivo over the short term (44). However, in recent studies no correlation could be demonstrated between the state of enzyme phosphorylation and cholesterol synthesis (45, 46), which argues against this hypothesis. The regulation of liver cholesterologenesis in other animals appears to be similar to that in the rat. For example, hepatic sterol synthesis is suppressed by feeding cholesterol to man, subhuman primates, mice, birds, fish, and amphibians (47). Likewise, the squirrel monkey exhibits suppression during a prolonged fast (1). Nevertheless, there are both qualitative and quantitative interspecies differences in the control mechanism of this enzyme (1, 41, 42, 47) as evidenced by the variable effects that bile acids have on hepatic cholesterol synthesis (see below).

Hepatic cholesterol derived from either endogenous or exogenous sources feeds several metabolic pathways (**Fig. 2**). It may be used by the cell itself for membrane synthesis, be excreted unaltered into bile, be converted to bile acids, be stored intracellularly as cholesteryl ester, or be incorporated into newly formed lipoproteins. When these pathways of cholesterol utilization were studied in animals using radioactive cholesterol precursors, it became apparent that the above products of cholesterol metabolism achieved different final specific activities, suggesting that the hepatic pool of the sterol was inhomogeneous. Balasubramaniam, Mitropoulos, and Myant (48) found such evidence for compartmentalization of the cholesterol in rat liver, and other workers (49) have further proposed that biliary cholesterol and bile acids arise from separate sterol pools. This phenomenon also appears to occur in man. A study of hepatic cholesterol metabolism in bile fistula patients (50) has indicated that there may be distinct precursor sites associated with the synthesis of bile acids and the secretion of biliary cholesterol; and about one-third and one-fifth, respectively, of the sterol channeled into bile acids and biliary cholesterol was derived from newly synthesized material. The remainder came from unesterified cholesterol circulating in lipoproteins since cholesteryl esters contributed only 11% to bile acid synthesis and trivial amounts to biliary cholesterol secretion.

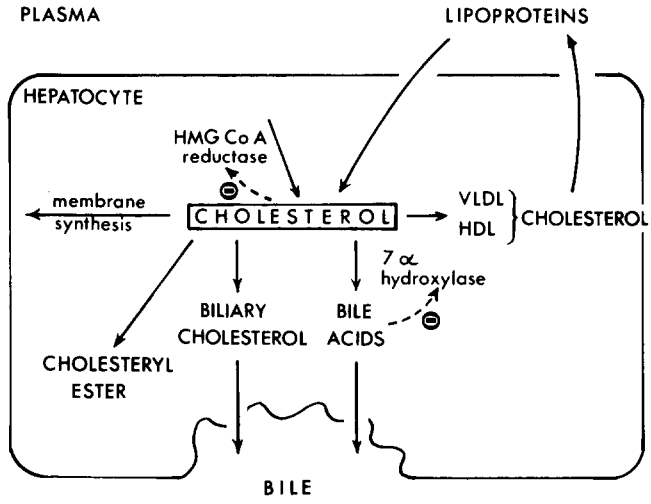

Fig. 2. Hepatic steroid metabolism. The hepatocyte derives cholesterol from circulating lipoproteins or by de novo synthesis and uses it for membrane synthesis, bile acid secretion, and lipoprotein formation. Excess sterol is stored in its esterified form. The rate-limiting enzymes of cholesterol and bile acid production, HMG CoA reductase and 7α-hydroxylase, are autoregulated via product inhibition.

In an attempt to define the origin of this lipoprotein cholesterol, Schwartz et al. (51) examined biliary steroid excretion in patients who had received injections of LDL (or HDL) differentially labeled with [^{3}H]cholesteryl esters and free [^{14}C]cholesterol. While their study was beset with problems of rapid exchange of sterol between lipoproteins, it appeared again that free cholesterol (particularly that in HDL) was the major substrate for biliary steroid production. In one patient, whose enterohepatic circulation was intact, there was a direct precursor-product relationship between plasma free cholesterol and the biliary sterol. It is of interest to note that in this patient, in contrast to biliary diverted subjects, the input of newly synthesized sterol into the biliary cholesterol pool was small.

Biliary cholesterol and bile acid formation

One possible means of exporting excess sterol from the liver is by direct secretion into bile (Fig. 2). Recent human studies (52) have shown that the content of cholesterol in bile from a fasting subject is positively correlated with hepatic HMG CoA reductase activity, and patients with elevated biliary cholesterol levels have a high hepatic cholesterol concentration. Other extensive investigations of biliary cholesterol metabolism in the rat (53), however, failed to show any relationship between bile sterol levels and the rate of cholesterol synthesis or lipoprotein assimilation by the liver. These contrasting findings demonstrate the major interspecies

variations that are a feature of biliary steroid metabolism (25).

Cholesterol in bile is maintained in solution by the detergent action of the bile acids and secretion of these two biliary products appears to be coupled (53). Bile acids leaving the liver are derived both from a preformed pool (delivered from the intestine in portal blood) and from conversion of cholesterol to the primary bile acids, cholic and chenodeoxycholic acid (25, 26) (Fig. 1). The rate-limiting enzyme in their synthesis is 7α-hydroxylase, a cytochrome P450-dependent microsomal monooxygenase that catalyzes the formation of 7α-hydroxycholesterol from cholesterol (26). This enzyme exhibits a diurnal variation that parallels that of HMG CoA reductase and, in general, the rates of cholesterol and bile acid synthesis vary in concert (25, 26). The mechanisms of interregulation of these two pathways are not yet defined and are at present controversial (1, 25, 26, 53–55). Biosynthesis of bile acids is subject to feedback inhibition. Ingestion of these compounds leads to a decrease in their rates of formation (25, 56) and in the activity of 7α-hydroxylase (26, 54, 56); this is, in part, independent of changes in microsomal cholesterol content (55). Likewise in man, feeding of one primary bile acid suppresses synthesis of the other (57, 58).

On the other hand, the mechanisms of the inhibitory effects of bile acid feeding on HMG CoA reductase are less clear and have been dealt with in detail in a number of review articles (1, 41, 42, 59). The argument centers on whether bile acids affect the activity of HMG CoA reductase directly, or indirectly as a consequence of their action on cholesterol absorption and transport. Certainly, when added in purified form, they do not modulate the function of the enzyme in vitro (60). Nor do they reverse the increased cholesterol production that follows biliary diversion in the rat, although chylomicron cholesterol can (61). These and other observations led Nervi and Dietschy (54) to propose that bile acid-induced suppression of cholesterol synthesis in the rat is secondary to enhanced sterol absorption. There are, however, experimental observations that cannot be explained on this basis. Hamprecht et al. (62) found that cholic acid feeding did suppress hepatic HMG CoA reductase activity in rats whose absorbed cholesterol had been diverted via an external thoracic duct fistula; and a similar effect was seen in animals whose cholesterol absorption had been blocked by feeding β sitosterol (63). Moreover, in humans, administration of chenodeoxycholate, but not cholate, inhibits HMG CoA reductase despite the fact that *a*) cholesterol absorption is the same on both treatments, and *b*) each acid actually diminishes the hepatic cholesterol pool (52). These observations are difficult to explain other than by supposing that bile acids decrease directly the activity of HMG CoA reductase in the liver.

Hepatic ACAT and lipoprotein synthesis

Cholesterol that is surplus to requirements is converted in cultured hepatocytes (40), as in other cells (4, 10), to a fatty acyl ester (Fig. 2) by the action of hepatic acyl coenzyme A:cholesterol acyltransferase (ACAT) (64). Feeding cholesterol to animals increases ACAT activity and promotes cholesteryl ester deposition in liver cells (64, 65). This may also occur in human liver, which has been shown to contain the enzyme (66).

In a recent study, Drevon, Engelhorn, and Steinberg (67) have suggested that, in addition to producing esters for intracellular storage, hepatic ACAT may play a role in the synthesis of cholesteryl esters for secretion in newly formed VLDL. This proposal was based on the observation that stimulation of ACAT activity in cultured rat hepatocytes promotes release of cholesteryl ester-rich VLDL. However, there is considerable interspecies variability in the degree of esterification of lipoprotein cholesterol secreted by the liver. In the rat, "nascent" VLDL, although markedly different in structure and composition from its plasma counterpart (2, 68, 69), contains substantial amounts of cholesteryl ester (68), reflecting the high hepatic ACAT activity in that species. In contrast, human VLDL cholesteryl esters are thought to be derived mainly from the action of plasma lecithin:cholesterol acyltransferase (LCAT) since they are virtually absent from the plasma of LCAT-deficient patients (70).

The synthesis and secretion of VLDL by the liver seems to be closely linked to cholesterol biosynthesis and HMG CoA reductase activity. Liver perfusion studies (71, 72) have shown that stimulation of VLDL production raises HMG CoA reductase; and in man, overproduction of VLDL (in type IV hyperlipoproteinemia) is associated with a similarly increased activity (73). Furthermore, secretion of VLDL lipids in the intact rat exhibits the same cyclic variation as hepatic HMG CoA reductase (74). On the basis of these findings, it has been suggested that a rise in VLDL synthesis depletes a regulatory hepatic cholesterol pool and, by release of feedback inhibition, increases the activity of the enzyme (72, 74). This association is specific to triglyceride-rich lipoproteins since the hepatic output of cholesterol in high density lipoprotein (the other major lipoprotein secreted by the liver (68)) is apparently independent of changes in triglyceride metabolism and microsomal HMG CoA reductase activity (72).

Effects of interruption of the enterohepatic circulation

Interruption of the enterohepatic circulation of bile acids can be achieved either medically by administration

of bile acid sequestrant resins or surgically by ileal bypass. Both treatments increase fecal bile acid excretion and induce a large, continuous drain on the bile acid pool. Other changes in hepatic and lipoprotein metabolism are ostensibly secondary to this event.

Effects on bile acid and cholesterol synthesis

The effects of bile acid sequestrants on hepatic sterol and bile acid secretion in man have been intensively studied in a number of laboratories. Early investigations by Moore, Crane, and Frantz (30), Moutafis and Myant (75) and Grundy, Ahrens, and Salen (32) demonstrated that the increased fecal loss of bile acids during drug therapy was balanced by stimulation of bile acid synthesis. Treatment of hypercholesterolemic subjects with the resin caused a 3- to 10-fold increase in bile acid secretion and promoted the conversion of cholesterol to bile acids (32). Further studies, however, revealed that each primary bile acid responds differently to the treatment. In normal (76) and type II hyperlipoproteinemic subjects (77), cholestyramine produces a 3-fold increase in cholic acid synthesis while that of chenodeoxycholate doubles. In consequence, the low ratio of cholate/chenodeoxycholate production, which is typical of type II hyperlipoproteinemic subjects (77), is restored to normal by the treatment. Patients with the type IV hyperlipoproteinemic phenotype, on the other hand, show a more modest response to cholestyramine. They already reportedly have elevated cholic acid production rates and any additional drug-induced increment in bile acid synthesis occurs principally in chenodeoxycholate (77). However, these data on hypertriglyceridemics should be interpreted with caution since methodological difficulties may confound the issue. Specifically, Davidson et al. (78) have evaluated the isotopic tracer technique used in the above study by comparing it with the standard sterol balance procedure. They found that, although both correlated well in normotriglyceridemic subjects, they were discrepant in patients with elevated triglycerides. In fact, in contrast to the raised bile acid production rates obtained in this group by the radioisotopic procedure (see above), balance studies failed to detect any anomaly in bile acid synthesis.

Following administration of sequestrant resins to animals, there is a general stimulation of both cholesterol and bile acid synthesis in the liver. This is reflected in changes in the activity of the rate-limiting enzymes of these metabolic pathways, HMG CoA reductase and 7α-hydroxylase, which both increase during cholestyramine treatment (33, 55, 56, 79). 7α-Hydroxylase appears to rise in response to a release of the feedback inhibition normally exerted by bile acids returning to the liver in portal blood and this increase in activity is, at least in part, independent of changes in substrate cholesterol concentration (26, 55). The mechanism of HMG CoA reductase stimulation, as may be appreciated from the above discussion, is not clear. Mitropoulos, Knight, and Reeves (80) could find no alterations in the phosphorylation status of the enzyme during cholestyramine therapy in rats, nor are there any differences in the immunological characteristics of the enzyme purified from normal and cholestyramine-fed animals (81). In consequence, it has been suggested (80) that increased conversion of cholesterol to bile acids during resin therapy depletes a microsomal regulatory pool of cholesterol and releases feedback inhibition of the reductase enzyme.

Effects on triglyceride and lipoprotein synthesis

In addition to its influence on biliary steroid metabolism, interruption of the enterohepatic circulation leads to changes in plasma cholesterol and triglyceride turnover. Using radiolabeled sterol, Clifton-Bligh, Miller, and Nestel (82) followed the early effects of colestipol treatment on plasma lipoprotein cholesterol specific activity decay curves and found evidence for an increased flux of newly synthesized cholesterol into plasma VLDL within a day of initiating therapy.

The synthesis of triglyceride is also changed. Nestel and Grundy (83) observed a 24% rise in the triglyceride synthetic rate and an increase in plasma VLDL triglyceride immediately following biliary diversion in type IV subjects. These findings suggest that triglyceride, as well as cholesterol production in the liver, is integrated in some way with bile acid metabolism. Angelin and coworkers have investigated this interrelationship in a series of studies (84–86). They found that, in type II and type IV hyperlipoproteinemic subjects, triglyceride and bile acid (particularly cholate) synthetic rates are strongly positively correlated. In seeking a link between VLDL (triglyceride) and cholate production, it has been suggested (86) that each may draw substrate from a common pool of newly synthesized cholesterol, expansion of which would stimulate both metabolic pathways. This is supported by the observations, first, that HMG CoA reductase activity and de novo cholesterol synthesis is high in subjects oversynthesizing VLDL (73, 87) and, second, that newly synthesized cholesterol contributes more to cholate than chenodeoxycholate production (88). As summarized in **Table 1**, other perturbations of bile acid metabolism also affect triglyceride turnover. Cholestyramine ingestion causes a stimulation of triglyceride synthesis in type II subjects, while chenodeoxycholic acid feeding has the opposite effect (85).

What possible mechanisms, then, can be offered in explanation of the above sequelae to bile acid sequestrant therapy? In several studies (74, 84–86) the "regulatory" effects of hepatic cholesterol pools have been

TABLE 1. Interrelationships between bile acid, cholesterol and lipoprotein metabolism in the liver

Subject/Treatment	Triglyceride Synthesis	Cholic Acid Synthesis	Chenodeoxycholic Acid Synthesis	Cholic Acid Pool	Chenodeoxycholic Acid Pool	HMG-CoA Reductase	Conversion of Cholesterol to Bile Acids
Type II/cholic acid			↓	↑	↓		↓
Type II/chenodeoxycholate	↓	↓		↓	↑	↓	↓
Type II/cholestyramine	↑	↑	↑	↑	↓	↑	↑
Type IV/none	↑	↑		↑	→ or ↑	↑	↑
Type IV/cholestyramine	→ or ↑		↑			?[a]	↑

[a] Effect not known.
This table is constructed from data in the following references: 52, 57, 58, 73, 77, 83–87, and 178.

invoked to link triglyceride, cholesterol, and bile acid metabolism. Following this argument, an increase in cholesterol conversion to bile acids with stimulation of 7α-hydroxylase leads to depletion of one regulatory pool of microsomal cholesterol that releases feedback inhibition of HMG CoA reductase and promotes cholesterol biosynthesis. This, in turn, expands a second pool of intracellular cholesterol, stimulating cholate (and chenodeoxycholate) synthesis, VLDL formation, and triglyceride production. However, there are problems with this proposal inasmuch as the same sterol pool that is substrate for 7α-hydroxylase (leading to cholate synthesis) is required both to increase and decrease. Therefore, while we cannot afford to ignore the potential importance of hepatic cholesterol levels, other regulatory factors may play a part in coordinating triglyceride, cholesterol, and bile acid metabolism. One candidate in this regard would be chenodeoxycholic acid which, when fed to humans (Table 1), decreases cholic acid synthesis (57), suppresses hepatic HMG CoA reductase activity (52), and lowers triglyceride production (85), the last being possibly due to inhibition of phosphatidic acid phosphatase (89). The other primary bile acid, cholic acid, has much less influence on cholesterol and triglyceride metabolism (52, 86, 89) and so these effects appear to be specific to chenodeoxycholate. Bile acid sequestrant therapy lowers chenodeoxycholate and it is tempting to speculate that some of the effects of this treatment are secondary to relative chenodeoxycholate deficiency. Of interest in this regard are the recent findings of the U.S. National Cooperative Gallstone Study showing that feeding of chenodeoxycholate has a significant hypotriglyceridemic effect (90). The more blunted response of type IV hyperlipoproteinemic subjects to bile acid sequestrant therapy (77, 85) may be due to the fact (Table 1) that many of the hepatic metabolic changes associated with cholestyramine therapy are already a feature of these subjects' lipoprotein and bile acid metabolism (73, 77, 85, 86). Further substantial increases in triglyceride and cholic acid synthesis, for example, may not be possible.

The sequestrant resin-induced changes in lipoprotein cholesterol synthesis which are discussed above appear to be specific for VLDL. HDL cholesterol levels in the plasma do not change with cholestyramine or colestipol therapy (82, 91) and in the metabolic studies of Clifton-Bligh et al. (82) there was no rapid influx of cholesterol into HDL similar to that observed for VLDL. However, since we (92) have noted that bile acid sequestrants do increase the synthesis of apolipoprotein A-I (the major HDL protein), we cannot exclude the possibility that the drug promotes hepatic HDL formation.

II. EFFECTS OF INTERRUPTION OF THE ENTEROHEPATIC CIRCULATION ON CHOLESTEROL, BILE ACID, AND LIPOPROTEIN METABOLISM IN THE GUT

Interruption of the enterohepatic circulation may, depending on its extent, result in changes in the composition and secretion rate of bile and produce alterations in the intestinal absorption of dietary fat. Dowling and co-workers (93), using a model system designed to permit controlled biliary diversion, have examined bile acid secretion in rhesus monkeys. They found that the animals could compensate for up to 20% biliary loss and maintain a normal bile acid pool size by increasing their production rate of the acids. However, when the loss of bile was greater than 33%, the compensatory synthetic capacity was exceeded and the bile acid secretion rate and pool size fell. This resulted in an increase in fecal fat excretion that correlated inversely with the bile acid pool.

Bile composition and lipid absorption following bile acid sequestrant resin therapy and ileal bypass

The "pharmacologic" biliary diversion achieved by administration of bile acid sequestrant resins does not normally broach the above compensatory threshold. Cholestyramine treatment of rats (53) does not change their bile secretion rate nor its content of bile acid, phos-

pholipid or cholesterol. In normolipemic man the total bile acid pool is not reduced significantly by cholestyramine administration (76, 94); in fact, in type II hyperlipoproteinemic subjects, it can even be raised (77). This explains why moderate cholestyramine ingestion (up to 16 g/day) does not predispose to fat malabsorption (95, 96), although at higher intake levels (above 32 g/day) there is a rise in fecal fat excretion (95, 97). In an attempt to measure directly its effect on intestinal cholesterol uptake, McNamara et al. (98), examined absorption of the sterol in a large number of hyperlipidemic subjects who were receiving cholestyramine (16 g/day). They were surprised to find that, in general, the patients had increased cholesterol absorption. However, when they measured uptake immediately following ingestion of the drug, they observed it had fallen, indicating a transient interference with cholesterol absorption. Fat malabsorption is a much more common sequel to ileal bypass surgery which, it has been reported, produces a sustained reduction in cholesterol absorption (23, 29, 99) and an increase in neutral steroid excretion (23, 31, 32, 99), although the latter is not a universal finding (100). This is likely to derive from a combination of effective loss of absorptive surface associated with a severely compromised enterohepatic circulation, which would lead to decreased efficiency of the remaining functional intestine. The reduced cholesterol absorption may contribute to the greater hypocholesterolemic action of ileal bypass surgery compared to resin treatment (99).

Bile acid sequestrant therapy markedly alters the composition of bile. While total bile acid content does not change (76, 77, 94), cholestyramine produces a dramatic increase in biliary cholate and a fall in chenodeoxycholate in most subjects (76, 77, 101, 102) with the notable exception (Table 1) of hypertriglyceridemic patients (77). These changes, as has been observed earlier, are partly due to the differential effect of the drug on the synthesis of the bile acids. But, in addition, cholestyramine causes a marked increase in the fractional removal rate of chenodeoxycholate, accounting for the drop in its pool size despite its increased synthetic rate (76, 77). The much greater rise in the fractional clearance rate of chenodeoxycholate (approximately 5-fold) over cholate (approximately 2-fold) (76, 77) probably reflects the higher affinity of the resin for di- than for tri-hydroxy bile acids (103). This phenomenon may also account for the fall in biliary deoxycholate (102) during drug treatment. Available evidence in animals (53) and man (102) indicates that the proportions of the other biliary constituents, phospholipid and cholesterol, do not change during cholestyramine therapy. Similarly, Moore, Crane, and Frantz (30) and others (32, 100) found no rise in the excretion of neutral steroids when treatment was instituted. Thus, except for isolated cases (104), the lithogenicity of bile does not appear to increase on sequestrant resin therapy nor is enhanced gallstone formation a feature of this drug treatment (104) or of ileal bypass surgery (23, 99). There are, however, subtle changes in biliary cholesterol metabolism in that, during sequestrant resin treatment, the proportion of newly synthesized sterol increases (32, 105).

Cholesterol and lipoprotein synthesis in the gut (Fig. 3)

Both liver and intestine are active in the synthesis of cholesterol so that, taken together, they account for the majority of corporeal cholesterol production (1, 5). However, regulation of cholesterol synthesis differs in each. In the rat, bile acids, in themselves, do not appear to affect liver sterol synthesis (54) but depress this activity in the intestine (1). Cholesterol feeding, on the other hand, inhibits hepatic rather than intestinal cholesterologenesis in that species (1, 59, 106). However, in hamsters (107), rabbits (108), and monkeys (1) dietary cholesterol, as well as bile acids, down-regulates intestinal sterol production. Human intestinal cholesterol synthesis has been examined in detail by Dietschy and Gamel (109). They found that, as in animals (1), there were regional variations in the rate of cholesterol synthesis, the ileum being most active. Fasting suppressed sterol production while cholesterol feeding had no effect. Consequently, bile acids appear to be the prime regulators of intestinal cholesterol synthesis and HMG CoA reductase activity (1, 59, 109). Cholestyramine feeding, ileal bypass surgery, or total biliary diversion, which all lower the concentration of bile acids in intestinal mucosal cells, lead to increases in sterol production by the gut (1, 32, 109). Panini et al. (110) have recently shown that the cholestyramine-induced rise in ileal HMG CoA reductase activity is particularly evident in the apical cells of the villi which are normally associated with cholesterol and bile salt absorption. Similarly in man, Grundy and co-workers (32) were able to obtain intestinal biopsies during cholesterol turnover studies and showed that acute feeding of cholestyramine caused a decrease in the specific activity of mucosal cholesterol below that of plasma or biliary cholesterol, indicating increased sterol synthesis in that tissue.

Human and animal intestine secrete lipoproteins in the density and size ranges of chylomicrons, VLDL, and HDL (**Fig. 3**). In the fasting state, apolipoprotein A-I is continuously synthesized and secreted (111), presumably in association with nascent HDL (112). A fat load triggers apolipoprotein B production and causes it to appear, with apolipoprotein A-I, in mesenteric lymph chylomicrons (2, 68, 113, 114). When these reach the

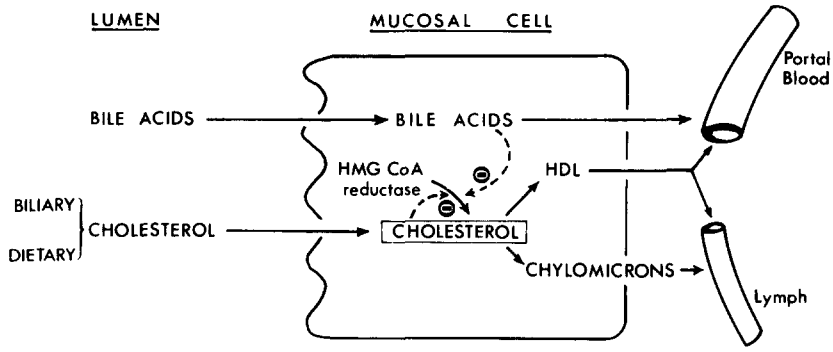

Fig. 3. Intestinal steroid metabolism. The intestinal mucosal cell acquires cholesterol from the lumen or by direct synthesis. The sterol is exported into the portal blood and chylolymphatic duct in association with HDL and chylomicrons. Endogenous sterol production is controlled by the influence of intracellular bile acids and cholesterol on HMG CoA reductase.

bloodstream, their exposure to plasma lipoproteins results in substantial changes in their lipid and protein complement; apolipoproteins A are lost and apoC and apoE are gained (68, 115). Wu and Windmueller (116) have shown that the rat small intestine synthesizes approximately 15% of plasma apolipoprotein B and 50% of apolipoprotein A-I; studies in chyluric patients have shown that the human intestine also produces substantial quantities of these apoproteins (114), although they may not be identical to their hepatic counterparts (117, 118).

When fasting rats are treated with cholestyramine or undergo biliary diversion, formation of tryglyceride-rich lipoprotein in intestinal mucosal cells is reduced (119) as is the concentration of triglyceride and cholesterol in thoracic duct lymph (120). Recent studies by Bearnot et al. (121) repeated these earlier observations and also found that biliary diversion caused an increased output of HDL and apolipoprotein A-I into rat intestinal lymph. These latter observations may pertain to our finding of a rise in plasma HDL and apoprotein A-I synthetic rate in type II subjects treated with cholestyramine (92). The animal studies mentioned above (119, 120) were performed in the fasting state and it is not yet known what effect sequestrant therapy and ileal bypass have on intestinal lipoprotein synthesis during fat absorption.

III. CHANGES IN PLASMA LIPOPROTEIN METABOLISM FOLLOWING INTERRUPTION OF THE ENTEROHEPATIC CIRCULATION

Measurable changes occur in the structure, composition and metabolism of the plasma lipoproteins within hours of ingestion of a pharmacologic dose of bile acid sequestrant resin. All lipoproteins, chylomicrons, VLDL, LDL, and HDL are affected to some extent and alterations are also detectable in the intravascular enzymes (e.g., lipoprotein lipase and LCAT) involved in plasma lipoprotein metabolism (**Fig. 4**).

The enterohepatic circulation and plasma lipid turnover

Interruption of the enterohepatic circulation by bile acid sequestrant therapy or ileal bypass surgery produces a stable, persistent reduction of plasma cholesterol in most human subjects by affecting both the synthesis and catabolism of the sterol. In a study of hypercholesterolemic subjects, Goodman and Noble (122) found increased rates of production and elimination of cholesterol during cholestyramine therapy but no significant change in the size of the "rapidly-miscible" pool which includes sterol in plasma, red blood cells, liver, and intestine. Further studies using colestipol produced similar results (105, 123). In the investigation by Miller, Clifton-Bligh, and Nestel (105), a significant correlation was observed between the increase in cholesterol catabolism from the rapidly miscible pool and fecal bile acid excretion. This finding provides further evidence for the proposal that bile acids are the major route of enhanced cholesterol elimination during sequestrant resin therapy. Ileal bypass surgery produces changes in cholesterol synthesis and catabolism comparable to those that follow bile sequestrant therapy (31). However, such changes are not invariably observed. Moutafis and Myant (75) have reported a study of two ostensibly homozygous familial hypercholesterolemic individuals who responded to cholestyramine with a marked increase in bile acid excretion but no change in plasma cholesterol or in the exchangeable cholesterol pool. Furthermore the half-life of radioactive cholesterol decay in their plasma was unaffected by treatment, while in other patients, as noted above, this parameter fell during drug therapy. Similar resistance to drug treatment and ileal bypass has also been observed in other apparently

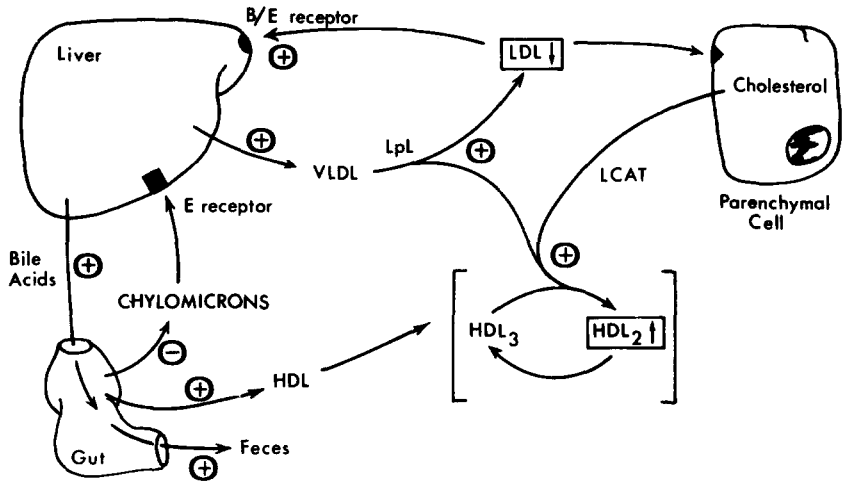

Fig. 4. Effects of bile acid sequestrants or ileal bypass surgery on plasma lipoprotein metabolism. Interruption of the enterohepatic circulation by the above therapy drains the hepatic bile acid pool and leads to the following events: 1), stimulation of VLDL secretion into the plasma; 2), promotion of VLDL → LDL conversion; 3), enhanced hepatic uptake of plasma LDL via high affinity receptors; 4), suppression of triglyceride-rich lipoprotein secretion by the gut (in the fasting state); 5), increased intestinal HDL secretion; 6), a rise in the plasma HDL_2/HDL_3 ratio, possibly secondary to activation of LpL and/or LCAT.

homozygous familial hypercholesterolemic patients (24, 32).

The bile acid sequestrants and ileal loop surgery have variously been reported to reduce (99, 124, 125), increase (82, 126), or leave unchanged (28, 91, 127) the plasma triglyceride level, reflecting the stimulatory effect which interruption of the enterohepatic circulation has on both the synthesis (see Section 1 above) and catabolism of plasma triglycerides (85, 125). Angelin and co-workers (85) observed that cholestyramine therapy increases the triglyceride fractional clearance rate in normotriglyceridemic (type IIa) but not in hypertriglyceridemic (type IV) subjects, while chenodeoxycholic acid feeding lowered this parameter in both groups. Since the catabolism of lipoprotein triglyceride occurs mainly in peripheral tissues by the action of lipoprotein lipase (2), it is possible that this enzyme is activated, albeit indirectly, during cholestyramine treatment or ileal bypass. This proposal gains some support from the observation that jejuno-ileal bypass, used as a treatment for obesity, promotes the clearance of synthetic triglyceride emulsions from the plasma (125). However, we do not yet know of any mechanism that links peripheral lipoprotein lipase activity with hepatic cholesterol and bile acid synthesis, and the changes observed in this enzyme may reflect an indirect effect of interruption of the bile acid circulation on the structure or composition of its triglyceride rich substrate.

The enterohepatic circulation and VLDL metabolism

Several studies have now documented an acute stimulation of synthesis and secretion of cholesterol (82) and triglyceride (83, 126) into VLDL following interruption of the enterohepatic circulation, and Witztum, Schonfeld, and Weidman (128) have demonstrated that the composition of VLDL changes during the first month of colestipol therapy. They found that in initially normotriglyceridemic patients there were transient rises in the triglyceride concentration and triglyceride/cholesterol and triglyceride/protein ratios in the VLDL density range that were associated with an increased particle size; these parameters reverted to near baseline values within a month. On the other hand, the hypertriglyceridemic subjects studied by Miller and Nestel (129) showed an equally rapid rise in VLDL lipids that did not resolve with time but was maintained at a level correlated with the initial plasma triglyceride concentration. Since increased triglyceride production is probably a long term feature of resin therapy (85), it would appear that those subjects who show a transient drug-induced hypertriglyceridemia can compensate for the enhanced triglyceride synthesis by increasing VLDL catabolism (85), while others, whose hypertriglyceridemia persists, possibly have saturated or defective VLDL removal mechanisms (as has been reported for type IV and type V patients (130)).

Despite our apparent understanding of the response of VLDL lipids to enterohepatic interruption, information is still lacking on the turnover of VLDL apoproteins in this situation. Since bile acid sequestrants increase VLDL triglyceride production, it might be expected that they would also have a similar effect on VLDL protein. This, however, does not necessarily follow. During resin treatment the triglyceride/protein ratio in VLDL increased (128), suggesting the triglyc-

eride secretion into the particle might rise without change in the production rate of its apoprotein component.

Effects of interruption of the enterohepatic circulation on LDL

One important objective of interrupting the enterohepatic circulation either by surgical or pharmacologic means is to lower circulating LDL levels in hypercholesterolemic patients with the expectation that this will slow or reverse the atherosclerotic process. This treatment has quantitatively and consistently been the most effective of the various cholesterol-lowering maneuvers. Patients whose compliance to the drug regimen is good can achieve 25–35% reductions in LDL cholesterol (27, 91, 127) and ileal bypass surgery can be even more effective, producing decreases greater than 45% (99, 100). However, this rule does not always apply. For example, in gross hypertriglyceridemia a transient rise in LDL lipids has been observed (129), possibly due to a much increased flux of VLDL into the plasma and stimulated conversion of VLDL to LDL.

The fall in LDL cholesterol that follows interruption of the enterohepatic circulation derives in part from a reduction in particle number (detected as a fall in apolipoprotein B, the major LDL protein (131)) and partly from a change in the composition of the lipoprotein. Several studies have recorded a significant fall in the cholesterol/phospholipid and cholesterol (both free and esterified)/triglyceride ratios in LDL in response to bile acid sequestrant therapy (82, 126, 127, 129, 131) or jejuno-ileal bypass surgery (125, 132). In addition, the cholesterol/apolipoprotein B ratio in the particle falls (125, 131) and, since the content of apoB per LDL is relatively constant (2, 133), this indicates a reduction in the cholesterol carried per lipoprotein particle.

Several mechanisms may be proposed to account for these effects. They may derive from secretion of VLDL with a lowered cholesterol/apolipoprotein B ratio which would then be converted (2) to similarly depleted LDL. Alternatively, the mechanism suggested by Sniderman et al. (134), whereby cholesterol is removed selectively from the particle in the splanchnic bed without apparent loss of apolipoprotein B mass, may be enhanced by interruption of the enterohepatic circulation. A third possible means of modifying the cholesterol content of the LDL particle is by inter-lipoprotein exchange of cholesteryl ester and triglyceride with VLDL (135). While this may have an effect in the early treatment phase when VLDL is elevated and has a reported increase in the triglyceride/cholesterol ratio (128), it cannot apparently operate later, at least in normotriglyceridemic individuals, when the altered LDL composition persists in the face of near normal VLDL levels and triglyceride/cholesterol ratios. This is borne out by the report of Vessby et al. (125) who found that after jejuno-ileal bypass in normolipemic obese subjects, LDL showed the same compositional changes as described above even although VLDL had neither risen in concentration nor had altered its triglyceride/cholesterol ratio in the long term.

LDL apolipoprotein B metabolism changes in response to bile acid sequestrant therapy. In an early study, Levy and Langer (136) reported that administration of cholestyramine to type II hyperlipoproteinemic subjects increased the fractional clearance of LDL apoprotein from their plasma without affecting its rate of synthesis. We have employed chemically modified LDL to further this investigation. Treatment of the lipoprotein with 1,2 cyclohexanedione abolishes its ability to interact with a high affinity receptor on cell membranes (137). Consequently, this material can be used as a tracer of receptor-independent LDL catabolism in vivo (12, 138–140) and, when its plasma clearance is compared to that of native LDL, we obtain a measure of LDL receptor activity in the recipient. Using this approach we found that heterozygous familial hypercholesterolemic (FH) subjects clear approximately half the normal fraction of the circulating LDL pool via the receptor pathway (138) while in a homozygous patient there was little, if any, detectable receptor-mediated LDL catabolism (140), as would be predicted from the cell culture studies of Goldstein and Brown (10, 12). Cholestyramine treatment of the heterozygous FH subjects increased specifically (by 110%) LDL catabolism by the receptor pathway and reduced its net uptake by receptor-independent routes (141). Thus, altered cell membrane receptor activity appears to be responsible for the hypocholesterolemic action of this drug. A study by Thompson et al. (140) of normal subjects whose LDL pool size had been reduced by cholestyramine or plasma exchange supports the contention that the increase which we observed in receptor-mediated LDL fractional clearance is the cause rather than the consequence of lowered circulating LDL levels. These findings explain why cholestyramine is generally ineffective in reducing plasma cholesterol in homozygous FH since subjects with this condition lack LDL receptors; response to sequestrant therapy occurs only in those who exhibit residual LDL receptor activity on their skin fibroblasts (142).

In a further study (143) in rabbits, we attempted to locate those tissues whose receptor activity is promoted by cholestyramine. As was observed in the FH heterozygotes, the drug lowered the circulating LDL level in the rabbits by promoting its clearance into the receptor pathway. Among all the tissues examined, only the liver demonstrated a specific increase (of 83%) in LDL re-

ceptor activity. Kovanen et al. (144) reached similar conclusions by directly measuring LDL receptor activity in liver membranes isolated from control and colestipol-fed dogs. Liver membrane receptor activity in the dogs (144) and hepatic receptor-mediated LDL uptake in the rabbits (143) correlates strongly and positively with the plasma LDL fractional clearance rate measured off and on drug treatment, suggesting that hepatic LDL receptor activity is a major determinant of the plasma LDL, and hence cholesterol, concentration. Recently, Hui, Innerarity, and Mahley (145) have been able to distinguish two lipoprotein binding activities on liver cell membranes from young dogs. One, termed the apoB/apoE receptor, recognizes both apoB- and apoE-containing lipoproteins, while the other, the apoE receptor, selectively binds apoE-rich lipoproteins such as chylomicron remnants and a subfraction of HDL called HDL_c (146). The apoB/apoE receptor is inducible by cholestyramine while the apoE receptor is relatively refractory to this treatment (145). This would explain earlier findings (147) that the rate of uptake of chylomicron remnant cholesterol by the rat liver remains unchanged during cholestyramine therapy.

Mechanism of the hypocholesterolemic effect of interruption of the enterohepatic circulation

The above observations shed light on the mechanism by which changes in hepatic cholesterol and bile acid metabolism are linked to the alterations in plasma lipoprotein turnover that follow interruption of the enterohepatic circulation. In fibroblasts and other cell types including hepatocytes, cholesterol depletion increases HMG CoA reductase activity, promotes cholesterol synthesis, and raises the number of LDL receptors on the cell membrane (4, 10, 40). It follows that similar changes should occur in liver cells in vivo in response to an expanded cholesterol requirement. Resin therapy and ileal resection induce a drain on the hepatocyte cholesterol and bile acid pools, leading to stimulation of HMG CoA reductase activity and promotion of cholesterol synthesis. The cell, according to the fibroblast model (4, 10), should preferentially use exogenous cholesterol and, in an attempt to do so, increases its expression of LDL receptors. If extrapolation from the rabbit and dog models can be trusted, then the above mechanism appears to apply to humans fed bile acid sequestrant resins. Certainly, in the reverse situation where chenodeoxycholic acid feeding reduces the hepatocyte requirement for cholesterol, plasma LDL cholesterol rises (90), possibly as a result of LDL receptor down-regulation. However, there are unexplained facets to the argument. For example, why should the liver, in response to cholestyramine treatment, secrete increased amounts of newly synthesized cholesterol in association with VLDL (see Section I) at a time when it is actively involved in uptake of the sterol in order to restore its depleted cholesterol pool? One possible explanation may reside in the finding that cholesterol, which is destined for different metabolic products, appears to be located in distinct pools within the hepatocyte. Thus, ingested LDL cholesterol may enter a separate pool from that being incorporated into VLDL prior to secretion into the circulation. Secondly, if the hepatocyte behaves according to the fibroblast model, why does it indulge in endogenous cholesterol production when it could acquire all the cholesterol it needs from plasma lipoproteins? Indeed, when a potent inhibitor of HMG CoA reductase is added to the bile acid sequestrant regimen (144), hepatic LDL receptor activity rises markedly, showing that the liver has the capacity for higher rates of lipoprotein uptake. It may be that the complexity of cholesterol metabolism in the hepatocyte requires cholesterol biosynthesis to rise in response to the bile acid drain. Or, as discussed above, it is possible that HMG CoA reductase activity rises because the concentration of an inhibitor (e.g., chenodeoxycholate) is reduced by interruption of the enterohepatic circulation.

If it is accepted, first, that LDL is catabolized as a unit as would appear to be the case in cultured cells (10), and secondly, that all of the drug-induced increment in receptor-mediated LDL catabolism occurs in the liver (143), it is possible to estimate the amount of extra cholesterol assimilated by that organ during treatment of FH heterozygotes with cholestyramine (141). It transpires that, on average in these patients, receptor-mediated LDL catabolism accounted for 280 mg/day of plasma cholesterol elimination before treatment and 380 mg/day on resin therapy. Thus, an additional 100 mg/day of cholesterol was available to meet the increased sterol requirement in the liver. Bile acid output, however, rises on average by 1–1.3 g/day as a result of resin therapy (30, 32, 100, 105) and so the increased acquisition of sterol from the receptor path fulfills only about 10% of the expanded demand. Interestingly, this value is of the same order as that calculated by Schwartz and co-workers (50) as the total contribution made by plasma esterified cholesterol to bile acid synthesis in patients with complete biliary diversion. Therefore, although stimulation of the LDL receptor pathway appears to be a prerequisite of the hypocholesterolemic action of the bile acid sequestrant resins, it only makes a minor contribution to the increased demand for sterol that these drugs make on the liver.

Effect of interruption of the enterohepatic circulation on HDL and LCAT

It is now widely held that HDL and LCAT operate in combination to promote removal of cholesterol from

tissues (148). Since we employ lipid-lowering agents like the bile acid sequestrant resins in the hope of achieving this objective, the effects of such treatments on HDL composition and metabolism have been examined with some interest.

Studies measuring only lipoprotein cholesterol levels commonly failed to show a change in HDL during resin therapy (82, 91, 127) or following jejuno-ileal bypass surgery (125, 132). However, when this fraction was examined in greater detail, it became clear that both its composition and metabolism are affected by such treatment. For example, administration of sequestrant resins to type II hyperlipoproteinemic subjects increased the ratio of the major HDL apoproteins, apolipoproteins A-I/A-II, in the plasma. This has been variously reported to result from a selective rise in plasma apolipoprotein A-I on the one hand (92) and a net fall in apolipoprotein A-II on the other (131).

We observed during cholestyramine treatment an increase of 84% in the plasma HDL_2 concentration with no change in HDL_3 (149); and ileal bypass surgery has been reported to produce a similar effect (150). Since HDL_2 has a higher apolipoprotein A-I/A-II ratio than HDL_3 (151), this drug-induced alteration in subfraction distribution should be accompanied by a rise in plasma apolipoprotein A-I/A-II due to a net increase in apolipoprotein A-I. Two possible factors could contribute to the increment in HDL_2. It might arise either from increased secretion of new particles into this density range or from changes in HDL metabolism leading to HDL_2 accumulation without the need for de novo synthesis. The first suggestion is supported by the observation that the higher levels of apolipoprotein A-I that are seen during resin therapy result from increased synthesis (92). Indeed, in a preliminary report Bearnot et al. (121) have indicated that the rat intestine secretes more apolipoprotein A-I in response to biliary diversion. But, since both liver and gut elaborate HDL precursor particles containing this apoprotein (68), each may contribute to this new synthesis.

In an alternative hypothesis, the level of HDL_2 may rise in the plasma of sequestrant resin-treated subjects following changes in the activities of lipoprotein lipase and LCAT. Lipoprotein lipase activity does appear to be enhanced by cholestyramine feeding (85) or following jejuno-ileal bypass (125). This may, by increasing the rate of VLDL catabolism, accelerate transfer of surface components from VLDL to HDL_3, with the formation of HDL_2 by the mechanism suggested by Patsch et al. (152). Likewise, LCAT can be activated by cholestyramine (see below) and also appears to be involved in the $HDL_3 \rightarrow HDL_2$ interconversion (153), but activation of these enzymes alone cannot account for the observed increase in apolipoprotein A-I synthesis that accompanies the rise in plasma HDL_2.

Clifton-Bligh, Miller, and Nestel (154) first showed that cholesterol esterification is stimulated by bile acid sequestrant therapy, but when this finding was re-examined (155, 156) it became apparent that the possible increase in LCAT activity only occurred in those subjects whose triglyceride levels were raised by treatment. In normal and type II hyperlipoproteinemic subjects who exhibited no long-term increase in plasma triglyceride, LCAT activity was unaffected (155). This is consistent with the recent observation by Fielding and Fielding (157) that LCAT activity appears to be rate-limited by the amount of lipoprotein present to assimilate the cholesteryl ester produced by the enzyme. Cholesterol esterification is thought to occur on HDL (148) with transfer of the product ester via cholesteryl ester transfer protein (135) to the acceptors, VLDL and LDL. If the activity of the enzyme in vivo is limited by the availability of these acceptor lipoproteins and by their saturation with cholesteryl ester (157), then, when the stimulation of triglyceride synthesis by resin therapy leads to increased circulating VLDL levels, LCAT activity should, and does, rise (154, 155, 156) in proportion to the increment in acceptor VLDL.

IV. BILE ACID SEQUESTRANT THERAPY AND ILEAL BYPASS SURGERY: CLINICAL IMPLICATIONS

Therapeutic interruption of the enterohepatic circulation is used to lower LDL cholesterol, with the expectation that this will confer on the recipient a reduced risk of developing atherosclerosis and ischemic heart disease. However, we do not yet know whether this expectation is realistic, since the metabolic regulatory factors influenced by intervention may not be the same as those that operate to keep LDL low in free-living, low risk individuals. The preceding discussion on the mechanism of action of bile acid sequestrants and ileal bypass surgery permits some observations on this topic.

Turnover studies in normal and hyperlipidemic individuals have shown that, while synthesis may play a role (158), the size of the circulating LDL pool is largely determined by its rate of catabolism as measured by the fractional catabolic rate (159–161). Moreover, detailed examination of the receptor-dependent and independent components of this catabolic process (138, 141) suggests that variation in LDL clearance is determined mainly by the receptor pathway, particularly in the liver (143, 144). If the observation of Hui et al. (145) that the activity of canine hepatic LDL receptors falls with

age, applies to humans then this may explain why LDL levels in man rise as he get older (162). Bile acid sequestrants in animals (143–145) and probably also in man (141) activate LDL receptors in the liver and promote catabolism of this lipoprotein by the pathway that apparently operates in the normal situation to moderate circulating LDL levels. Stimulation of hepatic receptors would therefore seem a reasonable means of achieving a reduction in plasma LDL. Moreover, cholestyramine suppresses LDL cholesterol clearance by the receptor-independent route (141) that has been implicated in the pathogenesis of atherosclerosis (163) and so bile acid sequestrants, on theoretical grounds, confer a double benefit when used as hypocholesterolemic agents.

Following their introduction into clinical practice, the bile acid sequestrant resins cholestyramine and colestipol soon became established as useful hypocholesterolemic drugs (91, 127). However, they have not yet been shown to reduce the incidence of ischemic heart disease in a population, although some studies have provided evidence that they may slow or even reverse the progress of the atherogenic process (164, 165). A major trial (22) is presently under way to examine their value in the treatment of initially asymptomatic hypercholesterolemic men.

The potential side effects of the resins on nutrient and vitamin uptake are not a matter for concern (166, 167) although sequestrants do appear to interfere with the intestinal absorption of certain other drugs (168, 169). Their main problem is that of poor patient acceptability since they are relatively unpalatable and may produce constipation and a feeling of gastric fullness (91, 127). Indeed, many trials using the drugs have suffered due to problems of patient compliance. Nevertheless, they still constitute the most useful hypocholesterolemic agents available at the present time. For a consideration of their therapeutic utility, the reader is referred to a number of recent review articles (91, 127, 166, 167). Clinical experience with ileal bypass surgery as a hypocholesterolemic maneuver is also covered in a number of articles (23, 24, 99, 100).

Combined drug therapy for hypercholesterolemia

Examination of the mechanism of action of the bile acid sequestrants has led to the suggestion that their hypolipidemic effect may be enhanced by addition of a second drug designed to prevent the rise in plasma triglyceride and the increase in cholesterol synthesis that often follows the introduction of resin therapy. Clofibrate (170, 171) and nicotinic acid have been used in this regard, and the last proved to be particularly valuable, producing dramatic improvements in several studies (172–174). LDL cholesterol in heterozygous familial hypercholesterolemic subjects is often normalized, HDL rises, and plasma triglyceride levels fall to below baseline values in response to this drug combination. We have studied (173) the effects of combined cholestyramine/nicotinic acid therapy on a number of atherosclerotic risk factors in a small group of FH heterozygotes. Their treatment improved the risk index ratios of HDL/LDL cholesterol, apolipoprotein A-I/B, and HDL_2/HDL_3, reflecting complementarity in the mechanisms of action of the two drugs. Nicotinic acid, by decreasing free fatty acid flux to the liver (175), inhibits hepatic VLDL and triglyceride synthesis (176), probably suppresses HMG CoA reductase (177), and so prevents the rise in cholesterol and triglyceride synthesis that is a feature of sequestrant resin therapy. This combination offers a medical alternative to ileal bypass in the treatment of severe heterozygous FH and, in subjects with this condition, Kane and co-workers (172) were able to demonstrate significant decreases in tendon xanthoma size after 8 months of combined therapy.

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