special report

Workshop on regulation of hepatic cholesterol and bile acid metabolism

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The National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) sponsored a workshop on the regulation of hepatic cholesterol and bile acid metabolism. The meeting was held in Bethesda, MD, October 19–21, 1979. Approximately 80 people including prominent scientists in the field and young investigators participated. Many investigators who were unable to attend the workshop have expressed an interest in the proceedings, and for this reason, we have attempted to summarize the highlights of each of the five half-day sessions.

I. Uptake of lipoproteins by the liver (Dr. John A. Glomset, Chairperson)

The major purpose of this session was to examine the mechanisms by which different lipoprotein species are taken up by the liver. Unfortunately, most studies to date have been carried out in the rat, and although it is questionable whether the results can be extrapolated directly to man, they do suggest new means for lipoprotein catabolism.

Pathways for uptake of remnants of chylomicrons and hepatic very low density lipoproteins (VLDL) were discussed by Dr. Allen D. Cooper and Dr. Claes-Henrick Florén. In rats, both types of remnants are cleared quickly by the liver, possibly by the same mechanisms. The liver apparently has a specific, high-affinity pathway for remnant removal. The uptake process is saturable. Partially-degraded lipoproteins (remnants) are taken up in preference to native VLDL or chylomicrons. The structural features of remnants that influence their recognition by the liver remain to be defined. Evidence was presented to support the concept that apolipoprotein E (apo E) on the remnant may promote hepatic uptake, while apo C-III may have an opposing effect. Dr. Cooper indicated that the receptor for chylomicron remnants probably is located on the plasma cell membrane, but the receptor itself has not been isolated. The observation that hepatic degradation of remnants is inhibited by chloroquine or colchicine suggests that their final catabolism degradation occurs within lysosomes.

A major unresolved question is the reason for the differences in uptake of VLDL remnants between normal man and other species (e.g., the rat). In many species, VLDL remnants are cleared expeditiously by the liver while hepatic uptake in normal man is very slow or non-existent. Whether this difference resides in the composition of VLDL remnants or in hepatic receptors remains to be determined.

The behavior of the liver in uptake of low density lipoproteins (LDL) remains controversial. Although it was long thought that the liver must be the major site of LDL removal, this concept came into question several years ago for two reasons: *a*) the observation that LDL removal proceeds uninterrupted in hepatectomized animals, and *b*) the discovery that cells of several types grown in tissue culture have the capacity to take up and degrade LDL. These findings raised the question of whether the liver plays any significant role in disposal of circulating LDL. Recently however, evidence has been obtained for considerable hepatic degradation of LDL. For instance, Dr. Richard Havel presented data showing that estrogen administration in rats augments the hepatic uptake of LDL. The nature of the LDL "receptor" is unresolved, but pathways of LDL removal by the liver are distinctly different from those removing chylomicron remnants. Additional proof of hepatic degradation of LDL was presented by Dr. Raymond C. Pittmann. He described a method developed with Dr. Daniel Steinberg for tagging LDL by covalent linkage with [^{14}C]sucrose. Since [^{14}C]sucrose cannot be hydrolyzed by lysosomal enzymes, it becomes trapped in lysosomes following uptake. By ascertaining the distribution of sucrose-tagged LDL in swine, Pittman and associates showed that as much as 40% of circulating LDL is cleared by the liver.

The possible function of apo E in regulating hepatic uptake of lipoproteins was discussed again by Dr. Karl H. Weisgraber. In association with Dr. Robert Mahley, Dr. Weisgraber has shown that an apo E-rich fraction of high density lipoproteins, designated HDL_c, is

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removed from the circulation much more rapidly than normal HDL_2 and HDL_3 which are both relatively poor in apo E. When ^{125}I-HDL was injected intravenously into dogs, about half of the injected dose was removed from the circulation in 5 min, and approximately half of this was extracted by the liver. These findings suggest that liver cells may have a receptor for apo E. Furthermore, when the lysine residues of apo E in HDL are modified by acetoacetylation and reductive methylation, the uptake of HDL by the liver is retarded suggesting that lysine residues are involved in the receptor-mediated disposal of HDL.

II. Regulation of hepatic synthesis of phospholipids (PL) and triglycerides (TG) by the liver (Dr. Robert K. Ockner, Chairperson)

This session was opened by Dr. Rosalind A. Coleman who discussed current knowledge of sites of glycerolipid synthesis in the liver cell. She presented evidence that enzymes of glycerolipid biosynthetic pathways are asymmetrically localized in the cytoplasmic face of endoplasmic reticulum (ER). Her interesting results raise a paradox about the assembly of the different components of VLDL which evidently occurs in the lumen of the ER. If triglycerides (TG) and phospholipids (PL) are synthesized on the cytoplasmic surface of the ER, they must transverse the bilayer membrane of the ER to participate in formation of VLDL. While the "flip-flop" mechanism may account for PL translocation, bilayer permeation by very nonpolar TG is more difficult to envision.

Next, Dr. Donald B. Zilversmit indicated that intracellular movement of PL from one organelle to another may be facilitated by PL-exchange proteins. These proteins are readily soluble and of low molecular weight. Some are specific for phosphatidylcholine (PC) or other PL, but others lack specificity for PL and even carry cholesterol. PL-exchange proteins have proven useful in the study of membrane asymmetry and PL translocation in model systems, but to date their biological roles are not well defined.

Dr. Sander J. Robins drew attention to the fact that the biliary PL is composed largely of PC containing palmitic and linoleic acids. Thus, biliary PL could be selectively synthesized or secreted from a distinct hepatic pool. His recent studies disclosed that PL secretion rates in rats can be mediated by availability of choline, among other factors. Both biosynthesis and biliary secretion of PC are stimulated by bile acids (BA), but the mechanisms are obscure. Despite the large quantity of PC secreted into bile and the constancy of its fatty acid (FA) composition, it has thus far not been possible to isolate or define a specific synthesis site within the liver cell for biliary PC.

Dr. Ockner discussed the influence of availability of FA in the synthesis of TG and PL by the liver. The production of TG seemingly is determined by the quantity of FA remaining unused after oxidation and utilization for PL biosynthesis. According to Dr. Ockner, sex steroids can have a major influence on TG formation. In mature female rats, FFA utilization and TG biosynthesis were more than twice as great as in males. Those sex-steroid actions likely are mediated in part by regulation of uptake of plasma free fatty acids (FAA); this may be accomplished by changing the cytosolic concentration of FA-binding protein (FABP). FABP is a low molecular weight, soluble protein that binds long-chain FA and acyl CoA. Its hepatic concentrations are greater in female rats than in males. The function of FABP in vivo evidently is that of a FA acceptor or carrier, but this remains to be ascertained with certainty.

Dr. Murry Heimberg next brought up the potential of thyroid hormone to modify hepatic pathways of FA oxidation and utilization. In isolated perfused livers, thyroid hormone augments oxidation of FA to ketone bodies and CO_2; the result is a fall off in TG synthesis. The raising of hormonal activity not only curtails output of VLDL-TG, but it also diminishes the size of VLDL particles and elevates the ratio of surface to core components.

Several reports in the literature have established that plasma VLDL-TG concentrations can be heightened in man by interrupting the enterohepatic circulation (EHC) of BA. Similarly, a rise in VLDL-TG concentrations also has been evoked by partial biliary diversion in the rhesus monkey by Dr. Ockner and coworkers, and their results support the concept that higher concentrations of VLDL-TG are secondary to intensified secretion of VLDL-TG. Dr. Scott M. Grundy presented data displaying a similar increment in VLDL-TG synthesis following BA diversion in man. The biochemical mechanism for stimulated production of VLDL-TG by interruption of the EHC has not been explained. However, considering the action of BA removal to promote VLDL synthesis and yet to depress LDL, there is unquestionably the need for more information on the critical interface between BA metabolism and hepatic production and catabolism of plasma lipoproteins.

III. Regulation of hepatic cholesterol synthesis (Dr. John M. Dietschy, Chairperson)

Dr. Dietschy first reviewed methods for measuring rates of hepatic cholesterol synthesis and the role of various lipoproteins in determining rates of cholesterol synthesis in the liver. It is now evident that the biosynthetic sequence for cholesterol is under the co-

ordinate control of a number of enzymes, but HMG CoA reductase is still clearly the rate-limiting enzyme. HMG CoA reductase recently has been shown to undergo reversible phosphorylation, a mechanism that may provide short-term control of enzymatic activity, but regulation over a longer period presumably requires new enzyme synthesis. For quantification of cholesterol synthesis in tissues, Dr. Dietschy showed that use of ^{14}C-labeled substrates can underestimate synthesis markedly, but through use of $^{3}H_2O$, accurate measurements can be made. His studies have revealed that in the normal rat, only about one-half of total body synthesis of cholesterol takes place in the liver, the remainder occurring in extrahepatic tissues. Synthesis of cholesterol by the liver is regulated in part by cholesterol delivered by lipoproteins. Most of the cholesterol entering the liver is derived from chylomicron remnants, but a small quantity may be obtained by uptake of LDL and HDL. Nevertheless, cholesterol that is newly absorbed and carried by chylomicrons still represents the major factor regulating activity of HMG CoA reductase.

Despite the importance of newly-absorbed cholesterol in the control of hepatic production of cholesterol, other factors also can modulate its synthesis. Dr. Heimberg presented tentative evidence that estrogens can stimulate cholesterol production in the rat; it is uncertain, however, whether sex hormones have a similar effect in human beings. As discussed by Dr. Grundy, obesity and increased caloric intake stimulate total body synthesis (and presumably hepatic synthesis) of cholesterol in man, and its production can be curtailed by caloric restriction. The same was demonstrated for other lipids made in the liver, namely VLDL-TG and biliary PL. However, as pointed out by Dr. Grundy and Dr. Barbara Howard, the stimulation of synthesis of VLDL-TG and biliary PL by obesity occurs only in whites and is not observed in American Indians. The reason for this racial difference has not been found.

The controversial question of whether bile acids directly regulate hepatic synthesis of cholesterol was brought into consideration by Dr. Sarah Shefer. She noted that removal of bile acids from the EHC can greatly stimulate cholesterol synthesis in the liver, and replenishment of the bile acid pool has the opposite effect. However she also specified that these changes do not prove that bile acids have a direct action on the enzymes involved in cholesterogenesis. More likely, their action is to indirectly affect cholesterol synthesis by modifying the size of the "metabolically-active" pool of cholesterol in the liver; this can be done by altering the rate of conversion of cholesterol into bile acids.

IV. Regulation of bile acid (BA) synthesis (Dr. Alan F. Hofmann, Chairperson)

Drs. Hofmann and William G. M. Hardison reviewed the role of BA as a means of eliminating cholesterol, and itemized factors regulating the conversion of cholesterol to BA. It was pointed out that interruption of the EHC causes a 4- to 10-fold increase in BA synthesis with a concomitant elevation in both cholesterol 7-α hydroxylase and HMG-CoA reductase. Normally, when the EHC is intact, only about 4% of BA in bile is derived from de novo synthesis. With BA feeding, synthesis of BA is suppressed about 50%. The suppression of synthesis is not structure specific; each BA normally present appears to inhibit its own synthesis as well as that of others with related structures. However, it is possible that ursodeoxycholic acid, the 7-β isomer of chenodeoxycholic acid, may not suppress the synthesis of other bile acids.

BA synthesis seems to be a continuous process. Nevertheless, Dr. William C. Duane has demonstrated in the rat the presence of a rhythmic diurnal variation in synthetic rates that may be an intrinsic property of the hepatocyte. Dr. Duane indicated that the diurnal rhythm of BA synthesis persists despite complete interruption of the EHC, short-term feeding, adrenalectomy, high doses of glucocorticoids, and ocular enucleation. Also, the peak production rates of cholic acid and chenodeoxycholic acid are separated by about 3 hours suggesting that factors in addition to 7-α-hydroxylation of cholesterol modulate changes in BA production.

Dr. Erwin Mosbach discussed chemical pathways for BA synthesis and delineated the subcellular compartmentalization of enzymes involved in this synthesis. The classical pathway involves 7α-hydroxylation of cholesterol followed by formation of the key intermediate, cholesterol-7α-hydroxy-4-ene-3-one. If the latter is 12α-hydroxylated, the product is destined to form cholic acid; if not it will proceed to chenodeoxycholic acid. The current concept is that BA precursors undergo nuclear changes before side-chain alterations. In the former sequence, the transformation of cholesterol to 5β-cholestane-3α,7α,12α-triol is catalyzed by microsomal and soluble enzymes. The first alteration on the side chain, 26-hydroxylation, probably involves a mitochondrial mixed-function oxidase. The oxidation of 5β-cholestane-3α,7α,12α,26-tetrol to the corresponding -26-oic acid proceeds via an intermediate aldehyde and is ascribed to the successive action of soluble ethanol and acetaldehyde dehydrogenases. Relatively little is known about the individual reactions in the subsequent steps leading

to cholic acid. The formation of chenodeoxycholic acid is thought to occur by the same pathway as that for cholic acid, except for the lack of 12α-hydroxylation. Alternate pathways for BA synthesis also have been proposed. One example is the (microsomal) 25-hydroxylation pathway of cholic acid biosynthesis; others are the (microsomal) pathways for chenodeoxycholic acid via either *a*) 26-hydroxycholesterol and 3α-hydroxy-5β-cholenoic acid, or *b*) 7α-hydroxycholesterol and 3α,7α-dihydroxy-5β-cholenoic acid. The quantitative significance of these alternate pathways of BA synthesis in the normal organism remains to be determined.

Progress on elucidating the intermediate steps in BA synthesis has been slow because of several problems. For example, the enzymes are difficult to isolate; the intermediates remain bound to cell organelles and are not easily trapped; they are difficult to synthesize; and labeled putative intermediates may not exchange with true intermediates when added in vitro or in vivo. Dr. Henry Danielsson discussed his excellent work in this field on the isolation, properties, and regulation of the key enzyme in BA synthesis, cholesterol-7α-hydroxylase.

Finally, Dr. Charles C. Schwartz opened the question of the relative contributions of hepatic newly-synthesized cholesterol and cholesterol from extrahepatic sources in the formation of BA. In isotope kinetic studies carried out in man, Dr. Schwartz and coworkers observed that radioactive unesterified cholesterol in plasma, and particularly that on HDL, readily makes its way into bile acids. This observation is compatible with the possibility that lipoprotein-free unesterified cholesterol, particularly that of HDL, is the preferred substrate for BA synthesis; however because of the problem of isotope exchange, it is not possible to make definitive statements about net transfer of cholesterol from plasma lipoproteins to the liver.

The sources of BA also were examined in rats by Dr. Steven Quarfordt. He used labeled mevalonate as a marker for newly-synthesized cholesterol. In animals with acute bile fistulas, approximately 20% of bile acids was judged to come from new hepatic synthesis. This value increased to 35% in rats with chronic bile fistulas.

V. Sources of biliary lipids and role of lipids in regulation of biliary secretion (Dr. Martin Carey, Chairperson)

This session was opened by Dr. Steven Turley, who, in association with Dr. John Dietschy, has examined the origins of biliary cholesterol in the rat. They used a method to measure hepatic synthesis of cholesterol which employed ^3H_2O. Several maneuvers were employed to alter hepatic synthesis of cholesterol and biliary secretion (e.g., bile acid infusion, cholestyramine feeding, and cholesterol feeding). Basically, these workers observed that while there was a direct correlation between the rate of hepatic cholesterol synthesis and the amount of newly-synthesized cholesterol in bile, extreme changes in the rate of liver cholesterol synthesis resulted in little change in total biliary cholesterol output.

Dr. Stephen Quarfordt discussed his work on the same problem using a different approach. He used acute infusions of triparanol to inhibit the conversion of desmosterol to cholesterol. This technique offers a means of detecting newly-synthesized cholesterol because measurement of desmosterol provides a direct estimation of new sterol synthesis. Dr. Quarfordt employed this method in rats with acute bile fistulas, and his results implied that about 28% of total sterol delivered into rat bile was recently synthesized in the liver.

Dr. Charles Schwartz next reported the potential sources of biliary cholesterol as determined from their isotope-kinetic studies of cholesterol metabolism in man. These studies were carried out by incorporation of radioactive unesterified cholesterol into HDL and LDL; HDL was labeled with [^{3}H]cholesterol and LDL with [^{14}C]cholesterol. When these were injected simultaneously, biliary cholesterol became labeled more rapidly from HDL-cholesterol than from LDL-cholesterol. These findings raise the interesting question of whether HDL-cholesterol might contribute its unesterified cholesterol to the liver for biliary secretion more readily than LDL. However, as indicated above, this could not be determined with certainty because of the problem of isotope exchange between plasma lipoproteins and the liver cell.

Dr. Richard N. Redinger described the use of trans-splanchnic methods to study relations between plasma and biliary lipids in the intact baboon. Measurements of biliary lipid outputs were determined by continuous bile sampling (5% of total secretion) from an exteriorized but physiologically intact EHC. Through use of strategically placed chronic indwelling catheters, it was possible to assess hepatic uptake, release, and/or secretion of various lipids in plasma lipoproteins. This ingenious but tedious method was described in considerable detail. When animals with an intact EHC were fasted, no trans-splanchnic changes could be detected for plasma lipoproteins, but biliary cholesterol decreased. When the EHC was interrupted by removal of bile acids, there was an enhanced uptake of HDL-cholesterol across the splanchnic bed, but no change in biliary cholesterol was found.

Finally, Dr. Martin Carey described his continuing studies on the physical chemistry of bile. In these studies he makes use of several physical methods including quasi-elastic light scattering spectroscopy and electron microscopy. Dr. Carey observed, in model systems containing high ratios of bile salts to lecithin, that two types of micelles are present; these are mixed micelles containing both lecithin and bile salts, and simple micelles with only bile salts. At low ratios of bile salts to lecithin, only mixed micelles were found. As the latter are diluted and approach maximum solubility of cholesterol, they expand in size, and at the phase limit they are transformed into liposomal vesicles. Electron microscopy carried out on human bile showed the existence of two types of particles; the particles of one species were small and probably represent the expected mixed micelles of lecithin and bile salts, but the other type of particle was much larger, approximately 600 Å, and is thought to be self-aggregation of bilirubin.