special report

Hepatic Cholesterol and Lipoprotein Conference. Report of a Conference

Peter A. Edwards* and Scott M. Grundy†

Departments of Biological Chemistry and Medicine,* University of California Los Angeles, Los Angeles, CA 90024, and Center for Human Nutrition,† 5323 Harry Hines Boulevard, Dallas, TX 75235

The 1988 Aspen Bile Acid/Cholesterol Conference was held in August in Aspen, Colorado. The meeting, chaired by Drs. Peter Edwards and Scott Grundy, brought together established investigators and younger investigators including graduate students and post-doctoral fellows to discuss four major research areas: fatty acid-binding proteins; protein secretion; regulation of cholesterol and bile acid synthesis; and mutations of apoprotein B.

SESSION I: FATTY ACID-BINDING PROTEINS

Robert Ockner, Session Chairman

Robert Ockner (University of California, San Francisco, CA) introduced the session with a summary of the background and current concepts of the structure, tissue distribution, regulation, and possible function of the fatty acid-binding proteins (FABP). It is now appreciated that FABPs, whose function remains undefined, are members of a family of eight or nine structurally related soluble 14–15 kDa proteins that bind fatty acids or retinoids in various tissues. Two major current issues were addressed.

In the first, the possible significance of two distinct FABPs (I-FABP and L-FABP) in intestinal mucosa was considered, as was the possible relationship of either or both to chylomicron triacylglycerol synthesis. A substantial body of circumstantial evidence supports such a relationship, including binding characteristics, tissue distribution, influence of dietary fat, and in vitro effects on enzyme reactions in which long chain fatty acids or their acylCoa thioesters are substrates. However, the human colon cancer-derived Caco-2 cell line, while synthesizing a wide variety of apolipoproteins and lipoproteins, fails to express I-FABP, suggesting that this protein is not required for these processes. Moreover, while abundance of both I-FABP and L-FABP responds to changes in dietary fat intake, these proteins are independently regulated, in that L-FABP is induced by fibrate drugs whereas I-FABP is not, suggesting that at least one of the two may subserve a different function. Thus, the relationship of either or both of the two enterocyte FABPs to chylomicron triacylglycerol biosynthesis, remains uncertain.

The possible role of L-FABP in peroxisomal fatty acid beta-oxidation was next considered. A relationship is suggested by the fact that both L-FABP and peroxisomes in liver and intestine are induced by clofibrate and other related agents. Recent studies of Brandes, Bass, and Ockner were described in which fibrate drugs were shown to induce L-FABP expression and peroxisomal beta-oxidation in primary hepatocyte culture. Significant differences in response of L-FABP and peroxisomes to fibrate drugs were observed, however, in time course, and the effects of extracellular matrix composition and of inhibitors of mitochondrial carnitine palmitoyl transferase I. The findings suggest that the mechanism by which fibrate drugs influence the expression of FABP and perioxsomal oxidation may differ.

Jeffrey Gordon (Washington University, MO) described two areas of current research in his laboratory, including studies of the molecular details of fatty acid/FABP interaction, and the regulation of gene expression and the maintenance of gradients of FABP expression in small intestine.

In the first, rat I-FABP was expressed in *E.coli* permitting preparation of large amounts of the native protein, which was found to be largely associated with palmitic acid. The protein was crystallized and its tertiary structure was defined. It consists of ten antiparallel beta-strands, organized into two nearly orthogonal beta-sheets. The structure resembles a clamshell ("beta clam"), with an "opening" formed by two of the beta strands and two short α helical segments. The interior, lined with the side chains of nonpolar amino acids, contains the bound fatty acid.

Gordon then described experiments in which various promoter elements from 5' nontranscribed region of the rat I- or L-FABP genes were linked to a reporter, the human growth hormone (hGH) gene. Expression of these fusion

genes was examined in transgenic mice. It was found that the normal "horizontal" (i.e., duodenum to colon) and "vertical" (crypt to villus tip) gradients appear to require different *cis*-acting sequences for their expression. For the L-FABP gene, horizontal gradients of gene expression were maintained in part by orientation-independent *cis*-acting suppressor elements. A 277 bp element from the rat I-FABP promoter was able to direct efficient gut-specific, epithelial cell-specific, and largely region-appropriate expression of the reporter gene. Studies in transgenic mice also suggested that there may be differences in the export pathways of human growth hormone within the gut epithelial cell population as a function of their location along proximal to distal axis of the small bowel.

Daniel Lane (Johns Hopkins, Baltimore) described two areas of current activity in his laboratory, involving the 422/aP2 protein. This protein, a homologue of FABP and the myelin P2 protein, is expressed in differentiated 3T3-L1 hepatocytes, but not in the undifferentiated pre-adipocytes. The studies described included a sequence analysis of the 5'-flanking region of the 422/aP2 gene and the identification of this protein as the target of the insulin receptor tyrosine kinase.

In the first area of activity, Lane and his colleagues have identified three potential glucocorticoid-responsive elements (GRE) and a fat-specific element (FSE) in the nontranscribed 5'-flanking region of the 422/aP2 gene. The GRE are located at positions − 393, − 1470, and − 1520, all remote from the FSE within which was identified an AP-1 binding site at position − 120. Using a CAT reporter gene, it was found that deletions or mutations near the AP-1 site resulted in increased CAT expression, suggesting the existence in that region of a repressor site. AP-1 itself and the c-fos, both of which bind to this site, result in increased expression. Other studies suggest that glucocorticoids act in this region by increasing cAMP, which in turn leads to increased AP-1 and c-fos expression. AP-1 and c-fos appear to result in increased expression of 422/aP2 by decreasing the activity of a *trans* inhibitor which represses 422/aP2 gene expression in pre-adipocytes via its interaction with sequences adjacent to the AP-1/c-fos elements.

Experiments leading to the identification of the 15 kDa phosphotyrosyl protein (pp15) in insulin-exposed 3T3-L1 adipocytes as the phosphorylated 422/aP2 protein were then described. Insulin interaction with its receptor results in phosphorylation of the 422/aP2 protein, which in turn is dephosphorylated by a membrane-bound vicinal dithiol-dependent enzyme. This dephosphorylation is required for transmission of the insulin signal to the glucose transport system. Inhibition of the dephosphorylating enzyme by vanadate, and of the requisite vicinal dithiol by phenylarseneoxide, resulted in accumulation of the rapidly turning over phosphorylated protein, which was then purified using two-dimensional gel electrophoresis and characterized. This observation constitutes an important direct demonstration in intact cells of the involvement of an FABP homologue in hormonally induced metabolic response.

Sam Sorof (Fox Chase Cancer Center, PA) described his studies of the induction of liver cancer by chemical carcinogens. Two cytosolic proteins have been identified as the principal targets of the metabolically activated carcinogen, N-2-fluorenylacetamide (2-acetylaminofluorine; FAA), i.e., the proteins to which the activated metabolites of the carcinogen become covalently bound. The early target protein (ETP) is a 14 kDa protein which has recently been identified as L-FABP. This protein was expressed in increased amount in hepatocytes undergoing normal mitosis, as well as under conditions of carcinogen-induced cell proliferation. Its increased expression in normal cells undergoing mitosis was evident at any location in the hepatic acinus where mitosis occurred, irrespective of the overall acinar gradient of the L-FABP expression. Jeffery Gordon noted that he had confirmed this interesting observation in his studies of the regulation of the L-FABP gene expression. The 150 kDa late target protein (LTP) is expressed in increased amounts after more prolonged exposure to the carcinogen. It contains a 55 kDa subunit whose expression bears a reciprocal relationship to the expression of L-FABP (ETP). The 55 kDa subunit also shares partial homology with L-FABP, as indicated by immunochemical cross-reactivity and similarity among oligopeptide fragments. The 55 kDa subunit does not appear to represent an oligomer of L-FABP (ETP). These observations, and those of a mammary-derived growth inhibitor bearing homology to the fatty acid binding proteins recently described by Bohmer et al. suggest a possible relationship of the FABP protein family to cell growth and differentiation.

SESSION II: PROTEIN SECRETION

Roger Davis, Session Chairman

Reid Gilmore (University of Massachusetts, Worcester, MA) discussed his recent studies on the intermediates required for nascent chain translocation across the endoplasmic reticulum. A guanine ribonucleotide was shown to be required for the translocation of nascent secretory proteins. Membrane integration and transport of lumenally exposed domains of integral membrane proteins were also shown to require GTP or nonhydrolyzable GTP analog. To further define the role of GTP in translocation, the translocation intermediate which accumulates in the absence of GTP during a post-translocational incubation of signal recognition particle (SRP)-ribosome complexes with microsomal membranes was characterized. The results show that the GTP-binding protein functions during an intermediate step in nascent chain transport, thereby coupling the initial SRP-SRP receptor-mediated targeting event to the subsequent

insertion of the nascent chain into the membrane. A series of translocation intermediates consisting of discrete-sized nascent chains was prepared by including microsomal membranes in cell-free translations of mRNAs lacking termination codons. The truncated mRNAs were derived from preprolactin and the G protein of vesicular stomatitis virus and encoded nascent chains ranging from 64 to 200 amino acids. Partially translocated nascent chains of 100 amino acid residues or less were insensitive to proteinase K digestion from the external surface of the membrane, while longer nascent chains were susceptible to protease digestion. It is concluded that the increased protease sensitivity of larger nascent chains is due to the exposure of a segment of polypeptide on the cytoplasmic face of the membrane. In contrast, low molecular weight nascent chains were remarkably resistant to protease digestion even after detergent solubilization of the membrane. This protease-resistant behavior of detergent-solubilized nascent chains could be abolished by the release of the polypeptide from the ribosome or by the addition of protein denaturants. Gilmore and his associates (T. Connolly and P. Collins) propose that the transport of nascent chains across the membrane is initiated by a direct contact between the signal sequence and proteinaceous components of the translocation apparatus.

Peggy Weidman (Princeton University, NJ) discussed the mechanisms involved in the transport of proteins through Golgi membranes. Using inhibitors in conjunction with a cell-free transport assay to dissect the pathway into vesicular intermediates, it was found that one of the inhibitors is the nonhydrolyzable nucleotide, GTPγS. GTPγS, in micromolar concentrations, affects the fusion of vesicles but not their formation. Morphological analysis of the intermediates that accumulated in the presence of GTPγS indicated that this nucleotide blocked transport after the transport vesicles have attached to the target compartment but prior to or during the uncoating of vesicles in preparation for fusion. This suggests that a GTP-binding protein is somehow involved in the process of vesicular transport, possibly in the regulation of vesicle uncoating. Another inhibitor of transport, N-ethylmaleimide (NEM), selectively inactivates a peripheral membrane component that is required for transport. The NEM-sensitive factor, NSF, can be extracted from the membranes and quantitatively restores the transport to NEM-inactivated Golgi membranes. On this basis, NSF has been purified to homogeneity. NSF is a tetramer of 76 kD subunits that acts enzymatically in the transport process. It is a peripheral membrane protein that is specifically released from membranes by Mg^{2+}-ATP and in the soluble form requires ATP for its stability. When NEM-treated Golgi membranes are incubated without NSF, vesicle intermediates accumulate that are associated with the Golgi stack but lack the cytoplasmic coats characteristic of intermediates accumulated in the presence of GTP γS. Both biochemical and morphological evidence suggest that NSF is required after vesicles have docked and lost their cytoplasmic coats and during the processing of vesicles for fusion. Additional evidence has been obtained showing the existence of two novel factors that are required for NSF to bind to membranes. One of these is an integral membrane receptor and the other is a cytoplasmic cofactor that acts stoichiometrically to promote a high affinity interaction between NSF and the receptor. This binding equilibrium is altered by Mg^{2+}-ATP. Since NSF is required for vesicle fusion, it seems likely that these two new components are also involved in the fusion process.

Vishwanath Lingappa from the University of California at San Francisco described variations on the theme of secretory and membrane protein biogenesis. The topic was introduced by summarizing questions concerning the mechanism for targeting proteins for their ultimate destination. "It is generally accepted that signal sequences are discrete regions of nascent chains capable of directing newly synthesized protein domains across the membrane of the endoplasmic reticulum. Similarly, it is a common notion that stop–transfer signal sequences serve to terminate translocation of such protein domains during the biogenesis of integral transmembrane proteins. However, the mechanisms by which these topogenic sequences direct initiation and termination of chain translocation remain unsolved problems. Two general paradigms exist as to the mechanism of translocation, initiation and termination. One is based on thermodynamic considerations and emphasizes protein-lipid interactions; the other is based on studies involving reconstitution of these processes in vitro. This view emphasizes protein-protein interactions, i.e., between topogenic sequences (ligands) and their binding proteins (receptors) as the basis for fidelity and efficiency in this intracellular protein traffic." Lingappa described his results obtained on the topology of engineered proteins containing various permutations of the order of topogenic sequences. The findings are consistent with certain receptor-mediated models and challenge other views of the mechanism of secretory and membrane protein biogenesis. Studies on the Scrapie prion protein reinforce the receptor-mediated view of protein topogenesis. Other studies on hepatitis B surface antigen suggest the evolution of multiple mechanisms by which a common protein domain can achieve secretory and transmembrane phenotypes. The combination of engineered and native probes of universal biosynthetic machinery may provide a promising avenue for understanding mechanisms of both conventional and unusual examples of intracellular protein topogenesis.

Roger Davis (University of Colorado) described recent experiments on the role of apoB in the assembly and secretion of VLDL. Initial studies of the effect of metabolic state on the synthesis and secretion of VLDL show that fasting is associated with a 50% decrease in the synthesis and secretion of small molecular weight apoB by cultured rat hepatocytes

and a corresponding decrease in the ability of these cells to secrete triglyceride. Conversely, hepatocytes obtained from rats fed a sucrose-rich diet show a twofold increase in the synthesis and secretion of both large and small molecular weight apoB as well as a corresponding increase in the ability to secrete triglyceride. These data suggest that the availability of apoB determines, at least in part, the capacity of the hepatocyte to secrete triglyceride-rich lipoproteins. Additional studies to examine the mechanism through which fasting and sucrose feeding alter the synthesis and secretion of apoB were undertaken. Isolation and characterization of the mRNA obtained from the livers of these rats showed that neither sucrose feeding nor fasting affected the concentration of mRNA using both a 5' cDNA probe as well as a 3' cDNA probe. In addition, it was found that the size of the mRNA was not altered by either fasting or sucrose feeding. These data suggest that the differences in the secretion of large and small molecular weight apoB as a result of metabolic state are not due to changes in the size or the content of mRNA. To further define the role of apoB in the assembly of VLDL, hepatic rough microsomes were obtained and characterized with respect to the topography of apoB. Rough microsomes that were subjected to trypsin were shown to have a dramatic 80–90% loss of large molecular weight apoB and a smaller loss of small molecular weight apoB. The rough microsomes were shown to remain intact as demonstrated by a retention of latency of mannose-6-phosphatase as well as the lack of any proteolysis of proteins known to be luminal (i.e., albumin). Additional studies showed that when rough microsomes were subjected to sodium carbonate, the majority of the large molecular weight apoB was retained with the pellet, whereas the majority of the small molecular weight apoB was in the supernatant. These data suggest that a significant fraction of large molecular weight apoB is a transmembrane protein in the rough endoplasmic reticulum. Integration into the endoplasmic reticulum may play a role in the assembly of VLDL, a process known to occur in the endoplasmic reticulum. Alterations in the fraction of membrane-bound apoB that enters the lumen of the endoplasmic reticulum may provide a mechanism through which VLDL apoB secretion is regulated independent of mRNA concentrations.

SESSION III: REGULATION OF CHOLESTEROL AND BILE ACID BIOSYNTHESIS

Peter Edwards, Session Chairman

Intracellular cholesterol homeostasis is maintained by feedback regulation of key proteins involved in the uptake and cellular biosynthesis of cholesterol. The genes encoding the LDL receptor (cholesterol uptake) and the houskeeping enzymes HMG-CoA synthase and HMG-CoA reductase (cholesterol biosynthesis) are primary targets for this feedback regulation. The first speaker, *Timothy Osborne* from the University of Texas Southwestern Medical Center at Dallas, indicated that coordinate regulation of all three genes is affected by a common DNA sequence element in the promoter-regulatory region upstream from all three structural genes. Each promoter is composed of a unique constellation of positive promoter elements with the common cholesterol regulatory sequence positioned in a different location in each promoter.

Osborne, together with Gregorio Gil, purified a protein (RPF-1) that binds to six separate sites in the HMG-CoA reductase promoter. One of the binding sites overlaps the sterol regulatory consensus element and they believe that this protein may be involved in the feedback regulation of HMG-CoA reductase transcription by cholesterol. The protein activity is resolved into a doublet of 33 and 35 kilodaltons by SDS electrophoresis, is composed of monomeric units in solution, and binds independently to each of the six binding sites in the HMG-CoA reductase promoter. The binding site specificity resembles the consensus sequence for the nuclear factor one (NF-1) protein. In fact, their protein (RPF-1) binds very avidly to a synthetic NF-1 binding sequence. Osborne proposed an attractive hypothesis to explain how an NF-1-like protein might be involved in feedback regulation by sterols: he proposed that this NF-1 activity behaves as a constitutive repressor protein and a cholesterol-sensitive protein relieves this inhibition when new sterol synthesis is required.

The second speaker was *Richard Tanaka* from the Squibb Institute for Medical Research in Princeton, NJ. He demonstrated that the activity of hepatic mevalonate kinase was increased when rats were fed diets containing cholestyramine +/- pravastatin (SQ 31,000) and was repressed when animals were fed cholesterol. Mevalonate kinase was purified to homogeneity and shown to have a molecular weight of 88,000, a subunit size of 39,120, and an isoelectric point of 6.2. Monospecific antibody was raised and used both to quantitate the kinase mass by immunoblots and to isolate a cDNA clone. Sequence analysis of the clone showed that it encoded a protein of 41,992 daltons and that it hybridized to an mRNA of 2.0 kb. Tanaka showed that, in response to the drugs and diets indicated above, kinase activity, kinase mass (measured by immunoblotting), and kinase mRNA levels (measured by Northern blotting) all changed in parallel. He concluded that the levels of mevalonate kinase in the rat were regulated by changes in enzyme synthesis. It seems likely that it will now be possible to determine the nature of the defect in mevalonate kinase that leads to the rare disease mevalonic aciduria.

Sarah Shefer (New Jersey Medical School) then discussed the purification and regulation of cholesterol 7α-hydroxylase. Shefer showed that in rat liver microsomes the 7α-hydroxylation of cholesterol was higher than for cholestanol (1.4-fold) or sitosterol (30-fold). The addition of the latter two sterols inhibited the hydroxylation of cholesterol in acetone-extracted microsomes. It was suggested that, in pa-

tients with sitosterolemia with xanthomatosis, the observed decrease in bile acid biosynthesis might be related to the inhibition of cholesterol 7α-hydroxylase by endogenous cholesterol analogues.

In a second series of experiments Shefer studied the effect of the structural orientation of the 7-hydroxyl group of cholic acid on the feedback regulation of cholesterol 7α-hydroxylase in bile fistula rats. [2-^{14}C]DL-Mevalonolactone was infused intraduodenally (154 μmol/h) prior to and during the infusion of sodium taurocholate (TCA) or sodium tauroursocholate (TUCA) to label the endogenously formed cholesterol and bile acids and to assure an adequate supply of newly synthesized cholesterol for bile acid formation. After 18 h infusion of TCA, bile acid synthesis decreased 90% and cholesterol 7α-hydroxylase activity decreased 71%. On the other hand, TUCA did not inhibit 7α-hydroxylase activity and bile acid synthesis. TCA, but not TUCA, increased hepatic cholesterol concentration from 2.0 to 3.1 mg/g liver. The results demonstrate that the regulation of bile acid synthesis is dependent on the enterohepatic circulation of TCA which inhibits cholesterol 7α-hydroxylase. Substituting the 7β-OH for the 7α-OH (TUCA for TCA) markedly reduces feedback inhibition of bile acid synthesis in the rat.

Shefer then reported on her studies to purify rat and human cholesterol 7α-hydroxylase. The enzyme was solubilized with sodium cholate, precipitated with polyethylene glycol and then purified by chromatography with agarose octylamine, hydroxylapatite and diethylaminoethyl Sepharose, and finally by anion exchange high performance liquid chromatography (HPLC). The purified enzyme has a molecular weight of 52000. Further studies should indicate whether this is a homogeneous protein.

Gerry Salen (New Jersey Medical School) then discussed the mechanism of side-chain cleavage in cholic acid biosynthesis (i.e., microsomal 25-hydroxylation versus mitochondrial 26-hydroxylation) using normal subjects and patients with the inherited lipid storage disease, cerebrotendinous xanthomatosis. Hepatic microsomes were extracted with acetone to remove endogenous substrates before addition of cofactors and radioactive substrates and measurement of product. The results show separate mechanisms for the side-chain oxidation of cholic acid and chenodeoxycholic acid in that cholic acid side-chain cleavage involves C-25-hydroxylated intermediates while the chenodeoxycholic acid pathway includes C-26-hydroxylated derivatives. According to these results, the bile acid synthetic block in CTX was located at the microsomal enzyme that catalyzes the conversion of 5β-cholestane-3α,7α,12α,25-tetrol to 5β-cholestane-3α,7α,12α,24S25-pentol.

In a second group of experiments, the quantitative conversion of 3α,7α,12α-trihydroxycoprostanoic acids (THCA) to cholic acid was assessed. This C_{27}-bile acid is a key intermediate in the mitochondrial pathway. Small amounts of THCA are found in normal (and CTX) bile. After intravenous pulse-labeling with (7β-^{3}H)THCA, the specific activity decay of biliary THC is linear, with a pool of 88 mg and a production rate of 18 mg/day. Inasmuch as 110 mg/day cholic acid was made in this subject, only 17% of synthesized cholic acid was derived from endogenous THCA. Another experiment was based on feeding 100 mg/day of labeled (7β-^{3}H)THCA to two subjects (control and CTX). Biliary cholic acid and THCA were isolated daily over the next week. The specific activity of biliary THCA was equal to dietary THCA in both subjects and was 3 times greater than that of cholic acid. Thus a portion of the injected THCA was converted to cholic acid but two-thirds of the daily production of cholic acid was derived by a path that did not include THCA as a precursor. Based on these experiments, it was concluded that THCA and the mitochondrial 26-hydroxy pathway are minor sources of cholic acid in both normal and CTX subjects.

Salen concluded that synthesis of chenodeoxycholic acid occurs via the mitochondrial 26-hydroxylation pathway but cholic acid synthesis takes place via the microsomal 25-hydroxylation pathway. Thus independent side-chain cleavage mechanisms exist for the formation of cholic acid and chenodeoxycholic acid in humans.

SESSION IV: MUTATIONS OF APOPROTEIN B

Thomas Innerarity, Session Chairman

The major conclusion to emerge from the session on mutations of apolipoprotein (apo) B is that alterations of the apoB gene can have profound influences on plasma cholesterol levels. Such mutations can cause either familial hypobetalipoproteinemia or hypercholesterolemia. Other work presented at this session convincingly demonstrated that abetalipoproteinemia is not caused by the mutation of the apoB gene.

James Scott of the Clinical Research Centre, Harrow, United Kingdom, opened the session by describing mutations in two patients with hypobetalipoproteinemia. One mutation, a cytosine-to-thymine substitution of codon 1306, changed this arginine codon to a stop codon. The other mutation, a deletion of guanine at codon 1795, changed the reading frame, resulting in a nonsense codon several codons downstream. The truncated apoB variants had a predicted length of 1,305 and 1,799 amino acids, respectively (the normal form of apoB-100 has 4,536 amino acids). Compared with apoB-100, the larger variant was found at greatly reduced levels in very low density lipoproteins (VLDL) and low density lipoproteins (LDL), whereas the smaller variant was not found in the plasma at all. According to Scott, the differences in size and behavior of these truncated apoB forms suggest that the smaller variant lacks a domain that is necessary for either lipoprotein assembly, lipoprotein secretion, or stability of the lipoprotein particle in the blood.

Using restriction fragment length polymorphism (RFLP) segregation analysis, Scott examined families with abetalipoproteinemia and found that the apoB gene did not segregate with the abetalipoproteinemia phenotype; therefore, the apoB gene could not be the cause of this disorder. Similar results were obtained by Li Sin Huang of the Rockefeller University: from linkage studies of five families, she also found that abetalipoproteinemia was not linked to the apoB gene.

Scott used RFLP to define haplotype of apoB in a random population of 295 49- to 65-year-old men from southern Britian. Distinct RFLP haplotypes of apoB were specifically associated with either obesity or elevated blood cholesterol levels. One RFLP, which gave rise to an arginine-to-glutamine substitution at residue 3611 in the putative receptor binding domain of apoB-100, was the most closely associated with elevated plasma cholesterol levels. The LDL from individuals with this mutation have not yet been tested for receptor binding.

James Scott also described the latest developments his lab has achieved on the mechanism by which the same gene forms both apoB-48 and apoB-100. As several groups have shown, apolipoprotein B-48 is generated by a novel RNA editing mechanism that leads to postranscriptional insertion of a stop codon in apoB mRNA. Studies performed in collaboration with N. O. Davidson in Chicago were designed to examine the mechanism by which thyroxine "turns off" production of apoB-100 in the rat liver. This shutdown occurs over 12 h, after which only apoB-48 is secreted by the rat liver. This modulation results from regulation of the stop codon insertion mechanism, previously described for the generation of apoB-48.

The second speaker was *Stephen Young* of the Gladstone Foundation Laboratories (University of California, San Francisco). Previously, Young had documented the existence of two abnormal apoB alleles in a familial hypobetalipoproteinemic homozygote. Moveover, he showed that the mutant apoB alleles segregated with the hypobetalipoproteinemic phenotype across three generations. One allele yields a truncated species, apoB-37, which is found in significant concentrations in both the VLDL and hibh density lipoprotein (HDL) fractions. Apolipoprotein B-37 is caused by a four-base pair deletion (a frame shift mutation) in the apoB gene beginning at codon 1728 for apoB-100. The second abnormal apoB allele yields trace amounts of another truncated apoB species, apoB-87, as well as markedly reduced amounts of the normal apoB species, apoB-100. This allele appears to yield a normal or near-normal amount of apoB-48. The molecular basis of this interesting allele is not yet understood, but Young speculated that a splice-site mutation could result in reduced amounts of apoB-87 and apoB-100. The normal triglyceride phenotype in this kindred is probably explained by the observation that both of the abnormal apoB alleles yield apoB species (apoB-37 and apoB-48) capable of forming large, triglyceride-rich lipoproteins.

Stephen Young also described another family with hypobetalipoproteinemia in which still another truncated apoB species, apoB-46, was identified. Using peptide antibodies to known sequences of apoB-100, it was determined that apoB-46 terminates in the vicinity of apoB-100 amino acid residues 2050–2100. The basis for this defect has not yet been determined, although it may be pertinent that apoB-46 appears to differ from apoB-37 in its distribution among the different density classes of plasma lipoproteins. For instance, unlike apoB-37, apoB-46 is found in very low levels in the HDL fraction.

Elaine Krul of the Washington University School of Medicine described two species of truncated apoB in kindred affected by hypobetalipoproteinemia. One of these, apoB-40, is slightly larger than the apoB-37 described by Young. The other, apoB-90, was found in LDL. Surprisingly, apoB-90 LDL bound with higher affinity to LDL receptors than did normal LDL.

Taken together, the studies of Scott, Young, and Krul, as well as published studies from the laboratories of Jan Breslow and Bryan Brewer, indicate that familial hypobetalipoproteinemia can be caused by structural defects in the apoB gene. It is unknown whether defects in the regulatory region of the apoB gene or mutations in gene products other than apoB can produce a similar clinical syndrome.

In another presentation, *Thomas Innerarity* of the Gladstone Foundation Laboratories described a genetic mutation of human apoB-100 that resulted in elevated levels of LDL. Individuals with this mutation, designated familial defective apoB-100, possess LDL that are defective in binding to the LDL receptor on normal human fibroblasts. Because their LDL cannot be cleared from the plasma by the normal mechanism, the hepatic LDL receptor pathway, affected individuals have appreciably higher LDL levels than the average for their age and sex. Studies on the families of these probands demonstrated that the disorder is transmitted as a co-dominant trait. All subjects found thus far are heterozygous for this defect, and their LDL are a mixture of two populations: one containing normal apoB-100, and one containing the abnormal form. The defective LDL population isolated from one heterozygote had less than 5% of normal receptor binding activity.

Thomas Innerarity also reported that Karl Weisgraber of the Gladstone Laboratories has found that a monoclonal antibody to apoB-100, MB47, binds with enhanced affinity to the LDL of subjects with familial defective apoB-100. MB47, whose epitope is between residues 3350 and 3506 of apoB-100, totally inhibits the receptor binding of LDL.

To determine the molecular defect responsible for familial defective apoB-100, genomic clones of DNA from affected subjects were sequenced in the region of the MB47 epitope by Brian McCarthy and his colleagues at Gladstone. They found that the affected individuals are heterozygous for a single amino acid substitution of glutamine for arginine at residue 3500. In 823 individuals, of whom half were moderately hypercholesterolemic, seven probands have been dis-

covered. These probands and all affected family members (a total of 22) have DNA with a mutation in the apoB-100 gene at codon 3500, and LDL that bind weakly to LDL receptors but avidly to the MB47 antibody.

Alan Attie of the University of Wisconsin continued the discussion of apoB-100 mutations that caused hypercholesterolemia by describing the most recent progress in his investigations of apoB-100 variants in pigs. He and his co-workers have sequenced the carboxyl one-third of the pig apoB gene and have found that it is about 80% homologous to the human apoB gene; however, the cysteine residues and the glycosylation sites are not well conserved. In addition to the normal pig apoB gene, Attie has sequenced much of apoB exon 26 from pigs homozygous for the $Lpb^{5.1}$ allele. These animals have a two- to fourfold elevation in plasma LDL. No mutations were found in the putative receptor binding region that were unique to this allele. Presumably, the apoB-100 mutation that causes the hypercholesterolemia is in some other region of the molecule. A mutation in the $Lpb^{5.1}$ allele alters receptor binding of pig LDL, as shown by in vitro receptor binding assays and in vivo turnover studies. The defect was most pronounced in the more dense LDL subspecies. However, pig LDL exhibited less defective binding to LDL receptors than did LDL from familial defective apoB-100 patients. Finally, Alan Attie described a yeast expression system in which partial sequences of apoB-100 can be expressed. This system should be useful for the study of the domain structure of apoB-100.

Major contributions for the conference were generously provided by the Colorado Trust. Additional funding was provided by Burroughs Wellcome Co., Bristol-Myers Co., Ciba Geigy Corp., Cholestech Corp., Council for Tobacco Research–USA, E. I. du Pont de Nemours and Co., Johnson and Johnson, Marion Laboratories, Inc., Merck and Company, Inc., Merrell Dow Pharmaceuticals, Inc., Pfizer, Inc., Sandoz Research Institute, E. R. Squibb and Sons, Inc., Syntex, Inc., and Warner-Lambert Co.