Meeting Summary

Workshop on Apolipoprotein Quantification

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On September 21 and 22, 1982 the Lipid Metabolism and Atherogenesis Branch of the National Heart, Lung, and Blood Institute sponsored a workshop on apolipoprotein quantification in Chevy Chase, Maryland. The purpose of the workshop was to bring together a large number of investigators with primary interests in epidemiology, genetics, and basic and clinical research to discuss recent advances in the knowledge of apolipoprotein metabolism, epidemiological correlations with cardiovascular disease, assay methods, structure and functions of apolipoproteins, and the possible needs for standards, reference materials, and standardization of immunoassay methods for apolipoproteins. The agenda was planned by Drs. Petar Alaupovic, John Albers, Bryan Brewer, Gerald Cooper, Ronald Goldberg, Moti Kashyap, Kenneth Lippel, and Gustav Schonfeld. More than 100 scientists attended the workshop; 46 made formal presentations or served as panel discussants. The participants included three speakers from Europe (Dr. Jean Charles Fruchart from France, Dr. Maryvonne Rosseneu from Belgium, and Dr. Gerd Utermann from West Germany), and Drs. Yves Marcel and Allan Sniderman from Canada. A question and answer period followed each presentation.

The summary presented here is organized into six sections: Epidemiology, Dyslipoproteinemias, Isolation of Apolipoproteins, Apolipoprotein Assay Methods, Antibodies (Polyclonal and Monoclonal), and Standardization of Apolipoprotein Assays and Materials — which correspond to the framework of the workshop. The entire proceedings of the workshop will be published in the near future as a separate NIH publication (NIH Publication 83-1266).

Four working groups met after the formal presentations were completed. Each group summarized the previous presentations and discussions, and made recommendations concerning the need for further epidemiological and clinical studies and for improved assay methods and reliable standards and reference materials. These recommendations are also presented here.

Session on Epidemiology

This session was chaired by Dr. Herman A. Tyroler. Dr. Gerardo Heiss reviewed 18 epidemiologic studies published between 1976 and 1982, which examined the association between apolipoproteins (apo) and ischemic heart disease (IHD). Different apolipoproteins singly and in combinations were reported from different investigations. Apo A-I, A-II, and B were studied most frequently. Apo A-I and A-II were generally found to be lower, while apo B, LDL-B, and VLDL-B were higher, in cases compared with controls. Apo D in two studies and apo E in one study showed no statistically significant differences between cases and controls. Apo D in two studies and apo E in one study showed no statistically significant differences between cases and controls. All but one of the studies reviewed were of case-control design, limiting the interpretation of the antecedent risk factor status of the apolipoproteins. Limited information was available to assess the association of each apolipoprotein controlling for other apolipoproteins, lipids, lipoprotein complexes, or for nonlipid risk factors that might have confounded the observed apo-IHD relationship.

Despite their methodologic limitations, the studies reviewed suggested consistent cross-study case-control differences for A-I and A-II as great or greater than those reported for HDL-C, which has been identified as one of the strongest correlates of IHD in several population-based cohort studies. Similar conclusions derive from comparisons of the apo B-IHD and LDL-cholesterol-IHD relationships, although based on fewer studies. The findings are plausible in that apolipoproteins are conceptualized as central components of the lipid transport and metabolism systems. Measurement techniques for reliable and valid determinations across epidemiologic studies have not been reported at a level comparable to those for lipid and lipoprotein quantification. Finally, no epidemiologic data were available for assessment of human experimental studies, i.e., no reports of apolipoprotein studies.

On balance, the studies reviewed suggested a positive association between apo B and IHD and a negative association between apo A-I and apo A-II.
with IHD, and that these associations appeared to be real (i.e., nonartifactual) based on their biological plausibility, their replication in different studies, and the strength of the observed relation.

**Session on Dyslipoproteinemias Characterized by Absent, Excess, or Aberrant Apolipoproteins**

Dr. Antonio M. Gotto was chairman for this session, and Dr. Petar Alaupovic set the stage with a review of apolipoprotein profiles of dyslipoproteinemic states. Dr. Alaupovic attempted to simplify the concept of the heterogeneity of lipoproteins and of apolipoproteins. For example, lipoprotein families have been viewed as a discontinuous system of density classes of electrophoretic bands. Instead, Dr. Alaupovic introduced the concept that lipoproteins should be viewed as chemically and metabolically in a state of equilibrium, as primary and secondary lipoprotein families, or simple and complex lipoproteins. The simple or primary lipoprotein particles contain only one type of apolipoprotein particle while the secondary or complex lipoproteins contain more than one. These entities are antigenically defined. Dr. Alaupovic introduced the concept of apolipoprotein profiling, a new approach to characterizing normal and abnormal lipid transport. He then described an apolipoprotein profiling system based on electrophoresis and immunodetection of apo A-I, A-II, B, C-I, C-II, C-III, D, and E, measured in whole plasma (not ultracentrifugally isolated fractions).

Among the interesting changes observed by Dr. Alaupovic and his associates were marked increases in the concentrations of apo A-I, A-II, B, and D in both boys and girls after birth. After puberty, the apolipoprotein that shows the greatest change is apo B, which continues to increase gradually into adulthood. The most characteristic effect of age noted was a continuous increase in the concentrations of apo A-I and apo B in both men and women.

Dr. Alaupovic then reviewed the various types of dyslipoproteinemias, which he divided into three categories, characterized as follows: 1) by the absence of one or more immunologically detectable plasma apolipoproteins; 2) by the deficiency of one or more apolipoproteins; and 3) by an increase in the level of one or several apolipoproteins. At least four recognized dyslipoproteinemias have been characterized by the absence of an apolipoprotein. One of these is abetalipoproteinemia, characterized by missing apo B, and significantly reduced apo A-I, A-II, C-I, C-II, C-III, and D; but interestingly, the levels of apo E are within the normal range. Possibly, the circulating apo E could account for the fact that the LDL receptors in leukocytes isolated from such patients are at least partially suppressed. A second condition is the absence of apo C-II, in which apo A proteins and apo B are also decreased. Because of the deficiency in the apo C-II activator of lipoprotein lipase, these patients may exhibit a Type I or Type V hyperlipoproteinemia pattern. A third deficiency state involves the absence of apo E. These individuals may have the clinical presentation of Type III hyperlipoproteinemia, but have no marked abnormalities in other apolipoproteins. This is in striking contrast to the fourth disorder, in which apo A-I and apo C-III are absent; apo A-II and C-I, C-II, D, and E are very low, while apo B is either normal or slightly elevated. This finding may explain the accelerated coronary artery disease present in these patients.

Dyslipoproteinemia is characterized by the deficiency of apolipoproteins, or by Tangier disease, hypobetalipoproteinemia, or by lecithin cholesterol acyltransferase (LCAT) deficiency. A number of dyslipoproteinemias are characterized by increased levels of apolipoproteins. Patients with Type I have reduced levels of apo A-I and apo B with an increase in apo C-III. Patients with Type IV have an increase in the ratio of apo C-I to apo C-III. In Type III and Type V, all of the apo Cs are increased, as are apo E and B. The ratio of apo C-I to C-III is an important marker, being relatively high in normal individuals but lower in Types III, IV, and V. Patients with familial hypertriglyceridemia, familial hypercholesterolemia, and familial combined hyperlipidemia may be distinguished by their molar ratios of apo B/apo C-III, apo B/apo E, and apo C-III/apo E. Other characteristic changes in apolipoproteins are also found in secondary hyperlipoproteinemias, especially in insulin-dependent diabetics, in which A-I and B are low and C-III and E are very high. In fact, elevations of apo C-III appear to be a hallmark of insulin-dependent diabetics, and a main feature in the apolipoprotein profile of patients with chronic renal failure.

Dr. Alaupovic's paper was complemented by the presentation by Dr. Ernst J. Schaefer, who spoke on disorders of lipoprotein metabolism associated with apolipoprotein deficiency. Dr. Schaefer reviewed the function of apolipoproteins, as: 1) structural components necessary for lipid binding and for the formation and transport of stable lipid particles; 2) activators of enzymes, particularly lipoprotein lipase (apo C-II) and LCAT (apo A-I and apo C-I); and 3) ligands for receptor-mediated uptake by lipoproteins, as in the case of apo B and E. He summarized apolipoprotein function and metabolism to set the stage for a discussion of hyperlipidemic deficiencies. Familial HDL deficiency is observed in hypoalphalipoproteinemia; in familial LCAT deficiency; in subjects with apo A-I<sub>MICRO</sub>; in a syndrome called fish-eye disease described by Carlson and Philipson in which there are massive corneal opacities; in dyslipoproteinemia; in hypertriglyceridemia; in HDL deficiency associated with planar xanthomas, and classically in Tangier disease. Dr. Schaefer referred to the familial deficiency of apo A-I and C-III in two sisters with severe corneal disease and to apo A-I absence associated with severe diffuse atherosclerosis. These studies point to an apparent necessity of apo A-I as a protective mechanism against atherosclerosis.
Dr. Schaefer reviewed apo E deficiency, a disorder associated with the accumulation of beta VLDL, in which there is a marked elevation of intermediate density lipoprotein and premature atherosclerosis. In normotriglyceridemic abetalipoproteinemia, apo B-48 was present, whereas in abetalipoproteinemia, both apo B-48 and B-100 from the liver were missing. Dr. Schaefer's primary conclusions were that: 1) HDL is a primary negative risk factor for premature coronary artery disease; 2) apo A-I is crucial for normal HDL formation but not essential for cholesterol esterification; 3) apo B-100 is necessary for LDL formation; 4) apo B-48 is necessary for chylomicron formation; 5) apo C-II is essential for lipoprotein lipase activation and chylomicron catabolism; and 6) apo E is crucial for hepatic uptake of chylomicron remnants. It is remarkable how our knowledge and understanding of the physiology and pathophysiology of lipoproteins have evolved from careful and systematic studies of the apolipoprotein carriers.

The presentation centered around the physiology, molecular biology, and the isolation of a cDNA clone. The complete amino acid sequence of apo E contains 299 residues. Breslow and associates selected the sequence between residues 218 and 222, which contains met-glu-glu-met-gly by relatively unambiguous codons. They then synthesized the corresponding cDNA sequence by the solid phase phosphoramidite method. They used the oligonucleotide to screen an adult human liver cDNA library in an attempt to isolate apo E cDNA clones. With the 14-base oligonucleotide, they performed hybridization and screened 10,000 clones with a cDNA library to identify 20 clones that were strongly hybridizing. Eventually, four of these 20 positive clones were examined by restriction enzyme analysis. DNA
was isolated in a sequence that contains the apo E polymorphic site at amino acid 158. Their important work supports the hypothesis that apo E polymorphism is due to mutations in the region of DNA coding for the apo E structural gene. Furthermore, these gene mutations are likely of great physiological importance and can be related to the pathogenesis of Type III hyperlipoproteinemia and premature atherosclerosis.

Session on Isolation and Characterization of Apolipoproteins

The session was chaired by Dr. H. Bryan Brewer and consisted of detailed reviews of the molecular and structural properties of the plasma apolipoproteins and their importance in the quantification of plasma apolipoproteins.

Molecular Properties of Plasma Apolipoprotein

Dr. Richard Jackson reviewed the current status of the physicochemical characterization of apo B. To date, the characterization of apo B has been incomplete due to the fact that apo B is insoluble in aqueous buffers. Detergents or denaturants have been used by the majority of investigators to solubilize this protein. However, removal of these reagents has resulted in the precipitation or aggregation of apo B. As a result, assays for quantification of apo B have not used lipid-free apo B, but have used apo B in intact plasma lipoproteins. Dr. Jackson described a recently developed method for the preparation of lipid-free, water-soluble apo B which involves the sequential use of sodium dodecyl sulfate, guanidine HCl, and urea. The final product is soluble in aqueous buffer, and can be utilized for the characterization of detergent-denaturant-free apo B. Water-soluble apo B may now be used in assay systems for the quantification of apo B, and for a detailed analysis of the physicochemical properties of this unique and important apolipoprotein.

Dr. James Osborne discussed the molecular properties of the major plasma apolipoproteins. Detailed characterization in aqueous solution has been possible for each of the isolated apolipoproteins except for apo B. Major studies have focused on apo A-I, A-II, C-I, and C-III. These studies have yielded a profile which is characteristic of plasma apolipoproteins.

Apolipoproteins undergo self-association with the formation of discrete oligomeric complexes. Concomitant with self-association is a major increase in conformation. The molecular interactions of the apolipoproteins are markedly influenced by solvent composition, temperature, and pressure. In addition to self-association, the plasma apolipoproteins undergo mixed-association between different apolipoproteins with the formation of discrete mixed oligomeric species.

Knowledge of the complex inter- and intramolecular properties of apolipoproteins is important in the preparation of standards as well as samples for apolipoprotein quantification. A detailed knowledge of the molecular properties of each apolipoprotein under the conditions of the assay is prerequisite to accurate quantitations. Great care must be taken to assure that the standards and unknowns have a similar conformation and state of association in order to obtain reproducible and accurate results.

Biological and Immunological Properties of Apo E

Over the last several years the E apolipoprotein has been extensively investigated. Apo E is now known to be coded for by three major alleles designated e2, e3, and e4, and the product of these alleles by three major E isoproteins, apo E-2, apo E-3, and apo E-4. These three major E apolipoproteins have been shown to differ in amino acid sequence by single amino acid residues. Recent data has suggested additional heterogeneity of the individual apo E isoforms. An increased frequency of the e2 and e4 alleles has been reported in patients with Type III and Type V hyperlipoproteinemia, respectively. The physiological function(s) of apo E have not been fully elucidated. It has been proposed that apo E interacts with a high-affinity hepatic receptor that is distinct from the B-100-E receptor, and that it is important in the hepatic clearance of chylomicron remnants.

Dr. William Bradley described studies on the cellular uptake of VLDL isolated from hypertriglyceridemic patients. It was shown that VLDL interacted with the LDL or B-100-E receptor on fibroblasts, and that the receptor-mediated uptake of VLDL was due almost exclusively to apo E. In endothelial cells the receptor-mediated uptake results in cell death, while in mouse peritoneal macrophages, uptake was associated with triglyceride accumulations and foam cell formation.

Apo E was very sensitive to cleavage by serine protease degradation both as a purified apolipoprotein and on intact lipoproteins. The cleavage produced by the serine protease thrombin occurred preferentially between residues 193-194 and was associated with marked reduction in the cellular uptake of VLDL. The apo E associated with VLDL was shown to be immunologically different from the apo E standard. In addition, the immunoreactivity of apo E on VLDL was altered following thrombin cleavage. These studies suggest significant conformational changes in apo E on a lipoprotein particle, and changes in conformation may be associated with changes in LDL receptor binding activity following thrombin cleavage. These studies were interpreted as indicating that immunological techniques may be used for apolipoprotein quantitation as well as to probe the immunological reactivity and functions of the apolipoprotein.

Dr. Joan Karlin discussed the problems associated with the development of radioimmunossays (RIA) for apo E. Detailed studies from several labora-
tories have recognized problems with the solubility and consistent immunoreactivity of apo E, and detergents or denaturants have been routinely used in the immunoquantitation of apo E. Dr. Karlin reported the development of an RIA for apo E in which detergents or denaturants were not used. Detailed chromatographic and immunochromatography indicated heterogeneity of the isolated standard and samples with different polyclonal antisera. The polydispersity of apo E on gel permeation chromatography was eliminated when a detergent was incorporated into the buffer. The combined results suggested that different apo E preparations have different molecular properties, and emphasized the importance that molecular properties have on the immunoreactivity of apolipoproteins.

Characterization of Lp(a)

Dr. Joel Morrisett detailed the purification and structural properties of lipoprotein (a) (Lp(a)). Lp(a) is a slow, pre-beta migrating apo B-containing lipoprotein which has been associated with premature cardiovascular disease. The synthesis and metabolism of Lp(a) are reportedly independent of other apo B-containing lipoproteins. The isolation of Lp(a) from plasma was improved by the inclusion of protease inhibitors in the buffers and initial separation of the plasma lipoproteins from plasma proteins (d > 1.21 g/ml). Apo(a), the unique apolipoprotein of Lp(a), and apo B were shown to be different proteins with a similar molecular weight of 490,000. Apo(a) was isolated often in association with apo B by one or more disulfide bonds. The structural properties of Lp(a) differed from LDL in several aspects. Lp(a) contained 8.5% carbohydrate, while LDL contained 2.3% carbohydrate. The secondary structure (alpha-helix) of the proteins of Lp(a) was significantly less, 29%, when compared with 48% for LDL and 71% for HDL. The motion of the fatty acyl chains in the surface monolayer of Lp(a) and LDL, which was assessed by electron spin response spectroscopy, was similar for Lp(a) and LDL lipoproteins. The ability of Lp(a) to regulate intracellular cholesterol biosynthesis was determined by quantitation of mevalonate formation in normal fibroblasts. Lp(a) reduced mevalonate formation in cultured fibroblasts; however, Lp(a) was less effective than LDL when compared on a cholesterol basis. The unique apolipoprotein, apo(a), on Lp(a) has permitted the development of highly specific and sensitive immunotechniques for the quantitation of Lp(a). The availability of these assays and an improved understanding of the structural and apolipoprotein composition of Lp(a) will now permit more definitive studies on the physiological function of Lp(a) and its role in atherosclerosis.

Session on Apolipoprotein Assay Methods

An overview of the immunoassay of apolipoproteins was presented by Dr. John J. Albers, session chairman. The measurement of the plasma apolipoproteins is of growing clinical interest. Recent evidence suggests that measurement of the apolipoproteins will be useful for the differential diagnosis of the dyslipoproteinemic states, the monitoring of the progress of therapeutic intervention, and the predicting of the risk of developing coronary heart disease. Various types of immunoassays are currently used for measuring apolipoproteins. Normal values for each of the apolipoproteins vary from laboratory to laboratory even among laboratories using the same method. The lack of comparability of results indicates a need for standardization of reagents and methods and preparation of common reference materials. The validation and standardization of apolipoprotein immunoassays represent a difficult challenge, particularly in view of the molecular characteristics of the apolipoproteins and their association with lipid-containing macromolecular complexes.

Dr. Angelo Scapu discussed the role of high performance liquid chromatography (HPLC) for the preparation of apo A-I and apo A-II and assessment of their properties during storage. HPLC represents a quick and accurate method for isolating lipid-free apolipoproteins. The technique is also a good analytical tool for assessing apolipoprotein purity and mode of self-association. The reverse phase and ion exchange modes should provide a means for assessing charge heterogeneity and the identification of isomers. HPLC is well suited as a key technique in a program considering apolipoprotein standardization.

Dr. Conrad Blum presented an overview of the radioimmunoassay of apolipoproteins. The major advantage of radioimmunoassay is its great sensitivity. An advantage of RID over the immunochromatographic techniques of radial immunodiffusion (RID) and electrophoresis (EIA) is that the slope of the displacement curves obtained in RID provides another dimension for validation of the assays. Assay validation must include nonimmunochemical procedures as well as consideration of stability of standards, consistency of results, and specificity of the determinations. In RID, antibody affinity is of paramount importance in determining sensitivity and precision. In contrast to RID and EIA, titer is relatively unimportant for RID.

Dr. Marian Cheung discussed radial immunodiffusion of apolipoproteins with particular reference to factors that contribute to assay variability. Radial immunodiffusion is chosen in preference to the other immunochromatographic methods because it is simple to perform and does not require any expensive instrumentation or special equipment and usually requires a minimal dilution of plasma or serum. The time to reach final precipitation ring size in RID is a function of the molecular weight of the particle contained in a given apolipoprotein. For this reason it is preferable to allow the apolipoproteins to diffuse to the endpoint when performing radial immunodiffusion of apolipo-
proteins. Aggregation and self-association affect not only the molecular size but also the immunoreactivity of an antigen. Differences in immunoactivity of an antigen as well as variability in antisera can also contribute to inconsistent results.

Electroimmunoassay (EIA) for apolipoproteins was presented by Dr. Paul Roheim. EIA is relatively simple and does not require expensive equipment. EIA, however, requires large amounts of antisera, cannot be automated, and therefore is difficult to apply to large-scale screening studies. Furthermore, the validity of the method requires that the antigen in samples and standards have the same physical state. Dr. Walter McConathy presented examples of the extension of EIA to cross-electroimmunophoresis. In this procedure two antibodies can be used in the gel to study different forms of the apolipoprotein. Combining electroimmunophoresis with analytical isoelectric focusing offers a powerful approach to the study of apolipoprotein polymorphism.

Dr. Jim Fesmire reported on the effect of freezing and storage of sera on apolipoprotein measurements. For quantitation of apolipoproteins by EIA, the optimal time limit for storage of serum at 4°C should be within 1 week of the collection date. Sera stored frozen at -20°C for 1 month with no additives exhibit little change in apolipoprotein levels (less than 10%). Sera stored in the lyophilized or dry state in the presence of preservatives will give reduced, but constant, apolipoprotein values for at least 8 months.

Immunonephelometry was discussed by Dr. Maria Lopes-Virella. Immunonephelometric assays for the major apolipoproteins are being developed. In the case of apo A-I the immunonephelometric assay has definite advantages over RIA. Immunonephelometry appears less useful than EIA when small numbers of samples are quantitated. The assay of apo B presents more problems because the apolipoprotein is associated with VLDL and LDL, which can disperse considerable amounts of light by themselves (VLDL) or after reaction with polyethylene glycol (LDL and VLDL). Dr. Maryvonne Rosseneu also discussed the immunonephelometric assay. The nephelometric assay of apolipoproteins presents several advantages over other immunoassays. It avoids the high dilution usually needed for RIA. Lyophilized apolipoproteins can be used as primary standards in the assay. The use of a detergent enables the quantitation of apo A-I and apo B in hypertriglyceridemic samples. The sensitivity of the assay is comparable to that of EIA and RID, and its reproducibility is comparable to that of other immunoassays. It is readily automated and applicable to screening programs and to population studies.

Dr. Jean Charles Fruchart presented data on enzyme immunoassay for human apo B. Enzyme immunoassays provide a specific and highly sensitive method for measuring apo B. The precision of this enzyme immunoassay equalled that reported for other assays. The greatest source of error is the great sensitivity, which necessitates a high sample dilution. However, this method should allow estimation of apo B in lipoprotein fractions or in experimental situations where small concentrations need to be measured. Dr. Evan Stein also discussed enzyme-linked immunoassays (ELISA) for apolipoproteins, including the advantages and problems. The initial results with an ELISA method for apo A-II appeared promising. The advantages of the ELISA system include high sample throughput, minimal maintenance of the system, elimination of radioactive isotopes, and a requirement of minimal technical skills and capital outlay. The use of monoclonal antibody in the ELISA system ensures a constant supply of unvarying antibody.

Dr. James Lee closed the session with a presentation on substrate-labelled fluorescence immunoassay (SLFIA). The SLFIA is a competitive binding method which uses an enzyme substrate for labeling the antigen. The results of an automated SLFIA for IgG demonstrated that the procedure possesses adequate precision, sensitivity, and accuracy to provide rapid quantitation of the major serum proteins.

Session on Antibodies

Dr. Angelo M. Scanu chaired this session. Dr. Thomas S. Edgington began with an overview of antibody properties and antigen-antibody interactions. He outlined the basic principles underlying these interactions and the importance of defining them in precise physicochemical terms. He also discussed the relationship between polyclonal and monoclonal antibodies, their main differences in properties, and their potential use as structural probes and/or apolipoprotein quantification. He stressed that the complexity of the antigen polyclonal antibody interactions is particularly significant in secondary and tertiary assays. Primary assays are best suited for monoclonal antibodies because these antibodies have a superfine specificity which is not exhibited by conventional polyclonal antibodies. This superfine specificity makes monoclonal antibodies well suited as structural probes but less ideal for immunoquantification assays.

Dr. John Albers briefly reviewed the preparation methods for polyclonal antisera and their use in the measurement of the two main apolipoproteins of HDL, apo A-I and apo A-II. He evaluated the influence that antisera variability can have on immunoquantification procedures and the need for obtaining pure antigens whether used as immunogens or as standards. Although apo A-I is a single polypeptide chain, it contains at least two distinct antigenic determinants, one for the carboxyl terminal and one for the amino terminal region. These regions may be variably exposed when apo A-I is a constituent of the high density lipoprotein class. Thus, there is a need for unmasking the buried antigenic determinants which can be achieved by either exposing the lipo-
The immunoreactivity against the A-II monomer may differ from that of the dimer. Similar to apo A-I, each form of apo A-II may have several antigenic sites not equally well recognized by the specific antibodies used. With regard to stability, the storage of antisera to apo A-I or apo A-II at -60°C in the presence of sodium azide (0.5 g/liter), chloramphenicol (0.01 g/liter), and gentamycin (0.005 g/liter) preserves the activity of the antibodies for at least 3 years. Thus, antibody activity may be affected by various factors; this variability may account for the differences in the absolute values of apo A-I or apo A-II when these antibodies are used in the immunoassay procedures.

Dr. Ronald Goldberg stressed the effect that antibody and apolipoprotein variability can have on apo lipoprotein immunoquantification. Difference in results may also depend on the immunoassay method. When he used five antisera obtained from different laboratories to measure the same preparation of apo A-II, the results varied almost threefold and were tant. This point was also emphasized by Drs. Karen Steinberg, Joan B. Karlin, and Simon J.T. Mao who subjected this lipoprotein to the action of lipolytic enzymes. Alternatively, the measurement of apo A-I in HDL may be achieved by the use of surface-specific antibodies isolated by HDL affinity chromatography.

Our present knowledge of the immunochemical quantitation of the C apolipoproteins in plasma was summarized by Dr. Moti L. Kashyap. He reviewed the advantages and limitations of the four immunochemical methods used, namely radioimmunoassay, electroimmunoassay, radial immunodiffusion, and enzyme immunoassay. He also described the recent developments in the preparation of pure apolipoprotein C standards, the mode of storage, and the roles played by various delipidated agents in the preservation of their immunogenicity. Overall, the amount of experience gained in the measurement of the C apolipoproteins appears to be comparatively less extensive than with the other apolipoproteins. The problem of obtaining suitable standards is well recognized and may relate to the fact that lengthy procedures are used in the preparation of these antigens by conventional chromatographic or electrophoretic techniques. More rapid methods, particularly those involving high performance liquid chromatography, may prove to be more suitable in the future. The problem of apo C quantification is also heightened when hyperlipidemic sera are examined; this applies to the three main forms of apo C. In such a case, delipidation of the specimens is required. Dr. McConathy also noted that it is very difficult to produce antibodies against apo C-I unless this apolipoprotein is appropriately coupled with albumin. A way to maximally expose the antigenic sites of apo C in VLDL was suggested by Dr. Mao, who subjected this lipoprotein to the action of lipoprotein lipase and Tween 20. In the absence of says, he found that they were directed against a minimum of five nonoverlapping antigenic determinants. The results indicated that monoclonal antibodies to apo B, although useful in mapping the LDL antigenic surface, may not be suited for the quantification of apo B in plasma, since this requires that the antibodies be well characterized and their specificity clearly defined.

In the ensuing discussion, the need for a constant source of a homogeneous LDL standard for immunoassay techniques was recognized. Future studies, however, should decide on the best choice of such a standard, particularly after knowledge has been gained on the number of antigenic determinants in apo B and on how many of them are fully expressed in LDL. Alterations of the LDL standard can occur with storage, and proteolytic events must be cautiously monitored and prevented by the use of appropriate and antiproteolytic agents. We must know more about the primary structure of apo B, its actual molecular weight, and the conformational changes attending its association with LDL. This lack of knowledge complicates the interpretation of the results currently obtained. For instance, when no immunoreactivity is seen, one cannot decide whether the given antigenic determinant is absent or if it is, in fact, present but not seen by the antibody either because the apolipoprotein has changed in conformation or is hidden by lipids.

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 Tween 20, delipidated preparations of apo C, once dissolved in aqueous buffers, form aggregates which are less immunoreactive than monomers. The complexity of the interactions between antibodies and apo C determinants was stressed in the ensuing discussion, which pointed to the need for a better knowledge of the physicochemical properties of apo C in solution and at the lipoprotein surface. When immunoassay techniques are used, there may be important changes in the partition and conformational properties of apo C, which may change the reactivity of the antigenic determinants. The use of detergents poses a problem. According to Dr. Joyce Gibson, Tween 20 interferes with immunoassay techniques involving apo C-II and C-III. Once the decision is made to use a detergent, it should be carefully selected and its optimal concentrations in the immunoassay determined.

With respect to the immunoassays for apo E, Dr. Richard Gregg analyzed current methods of isolation of apo E, production of specific antibodies against it, radioiodination, and the radioimmunoassay methods used in his laboratory. He presented data on the characteristics of the radiolabeled apo E, the assay characteristics, and the validation of the assay. He outlined the problems encountered in the assay as applied to a study of 59 normolipidemic subjects. Particularly important is the tendency of apo E to aggregate, with a potential masking of antigenic sites. This calls for the use of detergents both to eliminate nonspecificity of binding and to increase the exposure of the antigenic determinants hidden by their association with the lipoprotein particle. Solvent delipidation may lead to potential changes in the physicochemical properties of apo E and decreased solubility, accounting for a decrease in antigenicity. Moreover, apo E may undergo cleavage by proteases if the plasma is stored at 4°C for a prolonged period. Thus, the antibodies to apo E, which have been produced in several animal species, must be defined in terms of the method of immunization and the iodination procedure, and should be checked carefully for their reactivity against each apo E isoform. In general, apo E has properties that make its immunoquantitation difficult. This is exemplified by the wide range of mean plasma concentrations reported by different groups. Essential to the development of a reproducible assay is an understanding of the physicochemical properties of apo E and the availability of primary as well as secondary standards.

In the ensuing discussion, Dr. Conrad Blum commented on the problem of iodination of apo E. He found a difference in immunoprecipitability between antigen iodinated by chloramine T and by the iodine monochloride procedure. The latter insures a greater immunoprecipitability. However, even this technique poses problems in that the reducing agent usually present during the isolation of apo E can interfere with the iodination, which is essentially an oxidation reaction. In the presence of a reducing agent, less iodine will bind to the tyrosine residues. This does not apply to the chloramine T method. The early procedures for the radioimmunoassay of apo E involved the use of decylsulfate on the assumption that this detergent would maximally expose the antigenic determinants of apo E. However, Dr. Richard Havel now believes that decylsulfate is no longer needed for the actual radioimmunoassay procedure since similar results are obtained in the absence of this detergent. Decylsulfate, however, appears to be needed to store apo E in an active form. According to Dr. Havel, standards of apo E are stable up to 5 years when stored frozen in 100 mM decylsulfate. Samples of apo E iodinated by the iodine monochloride method become inactive in 2 or 3 days unless preserved in decylsulfate. However, Dr. Gregg found that iodinated specimens stored in decylsulfate retain their initial antigenicity for at least 4 months. According to Dr. Havel, when comparing normal values of apo E from laboratory to laboratory, we should take into account differences in gender since women have values about 20% higher than men.

In regard to the properties of apo E as an antigen, Dr. Carl Weisgraber summarized the experience of the Gladstone Laboratory and the use of cysteamine as an aid in apolipoprotein phenotyping. Such a chemical modification provides a convenient way to gain information on the number of cysteine variants in apo E. The most important advantage of the method is its sensitivity and its applicability to a large-scale study.

In the general discussion, it was stressed that more attention should be paid to examining the potential differences between plasma and serum and to the fact that when plasma is stored in a frozen state, thawing may create a clot adhering to the lipoprotein surface, with a potential loss of antigenic determinants. This concern was voiced by Dr. Joan Karlin and seconded by Dr. Edgington, who stressed the necessity of storing specimens in the presence of antiproteolytic agents to avoid having such enzymes as thrombin cause proteolytic fragmentation and changes in antigenicity of the apolipoproteins.

Overall, the section on polyclonal antibodies revealed that all the apolipoproteins discussed (A-I, A-II, B, C, and E) still present several problems in immunoquantification which relate to antigen isolation and purification, storage, radiolabeling of specimens for radioimmunoassay, antibody production and standardization, and the immunoassay techniques per se. These many uncertainties require a more coordinated effort to standardize procedures.

Dr. E. Dale Sevier discussed the methods of production and general properties of monoclonal antibodies. He pointed out that after only 7 years since its inception, this technology is offering clinical medicine a plethora of exciting developments. This technique relies on the ability to select useful cloned hybridoma and thus requires screening procedures to identify those hybridomas producing functional monoclonal antibodies. In general, once these anti-
bodies are produced, they are highly specific and present fewer problems in cross reactivity. If specific reactions are observed, contaminants can usually be readily removed by absorption procedures.

More selective monoclonal antibodies display homogeneous types of binding to their epitopes and provide an unending supply of homogeneous reagents instead of relying on an animal source. Such a scheme proved an unending supply of homogeneous reagents. In many respects, monoclonal antibodies have many advantages over the polyclonal; however, it is necessary to define the perfect antibody which can be useful in immunometric assays in which the label resides in the antibody and not in the antigen. This two-site immunometric assay takes advantage of the monoclonal antibodies and does not offer the problem of competition for binding sites, since each monoclonal antibody is selected to bind an independent site. This lack of competition also offers the possibility to carry out simultaneous assays. In many respects, monoclonal antibodies have many advantages over the polyclonal; however, it is necessary to define the perfect antibody which can be useful in screening procedures.

An interesting application of the monoclonal antibody technique was presented by Dr. Yves Marcel, who obtained 47 clones that reacted positively with LDL from mice immunized against normal plasma LDL in a density range of 1.030–1.050 g/ml. Of these clones, seven were studied in detail and were all capable of reacting against delipidated apo B but not against other plasma apolipoproteins. These specific antibodies were used to define the spatial relationship of the apo B antigenic determinants on the surface of the LDL particle. Using the cotitration method for cell surface antigens, he obtained a theoretical map which gave the relative position of the determinants, at least insofar as the recognition capacity by the monoclonal antibodies used. Stoichiometric binding studies using Fab fragments from three of the monoclonal antibodies suggested that apo B may be present in LDL as a 6 × 10^5 dalton protein, although the possibility of an apo B dimer was not ruled out. A model of LDL in which apo B consists of many small identical subunits was considered compatible with the immunological results.

Another interesting application of the apo B monoclonal antibodies was in identifying the domains of the apolipoprotein molecule which interact with the LDL receptor of human fibroblasts. Only a few monoclonals were able to block the interaction and thus block the intracellular events known to occur upon activation of the LDL receptor. The monoclonal antibodies were also used to investigate the antigenic heterogeneity of apolipoproteins B-100 and B-48, which are presumably under separate genetic control and have different origin. Two antibodies reacted only with B-100; two others reacted with both B-100 and B-48. The findings were interpreted to indicate that homologies exist between the two proteins (i.e., B-48 and B-100) and may have sequences in common. It has been previously reported that B-100 can generate two fragments named B-74 and B-26 by proteolytic cleavage. When these products were tested against the monoclonal antibodies and against apo B, different reactivities were observed. These results and the reactivities against B-100 and B-48 were combined to construct an antigenic map. However, Dr. Marcel cautioned that this map should not be taken as indicative of the position of the antigenic determinants on the protein sequence, since additional determinant identification is required for reaching a more definitive conclusion. Moreover, the interpretation of these results would be difficult if the reactivity of some of the epitopes is altered by their interaction with lipids. In fact, Dr. Marcel showed that LDL, VLDL, and partially delipidated VLDL reacted differently against the two monoclonal antibodies studied and against polyclonal antibodies. On the other hand, several examples illustrated the versatility of monoclonal antibodies in structural studies, particularly those related to the mapping of antigenic determinants at the surface of the lipoprotein molecule. It was also apparent, however, that the use of monoclonal antibodies in apo B quantification requires more investigation.

In 1980 the Cell Culture Department of the American Type Culture Collection was awarded a contract by the National Institute of Allergy and Infectious Diseases to develop, characterize, bank, and begin distribution of hybridoma cell lines. A progress report on the banking of hybridoma cells was given by Dr. Ann Hamburger, who described the main activities of the Center in the production of large amounts of high-affinity antibodies that are specific for immunogenic histocompatibility antigens, tumor and other surface antigens, viral and bacterial antigens, and single antigenic determinants on a variety of proteins, nucleic acids, and sugars. Dr. Hamburger outlined the characterization and banking procedures and provided details concerning the accessibility of these cell lines and the results on the characterization of the various cell lines. As of October 1, 1982, 116 lines had been submitted to the program, including 26 lines producing antibodies against murine H-2 determinants, five producing antibodies against human HLA determinants, six producing antibodies against cell surface antigen, four directed against mouse lymphoid cell surface determinants, five producing antibodies against tissue-specific or structural proteins, seven lines against viral antigens, and nine lines against antibodies to immunoglobulin determinants; 37 additional lines are in progress.

Although the laboratory program has been generally successful, a number of problems have been encountered, one of which relates to the presence of Mycoplasma in many of the hybridoma cell lines. It has proved difficult to grow these cells and to maintain their antibody production as compared with noninfected cultures. Other cell lines have failed to maintain antibody synthesis, which is probably related to growth conditions, chromosomal loss, or overgrowth by contaminating clones or variants arising during the large-scale propagation of the hybridomas.
Moreover, the antibodies occasionally do not retain the reported specificity. There is now a coordinated effort to characterize the cells more completely and to improve and standardize procedures in culture media. The reasonable success achieved by this unique nonprofit institute justifies hope for further expansion, particularly if appropriate support is received. Banking may also be considered for other hybridoma cell lines, although this would require more experience by the Center. The discussion also included questions about whether the bank should be made available to outside investigations and problems of regulating requests from institutions for cell lines, distributing the hybridoma cell lines, keeping an updated list of available clones, and making information available and accessible.

Session on Standardization of Apolipoprotein Assays

The session, on the standardization of apolipoprotein assays and materials, chaired by Dr. Petar Alanovic, consisted of two presentations which reviewed past and present experiences and the attempts at comparing and standardizing methods for the quantitative determination of apolipoproteins. Dr. Richard Havel presented the final report of a collaborative study undertaken by several laboratories to evaluate the immunoassay of human apolipoprotein A-I, in which investigators compared the values and stability of one purified apolipoprotein A-I preparation and two frozen reference pools. All laboratories of the Arteriosclerosis SCOR program, the laboratories of the Lipid Research Clinics, and the Molecular Disease Branch at NHLBI were invited to participate. Ten laboratories initially indicated willingness to participate, but only six provided complete data. Four laboratories used a radio immunoassay, one used an electroimmunoassay, and one used a radial immunodiffusion assay. Under the supervision of Dr. Bryan Brewer, NHLBI, an apolipoprotein A-I preparation isolated by Drs. Louis Smith and Henry Pownall, Baylor University, was distributed together with one serum pool with a normal triglyceride level (Pool A) and another serum pool with elevated triglyceride level (Pool B). Each sample was analyzed four times at 3-month intervals in duplicate. The results of these assays were considered to be quite encouraging in several aspects. The coefficient of variation among laboratories for Pool A was about 10% and for Pool B about 15%. This indicates that a normotriglyceridemic serum pool may be used as a satisfactory means for comparing results among laboratories even if they use different standards, antisera, and assays. It also appears that frozen apolipoprotein A-I and serum pools shipped on dry ice can be used as stable reference materials. There were, however, several apparent problems to be considered before any future standardization programs are planned and undertaken. The variability among laboratories was greater in the analysis of apolipoprotein A-I than in the analysis of serum pools, suggesting that immunoreactivity of standards varied for different immunoassays, including radioimmunoassay. There was no attempt to evaluate the extent of variation caused by differences on the specificity of various antisera to apolipoprotein A-I.

Dr. Maryvonne Rosseneu reported on the activities of the Lipid-Apolipoprotein Subcommittee of the Standardization Committee of the International Union of Immunological Societies (IUIS) which was established in Geneva in February, 1981. Some of the major goals of the Subcommittee include the evaluation of the presently available methodology for quantitative determination of apolipoproteins, the determination of resources needed for standardization of apolipoprotein assays, and a proposal of a suitable reference method and materials. The Subcommittee decided that the first priority was to standardize assays for apolipoproteins A-I and B. To this end, they surveyed the literature and prepared two manuscripts describing the status of methodology and feasibility of standardizing apolipoproteins A-I and B. Thirty American and European investigators active in this field who were members of the Subcommittee helped write these manuscripts, which were published in the March 1983 issue of Clinical Chemistry. The next steps to be undertaken by the Subcommittee include a survey of apolipoprotein methodology and the preparation and characterization of an apolipoprotein reference pool. This pool, to be used as an IUIS reference standard, will be prepared at the Centers for Disease Control. Some future projects being considered by the Subcommittee are: 1) the evaluation and standardization of the Lowry procedure for measuring apolipoproteins; 2) a recommendation for a standardized use of apolipoprotein ratios such as, A-I/B, A-I/A-II, LDL-C/apo B and HDL-C/A-I; 3) setting up reference methods for apolipoproteins A-I and B at the Centers for Disease Control; 4) a consideration of enzyme-linked immunoassay as a possible reference method for quantitative determination of apolipoproteins; and 5) a request for suggestions from members of the Subcommittee regarding the type and number of apolipoprotein measurements required for diagnosis of primary and secondary dyslipoproteinemias.

An apolipoprotein standardization laboratory has been established at the Centers for Disease Control and radioimmunoassay has been selected as an interim reference method for measuring apolipoproteins A-I and B. This interim reference method will be used to assign defined values of apolipoproteins A-I and B to the “fresh” serum pools. These serum pools will be available as a secondary standard and for comparison studies. Some participants questioned the use of radioimmunoassay as the reference method by pointing out that apolipoproteins occur in relatively high concentrations which can be determined by less sensitive and elaborate procedures. Enzyme-immunoassay was suggested as another potential reference method. However, a consensus
was not reached on this important aspect of stand-
dardization procedure. Dr. Albers noted that in most, if not all, discussions the emphasis is placed on la-
beled antigen as the standard. He suggested that with the availability of monoclonal antibodies these reagents may be considered as another suitable standard, eliminating any problems connected with the physical-chemical state of antigens. The discussion ended with remarks about the distinction be-
tween immunochemical concentration as measured by antibodies and physical mass of apolipoproteins.

Summary of Recommendations

Epidemiology Working Group

Dr. Herman A. Tyroler reported on the recommen-
dations of this group. Continuing clinical and epide-
miologic research is required to clarify the associ-
ation of the apolipoproteins with ischemic heart
disease (IHD). However, the currently evolving tech-
nology of apolipoprotein determinations argues
against their use in routine clinical practice or large-
scale population screening or community interven-
tion. Rapid, precise, and accurate assays for all the
apolipoproteins are not yet available. While qualita-
tive evaluation of individual patients is now practical,
quantitative evaluation of large groups of examinees
e.g., population studies must await further method-
ologic development. Some techniques can now be
applied to large numbers of samples even though
the apolipoprotein assay library is incomplete and
the technology is rapidly developing, but any study
that adopts today’s technology may be outmoded
tomorrow.

During this interim phase, case-control studies are
recommended with attention to an assessment of
multiple apolipoproteins, controlling for the known
associations of IHD with lipids, lipoprotein fractions,
and non-lipid risk factors. Specimens taken years
previously at the beginning of a study from normal
examinees who subsequently developed IHD and
those from appropriate controls who remained free
of IHD can be analyzed for apolipoproteins. This will
provide most of the information on the apo-IHD rela-
tionship in a large cohort using a relatively small
number of analyses (i.e., by use of a case-control
nested in a cohort study design). Implementation of
this recommendation requires knowledge of the ef-
facts of long-duration storage, which laboratory sci-
entists are beginning to acquire.

Continuing familial and genetic research studies of
apolipoproteins are recommended to elucidate he-
reditary syndromes and empirical risks for family
members, particularly if a proband has premature
IHD. These studies may be especially informative
when the proband and/or family members are nor-
molipidemic.

Studies of the effects of lifestyle on specific apoli-
oproteins are recommended; these include specific
nutrients, weight change, exercise, and alcohol.

Apolipoproteins Working Group

Dr. Bryan Brewer reported the recommendations
of this group, and noted that during the last several
years much progress has been made in developing
methods for the isolation and characterization of the
plasma apolipoproteins, and in the elucidation of
the molecular properties of the major plasma apo-
oproteins. Of particular interest have been the separa-
tion of apo B into B-100 and B-48, which appear to be
synthesized predominantly by the liver and intestine,
respectively, and the characterization of the apo E
and apo A-I isoproteins. Techniques have now been
developed to isolate all the major plasma apolipopro-
teins to virtual homogeneity by a variety of chemical
criteria. An assessment of the molecular properties
of the apolipoproteins, however, poses a much more
formidable task. The plasma apolipoproteins have
been shown to self-associate into discrete oligo-
meric complexes. Concomitant with self-association
are marked increases in conformation of the apoli-
oprotein. In addition, apolipoproteins form specific
mixed associations between different apolipopro-
teins. The mixed and self-associations of apolipopro-
teins are markedly influenced by solvent composi-
tion, lipid, temperature, and pressure. Because of
the complexity of mixed and self-associations of
apolipoproteins, and the marked conformational
changes with association, great care must be taken
in characterizing the isolated apolipoproteins. Pro-
tein aggregation, defined as “irreversible” protein
complexes, must be differentiated from oligomeric
species due to readily “reversible” association. Apo-
lipoprotein conformation, and therefore antigenic
sites, are significantly affected by protein aggrega-
tion as well as association. The unique molecular
properties of the apolipoproteins result in many prac-
tical problems related to apolipoprotein quantifica-
tion.

Apolipoproteins purified by standard laboratory
procedures for use as standards for apolipoprotein
quantification must be analyzed for the oligomeric
complexes of the apolipoproteins which undergo
self-association. A number of techniques, including
gel permeation chromatography, analytical ultracen-
trifugation, and high pressure liquid chromatog-
raphy, can now be used to readily determine the
The effects of buffers and method of storage on the molecular properties of the apolipoprotein standards must be carefully addressed. Unfortunately, at present no universal buffer that can be used for storage of all apolipoproteins has been identified.

The presence of the apolipoprotein for quantification as a constituent of a macromolecular complex in plasma presents an almost unique set of problems for the investigator involved in apolipoprotein quantification. In order for the immunochemical assays to provide accurate quantitative data, the apolipoprotein within the sample must be in the same molecular form as the apolipoprotein standard. As a result, some form of delipidation or dissociation of the apolipoprotein from the lipoprotein particle must be performed for the majority of the apolipoproteins currently being quantitated. A number of techniques, including heat, denaturants, or detergents, have been utilized to expose the hidden antigenic sites present on the apolipoproteins when associated with lipoprotein particles.

Each type of unknown sample (e.g., hypercholesterolemic or hypertriglyceridemic plasma) and each apolipoprotein being quantitated must be evaluated for the type of molecular species in solution under the assay conditions used and the comparability of apolipoprotein unknown and apolipoprotein standard. In addition, the immunoreactivity of different apolipoprotein isoforms must be associated with the antibody used by the investigator, and adequate protection of the sample for quantitation against proteolysis must be provided during sample collection and preparation. Because of the large number of variables present, and the number of apolipoproteins currently being quantitated, no general protocol has yet been developed which is applicable to all assay conditions or methods currently in use.

Additional research will be required for an improvement in our current methods for the preparation of samples, the optimal buffers for storage of samples as well standards, reagents for the inhibition of proteolysis, and the conditions which assure that the sample and standard have a similar antigenic exposure. In order to facilitate the standardization of different laboratories, and to provide reference reagents, it was strongly recommended that a secondary standard be made available for distribution. A systematic analysis of the physicochemical and immunological properties of the plasma apolipoproteins will ultimately provide the information necessary for the preparation and distribution of primary apolipoprotein standards by commercial or governmental laboratories.

Apolipoprotein Assays Working Group

In reporting on this group’s recommendations, Dr. John Albers noted that a committee of as many investigators as practical with expertise in apolipoprotein chemistry, immunochemistry, and apolipoprotein immunoassays should be organized to make decisions regarding what should be done to implement apolipoprotein standardization. More information is needed before specific recommendations can be made regarding apolipoprotein standardization. However, it was recognized that we need to establish a center to collect information and data and to produce apolipoprotein reference standards and antisera. One of the first steps in apolipoprotein standardization would involve preparation of “reference pools” or secondary standards that are stable over long-term storage conditions. It was recommended that a central laboratory provide “reference pools” to laboratories with experience in apolipoprotein immunoassay methods. The reference pools should be at several different levels, e.g., mid-range and above and below the normal range. The central laboratory would obtain values on these reference materials from each laboratory and collect information on the details of each method. It was also recognized that the numerical value obtained might be antibody- and method-dependent. For the values to be useful, each of the immunoassays would have to be validated. The values obtained on these “reference pools” could then be made available to investigators involved with apolipoprotein quantitation.

A primary reference standard that is stable over long periods under practical storage conditions is needed for use in the immunoassays. Little is known about the best method of storing purified apolipoproteins, and thus, more information is needed to decide the best method for the storage and transfer of apolipoproteins. Changes in the immunoreactivity of the primary standard may depend upon the specificity of the antibody and the immunoassay used. Stable secondary standards or reference pools can be prepared now. Apolipoproteins appear stable in plasma or serum stored at −70°C. Lyophilized plasma or serum is also a potential alternative for secondary standards or reference pools. More information is needed before specific recommendations can be made regarding the choice of additives to primary or secondary standards to minimize bacterial contamination, oxidation, and proteolytic degradation during storage and handling.

The NHLBI was encouraged to support a central laboratory for apolipoprotein standardization and to facilitate the formation of an apolipoprotein standardization committee.

Working Group on Antibodies

Dr. Angelo Scanu reported for this group that both polyclonal and monoclonal antibodies are very useful reagents that are likely to prove complementary to each other. Each has its own advantages and disadvantages. In both cases the properties and specificities have not been well defined and further studies in this direction are required. The monoclonal antibodies, because of their superfine specificitiy, are
likely to continue to prove useful in the probe of the antigenic distribution at the surface of the lipoproteins, but at the present time they appear to be less suited for screening purposes. Promising, however, are the studies of mixtures of monoclonals aimed at regenerating polyclonal antibodies. We need to better define polyclonal antibodies in terms of animal source, characteristics, specificities, stability, and the ability to recognize the same antigen in different lipoprotein particles. Standardization of both polyclonal and monoclonal antibodies appears to be a most immediate task. This can be achieved by having investigators produce their own polyclonals or monoclonals and submit them to a central laboratory where these antibodies will be tested and compared. Standardization should also be achieved by promoting interactions among laboratories, e.g., an exchange of reagents, particularly if they are characterized according to standardized procedures. The creation of a committee to oversee these interchanges and the operation of a center would be highly desirable. In spite of the current popularity of monoclonals, polyclonal antibodies are likely to continue to play an important role in immunoassay. It is clear, however, that monoclonals are new and exciting reagents, relatively simple to make and, if well characterized, reliable, with superfine specificity. They are well suited to automatic technology which does not require antigen labeling, a property not shared by polyclonal antibodies. The proposition of reconstituting polyclonals from a mixture of monoclonals is a very exciting one and more work should be encouraged in this direction.

It thus appears that, at this stage, more concerted efforts should be made by all laboratories involved in immunoquantification procedures to coordinate their efforts, ideally under the directives of a committee and with access to a laboratory capable of examining and comparing antibodies as well as antigens. Only after appropriate testing and standardization can these antibodies be of general use to the specific community and suitable for general screening purposes.

References
