Editorial

A System for Phenotyping Hyperlipoproteinemia

It has been well established that the lipids in plasma do not circulate free but combine in orderly arrangements with protein. Most of the lipid is combined with two major proteins, the α and β polypeptides, to form lipoproteins that extend over a wide density range, from greater than 1.21 to that of fat itself, about 0.9 Gm. per ml. Changes in this lipoprotein spectrum occur in many diseases. In many instances the lipoprotein abnormality appears to be the primary expression of a biochemical defect. These disorders are usually familial, relatively common, and of particular interest in relation to atherogenesis and coronary heart disease. Despite their relative importance, the present understanding of these hereditary defects is meager and their classification chaotic.

For the determination and study of such abnormalities a number of methods have been available for some time. The minimum is a determination of plasma cholesterol and glyceride content. The maximum in resolving power is offered by the ultracentrifuge, which is capable of measuring changes within small increments of the density spectrum. When one is concerned particularly with familial hyperlipoproteinemia, necessitating screening of large kindreds, neither of these approaches has proved ideal. Measurement of triglycerides is tedious. Furthermore, even after considerable experience, knowledge of both cholesterol and triglyceride concentrations is inadequate for distinguishing all of the familial syndromes. The ultracentrifugal technics also do not meet current needs because they are time-consuming, costly, and not sufficiently available to clinicians.

In the present state of knowledge of fat transport, most clinical studies require only rough quantitation of four groups of lipoproteins known to be related to independent metabolic processes and therefore possibly subject to specific inborn errors. These are the soluble alpha and beta lipoproteins and the two groups of lipoproteins or particles that transport, respectively, glycerides of exogenous or dietary origin (chylomicrons) and of endogenous, mainly hepatic, origin (pre-beta lipoproteins).

These lipoprotein groups can be separated by a rapid and simple method, a modification of established technics for paper electrophoresis. By its application to a large number of subjects with familial hyperlipoproteinemia, we have so far been able to detect what appear to be five different phenotypes, more than have been seriously considered in the past. It has also been possible to follow such patients through various dietary and metabolic studies much more easily than heretofore. Of particular value has been the ability to make a tentative diagnosis of fat or carbo-

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LIPOPROTEIN GROUPS
(PAPER ELECTROPHORESIS)

Normal

<table>
<thead>
<tr>
<th>CHYLOMICRONS</th>
<th>BETA LIPOPROTEINS</th>
<th>PRE-BETA LIPOPROTEINS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>α</strong></td>
<td><strong>β</strong></td>
<td><strong>pre-β</strong></td>
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</tbody>
</table>

Figure 1

The normal plasma lipoprotein pattern. This and all subsequent figures are schematic representations.

hydrate induction of hyperlipemia⁴ without the necessity of prolonged dietary studies.

Method

With the addition of 1 per cent albumin to the buffer, the electrophoretic method of Lees and Hatch separates plasma into four lipid-containing bands detectable by oil-Red-O staining. The particular advantage of the method is the ready distinction it provides between chylomicrons and pre-beta lipoproteins and thus some indication of the source of elevated plasma glycerides. At plasma glyceride levels below 100 mg. per 100 ml. neither of these bands is detectable by electrophoresis. Their usual migrations compared to the beta and alpha lipoproteins are shown schematically in figure 1. The relationships of all four lipoprotein bands to particles or lipoproteins isolated by other familiar technics have been established by appropriate studies⁵ and are shown in table 1.

Classification of Phenotypes

The abnormal lipoprotein patterns observed in propositi and their kindreds have been arbitrarily designated as types I-V. This is done partly for simplicity and partly because the current nomenclature for diseases in this category is inadequate to cope with their heterogeneity.

In subjects with hyperlipoproteinemia the pattern may be greatly altered by diet. Classification is therefore determined by the pattern observed in plasma drawn after a 12- to 16-hour fast and while the patient is on a "normal" American diet including 35 to 45 per cent of calories from mixed fats and 45 to 55 per cent from carbohydrates.

Correlation of the lipoprotein patterns or phenotypes with clinical manifestations and other genetic information in our patients as well as in apparently comparable patients reported by others reveals sufficient consistency within types and differences between types to indicate that each probably represents a different genotype. This has not yet been proved. We have already noted some heterogeneity of patterns within a given kindred although there are reasons to believe that this may reflect uneven distribution of several genes affecting lipoprotein concentrations. It

<table>
<thead>
<tr>
<th>Band</th>
<th>Synonyms</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>Particles of S₄ &gt; 400; secondary particles; primary particles²², ²³</td>
<td>Dietary fat</td>
</tr>
<tr>
<td>Beta lipoprotein</td>
<td>Lipoproteins of density 1.006-1.063 and S₄ 0-20; low-density lipoproteins</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Pre-beta lipoprotein</td>
<td>Lipoproteins of density &lt; 1.006 and S₄ 20-10²; very low-density lipoproteins; tertiary particles ²⁴</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Alpha lipoprotein</td>
<td>Lipoproteins of density &gt; 1.063; high-density lipoproteins</td>
<td>Endogenous</td>
</tr>
</tbody>
</table>

Circulation, Volume XXXI, March 1965
I FAMILIAL FAT-INDUCED HYPERLIPEMIA

Figure 2

Type I

is also likely that the number of possible patterns obtainable by this simple technic is too small for the number of possible mutations that eventually will be discovered.

The present usefulness of the classification system can perhaps best be demonstrated by brief description of each type. Their salient features are summarized in figures 2 to 6.

Type I

This fat-induced hyperlipemia is quite rare in its severe form. The first familial example was reported by Holt and co-workers in 1939. Approximately 35 reasonably certain examples have been reported or are known to us. The diagnosis is established by the presence of massive chylomicronemia on a normal fat intake. The syndrome is usually detected in childhood, frequently because of hepatosplenomegaly, eruptive xanthomas, or bouts of abdominal pain. These manifestations, like the often associated foam cells seen in biopsies of reticuloendothelial tissues, are not limited to the hyperlipemia of type I. Pancreatitis may be a complication; but, interestingly, evidence of accelerated atheromatosis has so far not been reported. Carbohydrate tolerance is characteristically normal.

Among all cases of familial hyperlipemia, these patients have almost uniquely low levels of plasma postheparin lipolytic activity (PHLA), presumably an indication of low activity of lipoprotein lipase in tissue. Proof that this is the inheritable cause of so severe a rate limitation in clearance of glyceride from plasma awaits direct assays of lipoprotein lipase in adipose tissue. It would also seem to require more than the present scanty evidence that this enzyme has a physiologic role in the liver, which apparently is an important site for the initial disposal of chylomicrons.

The type-I syndrome is due to a double dose of a rare abnormal allele. Parents and sibs may not have any fasting hyperglyceridemia although their plasma PHLA may be depressed below that of controls. It has been suggested that lesser defects in clearing of exogenous glyceride are common. Satisfactory evidence has not yet been presented showing that such familial defects exist in the American population.

Type II

This syndrome, characterized by increased beta lipoprotein concentrations (fig. 3), has been recognized for many years and is now best known as essential familial hypercholesterolemia. It is often accompanied by xanthomatosis and by accelerated atheromatosis. The mode of inheritance is still not firmly established, but the available evidence suggests that the type-II syndrome in all of its manifestations may be the expression of a single gene of relatively high frequency in many populations throughout the world. A double dose of the abnormal gene probably leads to

II FAMILIAL HYPER-BETA LIPOPROTEINEMIA

Figure 3

Type II
earlier expression of more severe xanthomatosis. There is still the possibility that several different mutations are involved. Whether the inheritable defect specifically involves the metabolism of cholesterol, phospholipid, the beta peptide, or beta lipoprotein as a unit has never been defined. In our experience and according to limited reports in the literature, glucose tolerance is usually normal in patients of type II and family history of diabetes is not abnormally frequent. This is of great importance in distinctions now to be made concerning types III to V.

Type III

For at least 10 years the literature has abounded with confusing examples of what has sometimes been referred to as "mixed" familial hypercholesteremia (type II) and hyperlipemia. Xanthomatosis, ischemic heart disease, and mild diabetes have frequently been present. Probably most such patients have had what is here termed the type-III lipoprotein pattern (fig. 4). Increased beta lipoprotein is accompanied by significant quantities of pre-beta lipoprotein. Considerable pre-beta lipoprotein may be present without lactescence, and type III is not necessarily accompanied by hyperlipemia. As suggested by Gofman and associates,14 the increased amounts of lipoproteins of Sf > 20 (comparable to pre-beta lipoprotein) tend more to be associated with tuberous and planar xanthomas. However, tendon xanthomas, especially typical of abnormally high concentrations of Sf 0-12 lipoproteins,15 are also often present in type III.

The type-III "syndrome" is a fascinating genetic and metabolic puzzle. In a kindred with a high incidence of coronary artery disease and diabetes, every member of a fairly large sibship may have this pattern; and it is clearly transmitted to subsequent generations. In some kindreds patterns of both type II and III may be present. It is also becoming evident that at least two "subtypes" of type III may be distinguished by paper electrophoresis. Some patients have well separated beta and pre-beta bands, consistent with increased amounts of Sf 0-12 and Sf > 20 low-density lipoproteins. In others Sf 12-20 lipoproteins are elevated as well, producing a pre-beta lipoprotein band that is an inseparable extension of the beta band. Such pattern variations, too, seem to be familial. Here is an instance in which the ultracentrifuge may provide valuable aid in sharpening classification.

It is conceivable that in some patients the type-III pattern represents only a "variant" in the expression of the type-II genotype. Several pieces of evidence, however, suggest that this interpretation is too simple, at least for all type-III families. First, hyperglyceridemia is not randomly distributed among hypercholesteremic families but tends to appear in most or none of the affected members.3 Second, subjects with pre-beta lipoprotein tend to be markedly susceptible to carbohydrate induction of hyperlipemia and, at the same time, to have abnormal glucose tolerance,3 abnormal tolbutamide responses16 or elevated levels of plasma insulin-like activity.16, 17 Third, a diabetic family history is more common among type-III than type-II patients.

It is tempting to speculate that in many patients the type-III abnormality might represent the addition of a "diabetic gene" to the type-II genotype. If this were true for most type-III patients, and considering the estimated gene frequency for diabetes,18 one would expect type II to be perhaps three or four times more common than type III. Appro-
propriate lipoprotein patterns and carbohydrate tolerance need to be determined concomitantly in a sufficiently large sample to help ascertain the reasonableness of this hypothesis.

Type IV

The presence of a familial increase in pre-beta lipoprotein alone may have been previously reported. It is impossible to be sure, for such a lipoprotein pattern may be associated with elevation of plasma glycerides alone or increases in both glycerides and cholesterol in severe examples. We have so far studied three or four kindreds in which all abnormal members tested have had this pattern. As with type III, the pre-beta lipoprotein concentration is abnormally sensitive to dietary carbohydrate intake, is associated with carbohydrate intolerance, and there may be a family history of diabetes.

More experience is required to determine the separateness of type IV from other syndromes and its relationship to diabetes. Further probing of the inherited defect is of considerable importance. In a survey of men who had had a myocardial infarction, without reference to familial occurrence, pre-beta lipoprotein has been shown to be extraordinarily frequent. That type IV is the predominant pattern indicating special proneness to coronary artery disease in young men is also suggested by retrospective studies utilizing measurements of $S_r$ subclasses or plasma cholesterol and glyceride analyses.

There are a number of potential sites of primary abnormality in the metabolism of endogenous glyceride and its transport from liver to adipose tissue as pre-beta lipoprotein. With respect to the abnormal ease of carbohydrate induction noted in the familial types III and IV, the primary defect may be increased diversion of glucose to glyceride in the liver or decreased direct utilization of glucose loads by the adipose tissue. In some instances enhanced flux of free fatty acids (FFA) from adipose tissue to liver could also cause increased pre-beta lipoproteinemia. Increased FFA mobilization is frequently considered a source of "stress-induced" hyperlipemia, but the operation of this mechanism in familial disorders remains to be demonstrated.

In any event, such hyperlipemia always indicates inadequate ability to clear pre-beta lipoproteins from plasma at rates commensurate with their synthesis. A primary defect in the ability to adjust to enhanced glyceride loads, probably operating in adipose tissue, is thus another possibility. Adequate glucose catabolism is required for the uptake of glyceride by adipose tissue. The association of defective glucose metabolism with carbohydrate induction of hyperlipemia enhances the attractiveness of this last hypothesis.

Type V

Three families have so far been uncovered in which a complex pattern suggestive of defects in metabolism of both exogenous and endogenous glyceride predominates. This has been called type-V (fig. 6). Such a pattern could be a different expression of single or
combined genotypes previously mentioned, or evidence of other mutations. As in type I there is ease of fat induction (severe chylomicronemia), bouts of abdominal pain, and sometimes modest decreases in PHLA in type V. Although patients with type-I syndrome are also susceptible to accumulation of pre-beta lipoproteins on low-fat, high-carbohydrate diets (making them "carbohydrate" as well as fat inducible), this is probably due to the severity of their defect in clearing glyceride in any form. There is no diabetic tendency in type I. In type V, on the other hand, glucose intolerance and family history of diabetes are common, and in this they closely resemble the easily carbohydrate-induced type IV. Hyperlipemia in type IV may sometimes become quite severe, and "chylomicrons" may accumulate at the origin on paper electrophoresis if the patient's fat intake is high. This chyli-
micron accumulation is always small relative to the pre-beta lipoprotein excess and is probably an expression of a well-known delay in clearing ingested fat in presence of any type of hyperlipemia. By type V, however, is meant a heavy accumulation of both chylomicrons and pre-beta lipoproteins on a regular diet. At present type V seems distinct from all the types previously mentioned. Involvement of more than one generation has been observed in all three kindreds, but no certain genetic mode is distinguishable. Further examples of type V will undoubtedly be uncovered with more extensive survey. Such patients would seem to be prototypes of many reported examples of "sporadic" hyperlipemia, which clears only partially with fat restriction and is associated with many of the ancillary features of type I.

Comment

All the possible permutations or features of this simple method for lipoprotein separation have not been discussed. Nothing has been said, for example, of alpha lipoprotein. This band is distinctly decreased in type I, as has been well known from earlier studies, and tends to be decreased in types III to V. Alpha lipoprotein is, in fact, responsible for the mig-

6. HOLT, L. E., JR., AYLWARD, F. X., AND TIMBRES,

Ideas and Experimental Proof

What if wise men had, as far back as Ptolemy,
Judged that the earth, Like an orange was round,
None of them ever said, Come along, follow me,
Sail to the West, and the East will be found.—Arthur Hugh Clough. 1819-1861.
Editorial: A System for Phenotyping Hyperlipoproteinemia
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