The Mysteries of Lipoprotein(a)

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Lipoprotein(a) [Lp(a)] is a macromolecular complex found in human plasma that combines structural elements from the lipoprotein and blood clotting systems and that is associated with premature coronary heart disease and stroke. It is assembled from low-density lipoprotein (LDL) and a large hydrophilic glycoprotein called apolipoprotein(a) [apo(a)], which is homologous to the protease zymogen plasminogen. Plasma Lp(a) concentrations vary 1000-fold between individuals and represent a continuous quantitative genetic trait with a skewed distribution in Caucasian populations. Variation in the hypervariable *apo(a)* gene on chromosome 6q2.6-q2.7 and interaction of *apo(a)* alleles with defective LDL-receptor genes explain a large fraction of the variability of plasma Lp(a) concentrations. Though of high theoretical and practical interest, many aspects of the metabolism, function, evolution, and regulation of plasma concentrations of Lp(a) are presently unknown, controversial, or mysterious.

LOW-DENSITY LIPOPROTEINS (LDLs) ARE THE MAJOR transporters of cholesterol in human plasma. They contain a single hydrophobic polypeptide chain, apolipoprotein B-100 (apoB-100), which has a molecular mass of 513 kD. This protein is one of the two principal ligands for the LDL receptor that facilitates the specific uptake of LDL primarily by parenchymal cells of the liver (*1*). In 1963, Berg (*2*) described genetic variation in LDL that was detected with heterologous antibodies from rabbits immunized with human LDL; he attributed this genetic variation to an antigen he called lipoprotein(a) [Lp(a)]. The Lp(a) antigenic property was later shown to reside in a distinct particle. It appeared that not all subjects had this lipoprotein in plasma, suggesting that it might be of only minor importance. Although Lp(a) was characterized in some detail already in the early 1970s (*3*), little attention was paid for almost two decades to this macromolecular complex that is assembled from LDL and a high molecular mass glycoprotein called the Lp(a) glycoprotein or apolipoprotein(a) [apo(a)] (*4*). Numerous studies suggested an association of plasma Lp(a) concentrations with atherosclerotic vascular disease (*5, 6*). However, only the recent discovery of the strong homology of apo(a) to plasminogen (*7, 8*) has stimulated intensive research in the genetics, metabolism, function, and disease association of Lp(a).

There are many questions, however, that await clarification or to which there are only partial answers. (i) What is the function of apo(a) and of Lp(a), if any? (ii) What is the mechanism underlying the strict genetic control of plasma Lp(a) concentrations? (iii) Where is Lp(a) assembled? (iv) Is there any relation between Lp(a) metabolism and that of LDL, and is Lp(a) catabolized by the same specific mechanism as other apoB-100–containing lipoproteins, that is, by the LDL-receptor pathway? (v) Is Lp(a) associated with premature myocardial infarction because it is atherogenic or thrombogenic, or both?

One major focus of research is on the genetics of Lp(a). Though the population distribution of Lp(a) concentrations in Caucasians is extremely broad and highly skewed, it is at the same time under rather strict genetic control. This strict genetic control of plasma Lp(a) concentrations and the association of high plasma Lp(a) concentrations with premature myocardial infarction are of particular relevance to predictive medicine.

Structure of Human Lp(a)

Lp(a) is different from other plasma lipoproteins in that it is assembled from two very different components (Fig. 1). One of these components, the hydrophilic apo(a), is unlike other known proteins of the plasma lipoprotein system and possibly should not even be classified as an apolipoprotein. The other component shares structural and functional properties with LDL; it has a density of approximately 1.04 grams per liter, contains apoB-100, and binds to the LDL receptor (*9*). Early studies showed that Lp(a) is not simply a variant of LDL distinguished by its antigenic properties. Rather Lp(a) differs from LDL in protein composition and electrophoretic mobility and by having a larger particle size (diameter 236 to 255 Å versus 200 to 225 Å), a higher buoyant density (1.05 to 1.08 g/liter versus 1.03 to 1.06 g/liter), and six times as much neuraminic acid (*3*). After simple disulfide reduction, Lp(a) dissociates into LDL and apo(a) (*9, 10*). This has led to the conclusion that apo(a) and LDL are covalently linked by disulfide-bridge formation. The LDL component of Lp(a) contains apoB-100 as the sole protein component, the cDNA-derived amino acid sequence of which has been elucidated (*11*). The protein conferring the characteristic properties to the Lp(a) complex is the highly glycosylated apo(a). Apparent molecular masses ranging from 200 to 700 kD have been reported for apo(a) and individuals with multiple apo(a) size isoforms have been described (*4*). Only recently it has been shown that this size heterogeneity is genetically controlled (*10*).

Sequencing of apo(a) at both the protein and cDNA level has revealed a high degree of homology to plasminogen (*7, 8*). Homology to plasminogen is also revealed by immunochemical studies that show cross-reactivity of apo(a) and plasminogen (*12, 13*). The immunochemical cross-reactivity of apo(a) and plasminogen and the size polymorphism of apo(a) have created problems for the quantification of Lp(a), but some of these have been overcome by the use of

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sandwich-type enzyme-linked immunoassays and combinations of antibodies to apo(a) and apoB (*14*) or monoclonal antibodies specific for apo(a) (*15*).

Plasminogen is a serine protease zymogen of the fibrinolytic system. It consists of a trypsin-like protease domain and five tandemly repeated homologous domains called kringles. Kringles are pretzel-like structures that are stabilized by three internal disulfide bridges. Kringle structures have also been identified in a variety of proteins of the blood coagulation and fibrinolytic systems, including prothrombin, tissue-type plasminogen activator (tPA), urokinase, and coagulation factor XII, and also in other proteins, for example, fibronectin (*16*). The apo(a) gene [*apo(a)*] contains a single serine protease domain that is 94% identical to the protease domain of the plasminogen gene. Further, it contains two types of plasminogen-like kringle domains. One of these is homologous to the fifth kringle domain of the plasminogen gene (kringle-5) and is present as a single copy. The other is homologous to the fourth kringle domain of the plasminogen gene (kringle-4) and is present in multiple copies. The size of the apo(a) mRNA was measured as 14 kb by RNA–gel blot hybridization of RNA from one human liver and the human hepatocarcinoma cell line Hep G2 (*8, 17*). This large mRNA encodes a protein of 4529 amino acids, including a 19-residue presequence (*8*).

There is considerable intragenic homology in apo(a). Out of the 37 repeats of the 342-bp kringle-4 domain in the only *apo(a)* yet sequenced, 24 ± 2 are identical. Overall the kringle-4 structures in *apo(a)* are highly conserved and have a high degree of homology with the kringle-4 domain of the plasminogen gene. There are, however, some specific differences between homologous structures of apo(a) and plasminogen. Unlike plasminogen, apo(a) cannot be converted to an active protease by tPA, urokinase, or streptokinase. This is because of a single amino acid substitution (serine for arginine) in apo(a) at the position that is cleaved in plasminogen when the zymogen is converted to the active protease (*8*). Also there is one extra unpaired cysteine residue in one of the kringle-4 repeats of apo(a). This residue is thought to facilitate the covalent binding of apo(a) with apoB-100 (*8*). In the connecting sequences between the kringle-4 units, including the end and the beginning of a kringle, there are six potential sites for O-linked sugars (four threonine and two serine residues). Further, there is one site for an N-linked sugar within each kringle structure. Thus in total there are 253 potential glycosylation sites in apo(a). It is not known which of these sites are actually glycosylated; however, Lp(a) is very rich in carbohydrate, staining strongly with the periodate-Schiff reagent and containing 28.1% carbohydrate by weight. Mannose, galactose, galactosamine, glucosamine, and sialic acid are present in an approximate molar ratio of 3:7:5:4:7, respectively (*18*).

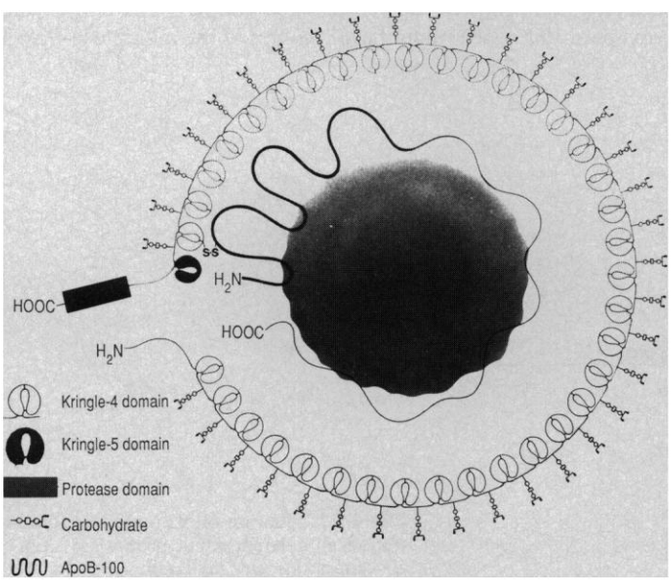

Fig. 1. Schematic model of Lp(a) (*4, 7, 8*). The central LDL particle, with its core of neutral lipids and the apoB-100 molecule, is attached to one molecule of apo(a). The mode of interaction of the LDL component with the apo(a) moiety and the spatial orientation of these components are unknown, as is the exact topology of the postulated (but not proven) disulfide bond (S-S) between apoB-100 and apo(a). The kringle domains may face out rather than in toward LDL. The regularity of carbohydrate chains also is a simplification.

Genetics of Lp(a)

Early studies by Berg (*2*) were consistent with Lp(a) being inherited as a simple dominant Mendelian trait under the control of two alleles, Lp^a and Lp^0. This view was challenged when several groups detected that the distribution of plasma Lp(a) concentrations is continuous but highly skewed (*3, 19*). Most Caucasians have plasma Lp(a) concentrations at the low end of the range, with 5% of Caucasians having a concentration of less than 1 milligram per deciliter (*15*). Mean and median concentrations are approximately 15 and 8 milligrams per deciliter, respectively. There are, however, strikingly different distributions in other ethnic groups ranging from bell-shaped in U.S. Blacks (*20*) and African Blacks to highly skewed in Chinese from Singapore (Fig. 2).

Several hypotheses have been advanced to explain the inheritance of plasma Lp(a) concentrations. Most groups agree in that one major locus is controlling plasma Lp(a) concentrations, but polygenic models have also been proposed (*19, 21*). The frequency (q) of a postulated major dominant gene for high Lp(a) concentrations was estimated as being 0.10. The nature of this major locus remained undefined, however, and it was difficult to see how the concentrations of such a complex molecule as Lp(a) could be determined in such a strict manner as to mimic a simple Mendelian trait.

The discovery of a genetic size polymorphism of apo(a) provided new insights into the genetics of the quantitative Lp(a) trait and allowed the identification of the *apo(a)* locus as the major locus that determines plasma Lp(a) concentrations (*10, 22*). Several apo(a) isoforms can be distinguished in total plasma from different individuals by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting with poly- or monoclonal antibodies against apo(a) (Fig. 3). These isoforms range in apparent molecular mass from approximately 400 to 700 kD. A single individual may have one or two major apo(a) isoforms or may be without a detectable apo(a) band. Together at least six different apo(a) isoforms have been distinguished but no individual exhibits more than two of them. These six isoforms have been designated F, B, S1, S2, S3, and S4 according to their relative mobility compared to apoB-100 (here F stands for fast, B for the position of apoB-100, and S for slow). Intermediates between these isoforms have been observed but will not be discussed here. The major apo(a) isoforms are specified by alleles at a single locus that has been identified as that of *apo(a)*. In addition, the existence of a null allele has been postulated to account for individuals with no detectable apo(a) and for the segregation of apo(a) types in families (*10, 22*). The definition of the null allele in the Lp(a) system is operational as it depends, at least to some extent, on the sensitivity of the immunoblotting assay. There is still debate on whether there are subjects who completely lack Lp(a) in plasma. The segregation of apo(a) types in 21 families including 192 subjects is consistent with the

genetic hypothesis (*10, 22*). The frequencies of apo(a) phenotypes and the calculated frequencies of *apo(a)* alleles as determined in a Caucasian population are shown in Table 1. These frequencies should be viewed as rough approximations because of the inherent problems of the system. Some "null types" in fact may have an apo(a) isoform, although this is not detectable by current methods. Some single-band types may in fact be heterozygotes but with one of the isoforms not detectable by the blotting method. According to this concept subjects with two apo(a) isoforms are heterozygotes, whereas those with a single band may be either homozygous or heterozygotes with one null allele.

Apo(a) Types and Lp(a) Concentrations

There is a strong inverse relationship between the apparent molecular mass of apo(a) isoforms and plasma concentrations of Lp(a). The mean Lp(a) concentration of individuals with apo(a) of the B-type is more than ten times as high as that in individuals with the S4-type (Table 1). Heterozygotes tend to have plasma Lp(a) concentrations that almost equal the sum of those of the respective single-band types (*23*). This indicates that *apo(a)* alleles affect Lp(a) concentrations in an additive manner. In support of this is the finding that the intensities of apo(a) isoforms on immunoblots in heterozygotes may be extremely different (*22*). The two apo(a) isoforms in heterozygotes reside on different particles that may be separated by density gradient ultracentrifugation (*24*). This suggests that heterozygotes have two particle species in plasma that differ in their metabolism. Hence the association of isoform molecular mass with Lp(a) concentrations is reflected even within a single subject. These findings provide a conceptual framework to explain the distribution of Lp(a) concentrations within populations. In Caucasians, the skewed distribution is a result of *apo(a)* allele frequencies and of the allele-specific effects on Lp(a) concentrations. Alleles that result in high Lp(a) concentrations, such as Lp^{B}, are rare in the Caucasian population, whereas those that result in low Lp(a) concentrations, such as Lp^{S4}, are frequent. A definitive answer as to whether the differences among populations (Fig. 2) are explained by differences in *apo(a)* allele frequencies or allele-specific effects, or both, is not yet available. Apo(a) phenotyping in Chinese individuals from Singapore, who have very low plasma Lp(a) concentrations (Fig. 2), did demonstrate an extremely high frequency of the Lp^{S4} allele which is associated with low Lp(a) concentrations in Caucasians (*22*). This suggests that the differences in the distribution of Lp(a) concentrations may be due to *apo(a)* allele frequency differences between ethnic groups. The reason why apo(a) types or concentrations are so different among human races is mysterious. The relation between apo(a) type and concentration is, however, not as simple as outlined. This is obvious from the true distribution of Lp(a) concentrations within a given apo(a) phenotype, for example, S2. There is not only a broad distribution of concentrations within the S2-type, but the distribution is also heterogeneous (*23*). Also the variation at the *apo(a)* locus does not explain the total variation of Lp(a) concentrations in the population. With the methodology of the measured genotype approach, it has been determined that about 40% of the variability in Lp(a) concentrations is explained by the measured variability at the *apo(a)* locus (*23*). This figure most certainly underestimates the true significance of the *apo(a)* locus as at present only the measured heterogeneity, that is, the size polymorphism of apo(a), can be related to Lp(a) concentrations. It is not known how the apo(a) size polymorphism affects plasma Lp(a) concentrations. It may be a direct consequence of the structural differences in the gene products or there may be linkage disequilibrium between structural and regulatory elements in *apo(a)*. Clearly the *apo(a)* locus is the major locus for determining Lp(a) concentrations in Caucasians, but still the calculated figure of 40% raises the question of what determines the rest of the variation in Lp(a) concentrations.

Linkage of Apo(a) and Plasminogen Genes

The plasminogen gene was localized to the long arm of chromosome 6 at q2.6–q2.7 by in situ hybridization and somatic cell hybrid studies with a probe to the plasminogen kringle-4 domain (*25*). In retrospect, this probe might have recognized the multiple kringle-4

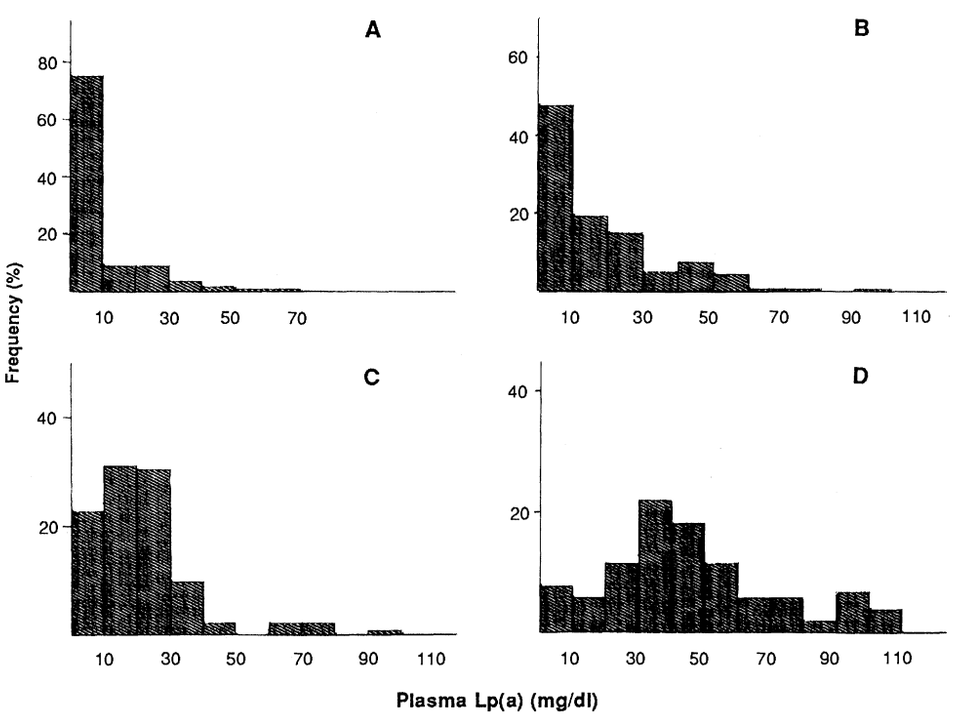

Fig. 2. Distribution of plasma Lp(a) concentrations in different ethnic groups (*46*). Lp(a) concentrations (in milligrams per deciliter) were measured by electroimmunodiffusion (*10*). (**A**) Chinese from Singapore (n = 112, mean Lp(a) concentration is 7.0 mg/dl). (**B**) Austrians, representing a Caucasian population (n = 162, mean Lp(a) concentration of 16.1 mg/dl). (**C**) Indians from Singapore (n = 145, mean Lp(a) concentration of 20.0 mg/dl). (**D**) Sudanese (n = 105, mean Lp(a) concentration of 45.7 mg/dl).

Table 1. Apo(a) isoforms and allele frequencies, and plasma Lp(a) concentrations (mean ± SD) in the Austrian population and in individuals with familial hypercholesterolemia (FH). Data from (*28*).

Isoform	Molecular mass (kD)	Allele	Allele frequencies		Phenotype*	Phenotype frequency (%)		Lp(a) concentration (mg/dl)	
			Population (n = 279)	FH patients (n = 102)		Population	FH patients	Population	FH patients
F	400	Lp^F	<0.002		B	1.1	1.0	61.7 ± 33.8	79.0
B	460	Lp^B	0.007	0.013	S1	2.9	3.9	34.4 ± 20.7	58.7 ± 32.0
S1	520	Lp^{S1}	0.017	0.034	S2	17.9	23.5	24.5 ± 24.2	62.8 ± 34.8
S2	580	Lp^{S2}	0.154	0.206	S3	20.8	16.7	10.2 ± 9.7	35.3 ± 31.6
S3	640	Lp^{S3}	0.209	0.163	S4	31.9	27.5	5.7 ± 7.6	23.4 ± 14.0
S4	700	Lp^{S4}	0.269	0.237	O	5.7	4.9	0.4 ± 1.3	2.0 ± 4.4
		Lp^0	0.344	0.345					

*Only single-band phenotypes shown.

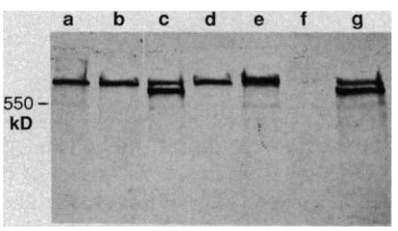

Fig. 3. Principal apo(a) phenotypes. Delipidated plasma samples from individual donors were subjected to 6.7% SDS-PAGE under reducing conditions. Apo(a) bands were visualized by immunoblotting with monoclonal antibody 1A2 to apo(a) (*47*). Single-band types representing homozygotes or individuals with one null allele are shown in lanes (a), (b), (d), and (e) (phenotype S2). Double-band types representing heterozygotes are shown in lanes (c) and (g) (phenotype S1/S2). In lane (f) is a null type.

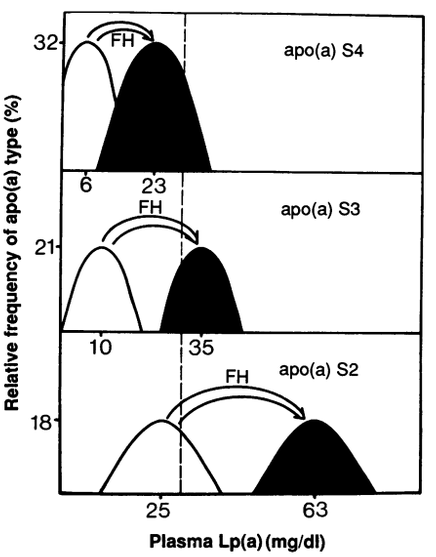

Fig. 4. Model demonstrating the multiplicative effects of the apo(a) and the LDL receptor loci on plasma Lp(a) concentrations. Numbers under the curves give the approximate mean Lp(a) concentration (in milligrams per deciliter) in a group (see Table 1). White areas under the curves represent the highly idealized distribution of Lp(a) concentrations for the respective phenotype in the population and black areas represent the corresponding distribution in individuals with heterozygous familial hypercholesterolemia (FH). The FH mutation (indicated by the arrow) does not result in a constant increase but in an approximately threefold increase in Lp(a) concentrations. The dashed vertical line indicates the threshold of 30 mg/dl, above which Lp(a) concentrations are believed to increase the risk for premature coronary heart disease.

repeats in *apo(a)* rather than the single plasminogen kringle-4 domain. Linkage studies that used as markers the apo(a) protein polymorphism, plasma Lp(a) concentrations, the plasminogen protein polymorphism, and a Sac I polymorphism recognized by the plasminogen kringle-4 probe unequivocally demonstrated linkage of the plasminogen gene and *apo(a)* (lod score >5.0 at a recombination fraction, $\theta = 0$) and established 6q2.6–q2.7 as the region of the *apo(a)* and plasminogen gene cluster (*26*).

Apo(a) Types and Lp(a) Concentrations in Familial Hypercholesterolemia

As Lp(a) contains apoB-100, one of the two principal ligands for the LDL-receptor, it might be anticipated that Lp(a) would bind to this receptor and be removed from plasma through the LDL-receptor pathway. Unfortunately, in vitro studies on the binding and uptake of Lp(a) by the LDL receptor have yielded controversial results (*27*), and it has been concluded by some that attachment of apo(a) to the LDL-like particle markedly reduces or abolishes binding to the receptor (*9*). The question of whether or not Lp(a) is removed through the LDL-receptor pathway in vivo may be addressed by studying individuals with familial hypercholesterolemia (FH), a common, dominant human disorder that results from multiple defects in the gene for the LDL receptor (*1*). As a consequence of the receptor defect, heterozygotes for FH have plasma LDL-cholesterol concentrations that are two- to threefold the normal value. If Lp(a) were also cleared by the LDL receptor in vivo, one would anticipate a similar increase in Lp(a) concentrations in individuals with defective LDL receptors. As Lp(a) concentrations in the general population are largely determined by apo(a) type, any effect of the receptor defect on Lp(a) concentrations will only be detected in subjects matched for apo(a) phenotype. Therefore, Lp(a) types and concentrations were compared in individuals with heterozygous FH and in a random population sample (Table 1) (*28*). Three major results emerged from this study. First, Lp(a) concentrations are indeed significantly increased in FH heterozygotes (Table 1), suggesting that the LDL-receptor defect does affect Lp(a) concentrations in the same manner as it increases LDL concentrations. Therefore, these results may be taken as indirect evidence that the LDL-receptor pathway is a crucial determinant of Lp(a) removal in vivo. Though straightforward, this interpretation may be incorrect. Given that the accumulation of Lp(a) in heterozygotes is a direct consequence of a failure of the mutant LDL receptor to interact with apoB-100 in Lp(a), one would expect a complementary situation when apoB-100 itself is defective. A mutant of apoB-100 that does not bind to the LDL receptor has recently been described (*29*). When six independent families with familial defective apoB-100 were analyzed for apo(a) isoform and Lp(a) concentrations, no effect of the apoB-100 mutation on Lp(a) concentrations was noticed (*30*). These data obviously contradict the conclusion that failure to interact with the defective receptor is the reason for the increased Lp(a) concentration in FH. What then causes the elevated Lp(a) concentrations in individuals with FH? At the moment this is still another mystery of the Lp(a) lipoprotein.

The second major result to emerge from the FH study was that the interaction of apo(a) type with LDL-receptor defect is multiplicative rather than additive in nature (Fig. 4). Such a situation is not commonly considered in statistical human genetics. The multiplica-

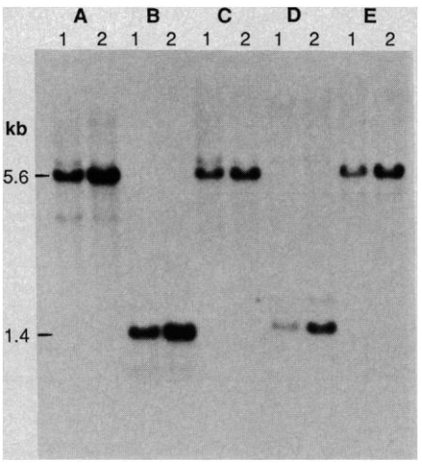

Fig. 5. Gel blot of genomic DNA from subjects with apo(a) types B/S2 (lanes 1) and S4 (lanes 2). DNA was digested with the enzymes Pvu II (A), Rsa I (B), Eco RI (C), Sac I (D), and Bam HI (E), and the fragments were hybridized with a 45-nucleotide oligomer corresponding to a sequence in the repeated kringle-4 domain of *apo(a)* (nucleotides 551 to 595). The signal intensities of the major fragments (5.6 or 1.4 kb) are different in the two phenotypes.

tive interaction of the *apo(a)* and LDL-receptor gene loci is deduced from the observation that Lp(a) concentrations were increased approximately two- to threefold in each common apo(a) phenotype (Table 1). The idea that plasma Lp(a) concentrations are determined by a major gene and a polygenic background therefore may be formally correct. We now know that the major gene is the *apo(a)* locus. One of the "polygenes" may be the LDL-receptor gene locus.

Finally, it emerged from the FH study that an increased Lp(a) concentration is a strong and independent risk factor for coronary heart disease in individuals with FH (*31*). FH individuals with coronary heart disease had on the average higher plasma Lp(a) concentrations (mean of 59.7 milligrams per deciliter) than those without (mean of 28.7 milligrams per deciliter). By multivariant analysis, plasma Lp(a) concentration was the best discriminant between FH individuals with and without coronary heart disease.

Thus the LDL-receptor defect has a dual effect on coronary heart disease morbidity by increasing both LDL and Lp(a) concentrations. Whether or not Lp(a) concentrations will be in a "pathological" range, however, depends on the apo(a) type. These findings may also have practical implications. Determination of plasma Lp(a) concentration might be considered in individuals with FH and possibly also other individuals with elevated LDL concentrations for a personal risk assessment.

Molecular Basis of Apo(a) Heterogeneity

It has been hypothesized that the size heterogeneity of apo(a) reflects differences among individuals in the number of kringle-4 repeats in *apo(a)*. Recently, indirect evidence for this hypothesis has been obtained. When genomic DNA from different individuals was digested with any of the restriction enzymes Pvu II, Eco RI, Bam HI, Rsa I, or Sac I, subjected to DNA-gel blotting, and hybridized to a 45-nucleotide oligomer corresponding to the highly repeated apo(a) kringle-4 region (nucleotides 551 to 595 of kringle-4A) (*8*), only one main fragment was obtained irrespective of the apo(a) phenotype of the individual (Fig. 5). Pvu II, Eco RI, and Bam HI generated fragments of identical size (5.6 kb) as did Rsa I and Sac I (1.4 kb). The intensity of the signal obtained with the oligonucleotide probe varied widely among individuals and was much stronger in individuals of phenotype S4 than in those with lower molecular mass isoforms (for example, B/S2; see Fig. 5). A clear correlation of the kringle-4 signal with the combined molecular mass of the apo(a) isoproteins of an individual was found in family studies (*32*). These results indicate that there is a high degree of internal homology also of the repeated introns in this region of *apo(a)*. This is uncommon and points to a very recent expansion of the locus. The results also indicate that differences in the number of kringle-4 repeats in *apo(a)* indeed underlie the size heterogeneity of apo(a). If so, one might also expect differences in the size of apo(a) mRNA between individuals that should correspond to the apo(a) isoform pattern in plasma. Heterogeneity of human apo(a) mRNA has been demonstrated by gel blotting of liver RNA. The mRNA sizes for apo(a) range from 10 to 14 kb, with some individuals exhibiting two mRNA species (*17*). A recent report (*33*) found that the size and type of apo(a) protein heterogeneity in baboons correlates with the heterogeneity of apo(a) mRNA from liver. However, in both species there were also discrepancies between the protein and mRNA types; for example, one liver mRNA species but two plasma apo(a) isoforms and vice versa. The reason for this discrepancy is unknown.

Evolution

A protein with the immunochemical properties and having a molecular mass similar to human apo(a) has been detected by immunoblotting in several species of nonhuman primates and Old World monkeys including the chimpanzee, orangutan, gorilla, rhesus monkey, and baboon. In all these species the protein is polymorphic (*33, 34*), and in several of them a lipoprotein with the characteristics of Lp(a) has been demonstrated (*35*). Neither Lp(a) nor apo(a) could be found in New World monkeys, rabbit, rat, or cow (*34*). These findings are complemented by the sequence comparison of human apo(a) with plasminogen. The degree of homology between the two structures suggests that they diverged some 40 million years ago (*8*), around the time when Old World and New World monkey lineages diverged.

These data suggest a scenario where apo(a) has evolved from plasminogen during early primate evolution by a series of events including an initial duplication, deletions (kringles-1 to -3 in the human lineage and kringles-1, -2, -3, and -5 in the rhesus lineage), duplications and deletions caused by out-of-register homologous recombination (kringle-4), gene conversion events, as well as point mutations and small deletions (*8, 36*). The presence of a size polymorphism in all primates studied thus far indicates that expansion and contraction of the locus by homologous recombination is probably still occurring.

A protein of the same apparent molecular mass as apo(a) that assembles with LDL and cross-reacts with antibodies to human apo(a) is present in the hedgehog (*37*). Notably, apo(a) is present in the hedgehog already as a lipoprotein and not just as apo(a). The existence of Lp(a) in the hedgehog raises several questions. (i) Has apo(a) been discovered by nature twice? (ii) Is apo(a) from the hedgehog by some mysterious accident cross-reacting with the primate protein, whereas this cross-reactivity does not exist in other species, which also might have the protein in plasma. Possibly, divergent kringle sequences have expanded independently in different animal species, resulting in a lack of cross-immunoreactivity and DNA hybridization but maintaining the same function. (iii) Finally, could it be possible that some species secrete Lp(a) into plasma, whereas others do not? It might be that in some species, including humans, there is erroneous secretion of apo(a) from cells. In order to have Lp(a) in plasma it is not only necessary to preserve an unusually large open reading frame of 13,644 nucleotides, including the presequence, for a protein that is targeted to the endoplasmic reticulum. The protein also has to meet the requirement for specific binding to LDL. For the human and rhesus proteins, this property is believed to require disulfide bond formation with apoB-100 in LDL and is ascribed to a single extra cysteine residue in one of the

kringle domains (*8, 36*). Together these structural requirements indicate that there must be selective pressure on the gene to maintain it in different species. Why then is it not present in other species?

Function of Lp(a) and Apo(a)

The physiological function of apo(a) or Lp(a) is unknown. Apparently even the near absence of Lp(a) from plasma does not cause a deficiency syndrome or any kind of disease. Also, Lp(a) and apo(a) seem not to be present in the plasma from most species evolutionarily below Old World monkeys. Nonetheless, various in vitro functions have recently been ascribed to apo(a) or Lp(a) (*38–40*). When considering the function of Lp(a) it should be borne in mind that apo(a) and LDL are complementary elements from two different functional systems. This has led to the suggestion that Lp(a) may bridge the two systems also in a functional sense (*41*). Structurally, apo(a) is a member of the protein superfamily that includes the regulatory proteases of the fibrinolytic and blood coagulation systems (*16*), many of which contain kringle structures. All of these proteins contain a protease domain with homology to the serine protease, trypsin. The function of the noncatalytic segments of these proteins is to mediate the binding to other molecules. In the case of apo(a), this other molecule might be LDL. For what reason does apo(a) bind to LDL? Or does apo(a) target a fraction of LDL to a specific binding site? It has been speculated that Lp(a) may bind to fibrin, thus delivering cholesterol to places of recent injury and wound healing (*41*). Indeed, it has been shown that proteolytical digestion of fibrin by plasmin not only results in the known binding of plasminogen but also of apo(a) to the degraded fibrin (*38*). Other suggestions are that Lp(a) may modulate the clotting process. Lp(a) and apo(a) do not exhibit proteolytic activity either alone or in the presence of tPA, urokinase, or streptokinase (*7*). It has been shown (*8, 36*) that two residues of the catalytic triad (His, Asp, and Ser) have been substituted in rhesus monkey apo(a), rendering it inactive. Karadi *et al.* (*12*) have found that Lp(a) prolongs the time required for fibrinolysis in an assay where fibrinolytic activity is stimulated with streptokinase. The mechanism for this appears to be an inhibition of the conversion of plasminogen to plasmin by streptokinase-mediated activation (*39*). Lp(a) inhibits plasminogen activation by competitive inhibition at low concentration and by noncompetitive inhibition at high concentration. Three recent studies have shown that apo(a) and Lp(a) compete with plasminogen for binding to the plasminogen receptor (*40*). These properties of apo(a) may explain the association of high Lp(a) concentrations with myocardial infarction. None of the latter in vitro functions described for apo(a) require the LDL moiety of Lp(a) or provide a reasonable explanation for its presence in the complex.

Metabolism of Lp(a)

By examining the apo(a) phenotype and concentration in individuals undergoing therapeutic liver transplantation and in the respective organ donors, it was found that a complete conversion of the apo(a) type of the recipient to that of the donor occurs after liver transplantation (*42*). Thus the major site of synthesis of plasma apo(a) appears to be the liver, a finding consistent with the presence of apo(a) mRNA in human, baboon, and rhesus monkey liver and in the Hep G2 cell line (*8, 17, 33, 36*). However, the demonstration of the major synthetic site for apo(a) unfortunately does not reveal where Lp(a) is assembled. LDL and apo(a) may be secreted independently from each other, and the assembly may take place in the plasma. Such a mechanism could explain why only a fraction of LDL, which varies from subject to subject, is complexed with apo(a). This fraction would be determined by the amount of apo(a) secreted rather than by the LDL concentration in plasma. From in vivo turnover studies of Lp(a) in humans, it has been concluded that differences in plasma Lp(a) concentrations among individuals are a result of differences in synthesis rather than by differences in catabolism (*43*). This conclusion, which is based on the study of only a few individuals of unknown apo(a) phenotype, requires confirmation in individuals of known apo(a) phenotype.

An alternative site for the assembly of apo(a), apoB-100, and lipids would be in the liver cell. The requirement for the formation of a specific disulfide bond between apo(a) and apoB-100 is a strong argument for an intracellular assembly of Lp(a) and suggests that formation of the bond could be catalyzed by specific thiolases that are located in the lumen of the rough endoplasmic reticulum. Unfortunately, the existence of this bond is not proven. The disruption of Lp(a) with reducing agents, which is taken as evidence for existence of the disulfide bridge, may simply be a consequence of the disruption of the kringle structures, each of which is stabilized by three internal disulfide bonds.

Studies on tissues from rhesus monkeys have disclosed that testes and brain, two organs that are separated from blood by barriers, contain mRNA for apo(a) but not for apoB (*36*). Thus in these organs, apo(a) may be secreted and function independently of LDL.

Even more mysterious is the catabolism of Lp(a). Both the site and mechanism of degradation of Lp(a) are presently unclear. The role of the LDL receptor is uncertain, and no other mechanisms or sites of degradation have yet been demonstrated for Lp(a). The asialoglycoprotein receptor may be one candidate for removal of this highly glycosylated particle from plasma.

Lp(a) and Atherosclerosis

It has been demonstrated that a positive family history of premature myocardial infarction is among the best single predictors for this trait (*44*). What are the genes that are responsible for this familial clustering? Certainly one is the dominant gene for familial hypercholesterolemia (*1*). Others are the apolipoprotein B and E genes; defects at these loci may result in type II and type III hyperlipidemia, respectively (*29, 45*), both of which are associated with premature coronary heart disease. It now emerges that variation at the *apo(a)* locus also relates to susceptibility to coronary heart disease. Numerous epidemiological studies have all found a positive association of plasma Lp(a) concentrations with premature myocardial infarction (*5*). For individuals with plasma Lp(a) concentrations exceeding 50 milligrams per deciliter and for those with concomitantly high LDL concentrations, the relative risk for early coronary heart disease may be increased up to sixfold. Together with the strong genetic control of Lp(a) concentrations, this has led to the conclusion that Lp(a) is an independent genetic risk factor for atherosclerotic vascular disease.

It is unclear whether increased Lp(a) concentrations are a significant risk factor in normolipidemic subjects. The relative risk for myocardial infarction increases significantly in subjects with high Lp(a) concentrations when LDL concentrations are also high (*6*). This increased risk is expected when two independent risk factors [LDL and Lp(a)] are present in one subject and is consistent with the findings in individuals with familial hypercholesterolemia. It will be of great practical importance to see whether Lp(a) is atherogenic also in the absence of elevated LDL and whether there is any "threshold" plasma Lp(a) concentration in normolipidemics. One of the many open questions is whether Lp(a) is involved in the long-

lasting process of atherogenesis because of its properties as a lipoprotein or whether it plays a role in the acute development of a thrombus due to its plasminogen-like apo(a) component. Or is Lp(a) a Janus with two bad faces?

REFERENCES AND NOTES

1. M. S. Brown and J. L. Goldstein, *Science* **232**, 34 (1986).
2. K. Berg, *Acta Pathol. Microbiol. Scand.* **59**, 369 (1963).
3. C. Ehnholm, H. Garoff, K. Simons, H. Aro, *Biochim. Biophys. Acta* **239**, 431 (1971); C. Ehnholm, H. Garoff, O. Renkonen, K. Simons, *Biochemistry* **11**, 3229 (1972); J. J. Albers, C.-H. Chen, F. Aladjem, *ibid.*, p. 57; K. Simons, C. Ehnholm, O. Renkonen, B. Bloth, *Acta Pathol. Microbiol. Scand.* **78**, 459 (1970).
4. G. Utermann and W. Weber, *FEBS Lett.* **154**, 357 (1983); J. W. Gaubatz, C. Heideman, A. M. Gotto, Jr., J. D. Morrisett, G. H. Dahlen, *J. Biol. Chem.* **258**, 4582 (1983); G. M. Fless, C. A. Rolik, A. M. Scanu, *ibid.* **259**, 11470 (1984).
5. W. Renninger, G. G. Wendt, P. Nawrocki, H. Weigand, *Humangenetik* **1**, 658 (1965); G. Dahlen, K. Berg, T. Gillnas, C. Ericson, *Clin. Chem.* **7**, 334 (1975); G. Kostner, P. Avogaro, G. Cazzolato, E. Marth, G. Bittolo-Bon, G. B. Quinci, *Atherosclerosis* **38**, 51 (1981); G. Dahlen, J. R. Guyton, M. Arrar, J. A. Farmer, J. A. Kautz, A. M. Gotto, Jr., *Circulation* **74**, 758 (1986); G. G. Rhoads, G. Dahlen, K. Berg, N. E. Morton, A. L. Dannenberg, *J. Am. Med. Assoc.* **256**, 2540 (1986); P. Költringer and G. Jürgens, *Atherosclerosis* **58**, 187 (1985).
6. V. M. Armstrong *et al.*, *Atherosclerosis* **62**, 249 (1986).
7. D. L. Eaton *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3224 (1987); H. Kratzin, V. W. Armstrong, M. Niehaus, N. Hilschman, D. Seidel, *Biol. Chem. Hoppe-Seyler* **368**, 1533 (1987).
8. J. W. McLean *et al.*, *Nature* **330**, 132 (1987).
9. V. M. Armstrong, A. K. Walli, D. Seidel, *J. Lipid Res.* **26**, 1314 (1985).
10. G. Utermann *et al.*, *J. Clin. Invest.* **80**, 458 (1987).
11. S. H. Chen *et al.*, *J. Biol. Chem.* **261**, 12918 (1986); E. H. Ludwig *et al.*, *DNA* **6**, 363 (1987); J. Knott *et al.*, *Nature* **323**, 734 (1986); S. W. Law *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8142 (1986).
12. I. Karadi *et al.*, *Biochim. Biophys. Acta* **960**, 91 (1988).
13. H. C. Guo *et al.*, *J. Lipid Res.* **30**, 23 (1989).
14. G. M. Fless, M. L. Snyder, A. M. Scanu, *ibid.*, p. 651.
15. C. Lackner, H. Dieplinger, G. Utermann, unpublished data.
16. L. Patthy, *Cell* **41**, 657 (1985).
17. R. M. Lawn, J. E. Tomlinson, J. W. McLean, D. L. Eaton, in *Lipoprotein(a)—25 Years of Progress*, A. M. Scanu, Ed. (Academic Press, New York, in press); H. J. Menzel and G. Utermann, unpublished data.
18. G. M. Fless, M. E. Zum Mallen, A. M. Scanu, *J. Biol. Chem.* **261**, 8712 (1986).
19. C. F. Sing, J. S. Schultz, D. C. Shreffler, *Ann. Hum. Genet.* **38**, 47 (1974); J. J. Albers, P. Wahl, W. R. Hazzard, *Biochem. Genet.* **11**, 475 (1974).
20. J. R. Guyton, G. H. Dahlen, W. Patsch, J. A. Kautz, A. M. Gotto, Jr., *Arteriosclerosis* **5** (no. 3), 265 (1985).
21. L. Iselius, G. Dahlen, U. de Faire, T. Lindman, *Clin. Genet.* **20**, 147 (1981); S. J. Hasstedt and R. R. Williams, *Genet. Epidemiol.* **3**, 53 (1986); S. J. Hasstedt *et al.*, *Am. J. Med. Genet.* **16**, 179 (1983); N. E. Morton, K. Berg, D. Dahlen, R. E. Ferrel, G. G. Rhoads, *Genet. Epidemiol.* **2**, 113 (1985).
22. G. Utermann, H. G. Kraft, H. J. Menzel, T. Hopferwieser, C. Seitz, *Hum. Genet.* **78**, 41 (1988); G. Utermann, C. Duba, H. J. Menzel, *ibid.*, p. 47; C. Sandholzer and G. Utermann, unpublished data.
23. E. Boerwinkle, H. J. Menzel, H. G. Kraft, G. Utermann, *Hum. Genet.* **82**, 73 (1989).
24. H. G. Kraft, H. J. Menzel, G. Utermann, unpublished data.
25. J. C. Murray *et al.*, *Am. J. Hum. Genet.* **40**, 338 (1987).
26. L. R. Weitkamp, S. A. Guttormsen, J. S. Schultz, *Hum. Genet.* **79**, 80 (1988); G. Lindahl *et al.*, *ibid.* **81**, 149 (1989); D. T. Drayna *et al.*, *Genomics* **3**, 230 (1988); S. L. Frank *et al.*, *Hum. Genet.* **79**, 352 (1988).
27. K. Maartman-Moe and K. Berg, *Clin. Genet.* **20**, 352 (1981); L. Havekes, B. J. Vermeer, T. Brugman, J. Emeis, *FEBS Lett.* **132**, 169 (1981); V. W. F. Krempler, G. M. Kostner, A. Roscher, F. Haslauer, K. Bolzano, *J. Clin. Invest.* **71**, 1431 (1982).
28. G. Utermann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4171 (1989).
29. T. L. Innerarity *et al.*, *ibid.* **84**, 6919 (1987); L. F. Soria *et al.*, *ibid.* **86**, 587 (1989).
30. C. Lackner, F. Hoppichler, T. L. Innerarity, R. W. Mahley, G. Utermann, unpublished data.
31. M. Seed *et al.*, unpublished data.
32. E. Gersdorf, H. J. Menzel, G. Utermann, unpublished data.
33. J. E. Hixon, M. L. Britten, G. S. Manis, D. L. Rainwater, *J. Biol. Chem.* **264**, 6013 (1989).
34. D. L. Rainwater, G. S. Manis, J. L. Vande Berg, *J. Lipid Res.* **30**, 549 (1989); F. Hoppichler, H. G. Kraft, G. Utermann, unpublished data.
35. D. L. Rainwater, G. S. Manis, R. S. Kushwaha, *Biochim. Biophys. Acta* **877**, 75 (1986); J. J. Albers, J. L. Adolphson, W. R. Hazzard, *J. Lipid Res.* **18**, 331 (1977); C. A. Nelson and M. D. Morris, *Biochem. Biophys. Res. Commun.* **71**, 438 (1976); L. L. Rudel, D. G. Greene, R. Shah, *J. Lipid Res.* **18**, 734 (1977); G. M. Fless and A. M. Scanu, *J. Biol. Chem.* **254**, 8653 (1979); C. A. Nelson, M. D. Morris, W. E. Greer, *Biochem. Med.* **29**, 214 (1983); K. Berg, *Ser. Haematol.* **1**, 111 (1968).
36. J. E. Tomlinson, J. W. McLean, R. M. Lawn, *J. Biol. Chem.* **264**, 5957 (1989).
37. P. M. Laplaud, L. Beaubatie, S. J. Rall, G. Luc, M. Sabourеau, *J. Lipid Res.* **29**, 1157 (1988).
38. P. C. Harpel, B. R. Gordon, T. S. Parker, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3847 (1989).
39. J. M. Edelberg, M. Gonzalez-Gronow, S. V. Pizzo, *Biochemistry* **28**, 2370 (1989).
40. M. Gonzalez-Gronow, J. M. Edelberg, S. V. Pizzo, *ibid.*, p. 2374; K. A. Hajjar, D. Gavish, J. L. Breslow, R. L. Nachman, *Nature* **339**, 303 (1989); L. A. Miles, G. M. Fless, E. G. Levin, A. M. Scanu, E. F. Plow, *ibid.*, p. 301.
41. M. S. Brown and J. L. Goldstein, *Nature* **330**, 113 (1987).
42. H. G. Kraft, H. J. Menzel, F. Hoppichler, W. Vogel, G. Utermann, *J. Clin. Invest.* **83**, 137 (1989).
43. F. Krempler, G. M. Kostner, K. Bolzano, F. Sandhofer, *ibid.* **65**, 1483 (1980).
44. P. N. Durrington, M. Ishola, L. Hunt, S. Arrol, D. Bhatnagar, *Lancet* **i**, 1070 (1988).
45. G. Utermann, *Am. Heart J.* **113**, 433 (1987).
46. C. Sandholzer, N. Saha, G. Utermann, unpublished data.
47. H. G. Kraft, H. Dieplinger, E. Hoye, G. Utermann, *Arteriosclerosis* **8**, 212 (1988).

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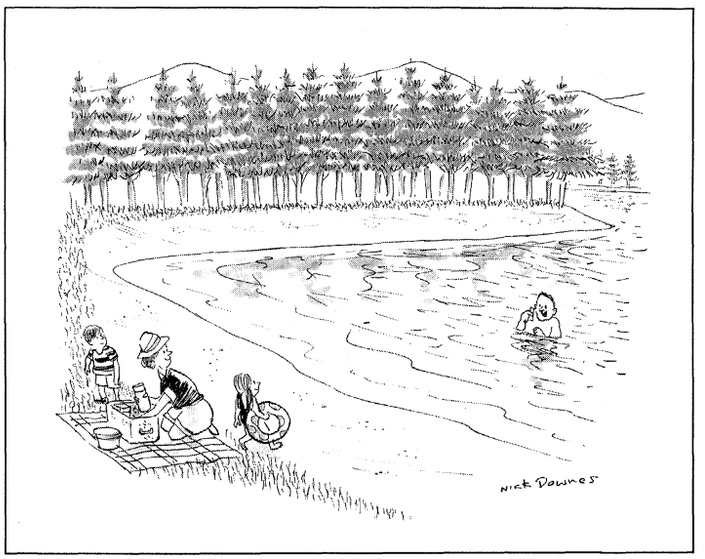

" Come on in—the pH is fine!"