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Historical Perspectives on Lipoprotein Research and Methodology

F.T. Lindgren

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HISTORICAL PERSPECTIVES ON LIPOPROTEIN RESEARCH AND METHODOLOGY

Frank T. Lindgren, Ph.D.

Research Medicine and Radiation Biophysics Division
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

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Historical Perspectives on Lipoprotein Research and Methodology

Frank T. Lindgren, Ph.D.

Lawrence Berkeley Laboratory
University of California
Donner Laboratory
1 Cyclotron Road
Berkeley, California 94720

Since the early history of lipoprotein isolation and characterization dates back more than 60 years, it would be helpful to describe some of the landmarks occurring before about 1965. This is particularly worthwhile for the younger scientists, since many libraries no longer provide access to these earlier journals, publications, and reprints. Although the first isolation of a lipoprotein fraction was done by Macheboef[1] in 1928, there were no physical-chemical characterizations of this lipoprotein at that time. It was simply called "ceonapse precipitated by acid" or CA, obtained by precipitation from horse serum using half-saturated ammonium sulfate. It was not until after the development of electrophoresis by Tiselius[2] that Macheboef reported this CA to be an α globulin. We now know this characterization to be the first isolated high density lipoprotein (HDL).

A most important development for characterization of lipoproteins was the invention, in 1924, of the ultracentrifuge by Svedberg and Rinde[3] who applied it to the study of gold colloids. Later, in 1927, Svedberg and Lysholm[4] developed a higher speed ultracentrifuge with an optical system to observe the migration of protein boundaries. Further refinements of the oil turbine high-speed drive and

the Lamm scale optical system[5] led to serum protein studies. In 1935, McFarlane[6] studied normal and pathological whole serum; some of the latter contained elevated cholesterol and other lipids. Figure 1 shows examples of these first analytic ultracentrifuge (AnUC) plots. What was of most interest was the presence of a density- and time-sensitive component in the region of the albumin boundary. Because of its labile nature, being influenced by time, by salt concentration and plasma dilution, it was called the "X protein". Others, such as von Mutzenbecker and Peterson[7] later verified these puzzling observations, but were unable to explain these anomalies. However, at Harvard during World War II a research group worked on blood plasma fractionation and plasma substitutes. Here Cohen, Oncley, Edsall, and their group isolated two distinctly different lipid-containing fractions from human plasma by low-temperature, low-salt ethanol precipitation. One was a dense (1.10 g/ml) α lipoprotein, and the other a high molecular weight (1.3×10^6 Daltons), low density (1.03 g/ml) β lipoprotein[8]. Also during this time, Pederson was making extensive AnUC studies of human and animal serum, which he published as a book[9]. These results all suggested that the β lipoprotein was the troublesome, density-sensitive "X protein". Up until this time all studies for over a decade had been unable to characterize this low density lipoprotein (LDL) and explain its behavior in the AnUC of plasma and serum.

Resolution of the "X Protein" and Development of Lipoprotein Flotation

It was some 41 years ago that John Gofman began to study the process of atherosclerosis at Donner Laboratory, University of California, Berkeley, at first with only a few graduate students. His objective was to study how cholesterol and other lipids were carried in the blood stream. At this time the first commercially available analytic ultracentrifuge (AnUC) was obtained from the Specialized Instrument Company, which later became part of the Beckman Corporation. This AnUC was designed by Ed Pickels while he was at the Rockefeller Institute and it was later equipped with an electrical drive and with the new continuous dn/dx Thovert Philpot-Svensson optical system[10]. This new optical system eliminated the tedious manual plots required of the Lamm scale method[5] and this had been one of the technical problems the earlier workers had had to contend with. Two graduate students, Harold Elliott and myself, were given the task of learning how to run this analytic ultracentrifuge and to verify the many anomalies in the neighborhood of the albumin peak. These anomalies seemed to increase in severity with time, and many samples exhibited a dip below the baseline, something that could not be explained by traditional multicomponent analysis (see Figure 2). We questioned such interpretations, and considering that the β-lipoprotein reported by the Harvard group had a low density of approximately 1.03 g/ml, slightly less than the density of whole plasma or serum, we suddenly asked an important question: does the macromolecular β-lipoprotein (or "X protein") see the density of the serum small molecular background, i.e.,

1.0063 g/ml, or does it see the density of the serum background plus the density increment of the much smaller serum proteins such as albumin? If the latter, the β-lipoprotein would ultimately accumulate on the radial side of the albumin boundary with time and I hastily conceived of a "pile-up" analysis to explain the distortions. Figure 3 shows how this analysis could result in a time-dependent dip below the baseline. If the pile-up were roughly Gaussian, then the schlieren optical system detecting dn/dx would result in a biphasic pattern that would be superimposed on the main albumin peak. If this pile-up occurred at different regions of the albumin boundary, all the bizarre anomalies could be explained (see Figure 4). At this moment, John Gofman, Harold Elliott and I were excited beyond belief. John said, as a corollary to the pile-up analysis, that a floating component must give rise to an inverse peak and the area over the peak would be proportional to concentration! As our AnUC run was almost finished, we shut it down, took another plasma sample aliquot and raised the salt background density with NaCl to about twice that of whole plasma, i.e. 1.063 g/ml. Eagerly, we waited for pump-down and to get the rotor and sample up to speed. Figure 5 shows the first flotation of the elusive "X protein" in the presence of whole serum. The rising inverse peak, essentially of total LDL, was confirmed and the slowly sedimenting albumin peak was symmetrical, without a trace of distortion or anomaly. After repeating this experiment many times and confirming that the area over the peak was related to lipoprotein concentration, we decided to write our first paper.

We eagerly wrote up this first paper and submitted it to the *Journal of Biochemistry*, expecting acceptance of this new interpretation and confirmatory data. However, after weeks passed we received the unbelievable news that the paper had been rejected by both reviewers. One reviewer thought we were confusing our interpretation with the Ogston-Johnson[11] type of anomaly. The other felt that since we were inexperienced in the field and since several ultracentrifugal experts had come to consistent yet different conclusions, that our manuscript should not be published until we had more definitive conclusions. Needless to say, we were frustrated and disappointed, but continued our arguments by correspondence with the editor. Finally, thanks to our perseverance and the scientific understanding of John Edsall, then the editor, our manuscript was accepted. In June of 1949 our first paper[12] was published and it was the only plasma lipoprotein paper published that year. Since 1927 there had been only some four plasma lipoprotein papers published[1,6,7,8]. Thus, with this landmark paper the decade-long mystery of the elusive "X protein" was resolved and a new era of characterization and quantification of lipoproteins by AnUC flotation began.

While awaiting publication of our first paper, we began to apply the new technique of lipoprotein flotation to normal and cholesterol-fed rabbits, as was first done by Anitschkow[13]. Also, we studied normal humans and patients with proven cardiovascular disease. This study of Gofman et al.[14] was sent to *Science* and was quickly accepted and published, in marked contrast with our first paper.

These findings indicated that, in the rabbit developing atherosclerosis, there was a minimal increase in LDL but a marked increase of the cholesterol-rich, S_f^o 10-30-class lipoproteins. By present nomenclature, these would be described as intermediate density lipoproteins (IDL) and the smaller, higher density class of very low density lipoprotein (VLDL). These studies further showed that the coronary patients, when compared to normal subjects, had elevated S_f^o 12-20, or IDL. The general features of these findings are shown in Figure 6. Thus, the early studies at Donner had identified an "atherogenic class" of low density lipoproteins by quantitative AnUC flotation which would alert and stimulate the scientific community that was interested in coronary artery disease (CHD). After these two initial papers, other scientists, at first notably at NIH, began to isolate by ultracentrifugation, to characterize, and to study plasma lipoproteins. These included the early Donner studies by Shore, Nichols, and Freeman[15] on "clearing factor" identified as a lipolytic mechanism and analagous studies at NIH by Brown, Boyle, and Anfinson[16] that identified the enzymatic transformation of chylos and VLDL by what is now called "lipoprotein lipase". Two years later, and almost simultaneously, three groups, Havel et al.[17] (NIH), Hillyard et al.[18] (UC Berkeley), and Lindgren et al.[19] (Donner Laboratory), published, with slight differences, sequential flotation and isolation of all the major plasma lipoprotein classes. The latter study[19] also identified the basic nature of the lipoprotein transformations induced by IV-heparin injection.

At first, the lipid composition of each major lipoprotein class was studied. These major lipoprotein classes were found to contain all the major lipids, triglycerides, phospholipids, free cholesterol, cholesteryl esters, and small amounts of free fatty acids, as shown in Figure 7. The other variable component was peptide, varying from some 2% for chylomicrons, 10-15% for VLDL, 25% for LDL, and as much as 55% for HDL. For some time, little was known about the nature of the peptide moiety, but about 1956 studies at both NIH by Avigan et al.[20] and at Donner by Shore[21] began to characterize the major peptides of VLDL, LDL, and HDL by their specific N-terminal amino acids. The concept evolved that perhaps the nature of the peptide determined what kind of a lipoprotein might be synthesized, say in the liver, organs or other synthetic sites. Soon, as the analyses became more sensitive and quantitative, it became clear that the occurrence of the peptides was much more complicated than, for instance, glutamic-N-terminal for LDL and VLDL, and aspartic-N-terminal for HDL. Both N-terminal and C-terminal amino acids were characterized, some in subfractions of the major lipoprotein classes. The first solubilization of apo-HDL was achieved by Scanu et al.[22]. Later, the approximate molecular weights of the isolated and solubilized apolipoproteins were characterized. After this, amino acid sequencing began, first with apoAII by Brewer et al.[23] and then later with other smaller peptides by others. More recently, the largest apolipoprotein (apo)B-100, considered the major atherogenic peptide, was solubilized as a monomer and this led to difficult but successful cooperative sequencing studies by several groups.

All of this fully documents the early Donner and NIH concepts that the nature of the peptide is the fundamental determinant of the lipoprotein macromolecule.

Additionally, another area of landmark lipoprotein development was concerned with how do the various lipoproteins leave the bloodstream, and for what purpose. It was the novel approach of Goldstein and Brown[24] who introduced the concept of lipoprotein cell receptors that led to the understanding of how LDL are normally bound at the cell surface and internalized by "receptors", and how the defective receptor in familial hypercholesterolemia leads to massive plasma LDL elevation and premature CHD. These cell "receptor" peptides also have now been isolated and sequenced. Most of the presently known and sequenced plasma apolipoproteins, their properties and functions are given in Table 1.

Some of these developments, such as the final sequencing of the known apolipoproteins, were done simultaneously with the recent cell biology breakthroughs and developments. However, recently the genetic features of "lipoprotein diseases" now are being recognized, appreciated, and fully studied. But this concept was recognized earlier at NIH by the Fredrickson types[25] as a basis for categorizing lipoprotein abnormalities, which are still used as clinical categories. Earlier, Gofman et al.[26] had postulated the severe clinical types for *xanthoma tendinosum* (type II) and *xanthoma tubersum* (type III or dysbetalipoproteinemia).

Since about the mid-70's, there has been an enormous expansion of the lipoprotein field. Therefore, in this short chapter it would be impossible to describe and acknowledge these most recent and numerous "landmarks". One positive reason for this explosion has been the free, intense, and extensive collaboration and communication among lipoprotein-oriented scientists. Many scientists, not just those specifically cited here, have been crucially responsible for the development of the field of lipoproteins, their vital role in the life process, and their understanding of and contribution to the amelioration of premature coronary artery disease. Among those early pioneers were Svedberg and Ed Pickels, who provided the technology of the AnUC and particularly the convenience of the preparative ultracentrifuge. Today nearly every laboratory has one or more preparative machines. Lastly, Gofman was the conceptual pioneer for his interpretations and early lipoprotein developments. Those mentioned here and the countless others documented in chapters, books and symposia[27-35] helped build the "lipoprotein field" as we know it today. This year over a thousand lipoprotein papers and abstracts from throughout the world have been published, in marked contrast with the landmark year 1949.

ACKNOWLEDGEMENTS

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FIGURE AND TABLE LEGENDS

Figure 1. Early sedimentation patterns by McFarlane showing the apparent labile nature of the "X protein". Reprinted with permission from Figure 2, Ann. N.Y. Acad. Sci. 348, 1-15, 1980.

Figure 2. Donner reproduction of albumin boundary distortions and the "dip phenomenon". Reprinted with permission from Figure 3, Ann. N.Y. Acad. Sci. 348, 1-15, 1980.

Figure 3. Analysis of the pile-up hypothesis that could explain the dip phenomenon. Reprinted with permission from Figure 4, Ann. N.Y. Acad. Sci. 348, 1-15, 1980.

Figure 4. Analysis of where the pile-up of β lipoproteins could explain all observed distortions of the albumin peak. Reprinted with permission from Figure 5, Ann. N.Y. Acad. Sci. 348, 1-15, 1980.

Figure 5. First flotation of the β lipoproteins (LDL) as an inverse peak obtained by raising the density to 1.063 g/ml. Reprinted with permission from Figure 6, Ann. N.Y. Acad. Sci. 348, 1-15, 1980.

Figure 6. Ultracentrifugal flotation diagrams of the normal rabbit (a') and the rabbit pattern developing hypercholesterolemia and atherosclerosis (b', c').

Analagous human patterns are shown to the left (a, b, and c). Reprinted with permission from Science (copyright 1950 by the AAAS) from Figure 1, Gofman, J.W., Lindgren, F.T., and Elliott, H. J. Biol. Chem. 179, 973 (1949).

Figure 7. Lipid and lipoprotein composition of the major lipoprotein classes.

Figure 8. Plasma lipoprotein classes as currently characterized by AnUC.

Figure 9. Occurrence of the many known and characterized apolipoproteins among the major lipoprotein classes.

Table 1. The major plasma apolipoproteins: molecular weight and function.

Table 1. The major plasma apolipoproteins: molecular weight and function.

Apolipoprotein	Molecular Weight	Physiological function
B-100	549,000	Lipoprotein liver biosynthesis and LDL receptor recognition
B-48	264,000	Triglyceride transport from the intestine to blood stream
A-I*	28,300	Activation of lecithin:cholesterol acyltransferase (LCAT)
A-II*	17,400 (diamer)	LCAT activity, activates hepatic lipase *in vitro*
A-IV*	46,000	Activates LCAT *in vitro*
C-I*	6,600	Activates LCAT
C-II+	8,800	Activates lipoprotein lipase
C-III	9,200-9,700	Unclear
D*	22,000	Cholesteryl ester transfer protein? LCAT activity?
E	35,000-39,000	Chylomicron remnant and LDL receptor recognition
F	———	Unknown
G	———	Unknown
H+	43,000 (SE)	Modulates lipoprotein lipase in presence of CII

* LCAT activity; + lipase activity

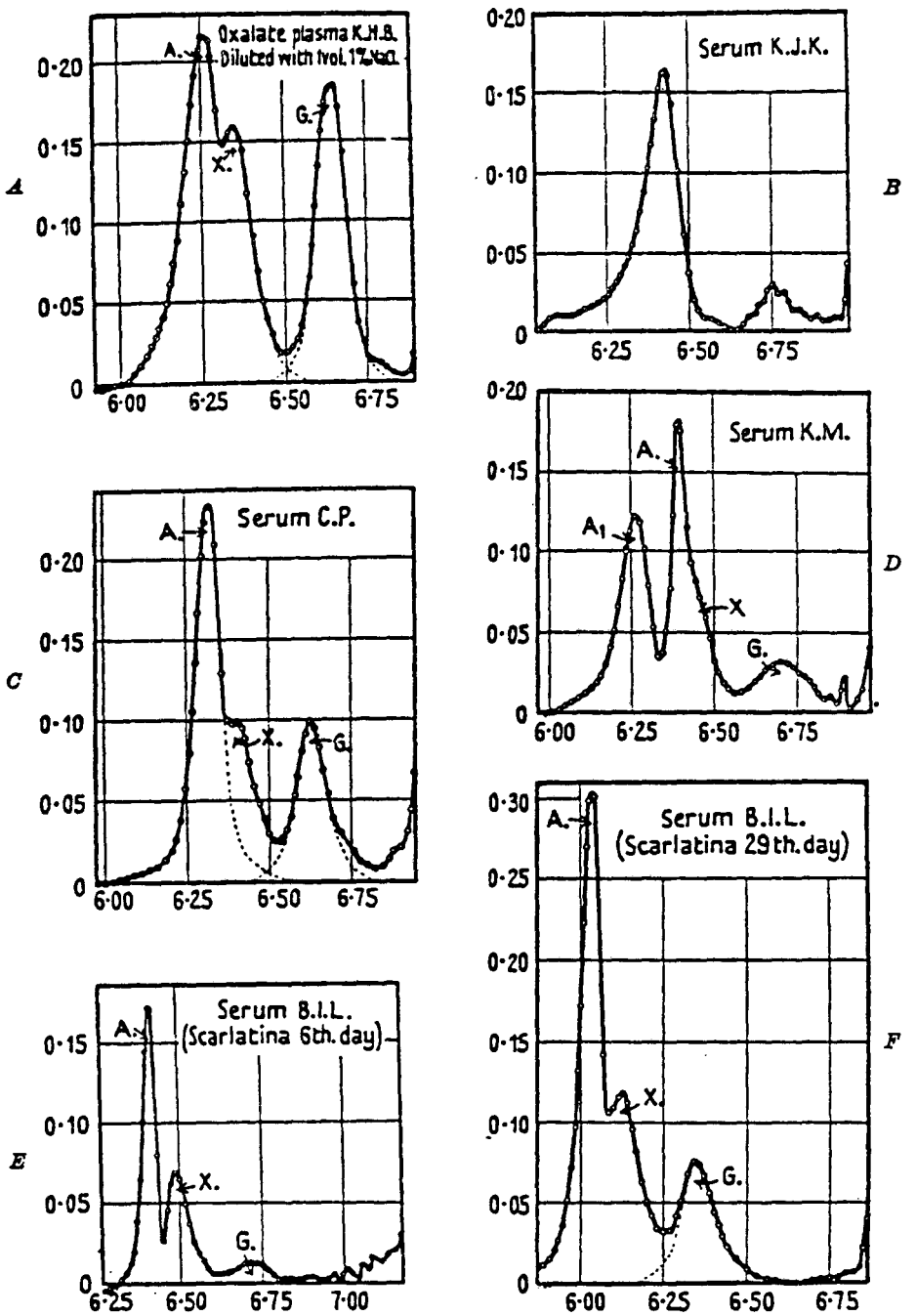

FIG. 144. Sedimentation diagrams from different pathological sera and plasma obtained by means of the refraction method

A. Pulmonary tuberculosis. *B*. Nephritis. *C*. Rectal carcinoma. *D*. Malignant tumor of bile duct. *E*. Scarlatina 6th day. *F*. Scarlatina 29th day (same person).

(McFarlane, 1935, *c*.)

XBL 8910-3766

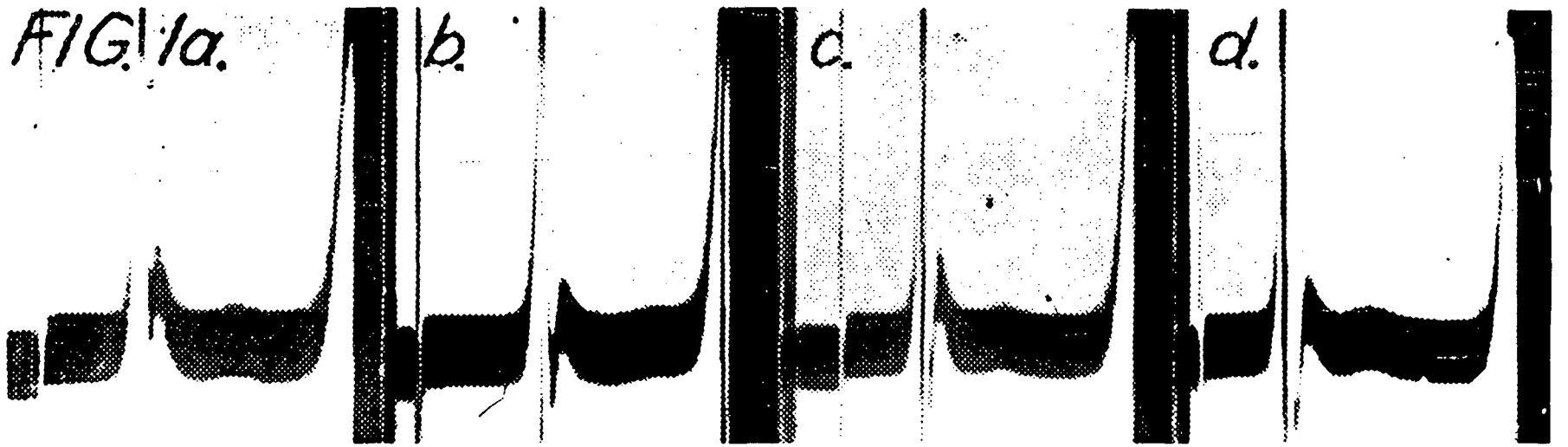

FIGURE 2

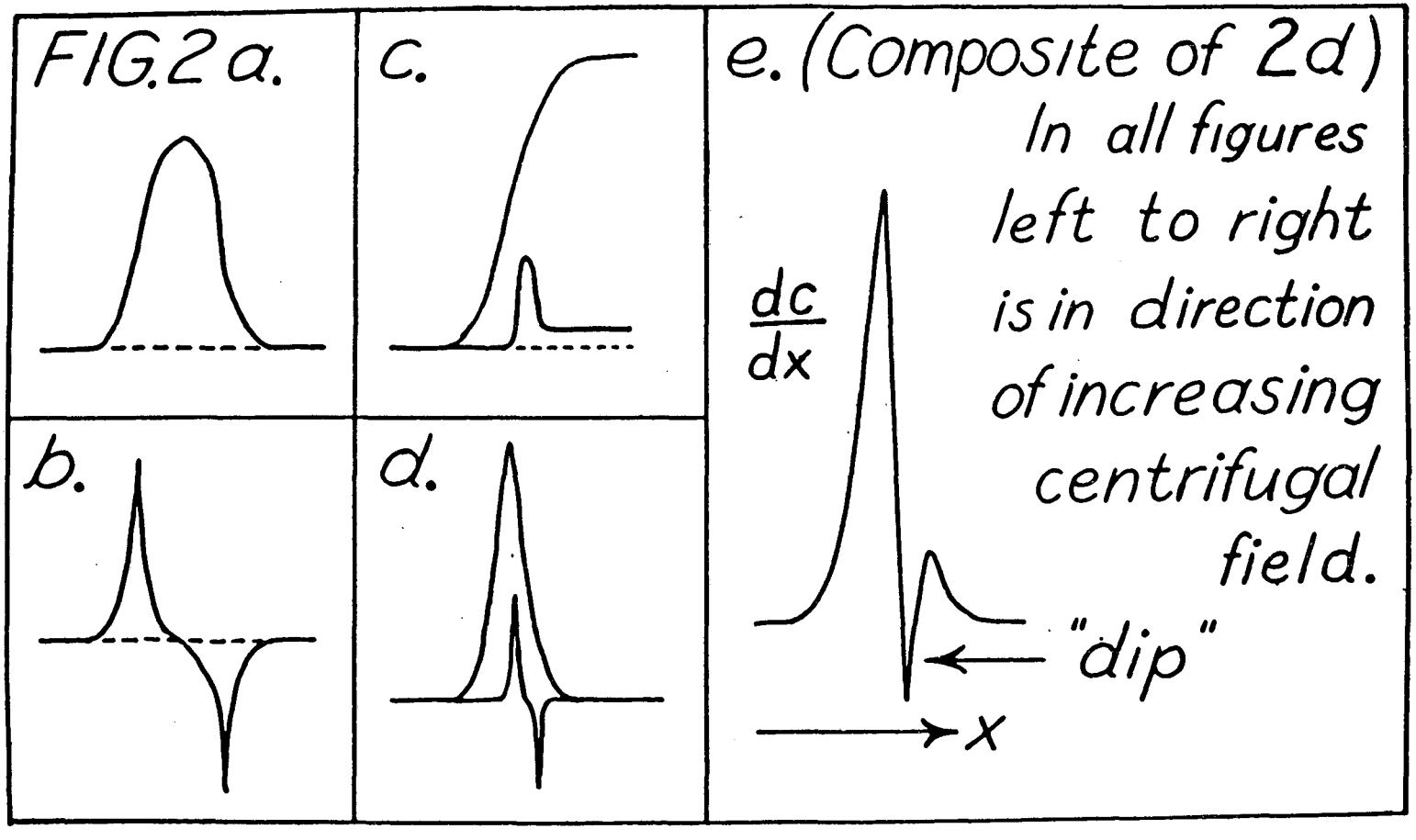

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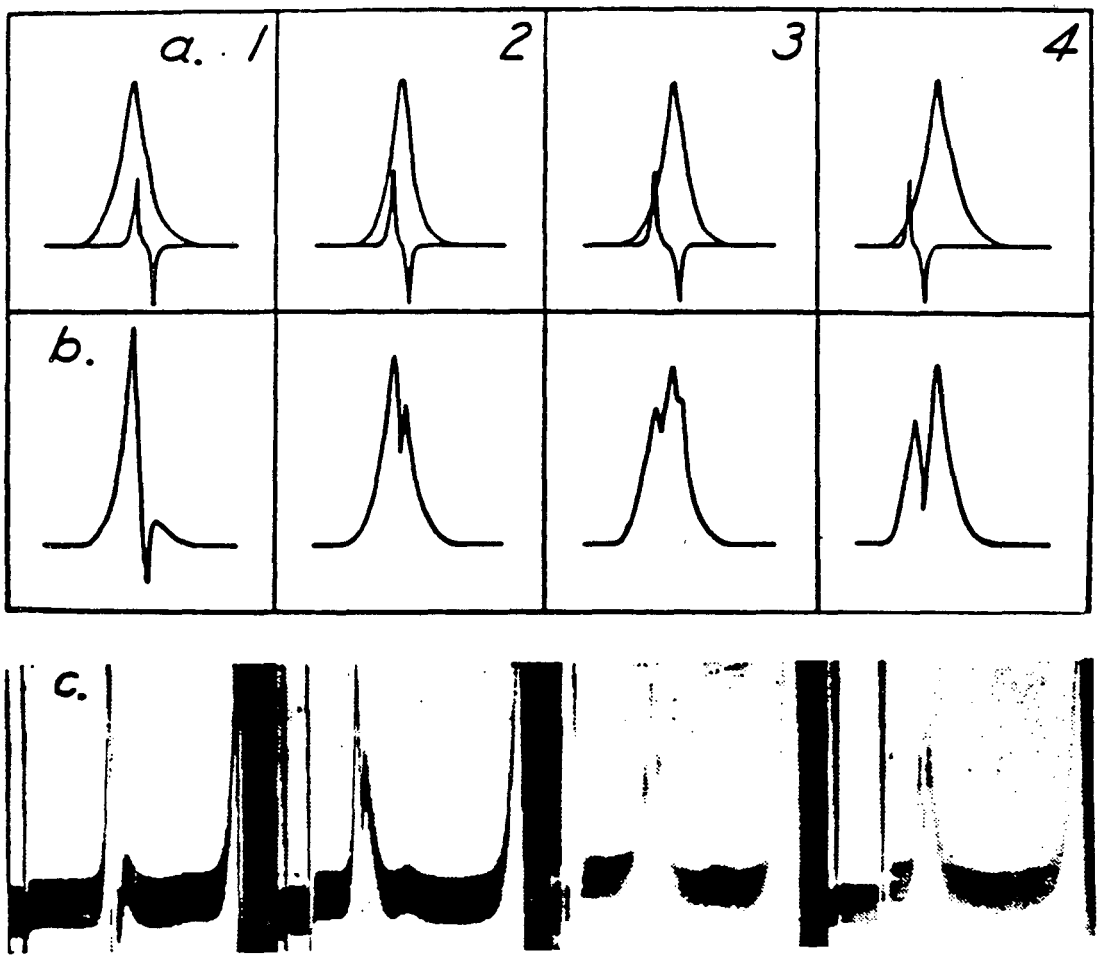
FIGURE 3

FIGURE 4



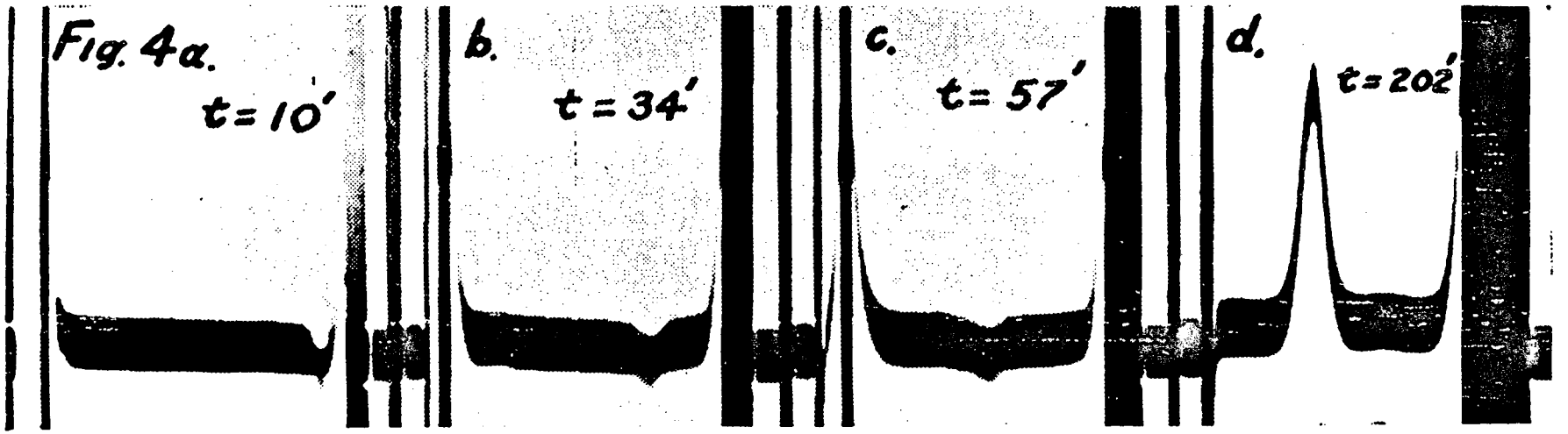
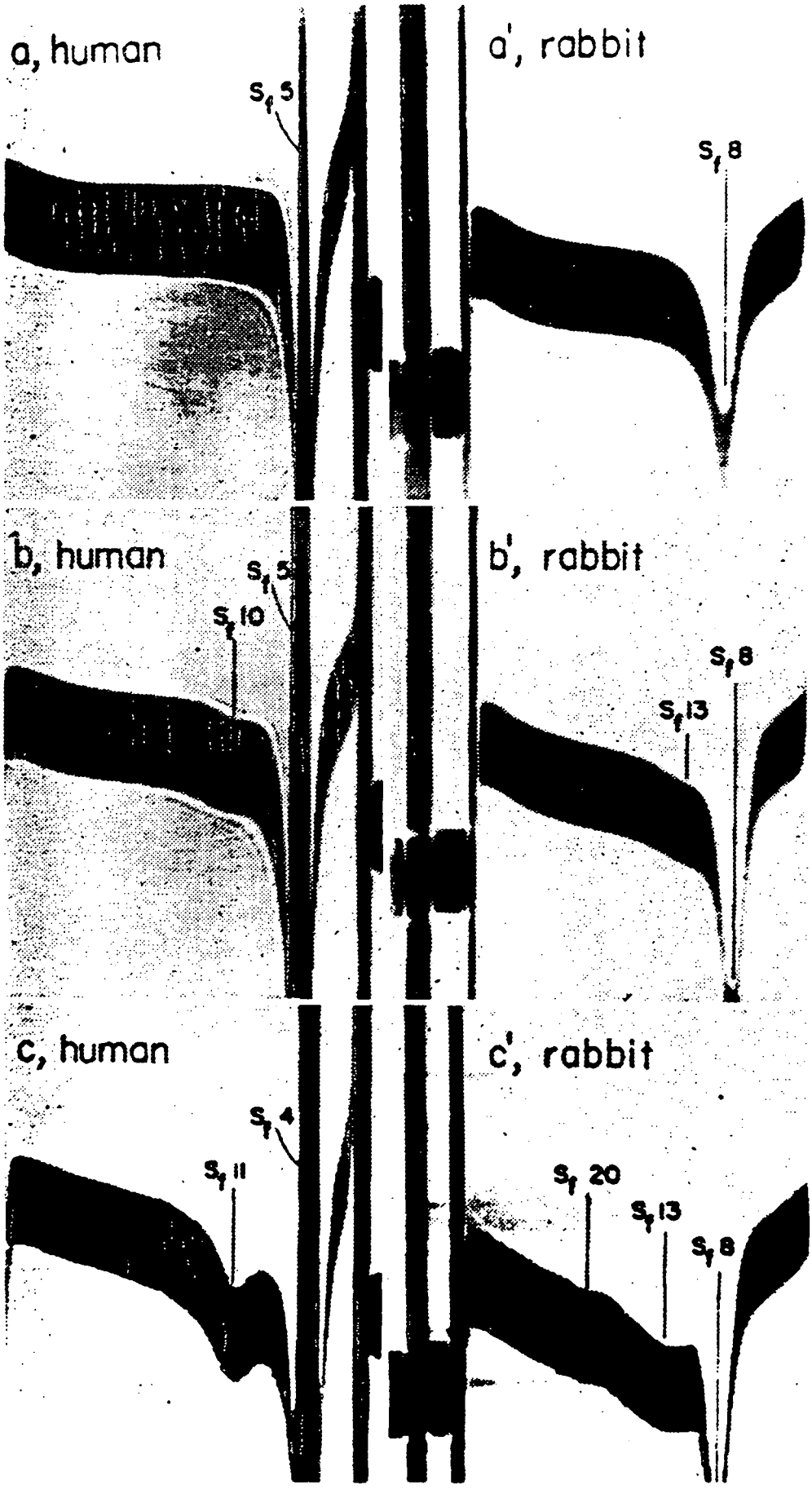


FIGURE 5

FIGURE 6


a, human
S_f 5
a', rabbit
S_f 8
b, human
S_f 5
S_f 10
b', rabbit
S_f 8
S_f 13
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S_f 4
S_f 11
c', rabbit
S_f 20
S_f 13
S_f 8

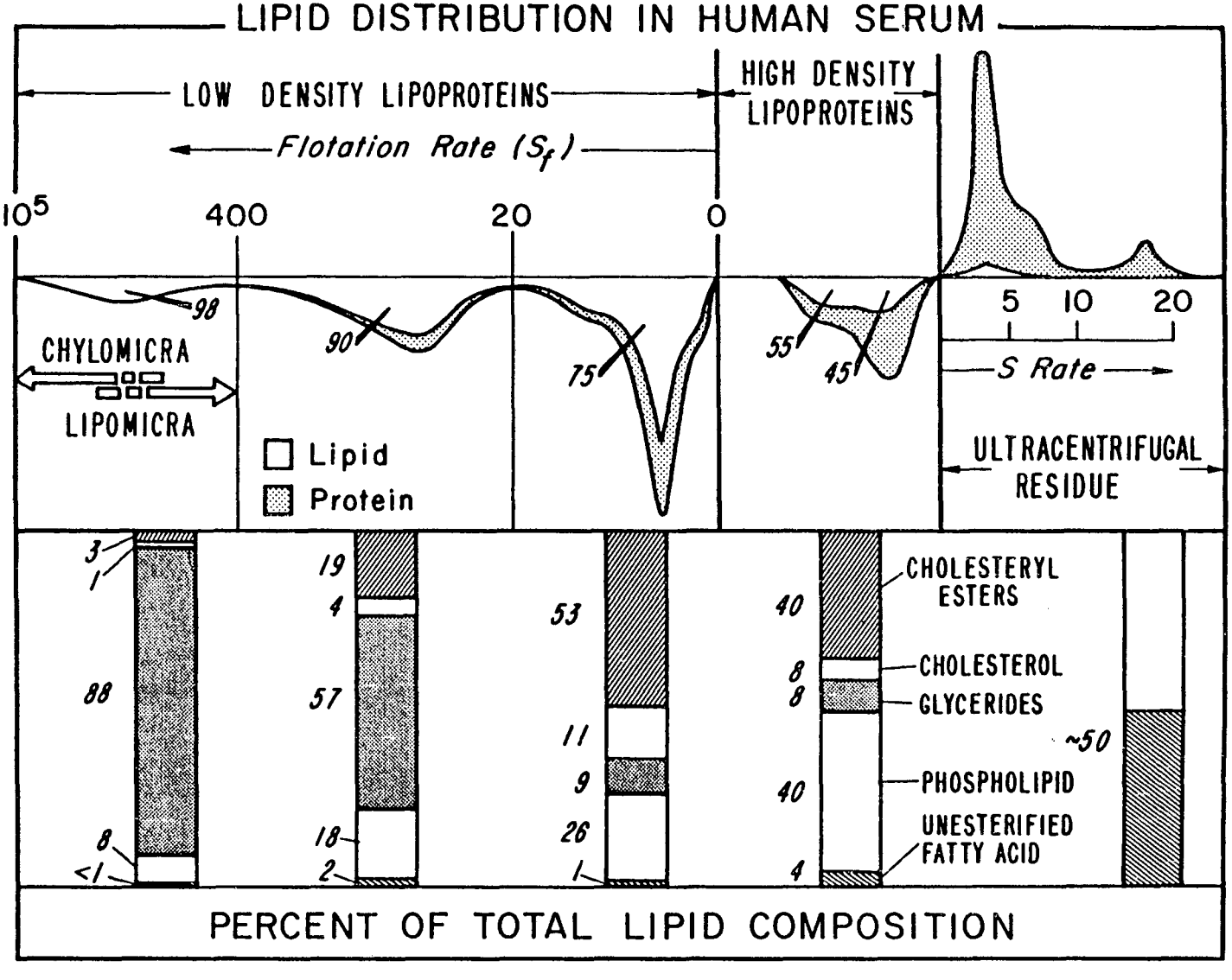
LIPID DISTRIBUTION IN HUMAN SERUM
LOW DENSITY LIPOPROTEINS
HIGH DENSITY LIPOPROTEINS
Flotation Rate (S_f)
10^5
400
20
0
5
10
20
S Rate
98
90
75
55
45
CHYLOMICRA
LIPOMICRA
Lipid
Protein
ULTRACENTRIFUGAL RESIDUE
3
1
88
8
<1
19
4
57
18
2
53
11
9
26
1
40
8
8
40
4
CHOLESTERYL ESTERS
CHOLESTEROL
GLYCERIDES
~50
PHOSPHOLIPID
UNESTERIFIED FATTY ACID
PERCENT OF TOTAL LIPID COMPOSITION
XBL768-9174

FIGURE 7

ULTRACENTRIFUGAL ANALYSIS, Lp(a) CONCENTRATIONS AND OCCURRENCE

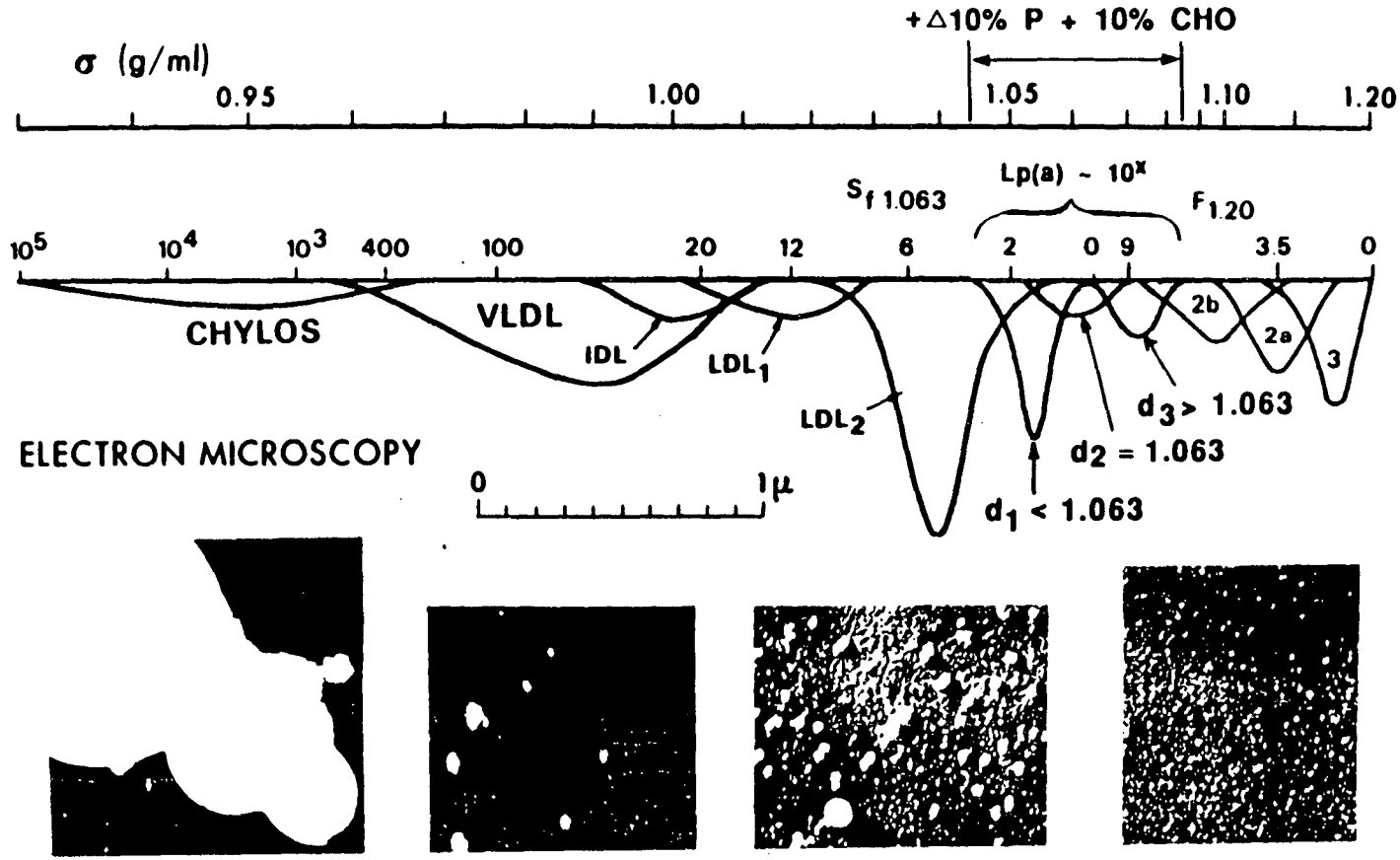

FIGURE 8

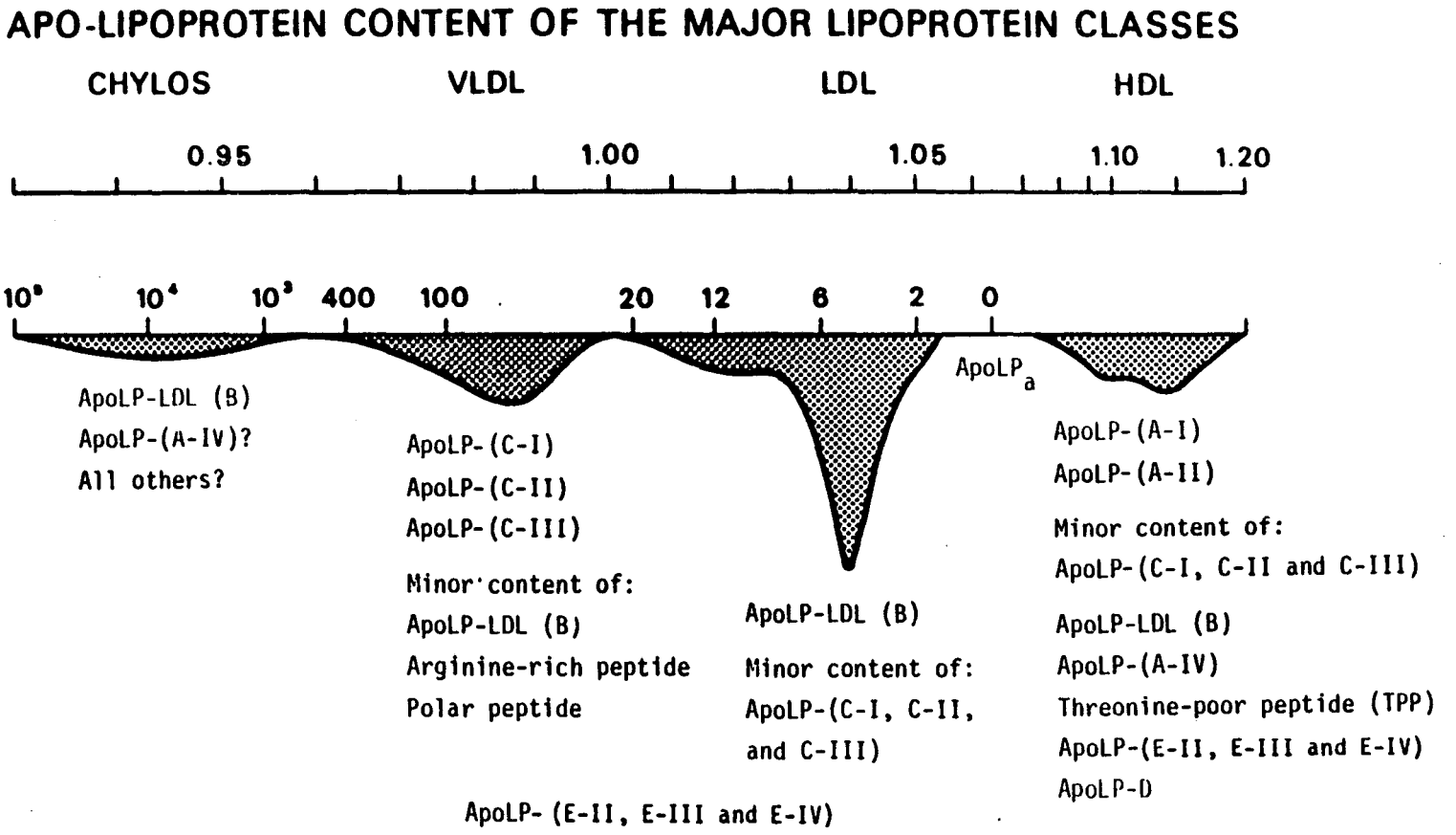
APO-LIPOPROTEIN CONTENT OF THE MAJOR LIPOPROTEIN CLASSES
CHYLOS
VLDL
LDL
HDL
0.95
1.00
1.05
1.10
1.20
10⁸
10⁴
10³
400
100
20
12
6
2
0
ApoLP-LDL (B)
ApoLP-(A-IV)?
All others?
ApoLP-(C-I)
ApoLP-(C-II)
ApoLP-(C-III)
Minor content of:
ApoLP-LDL (B)
Arginine-rich peptide
Polar peptide
ApoLP-LDL (B)
Minor content of:
ApoLP-(C-I, C-II, and C-III)
ApoLP-(E-II, E-III and E-IV)
ApoLP$_a$
ApoLP-(A-I)
ApoLP-(A-II)
Minor content of:
ApoLP-(C-I, C-II and C-III)
ApoLP-LDL (B)
ApoLP-(A-IV)
Threonine-poor peptide (TPP)
ApoLP-(E-II, E-III and E-IV)
ApoLP-D
XBL 7910-3826

FIGURE 9

LAWRENCE BERKELEY LABORATORY
TECHNICAL INFORMATION DEPARTMENT
1 CYCLOTRON ROAD
BERKELEY, CALIFORNIA 94720