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A GUIDEBOOK TO LIPOPROTEIN TECHNIQUE

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Preface

Charles Greville, writing in 1839 of a book by Lady Blessington, sourly remarked that 'while it is very difficult to write good books, it is not easy to compose even bad ones'. In writing this guide to lipoprotein technique, we have come to appreciate the truth of this observation, not only because writing is a difficult art, but because the plasma lipoproteins are a peculiarly intractable subject. To begin with, there is no such thing as 'a lipoprotein'. Like 'wine', lipoproteins abound in an almost infinite variety, many of them differing in only the most subtle ways. The realisation of this complexity and the need to isolate the 'purest' possible lipoproteins for detailed studies of their chemistry has, since about 1945, led to the development of many methods for their isolation, identification and estimation. The newcomer to the field is therefore faced with a choice that he may find difficult to resolve unaided. It is primarily to such a novice that this guidebook is directed; we shall have comparatively little to say to the experienced worker in a large, well-established lipoprotein laboratory.

Unfortunately, the most powerful of the available techniques are often those which the most sophisticated and expensive equipment. They are therefore not always available to small teams or to isolated investigators. Moreover, they are often time-consuming and not well suited to routine clinical analysis. Consequently, many simple methods have been developed which will give the rough-and-ready results that often suffice for para-scientific experiments. In this book, we have tried to present a range of techniques that can be used for work at these different levels of sophistication. At the same time, we have

Glossary of lipoprotein nomenclature as used in this manual

For details of the different systems refer to Section 1.1.

(1) The density nomenclature will generally be used, either in specific terms, e.g. 'the fraction of density 1.02–1.04 g/ml', or in the orthodox generalised forms HDL, LDL, VLDL.

(2) Where it is more appropriate to use the electrophoretic nomenclature, the terms α-, β-, and pre-β-lipoprotein will be used.

(3) Fractions that are isolated by methods that do not define the product will be referred to the most relevant term of the electrophoretic nomenclature in quotation marks, as follows: 'α-lipoprotein', etc.

(4) Lipoproteins that are defined by their protein moieties will be named according to a modification of the Alaupovic system i.e. lipoprotein A, lipoprotein B, etc. (LP-A, LP-B, etc.).

Note that LP-A, LP-B, LP-C etc. each contain only the mixture of proteins that is designated A, B or C, etc. respectively. Lipoproteins formed from the individual proteins that compose these mixtures will be referred to as: lipoprotein A-I, lipoprotein A-II, lipoprotein C-I, etc. (LP-A-I, LP-A-II, etc.). In principle, this system can be extended to particles that bear more than one apo-lipoprotein, e.g. LP-B, C or LP-A, C, E, etc.

Likewise, it is possible to construct names for such complex particles as may be formed by the association of those that carry only one protein apiece, e.g. LP-B:LP-C, or LP-B:LP-C:LP-E, etc. or by the association of particles that carry more than one protein, e.g. LP-B, C:LP-C, E, etc.

Glossary of apo-lipoprotein nomenclature as used in this manual

For details refer to Section 1.2.

The prefix 'apo-' will be added to any term that includes the word lipoprotein or its abbreviation, e.g.

apo-high-density lipoprotein (apo-HDL), etc.
apo-lipoprotein A (apo-LP-A), etc.
apo-lipoprotein A-I apo-LP-A-I), etc.
apo-α-lipoprotein, etc.

Some of these protein moieties will be heterogeneous, for example apo-HDL, apo-LDL, apo-LP-A. Others will consist of a single protein, for example, apo-LP-A-II. Some of the apo-lipoproteins, of which A-I and E are the best known examples, are composed of a mixture of several closely similar proteins that are usually referred to as 'polymorphs'. It is arguable that this term is not properly used to describe a group of proteins whose common feature is certain immuno-reactive properties i.e. they are at least partially iso-immunogenic. Some authors have used the term 'isoform' instead of polymorph, but this is also not without its objections. In the absence of an entirely satisfactory, agreed nomenclature for these substances we shall generally use the more common term 'polymorph'.

tried to present even the most advanced techniques in their simplest and most widely accessible forms. But it must be clearly understood that very simple methods cannot be used for work of the highest calibre; for example, it has to be accepted that it is very difficult to do significant work on lipoproteins without a preparative ultracentrifuge.

In the end, it is the user who must select the technique most appropriate for his needs and, to this end, we have devoted a part of our text to a discussion of the relative merits and limitations of the methods available. The method having been chosen with the aid of this digest, the experimental details can then be obtained from the relevant section of the book. In these sections, we have tried to present every significant detail. But significance in this sense is subjective and some may think that our instructions are sometimes self-evident. Our excuse is that, in a world in which a post-doctoral researcher has been known to try running a single tube in the preparative ultracentrifuge, it is unwise to assume too high a level of expertise. Moreover, although techniques are sometimes described in careful detail by their originators, later users tend to degrade them by omitting steps, or by changing conditions without checking that it is valid to do so.

In composing this guide, we have inevitably drawn on the work of many scientists, as the list of references will show. However, we are glad to acknowledge the special influence that has been exerted by Dr. F.T. Lindgren and by Dr. P. Alaupovic, and their respective colleagues, both on the development of our subject and on our treatment of it. Many other colleagues have contributed by passing on practical tricks of expertise that are often vital to the success of an experiment, but are not so often made public. In this context, we are particularly indebted to Dr. Sonia Goldstein and to Dr. M.J. Chapman for their encouragement and for help with those sections that deal with their special interests. Finally, it is a pleasure to recognise the willing and expert help of the librarians and photographers of the Middlesex Hospital.

Contents

Brief introduction to the plasma lipoproteins

One of the characteristics of vertebrate animals is a highly developed system for the production of stable dispersions of fat in water. This ability plays an important part in the transport of fat from the gut to the bloodstream and in the intravascular transport of energy. It is also used by mammals in the production of milk. In each case, the dispersion of the fat is brought about by surrounding small globules with a hydrophilic shell that consists mainly of phospholipid and protein. The particles that perform the transport function in blood have come to be known as lipoproteins, and are present in essentially the same form in all vertebrates. It appears that the main difference between these plasma lipoproteins and the particles produced by the mammary gland is their smaller average size. In this respect the plasma lipoproteins have much in common with the microemulsions of Schulman (see Bowcott and Schulman, 1943; Prince, 1977), whereas the particles from milk are more akin to the conventional emulsions that are optically opaque.

The way in which plasma lipoproteins are synthesised is unknown. At first sight, the production of the interface between the particle and the surrounding medium would require considerable energy but, in many cases, appropriate mixtures of lipids and emulsifiers can be induced to form microemulsions spontaneously in the laboratory, and this may be a clue to the mechanism of lipoprotein synthesis by living cells. Whatever their genesis may be, the general structure of the plasma lipoproteins seems clear. They consist of a core of hydrophobic material that is surrounded by a hydrophilic envelope. The core is mainly composed of triglyceride and cholesteryl esters, although in

lipoproteins from some of the more primitive vertebrates it may also contain hydrocarbons and glyceryl ethers. The envelope is composed of phospholipid and a mixture of various specific proteins (apo-lipoproteins), together with some unesterified cholesterol. As with any emulsion, these substances are bound by non-covalent forces and their proportions are variable, at least within fairly wide limits. Moreover, the lipoproteins produced by biosynthesis, like the emulsion particles made in the laboratory, are not uniform in size.

Unfortunately for the biochemist, this heterogeneity at the site of synthesis is not the only source of variation in the size and composition of the plasma lipoproteins. These substances are unusual, if not unique, in that they undergo a considerable degree of metabolic alteration while they are circulating in the blood. Although some of this modification may take place while the particles are passing through the liver, it is now generally thought to be mainly brought about within the extra-hepatic vascular bed. This is a process that results in a progressive decrease in the size of the lipoprotein particle at a rate that is itself related to the size of the particle. Thus, a steady state distribution of lipoproteins is established in the plasma, in which the particles are of similar chemical structure but differ in size and composition. These differences in composition are, in turn, the cause of variation in such constitutive properties as density and electric charge. The extent of this variation in the major properties of the lipoproteins is summarised in Fig. 1.1 over the whole range that is normally found in plasma. Note that the abscissae are not necessarily on a linear scale and that, because the electrophoretic mobility of the lipoproteins is dependent on the experimental system used, it is expressed as a fraction of the mobility of albumin. It is these differences in the properties of the lipoproteins that are used to separate them into different fractions. But it will be evident from the figure that, because the distribution of the particles is essentially continuous, the differentiation of these fractions will be quite arbitrary.

It is important to note that there is no fixed relationship between the size of a lipoprotein and its composition, and that in consequence there is no fixed correlation between size and the density or relative

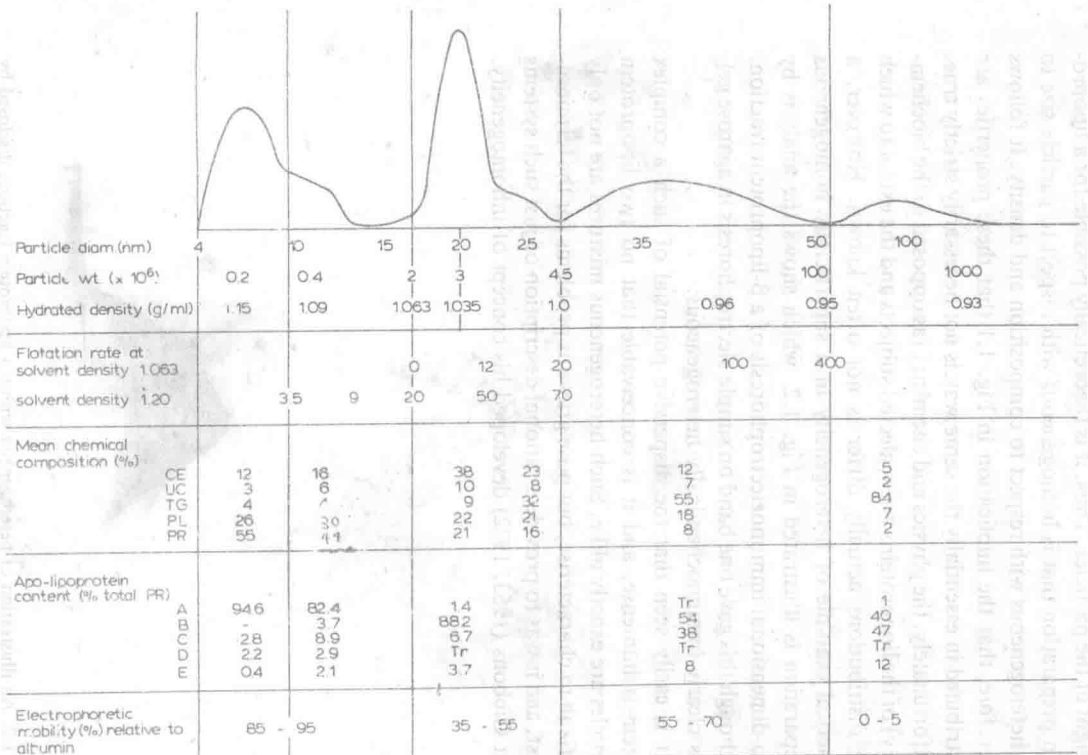

Fig. 1.1. The major physical and chemical properties of human plasma lipoproteins. The upper diagram shows a characteristic quantitative distribution of the different particles. Abbreviations: CE, cholesteryl ester; UC, unesterified cholesterol; TG, triglyceride; PL, phospholipid; PR, protein.

weight of the particles. Thus, it is theoretically possible for a lipoprotein preparation that is homogeneous with respect to particle size to be heterogeneous with respect to composition and density. It follows therefore, that the implication in Fig. 1.1 that these properties are distributed in essentially the same way is not necessarily strictly true. Unfortunately, the physics and chemistry (as opposed to the biochemistry) of the lipoproteins are neglected subjects and the extent to which the distributions actually differ is not often known. However, a practical example of heterogeneity in a superficially homogeneous preparation is illustrated in Fig. 1.2, which shows the analysis by two-dimensional immunoelectrophoresis of a β-lipoprotein fraction. Although this gave one band on simple electrophoresis in agarose gel, it is clearly immunochemically heterogeneous.

It is easily seen that the dispersive potential of such a complex system is immense, and it is conceivable that no two lipoprotein particles are exactly alike. Such heterogeneous mixtures are not only difficult to characterise, but pose grievous problems for the terminologist, and it was to provide a rational description of just such systems that Gibbons (1963, 1972) developed his concept of inhomogeneity.

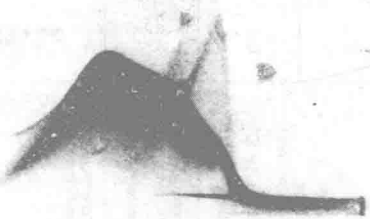

Fig. 1.2. An illustration of heterogeneity within a lipoprotein fraction, disclosed by the method of two-dimensional immunoelectrophoresis (Section 5.7).