The Plasma Proteins

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VOLUME I

Isolation, Characterization, and Function

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PREFACE

Plasma proteins have long been favored for study by the biochemist and the clinician because they are the most readily available group of proteins having a wide variety of natural functions and biological properties. In fact, the word "protein" was originated by Mulder after his chemical analyses of blood fibrin and other substances. Increasing awareness of the complexity of the plasma proteins has fostered the development of numerous methods for their fractionation and characterization, which have had general applicability to other mixtures of proteins. The widespread acceptance of physicochemical techniques such as electrophoresis, and of protein fractionation methods such as ethanol precipitation, came about as the result of their successful application to the plasma protein system. Present developments owing to even finer methods of resolution increasingly emphasize the complexity of the plasma protein system, and the newer methods permit a more accurate association of specific physiological properties with individual components. It is this exciting advance in the resolution of the plasma proteins, coupled with greater understanding of their function and metabolism, that has prompted the writing of this treatise.

Although many reviews have been written on individual plasma proteins, on certain of their functions, or on alterations in specific disease, no previous work has described this system as a whole. The purpose of this treatise, therefore, is to present an authoritative, interpretative, and integrative account of the plasma proteins, both for the individual purified components and as a dynamic system. The work is divided into two volumes. Volume I, "Isolation, Characterization, and Function," largely considers the plasma proteins as a physicochemical system, describes the isolation and characterization of the major components and certain subgroups, and also their functional differentiation. Volume II, "Biosynthesis, Metabolism, Alterations in Disease," gives greater emphasis to the physiological role and metabolic interrelationships of the plasma proteins in the normal state and in disease. Since certain chapters could properly be placed in either volume, the division is somewhat arbitrary, but not without design.

In order to achieve the comprehensive scope of a treatise without unnecessary length or duplication, the work was organized on an interdependent basis by exchange of outlines and through cross reference. By taking cognizance of the description of methods for the isolation and characterization of plasma proteins in the first five chapters, later authors could detail the special characteristics of their own subject in a way that was at once self-sufficient, yet sophisticated. Each author submitted a chapter outline

for circulation to all contributors. Where material of a similar nature was relevant to several chapters, it was assigned by agreement among the editor and the contributors on the basis of emphasis and significance.

The coverage is intended to be inclusive rather than circumscribed by the contributors' interests. By good fortune, three of the authors were abroad on sabbatical leave and had an opportunity to confer with European colleagues as well as with each other. Other authors brought their manuscripts to Florida, and some were visited by me. This high degree of personal contact and exchange among contributors has aided in averting some of the pitfalls which plague volumes written by many contributors. Furthermore, both a simultaneity of outlook and an up-to-date content were achieved by the excellent cooperation of all contributors; this permitted the completion of the entire treatise within 18 months of the first invitations to authors and publication of the first volume within one year of the first deadline for manuscripts. Yet, it is to be hoped that when further progress outstrips the new advances described herein, the wealth of tabular data and the comprehensive treatment given will assure enduring value for this treatise. The integration of the text achieved through the exchange described above was implemented further through frequent cross reference. For this reason the chapters are numbered consecutively for the entire treatise although each volume is paged and indexed separately.

In this treatise the emphasis has been on human plasma proteins. However, reference is frequently made to other species, and the long neglected subject of the comparative biochemistry and embryological development of plasma proteins is covered by a separate chapter. The red cell and nonprotein components of blood are dealt with only in relation to plasma proteins.

Because of the supernumerary names of many of the lesser constituents and the uncertain identity of others the nomenclature of plasma proteins remains confusing. In these volumes an attempt has been made to achieve consistency in terminology, preference is given to certain names and to the new international nomenclature for blood clotting factors. The problem of nomenclature ought soon to be handled by international agreement, perhaps through the Protein Commission of IUPAC.

Many have aided by criticism of the plan, reading chapters, releasing copyrighted material, etc. I will, however, single out for acknowledgment only John Ische, formerly Reference Librarian at the J. Hillis Miller Health Center Library. Mr. Ische verified in detail the bibliography for each chapter, thus assuring the usefulness of this treatise as a reference work. To him and to all others who have aided or encouraged this work I extend my sincere thanks.

Gainesville, Florida FRANK W. PUTNAM
January 1960

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Chapter 1

Introduction

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"For the life of all flesh, is the blood thereof," Leviticus *17:* 14.

I. Scope

Plasma, the viscous medium through which the red cells are transported and the tissues nourished, reflects and guards the internal milieu. Through its myriad protein components the plasma contributes to nitrogen needs, to defense against invasion and injury, to maintenance of body pH and osmotic balance, and to regulation of cellular activity and function. As components of a metabolically dynamic system, the plasma proteins fluctuate in disease, usually nonspecifically, and their determination and fractionation has been the subject of thousands of papers in the past century. Several monographs on plasma proteins have appeared in other languages (2–4, 25, 31), usually with the theme of variations in disease as measured by a particular technique or approach. Symposia on various aspects of the plasma proteins (10, 22, 29, 32) and brief monographs (21, 30) have also been published. Yet, heretofore, no book has been available in English or other languages that treats broadly and collectively of the many types and manifold functions of the plasma proteins. It will be the objective of this collectively written treatise to describe from the modern, experimental point of view the purification and characterization of plasma proteins, their metabolic function and activity, and their derangement and dysfunction in disease.

II. Historical

Though serum must have been known in antiquity because of its syneresis from the clot, it was to whole blood that recuperative and rejuvenative

powers were attributed in ancient times. Thus, the early Egyptians are alleged to have bathed in blood and the Romans to have drunk the flow from the dying gladiators. However, in Hebraic law, sanctions were placed against the "eating of blood," and similar religious scruples were raised against early attempts at transfusion.

Long before the functions of plasma became known, its proteins were studied because of their availability and ease of isolation. Thus, blood "albumin" and blood fibrin were first analyzed by Liebig and Mulder in the late 1830's when the term "protein" had just been introduced. However, the study of the components of serum began only about a century ago when several authors observed the formation of a precipitate upon dilution of slightly acidified serum.[1] Panum in 1851 designated the precipitate "serum casein" to distinguish it from serum albumin, but Schmidt in 1862 introduced the name of globulin for the protein fraction that was insoluble in water. Heynsius in 1869 demonstrated that the water insoluble precipitate was similar to that obtained on saturation with sodium chloride, and later workers employed different salts for the precipitation of globulins. The introduction of fractional precipitation with ammonium sulfate as a means of separating the serum proteins is attributed to Hofmeister. Although the isolation of the globulin fractions was painstakingly investigated but never completely succumbed to salt fractionation, a procedure for the crystallization of horse serum albumin by precipitation with ammonium sulfate was reported as long ago as 1894 (6).

At the turn of the century it became obvious that the "total globulin" could be fractionated both by dialysis against water and by salt precipitation. The term "euglobulin" was introduced for the fraction that precipitated on dialysis against water or between 0.28 and 0.33 saturated ammonium sulfate; "pseudoglobulin" designated the water-soluble fraction or that which precipitated between 0.34 and 0.46 saturation with ammonium sulfate. Later a profusion of terms developed as other fractionating procedures were devised. Today, a similar situation exists regarding the blood coagulation factors and many of the minor plasma proteins such as the glycoproteins.

It is not surprising that fractionation of such a complex mixture of proteins as is serum has led to much confusion, especially prior to the development of physical chemical apparatus for characterization of the fractions. About 1920–25, serum fractionation procedures such as the Howe sodium sulfate method (11), were added to the clinical chemistry laboratory manual, and interest in the variation of plasma components with disease was awakened. Euglobulin and pseudoglobulin were redefined by Howe in terms of solubility in sodium sulfate solutions, but he was aware that the

[1] For detailed historical reviews and references, see Hammarsten (8), Howe (12), and Pedersen (21).

precipitates were mixtures of proteins. Many other systems for charac terization of plasma proteins by fractional precipitation with salts were proposed; these have been reviewed by Gutman (7). From this point on, the history of the plasma proteins is virtually to be found in the listing of the chapters of this book.

III. Development of Physicochemical Instrumentation and of Inclusive Fractionation Procedures

The identification, characterization, and separation of the plasma proteins was greatly facilitated by the advent of the Svedberg analytical ultracentrifuge and the Tiselius moving-boundary electrophoresis apparatus (Chapter 3). Indeed, the impetus for the technical development of this equipment came largely from the problems arising in the study of serum. The first investigation of serum in the ultracentrifuge was carried out at Uppsala by Pedersen in 1930, but the centrifugal field was too low to enforce separation of the albumin and globulin. With improvement of the ultracentrifuge, von Mutzenbecher (19) achieved separation into two major components (4.5 S and 6.8 S), plus a small amount of a high molecular weight material (17 S).[2] McFarlane (17) was the first to investigate thoroughly with the ultracentrifuge the normal sera of man, the horse, and the cow. He also began the study of pathological human sera, and of artificial mixtures of albumin and globulin. In his work and in that of Pedersen's (21), a great deal was made of an "X-protein," which varied with the dilution of the serum and the density of the solution. The "X-protein" was always associated with lipid, and somewhat later Gofman and his associates (16) found the explanation for this artifact in the flotation behavior of low density lipoproteins (Chapter 11). By use of the original Svedberg ultracentrifuge, Pedersen (21) also discovered fetuin, a low molecular weight protein with the mobility of an α_1-globulin found in fetal bovine, equine, and sheep serum. However, the most important result of the early ultracentrifugal work was that it led to the abandonment of the concept of proteins as heterogeneous colloidal systems in the light of evidence for their definite molecular weights.

During the same period at Uppsala, Tiselius (28) developed the moving boundary electrophoresis apparatus enabling precise measurements on purified proteins and on mixtures such as serum. In so doing, he discovered and named the α-, β-, and γ-globulin fractions. Subdivision of the globulins into α_1, α_2, β_1, β_2, γ_1, γ_2, etc. resulted from further improvement in the Tiselius apparatus and better choice of buffers by later workers.

As apparatus for physicochemical characterization of proteins became

[2] Throughout this book, 10^{13} times, the fundamental unit for the sedimentation constant (in the c.g.s. system) will be called the Svedberg unit (S).

more widely available, other investigators such as Kendall (14), Hewitt (9), Neurath (20, 24), McMeekin (18), and Green (5) undertook the purification of serum albumin and the fractionation of globulins using ammonium sulfate. Unfortunately, confusion resulted because of the variety of procedures and nomenclature applied by different workers using the salting-out methods. In 1941, an electrophoretic study by Svensson (27) of the fractionation of horse, cow, swine, and rabbit serum demonstrated the heterogeneity of many of the globulin fractions obtained through different procedures, and this aided in interrelating the products of various investigators. Subsequently, under the pressure of the wartime needs for stable processed plasma proteins, the cold-ethanol method for the inclusive fractionation of plasma was developed in the laboratory of E. J. Cohn (1).

Whereas earlier procedures had been designed primarily for the isolation of a single component, the ethanol method was an inclusive scheme planned for the simultaneous isolation of a large number of components in a highly reproducible manner (for the principles and details see Chapter 2). The impact of the new procedure on the history of plasma proteins was even greater than that of the development of the ultracentrifuge and electrophoresis apparatus. Human serum albumin was not only crystallized for the first time, but now made available for military use at the rate of some 400,000 transfusion units a year. An immediate result was a great burst of investigations of the physical properties of serum albumin, which have made this the most thoroughly characterized of the proteins from the physicochemical—if not the structural—point of view (Chapter 6). Fibrinogen and γ-globulin became, as it were, highly purified by-products that rapidly found clinical use. Variation in the methods with introduction of the use of specific metallic ions permitted the finer fractionation of plasma and the purification of minor components (13). Indeed, much of the substance of this book could not have been written in the lack of an inclusive plasma fractionation scheme, for the latter serves as a characterization procedure as well as a source of products for study.

IV. Recent Advances

The accumulated volume of articles on the preparation and the properties of plasma proteins that appeared shortly after World War II began to abate just as precision electrophoresis apparatus and the analytical ultracentrifuge became commercially available on a wide scale. This, coupled with the arrival of the simple and inexpensive method of paper electrophoresis, led to a tremendous upsurge in studies of the variation of plasma proteins in disease. Although this topic is summarized in Chapter 17, the bibliography is too great even for listing, let alone for critical analysis of individual papers. Less extensive study, however, has been devoted to

characterization of the abnormal plasma proteins found in disease (Chapter 18). The refinement of the technique for zone electrophoresis, particularly the starch block method of Kunkel (15), contributed to ease of isolation of components, especially for those having specific biological activity. The application by Gofman's group (16) of the preparative ultracentrifuge coupled with the principle of flotation analysis in the analytical ultracentrifuge enabled the isolation and characterization of serum lipoproteins that were unobtainable by the ethanol method. Because of the implication of serum lipoproteins with atherogenesis, a whole literature of clinical studies has grown up in the past decade (Chapter 11). Ultracentrifugal methods have likewise been indispensable to clarification of the problem of normal high molecular weight antibodies and the pathological macroglobulins (Chapter 8). The newest fractionation technique, and one of great promise and already of wide application, is that of chromatography on cellulose ion-exchange resins as developed by Sober and Peterson (Chapter 4).

With the advent of the newer techniques and wider knowledge of plasma proteins, investigation could be focused on problems of metabolic and medical interest, for example, the factors involved in blood coagulation (Chapter 14), the functions of the metal-binding proteins (Chapter 10), and the significance and clinical evaluation of plasma enzymes (Chapter 12) and of plasma hormones (Chapter 13). An example of potentially great importance is the recent discovery of the genetic control of serum proteins (Chapter 19). This virtually began in 1955 with Smithies' (26) development of the method of starch-gel electrophoresis and his finding of familial differences in haptoglobins. Indeed, it has been said to be startling to immunologists that the first clear-cut evidence for hereditary serum protein groups in normal humans came from electrophoretic rather than serological methods. Genetic differences in serum proteins are now found in animals as well as humans. Although this treatise is focused on the human, phylogenetic comparison of plasma proteins is a subject of great interest, particularly in regard to the development of plasma cells and other possible sites of globulin synthesis (Chapter 15).

V. Trends for the Future

The great advances in knowledge of the plasma proteins during the last two decades should not obscure the present deficiencies in two uncharted areas of the first importance, namely, protein biosynthesis and structure. Isotopic techniques have contributed greatly to the understanding of serum protein metabolism and turnover (Chapter 16), but developments in understanding of the mechanism of biosynthesis are more slow in coming. However, progress is being made through the application of tissue culture and of cellular partition techniques. Likewise, only the barest details such