ADVANCES IN PROTEIN CHEMISTRY

EDITED BY

M. L. ANSON
Thomas J. Lipton, Inc.
Hoboken, New Jersey

JOHN T. EDSALL
Harvard University
Boston, Massachusetts

KENNETH BAILEY
University of Cambridge
Cambridge, England

VOLUME VI

With Cumulative Subject Index for Vols. I–V

1951

ACADEMIC PRESS INC., PUBLISHERS
NEW YORK, N. Y.

Copyright 1951, by
ACADEMIC PRESS INC.
125 EAST 23RD STREET,
NEW YORK 10, N. Y.

All Rights Reserved

No part of this book may be reproduced in any form, by photostat, microfilm, or any other means, without written permission from the publishers.

PRINTED IN THE UNITED STATES OF AMERICA

CONTRIBUTORS TO VOLUME VI

JOHAN BJORKSTEN, *Bjorksten Laboratories for Sponsored Industrial Research, Madison, Wisconsin*

PAUL DOTY, *Department of Chemistry, Gibbs Memorial Laboratory, Harvard University, Cambridge, Massachusetts*

JOHN T. EDSALL, *University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University, Boston, Massachusetts*

*HARRY L. FEVOLD, *Food Research Division, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois*

SEYMOUR LIONEL HALPERN, *Mount Sinai Hospital, New York, New York*

EPHRAIM KATCHALSKI, *Weizmann Institute of Science, Rehovoth, Israel*

RAYMOND MICHEL, *Collège de France, Paris, France*

HERBERT POLLACK, *Mount Sinai Hospital, New York, New York*

JEAN ROCHE, *Collège de France, Paris, France*

HEINRICH WAELSCH, *Departments of Biochemistry, New York State Psychiatric Institute, and Columbia University, New York, New York*

RALPH W. G. WYCKOFF, *Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Maryland*

*Present address: *Baxter Laboratories, Inc., Morton Grove, Illinois.*

EDITORS' PREFACE

Our objectives in the preparation of this series were described in the Editors' Preface to Volume I, and they remain today in all essentials the same. Now, even more than then, protein chemistry has become a focal point of absorbing interest to scientific investigators in very diverse fields, ranging from fundamental structural chemistry to numerous applications in medicine, nutrition and industry. We have attempted, and shall continue to attempt, to present contributions which cover the whole gamut of these activities, while stressing basic studies in protein chemistry as the essential foundation of further advance. We have also endeavored to obtain contributors truly representative of the international character of research in this great area, so far as this is possible in the divided world of today. Volume VII, which is due to appear in 1952, will contain at least three contributions from Great Britain, one from France, and one from Germany; and contributions from Scandinavia will appear in later, as they have in earlier, volumes.

It is hoped that, as the successive volumes of this series appear, they will as a whole in time provide something approaching a comprehensive picture of the present status of protein chemistry. This aim is as yet by no means achieved, but some progress has been made in that direction, in these six volumes. To make this material more accessible, we have included in this volume a Cumulative Index to the first five volumes, as well as a separate index to Volume VI. We are indebted to Dr. Martha Sinai for much devoted work in the arduous task of compiling the Cumulative Index.

April, 1951

M. L. Anson
K. Bailey
J. T. Edsall

此为试读,需要完整PDF请访问: www.ertongbook.com

CONTENTS

Natural and Artificial Iodoproteins

By Jean Roche and Raymond Michel, *Collège de France, Paris, France*

Glutamic Acid and Cerebral Function

By Heinrich Waelsch, *Departments of Biochemistry, New York State Psychiatric Institute, and Columbia University, New York, New York*

Cross Linkages in Protein Chemistry

By Johan Bjorksten, *Bjorksten Laboratories for Sponsored Industrial Research, Madison, Wisconsin*

The Relation of Protein Metabolism to Disease

By Herbert Pollack and Seymour Lionel Halpern, *Mount Sinai Hospital, New York, New York*

The Electron Microscopy of Macromolecules

By RALPH W. G. WYCKOFF

Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Maryland

Contents

I. Introduction

Our developing ability to see particles of macromolecular dimensions with the help of the electron microscope permits a study of organization within biological structures on a new and more fundamental level. Heretofore, the cell and its optically visible components and products have been the units in terms of which the organization of living matter has been discussed; but we are now beginning to see the macromolecules that are essential parts of these cells and the way they are organized to carry out its functions. The cell of course will never lose its importance as the unit of vital activity, but it is inevitable that before long conventional cytology will be supplemented by a kind of molecular cytology that describes the macromolecular architecture of cells, and the way this

architecture is built up during growth and altered by the cell's metabolic activities.

II. Factors Determining Macromolecular Visibility

1. *Resolution*

Present day electron microscopes have a resolving power that is more than adequate for the visualization of particles having the size of all but the smallest proteins. In addition, the smallest known viruses provide spherical objects with diameters as small as 100–150 A. and weights in the range between one and four millions. Molecules of some hemocyanins, easily seen in the electron microscope, are at least this big; others have molecular weights in the hundred thousands and sizes comparable with molecules of the plant and animal globulins, including the larger enzymes. All these are not hard to photograph. Examining still smaller molecules is primarily a problem of incorporating them into satisfactory preparations. It is hard to determine precisely the practical lower limit of existing electron microscopic vision because of this difficulty in getting preparations suitable for its measurement. The theoretical lower limit of resolution of the electrons used for microscopy is only a fraction of an angstrom unit—far less than the diameter of an atom. Existing electron lenses are, however, completely uncorrected and can only yield satisfactory images after being so closely apertured that the resulting optical system achieves a mere fraction of this higher limit of resolution. The average present electron microscope of precision, such as the RCA Type EMU with which most work is done in this country, should have a resolution of better than 100 A. under the most ordinary conditions of operation. If it is equipped with a good objective lens that has little astigmatism, either inherently or by correction, its average working resolution will probably be in the neighborhood of 50 A. For better resolution than this the microscope must be in the hands of an operator of more than average experience and the preparations must be of more than average thinness. Under such circumstances resolution of the order of 20 A. can be attained and resolutions of half this value have been claimed under certain conditions.

2. *Contrast*

While resolution sufficient to portray minute particles in their true shapes is needed, it is not the only factor essential for the photography of macromolecules. The other factors must be understood in terms of physical considerations that are different from those important in optical microscopy. We see things under the optical microscope by reason of

various amounts of light absorption in a preparation or through differences in refractive index over a transparent object. Neither of these is important in establishing what detail is seen in a satisfactory electron microscopic image. The degree of detail is determined by the different scattering powers for electrons of different parts of a preparation. The amount of this scattering depends on the number and weight of the atoms involved. Dense objects and especially those containing heavy atoms scatter strongly and appear relatively opaque under the electron microscope. Macromolecules of biological origin consist almost exclusively of very light atoms which scatter electrons poorly. The amount of this poorly scattering material diminishes rapidly as a function of molecular size. In almost all cases they must be supported for microscopy on a substrate which also scatters electrons. When therefore we look at smaller and smaller molecules a point is soon reached where the molecule does not scatter enough more than this substrate to be clearly distinguished from it. Such a light molecule is thus practically invisible because its image does not contrast appreciably with that of its support, and experience shows that the limit of visibility through this lowered contrast is reached with objects that greatly exceed in size the limit of resolution of the microscope as outlined above. If nothing could be done to circumvent this difficulty, there would be little hope of finding out much about biological macromolecules.

Fortunately, however, it is possible to enhance preferentially the scattering and visibility of macromolecular particles. In some cases this can be done by incorporating heavy atoms into the molecular particles, either through chemical reaction or by adsorption. Seemingly a more broadly useful procedure for enhancing contrast is metal shadowing. This is accomplished by evaporating a metal obliquely and in an exceedingly thin layer upon the surface of a preparation. The metal thus deposited is thickest on those aspects of an object that face the evaporating atoms and is completely absent from parts of the substrate on the far side of tall objects. The scattering power from such an unevenly distributed deposit varies so as to bring out with added clarity the micro detail of an object, including its macromolecules. The most satisfactory and informative electron micrographs yet made of macromolecules are ones that have been shadowed. All those shown in connection with this chapter have been so treated.

Definite conditions must be fulfilled if metal shadowing is to reveal small macromolecules. The layer of deposited metal should be so thin that it does not perceptibly disturb the shape of the molecular particles. This means that the metal should be of high atomic weight and consequent scattering power. It should be chemically inert and its atoms

此为试读,需要完整PDF请访问: www.ertongbook.com

must not be able to move about and recrystallize after deposition, even under the electron bombardment incident to microscopy. A number of materials meet these requirements well enough to use for shadowing the larger macromolecular particles, but only the noble metals have the chemical inertness and high melting points required for the best photography of small molecules. Platinum is especially satisfactory for this purpose, but there are serious difficulties in evaporating a substance of so high a melting point without damaging, through radiation, the preparation being shadowed—and these difficulties have not yet been fully met.

3. *Substrate*

The ability to see small molecules is as dependent on the smoothness as on the thinness of the substrate that is supporting them. It is evident that for good visibility they can scarcely be smaller than the molecular texture of the supporting membrane. Known plastics have a texture of their own that is of the order of 100 A.; they, therefore, have a limited usefulness when studying molecules smaller than this. To examine smaller molecules we should if possible employ the smoother backgrounds that are provided either by exceedingly thin metallic films or by atomic replicas. They are both technically far more difficult to use, but for the present their roughness is not the limiting factor in seeing small particles.

4. *Specimen*

The maximum of resolution of which a microscope is capable can only be realized from preparations of extreme thinness. Most electrons lose energy when they are scattered. Since electron lenses cannot focus electrons of different velocities in the same plane (*i.e.*, have no "color" correction), these slowed-up electrons strike the image plane in such a way as to diffuse and damage the image produced. Some which are bent through especially large angles can be screened out by close aperturing of the objective lens, and this does greatly improve the quality of the final image. But under all circumstances the ability to perceive small detail falls rapidly with increase in specimen thickness, and this fact must be given great weight when seeking to see macromolecular particles.

The most serious limiting factors now reside in the substance itself. One of these lies in the need for having clean molecules to look at. Experience shows that a relatively small amount of a low molecular weight impurity may be sufficient to obscure completely the smaller macromolecules and to smear over and disturb the appearance of the larger ones. The monomolecular films formed on the air-liquid interfaces of solutions of all but the big molecular proteins interfere very seriously

with the visualization of their molecules. In some cases we know how to evade the obscuring effects of these films, but they are one of the most serious problems we now face in the photography of protein molecules.

III. Visualization of Macromolecules

Macromolecular particles of many different sorts are now accessible to observation and photography. In general they are of two kinds: (a) proteins and other substances that can be obtained as solutions, or suspensions, which can be purified and sometimes crystallized and (b) parts of a more or less insoluble macromolecular fabric. To the first group belong the viruses, the respiratory proteins of vertebrates and invertebrates, seed globulins, and proteins constituting animal sera or derived from tissue extracts. The majority of these substances seem to have spherical or short rod-like molecules. Macromolecular particles of the second group are typified by those building the cellulosic structures of plants and the muscle, connective tissue and nerve of animals. They usually are long filaments.

Resulting Problems

The present problems arising from the ability to see these macromolecules with the electron microscope deal mainly with (a) a description of the shapes and sizes of members of these two groups, (b) the arrangement of their particles as they occur in nature or in the solids they form under laboratory conditions, (c) the changes they undergo as a result of chemical reactions in which they participate and (d) the mechanisms whereby they are produced in nature. For many of these substances a determination of their particle dimensions must be considered largely of academic interest or as information essential to an attack on the other problems; but for viruses such knowledge has very immediate and practical value for their recognition and for the study of the way they cause disease. We are only beginning to penetrate the molecular architecture of the fixed tissues of the animal body and of the cellulose that is the principal fixed tissue of plants. But it is already obvious that the determination of this architecture will give quite a new insight into how these tissues function and a study of how this architecture is modified with time will provide a new approach to many problems of degenerative disease and of the aging process.

Though many of the macromolecular particles derived from living matter serve as frameworks and fabrics to support more actively metabolizing components, others, such as the respiratory proteins and the enzymes, fulfill their purposes by being centers of intense chemical activity. It is bound to be an increasing concern of electron microscopy to

observe the changes that these molecules produce in their environment and that they themselves undergo as their chemical reactions proceed. It is impossible at this time to perceive more than the broadest outlines of this visual chemistry or even to be sure of the directions in which it will most actively develop, but that such a chemistry will inevitably arise and that it will yield exciting new prospects over the way living matter functions is certain beyond doubt.

The mechanisms through which these macromolecular particles are produced in nature are intimately tied up with the way both they and the living organisms that originate them function. As yet we have no good picture of how large molecules, some of several million molecular weight, can be so constructed that each is exactly like all the others of a given substance. But we are beginning to perceive details of the mechanism whereby the viruses develop within their living hosts, and it is not beyond hope that this accumulating knowledge, besides clearing up many of the mysteries surrounding the fundamental nature of these agents of disease, will indicate how we should proceed to find out more about the benign macromolecular entities, of similar size, that are to be found throughout the living world.

IV. Macromolecular Morphology

1. *Historical*

Until a generation ago very little was known about the elementary particles of the materials dealt with here. Over the past years the ultracentrifugal studies of Svedberg supplemented by measurements of rates of diffusion, viscosity, double refraction of flow and other physicochemical characteristics have, however, given much information about the weights and probable shapes of such of these molecules as can be obtained in solution. The electron miscroscope has for the most part confirmed this information and has extended our knowledge to many substances whose particles are altered by attempts to put them into solution.

There are two phases to be recognized in the electron microscopy of macromolecular particles. One is what might be termed the historical phase, the other is the phase of present-day development. During the first few years in which electron microscopes were available, beginning in the later 1930's, a considerable number of macromolecular objects were examined (*cf.* 1 and 3 for early references). The first adequate microscopes, manufactured by Siemens, were all in Germany; during these earlier years of the war, they were used to look at most of the materials we still find it most profitable to study. The same sort of exploratory work was carried out in this country (*cf.* 46 for many early references)

after the first RCA microscopes were built. The methods of specimen preparation developed during these early years were not well adapted to show small organic particles such as the biological macromolecules; therefore, though many particles of macromolecular dimensions were seen during this early work both here and abroad, the visualization was usually not clear enough to add much to the knowledge we already had of them.

Subsequent development of methods for reinforcing the scattering from small and light particles, notably shadowing, made the electron microscopic images of macromolecules so much clearer that it has proved well worthwhile to repeat and greatly extend the earlier explorations. In all preliminary investigations of this sort, designed to find out the range and limitations of a new method, the objects for study will in the main be those that are especially easy to prepare and that have already been examined by other methods. We therefore continue to look at the same kinds of macromolecular particles that were first put under the electron microscope: for example, the hemocyanins, plant globulins, the viruses, muscle, connective tissue, fibrin, etc. As our understanding of these and similar "classical" objects of investigation is increased through improved experimental procedures and increased experience, the field of investigation will inevitably be widened.

2. *"Spherical" Macromolecules*

As already stated nearly all the macromolecular particles thus far visualized are either nearly spherical, or they are filaments of great length. Where comparisons can be made they have dimensions that for the most part accord well with the ultracentrifugal data, but some are not as asymmetric as these less direct methods of measurement had led us to expect.

A number of the plant virus proteins have spherical macromolecules with diameters that range between *ca.* 150 and 300 A. Some of these, like the tomato bushy stunt, the tobacco necrosis and the turnip yellows viruses, seem to be strictly spherical; others, like the Southern bean mosaic virus (Fig. 1) and the non-infectious Rothamsted protein, are nearly, but not strictly, spherical. Some of the smaller animal viruses such as those causing the encephalomyelitides, papillomas in rabbits and warts in man, poliomyelitis and related diseases, have macromolecular infectious units that are nearly or exactly spherical particles with diameters in the range between 500 and 100 A. Aside from these products of virus activity the hemocyanins and erythrocruorins, as respiratory proteins of invertebrates, have the largest centro-symmetrical molecular particles yet seen. Molecules of the hemocyanin of the horseshoe crab,

Limulus polyphemus, have sppeared after ultracentrifugal purification as spherical objects *ca.* 160 A. in diameter (40). Careful examination of their electron microscopic images led to the rather surprising result that they were not of identical size but had a broad gaussian distribution about a mean value. The reason for this is not altogether clear, but it could be due either to an irregular flattening of the molecules during drying or to a minimal denaturation and alteration in molecular shape brought about by the procedures used in purification.

FIG. 1. A dried deposit from a purified suspansion of the Southern bean mosaic virus protein shewing an orderiy grouping of its elementary particles. In many regions the packing is the closest possible and yields an hexagonal net, in others it is the more open arrangement of a square net. Especially over the close-packed areas the particles are often more than one layer deep. Platinum shadowing. Magnification = 32,000 ×.

Interesting photographs (26) have been made of very cautiously purified hemocyanin of the sea snail, *Busycon canaliculatum*. The molecules found in such carefully purified solutions are cube-like objects* having a well-defined inner structure. Inspection seems to show that each cube is a group of four short rods. This is one of the hemocyanins which Friksson-Quensel and Svedberg (5) have sliown by ultracentrifuga-

* Electron micrographs of this and many other macromoleoular particles have been collected in a recently published bcok by the writer (Electron Mieroscopy, Interseience, New York, 1949). Thesethave not been reproduced here nor have all the bibliographie references given there been repeated.

tion to possess molecular weights that split into simple fractions when the pH is raised. In agreement with this, alkaline solutions do not show these cube-like aggregates, but instead have rod-like molecules which appear to be the components of the cubes. If this hemocyanin is not freshly taken from the animal, or if it is subjected to chemicals or to repeated ultracentrifugations for purification, these cube-like particles are no longer seen. Instead the molecules seem to be roughly spherical objects of about the same size. It is possible that they represent the first stage in denaturation of the original molecules.

Plant globulins, of which edestin is the most familiar example, offer a series of middle-sized macromolecules which can easily and profitably be observed in the electron microscope. Edestin was in fact one of the first molecules to be photographed (36) and both then and later (10) has shown itself as an approximately spherical particle *ca.* 70 A. in diameter. In this case ultracentrifugal data (38) had pointed to a somewhat elongated particle, and we are not at this moment in a position to explain this difference of result.

The molecules of the serum globulins, with half the molecular weight of the plant globulins, are not much smaller and in some sera, such as those of the horse, there are antibody molecules that are considerably heavier and very easy to photograph.

As previously indicated, the resolution of good present-day electron microscopes is sufficient to allow us to see molecules of weights somewhat under 50,000. It thus has become feasible to try to look at molecules of hemoglobin and of the most important enzymes. There have as yet been few serious attempts to examine these smaller and in some respects more interesting molecules. As earlier suggested, useful knowledge about them will only follow when satisfactory methods of preparation are found; and this in its turn will depend on being able to spread them over a sufficiently thin and smooth substrate in such a way that the molecules are unobscured by the monomolecular films that ordinarily form on the surfaces of their aqueous solutions. The more active cultivation of this field has been delayed by the numerous rewarding investigations that can more easily be made involving larger particles.

Macromolecular particles of all sorts are seen within the protoplasm of living cells. Until recently it has been possible to examine them only after cellular disruption; under such circumstances it has seldom been possible to identify what is seen and to determine its relationship to the grosser cellular components. This situation is being changed by our increasing ability to obtain sections thin enough for satisfactory electron microscopy. Both spherical and filamentous macromolecular particles of a wide variety of sizes are evident in sections through tissue cells.

此为试读,需要完整PDF请访问: www.ertongbook.com