Introduction [1]

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> The practical counsel to be derived from Pythagoras is to measure, and thus to express quality in terms of numerically determined quantity. But the biological sciences, then and till our own time, have been overwhelmingly classificatory. . . . If only the schoolmen had measured instead of classifying, how much they might have learnt.
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> —Alfred North Whitehead

CLASSIFICATION AND DESCRIPTION in the biological sciences have proceeded from taxonomic and morphologic studies on whole animals and plants to the study of successively smaller parts of living systems, culminating in the visualization and description of individual protein and nucleic acid molecules. Biochemical studies, beginning with the isolation and identification of urea, sugars, amino acids, and lipids, have proceeded to the analysis of larger and more complex molecules leading, within the last decade, to the nearly complete analytical description of certain protein and nucleic acid molecules. Thus are joined classical chemistry and classical biology, and the approaches of each discipline will now pervade the other. Modern crystallography allows many small molecules and some large ones to be visualized as biologists would like to see them. At the same time, larger multimole cular structures such as chloroplasts, ribosomes, mitochondria, and chromosomes are beginning to occupy the attention of the chemist. As a result a conceptual continuum is emerging that extends from the atoms of nuclear physics to the living animal of the behaviorist.

The advances that have made this juncture possible depend in a very real way on the development of techniques, and molecular biology, more than any previous branch of biological science, has been critically dependent on instrumentation. If separation and analysis are to proceed

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[1] This research performed under The Joint National Institutes of Health-Atomic Energy Commission Zonal Centrifuge Development Program which is supported by the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and the U.S. Atomic Energy Commission.

[2] Operated for the U.S. Atomic Energy Commission by the Nuclear Division of Union Carbide Corporation.

to even higher orders of complexity, certain new basic tools must be developed. The ordered fabric of protein and nucleic acid molecules found in living cells cannot, in most cases, be unravelled by the same techniques as those used for the separation and analysis of low molecular weight substances. A case in point is protein synthesis. The initial studies on the incorporation of amino acids into the proteins of whole cells were followed by others on the localization of synthetic activity in isolated cell fractions. "Microsomes" were implicated in protein synthesis, and further fractionation suggested that ribosomal particles were the synthetic units. By use of gentler methods for breaking cells and with centrifugal separation systems having improved resolution, it was found that the active particle is a complex structure (the polysome) composed of a long ribonucleic acid molecule to which variable numbers of ribosomes are attached. The mode of attachment of the polysome to membrane surfaces now occupies the attention of a number of investigators in this field, and methods are required for isolating portions of the membrane where attachment occurs. Thus the pursuit of a biochemical problem has led to the isolation of structures of increasing complexity and increased sensitivity to the physical and chemical procedures employed.

Except for storage materials, most cell structures appear to be composed basically either of filaments or membranes. The analysis of the organization of two-dimensional structures is more difficult than the analysis of sequence in linear systems. However, as first discussed by Tiselius (*1*), "every fragment derived from (an) original structure and containing more than one structure element offers a piece of information about that structure if we know the size and the composition (not necessarily the structure) of the fragment." The preparation, separation, and analysis of small fragments of cell structures, therefore, may offer a key to the patterns that underlie the complex behavior of the intact organelle.

We require not only high-resolution techniques for separating the microscopically identifiable formed elements of cells, but also methods for separating their fragments which are applicable to particles as small as the smallest unit of interest—in this case, individual protein molecules. Since the initial step in certain studies involves the separation of a distinct cell type from a population of cells, our requirements may be broadened to include separation methods that cover the entire range of particle sizes extending from whole cells to proteins. Initially, zonal centrifuge systems have been and are being developed for this purpose, as is evident from the contents of this Monograph. However, additional methods are being explored concurrently, since no one separation technique will be applicable to all fractionation problems we must eventually consider.

Two fractionation problems were sufficiently urgent to catalyze zonal centrifuge development: 1) the search for trace amounts of virus in pathological tissue samples, including neoplasms, and 2) the preparation of vaccines that contain only virus particles, specific cell antigens, or viral subunits. The former interest led to support from the National Cancer Institute, and the latter to a program of virus isolation sponsored

by the National Institute of Allergy and Infectious Diseases. Emphasis on virus isolation, however, does not diminish either our interest in, or the application of the systems to, the isolation of other subcellular particles. The fractionation of particle populations with heterogeneous physical properties can best be done—and can only be done with confidence—after it has been demonstrated that polydispersity, when observed, is real and not due to an inherently low resolution of the isolation system.

If our ultimate interest is in the quantitative visualization of the cell as a spectrum of separated particles, it is evident that the separation of macromolecular species is only half the story. The low molecular weight substances found in cells, including amino acids, nucleotides, sugars, lipids, and numerous metabolic intermediates also should be quantitated with automated analytical systems, only a few of which have been developed at this writing. Two such systems, a carbohydrate analyzer and a high-pressure nucleotide analyzer, are described in this Monograph.

Conceptually, an effort to fractionate a system as complex as the cell is an extension of the simple principle that a complex structure is to be understood in terms of its parts. In addition, it should be noted that the basic philosophy of the analysis of complex mixtures has undergone a revolution in the last two decades. In place of a battery of presumably specific analytical procedures, one for each component to be tested, emphasis now is on high-resolution separation techniques, with the results of the separation monitored by a sensor which is sensitive to mass, radioactivity, color, or a chemical group. The U.S. Atomic Energy Commission's Oak Ridge installation was originally organized to achieve certain difficult separations on a large scale. These include the separation of the isotopes of uranium by the electromagnetic process, by gaseous diffusion, and by other methods; the separation of plutonium from fission products and the fractionation of the fission products themselves; the isolation of radioisotopes in pure form for experimental and therapeutic use; the separation of experimental quantities of all the major stable isotopes of all the naturally occurring elements of the atomic table; and, most recently, the construction of facilities for producing and purifying quantities of the transuranium elements. The zonal centrifuge development program is therefore an extension of a large existing program into biological areas.

There are a number of reasons why systems for the separation and analysis of all the separable constituents of cells should be pushed to the limits of available theory and technology. One is that detailed information on the structure and composition of cells and on changes occurring from injury, disease, or during the cell cycle would throw into bold relief our need (and hopefully provide information) for an adequate theory or model of the cell into which available data may be fitted. Additional reasons related to basic biochemical or clinical studies are not difficult to enumerate. However, the most valid and compelling reason for creating new methods for probing living cells still remains our simple curiosity.

This Monograph marks the completion of the joint NIH-AEC Zonal Centrifuge Development Program. The program has served as a pilot study for a broader effort concerned with instrumental approaches to Molecular Anatomy (the Man Program), which will be continued as a joint NIH-AEC effort.

In the pursuit of the studies reported in this volume, assistance and counsel have been generously given by many individuals. It is a pleasure to record the support and encouragement of Drs. C. E. Larson, A. M. Weinberg, J. A. Swartout, A. Hollaender, J. L. Liverman, and C. S. Shoup at Oak Ridge; Drs. C. L. Dunham and J. S. Kirby-Smith of the Atomic Energy Commission; Drs. Gordon Zubrod, W. Ray Bryan, P. Kotin, and Robert Stevenson of the National Cancer Institute; and Drs. Robert Huebner, R. M. Chanock, M. Mufson, and Helen Coates of the National Institute of Allergy and Infectious Diseases. Without their assistance and cooperation, the complex problem of organizing an interagency program could not have been solved.

The enthusiastic, competent, and original contributions of members of the Technical Division of the Oak Ridge Gaseous Diffusion Plant have been important to the centrifuge program. This group, under Paul Huber, included P. R. Vanstrum, E. F. Babelay, H. P. Barringer, A. S. Berman, E. C. Evans, N. Cho, W. J. Wilcox, Jr., E. J. Barber, J. G. Green, C. E. Nunley, D. A. Waters, J. W. Amburgey, Jr., and L. L. McCauley. Special assistance in electron microscopy under W. W. Harris has been provided by T. W. Bartlett and F. L. Ball. The development of new instrumentation is due to the efforts of H. D. Culpepper, R. H. Stevens, G. R. Jamieson, G. M. Lindner, and M. J. Bartkus. Computer studies and programming were carried out by Barbara Bishop, Dr. K. O. Bowman, Elizabeth L. Rutenberg, and E. F. Martin, Jr., and metallurgical studies by W. S. Dritt, K. T. Ziehlke, and C. E. Weaver. Work on the chemistry of gradient materials was done by C. W. Weber, R. R. Wright, W. S. Pappas, and J. A. Carter. We cannot fully record our indebtedness to all the many additional staff members who have assisted in the solution of technical, supply, legal, design, and fabrication problems.

In the Biology Division of the Oak Ridge National Laboratory the research programs of Drs. W. D. Fisher, G. M. Padilla, I. L. Cameron, Peter Mazur, G. L. Whitson, and G. B. Cline have contributed directly to the experimental evaluation of new rotor systems. In addition, Drs. A. A. Barber and M. L. Barber (University of California, Los Angeles), Dr. Charles Burger (Upstate Medical Center, New York), Dr. Carl Price (Rutgers University), Dr. Herbert Schuel (Oakland University, Michigan), Dr. Charles Reimer (Eli Lilly Research Laboratories), Drs. Jacob Holper and John Garin (Abbott Laboratories), and Mr. Irving Toplin (Charles Pfizer and Company) have contributed in many important ways to the development of the program.

The difficult task of operating new experimental systems has been performed by R. E. Canning, C. T. Rankin, Jr., E. L. Candler, Jr., Tom Grizzard and Hunter Elrod. Without their skillful assistance

many of the difficult experimental problems would not have been solved. The existence of this volume is largely due to the editorial assistance of Domenic Fuccillo and to the assistance of Mrs. Margie Harding in manuscript preparation.

Finally, I would like to thank Dr. Jesse Beams of the University of Virginia and Dr. Rodes Trautman, Plum Island Animal Disease Laboratory, U.S. Department of Agriculture, Greenport, Long Island, New York, for their advice and encouragement during the entire course of this program and for reviewing the manuscripts which comprise this Monograph.

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(*1*) TISELIUS, A.: Some general relations between the composition of fragments and the structure from which they originate. I. Random fragmentation. Arkii för Kemi 15: 171–180, 1959.