VIERTER INTERNATIONALER KONGRESS FÜR ELEKTRONENMIKROSKOPIE

FOURTH INTERNATIONAL CONFERENCE ON ELECTRON MICROSCOPY

QUATRIÈME CONGRÈS INTERNATIONAL DE MICROSCOPIE ÉLECTRONIQUE

BERLIN 10.–17. SEPTEMBER 1958

VERHANDLUNGEN

HERAUSGEGEBEN VON

W. BARGMANN · G. MÖLLENSTEDT · H. NIEHRS
D. PETERS · E. RUSKA · C. WOLPERS

BAND II

VERHANDLUNGEN BAND II

BIOLOGISCH-MEDIZINISCHER TEIL

HERAUSGEGEBEN VON

W. BARGMANN · D. PETERS · C. WOLPERS

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Electron microscopy in morphology and molecular biology

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Electron microscopy, in scarcely more than two decades, has led to revolutionary new concepts of cell structure and the mechanism of basic life processes. In many instances biological systems have been observed at or near the molecular level. These advances become the more significant because of profound parallel discoveries in biochemistry, biophysics, and biophysical chemistry in about the same period. The fusion of these sciences into a single unified effort has already been begun, leading toward what may appropriately be called molecular biology, a term early employed by one of the pioneers in the field, W. T. ASTBURY.

It will be our task to bring these recent advances into perspective with the fundamental cytological discoveries of the preceding century and to indicate the lines along which further profitable advances may be expected. In this brief paper we can provide only a perspective and a point of view, sketched in roughest outline. However, because it is urgent that contemporary morphologists and molecular biologists have a clear understanding of the limitations and potentialities of the various approaches to the study of life processes, I am grateful to Professor ERNST RUSKA and the Program Committee of this International Congress on Electron Microscopy for giving me the opportunity to express my point of view on this timely subject.

Exactly a century ago, here at the University of Berlin, RUDOLF VIRCHOW (*85*) gave a lecture, *„Die Cellularpathologie,"* in which he brought together the evidence that the unit of life, with which pathology must deal, is the cell. He had previously (*84*) announced his dictum *omnis cellula e cellula*. This point of view, which has been orthodox biological dogma for so long, was by no means universally accepted at the time, especially by those who sought the ultimate living units in subcellular, possibly molecular, particulates. Even to eminent cytologists, such as MARTIN HEIDENHAIN, the cell was but one level of complexity in an organizational hierarchy in the realm of living things. VIRCHOW, however, regarded the organism as an integrated federation of its constituent cells. For the effective development of pathology and of medical research generally he emphasized the necessity of integrating histology, pathology, and physiology, rather than pursuing each of these disciplines as sciences self-sufficient in its own right. For a provocative essay on VIRCHOW's contribution in relation to the present-day situation see BARGMANN (*4*).

We stand in need of a similar message today. If we are to achieve a "molecular biology", there must be a joining of forces among physicists, chemists, and biologists in a common assault on the fundamental problems of life science, of which medical science is but a branch. PAULING's (*58*) development of the concept of the "molecular disease" is a landmark on the road leading toward such integration. The amazing developments in molecular genetics, virus research, and the proof that a purified molecular entity (DNA) can be infective, focus attention upon molecular codes in transferring genetic — and also pathological — information. Despite these promising successes in the analytical approach to what may be termed biophysical and biochemical communications theory at the molecular level, there is need constantly to bear in mind the importance of the organization of the whole — not just the cell or tissue, but the organism as a whole — probably even the organization of the individuals within the biological community.

The electron microscope permits high resolution (10 Å) examination of sections of entire cells. Within the limits of the biochemical indeterminacies involved, the electron microscope is capable

of dealing with subcellular particulates at the molecular level and, at the same time, with the entire cell as a whole (seen in section, of course). It should be possible, therefore, with the electron microscope to employ not only the analytical, but also the "systems" approach, so vital in dealing with biological problems.

The electron microscope has also revealed invaluable information concerning the interaction properties of highly organized macromolecular systems. Such data, together with other biophysical and biochemical evidence, should lead to an understanding of some of the intrinsic properties of systems which are at the basis of life processes. We shall try, in this paper, to bring some of these problems into focus.

I. A century of cytology — Historical perspectives

Cell and tissue structure has been studied primarily with three basic motivations:

1. As morphology for its own sake, independent of applications in physiology, pathology, medicine, developmental biology, biophysics, or biochemistry. As such, the work should be judged only as to its excellence as morphology, not by the extent to which it may have solved a basic problem, say, for example, the nature of gene action, muscle contraction, or nerve conduction.

2. To provide a basis for understanding function, either normal, as in physiology, or abnormal, as in pathology. This is the classical, pragmatic approach; the many basic contributions that have arisen from this motivating force explain why morphological studies have occupied a place of prominence in medical research and teaching for more than a century.

3. To search for fundamental life principles, such as those which may be involved in the replication and ordering processes by which physical and chemical information constituting the molecular message of life for the individual and for the continuity of living organisms is transferred within the microcosm of protoplasm. The motivation of this approach may be essentially independent of immediate or eventual applications in the biomedical sciences. It is found frequently in the work of physicists who have recently entered the field of biophysics and who, like physicists generally, search for ultimate causes.

The historical perspective will be better understood if interpreted in terms of the basic aims — such as those mentioned above — that motivated the investigators as well as by the actual results achieved. Clarity in this regard is important also for present-day investigators, not only for more effective orientation of their own efforts, but in better assessing the significance of the work of others in the broad area of the biomedical sciences.

With the advent, in mid-nineteenth century, of methods of fixation, staining, sectioning, and other histological techniques, there was a turning-away from the study of the living cell which characterized the biology of the early decades of the nineteenth century. There followed, in the Golden Age of Cytology (1870—1890), many noteworthy discoveries concerning the major organelles of the cell, such as the nucleus, nucleoli, chromosomes, mitochondria, Golgi apparatus, ergastoplasm, cell membranes, and so forth. Rapid strides were made also in the histological characterization of the specialized structures of particular tissues, such as muscle, nerve, and glands. By and large, these discoveries involved structures sufficiently interbonded chemically to permit reasonably satisfactory fixation and preservation. These historical discoveries were of salient importance in providing a structural basis for understanding physiological and pathological function. However, they also ushered in an intense search for the physical basis of life itself in terms of subcellular particulates and pseudo-crystalline aggregates [called micelles by NAEGELI (*53*)]. There sprang up what might be characterized as the fibrillar, membranous, and granular schools of thought concerning that which is actually "alive" in the cell. Many new terms were coined to describe these hypothesized vital units, most of which have long since been forgotten. Replication and biochemical determination (coding) were considered primary life criteria. The followers of the granular theory, particularly ALTMANN (*1*, *2*), proposed "*omne granulum e granulo*," paraphrasing VIRCHOW's earlier dictum "*omnis cellula e cellula.*"

Unfortunately in this rush to discover the physical basis of life in terms of microscopically resolvable objects, the investigators — competent morphologists though they were — neglected to evaluate the effect of the fixatives and microtechnical processes upon highly metastable proto-

plasmic systems. The artifactitious nature of many of the structures became glaringly obvious to the physiologists and biochemists and eventually even to the morphologists themselves. There followed an unfortunate eclipse of morphology during the early decades of this century in which morphological descriptions of fixed and stained preparations were given scant attention by physiologists.

Since the limit of light-microscope resolution is approximately 0.2 μ (2,000 Å), it was assumed that henceforth any information concerning smaller objects would have to derive from indirect evidence, as by the application to biological problems of polarization optics. This method, which could be applied to fresh, unfixed material, provided information about molecular orientation and the regularity of the internal organization of the micelles. For a useful introduction to this literature, the reader is referred to the books of W. J. SCHMIDT (*68*, *69*) and FREY-WYSSLING (*26*, *27*). It is an interesting commentary on this early work that conclusions about macromolecular organization of cells have been confirmed in all cases in which electron microscopy has subsequently been applied.

Another powerful indirect method, that of X-ray diffraction, developed through the theoretical work of the first speaker of this session, Professor VON LAUE (*28*). Beginning with the early applications of this technique to the study of tissue structure by investigators such as HERZOG (*35*) and SPONSLER (*80*, *81*), X-ray diffraction has culminated more recently in extraordinarily fruitful investigations of the internal structure of proteins, nucleic acids, and other biologically significant macromolecules; for a valuable non-technical description of recent discoveries see PERUTZ (*59*).

The diffraction principle has now been adapted to the electron microscope, making it possible to obtain electron diffraction patterns of selected delimited areas observed directly in the electron microscope. This ability to identify objects by their diffraction pattern offers promise of reducing one of the main limitations of electron microscopy, namely that of identifying, crystallographically or chemically, extremely small objects observed in specimens with high-resolution electron microscopy.

The development of the electron microscope as a practical tool for the study of biological structures far smaller than could be resolved by the light microscope ushered in a new era in cytology or "ultrastructure" research. No longer was it necessary to rely on indirect methods to deduce the structure of "submicroscopic" objects. By the late 1930's it became clear that resolutions of the order of 10 Å would be feasible. Though World War II retarded development in this field, electron microscopes practical for use by non-physicists were manufactured in fairly large quantities by the mid-forties. The degree to which these technical developments have now been pushed in many countries, with the productions of a gratifying array of types of instruments, is graphically illustrated in the exhibits at this Congress. Until methods of obtaining ultrathin sections were developed in the 1940's, most of the relatively high resolution electron microscopy of biological materials was performed upon fragmented specimens, such as biological fibers and other macromolecular preparations, deposited as dispersed particulates on the grid film. Since biological materials are composed largely of atoms of low atomic number, it became necessary to develop "electron stains", i.e., compounds which combine specifically with the biological materials and which contain elements of high scattering power. The heteropolyacids, such as phosphotungstic acid, as well as uranyl salts, and osmium tetroxide, were found valuable for such staining. Heavy metal evaporation, to produce shadows, was another significant milestone in these early developments. The use of plastics for embedding fixed tissues and the mechanical improvements of microtomes, which permit reliable sectioning to as thin as 100 Å, opened the door for biological application generally. Within the span of one decade the application of electron microscopes, capable of reliable and reproducible resolutions of 10 to 15 Å and sufficiently simplified for use by biologists, revolutionized cytology.

II. Electron microscopy and the new golden age of protoplasmic ultrastructure research

As progress in cytology was accelerated a century ago when methods of fixation and staining were first invented, so in the mid-twentieth century, particularly after the importance of main-

taining appropriate p_H of the fixative became appreciated, startling new discoveries revealed the structure of cellular organelles, especially those whose constituent molecules are so interbonded as to render them insoluble and stable after fixation, embedding and sectioning. A significant difference exists, however, between these investigations of cellular ultrastructure and those made in the last century by the light-microscope cytologists. The chemical nature and probable function of the structures observed with the light microscope had to be inferred from the rudimentary histochemical criteria then available and by attempting to arrange the structures in a meaningful, functional sequence from comparative studies and from their apparent behavior in different phases of physiological processes, such as secretion and contraction. Some twenty years ago it was discovered that cells could be macerated and, by appropriate methods of fractionation and differential centrifugation, relatively pure fractions of individual organelles, such as mitochondria and microsomes, could be obtained. The isolated organelles were therefore available for direct chemical investigation. By these methods it was shown that mitochondria are the site of oxidative phosphorylation and electron transport, leading to the formation of "energy rich" compounds, such as adenosine triphosphate (ATP), which are the biochemical fuel of the cell. Mitochondria thus became recognized as the "power plants" of the cell. By detailed biochemical investigation of mitochondria and their fragmented membranes *in vitro*, some deductions may be made about the way in which the enzyme assemblies are arranged within the planes of the lipoprotein membranes, so as to carry out the coordinated reactions of the citric acid (Krebs) cycle [see (*49*)]. From comparative studies and other indirect evidence the cytologists of a generation ago had concluded that the function of the mitochondria must be respiratory; this is now verified by the biochemical studies mentioned.

In the microsome fraction, containing fragmented bits of the intracytoplasmic membrane systems (endoplasmic reticulum, ergastoplasm), biochemists have demonstrated a system which, when associated with particles rich in ribonucleic acid ("ribosomes"), constitutes the mechanism by which important biomolecular compounds, such as proteins and steroids, are biosynthesized. The early light-microscopists realized that these regions in the cell, being basophilic, must contain an organic acid. This acid was proved by CASPERSSON (*10*) and by subsequent investigators to be nucleic acid. Moreover, since the basophilic material, as in the Nissl substance of nerve cells and in the basal area of secreting gland cells, occurs in the regions of active secretion or metabolism, it was concluded by the light microscopists that the basophilic material must be involved in biosynthesis.

This new knowledge from fractionated and purified organelles, or particulates derived therefrom, has made the current developments in the electron microscopy of cells interesting to biochemists; their active collaboration will doubtless help descriptive morphologists avoid misinterpretation based on fixation and other microtechnical artifacts.

Similar considerations apply also to cytological and histological studies of tissues, the major macromolecular constituents of which have been isolated and subjected to detailed biochemical and physicochemical studies. Thus mechanical properties as manifested by connective tissue are coming to be much better understood by virtue of investigations of isolated collagen macromolecules; interpretation of muscle has been enormously facilitated by physicochemical studies on isolated fibrous proteins of muscle; vast strides have been made in an understanding of the genetic mechanisms through a study of purified DNA.

The new discoveries in the biochemistry and physiology of cellular organelles place in new perspective the early cytologists' speculations about the physical nature of life and subcellular "living" entities. Though the membrane-limited structures (endoplasmic reticulum) of cytoplasm are not the vital units postulated by the nineteenth-century proponents of the membrane theories, they are, nevertheless, essential constituents of the biosynthetic mechanism without which life in the cell would be impossible. Similarly, the vital granule ("bioplast") theory of ALTMANN is now superseded; though the granules (mitochondria) are essential as generators of the biochemical fuel of the cell, they are not themselves "living" structures. Life requires the complex functional and structural organization of the types of these subcellular constituents, each of which subserves a specialized role vital to the cell function.