PROGRESS REPORT

NASA Grant NGL 14-001-012

for the period:
January 1, 1968 through
August 31, 1969

H. Fernandez-Moran, M.D., Ph.D.
Department of Biophysics
The University of Chicago

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July 25, 1969

Miss Winnie M. Morgan
Technical Reports Officer
Office of Grants and Research Contracts
Code SC
National Aeronautics and Space Administration
Washington, D.C. 20546

Dear Miss Morgan:

We have completed and are sending, under separate cover, 10 copies of our Progress Report for NASA Grant NGL 14-001-012, covering the period from January 1, 1968 to August 31, 1969.

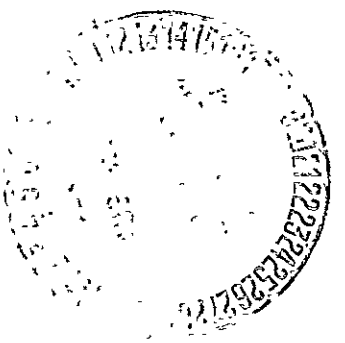
Your continued help in administering our grant is highly appreciated.

Kindest regards.

Sincerely yours,

H. Fernandez-Moran, M.D., Ph.D.
Professor of Biophysics

HFM/jr



PROGRESS REPORT

NASA Grant NGL 14-001-012

Title of Project: Investigations in Space-Related Molecular Biology Including Considerations of the Molecular Organization in Luster Sounding Rocket Program.

Period of Project: January 1, 1968 - August 31, 1969

Institution: The University of Chicago

Principal Investigator: Humberto Fernandez-Moran, M.D., Ph.D.
Professor of Biophysics
Department of Biophysics

Progress Report
NASA Grant NGL 14-001-012

Title of Project

Investigations in Space-Related Molecular Biology Including Considerations of the Molecular Organization in Luster Sounding Rocket Program.

Period of Project

January 1, 1968 - August 31, 1969

SUMMARY OF RESEARCH ACCOMPLISHMENTS

Significant developments in our research and training program in space-molecular biology, carried out at our facilities for high resolution electron microscopy include:

1. Continued investigation of particles obtained from the Luster Sounding Rocket Experiment and development of techniques for non-destructive analysis of extra-terrestrial matter. Our facilities offer a unique environment for precise examination of material from extraterrestrial regions, due to the following aspects:

    a. clean-room conditions.

    b. high voltage electron microscopy (with superconducting lenses at liquid helium temperatures), yielding:

        (1) increased penetration power
        (2) reduced radiation damage and specimen contamination
        (3) improved resolution and higher diffraction accuracy
        (4) ultra-high vacuum of 10^{-7} to 10^{-10} mm Hg
        (5) low temperatures of 20 to 1.85°K, provided by closed cycle superfluid helium refrigerator.

    c. advanced preparation techniques, including diamond knife with ultramicrotome for thin sectioning.

2. Continued development of high-resolution electron-optical information storage and retrieval, including:

    a. methods for ultraminiaturization and information

storage and retrieval at the molecular and near-atomic level.

b. instrumentation for a portable high resolution information retrieval system uniquely adapted to lunar and planetary missions.

3. Development of cryo- and high voltage electron microscopy with superconducting lenses and closed cycle liquid helium refrigerator, using improved instrumentation and preparation techniques, resulting in attainment of 2-3Å point-to-point resolutions and imminence of direct readout of molecular structures.

4. Collaborative electron microscopic studies of DNA conformations associated with membranes in chloroplasts.

5. Collaborative investigations, yielding a preliminary electron microscopic characterization of a highly purified hemagglutinin from the hemolymph of Limulus polyphemus.

6. Collaborative studies on RNA polymerase to determine its participation in the differential RNA transcription upon DNA templates and better understand membrane biosynthesis via the association of nucleic acids and the protein synthetic machinery with cell membranes.

7. Extension and verification of our early concept of organized water as an integral structural component of biological systems by other investigators.

Progress Report
NASA Grant NGL 14-001-012

Principal Investigator	Grant Number
Dr. Humberto Fernandez-Moran	NGL 14-001-012
Institution	**Period of Project**
The University of Chicago	January 1, 1968 - August 31, 1969

Title of Project

Investigations in Space-Related Molecular Biology Including Considerations of the Molecular Organization in Luster Sounding Rocket Program.

Our activities during this grant period were carried out according to the program set forth in our renewal proposal and include:

I. Specific Research Program.

A. Continued Participation in the Luster Project and Related Projects for Electron Microscopic and Electron Optical Analysis and Identification of Extraterrestrial Matter:

As described in previous reports, we are continuing our investigations on particles obtained from the Luster Sounding Rocket Experiment (during the Leonid Meteor Shower of November 16, 1965 and the Orionid Meteor Shower of October 27, 1966) with particular emphasis on improving and refining non-destructive techniques for collecting and identifying extraterrestrial matter.

The sophisticated instrumentation available in our clean-room laboratories, (including diamond knife with ultramicrotome for thin sectioning, and high-voltage, low-temperature electron microscopy, using closed cycle liquid helium refrigerator system--which yields increased penetration power

and reduced radiation damage), is now providing more precise information in the comparative analysis of our Luster particles and the three types of micrometeorite particles described earlier by Hemenway and Soberman. (See photographs.)

Because our facilities comprise a unique academic and physical environment (suitable for maintaining ultra-high vacuum, 10^{-7} to 10^{-10} mm Hg, and low temperatures, 20°to 1.85°K, characteristic of the lunar surface), it is our privilege and hope to continue to attract interested investigators from local and international institutions for examining material from extraterrestrial regions, including the moon.

B. High-Resolution Electron-Optical Information Storage and Retrieval:

1. Methods for ultraminiaturization and information storage and retrieval at the molecular and near-atomic level are being further developed.

Pursuit of this technique could result in the potential recording of several million books on a single page for read-out with a miniature electron microscope and television display.

Integrated circuits and computer elements of submicron sizes could also be produced by related electron optical techniques.

In the optimal projection of this concept, one can foresee integrated ultramicroelectric circuits, near the size of macromolecular assemblies, being incorporated into key junctional sites of living nerve membranes without causing serious perturbation.

These "submicroscopic prosthetic sensors," whose envelopes would be composed of biosynthetically produced protein coats so as to form integral components of the nervous system, could be of unique service. Because they could be produced in large number and inserted throughout the central

nervous system, the sensors could effect a direct operational link at the macromolecular level between the central nervous system and man-made information processing systems, such as computers of commensurate complexity.

2. We have recently perfected the instrumentation for a portable high resolution information retrieval system uniquely adapted to lunar and planetary missions.

A portable pocket-sized microprojector, tentatively designated "Pico-Pathfinder," has been developed, constructed, and tested in our laboratories. The unit weighs less than 1 pound and embodies its own electrical power supply, i.e., a one-dollar flashlight. (See photographs.)

The instrument should permit accurate high-contrast projection images of demagnified moon maps and related information onto any suitable surface. Since this system will work reliably in a high-vacuum, low-temperature environment characteristic of the lunar surface, it could serve as a vital aid in orientation and localization of selected areas in the exploratory phases of the Apollo mission.

Moreover, the combination of ultraminiaturized information storage and retrieval using highly refractory thin film substrates of novel design can be used to selectively transmit critical information from lunar vehicles and space platforms back to Earth in an unprecedented from (e.g., space courier modules), well beyond present telemetering techniques, under conditions of minimized contamination hazards. (See ref. Potential Application of Electron-Optical Methods to Storage of Information for Direct Retrieval, in Biology and the Exploration of Mars, 1966, p. 503.)

C. Development of Cryo- and High Voltage Electron Microscopy with Superconducting Lenses and Closed Cycle Liquid Helium Refrigerator, Using Improved Instrumentation and Preparation Techniques, Resulting in Attainment of 2-3Å Point-to-Point Resolutions and Imminence of Direct Readout of Molecular Structures:

1. Improved point cathode sources with single-crystal filaments and a new type of molybdenum gun

are used to provide stable coherent microbeam illumination of high brightness, small spot-size, and low energy spread.

2. High resolution phase contrast imaging is carried out with zone plate apertures, enhanced wish short focal length objective lenses to resolve structures of 5-10Å in unstained biological molecules.

3. A 200 kV high voltage electron microscope (Hitachi Perkin-Elmer HU-200E) was successfully installed in our laboratories under optimum environmental conditions.

The increased penetration power, reduction in radiation damage, and improved resolution available with this instrumentation are particularly valuable for examining extraterrestrial particles not suitable for conventional electron microscopy.

Resolutions of 3.6 to 6.0Å were obtained in crystalline lattices and 4Å point resolutions in 250-350Å-thick biological specimens. High resolution electron diffraction studies carried out with the 200 kV microscope yielded a remarkable 50 to 100 diffractions for biological specimens as compared with the typical 5 to 10 diffractions obtained from lower voltage microscopes. (See photographs.)

4. The development of cryo-electron microscopes operating with high-field superconducting solenoid lenses at liquid helium temperatures represented one of the most significant advances, providing superstable lenses, ultrahigh vacuum, minimized specimen damage, contamination, and thermal noise, and enhanced image contrast. Earlier observations of biological specimens under these conditions revealed new electron optical phenomena.

In order to pursue the promising superconducting work on a systematic and more practical basis, we have installed and adapted to our facility a closed cycle liquid helium refrigerator system specially designed for our laboratories in collaboration with Prof. Samuel Collins of

A.D. Little, Inc.. Prior to shipment, the system was tested out successfully at 1.85°K with 10 watts refrigeration capacity. During the extensive tests which are being carried out on this equipment, we recently produced liquid helium at 1.82°K, maintaining superfluid for one hour, with potential for three hours. When the final testing is completed, we will be able to operate continuously in this range of 1.85° to 2.0°K, with special 36-foot transfer lines.

The superfluid helium produced at these temperatures is not only critical for research work with superconducting lenses, but also provides ultrahigh vacuum and improved specimen preservation.

The work that we are now carrying out with this closed cycle liquid helium refrigerator system will provide experience which will prove to be of great value to all other workers in this field both in the United States and abroad. If successful, it will break the present serious economic barrier which has prevented many other laboratories from using this uniquely important tool. They will then be in a position, with expenditures that are not much greater than those of a commercial electron microscope, to produce liquid helium and work with it without loss of this precious natural resource.

5. We are now equipped with a unique opportunity to apply simultaneously the advantages of superconducting microscopy and high voltage electron microscopy to biological and exobiological investigations. The new installations have provided us with the only existing high voltage electron microscope with superconducting lenses operating at closed-cycle liquid helium temperatures.

During preliminary experiments with this facility, we discovered an anomalous transparency effect for 200,000 volt electrons in thick (ca. 1000-2000Å) lead and niobium films at liquid helium temperatures. This remarkable effect extends previous observations by Boersch and our own group at 30-75 kV. It poses interesting theoretical and experimental questions,

particularly since it is coupled with a marked decrease in radiation damage in both organic and inorganic specimens. See photographs.

6. A unique and promising project of Dr. Robert Nathan (Jet Propulsion Laboratory, California Institute of Technology), as reported in his paper "Computer Enhancement of Electron Micrographs," combines the digital computer and associated video camera scanner equipment with the electron microscope to enhance electron micrographs and strive for atomic resolution in bio-organic compounds.

The experiments have been carried out using a high resolution electron micrograph of negatively stained catalase, taken in our laboratories. See Supporting Material and photographs.

As stated in the paper, electron microscopes are now available which, through the use of highly stabilized lens currents and high voltage, can maintain an image stationary to 1Å or less for a period of many seconds, even though spherical aberration may still be present to the extent of limiting resolution to 4Å.

According to Dr. Nathan's technique, it is possible to recover from this stationary image 1Å resolution by computer transformation to the Fourier or spatial frequency domain. After the computer corrects for phase error, a transformation would be made back to the real plane to reconstruct a higher resolution image.

D. Electron microscopy studies of DNA conformations associated with membranes in chloroplasts were carried out with Dr. Christopher Woodcock and described in our paper, "Electron Microscopy of DNA Conformations in Spinach Chloroplasts," J. Mol. Biol., 1968, 31, 627-631. The paper reports the successful application of new techniques for the isolation and purification of nucleic acids on a micro-scale which enabled us to visualize quantities of DNA and RNA which are several orders of magnitude too small for density gradient analysis. We were able to detect by electron microscopy the DNA associated with each chloroplast, following

disruption by osmotic shock. DNA molecules extracted from purified chloroplasts were also analyzed. Examination of these preparations under controlled conditions has consistently revealed two major DNA conformations and a characteristic association with the chloroplast membrane system. See photographs.

E. In collaboration with Dr. Gerald Edelman and John Marchalonis of the Rockefeller University, a preliminary electron microscopic characterization has been carried out on a highly purified hemagglutinin from the hemolymph of the horseshoe crab, Limulus polyphemus.

The present studies indicate that the hemagglutinin is a ring-shaped structure with a diameter of 100Å and a central core of 20Å to 40Å. The height of the molecule was tentatively estimated to be 65Å and multiple views suggest the presence of 6-fold symmetry and subunit organization. The physical-chemical data are in good agreement with the preliminary results of the complementary studies of the hemagglutinin by high resolution electron microscopy.

Because of the uniformity of the hemagglutinin preparations and the availability of increased resolution, it is now feasible to attempt structural analysis of the subunits of this protein. In addition, the mode of attachment of the hemagglutinin to red cell membranes should be amenable to study by combined biochemical and electron microscopic techniques. See photographs.

F. We have participated in collaborative studies on RNA polymerase to determine its participation in the differential RNA transcription upon DNA templates and better understand membrane biosynthesis via the association of nucleic acids and the protein synthetic machinery with cell membranes.

Dr. Richard Gumport, under Dr. Samuel Weiss, of the Biochemistry Department, carried out in our laboratories electron microscopic investigations

on the binding of RNA polymerase to circular forms of DNA (e.g., from ØX 174), as part of his doctoral thesis work.

Resultant electron micrographs of Kleinschmidt preparations demonstrated characteristic molecular complexes derived from the reaction of this DNA with E. coli RNA polymerase upon addition of the four regular nucleoside triphosphates: ATP, CTP, GTP, UTP. See photographs.

G. A major aspect of our biological investigations has been the concept of organized water as an integral structural component of biological systems, particularly membranes.

A comprehensive survey of this subject was given in our paper, "Membrane Ultrastructure in Nerve Cells" (in The Neurosciences, G.D. Quarton, M. Melnechuk, and F. O. Schmitt, editors, Rockefeller University Press, New York, 1967, p. 281). Several mechanisms of membrane function were hypothesized in terms of ordered water lattices.

These ideas have now been extended and experimentally verified by investigators at the Astropower Laboratory, McDonnell Douglas Astronautics Company. Our postulated structural parameters have led to the formulation of a new model of the cellular plasma membrane and to the concept of polywater, as will be described in the following abstracts.

It is reported in the Douglas paper, entitled "Characterization of Ordered Water in Hydrophilic Membrane Pores," that ordered water, whose presence in biological membranes is often postulated, has been characterized in cellulose acetate and porous glass desalination membranes.

The authors found that above a certain critical diameter, each membrane pore is lined with a hydration sheath (ca. 22Å thick) of highly hydrogen-bonded water in which salt is essentially insoluble. In a pore with an ideal critical diameter of about 44Å, ordered water almost entirely fills it, having an average viscosity of 0.36 poise,

about 39 times greater than that of ordinary water at 23°C.

A rate process of the permeation of salt-free ordered water through an ideal desalination membrane can be calculated in good agreement with experimental data due to the simple assumption of approximate equality between hydration sheath thickness and the average diameter of a "flickering cluster."

The thickness (t) of the hydration sheath is about half the critical diameter (2t) of a pore that will reject 100% of salt from the pressurized feed water.

It was noted that a Loeb-Sourirajan cellulose acetate membrane, as cast, has an extremely high water permeation rate and little tendency to reject salt. Heat treatment in water at 80-90°C lowers the water permeation rate and drastically increases salt rejection.

Lateral shrinkage of only 11% in the membrane plane, after 15 minutes at 87°C, increases salt rejection from less than 10% for the "as cast" membrane to 95%. It follows that twice the average pore radius (r_p = 21.3Å) previously estimated should be nearly equal to the ideal critical pore diameter (2t) for desalination.

The authors calculated from the known volume of a water molecule (29.9Å^3) and from the value $t \simeq r_p \simeq 21.3$Å, that the number of molecules n_{cl} in the average cluster of water in a cellulose acetate membrane pore is about 162 at room temperature T = 296°K.

The authors found that the activation energy for viscous flow of water supercooled to 255°K, calculated by the empirical relationship of Miller, turns out

to be 6015 cal/mole, in excellent agreement with the asymptotic experimental value 5950 cal/mole reported by Keilen et al for permeation of water through a Loeb-Sourirajan type cellulose acetate desalination membrane of 53.5% water content and 99.5% salt rejection capability.

In comparison of theoretical and experimental water permeation rates through cellulose acetate desalination membranes, the authors found that for 1% NaCl feed at 296°K subjected to 1500 psi operating pressure (i.e., 1381 psi driving pressure), a rate of 28 gfd was calculated in reasonable agreement with a published value of 19.7 gfd for an ultrathin, 2400Å, cellulose acetate membrane. This rate corresponds to a 0.5 hour run in which 98% salt rejection was obtained and in which a 100Å millipore filter support (dull surface in contact with the membrane) may have reduced the water permeation to a value somewhat below theoretical.

It seems to the authors that the characteristics of ordered water in a porous glass desalination membrane are very similar to those in a cellulose acetate desalination membrane.

The ultrafiltration data presented, showing that salt is essentially insoluble in ordered water, presumably because its highly hydrogen-bonded structure cannot accommodate ions, suggests that ordered water in a cellular membrane could have a high electrical resistivity approaching the value 10^8 to 10^9 ohm-cm proposed for pure ice.

In the second Douglas paper, entitled "Ultrastructure of Hexagonal Subunits and Variable Aperture Pore Function in Plasma Membranes," the authors report that development of the concept advanced by Fernandez-Moran (i.e., that the selective permeability of nerve membranes might be envisaged in terms of molecular "pores" lined with highly ordered water, resembling "ice-like" hydration sheaths or crystalline hydrate lattices), was prevented by inadequate knowledge of the precise molecular nature of ordered water.

Such knowledge has now been extended by the McDonnell Douglas group as a result of their studies on the transport properties of ordered water in the pores of cellulose acetate and unfired Vycor glass desalination membranes.

Arrays of hexagonal subunits, ca. 90-95Å diameter, were revealed by high resolution electron microscopy in a black lipid membrane of the type in which Mueller and Rudin induced nerve-like electrical activity by addition of the circular polypeptide antibiotic, alamethicin.

The subunits appear to be related to those reported in junctional sites where adjacent membranes of living cells are known to be in electrical contact. Molecular models of the subunits strongly suggest that they act, in association with adsorbed allosteric proteins, as variable aperture pores in the cellular membrane. The arrangement and movement of cholesterol molecules in these models resemble those of the leaves of a between-the-lens shutter of a modern camera.

It was found that for optimum desalination, the membrane pores should be about 40-45Å in diameter, about twice the thickness, t, of the hydration sheath of salt-rejecting ordered water at the hydrophilic surface of the membrane. The Poiseuille flow of water through the membranes was measured and showed that the mean viscosity of this ordered water is about 37-40 times greater than that of ordinary bulk water at room temperature.

"The insolubility of salt in ordered water suggests that its electrical conductivity might be very low approaching the value 10^{-8} to 10^{-9} ohm^{-1} cm^{-1}, proposed for pure ice."

One of our micrographs of ultrathin submicroscopic nerve fiber ending served as reference to suggest the presence of unordered water channel plus surrounding hydration sheath as components of an electron dense membrane. This result led to their pursuit of similar components in an ultrathin black lipid membrane of the kind that has been proposed as

a prototype of the plasma membrane of cells and the membraneous elements of mitochondria, nuclei, chromosomes, endoplasmic reticulum, Golgi apparatus, and chloroplasts.

Electron microscopic evidence is given that a black lipid membrane, with protein adsorbate, can have an hexagonal structure resembling the hexagonal liquid-crystalline phase discovered by Luzzatti and Husson in a brain phospholipid-water system.

In resting configuration the hexagonal subunit of an axon membrane is postulated to have a pore of unordered water of diameter x≈4.0Å. This is shown in their figure of molecular model of lipid water components of an hexagonal subunit in a plasma membrane. The figure shows how a slight angular displacement of each cholesterol "shutter leave," each moving cooperatively with its neighbor, could increase the radius of the central pore of unordered water from 4.0Å in resting configuration to the "equivalent pore diameter" of ~8.4Å, cited earlier for the squid axon. During this configurational change the ordered water sheath thickness, t, would remain nearly constant at ~22Å and each phospholipid complex would unbend slightly about a natural bending point situated near the outer end of the cholesterol leaf.

The authors discuss the Hodgkin and Huxley nerve excitation theory based on the postulate of two types of specific pores or ion-gates, one for sodium and one for potassium. They indicate that his theory is now challenged, in particular by Watanabe, Tasaki and Lerman, who obtained normal-like action potentials with normal polarity from isolated squid giant axons perfused internally with dilute (e.g., 30 mM) sodium phosphate solutions and externally with 100 mM calcium chloride. They noted that no potassium was present and that the sodium ion gradient was opposite to that found normally, concluding that the data are inconsistent with the Hodgkin-Huxley theory that initiation of an action potential is the consequence of a membrane permeability increase "specific" to sodium ions. The molecular model of the hexagonal subunit developed by the authors,

however, makes it possible to resolve this apparent discrepancy while still preserving the essentials of the Hodgkin-Huxley theory.

A clue to the nature of the drive required to turn the cholesterol leaves cooperatively in a manner that opens and closes the central pore in an axon plasma membrane is given by the discovery of Mueller and Rudin that the antibiotic alamethicin induces nerve-like electrical behavior when added to one side of a black lipid membrane (e.g., lecithin-decane-squalene) separating two aqueous solutions. The alamethicin-treated membranes exhibit increased cation conductance and action potentials on electrical stimulation. The steady state conductance increases as the sixth power of both the alamethicin concentration and the cation concentration and also as the sixth power of the potential across the membrane (or the sixth power of the positive charge on the alamethicin side of the membrane). The authors indicate from this data that the reaction involved in development of the conductance is a highly cooperative phenomenon involving six alamethicin molecules and six cations. Mueller and Rudin suggested that this phenomenon might be allosteric in nature, involving intramolecular chelate bond configurations, and these authors further develop this concept in terms of the hexagonal subunit model.

In an actual cellular plasma membrane, the molecule that provides the drive for the ion gate is probably much larger than alamethicin. Mueller and colleagues have discovered a proteinaceous excitability inducing material (EIM) of cellular origin, that lowers the electrical resistance of black lipid membranes, develops resting membrane potentials in ionic gradients and produces nerve-like electrokinetic phenomena.

A high resolution electron micrograph of bovine L-glutamate dehydrogenase (GDH), taken in our laboratories, served in the authors' studies on black lipid membrane as an approximation of the arrangement of the excitability inducing material molecules in an ion-gate. This enzyme has a M.W. of 310,000

corresponding to an oligomer which may contain six identical subunits and six active sites. The authors' figure shows a circular aggregate of GDH containing more than six subunits but it appears that each GDH subunit might be ~30Å in diameter and that about six of the GDH subunits surround the central void of the aggregate to form an oligomeric ring or rosette ~50Å i.d. and ~105Å o.d., that would just fit over the lipid ring of the plasma membrane hexagonal subunit shown in their previous figure 4.

Recent experimental observations indicate allosteric cooperative interaction between the subunits of the GDH oligomer. Such interaction would be consistent with a postulated role of the oligomer as one specialized type of mechanical drive of an hexagonal subunit of a nerve membrane. Interaction of the GDH oligomer with glutamate and NAD coenzyme substrate could allosterically open the hexagonal subunit causing a localized depolarization of the membrane; release of product α-ketoglutarate and NADH could close the subunit.

Such a transient electrical behavior has been observed for ox brain black lipid films treated with GDH; addition of substrates causes a transient decrease in membrane impedance, which effect can be demonstrated repeatedly. This effect is of great interest "because of the role postulated for glutamate as an excitatory transmitter substance in the mammalian nervous system and because of the relationship, by decarboxylation, of glutamate to gamma-aminobutyric acid, GABA, which appears to function as an inhibitory transmitter substance. A similar effect was obtained with acetylcholine esterase and acetylcholine, a known excitatory transmitter substance."

The authors discuss how a rhodopsin molecule and about five EIM molecules, adsorbed on the lipid ring, might act as an allosteric shutter relase and mechanical drive for a hexagonal ion gate in the unit disk of a vertebrate photoreceptor membrane. The EIM molecules undergo a cooperative allosteric transition that turns the underlying cholesterol "shutter leaves" in an angular direction that increases the aperture of the ion-conducting unordered

water pore. To effect this overall change, the rhodopsin molecule must also undergo an analogous allosteric transition in synchrony with the EIM molecules. However, this transition, and therefore the opening of the ion gate, is blocked by the bent and twisted form of the 11-cis retinal isomer. Adsorption of only one photon converts this chromophore to the straight all-trans isomer, thereby removing the steric hindrance to the overall configuration change that opens the ion gate and triggers the electrical activity of the retinal rod.

Additional recent references support the concept of ordered water in biological systems.

For example, it is reported in "Polywater," by Lippincott et al from Science, Vol. 164, p. 1482, June 27, 1969, that the infrared and Raman spectra of a form of water prepared in fused quartz capillaries and previously designated as anomalous water have been obtained. According to interpretation of the new data, new and previously unreported strong symmetric O-H-O bonds are formed, isoelectronic with FHF^-. These bonds are regarded as responsible for the remarkable properties of the material and have considerable covalent character. They are so strong that they cannot be considered as normal O···H hydrogen bonds.

Several structures are proposed which are consistent with the spectral data and the remarkable properties and stability of the material. It is concluded that the material is a true polymer of water, and, therefore, is named polywater.

Another paper, entitled "Icelike Cell Water Shakes Biotheories" in Scientific Research, June 23, 1969, p.9, reports that researchers at the U.S. Naval Air Development Center in Johnsville, Pa. and at the Texas Children's Hospital of the Baylor University College of Medicine in Houston discovered that most of the water in muscle and brain tissue is not in its familiar liquid state, but instead appears to possess a highly ordered structure that can best be described as semicrystalline, or ice-like.

Using nuclear magnetic resonance spectroscopy to study biological water, their studies indicate that tissue water has a significantly greater degree of crystallinity than liquid water. If these findings are confirmed, they may revolutionize old theories of how ions are transported across membranes and how electrical impulses are transmitted along nerve fibers.

They further report that the conventional theory of membrane transport has led to a contradiction which can be avoided if tissue water is treated as a solid-state system.

II. Training Program:

In addition to specific research programs, the laboratory has served as a center for information exchange to investigators and students in the United States and other countries. Events during the past grant period have included:

A. Visiting Scientists to learn electron microscopic techniques and/or consultation on research projects of mutual interest:

1. Dr. Hermann Träuble, Max Planck Institute, Gottingen, West Germany, Summer, 1968.

2. Dr. Fred Gamble, Stanford University, Palo Alto, California, February, 1968.

3. Dr. Hans Schier, Sprague Electric Company, North Adams, Massachusetts, Spring, 1968.

4. Dr. Richard Gumport, University of Chicago, 1967-1968, consultation on doctoral thesis.

5. Mr. John Wooley, Graduate Student, University of Chicago, 1968-1969.

B. Lectures Presented by H. Fernandez-Moran:

1. "Physics of Nerve and Synaptic Actions," invited lecture at the Semi-Annual Symposium, American Physical Society, New York State Section, Syracuse University, March 30, 1968.

2. "High Resolution-Low-Temperature Electron Microscopy," Guest Lecture before Sigma Xi Chapter, Iowa State University, April 1, 1968.

3. "Humanity and Science at the Crossroads," keynote address for the IR-100 Awards Banquet, sponsored by INDUSTRIAL RESEARCH during the National Conference on Industrial Research, Conrad Hilton, New York, October 4, 1968.

4. "High Resolution Electron Microscopy Applied to the Study of Nerve Membranes, presented at the Neurosciences Research Program Work Session, February 9-11, 1969, M.I.T., Boston, Mass.

5. George A. Miller Visiting Lecturer, University of Illinois, Champaign-Urbana, Illinois. a. "Recent Advances in High Resolution Electron Microscopy"; b. "Electron Microscopy at Liquid Helium Temperatures"; c. "The Role of the University of the Evolution of Latin America"; d. "Molecular Organization of Cell Membranes," March 24-27, 1969.

6. Presentation before NASA Congressional Hearing, March 31, 1969, Washington, D.C.

7. "Three Decades of Membrane Research," banquet of the Symposium on Ion and Water Transport Through Biological Membranes, co-sponsored by the Office of Saline Water and the Albert Einstein College of Medicine of Yeshiva University, June 17, 1969.

8. "High Voltage, Low Temperature Electron Microscopy," presented at the International Conference on Current Developments in High Voltage Electron Microscopy, United States Steel Corp., Monroeville, Penn. June 17-19, 1969.

9. Presentation before NIH Advisory Council, June 20, 1969, Washington, D.C.

C. Professorship Awarded H. Fernandez-Moran:

Dr. H. Fernandez-Moran was invited to become the A.N. Pritzker Professor of Biophysics in the Division of Biological Sciences and Pritzker School of Medicine, October, 1968.

III. Publications and Supporting Material for the Period January 1, 1968 through August 31, 1969.

A. Publications:

1. H. Fernandez-Moran, J.J. Marchalonis, G.M. Edelman, "Electron Microscopy of a Hemagglutinin from *Limulus polyphemus*, J. Mol. Biol., 1968, Vol. 32, 467-469.

2. C.L.F. Woodcock and H. Fernandez-Moran, Electron Microscopy of DNA Conformations in Spinach Chloroplasts," J. Mol. Biol., 1968, Vol. 31, 627-631.

3. H. Fernandez-Moran, The World of Inner Space, Science Year 1968, The World Book Encyclopedia Science Annual, p. 216.

4. H. Fernandez-Moran, Humanity and Science at the Crossroads, keynote address presented at the banquet of the National Conference on Industrial Research and I-R 100 Awards Program, October 4, 1968.

5. a. H. Fernandez-Moran, The Electron Microscope-Part 1: Toward the Center of Life, Laboratory Management, January, 1969.
   b. H. Fernandez-Moran, The Electron Microscope-Part 2: Big Future for Demagnification, Ibid.

6. H. Fernandez-Moran, High Resolution Electron Microscopy Applied to the Study of Nerve Membranes, presented at the Neurosciences Research Program Work Session, February 9-11, 1969, M.I.T., Boston, Mass. To be published in the NRP Bulletin.

B. Supporting Material:

1. Scientific Research, January 8, 1969, article about H. Fernandez-Moran.

2. "Meet U. of C's Renaissance Man," Chicago Sun Times, July 11, 1968.

3. R.D. Schultz, S.K. Asunmaa, F.D. Kleist, Ultrastructure and Variable Aperture Pore Function of Hexagonal Subunits in Plasma Membranes, Douglas Paper 10,246, March 1969, McDonnell Douglas Corp., to be presented to the Second International Meeting, International Society for Neurochemistry, Milan, Italy, September, 1969.

4. R.D. Schultz, S.K. Asunmaa, G.A. Guter, F.E. Littman, Characterization of Ordered Water in Hydrophilic Membrane Pores, Douglas Paper 10,247, March, 1969, Ibid.

5. H. Fernandez-Moran, The World of Inner Space, The University of Chicago Magazine, Vol. LXI, No. 5, March/April, 1969 (reprinted from Science Year, 1968, The World Book Encyclopedia).

6. University of Chicago; Office of Public Information, Press Release, December 3, 1968.

7. H. Fernandez-Moran, Die Erforschung der Innenwelt des Menschen mit Hilfe des Elektronenmikroskopes, Med. Welt, 20 N.V., 1969, 16.

8. Robert Nathan, Computer Enhancement of Electron Micrographs, Jet Propulsion Laboratory, California Institute of Technology.

Progress Report
NASA Grant NGL 14-001-012

Acknowledgements

To my colleagues: Prof. S. Bennett, Dept. of Anatomy; Prof. F. Schmitt, Neurosciences Research Program, M.I.T.; Dr. W. Sweet, Mass. Gen. Hosp.; Prof. S. Weiss, Dept. Biochemistry; Prof. A. Crewe, Dept. Physics, Dr. T. Halpern, Argonne Nat'l. Lab.; Dr. H. Träuble, Max Planck Institute, Gottingen; Prof. L. Meyer and Dr. R. Szara, Low-Temperature Labs; and Dr. M. Neumann, Dept. Physics, I am indebted for their encouragement, generous collaboration, and discussions.

We are especially grateful to Prof. Samuel Collins of A.D. Little, Inc. for design of our closed cycle superfluid helium refrigerator and for his continued interest. Special thanks are also due his colleagues, Mr. Richard Osburn and Mr. Milton Streeter for their essential contribution in the testing and development stages of this project.

We are greatly obliged to our staff members for their continued help: in particular, we thank Mr. C. Hough for valuable help in high resolution, light optical demagnification development work, Mr. M. Ohtsuki for expert high voltage and high resolution electron microscopy, Mr. H. Komori for valuable help in electron microscopic examination of Luster specimens, and Mr. C. Weber and Mr. G. Bowie for photographic reproduction work.

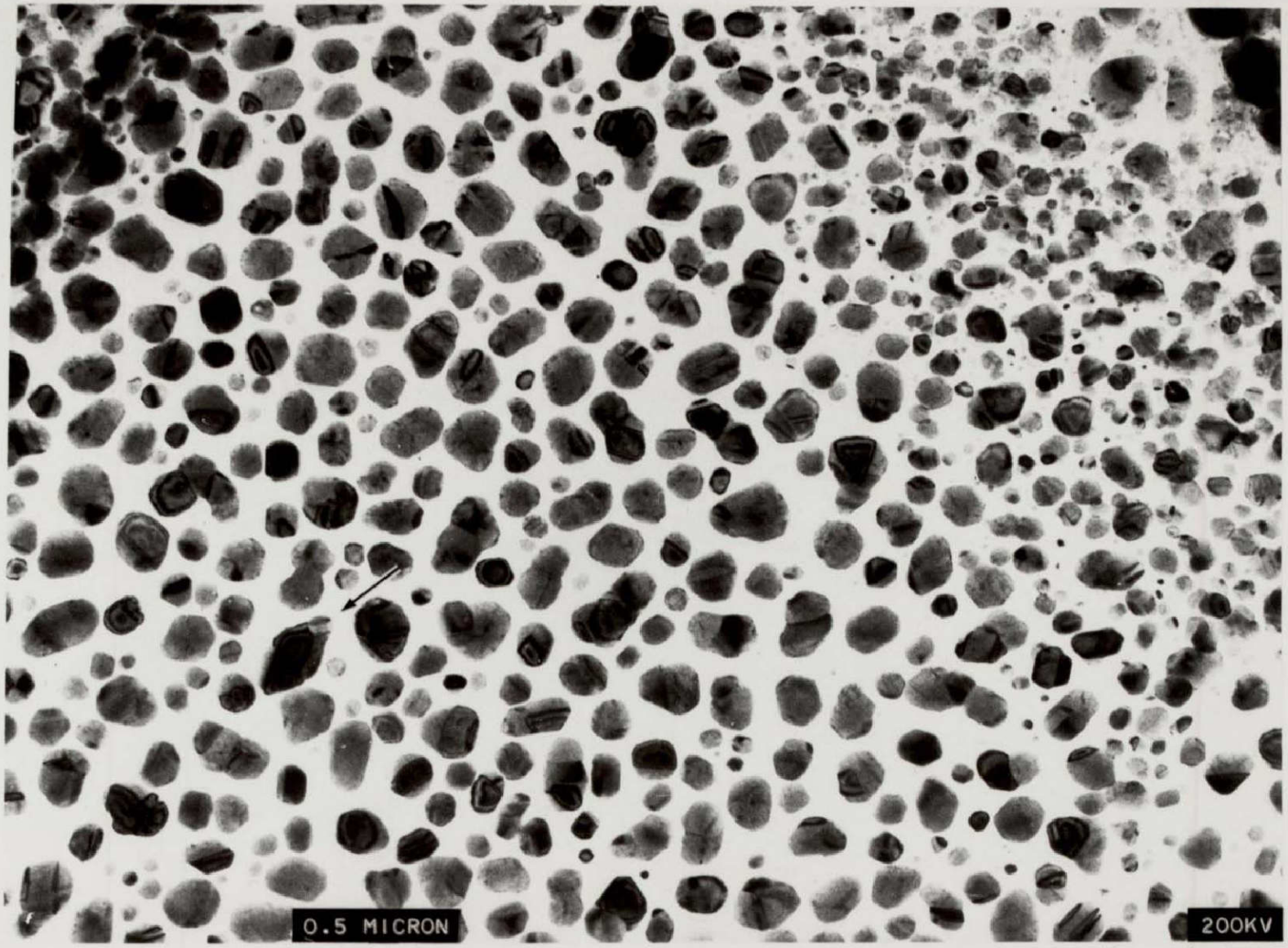
We are also indebted to Mr. R. Vicario for valuable help in the development and engineering aspects of our program, as well as to Mr. H. Krebs, Mr. J. Costa, Mr. H. Akerhaugen, and other members of the Central Development Shop for valuable assistance in the course of our work.

We also wish to thank Mrs. V. Iglesias, Miss A. Hibino, and Miss M. Hanaoka for technical assistance, specimen preparation and maintenance of the clean-room laboratory.

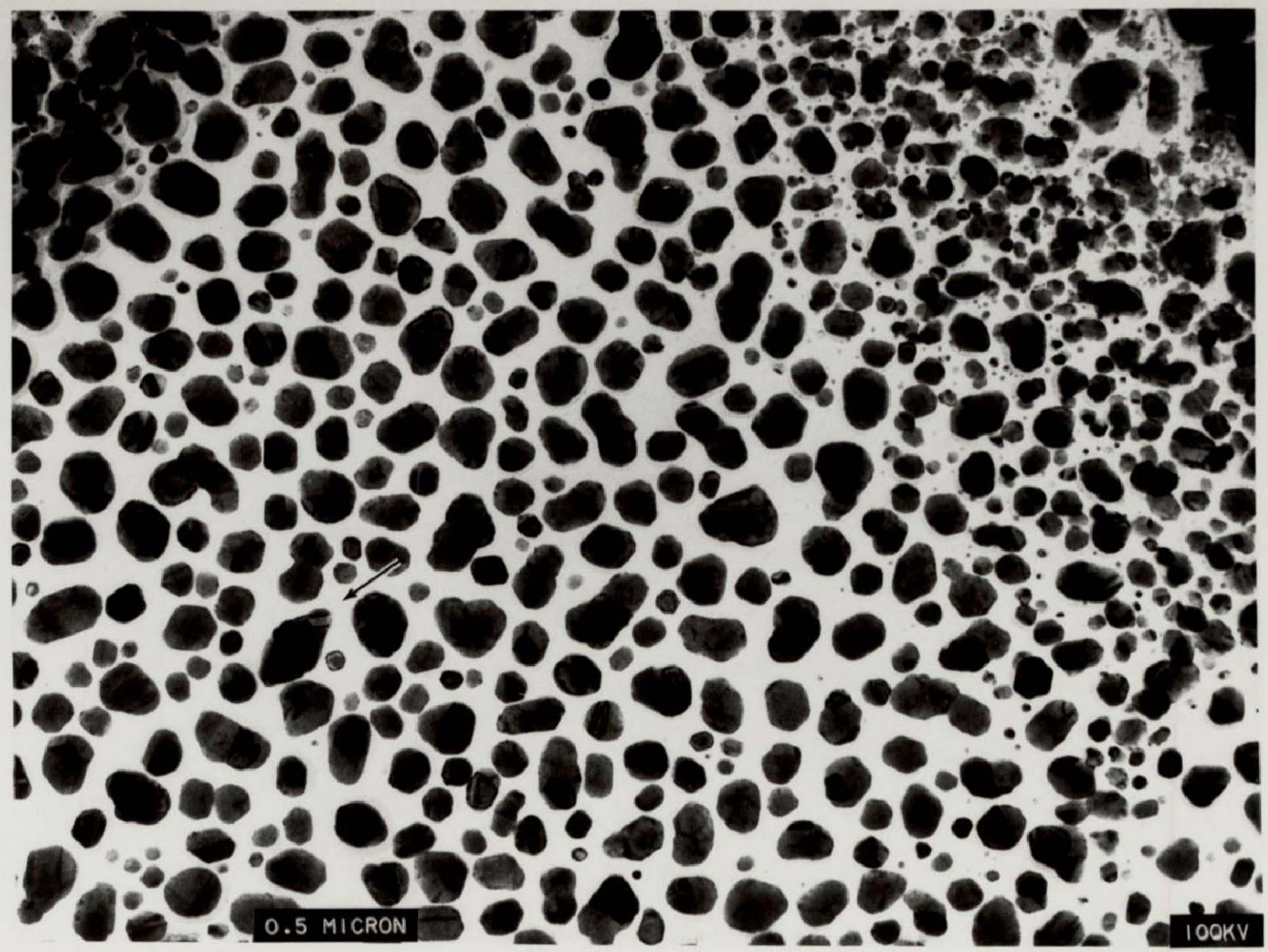
Special thanks to Miss J. Richardson for scientific writing, to Mrs. S. Erikson, Miss C. Benitez, and Mrs. G. Kilgore for valuable administrative and clerical assistance.

Our sincere appreciation is expressed to Dr. George Jacobs, Bioscience Programs, NASA, for his counsel and far-sighted support.

Our work was partially supported by the Pritzker Fund, the L. Block Fund, and the Otho Sprague Memorial Fund of the University of Chicago and by Grant GM 13243 from the National Institutes of Health, General Medical Sciences, in addition to the generous support from the National Aeronautics and Space Administration.



HIGH VOLTAGE HU 200KV ELECTRON MICROGRAPH OF SUBMICROSCOPIC MICROMETEORITE PARTICLES FROM ORIONID SHOWER 1966/69



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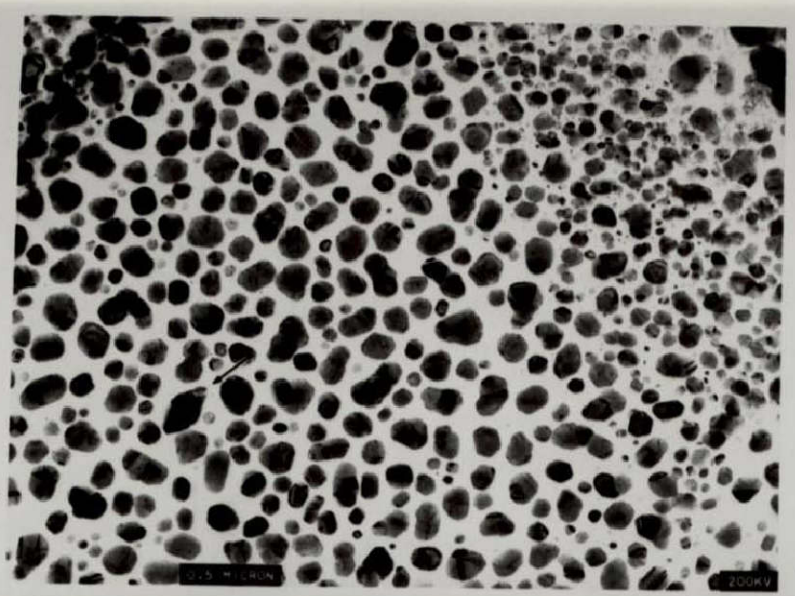

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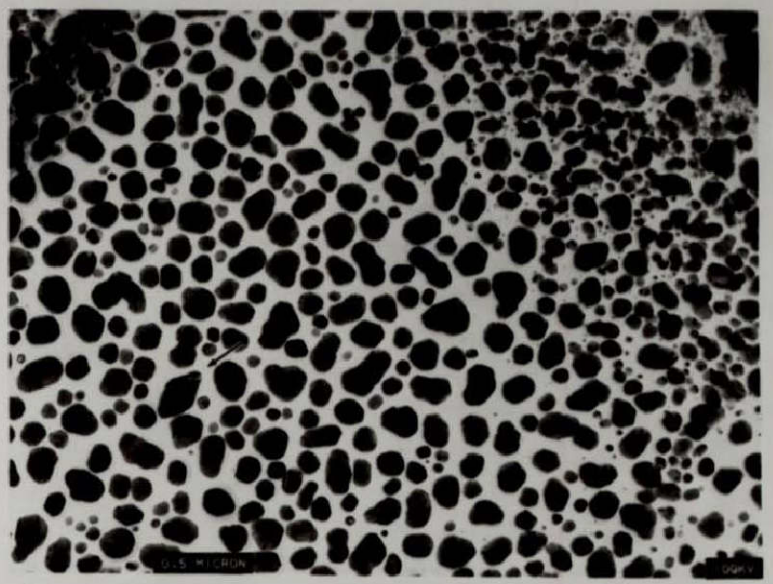

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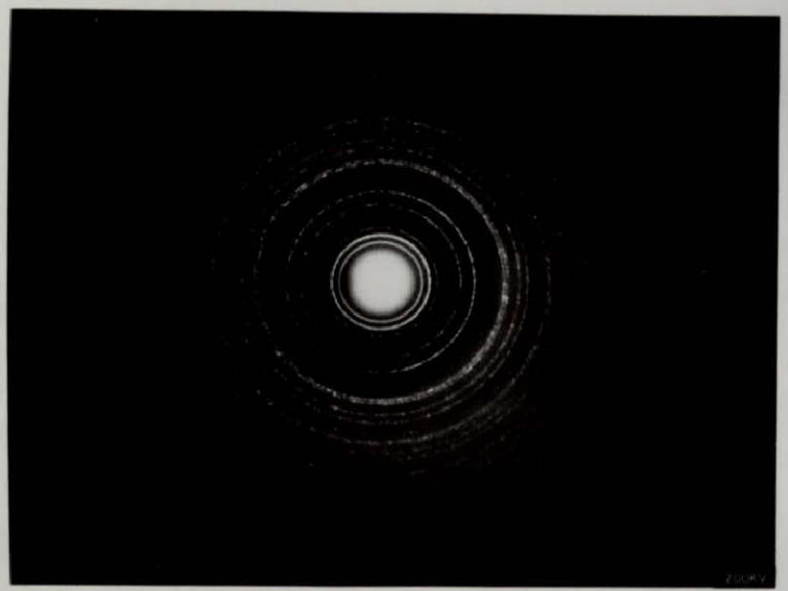
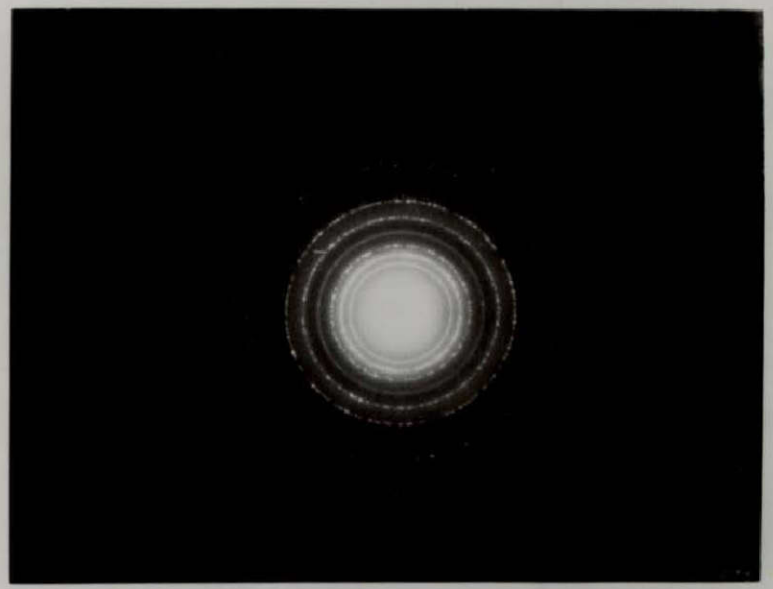
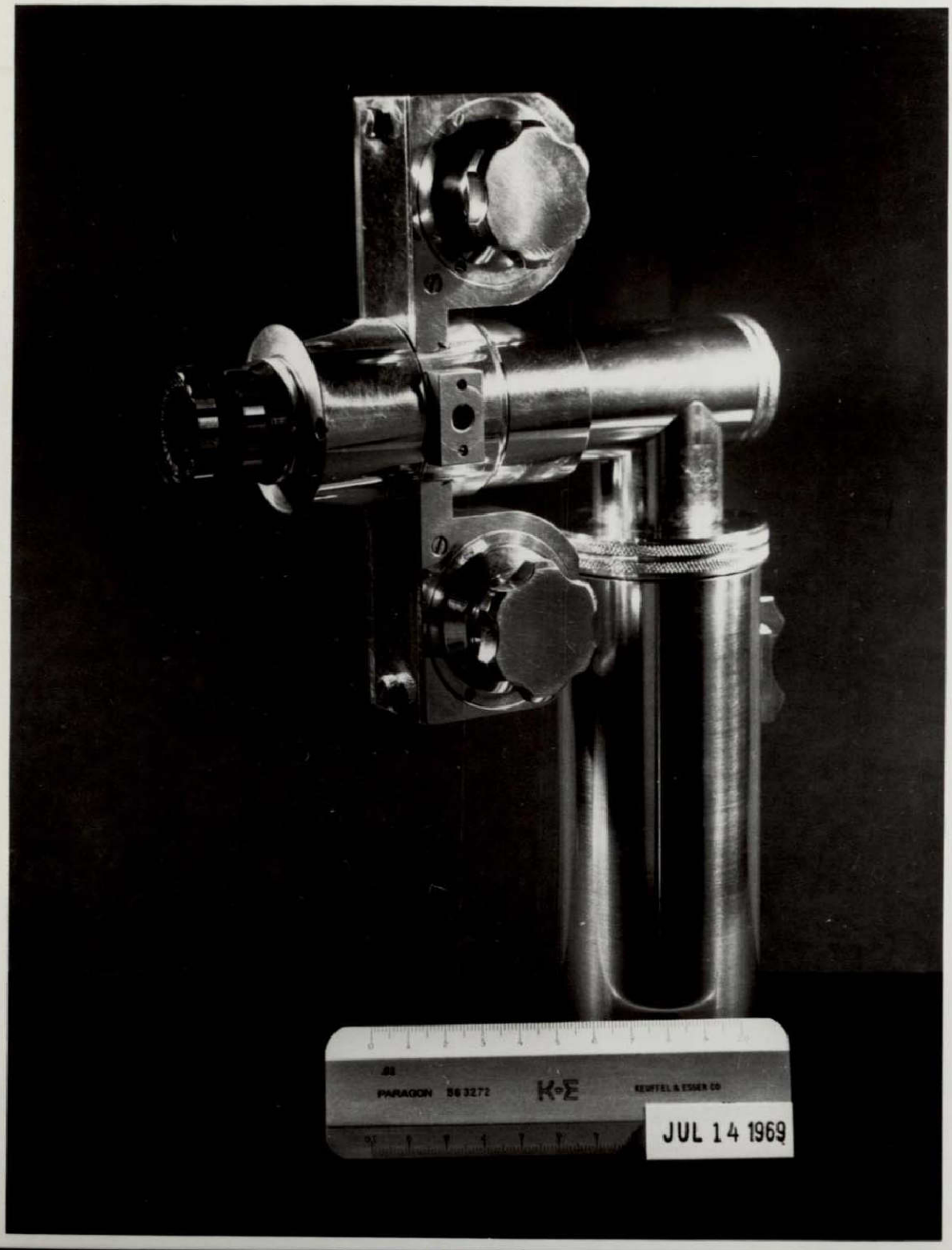
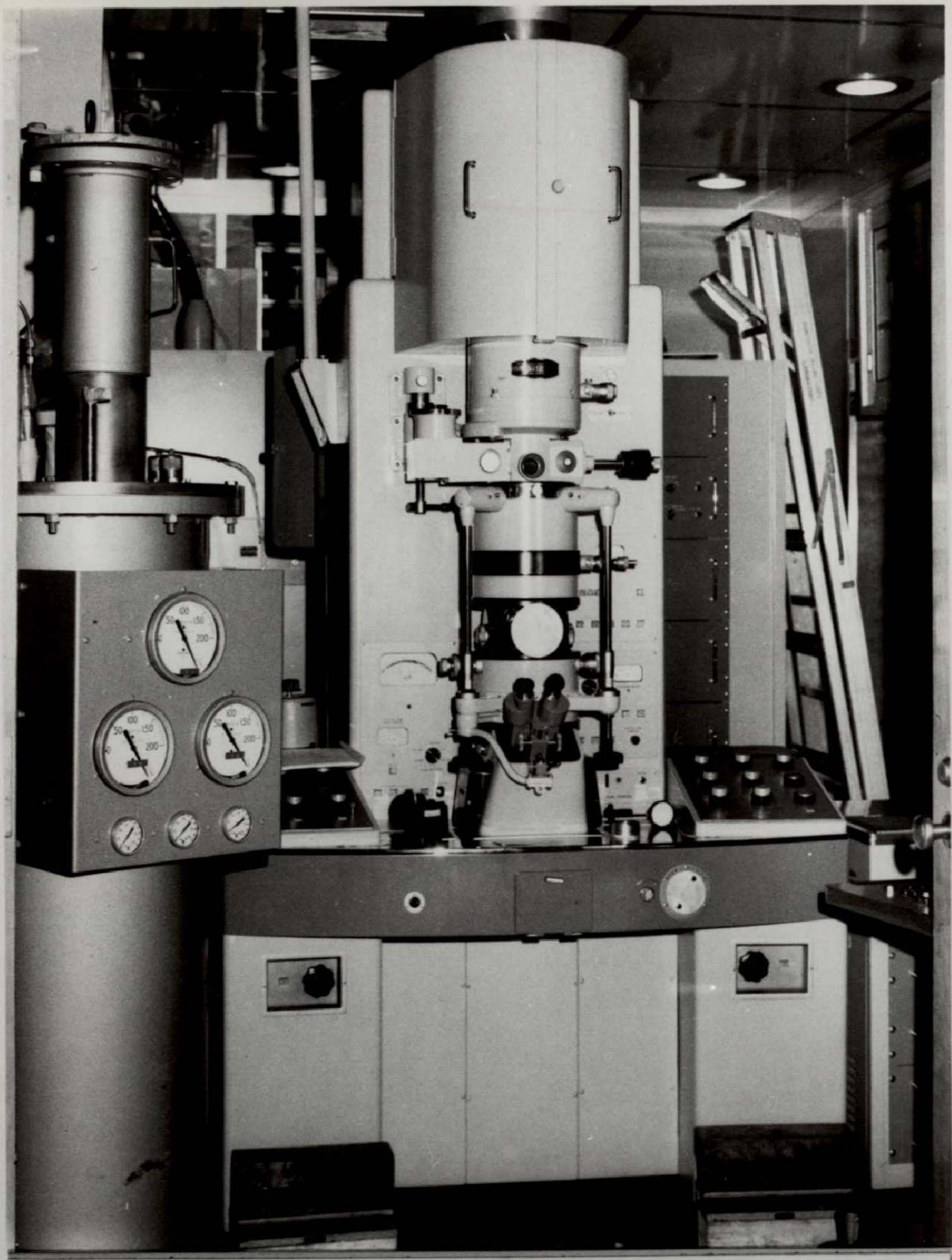




ELECTRON MICROGRAPHS AND ELECTRON DIFFRACTION PATTERNS OF MICROMETEORITE PARTICLES FROM ORIONID SHOWER LATER
PROVED SHOWING IMPROVED RESOLUTION AND REDUCED RADIATION DAMAGE OBTAINED WITH HIGH VOLTAGE ELECTRON MICROSCOPY



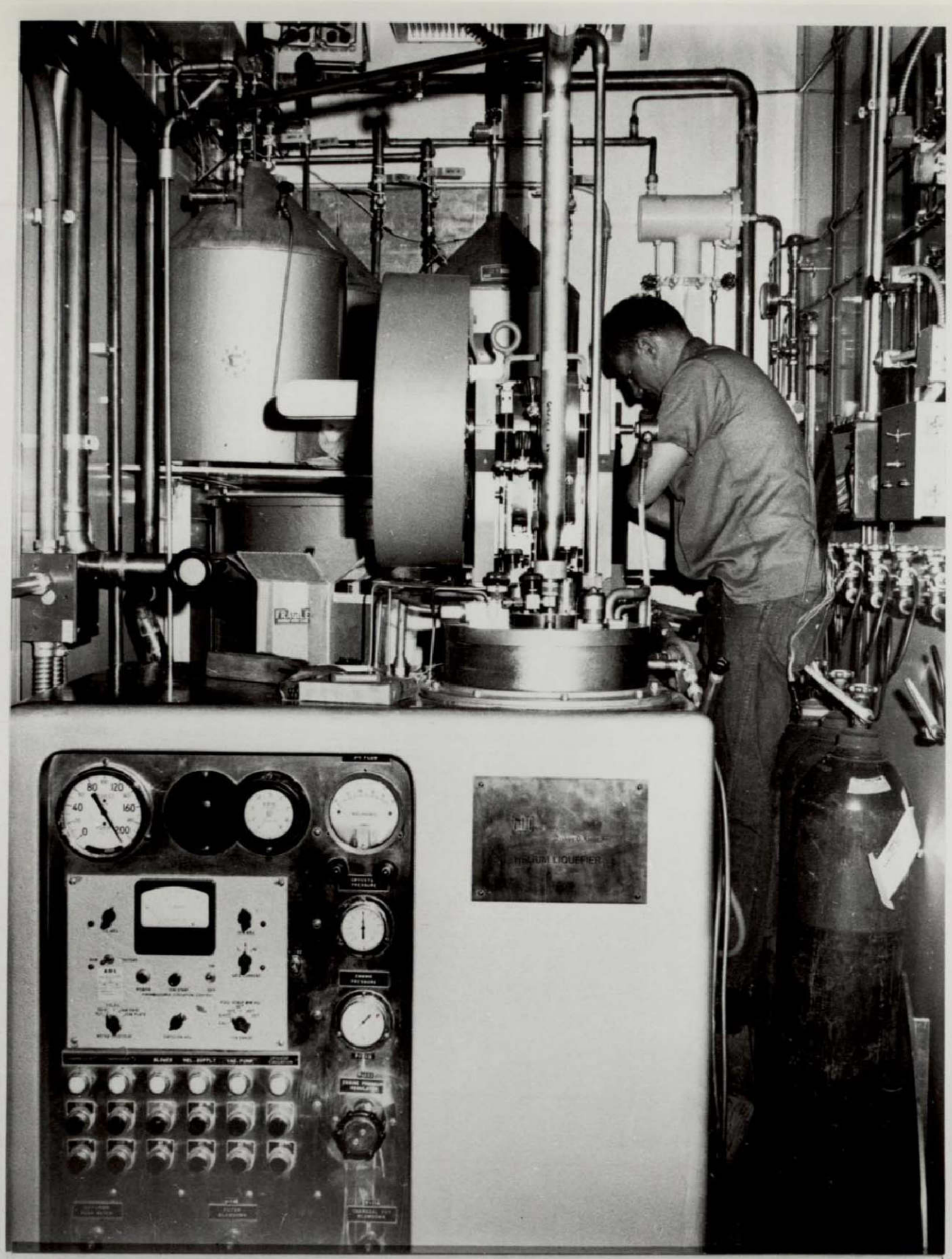
IMPROVED COMPOUND 'PICOPROJECTOR-PATHFINDER' FOR MAGNIFICATION OF DEMAGNIFIED MOON MAPS ON HIGH RESOLUTION 'ULTRA-MICRO FILM'.



INSTALLATION OF CLOSED CYCLE SUPERFLUID LIQUD HELIUM REFRIGERATOR

WITH COLLINS/ADL HEAT EXCHANGER AND ACCESSORIES FOR SUPERCONDUCTING

HIGH VOLTAGE CRYO ELECTRON MICROSCOPE 1969



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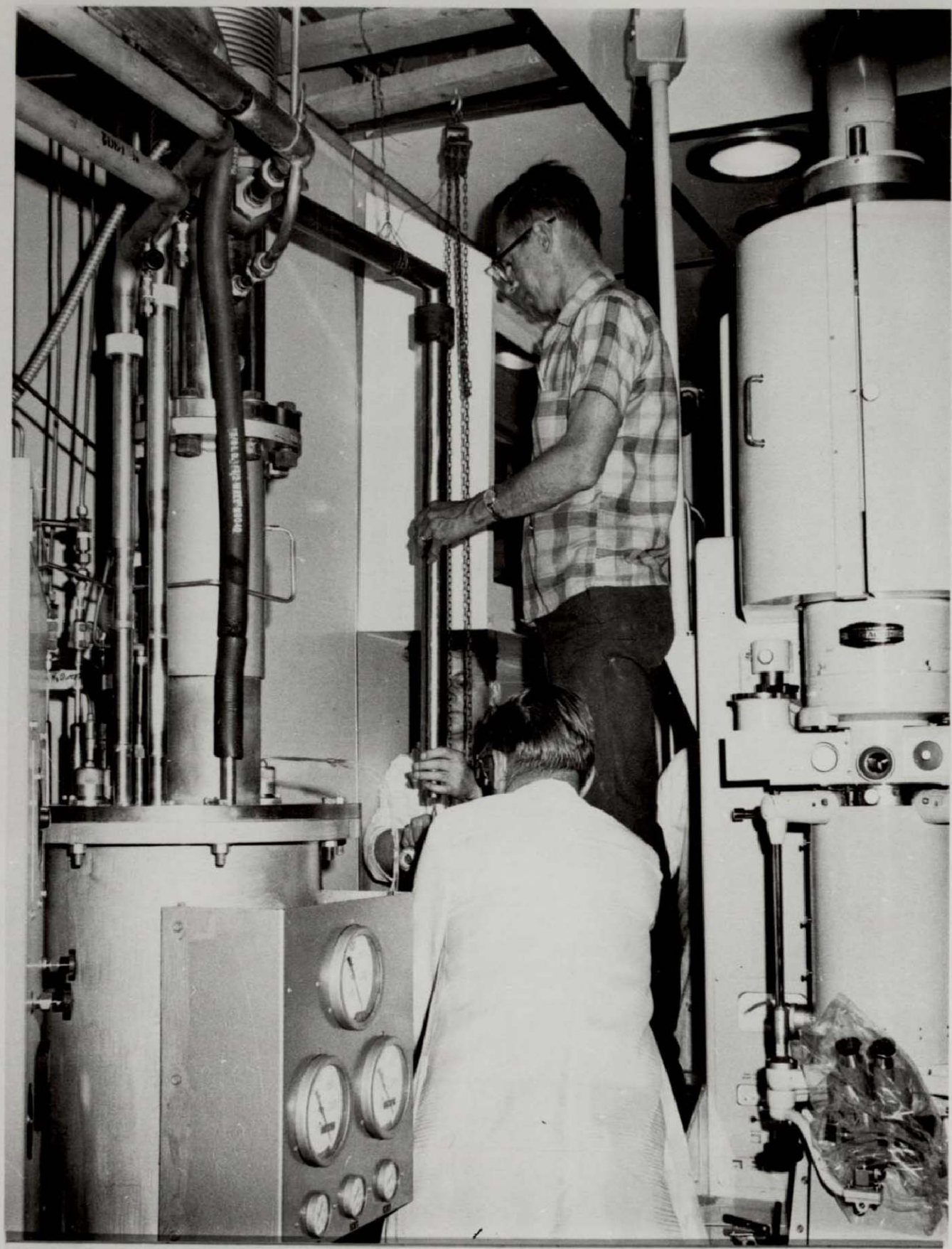

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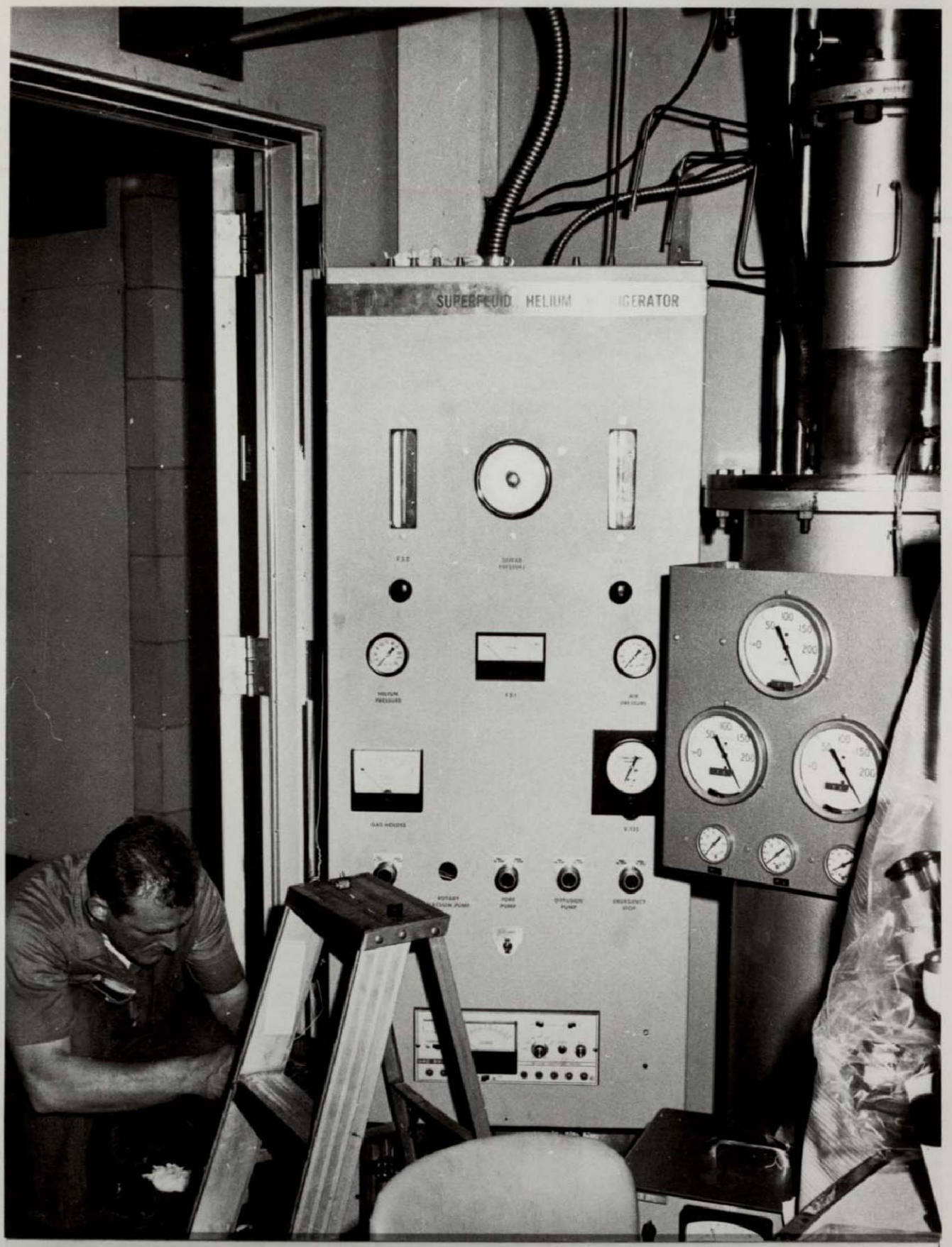

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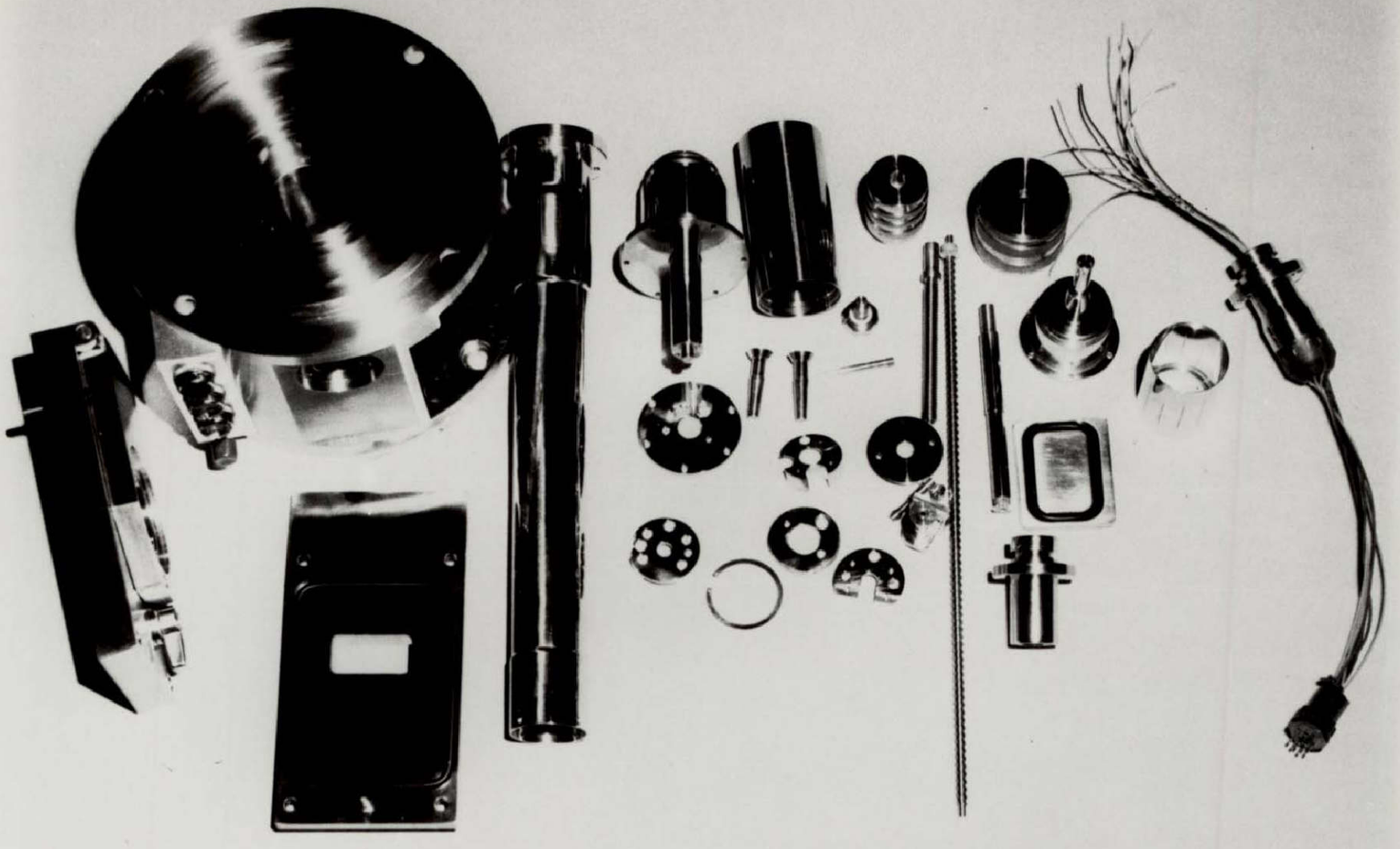

COMPONENTS OF SPECIAL SPECIMEN STAGE FOR OPERATION IN ULTRAHIGH VACUUM AND LIQUID HELIUM TEMPERATURES OF SUPERCONDUCTING CRYO ELECTRON MICROSCOPE

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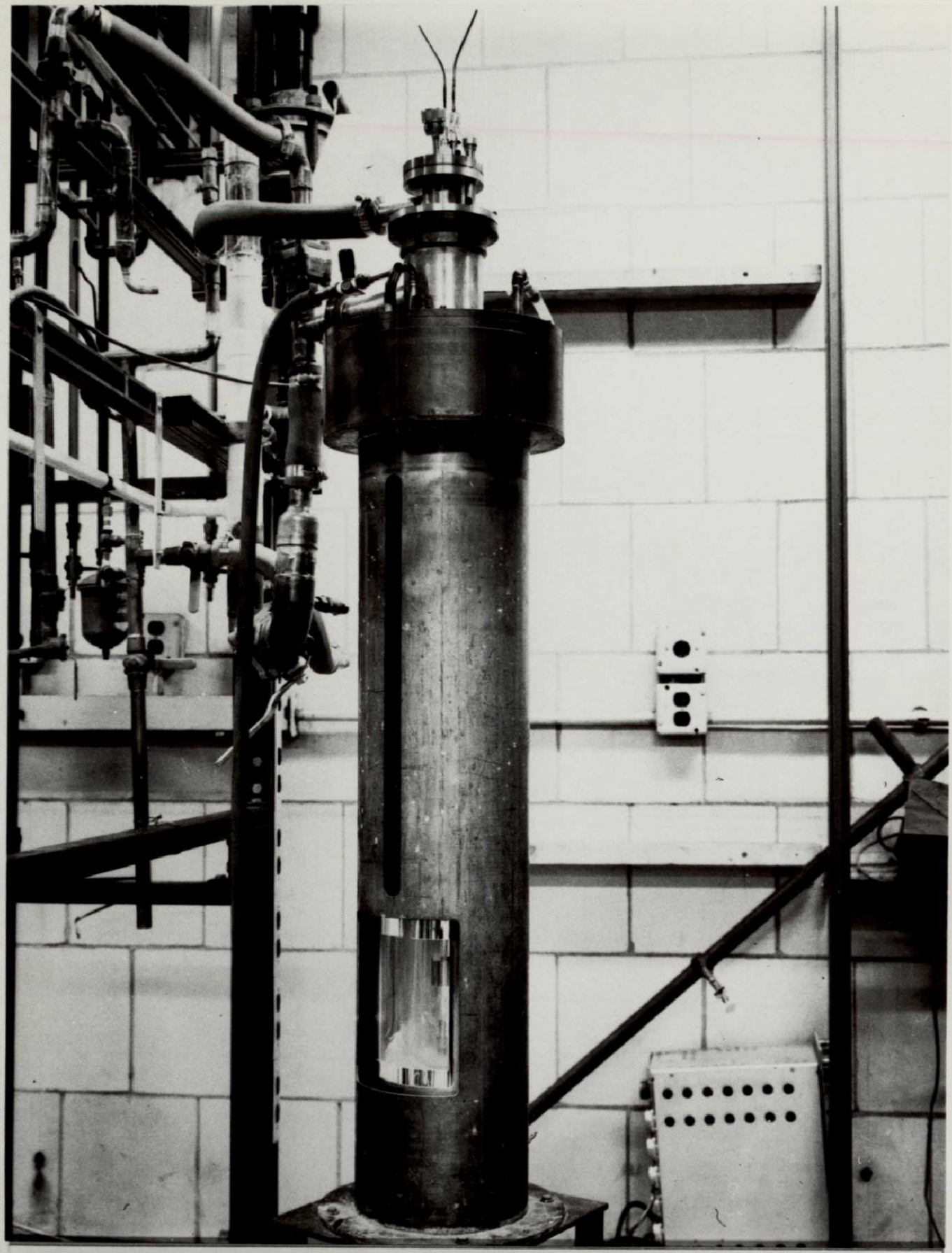
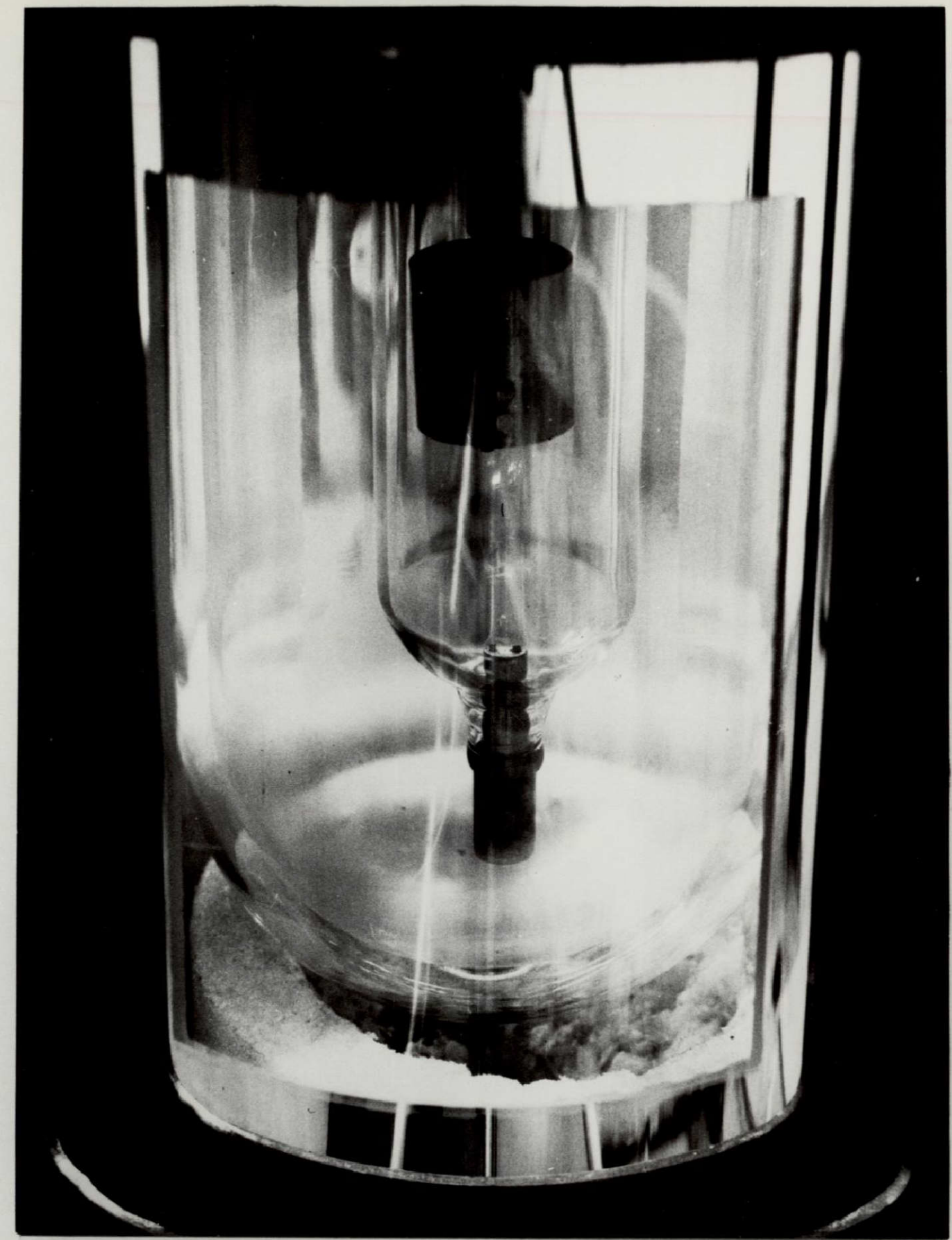


SPECIALLY DESIGNED CRYOSTAT AND ACCESSORIES FOR EVAPORATION OF THIN FILMS IN ULTRAHIGH VACUUM AT LIQUID HELIUM TEMPERATURES



SPECIALLY DESIGNED CRYOSTAT AND ACCESSORIES FOR EVAPORATION OF THIN FILMS IN ULTRAHIGH VACUUM AT LIQUID HELIUM TEMPERATURES

LIQUID HELIUM IN DEWAR PRODUCED IN COLLINS/ADL CLOSED CYCLE SUPERFLUID

LIQUID HELIUM REFRIGERATOR WITH 36 FT TRANSFER LIINES 1969

INSTALLATION AND SERVICE REPORT

CLIENT: University of Chicago Department of Biophysics

Address 5640 Ellis Ave., Chicago, Ill. 60637

Phone 312/667-4700 Ext.8362

Personnel Dr. Fernandez-Moran Title (Tech) Lab, Ext.8363

Mr. Ralph Vicario

Mrs. Erickson(Sec) Ext. 8361

Equipment Type/Model CHL/Special 1.85K Cabinet 2840-1-83

Serial Numbers Compressors 2424-53, 3016-2-437

Gas Holder D1617-28

Cryogenic Adsorbers 30141-5-199, 30185- 9-213, 30185-10-214

Pure Helium Recovery Box 30173-1-208

Draw off Recovery Tanks 30184-1-271, 30184-2-273, 5800/143516

Heracus-Engelhard Vacuum Pump 5800/143522

SERVICE CASE: 39200-10

E.P. 1041-69

Client Case

Service Dates

Installation Complete

Tests Satisfactory

Shipment Complete

ACTION:

Billing To Client Yes

Cancel Insurance Yes

Parts Shipment (Paid or Warranty)

Return Trip Necessary Yes

Sales Follow Up No

BILLING DETAILS:

Account No.'s	Labor Days	Labor Dollars	Expenses	Parts	Total
39200-10	See case status reports				

COMMENTS:

See attached report

DISTRIBUTION:

A. Latham/H.Howell
Sales Adm./Spare Parts
Engineering (2)
Sales
Manufacturing (2)
W.F. O'Neil
Service File
Service Mgr./Controller/R.E.File

SERVICEMAN (Signature)

SUMMARY

The Superfluid Refrigerator at the University of Chicago produced approximately 2.5 liters of superfluid helium at 1.82°K. in a glass dewar attached to the superfluid transfer line. The superfluid was maintained for one hour but could have been maintained for three hours. The control panel for the Superfluid Refrigerator was installed by university personnel prior to this visit.

To investigate a heat leak in the system and instrumentation problems in the microscope dewar, a glass dewar was installed on the superfluid transfer line. This permitted visual observations of the liquid collected and superfluid produced, to insure that the instrumentation was operating properly. Because of the heat leaks in the system it has been necessary to produce liquid helium in the Collins Helium Liquefier for cooling the heat exchanger below the inversion point so liquid could be accumulated in the glass dewar. This discontinuous process was made continuous by injecting liquid helium directly into the internal dewar of the liquifier at a rate equal to the heat leak in the system. The boil-off gas from this technique is automatically stored in the refrigeration system.

Concurrent with these experiments bench measurements were made on the microscope dewar to evaluate its heat leakage and the accuracy of new instrumentation installed.

The system performance was improved by adding liquid nitrogen shielding coils to the outside of the thirty-foot transfer tubes.

A leak to vacuum in the radiation shield liquid nitrogen line inside the heat exchanger was repaired.

A valve spring that failed in one of the compressor heads was replaced.

HISTORY

(See Installation and Service Report, March 11, 1969)

Since the control panel had not yet been installed and problems existed with the instrumentation in the dewar, it was decided to operate the superfluid refrigerator in a glass dewar so visual observations could be made on the system performance. This was an essential supplement to the work performed during the initial operation at the factory. Concurrent with these experiments, bench tests could be made on the microscope dewar to determine the boil-off rate and to calibrate new instrumentation.

SERVICE PERFORMED

Prior to this visit, university personnel had accomplished the following:

1. Installed, wired, and piped the control panel.
2. Installed the orifice for measuring the flow on the discharge of the blowers.
3. The superfluid line was connected to the glass dewar.
4. Cylinders of nitrogen gas for pressurizing the liquid nitrogen storage dewars were installed in the change room.
5. A ladder was installed to provide access to the cryogenic adsorbers.

The following changes were made in the system during these visits:

1. Installed the Brink Mist Eliminator in the discharge line of the compressor in the attic.
2. Repaired a leak to vacuum in the liquid nitrogen precooling coil on the radiation shield.
3. Made some wiring modifications and replaced several components in the control panel.
4. Installed a solenoid valve on the water cooling line to the vacuum pumps (It is wired in parallel with V-601).
5. Installed a 3/8" cooper line on the outside of the transfer line to reduce the heat load on the lines. One inch of armaflex-type insulation was applied over the coils.

6. Installed carbon resistors in the glass dewar to indicate the temperature and the liquid level.

OPERATION

Many runs were made in an attempt to locate and eliminate a heat leak that prevented liquefying directly in the glass dewar. Liquid helium could be made in the Collins Helium Liquefier and then be used to precool the heat exchanger below the inversion temperature so liquid could be accumulated in the glass dewar. This technique is reliable and repeatable, but the liquid can only be maintained for about 4 hours. This technique, combined with the vacuum pumps, resulted in achieving superfluid at 1.82°K. The superfluid was maintained for 1 hour but could have been contained for approximately 3 hours.

RECOMMENDATIONS

University of Chicago

1. Water treatment is required for the cooling system on the vacuum pumps.
2. A water separator should be installed in the control air line to the inlet valve of the vacuum pumps.
3. Install a room thermostat in the vacuum pump room to start the ventilation system on heat rise in the room.
4. Install a 50 psig relief valve on the liquid nitrogen line.

CONTINUATION OF THE INSTALLATION

The present mode of operation can be improved by finding and minimizing the system heat leaks so that liquid helium and superfluid helium can be produced directly in the glass dewar and be maintained for extended periods of time without loss of helium gas.

After completion of the experiments in the glass dewar, and the bench tests of the microscope dewar, it will be necessary to reinstall the microscope dewar. The superfluid refrigerator will then be checked out and operated with this dewar.

It is estimated that it will take several weeks to complete the experiments in the glass and microscope dewars. If problems are encountered, several months could be required before the refrigerator is operating in the dewar on the microscope.

CRYO INCORPORATED

INSTALLATION AND SERVICE REPORT

CLIENT: University of Chicago

Address: Department of Biophysics
5640 Ellis Avenue
Chicago, Illinois 60637
Phone 312-667-4700

Personnel: Dr. Fernandez-Moran 8362
Mrs. Erickson (Sec.) Title 8361
Mr. Ralph Vicario (Tech.)
Laboratory Ext. 8363

Equipment Type/Model: CHL/Special 1.85K

Serial Numbers: Cabinet 2840-1-83
Compressors 2424-53, 30169-2-437
Gas Holder D1617-28
Cryogenic Adsorbers 30141-5-199, 30185-9-213
30185-10-214
Pure Helium Recovery Box 30173-1-208
Draw Off Recovery Tanks 30184-1-27?, 30184-2-27
Heraeus-Engelhard Vac. Pump 5800/143516
5800/143522

SERVICE CASE:

Installation Complete

E.P.

Tests Satisfactory

Client Case

Shipment Complete

Service Dates: 11/13/68 - 11/22/68 12/9/68 - 12/18/68 1/20/69 - 2/14/69

ACTION:

Billing To Client: Yes

Return Trip Necessary: Yes

Cancel Insurance: Yes

Sales Follow Up: No

Parts Shipment (Paid or Warranty): Paid

BILLING DETAILS:

Account No.'s	Labor Days	Labor Dollars	Expenses	Parts	Total
39200-10	See Case Status Reports				

COMMENTS:

NOTE: Address all shipments to 5641 South Ingleside Avenue

DISTRIBUTION:

A. Latham/R.Howell
Sales Adm./Spare Parts
Engineering (2)
Sales
Manufacturing (2)
Contracts
Service File
Service Mgr./Controller/R.E.File

SERVICEMAN (Signature)

Liquefier Cabinet, three Cryogenic Adsorbers and associated equipment was installed. In both cases it was only necessary to discuss the layout, the equipment and piping so University Central Shop personnel could proceed with the installation.

The Liquefier Assembly was lifted out of the cabinet and the RDT flex line was removed and replaced with a plug. The heat exchanger was not removed from the #3 flange. The flex line was removed because the RDT tube cannot be installed in the cabinet in the existing room.

The schematic electrical drawing was translated into a field wiring sketch for the electrical contractor to lay out and bid on the job. This was later translated into a field wiring diagram.

The oil was drained out of the blowers and replaced with new oil.

The original drawing did not show a flow switch on the water and pressure switch on the air to protect the blowers. These have been installed and will be wired in the near future.

The final step of Phase I involved checking the wiring, pressure checking, and cleaning the system prior to liquefying helium in the cabinet.

Phase II. The transfer lines were shipped in two sections for final assembly in the field. Installing the refrigerator-end of the transfer lines into the couplings disclosed the following problems:

(1) The Crosshead could not be rotated because the evacuation valve on the return transfer line ("K") was in the path of the #1 engine connecting rod.

(2) The #2 engine crank shaft connecting rod journal rubbed on line ("K").

(3) The coupling nut on line ("K") would not slide past the weld at the top of the reducer on supply line ("J").

The following changes were made to alleviate the above problems:

(1) The vacuum valve was removed from the mounting boss on line ("K") and replaced by a plug. A hole was drilled and tapped into the front of the boss. A nipple was soft-soldered and screwed to the transfer line boss and the vacuum valve.

(2) The ("K") line interference was eliminated by filing 1/8" off the crank shaft journal of the #2 engine connecting rod.

(3) The upper weld on the line ("J") reducer was sanded down with emery cloth. This combined with a spacer clamp on the transfer tubes eliminated the interference.

The helium coupling assemblies were installed on the heat exchanger without difficulty. Both joints were made with soft solder because the previous connections were of this material. The transfer lines were inserted into the couplings and lined up with the mating sections perfectly. The lines were cut to length, inner line silver soldered, insulation sewed on and the outer jacket coupling installed.

The superfluid heat exchanger was opened for visual inspection and to repair several minor problems. A copper shield was placed over the hole in the radiation shield where the shipping mount had been connected to the heat exchanger. The liquid nitrogen line precooling the radiation shield was relocated slightly to eliminate it contacting the mylar on the heat exchanger.

A copper shield was fabricated to cover the hole where the nitrogen line goes from the outside of the radiation shield into the heat exchanger. The outside of the radiation shield and heat exchanger vacuum jacket was cleaned. Some diffusion pump oil had collected on the shield and in the jacket. Mylar was placed over the outside of the radiation shield prior to reassembly. The diffusion pump was disassembled, cleaned and reassembled.

The Brink Mist Eliminator had a leak between the flange and the gasket of the Brink element. The eliminator was shipped (collect) back to Monsanto on February 13, 1969 for repair.

An abortive attempt was made to clean up the system by evacuating the total system with a vacuum pump. The valves to the blowers were opened and the blower seals do not seal under vacuum. After closing the valves to the blowers, the system was readily evacuated and cleaned up.

OPERATION

Phase I. The system was checked out, cleaned up and cooled down. The liquefaction rate in the cabinet was 8 to 8.5 liters per hour.

It was necessary to run the blowers while replacing the oil. The high noise and vibration level produced numerous complaints from people in adjacen labs, so a sound attenuating room was erected over the blowers. The blowers blanked off at 6 microns.

Phase II. The system was cleaned up and cooled down. Several problems, helium leaks, vacuum leaks, contamination, and insufficient nitrogen supply aborted the first few runs. After surmounting these difficulties, the

liquefier and heat exchanger cooled down and liquid was collected in the microscope dewar. In four different runs liquid conditions were maintained in the microscope dewar for from 2 to 4 hours. These conditions could have been maintained for longer durations, if desirable.

TRAINING

Training sessions were conducted in the theory of the liquefier and refrigerator for 4.2°K operations. Two technicians have been trained to operate the liquefier and one to operate the refrigerator heat exchanger combination for 4.2°K. The training is not complete and will be continued in future visits.

EQUIPMENT CONDITION

(1) The flare on the supply line of the left supply flange was almost non-existent. The line was reflared.

(2) The General Electric Starters for the blowers arrived with one of them wired for 220V internal control in place of the 110V external control that was ordered. General Electric supplied the necessary parts for the electrical contractor to modify the Starter.

(3) The client supplied the diffusion pump oil because that shipped was full of pieces of "red rubber."

(4) The #2 Compressor fourth-stage pressure gauge was replaced.

(5) There was a leak around the nut on the #2 blower. The clearance hole in the casting for the stud had excessive and eccentric clearance. The leak was eliminated by brazing a washer with an O-ring groove to an acorn nut to replace the original nut and washer.

(6) One belt-guard support on the #2 blower failed. This probably failed during installation. A new support was made and installed.

WORK TO BE COMPLETED

University of Chicago

(1) Nitrogen gas for pressurizing liquid nitrogen dewar should be installed (check with dewar manufacturer for pressurizing coil).

(2) Revamp the liquid nitrogen piping near and in the change room.

(3) Install the third liquid nitrogen dewar.

(4) Install a wall rack for the helium cylinders.

(5) Install the Brink Mist Eliminator under the sink in liquefier room. (Shipped back to Monsanto for repair February 13, 1969.)

(6) Install on the heat exchanger a styrofoam insulating block on th 77°K helium line for the microscope dewar shield.

(7) Change the water and air piping for blowers in Room 207.

(8) Check the operation of ventilation system for the blowers.

(9) Install vibration dampening on 6" blower vibration isolators.

(10) Install more rubber around 6" vacuum line in the ceiling of the blower attenuation room in 207.

(11) Install a 1/4" airline from Room 207 to the microscope room for controlling the butterfly valve on the blowers.

(12) Install a 1/4" line for the compressor fourth stage pressure gauge from the attic to microscope room.

(13) Check the operation of the ventilation system for the liquefier compressors in the attic.

(14) Install lights and steps for reading compressor gauges.

(15) Attach a ladder to the Gas Holder for access to the cryogenic adsorbers.

(16) Fix the relief valve on the Gas Holder.

(17) Vacuum inside of Gas Holder.

(18) Install a Korofund pad under the Gas Holder if necessary to eliminate vibration.

500 INCORPORATED

(1) Complete the electrical drawing showing the control panel.

(2) Provide a complete set of spare parts.

Richard A. Osburn

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20 ACORN PARK, CAMBRIDGE, MASSACHUSETTS 02140 • TELEPHONE (617) 491-5700 • TELEX 094-6210

August 16, 1968

Dr. Fernandez-Moran
University of Chicago
5640 Ellis Avenue
Chicago, Illinois

Dear Dr. Fernandez-Moran: 39510

On August 14, Dr. Collins and myself were successful in liquefying helium in the glass dewar which is a mock-up of your electron microscope superconducting magnets at the rate of about 2.5 liters per hour. Furthermore, we lowered its temperature to 1.8°K for about 10 minutes using the two Heraeus vacuum pumps maintaining 13.5 watts heat input and to 1.85°K for 15 minutes maintaining 10 watts. After about 30 minutes operation, we were forced to shut down because of freezing out water vapor coming from the Heraeus vacuum pumps. Our next task will be to dry these pumps before conducting our tests.

We find it necessary to use a special liquid nitrogen cooled trap between these pumps and the refrigerator particularly so when set up in your laboratory where the pipe lines are much longer.

Sincerely,

Milton H. Streeter

MHS:caj

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20 ACORN PARK, CAMBRIDGE, MASSACHUSETTS 02140 • TELEPHONE (617) 491-5700 • TELEX 0944-5-2-1-0

921-436

October 30, 1968

The University of Chicago
Department of Biophysics
5640 Ellis Avenue
Chicago, Illinois 60637

Attention: Dr. Fernandez-Moran

Dear Dr. Fernandez-Moran:

This letter will confirm our telephone discussion of October 14, and review the status of the superfluid helium refrigeration system which we are supplying to you.

I am very pleased with the results of the tests which were conducted on your behalf during the week of September 23, by Dr. Collins and Mr. Streeter. As Mr. Streeter reported to you, continuous refrigeration of 20-watts was achieved while maintaining superfluid helium in the external dewar. These tests demonstrated beyond question the feasibility of providing superfluid helium refrigeration outside of a refrigerator. I believe this is the first time that refrigeration of this magnitude has been produced in this manner.

We are looking forward to the application of the refrigerator to your superconducting microscope with a great deal of enthusiasm. Mr. Streeter will be available to assist you with the tests after you have completed the installation of all of the equipment.

At the request of Mr. Walsh, we have submitted a quotation to you for step starters for the 30 hp vacuum pump motors. We have requested that these be shipped directly to you from the General Electric Company. They expect to ship them to you on November 14. In the meantime, we have shipped the starters which were used during our tests.

With the exception of the refrigerator-to-heat exchanger transfer lines, all equipment has now been shipped. As soon as you have installed the refrigerator and heat exchanger, we will make final measurements for these lines and complete the fabrication.

I have enclosed a milestone chart which summarizes the current schedule. It now appears that testing at your laboratory will take place early in December. Final tests should be complete in December or early January.

DATE June 27, '69 (25th) OPERATORS Helmuth, [illegible], Coburn

NAME OF EXPERIMENT

HELIUM LIQUEFACT...

COLLINS HELIUM CRYOS...

TIME	HELIUM THERMOMETER °K - KELVIN	GAS HOLDER RATE OF FALL IN./MIN.	LIQUIFYING RATE LITERS/HR.	MAGNEHELIC GAUGE IN. H_2O	ENGINE INLET PRESSURE P.S.I.	SUPPLY PRESS (SIDE CABINET) P.S.I.	CRYOSTAT PRESSURE +P.S.I. - "HG	#1 ENGINE SPEED R.P.M.	#2 ENGINE SPEED R.P.M.	JACKET VACUUM mm Hg	PRECOOLING (✓)	HELIUM C... STAGE PRESS... #1 COMPRESSOR FIRST	SECOND	THIRD	FOURTH	FIRST
9:23	200+	-Sl		20	210	210	2.5	215		Op	✓				235	-1
9:40	200+			18	200	202	2.1	235							235	
10:20	118			14	210	223	2.7	210				11	35	78	235	12
11:12	68	44.5		15	235	245	3.0	360		✓	✓				275	
11:53	53	51.1		0	225	230	2.0	250							260 / 233	
12 30	11	56.3		0	198	206	1.8	175		✓	✓					
1300	7	63.3		0	175	186	2.0	150							220	
1313		120.5														
1320		94.5							* then things warmed up a bi[t]							
1345		93.9														
1410		101.2														
1929		48.1	5.5	13											210	
2030		Ran in the bottle for 2 hours + making liquid at the...														

ADL 761

REFRIGERATION RUN

SERIAL NO. _____

Arthur D. Little, Inc.

EXPERIMENT NO. _____

TOTAL RUNNING TIME TO START OF THIS RUN: _____ HR. _____ MIN.

SOR DATA				RDT SYSTEM				HELIUM REFRIGERATOR DATA						NOTES ON METHOD OF CLEAN UP, APPEARANCE OF LIQUID HELIUM, SHUT DOWNS, ETC.
E - - P.S.I.				DEWAR SIZE					HELIUM THERMOMETERS					
#2 COMPRESSOR				LITERS										
FIRST	SECOND	THIRD	FOURTH	DEWAR PREPARATION T1	DEWAR PRESSURE P.S.I.G. P2	HELIUM LEVEL GAUGE T3	HELIUM LEVEL MEASURED P1	WATTS P2	SUPPLY PRESSURE P.S.I.G. P3	RETURN PRESSURE P.S.I.G. Pc	TEMP. COOLING STREAM °K - - KELVIN CFM F1	TEMP. RETURNING STREAM °K - - KELVIN SCFM F2	F3	
-Pencil reading				200+	200+	200+	129	173	26	6.5/10	0	0	0	
				200+	200+	200+	210	206	0	5/10	0	0	0	2nd L.N. on He coil @ 1.8°K
2	?	76	275	200+	95	200+	170	170	66	1/10	11.5/12.5	0	0	225 psig on Control panel
				135	70	83	205	202	15	1/10	10	0	0	Cooling down rapidly
				55	37	45	206	204	10	1/10	9.5/10.5	1.5	1	Changed lines on cold head
				20	12	15	176	165	4	5 x 10⁻⁶	10	4.5	3.0	Cooling down manually
				35	10	16	165	152	1	1/10⁻³	7.0	3.3	2.0	
				12	8	10	165	152	1	1/10⁻⁵	9.5	4.8	3.3	
bit	then	went	back	to	bypassing.									Closed V-107, Throttle valve V-10
				12	10	11	175	170	1	8.5/10⁻³	8	2	0	
				16	9	10	173	166	1	9.5/10⁻³	9	2	2	
										3/10⁻⁵				
tempted to move it to the glass dewar unsuccessfully - back to CH4														

FOLD

FOLD

HELIUM LIQUEFACTION OR REFR
COLLINS HELIUM CRYOSTAT SERIA

DATE 6/18/69 OPERATORS ______ ______
NAME OF EXPERIMENT ______

TIME	HELIUM THERMOMETER °K -- KELVIN	GAS HOLDER RATE OF FALL IN./MIN.	LIQUIFYING RATE LITERS/HR.	MAGNEHELIC GAUGE IN. H_2O	ENGINE INLET PRESSURE P.S.I.	SUPPLY PRESS (SIDE CABINET) P.S.I.	CRYOSTAT PRESSURE +P.S.I. -- "HG	#1 ENGINE SPEED R.P.M.	#2 ENGINE SPEED R.P.M.	JACKET VACUUM mm Hg	PRECOOLING (✓)	HELIUM COMPRESSOR DAT… STAGE PRESSURE -- P.S… #1 COMPRESSOR FIRST	SECOND	THIRD	FOURTH	#2 C… FIRST	SECOND
2051																	
2102															205		
2110																	
2124																	
2215	5			0	180	190	23	150							210		
								Shut down							DOR Tank		
6/19/69 930				Start up											DOR Tank		
934	5			0/4	235	240	2.2	200							270		
955															245		
11:30	5	—	-	0/3	180	200	9.2	170	✓	✓	✓	12	34	73	240	17	33
1333	5	1:43	8	5/11	180	194	2.5	150				—			215		
1410			8.5														
1420			8.7														
1418																	
6:35	PM				— line (bypass) [illegible] no gas passed through [illegible]												
7:30	PM		C														

FOLD

ADL 3?1

ERATION RUN

INCORPORATED

NO. 83 UPDATED RRDT EXPERIMENT NO. 1

TOTAL RUNNING TIME TO START OF THIS RUN: _____ HR. _____ MIN.

PRESSOR THIRD	PRESSOR FOURTH	RDT SYSTEM DEWAR SIZE LITERS: DEWAR PREPARATION	DEWAR PRESSURE P.S.I.G.	HELIUM LEVEL GAUGE	HELIUM LEVEL MEASURED	HELIUM REFRIGERATOR DATA: WATTS	HELIUM THERMOMETERS: SUPPLY PRESSURE P.S.I.G.	RETURN PRESSURE P.S.I.G.	TEMP. COOLING STREAM °K -- KELVIN	TEMP. RETURNING STREAM °K -- KELVIN	NOTES ON METHOD OF CLEAN UP, APPEARANCE OF LIQUID HELIUM, SHUT DOWNS, ETC.
3-6											UNIV OF CHICAGO
											1.8° UNIT 39510
											COLLINS PROTOTYPE
											SLOW SPEED X HEAD
											ORIGINAL DEWAR-6 ETERS
											ADDED - DIFFUSION PUMP
	4 CYCLES	-	6-17-68								REMOVED.
							275				EXTENDED J T HT EXCH.
SOLID	HEAT EX. CLOSED										RRDT - 0111-00
376	252						272				DEWAR # 186-1
✓	✓						✓				RRDT FROSTING
✓							✓				
											RRDT BEGINNING TO CRYOPUMP
		RR COLD	0	0						VMC	REMOVED VALVE HANDLE TO LOWER RRDT
✓	✓	✓	3.6	[illegible]			260	¼ FLR CAP			SEALING VAC. TUBE CRYOPUMPING
✓	240		3.7	✓			258				
SOLID	LEAKING							INSTALLED			FAN ON FLYWHEEL
✓	✓		4.4	1			210				

FOLD

REFRIGERATION RUN

Arthur D. Little, Inc.

SERIAL NO. 1

EXPERIMENT NO. _____

TOTAL RUNNING TIME TO START OF THIS RUN: _____ HR. _____ MIN.

OR DATA				RDT SYSTEM			HELIUM REFRIGERATOR DATA						NOTES ON METHOD OF CLEAN UP, APPEARANCE OF LIQUID HELIUM, SHUT DOWNS, ETC.
-- P.S.I.				DEWAR SIZE				HELIUM THERMOMETERS					
#2 COMPRESSOR				LITERS									
FIRST	SECOND	THIRD	FOURTH	T1	T2	T3	P1	P2	P3	Vac	F1	F2	F3
				13	11	19	80	140	11	$9/10^{-4}$	3	1	6 — 51 Ω
				12	9	15	65	146	9	$1/10^{-4}$		1	6
				13	9	10	50	158	5	$1.2/10^{-6}$		1	5.7 — 492 Ω
				14	8	10	50	155	5	$1/10^{-6}$	3/0	1	5.8
				11	9	11	40	163	5	$3/10^{-7}$		1	2 — 463 Ω
Tank	55 psig												
													Howard
Tank	60 psig												Measured 633 Ω resistor
			0	175	155	140	0	0	0	$1/10^{-4}$	0	0	0 at 77K = 93.6 Ω in liquid
				150	200	150	25	95	12	$1.4/10^{-4}$		+	+
17	35	70	250	25	12	15	0	175	1	$6/10^{-7}$	5/0	0	0 — 755 Ω
				41	45	41	0	0	0	$2.9/10^{-5}$	0	0	0 — 585 Ω
													1210 Machine after operation
													½ hr. is producing 8.5 ℓ/hr.
													Check system for air
													Leak - Found one on
													line between CHC + 1.8 °K
				180	5	9	60	90	5	$9/10^{-7}$	liquid		Opened V.T. Valves 10%
										5×10^{-7}			Heat exchange appears 7%
				14	7	9	150	150	5	4.5×10^{-7}			Made about 1 ℓ of liquid,
Vacuum											3/4	3.8	3.5 Holding steady.
				13	9	12	145	160	2		4-5	1.9	2.0 Still liquid in Dewar.

FOLD

FOLD

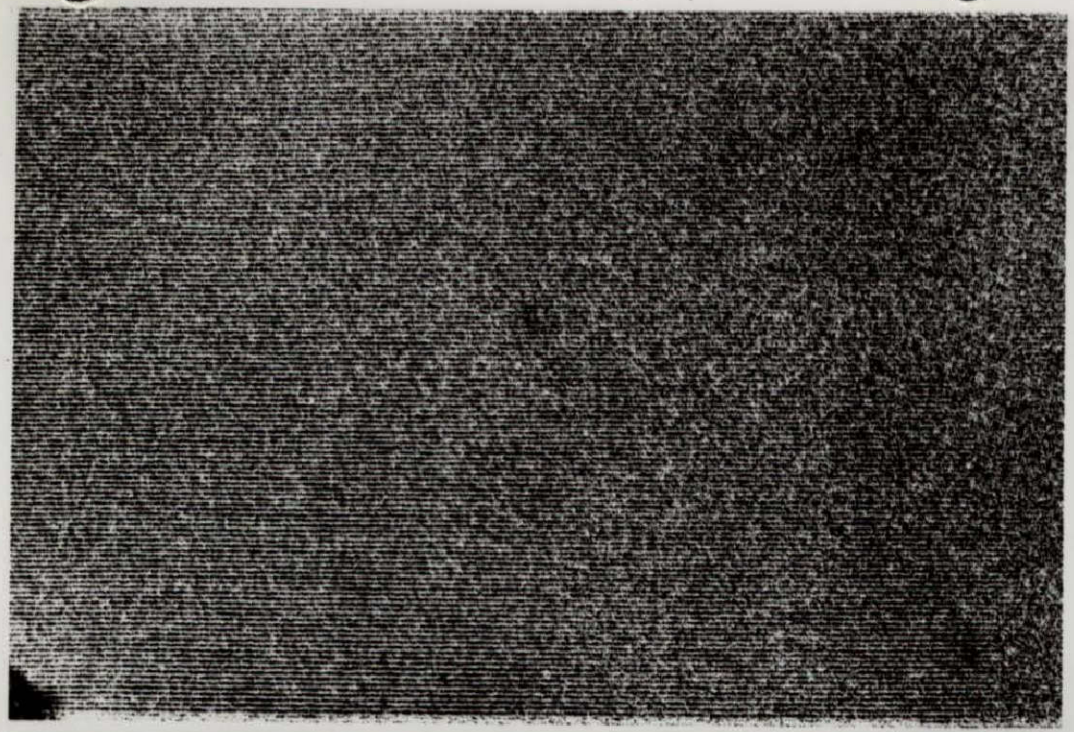
HELIUM LIQUEFACTION OR REFRI

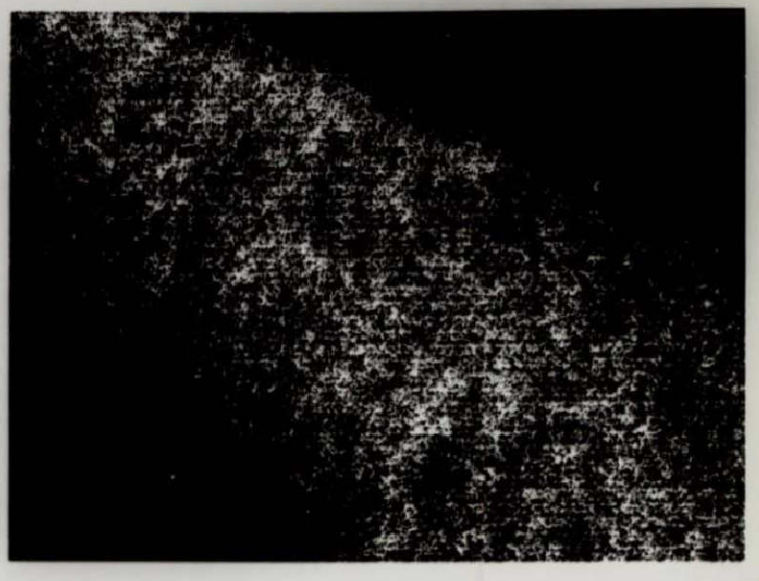
COLLINS HELIUM CRYOSTAT SERIAL

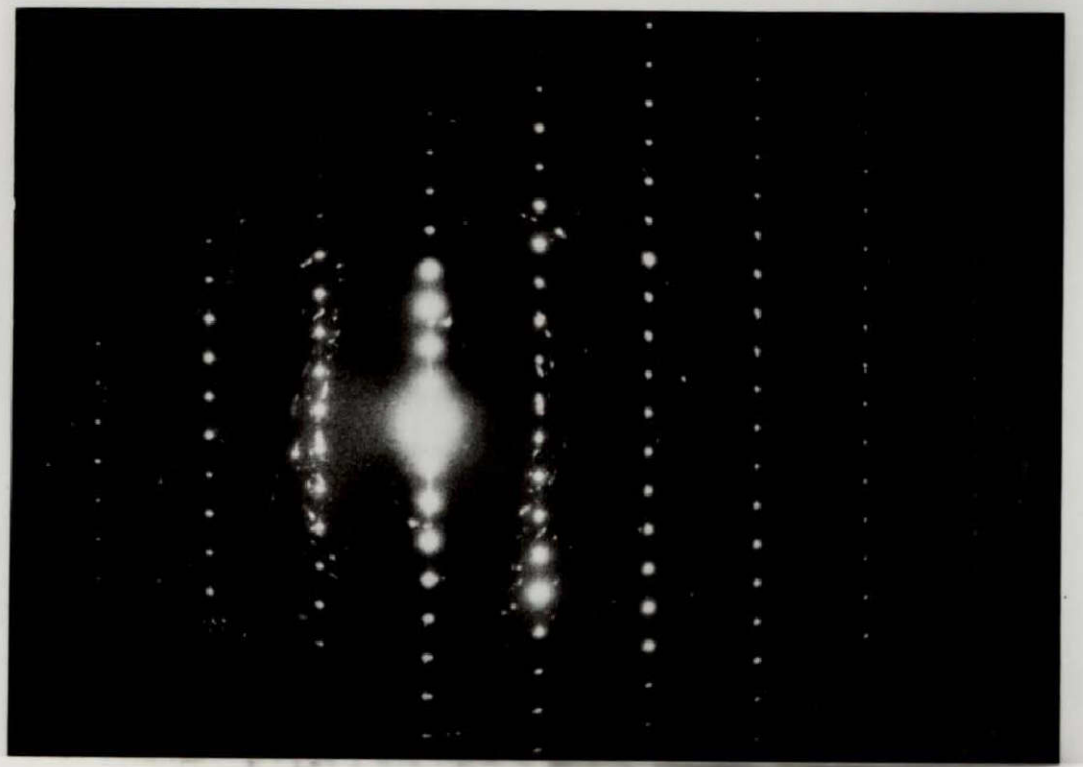
DATE 6-18-68 OPERATORS B.D.A.H.

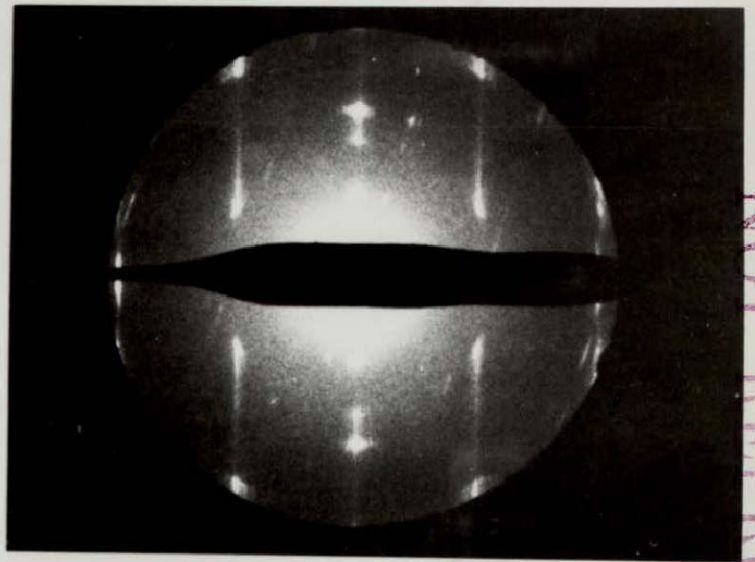
NAME OF EXPERIMENT Initial Cool Down

TIME	HELIUM THERMOMETER °K -- KELVIN	GAS HOLDER RATE OF FALL IN./MIN.	LIQUIFYING RATE LITERS/HR.	MAGNEHELIC GAUGE IN. H_2O	ENGINE INLET PRESSURE P.S.I.	SUPPLY PRESS (SIDE CABINET) P.S.I.	CRYOSTAT PRESSURE + P.S.I. -- "HG	#1 ENGINE SPEED R.P.M.	#2 ENGINE SPEED R.P.M.	JACKET VACUUM mm Hg	PRECOOLING (V)	HELIUM COMPRESSOR DAT: STAGE PRESSURE -- P.S. #1 COMPRESSOR FIRST	SECOND	THIRD	FOURTH	#2 CC FIRST	SECOND
						SYSTEM	CIRCULATED						AND		EVACUATE		
1030							"						"		BLOWN	DOWN	
1040	AMB			START		COOL	DOWN						ALL VALVES		TO	SUPPLY	
1109	180			2.0	238	244	2.3	260				10.5	35	78	250	10	
1113	160			2.0	236	240	2.6	260		5×10^{-3}		✓	✓	✓	✓	✓	
1120	120			2.0	233	239	2.5	250		5×10^{-3}		✓	✓	✓		✓	
1125	80							CLOSE	34	PASSES							
1134	40			2.0	227		2.6	220									
1135	30							R.T.D		INTO		LSD	186-1				
1145	14			3.0	222	242	3.5	195		5×10^{-6}		✓	✓	✓	✓	✓	
1200	10.5			3.0	218	237	3.8	190		3×10^{-6}		✓	✓	✓	245	✓	
1215								O-RING	UNDER	1/16"		SUPPLY	MAG				
1345	11.5	20"	5.9	14.8	225	242	4.0	190		5×10^{-6}		✓	✓	✓	✓	✓	
1430							SHUT	DOWN		ON	RECHARGE						





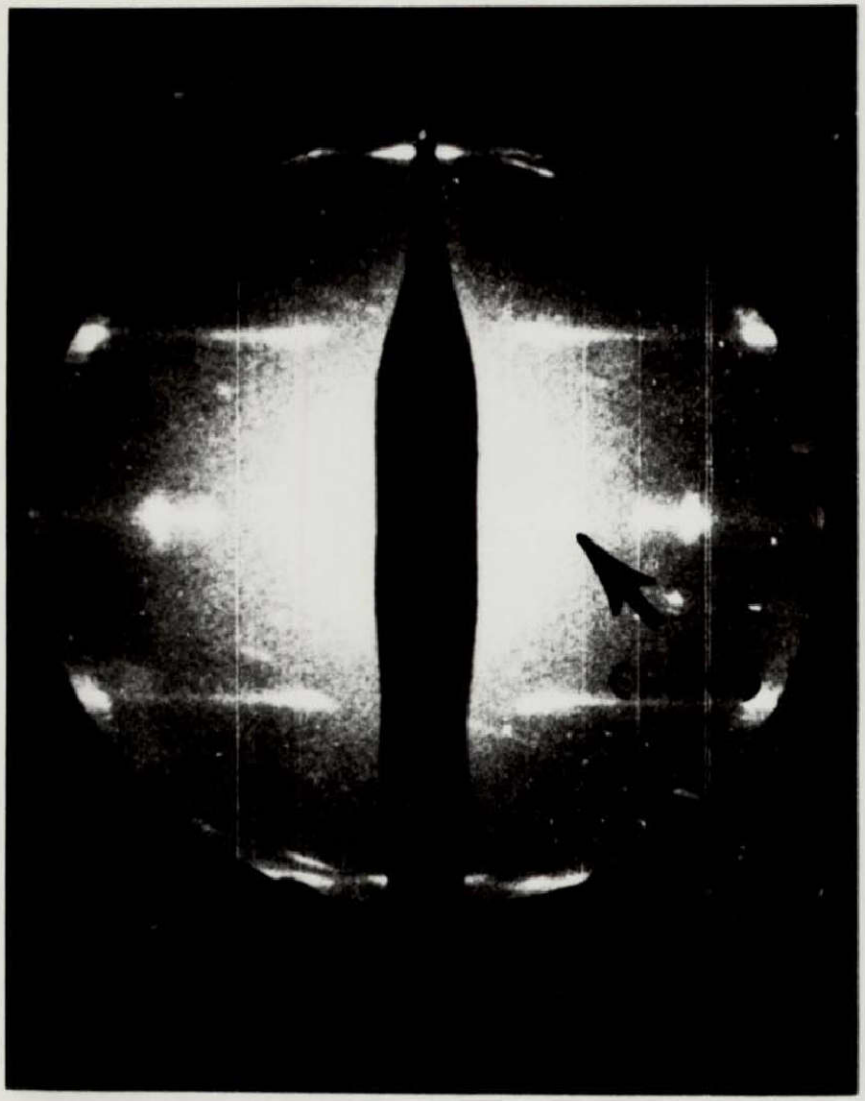




CU-PHTHALOCYANINE — (a) PHASE CONTRAST DARK FIELD

SELECTED AREA DIFFRACTION PATTERN

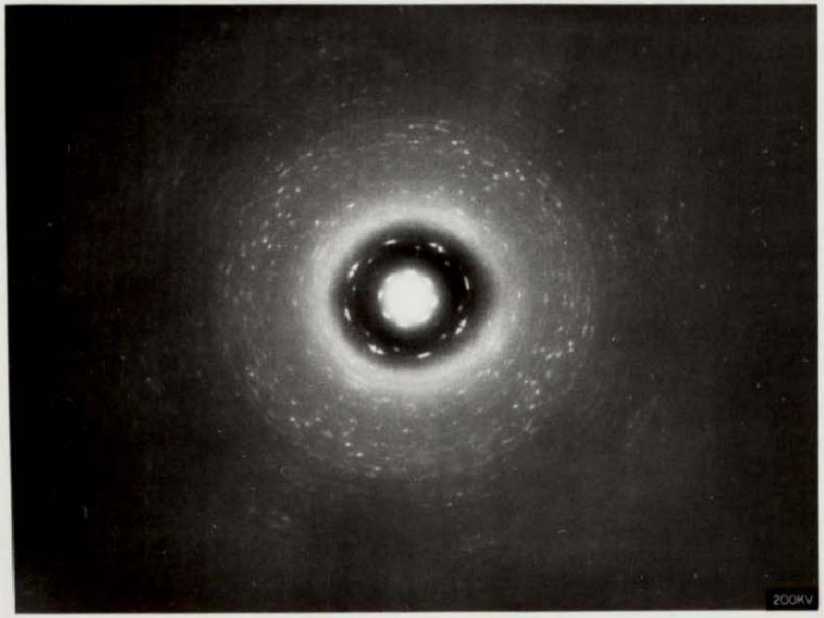
CALIBRATION - HIGH RESOLUTION ELECTRONMICROGRAPH AND ELECTRON DIFFRACTION OF CRYSTALLINE LATTICE, 200KV

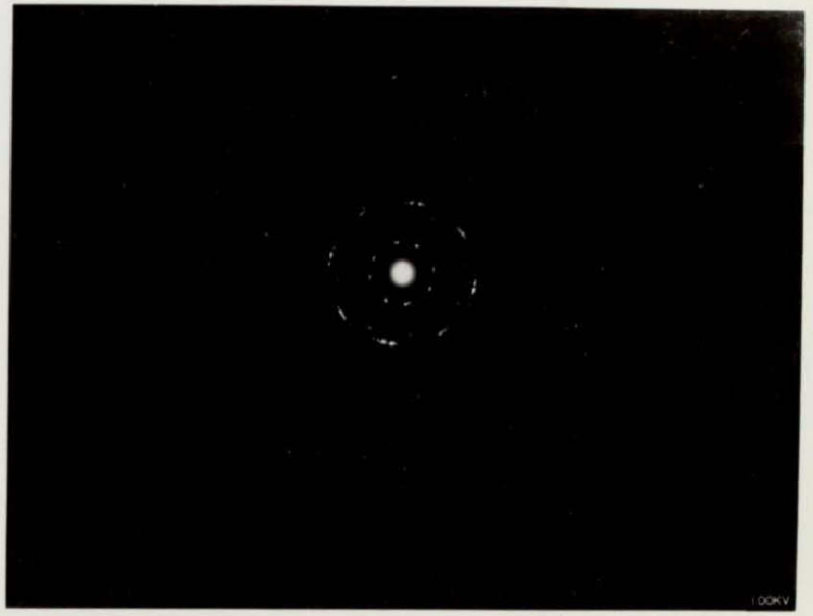


6.25A

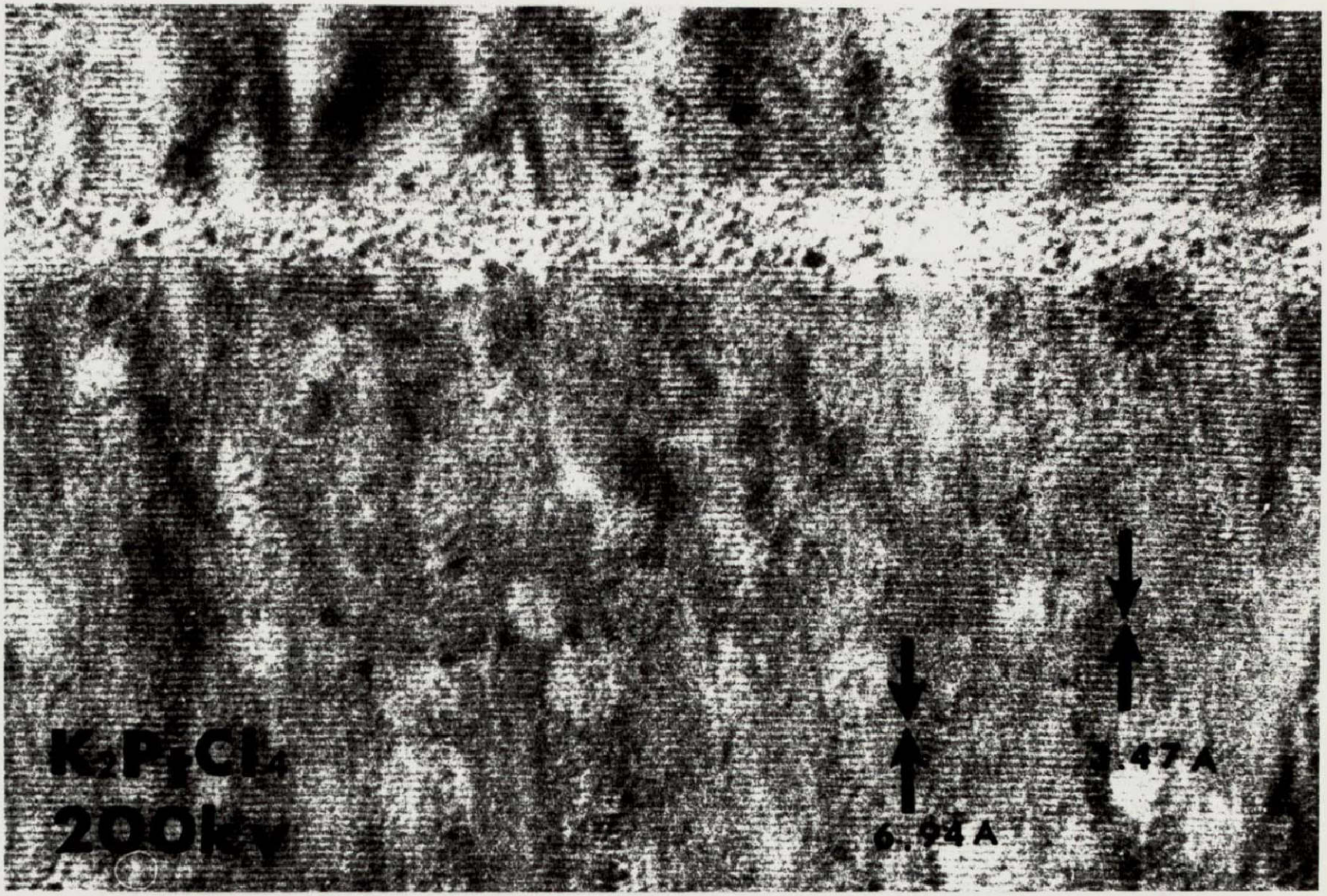
200kv

CALIBRATION HIGH RESOLUTION ELECTRON MICROGRAPH AND ELECTRON DIFFRACTION OF CRYSTALLINE LATTICE. 200 KV



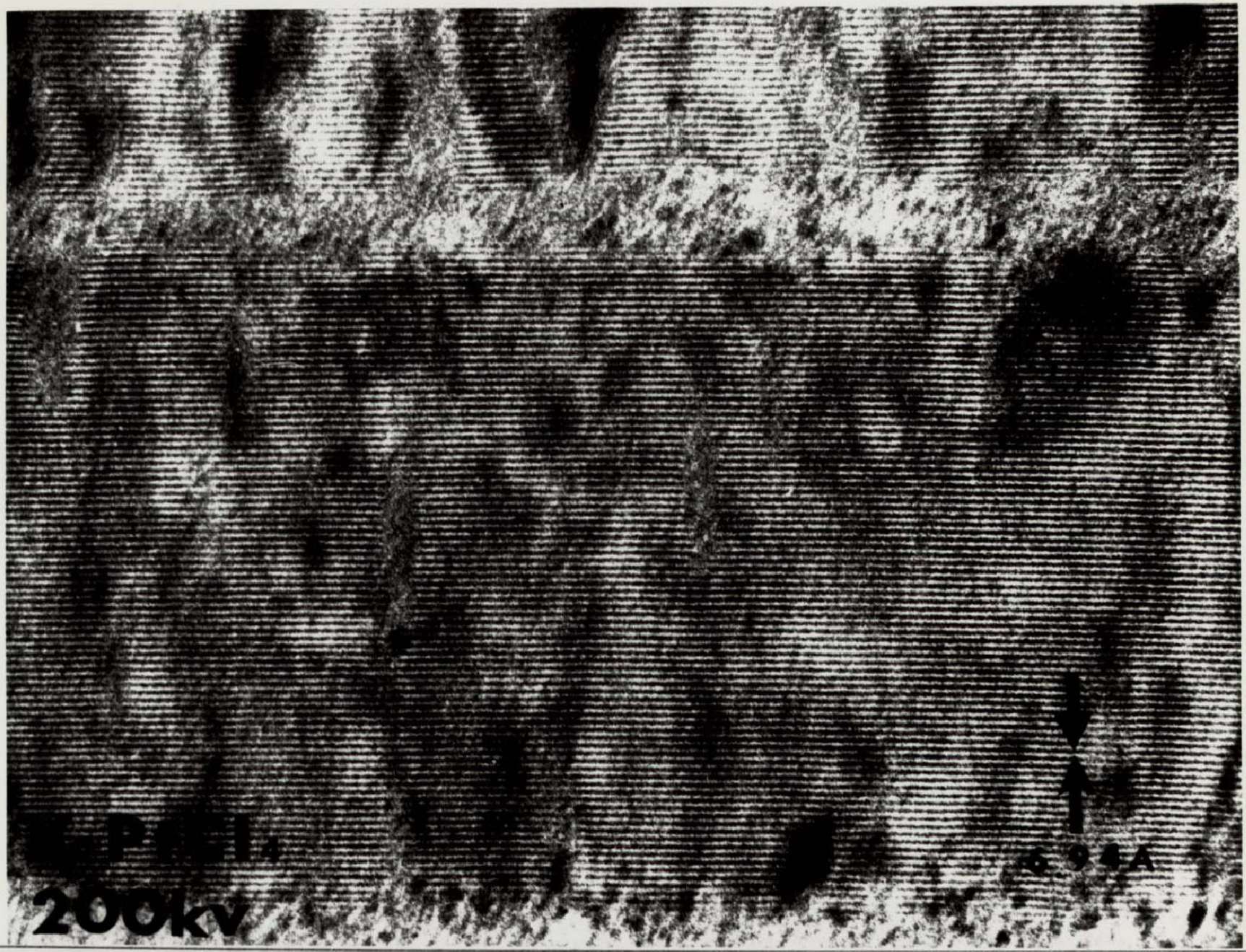


ELECTRON MICROGRAPHS AND ELECTRON DIFFRACTION PATTERNS OF CU-PHTHALOCYANINE CRYSTALLINE LATTICE SHOWING IMPROVED PENETRATION AND REDUCED RADIATION DAMAGE OBTAINED WITH HIGH VOLTAGE ELECTRON MICROSCOPY



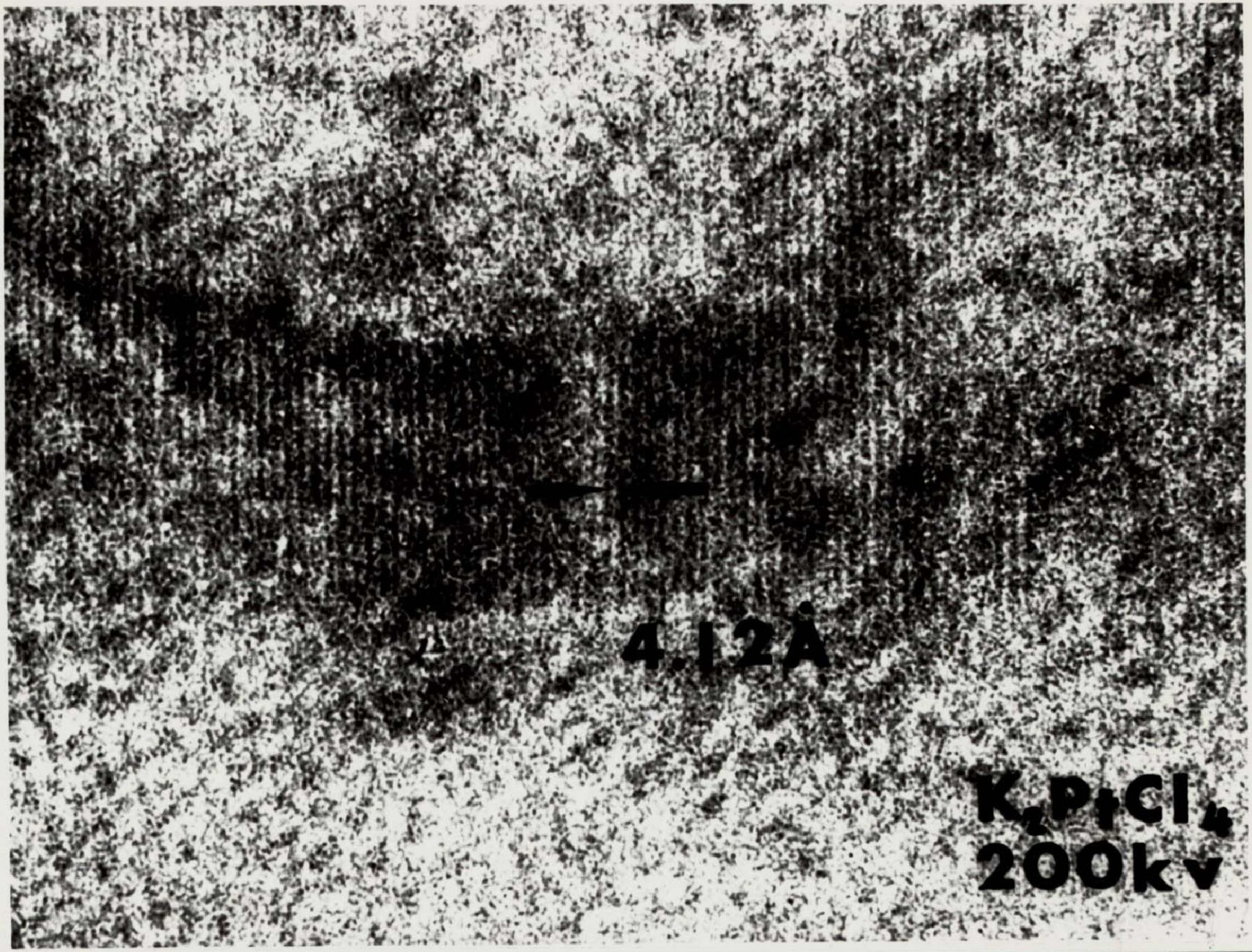
HIGH RESOLUTION 2-4 ANGSTROM ELECTRON MICROGRAPH OF CRYSTALLINE LATTICE

RECORDED WITH HIGH VOLTAGE H.U. 200KV MICROSCOPE



HIGH RESOLUTION 2-4 ANGSTROM ELECTRON MICROGRAPH OF CRYSTALLINE LATTICE

RECORDED WITH HIGH VOLTAGE H.U. 200 KV MICROSCOPE

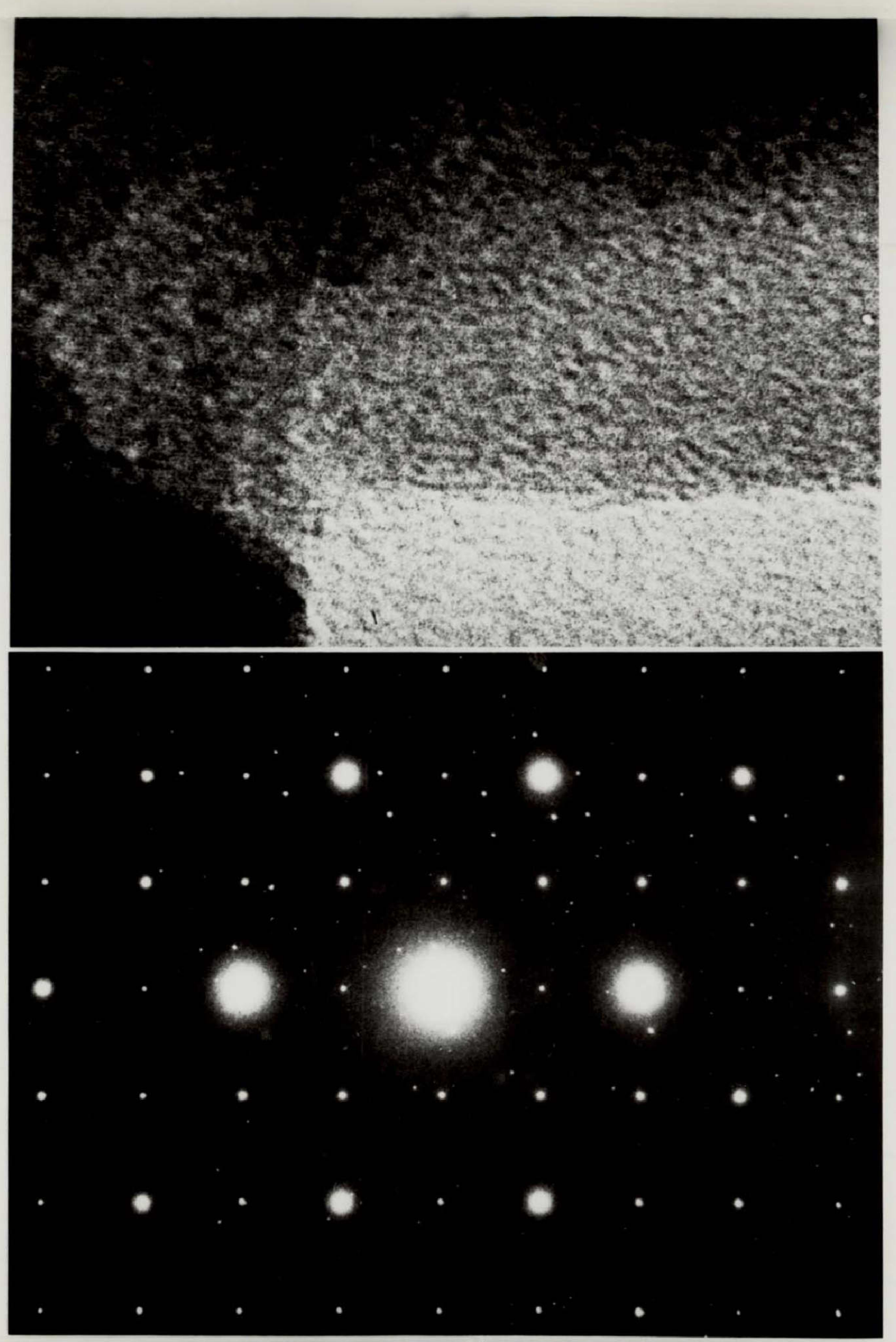


HIGH RESOLUTION 2-4 ANGSTROM ELECTRON MICROGRAPH OF CRYSTALLINE LATTICE

RECORDED WITH HIGH VOLTAGE H.U. 200 KV MICROSCOPE

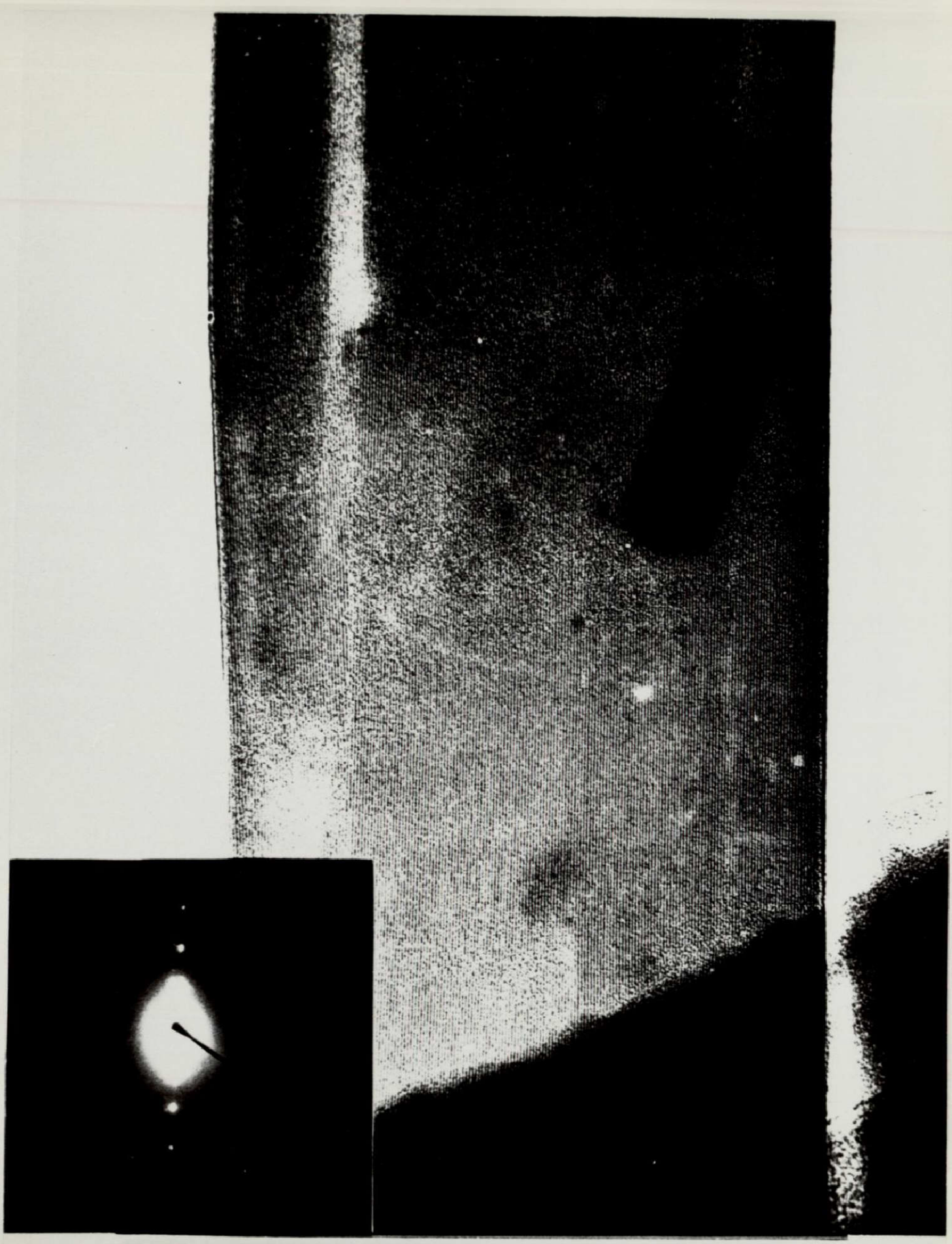
HIGH RESOLUTION 2-4 ANGSTROM ELECTRON MICROGRAPH OF CRYSTALLINE LATTICE

RECORDED WITH HIGH VOLTAGE H.U. 200 KV MICROSCOPE

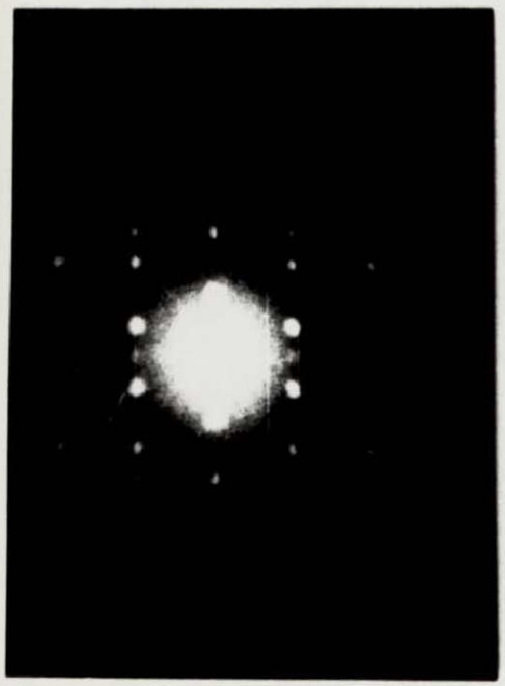


CALIBRATION HIGH RESOLUTION ELECTRON MICROGRAPH AND ELECTRON DIFFRACTION OF CRYSTALLINE LATTICE. 200 KV

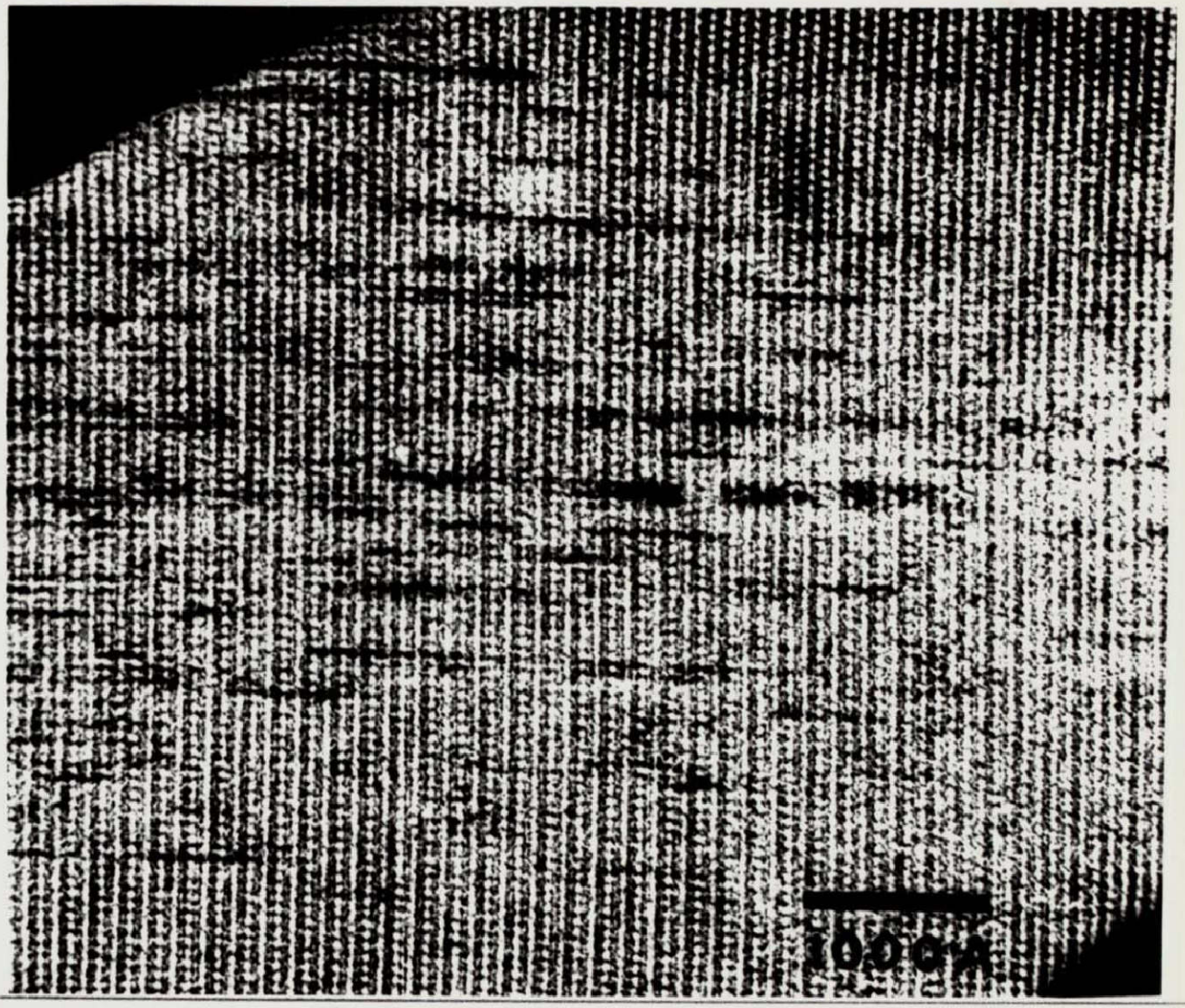
MOLYBDENUM TRIOXIDE 110 WITH 3.81 ANGSTROM RESOLUTION



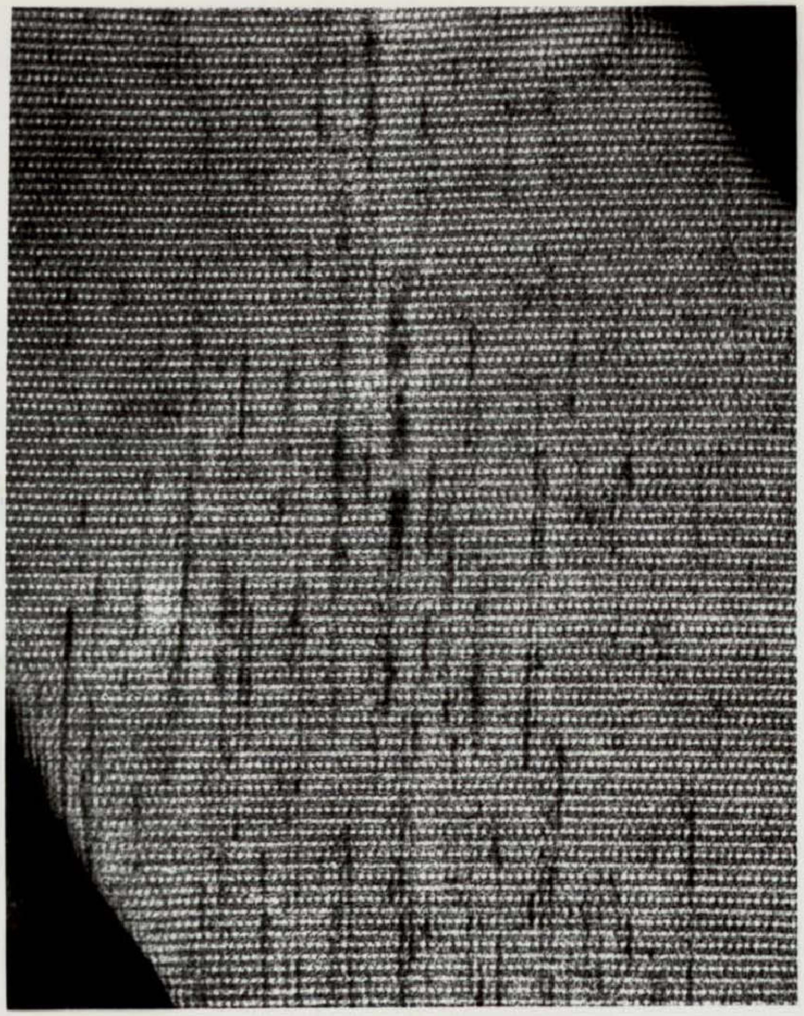
CALIBRATION HIGH RESOLUTION ELECTRON MICROGRAPH AND ELECTRON DIFFRACTION OF CRYSTALLINE LATTICE. 100 K V

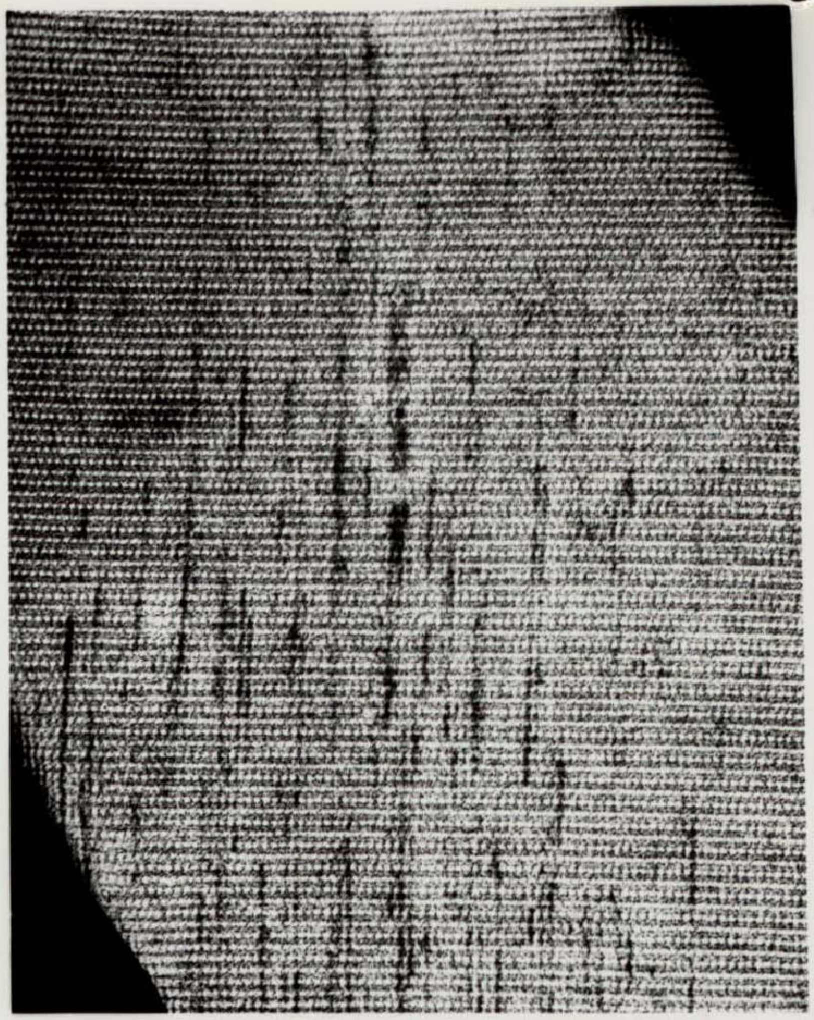


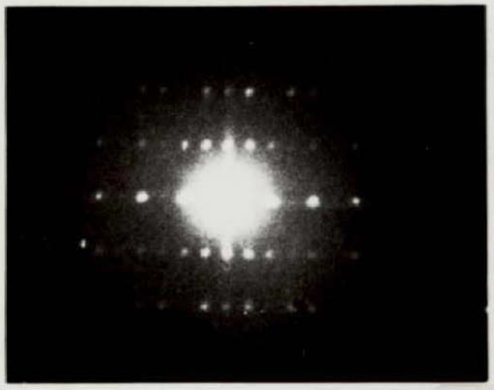
200kv

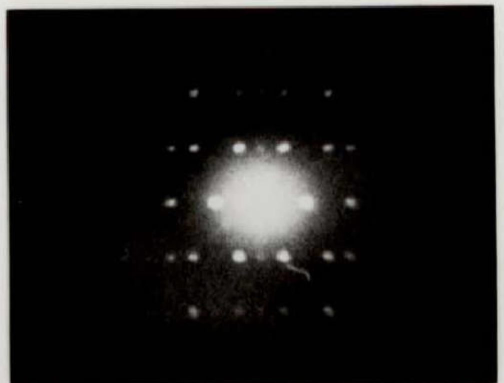


HIGH RESOLUTION 4-10 ANGSTROM ELECTRON MICROGRAPH OF THICK 500 ANGSTROM CATALASE CRYSTAL RECORDED WITH HIGH VOLTAGE H.U. 200KV MICROSCOPE

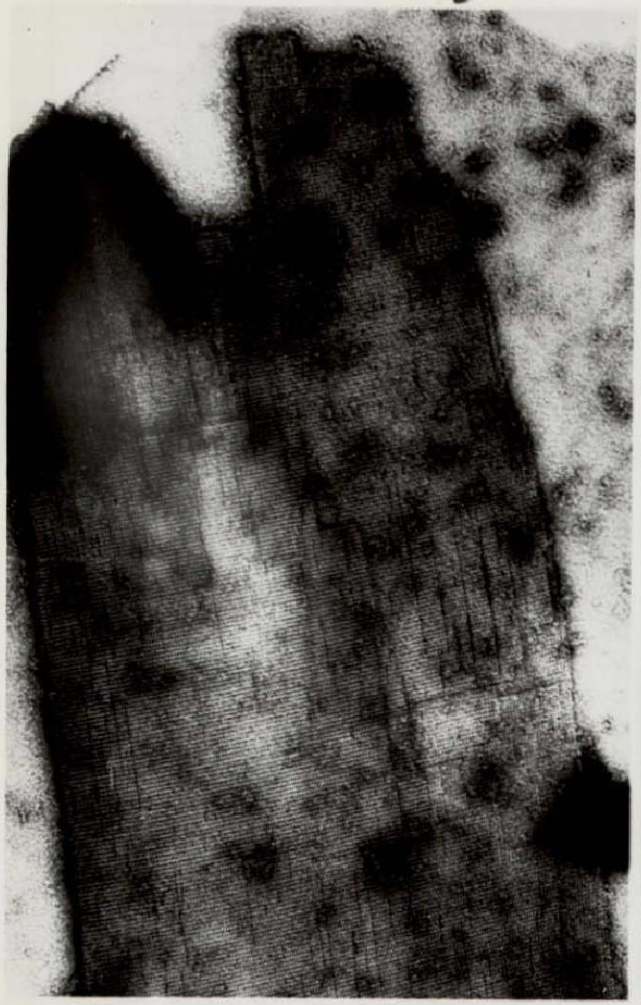


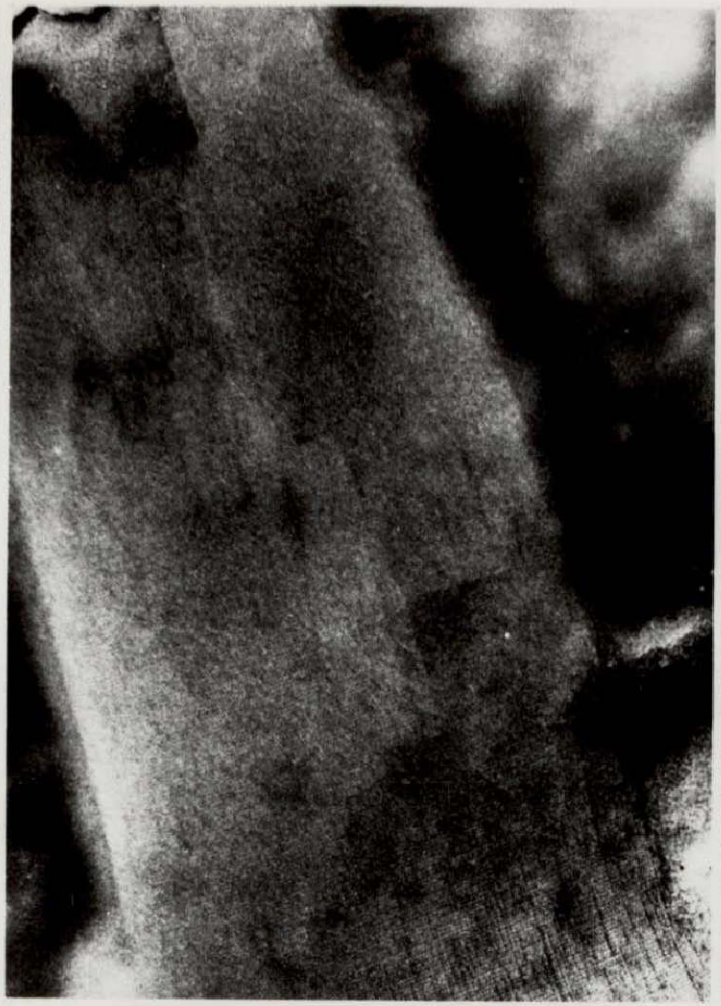


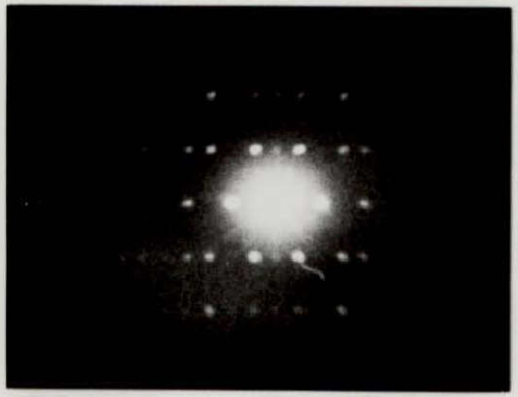


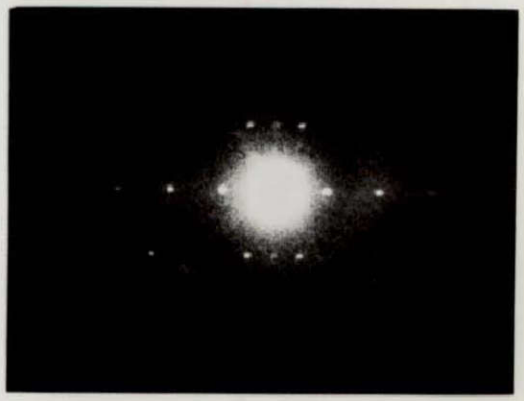


CALIBRATION: HIGH RESOLUTION ELECTRON MICROGRAPH AND ELECTRON
DIFFRACTION OF CRYSTALLINE LATTICES, 200 KV
CATALASE CRYSTALS WITH SMALL ANGLE PATTERN



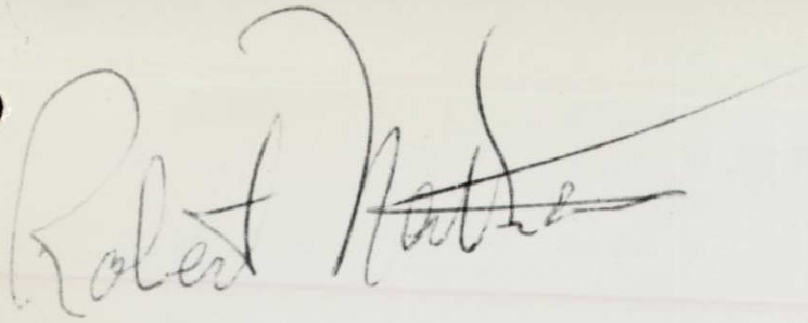






CALIBRATION — HIGH RESOLUTION ELECTRON MICROGRAPH AND ELECTRON DIFFRACTION OF CRYSTALLINE LATTICE — 200KV

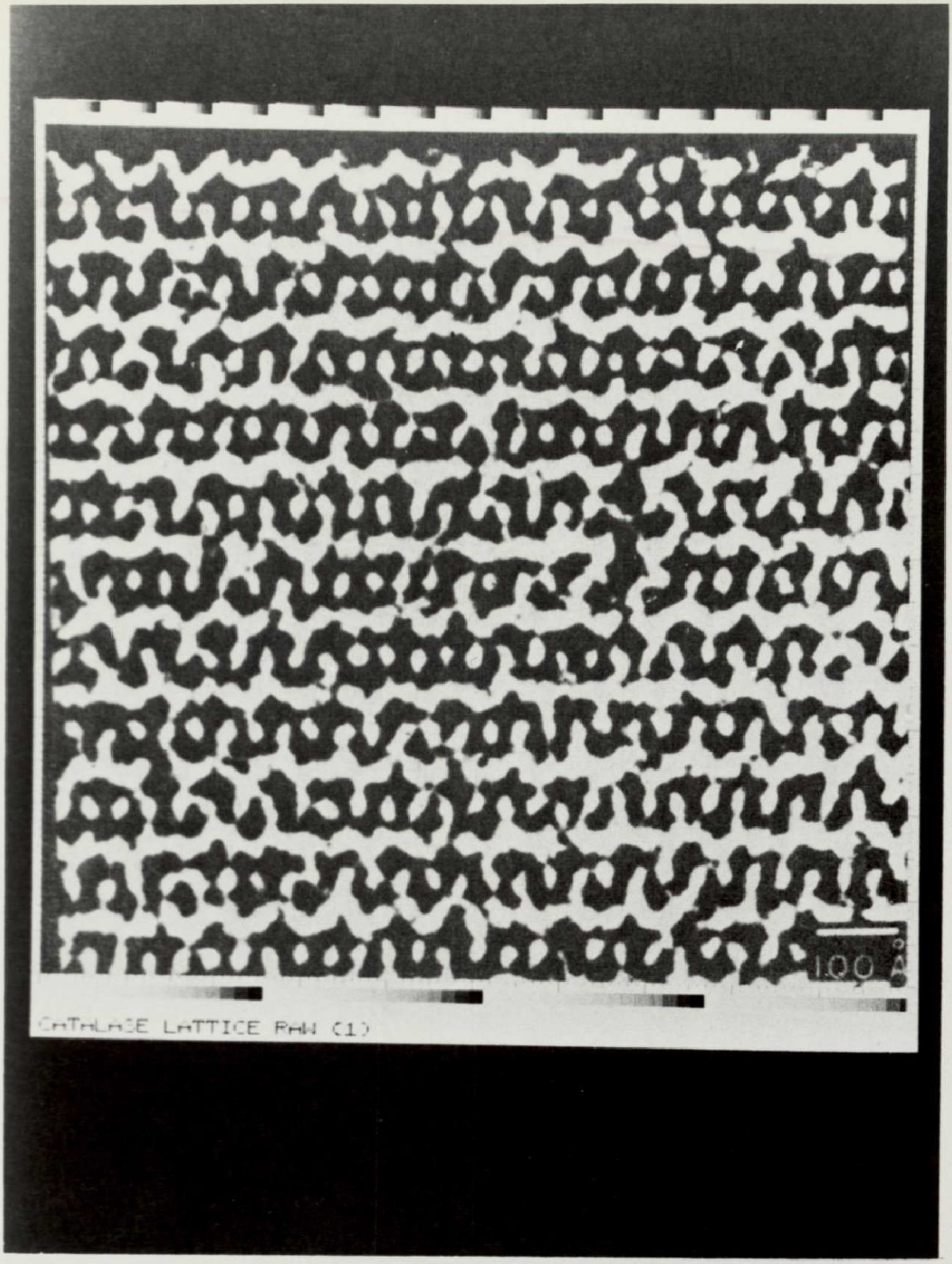
DARK FIELD PHASE CONTRAST WITH SPECIAL APERTURE



COMPUTER ENHANCEMENT OF ELECTRON MICROGRAPHS

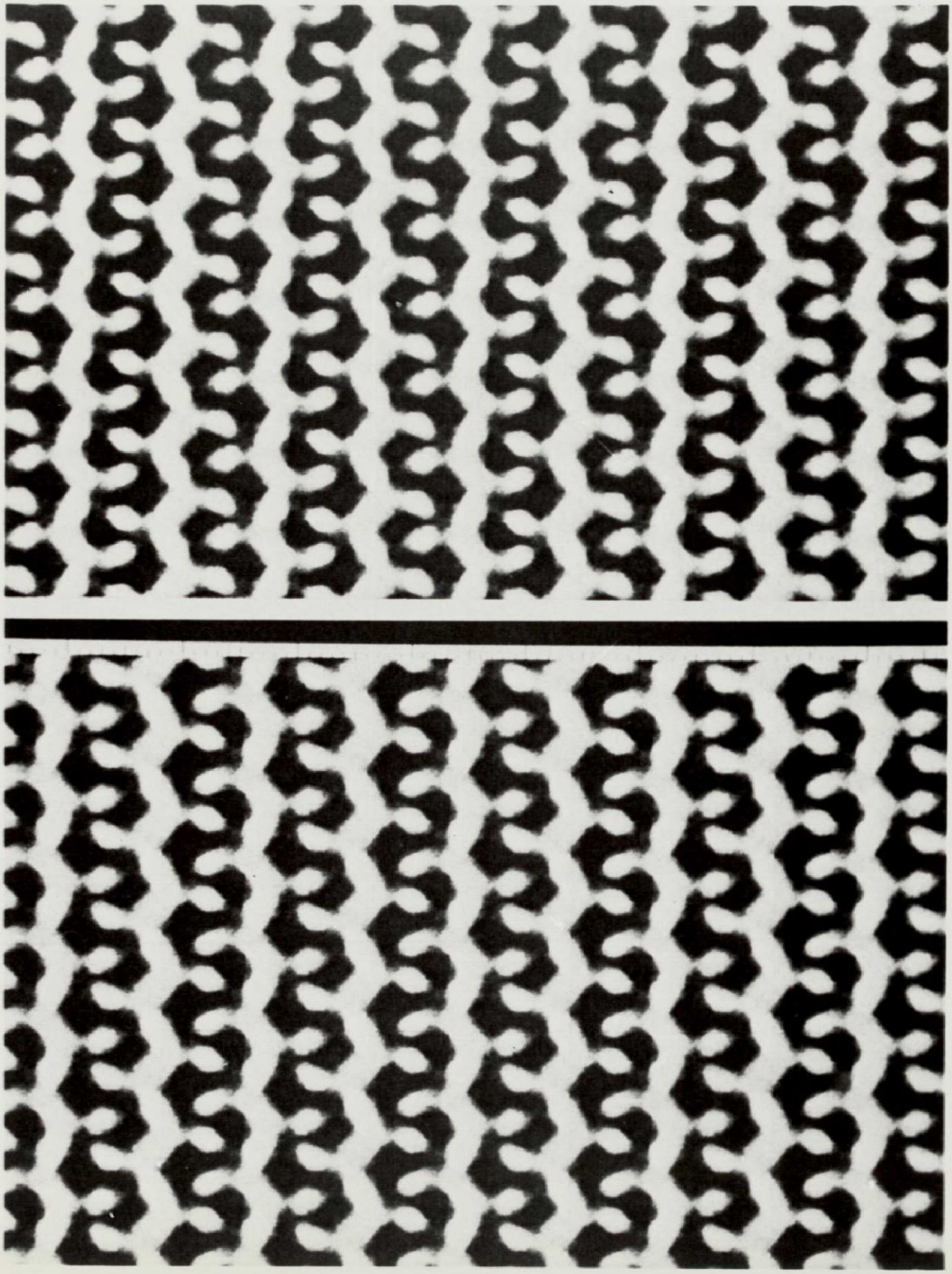
Robert Nathan, Ph.D

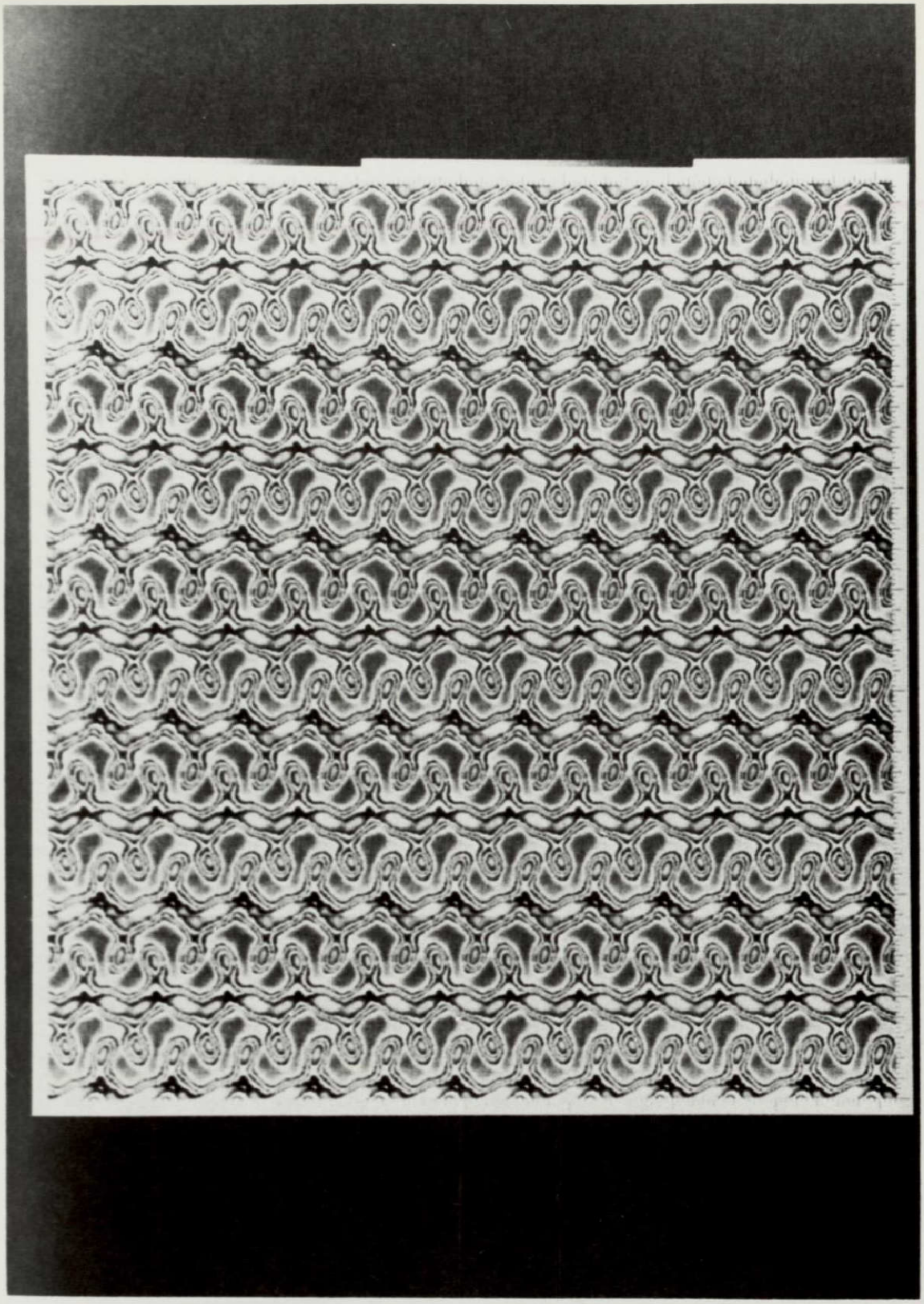
Staff Scientist
Jet Propulsion Laboratory of the
California Institute of Technology



9. Raw Catalase Micrograph - Negatively stained

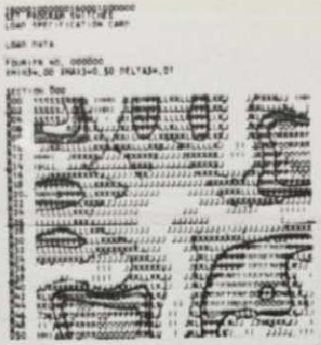
12. Stereo pair is result of rotating image by 180Å and placing it next to original.



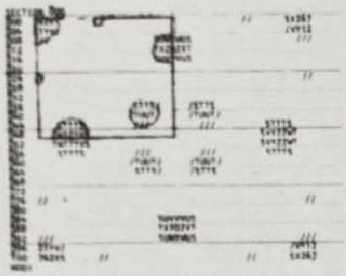


High order digital bits removed and contrast stretched on remaining bits gives artificial contouring and brings out detail.

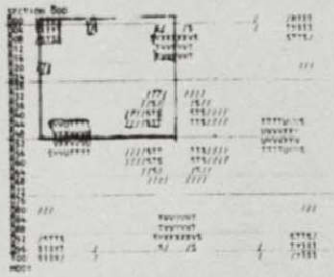
1/4 UNIT CELL-LOW RESOLUTION



1/4 UNIT CELL-SIMULATION OF HIGH RESOLUTION



FULL UNIT CELL-LOW RESOLUTION



FULL UNIT CELL-SIMULATION OF HIGH RESOLUTION

COMPUTER RECONSTRUCTION FROM FOURIER TRANSFOM OF DIFFRACTION MAXIMA

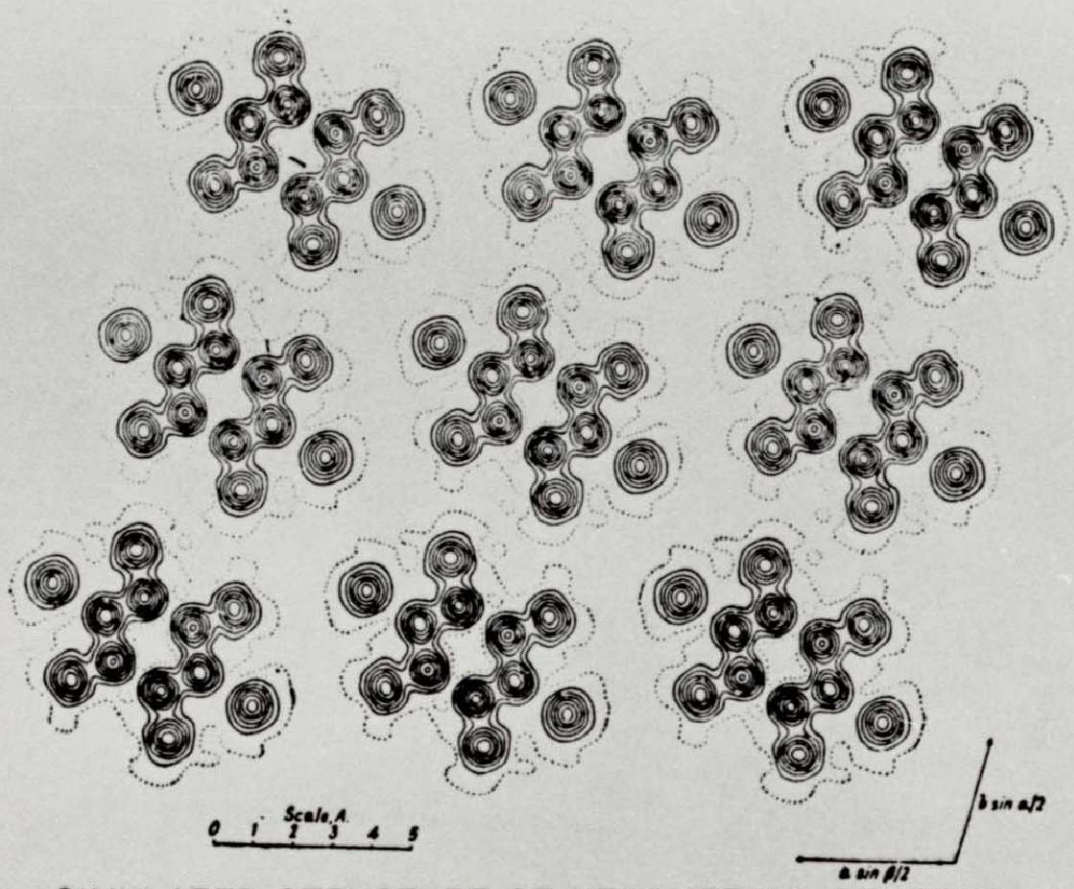
20. Computer Reconstruction From Fourier Analysis of Images in Figures 19 d and f.

(a) Reconstitution of 1/4 cell repeat unit as seen in Figure 19d (low resolution image)

(b) Reconstitution of full unit cell from Fourier components obtained from analysis of Figure 19d. Note loss of image detail.

(c) Reconstitution of 1/4 cell taking phase and magnitude of Fourier components from both on-axis (Figure 19d) and off-axis (Figure 19f) images to form single high resolution image resembling Figure 19 b.

(d) Full unit cell simulation of high resolution (See Figure 20 c). Note increased sharpness and appearance of non-round shape.



SIMULATED STRUCTURE OF HEXAMETHYL BENZENE AS DETERMINED BY X-RAY CRYSTALLOGRAPHIC METHODS

21. Atomic resolution obtained by X-ray methods for simpler substances. This resolution is goal of present effort on electron microscope, but is expected to be applied to more complex organic substances.

DOUGLAS PAPER 10,247

CHARACTERIZATION OF ORDERED WATER IN HYDROPHILIC MEMBRANE PORES

MARCH 1969

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TO BE PRESENTED TO THE
SECOND INTERNATIONAL MEETING
INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY
MILAN, ITALY
SEPTEMBER 1-5, 1969

MCDONNELL DOUGLAS
CORPORATION

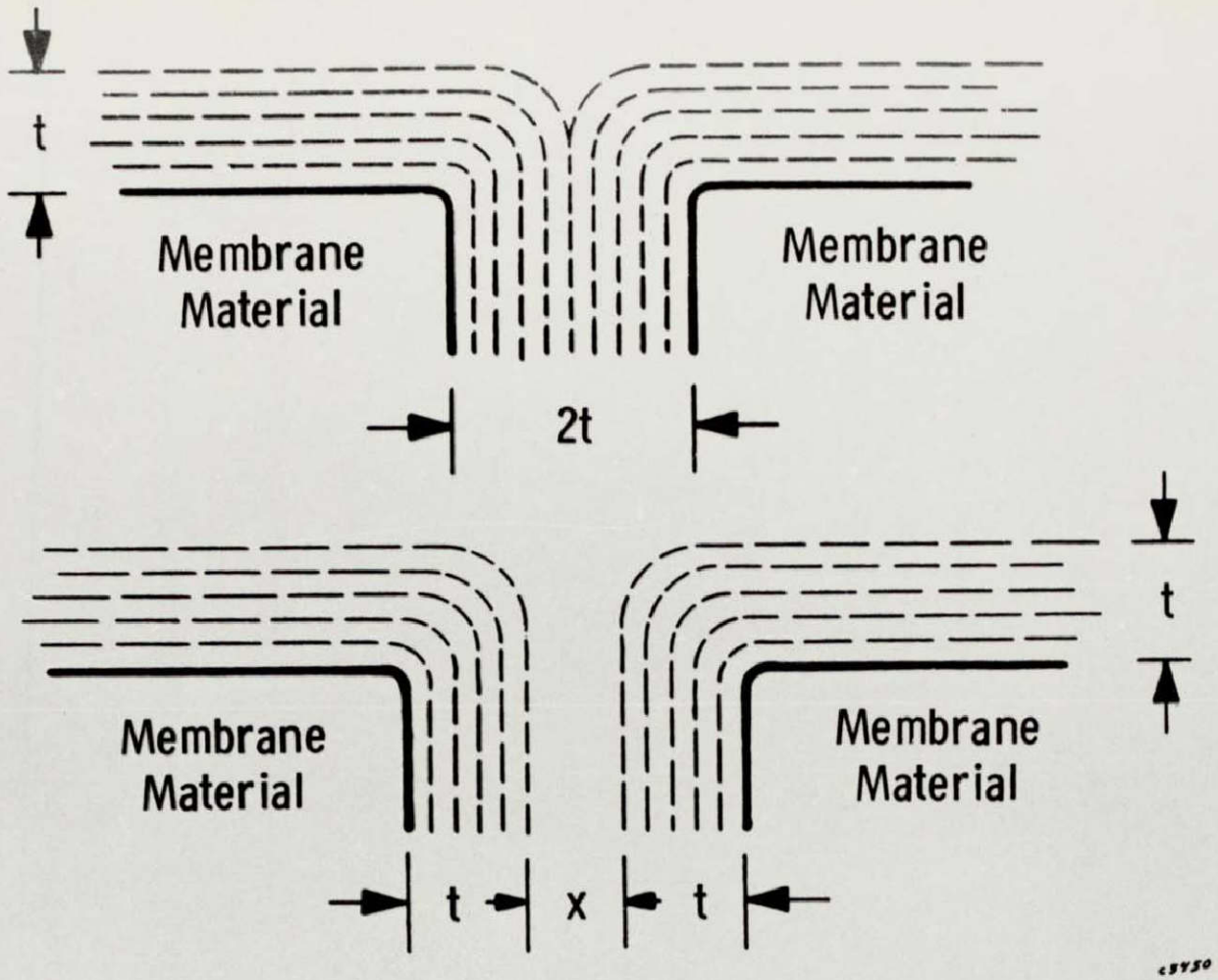

FIGURE 1. Diagrammatic representation of ordered water sheath (thickness, t) in the vicinity of a pore in a hydrophilic membrane. Top diagram shows critical diameter (2 t) of hole filled with ordered water that will exclude all ions; bottom diagram shows hole lined with ordered water that will allow ions of width ($<x$) to pass through unordered water channel of diameter (x).

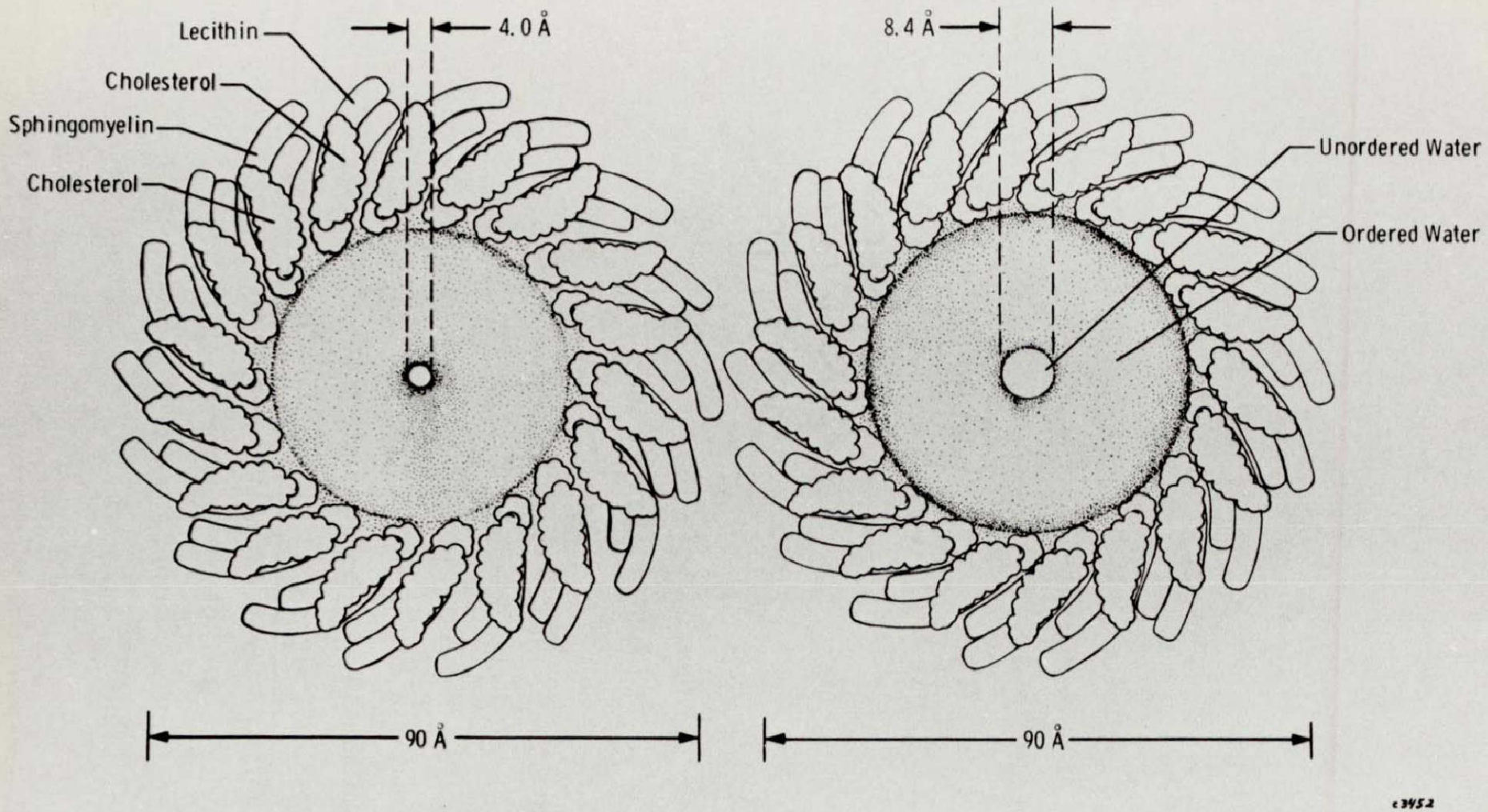

FIGURE 4. Molecular model of lipid water components of an "hexagonal" subunit in a plasma membrane. Left diagram shows top monolayer in partially closed "resting" configuration. Right diagram shows top monolayer in open configuration. Each subunit contains two monolayers back to back, with the cholesterol leaves outside.

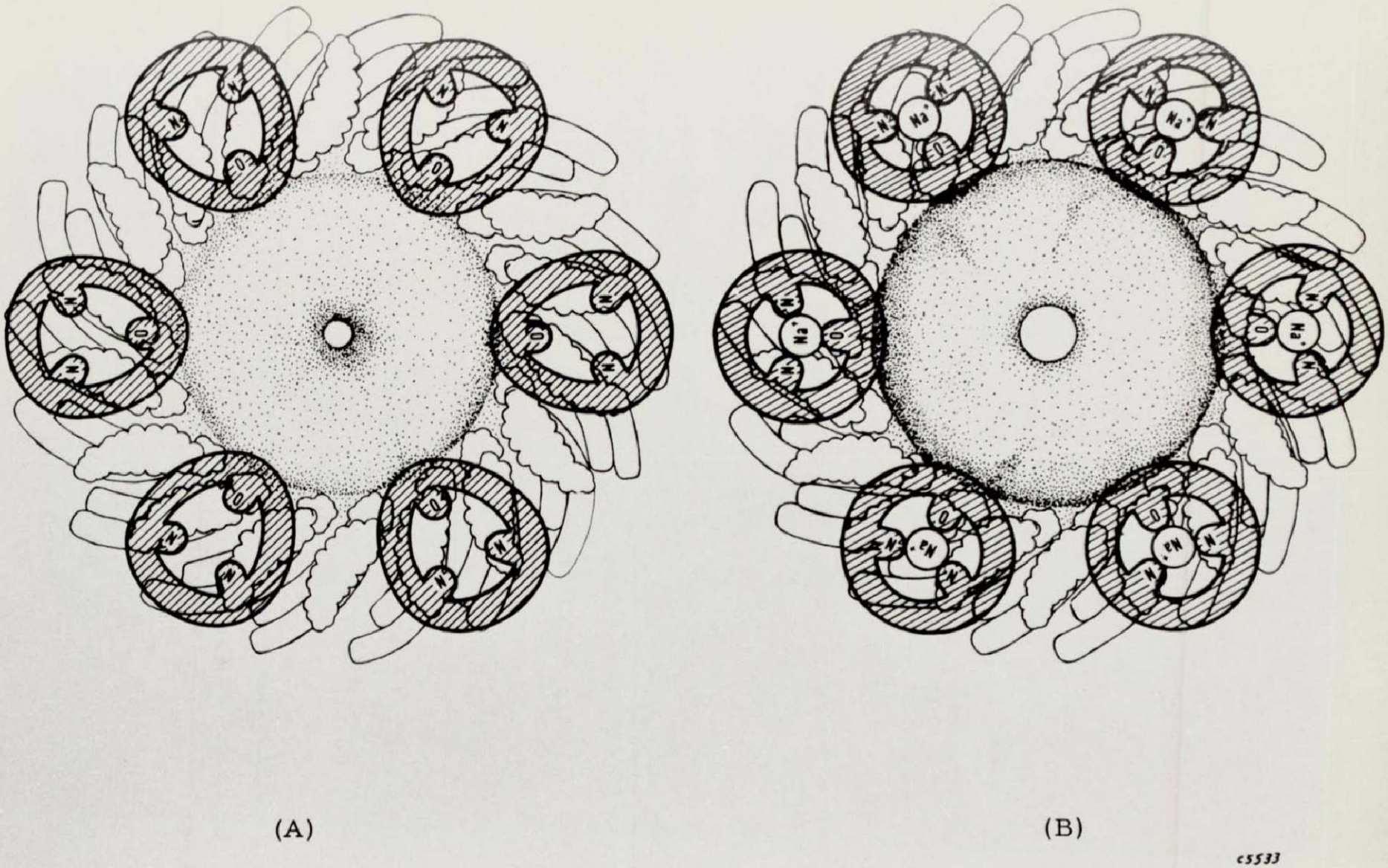

FIGURE 7. Schematic representation of (A) six alamethicin molecules adsorbed on hexagonal subunit in resting configuration (B) allosteric chelation interaction of six $Na(H_2O)_3^+$ ions with six alamethicin molecules to effect an open ion-gate configuration.

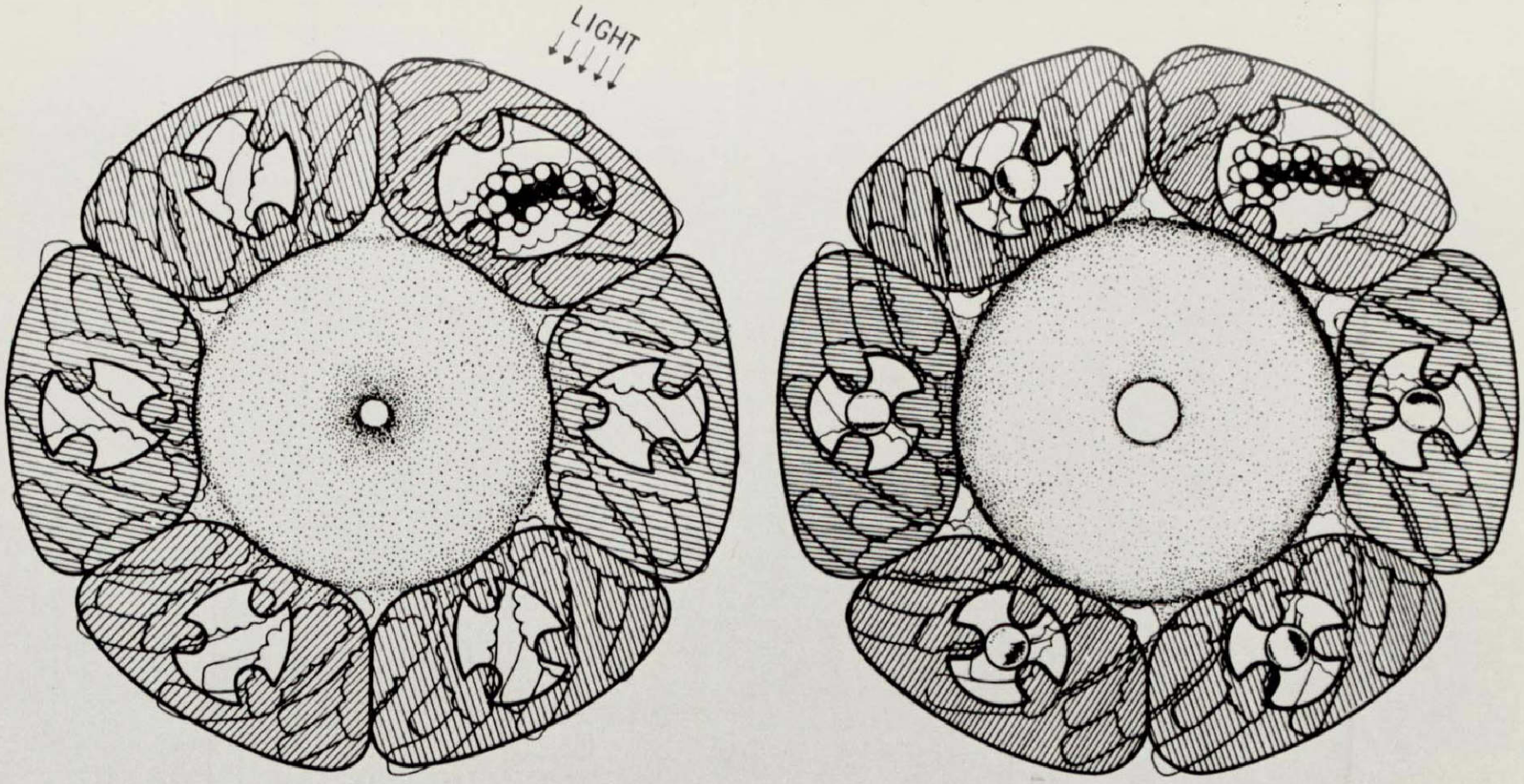

FIGURE 8. (A) Hypothetical arrangement of one rhodopsin molecule and several EIM (or EIM-like) molecules adsorbed on the interior surface of a lipid ring of a hexagonal subunit in the retinal rod membrane.
(B) Representation of the ion-gating allosteric transition resulting from the adsorption of one photon by 11-cis retinal of a rhodopsin molecule and the chelation of sodium ions by the EIM (or EIM-like) molecules.

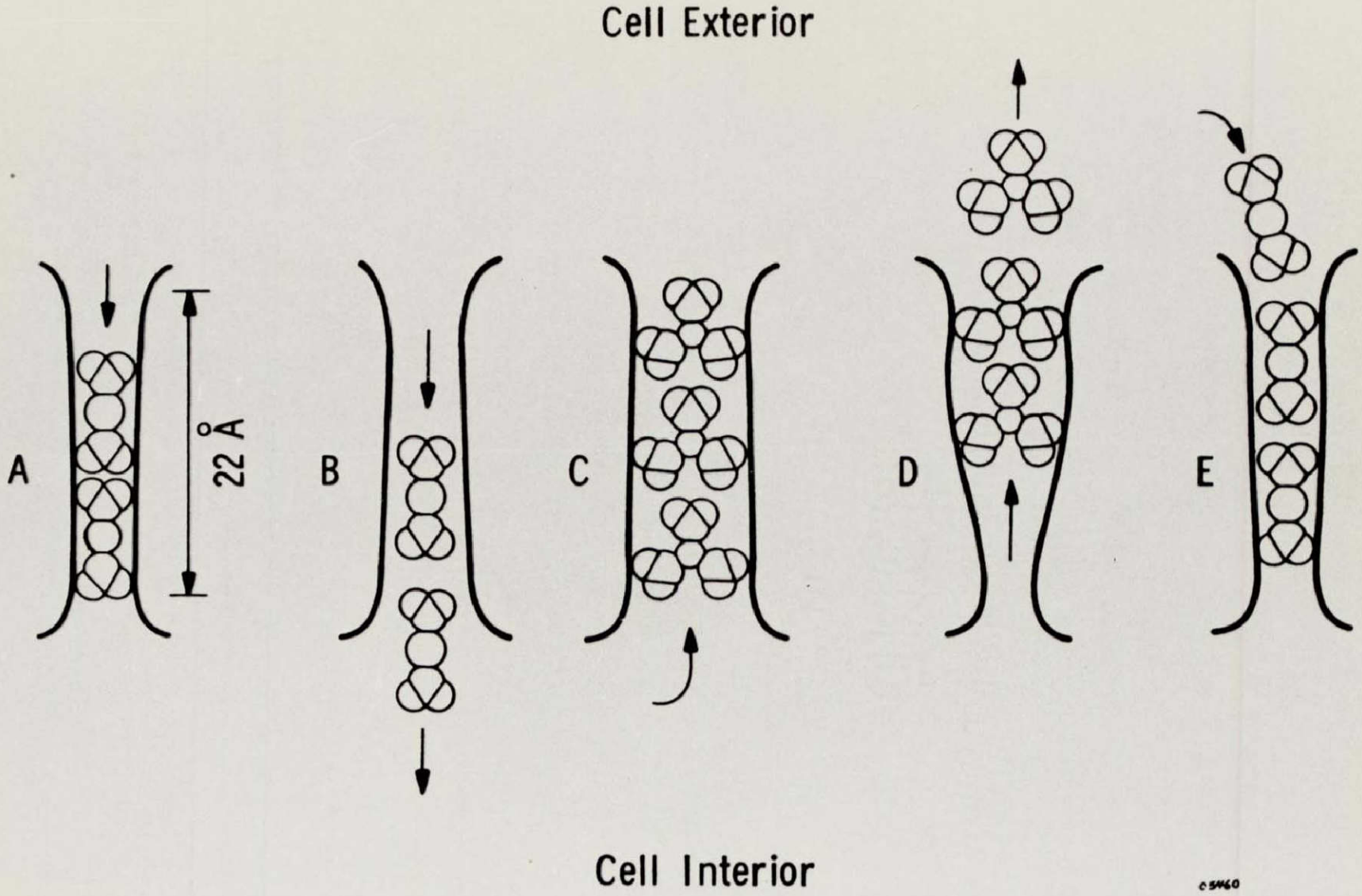

FIGURE 9. Sequential configurational changes of the pore of unordered water of an hexagonal subunit during the operation of the sodium pump.

DOUGLAS PAPER 10,246

ULTRASTRUCTURE AND VARIABLE APERTURE PORE FUNCTION OF HEXAGONAL SUBUNITS IN PLASMA MEMBRANES

MARCH 1969

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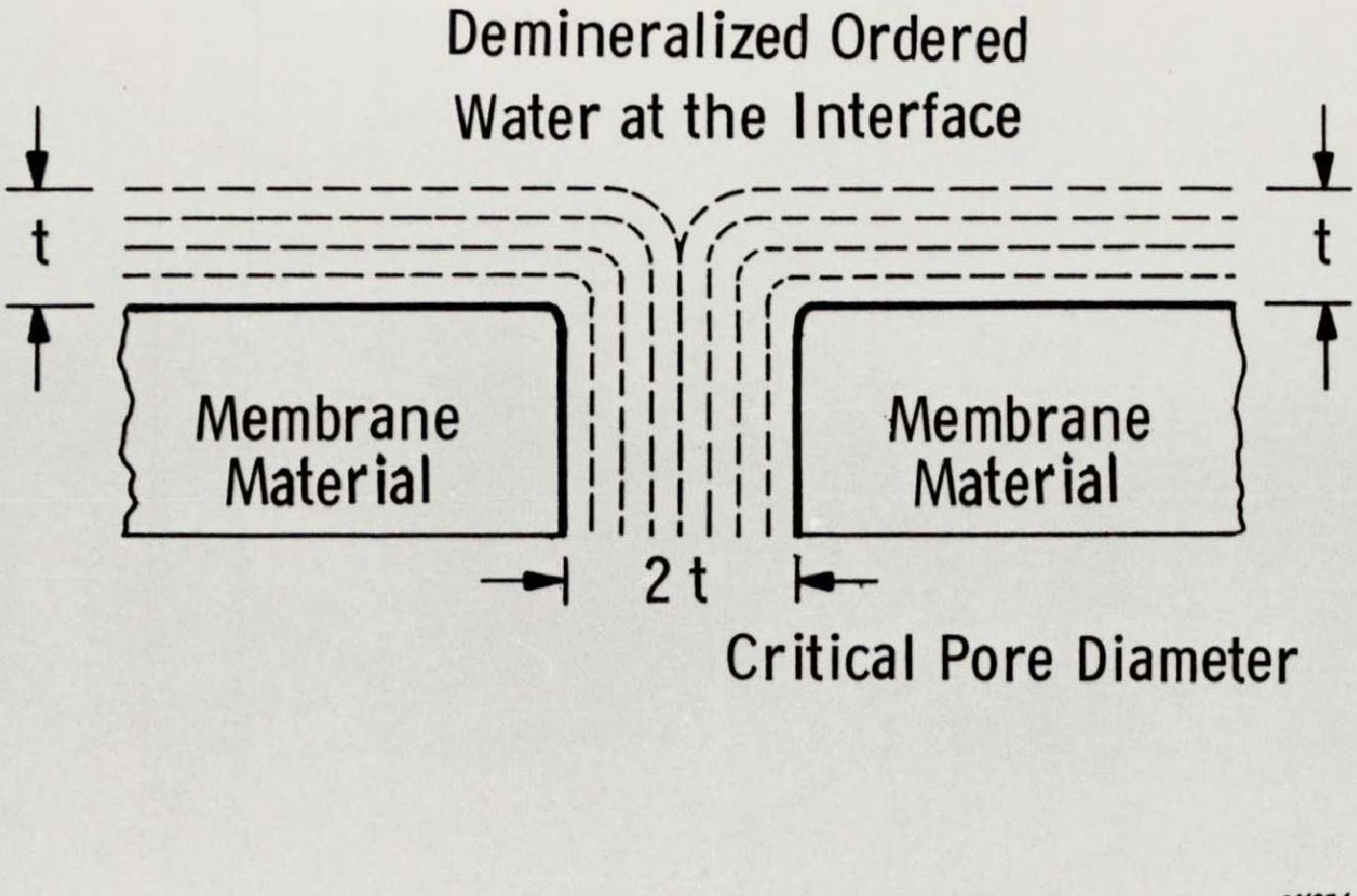


Figure 1. Relationship between salt-free ordered water hydration sheath at hydrophilic membrane interface and critical pore diameter for desalination.

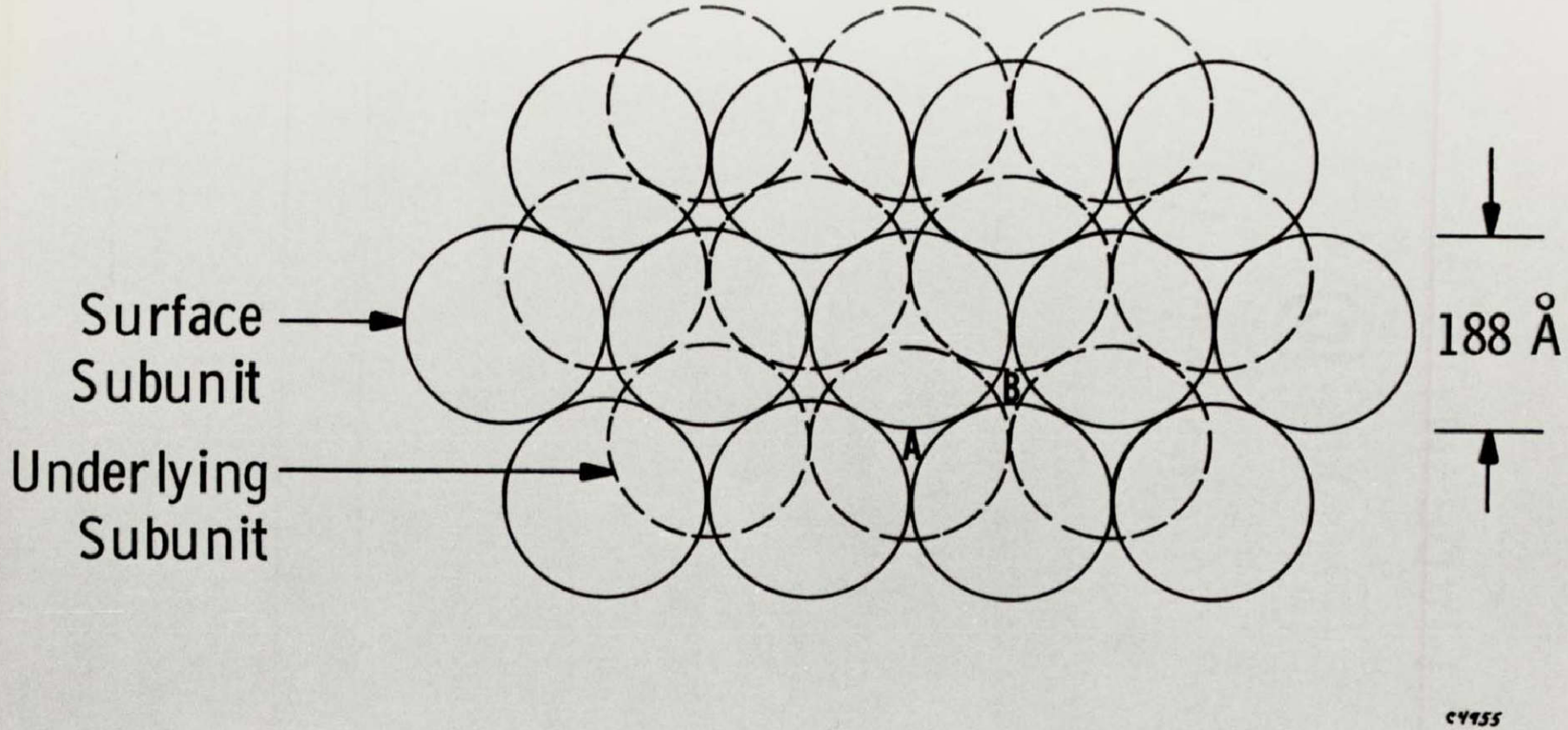


Figure 4. Active desalination layer of cellulose acetate membrane idealized as assembly of close packed 188 Å diameter spheres. Ordered water permeates through interstitial pores (A and B).

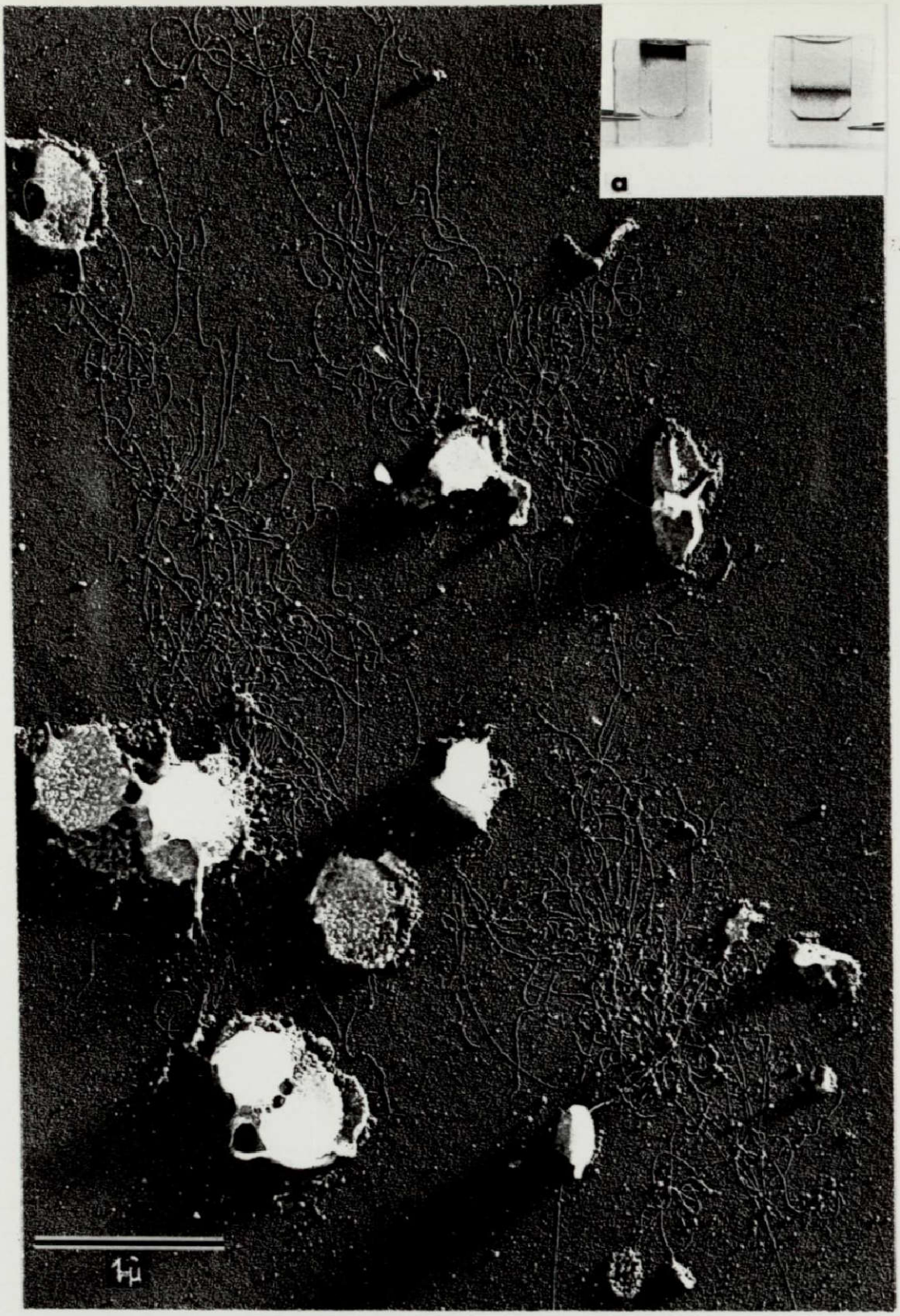


Plate Ia) Microcuvettes before and after centrifugation.

Plate Ib) Portion of an osmotically disrupted chloroplast showing DNA strands associated with membranes. Platinum shadowed. (X 30,000)

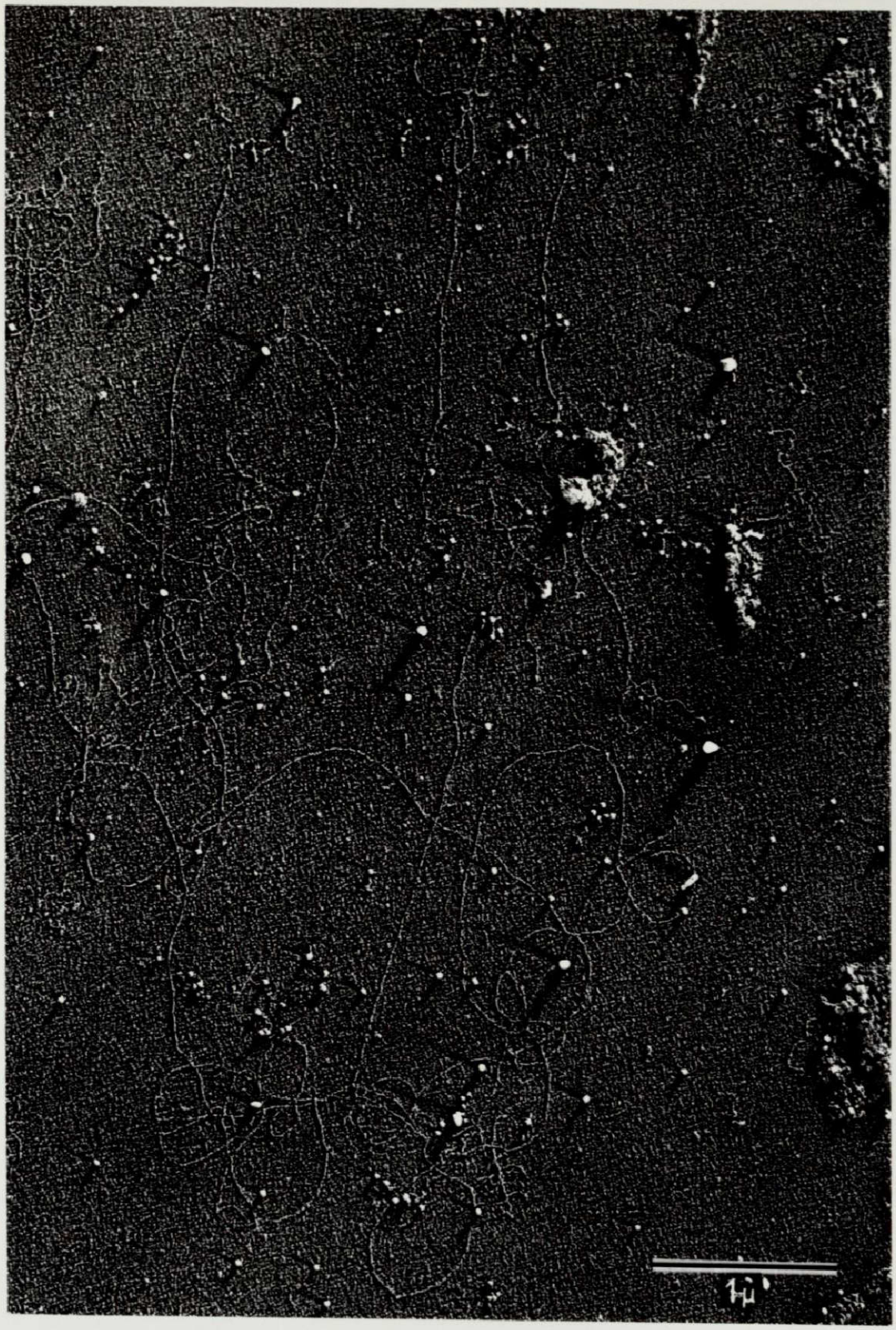
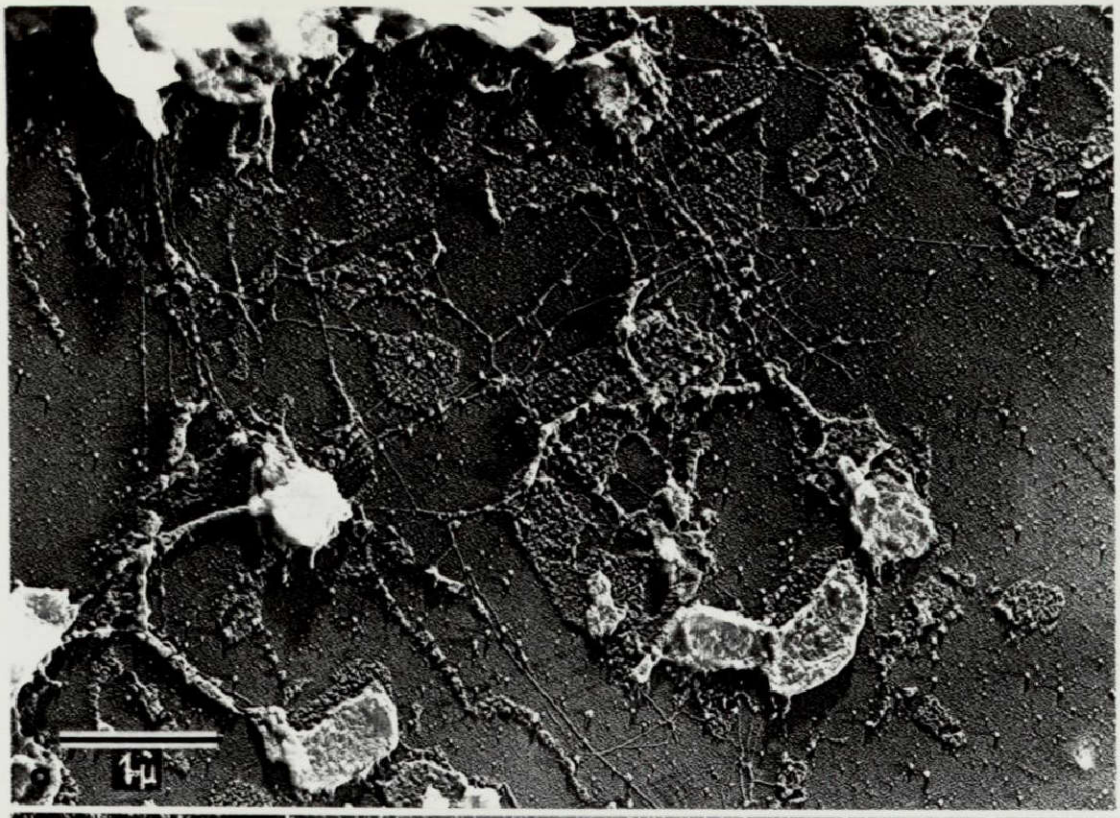


Plate II) Portion of a fully dispersed chloroplast showing typical DNA conformations. Platinum shadowed on carbon-mica substrate. (X 29,000)



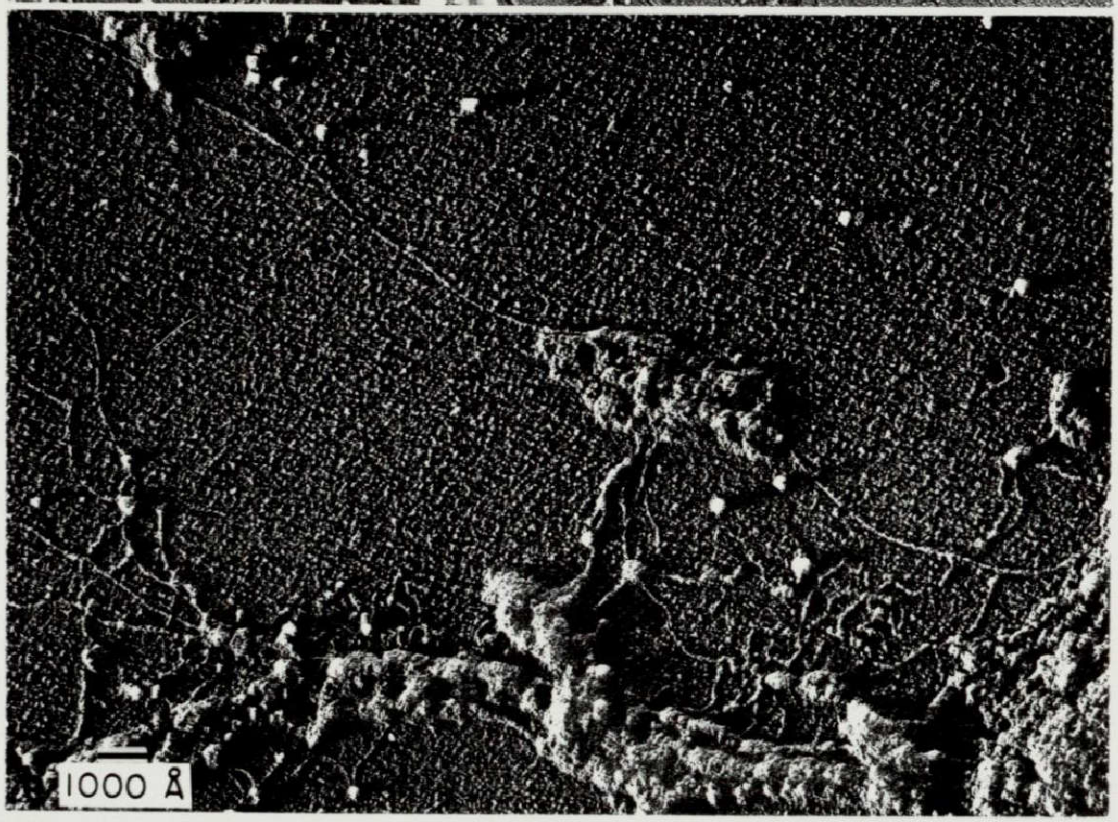


Plate IIIa) Part of dispersed chloroplast showing DNA associated with granal and intergranal membranes. (X 20,000)

Plate IIIb) "Linear" and "mesh" forms of DNA attached to membranes. (X 62,000)

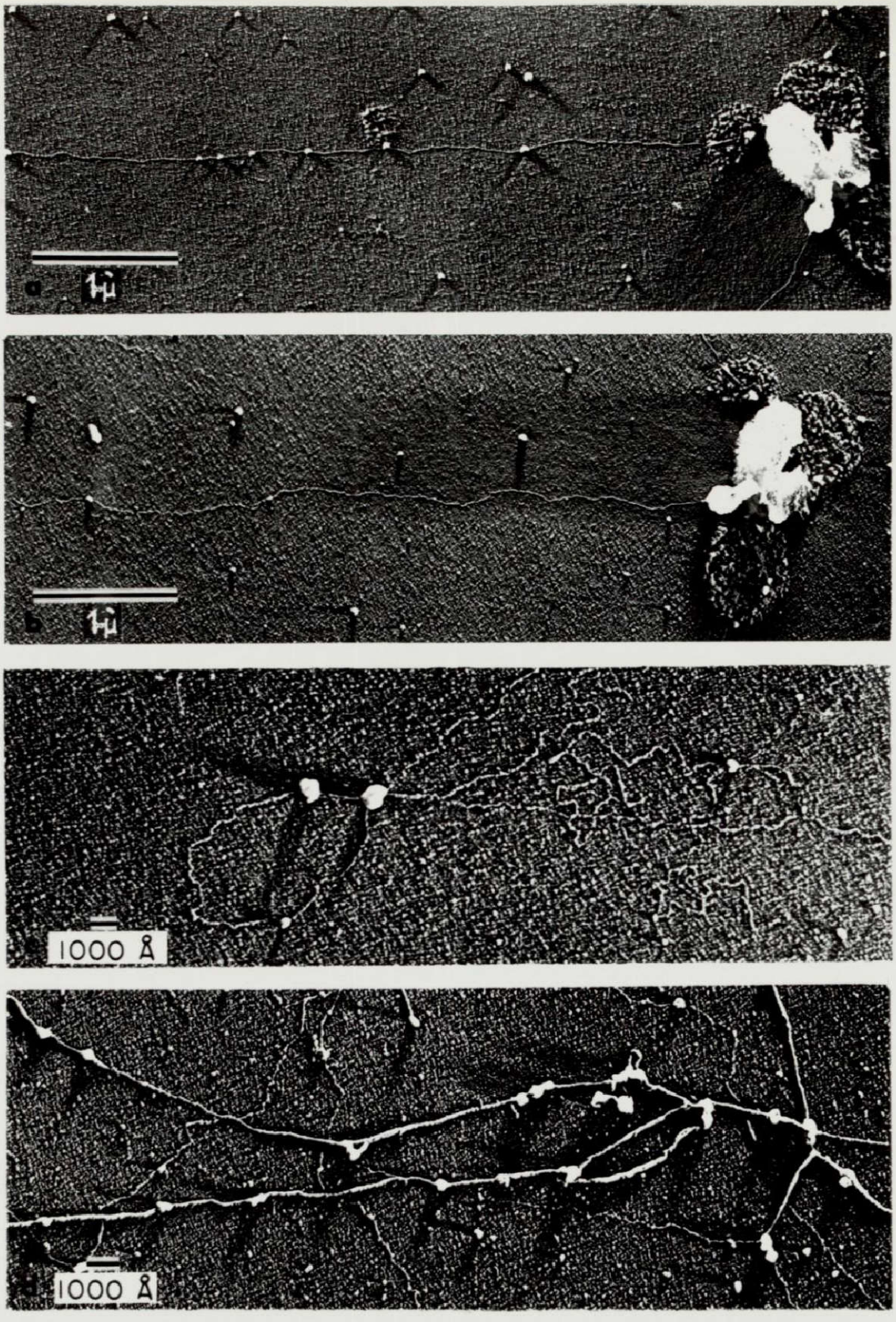


Plate IVa),b),c) DNA molecules extracted from purified chloroplasts. Platinum shadowed. (X 23,000)(X 40,000)

Plate IVd) DNA extracted from the nuclear fraction. Platinum shadowed. (X 50,000)

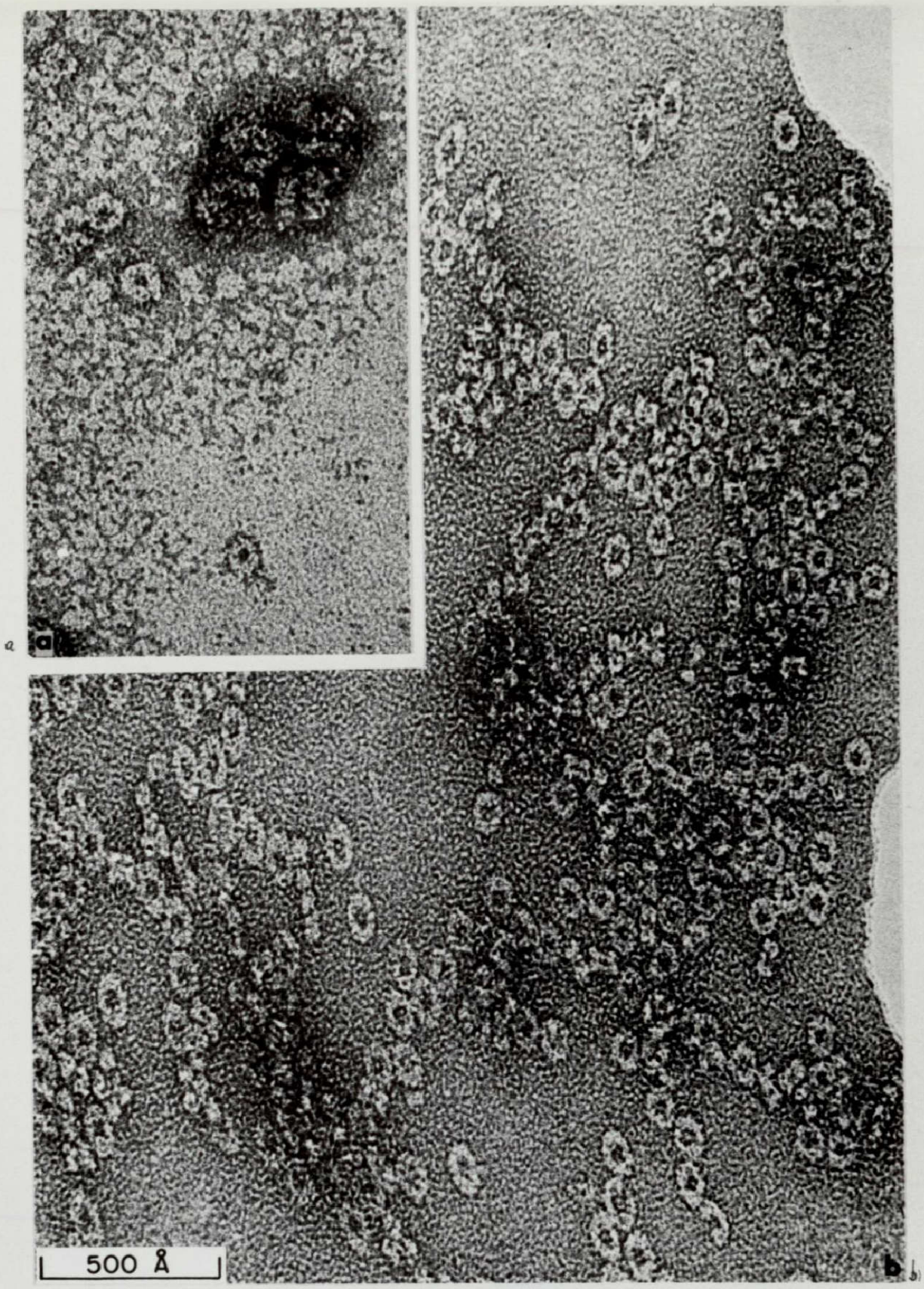


Plate I. (a) Hemolymph of Limulus polyphemus stained with uranyl acetate showing, in addition to hemocyanin molecules, typical ring structures about 100Å in diameter.

(b) Hemagglutinin isolated from Limulus polyphemus stained with uranyl formate showing uniform 100Å particles with same kind of ring structure. X 550,000.

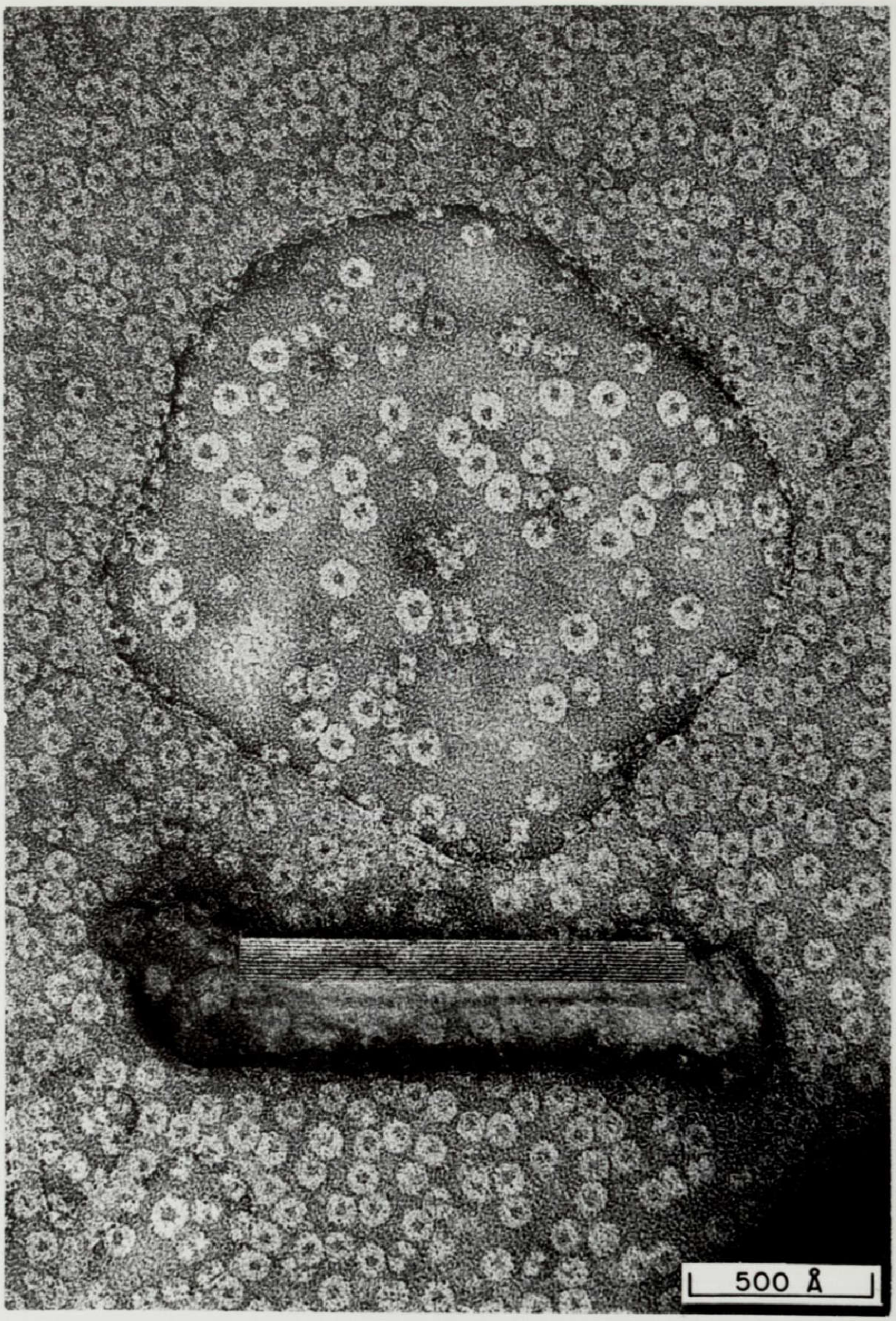


Plate II. Limulus hemagglutinin fixed with glutaraldehyde, stained and embedded in ultrathin film of uranyl formate extending unsupported over hole of fenestrated carbon film. In central areas the ring-shaped molecules are often distended, displaying their fine structure in various orientations. X 550,000. Asbestos filament (insert: X 1,000,000) exhibits lattice period of 7.3Å which serves as accurate calibration standard.

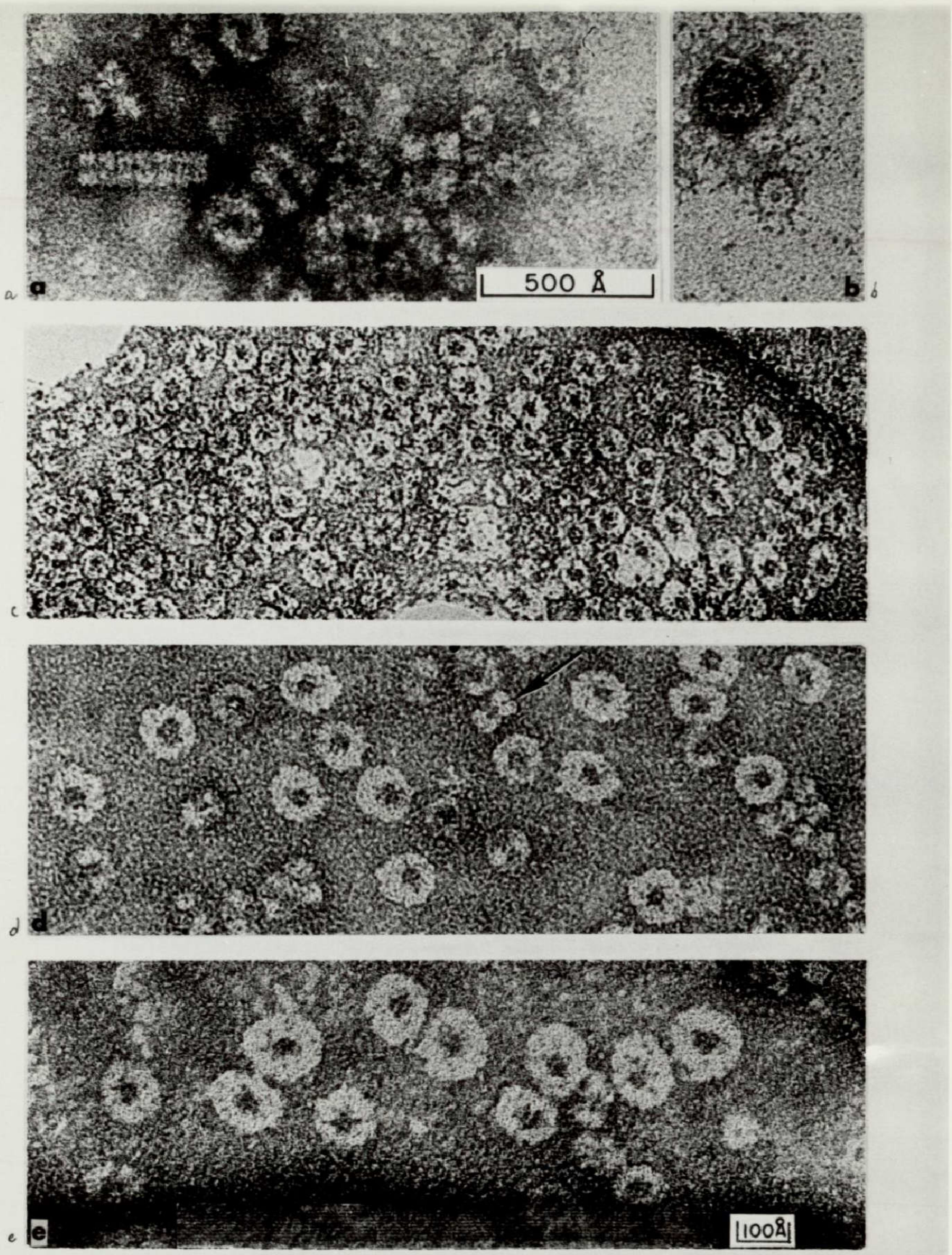
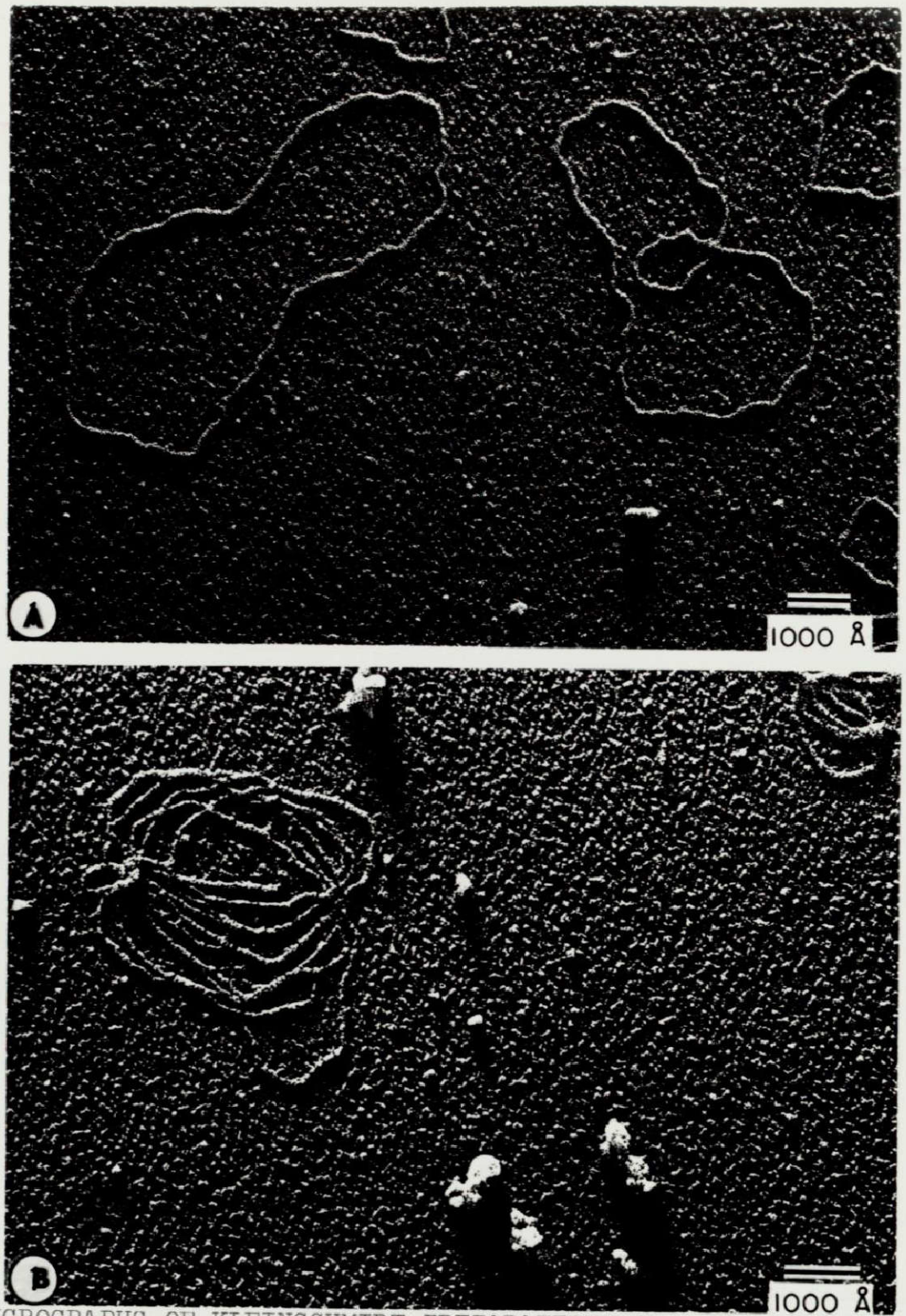


Plate III. Structural details of ring-shaped molecules in (a) fresh Limulus hemolymph stained with phosphotungstate, and uranyl acetate (b); (c) Limulus hemagglutinin stained with uranyl formate without prior fixation, and after fixation with glutaraldehyde (d,e). X 550,000; X 800,000.

ELECTRON MICROGRAPHS OF KLEINSCHMIDT PREPARATIONS SHOWING: (a) CIRCULAR DNA OF ØX 174rf 21S; (b) MOLECULAR COMPLEXES RESULTING FROM THE REACTION OF THIS DNA WITH E. COLI RNA POLYMERASE UPON ADDITION OF THE FOUR REGULAR NUCLEOSIDE TRIPHOSPHATES: ATP, CTP, GTP, UTP. AFTER FIFTEEN MINUTES AT ROOM TEMPERATURE CHARACTERISTIC CONVOLUTES FORMED BY FILAMENTS OF VARYING WIDTHS ARE FOUND IN CLOSE ASSOCIATION WITH THE DNA-ENZYME COMPLEX. THESE CORRELATED ELECTRON MICROSCOPIC AND BIOCHEMICAL STUDIES, CARRIED OUT IN COLLABORATION WITH DR. RICHARD GUMPORT AND DR. SAMUEL WEISS AT THE UNIVERSITY OF CHICAGO, ARE EXPECTED TO YIELD FURTHER INFORMATION ON THE PARTICIPATION OF RNA-POLYMERASE IN THE DIFFERENTIAL RNA TRANSCRIPTION UPON DNA TEMPLATES.



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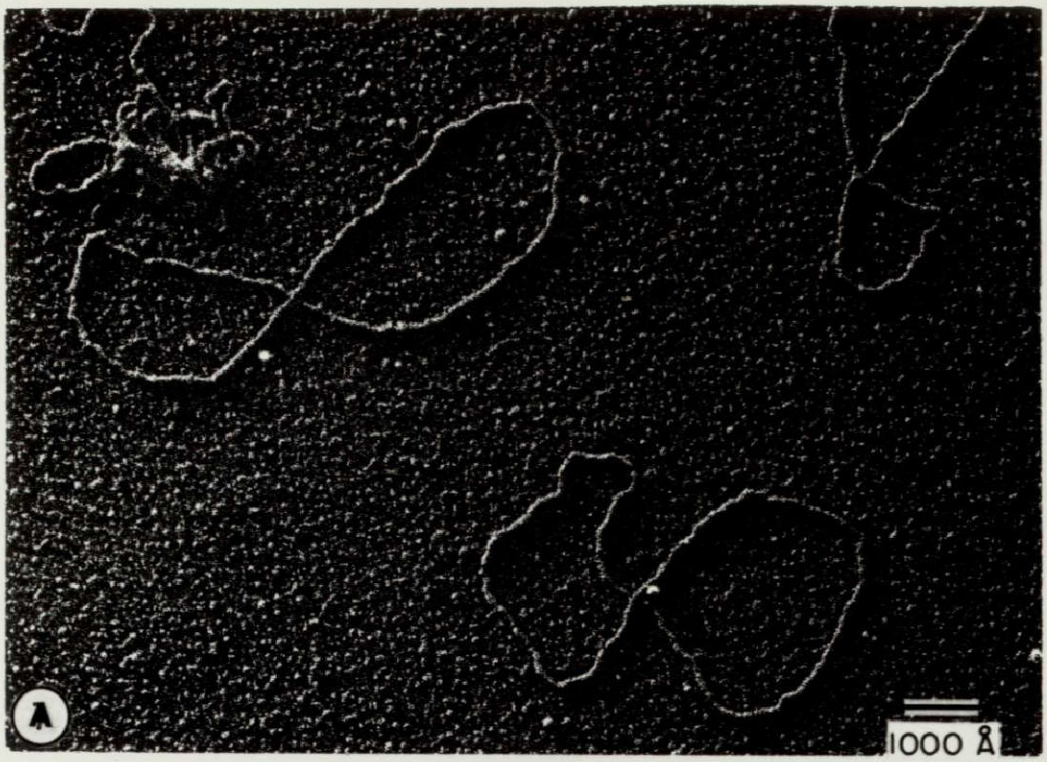

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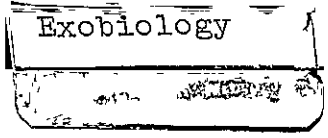
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Electron Microscopy of DNA Conformations in Spinach Chloroplasts

C. L. F. Woodcock and H. Fernández-morán

J. Mol. Biol. (1968) **31**, 627–631

Electron Microscopy of DNA Conformations in Spinach Chloroplasts

Chloroplasts have been shown to contain DNA by experiments involving density gradient ultracentrifugation (Chun, Vaughan & Rich, 1963; Shipp, Kieras & Haselkorn, 1965; Tewari & Wildman, 1966), and by electron microscopy of tissue sections (Ris & Plaut, 1962; Kislev, Swift & Bogorad, 1965; Bisalputra & Bisalputra, 1967).

For the reliable identification of chloroplast DNA it is essential to obtain chloroplasts free from nuclear DNA contamination. It was therefore necessary to devise techniques for the preparation of clean, intact chloroplasts.

In this communication we report the successful application of techniques for the isolation and purification of spinach leaf chloroplasts on a microscale. This has enabled us to visualize by electron microscopy the DNA associated with each chloroplast, following disruption by osmotic shock. DNA molecules extracted from purified chloroplasts were also analyzed. Examination of these preparations under controlled experimental conditions has consistently revealed two major DNA conformations and a characteristic association with the chloroplast membrane system.

Young leaves of *Spinacea oleracea* var. Viroflay were harvested and chilled to 0°C for all preparations. In order to eliminate grinding and homogenizing, which are major contamination sources, chloroplasts were taken from single razor cuts made in the leaf. As shown in Fig. 1, the leaf was placed lower side down on 0·05 ml. buffer (solution A, Jensen & Bassham, 1966), and a glass capillary tube used to take up the diluted sap. By this gentle procedure, which essentially taps the native cell contents, the ratio of intact nuclei to chloroplasts was one to 150, similar to that in mesophyll cells. By contrast, after five seconds homogenization of the leaves by the usual macromethods, only one intact nucleus per 1000 chloroplasts could be observed, thus showing that 75% of nuclei had been fragmented. 75% of the chloroplasts isolated by the micromethod were of the class I type (Spencer & Unt, 1965) which are thought to have the outer membrane intact.

To separate chloroplasts from nuclei, specially constructed glass microcuvettes (Fernández-Morán, 1957) with an internal volume of 0·1 ml. were used, in which gradients of 50 and 30% sucrose in buffer were set up. 0·01 ml. of chloroplast suspension, containing about 10^6 class I chloroplasts, was layered on top, and the cuvettes centrifuged at 12,000 *g* for 60 minutes in a swinging bucket rotor (type HB4, Sorvall) specially adapted for microcuvettes. After this time, a distinct chloroplast band and a pellet were formed (Plate I(a)). Feulgen staining of these two fractions by the method of Leuchtenberger (1958) showed that the nuclei were confined to the pellet, which also contained the larger chloroplasts.

For the extraction of DNA, the chloroplast bands from about ten microcuvettes were pooled, an excess of buffer added, and the chloroplasts pelleted. They were then resuspended in 0·5 ml. BPES medium (0·5 M-Na_2HPO_4–0·5 M-EDTA–5M-NaCl (16 : 20 : 36 by vol.) in 1 liter water, adjusted to pH 9·0), and placed in a dialysis bag with 5·0 mg/ml. pronase (Worthington Biochemical Corporation) which had

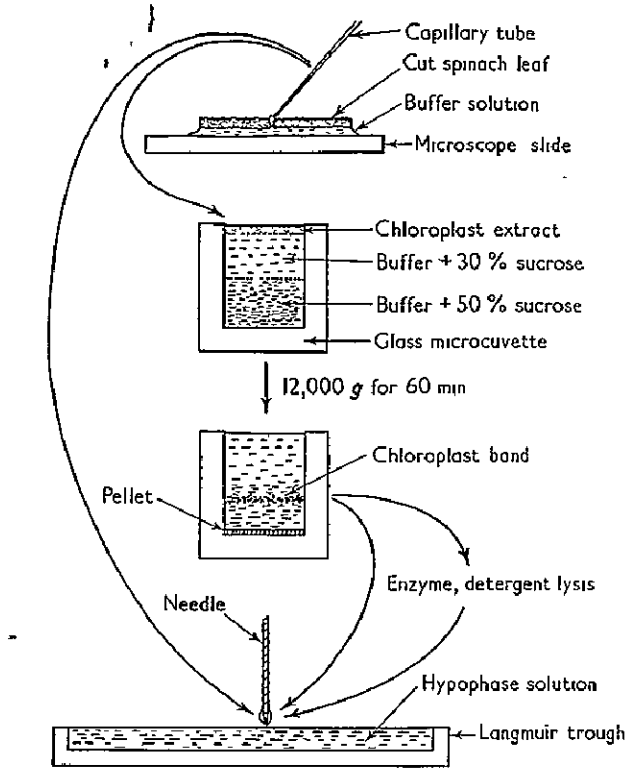

FIG. 1. Scheme for the extraction, purification, and spreading of chloroplasts, and chloroplast DNA.

previously been heated at 80°C for ten minutes to destroy nucleases. Dialysis was carried out against a large excess of BPES medium containing 1% sodium dodecyl sulfate at 50°C for 18 hours. Fresh BPES was then added, and the dialysis continued in the cold for five hours, after which the NaCl concentration was gradually reduced during a further four hours to 0·05 M. The dialysate was collected and reduced by evaporation to 0·1 to 0·2 ml.

The preparation of chloroplasts for electron microscopy was based on the osmotic shock method used for the release of viral DNA (Kleinschmidt, Lang, Jacherts & Zahn, 1962). About 10^3 purified chloroplasts in 0·005 ml. buffer, containing 100 μg/ml. cytochrome *c*, were transferred to a polished and flamed steel needle which was slowly lowered through a clean water surface containing a few talc particles (Fernández-Morán, 1948). The resulting protein film, occupying a circular area one to two centimeters in diameter, was transferred to carbon-coated electron microscope grids, or to thin carbon films deposited on mica squares. The carbon films were prepared by evaporation onto freshly cleaved mica in an ultra-high vacuum (Fernández-Morán, van Bruggen & Ohtsuki, 1966). The specimens were subsequently stained while wet with 1% uranyl formate, or dried from ethanol, and shadowed with platinum from two sources at right angles. As a control, 0·1 mg/ml. DNase (Worthington Biochemical Corporation) was added to the chloroplast suspension immediately prior to spreading. Samples of the pelleted fraction were prepared in the same way. DNA extracted from the chloroplast and nuclear fractions was also spread in a protein monolayer, but on a 0·1 M-ammonium acetate hypophase.

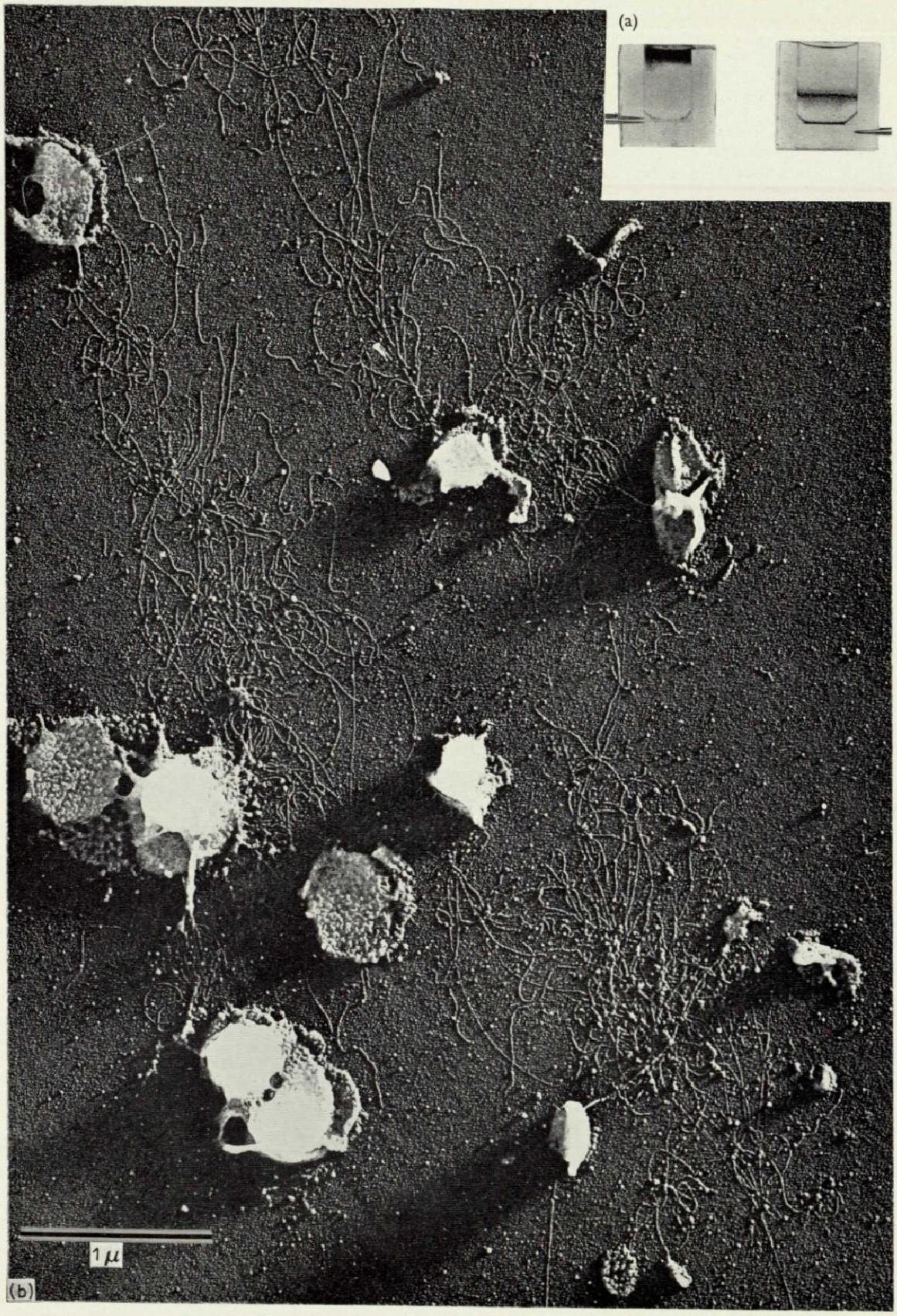

PLATE I. (a) Microcuvettes before and after centrifugation.
(b) Portion of an osmotically disrupted chloroplast showing DNA strands associated with membranes. Platinum shadowed. (× 30,000).

[*facing p. 628*

PLATE II. Portion of a fully dispersed chloroplast showing typical DNA conformations. Platinum shadowed on carbon–mica substrate. (× 29,000).

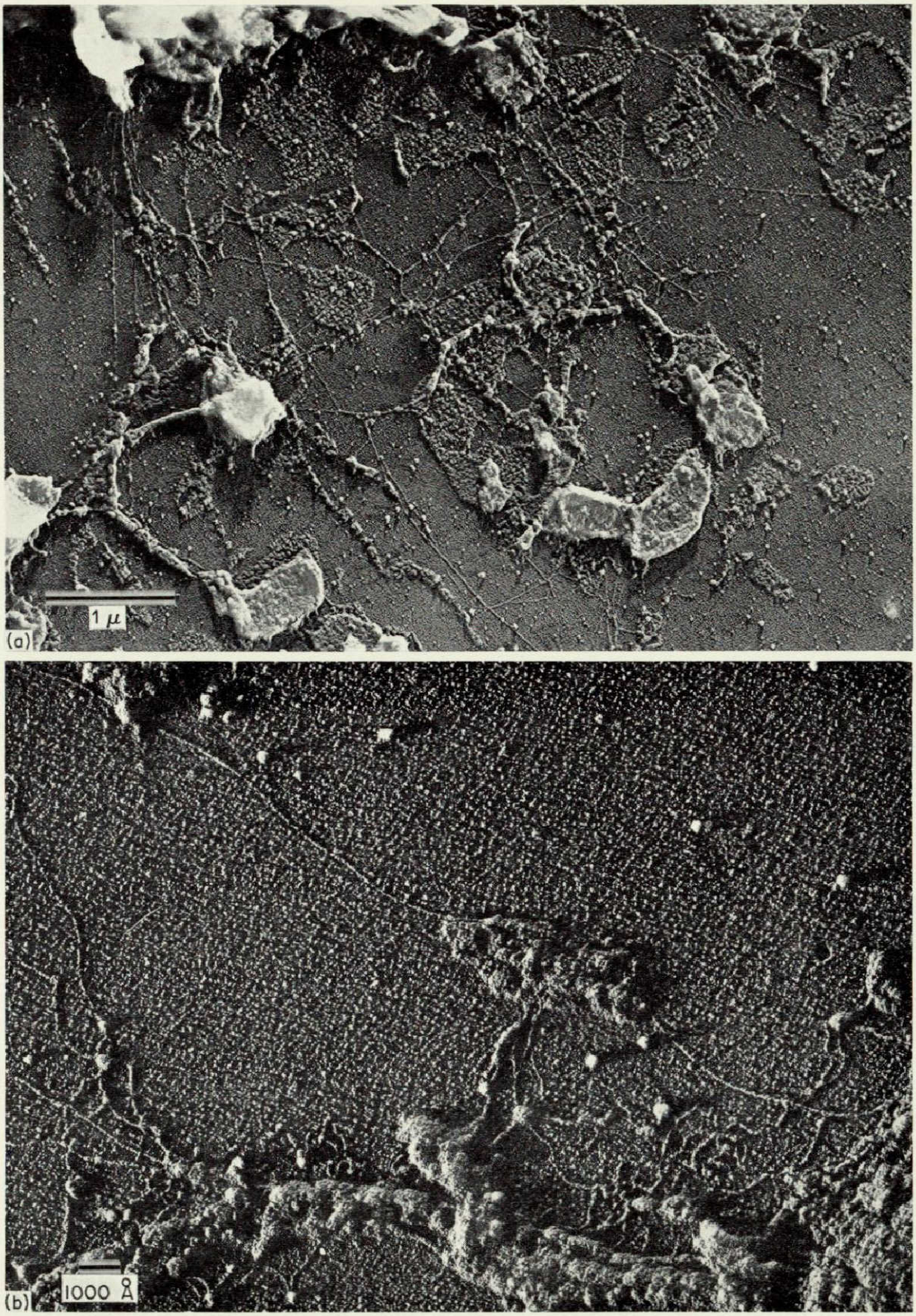

PLATE III. (a) Part of dispersed chloroplast showing DNA associated with granal and intergranal membranes. (× 20,000).

(b) "Linear" and "mesh" forms of DNA attached to membranes. (× 62,000).

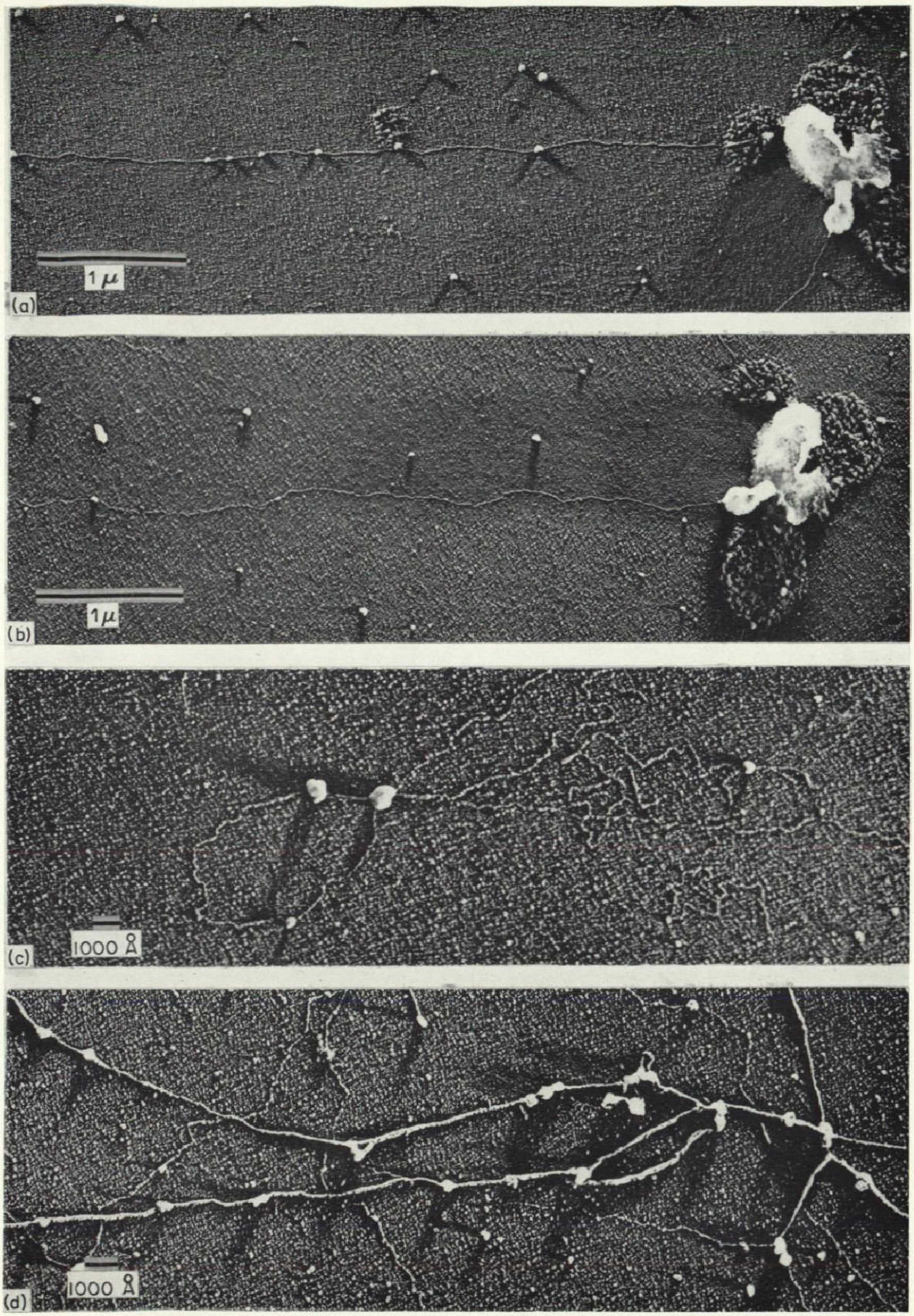

PLATE IV. (a), (b), and (c) DNA molecules extracted from purified chloroplasts. Platinum shadowed. ((a) and (b) × 23,000) ((c) × 40,000).

(d) DNA extracted from the nuclear fraction. Platinum shadowed. (× 50,000).

Electron micrographs were recorded with Siemens Elmiskops I and Ia, provided with highly regulated power supplies, improved pointed filaments (Fernández-Morán, 1960) and liquid nitrogen anticontamination devices. A diffraction grating replica (2160 lines/mm) was used to calibrate the microscopes. To measure DNA lengths a comparator (Nikon Shadowgraph) was used.

The spreading of an osmotically shocked chloroplast in a protein monolayer unfolds this complex organelle so as to reveal the DNA moiety and its relationships with the membrane constituents. The micrographs yield new information, unobtainable from thin sections, and in many ways analogous to the clear display of viral and bacterial DNA (Kleinschmidt *et al.*, 1962; Bode & Morowitz, 1967).

All transitions were observed from chloroplasts with intact envelopes to those in which the contents were completely dispersed. In the latter, characteristic thin filaments were found extending between the granal and intergranal lamellae, and the particulate matrix components (Plates I, II and III). These thin filaments occurred in 97% of dispersed chloroplasts, but were entirely absent from DNase-treated specimens. Since the use of DNase in combination with electron microscopy is recognized as one of the most sensitive methods for detecting DNA, these distinctive filamentous structures were considered to be DNA macromolecules.

Two main conformations of the DNA were observed: linear segments, about 5 to 20 μ in length, and 20 to 50Å in diameter (Plates 1(b) and II); and meshworks of thinner (about 15 Å) filaments, looped and interconnected with sharp angular bends (Plate III).

Transitions between the linear L forms and the mesh M regions were observed, often forming an interconnected whole. The maximum length of DNA recorded from a single chloroplast was 150 μ (Fig. 2(a)), but this probably does not represent the

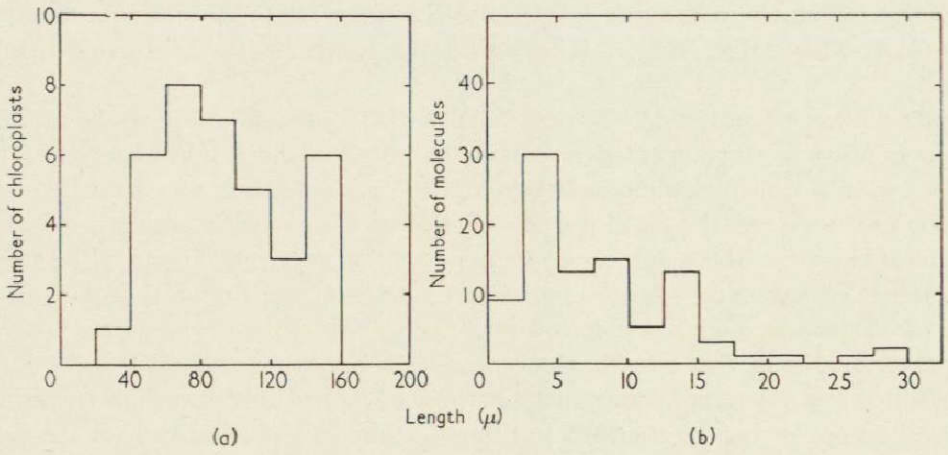

FIG. 2. (a) Size distribution of DNA released from fully dispersed, osmotically shocked chloroplasts.
(b) Size distribution of DNA extracted from purified chloroplasts.

whole of the DNA moiety, since some may have remained attached to or hidden by membranes. Whereas the linear forms resembled double-stranded DNA, the thinner and convoluted M regions were strikingly similar to the single-stranded DNA described by Freifelder & Kleinschmidt (1965). Both DNA forms were found in association with the granal and intergranal membranes; but the M regions in particular

seemed to be in intimate connection as though they formed integral parts of certain membrane areas (Plate III(a) and (b)).

Discrete DNA molecules of varying form were extracted from the chloroplast band, the size distribution being shown in Fig. 2(b). The majority of the extracted filaments were linear with modal lengths of 3 to 5 μ (Plate IV(a) and (b)). In addition, highly convoluted segments, reminiscent of the M regions, were observed (Plate IV(c)). The molecules were frequently associated with electron-dense particles which were resistant to DNase, and thought to be chloroplast breakdown products, and portions of membranes (Plate IV(a), (b) and (c)).

Digestion of the pelleted fraction failed to resolve the nuclear DNA into separate molecules. Instead, a mass of interconnected strands of varying width (20 to 200 Å) was obtained (Plate IV(d)).

In evaluating these results, numerous artifact possibilities, including contamination with non-chloroplast DNA, could be largely excluded as significant factors.

Although the chloroplast band from the microcuvettes appeared to be uncontaminated with nuclear material, it was possible that small fragments, undetectable with the light microscope, could be present. Since such contamination would affect intact chloroplasts to the same extent as dispersed ones, the number of intact chloroplasts associated with DNA strands provides a measure of the non-chloroplast DNA present. In these experiments, DNA strands were observed in 97% of well-dispersed chloroplasts, 38% of poorly dispersed chloroplasts, and 0% of intact chloroplasts, showing that contamination with nuclei (or other sources of DNA) was negligible.

Estimates of the total amount of DNA per chloroplast have been in the order of 10^{-15} to 10^{-16} gram, corresponding to molecular weights of about 5×10^7 to 5×10^8. Assuming the β-form of the double helix, this would correspond to lengths of between 30 and 300 μ. A minimum estimate for the size of the spinach chloroplast DNA may be obtained from the maximum length of DNA associated with a single disrupted chloroplast. This value, 150 μ, falls within the range estimated by biochemical methods.

Whether the chloroplast DNA moiety exists in the organelle as a single molecule or as a number of smaller entities remains to be established. Although strict precautions were taken to exclude nucleases and to avoid shearing forces during manipulation of the extract, it is still possible that breaks were artificially induced in the molecules. However, there appears to be a correlation between the modal lengths of extracted DNA molecules and the individual lengths of the L and M regions in the DNA of osmotically shocked chloroplasts.

Extracted DNA of *Tradescantia* chloroplasts also appeared as short lengths (Grun, 1967), but it has yet to be determined whether the looped configurations correspond to our M forms, or can be ascribed to other factors. The possibility that the interconnected L and M forms of the chloroplast DNA may correspond to double- and single-stranded regions, respectively, is an attractive working hypothesis. In this connection, it is of interest that two distinct chloroplast-associated DNA components have been distinguished biochemically in spinach (Chun *et al.*, 1963).

In embedded and sectioned chloroplasts, 25 Å DNA fibrils were observed in the low-density areas of the matrix (Bisalputra & Bisalputra, 1967). Our results indicate a closer association between the DNA and the internal lamellar system than is apparent from sectioned material.

At present the DNA is visualized as being intimately associated in specific regions

with the membrane system. A highly organized spatial orientation can be anticipated, assuming that the DNA and the self-duplicating mechanism of the chloroplast must somehow conform to the paracrystalline arrangement of the membranes. Moreover, as the chloroplast is a self-propagating, multi-component structure, the genome which ensures its form and continuity may have commensurate complexity, which we are only now beginning to elucidate.

Further work along these lines is continuing and will be reported in later papers.

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ULTRASTRUCTURE AND VARIABLE APERTURE PORE FUNCTION OF HEXAGONAL SUBUNITS IN PLASMA MEMBRANES

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CORPORATION

ULTRASTRUCTURE AND VARIABLE APERTURE PORE FUNCTION OF HEXAGONAL SUBUNITS IN PLASMA MEMBRANES

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ABSTRACT High resolution electron micrographs have revealed arrays of "hexagonal" subunits, ~90-95 Å diameter, in a black lipid membrane of the type in which Mueller and Rudin induced nerve-like electrical activity by addition of the circular polypeptide antibiotic, alamethicin. The subunits appear to be related to those reported in junctional sites where adjacent membranes of living cells are known to be in electrical contact. Molecular models of the subunits strongly suggest that they act, in association with adsorbed allosteric proteins, as variable aperture pores in the cellular membrane. The arrangement and movement of cholesterol molecules in these models resemble those of the leaves of a between-the-lens shutter of a modern camera. The possible roles of the hexagonal subunits in ion-gating, nerve conduction, sensory reception and active transport are discussed in some detail. Ordered water, whose properties have recently been characterized by this laboratory, is an important structural component of the hexagonal subunit.

INTRODUCTION

Fernández-Morán (1) suggested that the selective permeability of nerve membranes might be envisaged in terms of molecular "pores" lined with highly ordered water, resembling "ice-like" hydration sheaths or crystalline hydrate lattices. Lack of knowledge of the precise molecular nature of ordered water has, heretofore, prevented the development of that concept. Recently, however, this laboratory completed a study (2) of the transport properties of ordered water in the pores of cellulose acetate and unfired Vycor glass desalination membranes. With the aid of electron microscopy,

it was found that for optimum desalination these pores should be ~ 40-45 Å in diameter, which is about twice the thickness, t, of the hydration sheath of salt-rejecting ordered water at the hydrophilic surface of the membrane (Fig. 1). Measurements of the Poiseuille flow of water through such membranes, showed that the mean viscosity of this ordered water is about 37-40 times greater than that of ordinary bulk water at room temperature. The simple assumption of approximate equality between the hydration sheath thickness, t, and the average diameter of a "flickering cluster" (3) permitted a rate process calculation of the permeation of ordered water through ideal membrane pores, in good agreement with the experimental data. In that calculation (2) the following parameters of ordered water at 23°C were estimated:

t	=	hydration sheath thickness	= 21 - 22.3 Å
d_{cl}	=	average cluster diameter	= 21 - 22.3 Å
n_{cl}	=	average cluster size	= 162 - 191 molecules
$\Delta H^{\ddagger}$	=	activation enthalpy for flow	= 6.0 - 6.3 kcal/mole
$\Delta S^{\ddagger}$	=	activation entropy for flow	= 5.6 - 6.5 cal/mole·°K
$\Delta F^{\ddagger}$	=	free activation energy for flow	= 4.4 kcal/mole
ΔE_{vap}	=	energy of vaporization	= 10.6 - 10.7 kcal/mole
η	=	viscosity	= 0.35 - 0.37 poise

The insolubility of salt in ordered water suggests that its electrical conductivity might be very low approaching the value 10^{-8}-10^{-9} $ohm^{-1}cm^{-1}$ proposed (4) for pure ice.

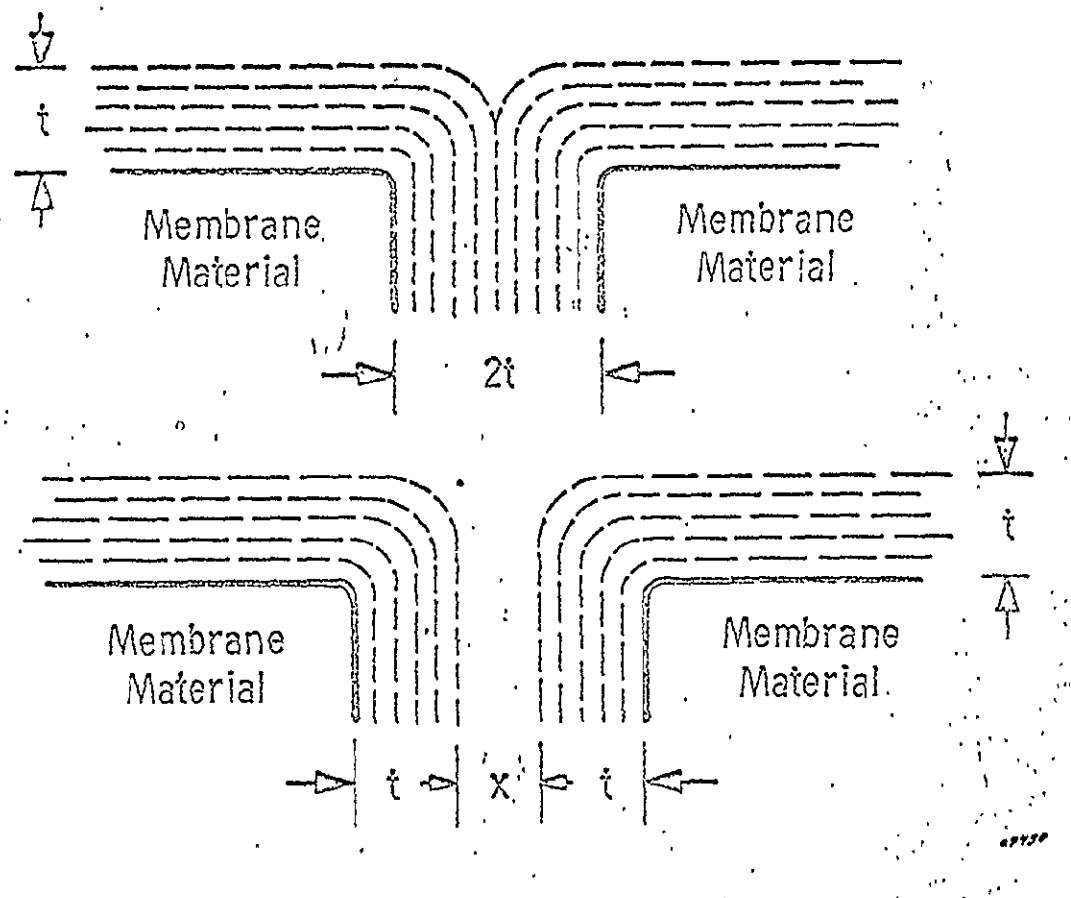


FIGURE 1. Diagrammatic representation of ordered water sheath (thickness, t) in the vicinity of a pore in a hydrophilic membrane. Top diagram shows critical diameter (2 t) of hole filled with ordered water that will exclude all ions; bottom diagram shows hole lined with ordered water that will allow ions of width (<x) to pass through unordered water channel of diameter (x).

A pore in open configuration in a nerve membrane may now be approximated, in the manner illustrated in Fig. 1, as an essentially unordered water channel, of diameter $x \simeq 8.4\text{-}8.5$ Å surrounded by a hydration sheath of ordered water of wall thickness $t \simeq 21\text{-}22$ Å. The value $x \simeq 8.4\text{-}8.5$ Å is the "equivalent pore" diameter in a squid axon axolemma, derived by Villegas and Barnola (5) from solute penetration data. About the same equivalent pore diameter has been reported for the human erythrocyte (6), for the luminal surface of the mucosal cells of a rat intestine (7), and for the walls of frog single muscle fibers (8). The unordered water channel, itself, would be difficult to resolve by electron microscopy. However, the channel plus the surrounding hydration sheath have a diameter estimated to be about 50 to 60 Å, which should readily be resolved, as an electron dense membrane component, when surface-stained by a heavy metal salt. Close examination of an electron micrograph by Fernandez-Moran, Fig. 9 of reference (1), does suggest the presence of such components in a specially prepared ultrathin submicroscopic nerve fiber ending, stained with uranyl formate. It was, therefore, of interest to look for similar components in an ultrathin black lipid membrane of the kind that has been proposed (9) as a prototype of the plasma membrane of cells and the membraneous elements of mitochondria, nuclei, chromosomes, endoplasmic reticulum, Golgi apparatus and chloroplasts.

Heretofore, it has been thought that all black lipid membranes have a lamellar bimolecular leaflet structure similar to that in the Robertson "unit-membrane" model (10), which incorporates elements of the earlier Gorter-Grendal (11) and Danielli-Davson (12) models of the cellular membrane. However, the next section of our paper gives electron microscopic evidence that a black lipid membrane, with protein adsorbate, can have an hexagonal structure resembling the hexagonal liquid-crystalline phase (Fig. 2) discovered by Luzzatti and Husson (13) in a brain phospholipid-water system. The hexagonal subunits of this membrane may be related to those observed by Robertson (14-16) in the synaptic membranes of Mauthner cells in the goldfish brain, by Benedetti and Emmelot (17, 18) in tight intercellular junctions from isolated rat-liver plasma membranes, and by Revel and Karnovsky (19) and Kreutziger (20) in tight junctions from the mouse heart and liver. These sites, at which a honeycomb array of hexagonal subunits can be demonstrated, are regions in which cells have been shown to be in electrical contact (21-24).

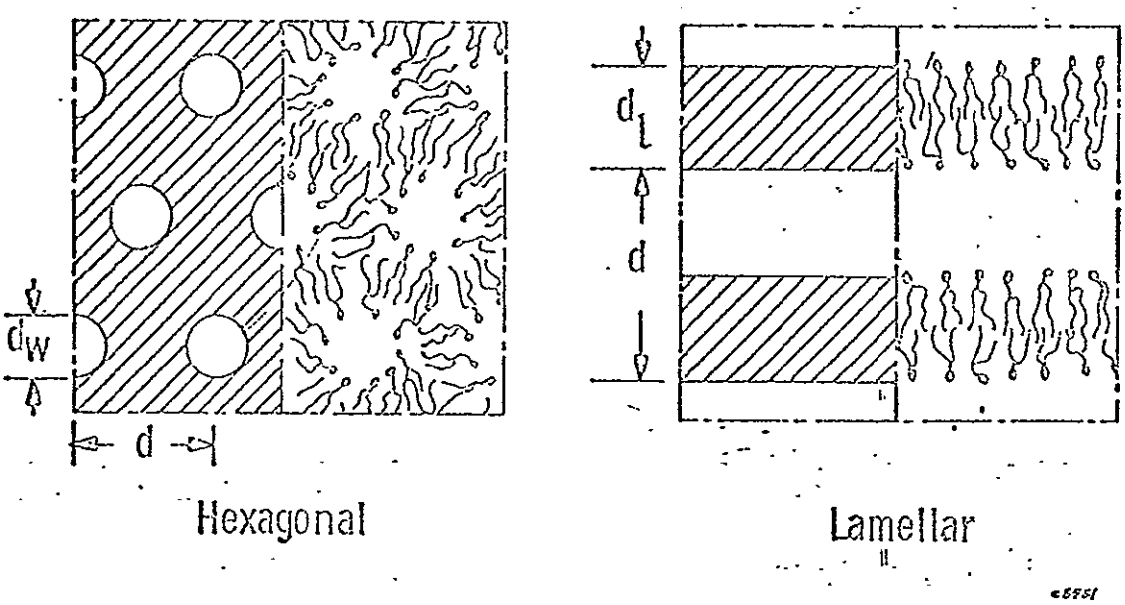


FIGURE 2. Rough schematic diagram of the liquid-crystalline phases of a brain phospholipid-water system, redrawn from Luzzatti and Husson (13).

EXPERIMENTAL SECTION

Electron Microscope Studies

Mueller and Rudin (25) had previously reported that a protamine-stabilized membrane made from purified lecithin (10% in decane-squalene, 2:1) could be readily induced to show nerve-like action potentials by alamethicin, a cyclopeptide antibiotic. Because of the indirect evidence that variable aperture pores or ion-gates play a key role in nerve conduction, it seemed reasonable that some of the ultrastructure of such ion-gates might actually be seen in this artificial membrane.

The membrane was prepared by placing a drop of the lipid solution on the surface of 50 ml of a solution of 10 μ g/ml of protamine in 0.1 M NaCl, contained in a Petri dish. After evaporation of the decane, a portion of the resultant ultrathin surface film was collected on an electron microscope grid, which had been precoated with cellulose nitrate and carbon. The collection procedure involved gently touching the surface film with the grid. For negative staining, a drop of 1% sodium phosphotungstate (pH7) was placed on the grid and subsequently withdrawn by adsorption into filter paper. All operations were performed at room temperature ($\sim 23\,^{\circ}C$) in a clean-room.

Fig. 3 is a photo-enlargement of an electron micrograph of a stained membrane taken with a Hitachi HU-11 microscope at 39,000 X electron-optical magnification using 100 Kev acceleration voltage, a low beam intensity and a cold trap to prevent contamination. Overall, a distorted hexagonal subunit pattern is evident. Only a few of our attempts to obtain this pattern were successful, possibly because this hexagonal lipid-water phase is just barely stabilized at room temperature by the low concentration of protamine. The larger dark areas, about 200-300 Å in diameter, are staining artifacts, essentially areas where excess stain was not removed successfully. Note the areas, marked S, where the original print showed a pattern similar to the rest of the film, but almost obscured by an overlay of excess stain. The black area marked X is a defect in the original photographic emulsion. Several thin lines, denoting ~ 240 Å, have been drawn to emphasize regions where particularly regular patterns of subunits occur.

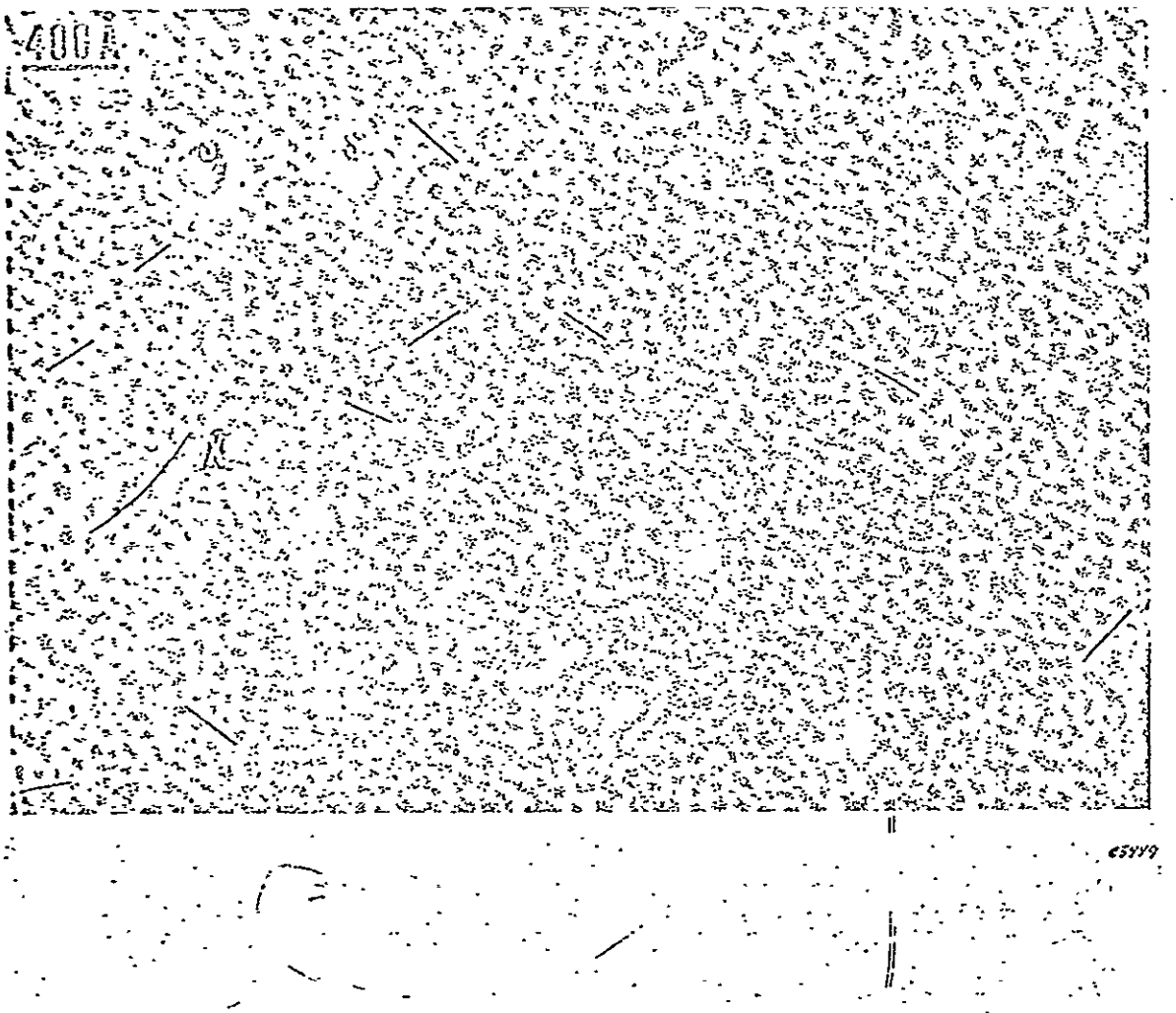


FIGURE 3. Transmission electron micrograph of protamine-stabilized black lipid membrane made from purified ova-lecithin (10% in decane-squalene, 2:1) stained with 1% sodium phosphotungstate (pH7)

Here the subunit center-to-center distance is about 90-95 Å and the darkly stained electron-dense center is about 37-45 Å. This dark center presumably delineates a core of ordered water stabilized by the polar ends of the lipid molecules. The electron micrograph may be compared with the rough schematic Luzzati-Husson (13) diagram (Fig. 2) of the hexagonal phase of a brain phospholipid-water system. Note, however, that the squalene-lecithin membrane is an ultrathin film, perhaps 35 Å thick, while the Luzzati-Husson diagram refers to an hexagonal array of indefinitely long cylinders. In the diagram, the blank white circular areas represent water; the small black circles represent the polar ends of the lipid molecules; the wiggly lines represent the lipid hydrocarbon tails.

Squalene, although non-polar, is biologically a precursor of cholesterol and its folded form is structurally related to that molecule. However, squalene is an oil at room temperature, while anhydrous, polar, cholesterol is a crystalline solid which melts at 149°C. This difference may help explain why our attempts to demonstrate an hexagonal subunit pattern in a protamine-stabilized lecithin-cholesterol black lipid membrane have not yet been successful. There is an indication from our work and from the work of Luzzatti and Husson (13) that the hexagonal arrangement in protein-free cholesterol-containing phospholipid membranes is stable only above 37°C and that transition to a lamellar arrangement occurs below this temperature. Stabilization of the phospholipid membrane in an hexagonal arrangement at room temperature apparently requires the presence of the proper amount and type of adsorbed protein or glycoprotein. It is reported (9) that protein-free black lipid membranes have appreciable surface tension and tend to shatter like soap bubbles when broken. In contrast, membranes made from total tissue containing intact proteolipids can show very little surface tension. These observations suggest that artificial, protein-free, black lipid membranes do have the Gorter-Grendal-Danielli-Davson lamellar bimolecular leaflet structure but that proteolipid membranes (particularly, plasma membranes of living cells) actually have the hexagonal structure. A discussion of the flexibility limits of lipid bimolecular leaflets will be found in the first chapter of a recent book by Stein (26). A detailed criticism of the bimolecular leaflet "unit membrane" as a model for the cell membrane has been given by Korn (27).

THEORETICAL SECTION

Molecular Model of an Hexagonal Subunit

Vandenheuval (28) developed an extraordinarily detailed molecular model of the lamellar bimolecular lipid leaflet. As part of that model, he also developed submodels of the cholesterol-lecithin complex and the cholesterol-sphingomyelin complex based on the principle of maximum van der Waals interaction between the component molecules. These submodels will now be utilized to develop a detailed model of an hexagonal subunit of a cellular plasma membrane, with particular emphasis given to the axon membrane of the nerve cell.

In resting configuration (Fig. 4) the "hexagonal" subunit of an axon membrane is postulated to have a pore of unordered water of diameter $x \simeq 4.0$ Å. This diameter would just about permit the doubly hydrated potassium ion, $K(H_2O)_2^+$ to pass through the membrane but would prevent passage of the larger triply hydrated sodium ion $Na(H_2O)_3^+$. The scale drawings of these ions (Fig. 5) are based on hydration numbers from Hindman (29) and bond angles, bond distances, ionic and van der Waals radii from Pauling (30). The coordination bonding radius of the oxygen atom in H_2O is approximated as 1.25 Å, the same as in $K_2CuCl_4 \cdot 2H_2O$. Surrounding the unordered water pore is an annular sheath of ordered water (sheath thickness $t \simeq 22$ Å) stabilized by the polar ends of phospholipid complexes. Fig. 4 shows how eighteen of these complexes could be arranged in symmetrical fashion around this sheath to yield one of the two monolayers of an hexagonal subunit. The monolayer arrangement is shown for nine cholesterol-lecithin complexes intercalated with nine cholesterol-sphingomyelin complexes. This arrangement, although not representative of the actual lipid composition of the axon membrane, serves to illustrate the type of close packing that might occur. The hydrocarbon tails of the lipids are flexible and could extend beyond the nominal 90 Å diameter of the subunit to intermesh with the corresponding tails of other subunits, thereby imparting structural strength with high flexibility to the overall hexagonal array. Figs. 6A and 6B, which suggest

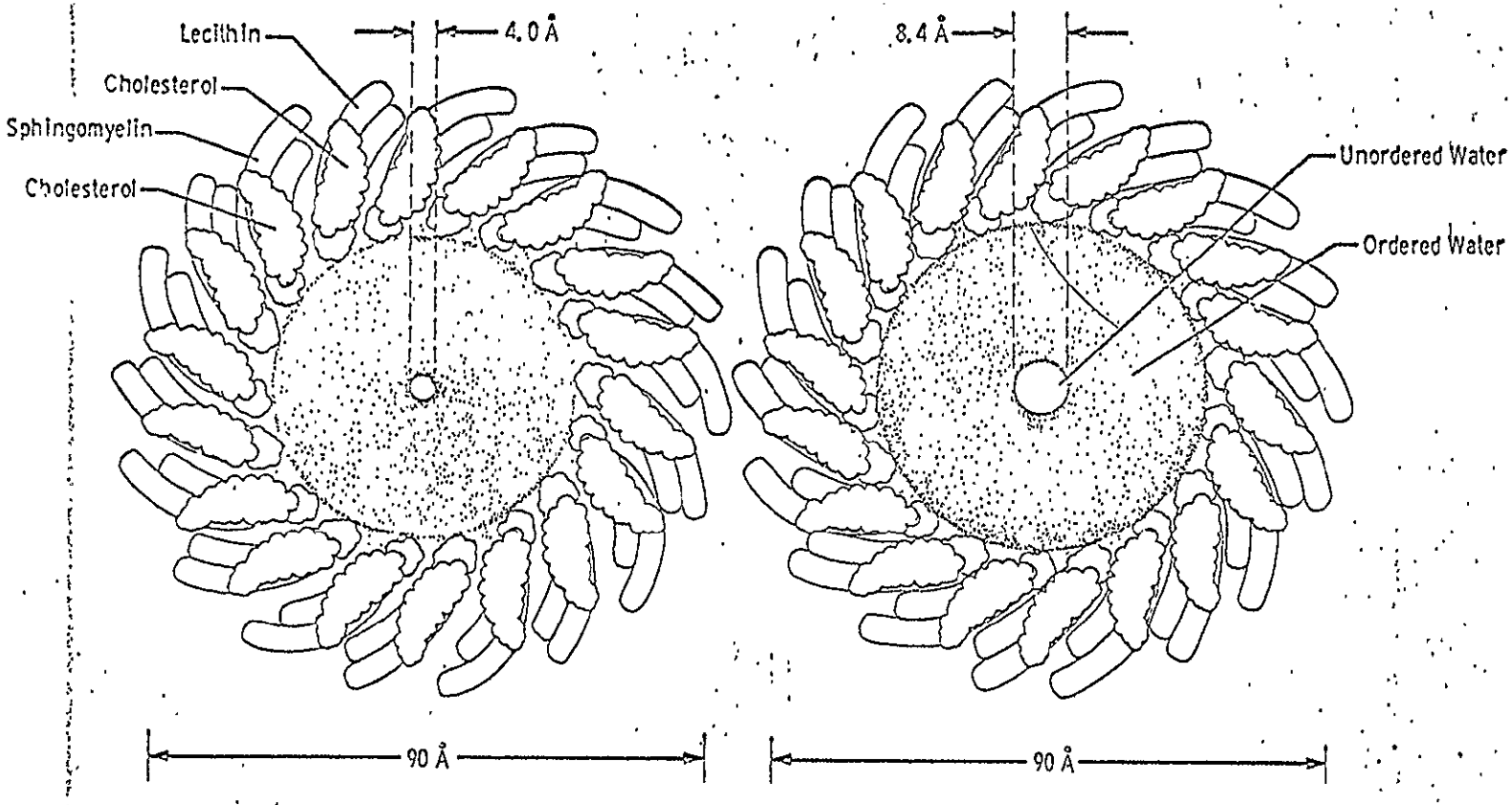


FIGURE 4. Molecular model of lipid water components of an "hexagonal" subunit in a plasma membrane. Left diagram shows top monolayer in partially closed "resting" configuration. Right diagram shows top monolayer in open configuration. Each subunit contains two monolayers back to back, with the cholesterol leaves outside.

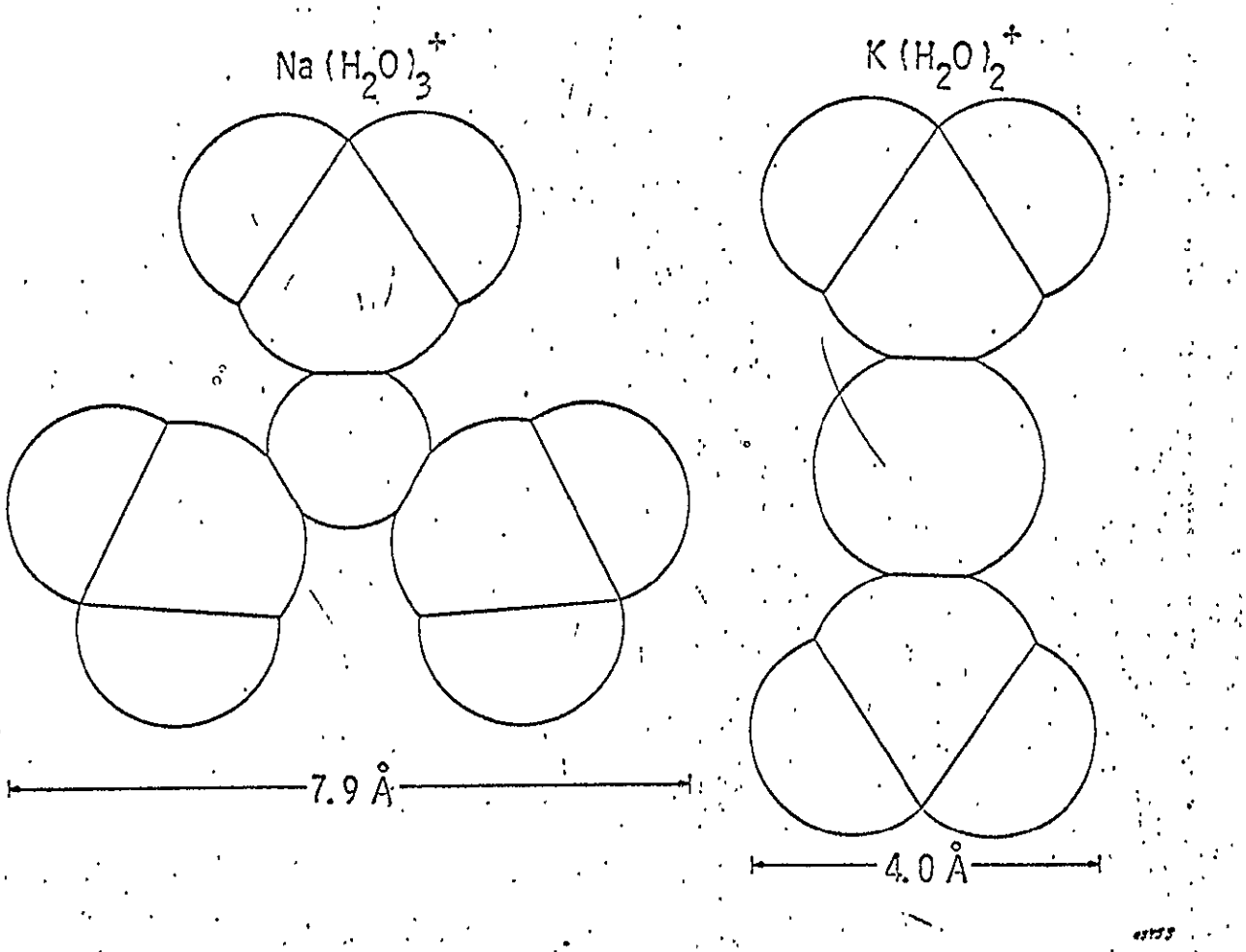


FIGURE 5. Molecular models of hydrated sodium and potassium ions.

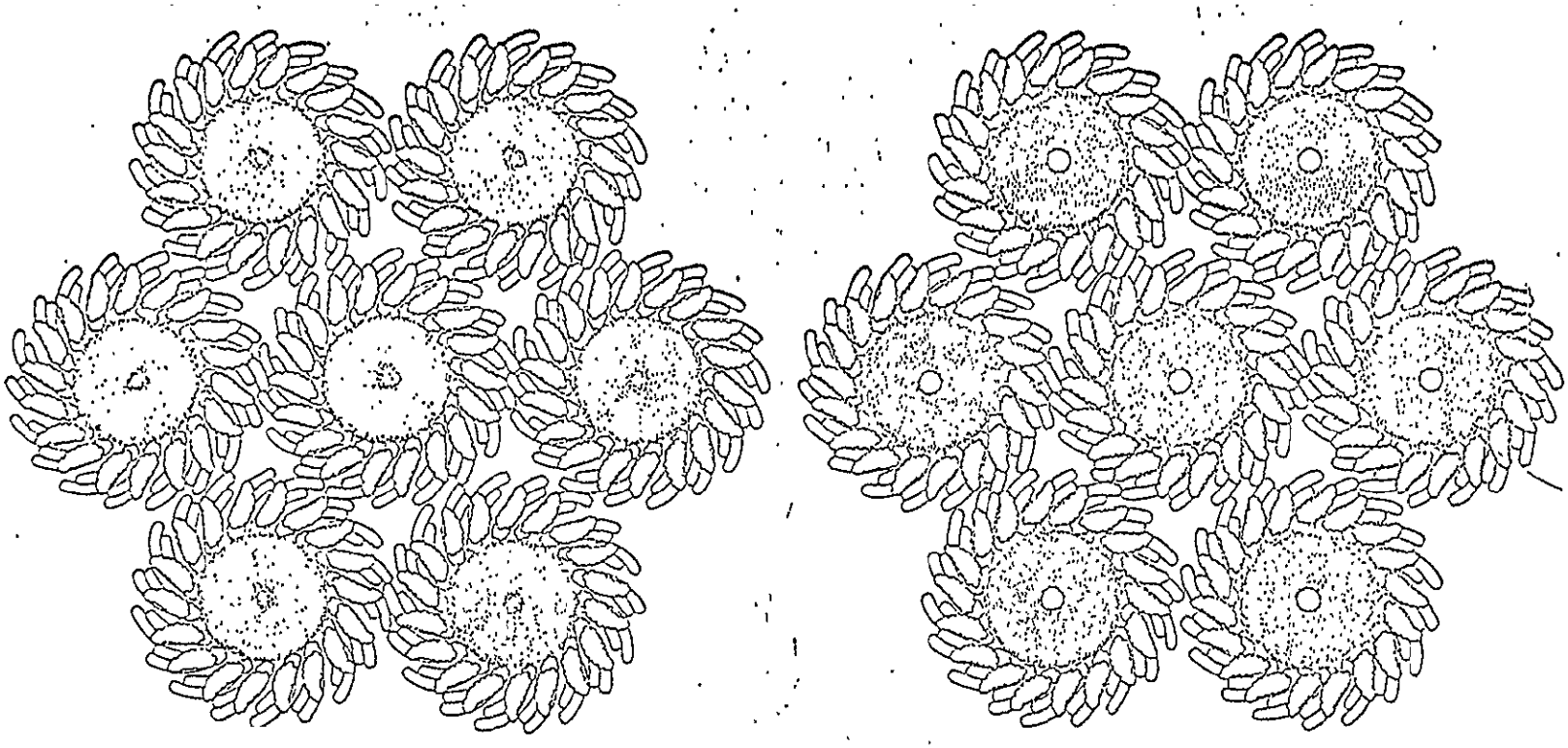


FIGURE 6. "Paste-up" approximation of close packing of lipid rings of hexagonal subunits (A) in resting configuration (B) in open configuration.

this intermeshing, were each made from a paste-up of photocopies of a single drawing of a subunit. The reader can judge for himself that reasonable individual adjustments in the positions of the flexible hydrocarbon tails would permit closer packing of the subunits to yield a 90-95 Å center to center distance. The closer packing would almost eliminate the apparent triangular interstices, each situated between three neighboring subunits. However, even with closer packing, these interstitial areas, filled with hydrocarbon tails in a fluid state, could conceivably be a path for nonelectrolyte hydrocarbon-soluble molecules to pass through the cellular membrane. Closer packing would also cause some geometric distortion of each subunit resulting in an hexagonal appearance; hence the term "hexagonal."

Those familiar with photography, will immediately recognize the striking similarity between the arrangement of the cholesterol molecules of the hexagonal subunit monolayer (Fig. 4) and that of the leaves of a between-the-lens shutter of a camera. The figure shows how a slight angular displacement of each cholesterol "shutter leave," each moving cooperatively with its neighbor, could increase the radius of the central pore of unordered water from 4.0 Å in resting configuration to the "equivalent pore diameter" of ~8.4 Å cited earlier for the squid axon. During this configurational change the ordered water sheath thickness, t, would remain nearly constant at ~22 Å and each phospholipid complex would unbend slightly about a natural bending point situated near the outer end of the cholesterol leaf.

The remarkable electron micrograph by Robertson (16) of a cross-section of an hexagonal subunit array in a synaptic disk from a Mauthner cell club ending shows each of the two cell membranes at the synapse to consist of a layer with unstained regions ~23 Å thick sandwiched between two stained layers each also ~23 Å thick. [See also Benedetti and Emmelot (17).] The stained layers presumably contain protein and glycoprotein; the unstained regions of the central layer are probably identifiable with the nonpolar parts of the phospholipid ring. It appears, therefore, that the lipid ring of an hexagonal subunit consists of two monolayers, each ~11 Å

thick, as determined from the Vandenheuval phospholipid complex sub-models (28). Each monolayer would have the arrangement shown in Fig. 4, but the two monolayers would be back-to-back, with the cholesterol leaves toward the inner and outer cellular surfaces. The protein and glycoprotein layers in contact with these leaves contain the allosteric machinery for opening and closing the subunit. Some details of operation of this machinery will be considered next.

The Hexagonal Subunit Ion Gate in the Axon Membrane

Hodgkin and Huxley (31, 32) demonstrated that the propagation of the nerve impulse coincides with changes in the ion permeability of the axon membrane. Their research led to the development of a nerve excitation theory based on the postulate of two types of specific pores or ion-gates, one for sodium and one for potassium. [See Katz (33) and Baker (34).] Normally, the nerve axon interior is rich in potassium ions and poor in sodium ions while the reverse is true of the exterior fluid. According to the theory, the axon membrane attains its negative resting potential by permitting positive potassium ions to flow outward through a "potassium gate" which is relatively impermeable to other ions. Immediately in advance of the nerve impulse or action potential a "sodium gate" opens, permitting positive sodium ions to pour into the axon, producing a localized positive charge in the interior. In the wake of the impulse, the "sodium gate" closes, whereupon the "potassium gate" opens, allowing K^+ ions to flow out, thereby restoring the normal negative resting potential of the axon interior.

The above theory of the nerve impulse has been subjected to searching criticism by Tasaki and colleagues (35). The most serious challenge has come from Watanabe, Tasaki and Lerman (36) who obtained normal-like action potentials with normal polarity from isolated squid giant axons perfused internally with dilute (e.g. 30 mM) sodium phosphate solutions and externally with 100 mM calcium chloride. Noting that no potassium was present and that the sodium ion gradient was opposite to that found normally, these investigators concluded that their data are inconsistent with the Hodgkin-Huxley theory that initiation of an action potential is the consequence of a membrane permeability increase "specific" to sodium ions. Fortunately, the molecular model of the hexagonal subunit developed in the previous section makes it possible to resolve this apparent discrepancy while still preserving the essentials of the Hodgkin-Huxley theory.

The use and meaning of the aforementioned word "specific" will be restricted; the "potassium gate" of the axon or membrane will be identified with the partially closed resting configuration of the hexagonal subunit (Fig. 4);

the "sodium gate" will be identified with the open configuration. In the resting configuration $K(H_2O)_2^+$ ions can just about squeeze through the 4.0 Å diameter pore of unordered water. Because of their concentration gradient, these ions will diffuse outwards until the negative resting potential, sufficient to retard this diffusion, has been built up in the axon interior.

It might be expected that negative Cl^- ions, unhydrated and only 3.6 Å in diameter (29), would contribute to the resting potential by diffusing inwardly from their high concentration outside the axon. Actually, under normal circumstances, inward diffusion of Cl^- ions contributes little to the resting potential (34). The probable reason is that the unordered water pores of the hexagonal subunits in resting configuration are effectively plugged by $K(H_2O)_2^+$ ions. Radiotracer diffusion experiments by Hodgkin and Keynes (37) indicate that the axon membrane pore for potassium ion diffusion is so narrow that these ions have to move through in single file, with an average of ~ 2.5 molecules able to fit in the pore at any one time. (Fig. 9A, to be discussed later, shows how this value yields a minimum pore length of ~ 22 Å in reasonable agreement with the earlier estimate for the axial thickness of the lipid ring of the hexagonal subunit.) In order for a labeled $K^*(H_2O)_2^+$ ion to diffuse through the membrane, it must first enter a 4 Å pore, either by filling a vacancy or by expelling an ion on the opposite side; it must then be hit successively from the same side an average of 2.5 times, with a force sufficient to expel an ion on each collision. A Cl^- ion from the axon exterior that enters a partially plugged pore has a diameter too small to be wedged in like $K(H_2O)_2^+$ and will not be able to pass through to the interior by means of a series of collisions. However, in the absence of $K(H_2O)_2^+$ ions, in the aforementioned axon perfusion experiments by Watanabe, Tasaki and Lerman (36), these pores are unplugged and can permit inward diffusion of Cl^- ions from an external $CaCl_2$ solution to set up a normal appearing negative resting potential in the axon interior. During the action potential under these artificial conditions, the ~ 8.4 Å diameter unordered water pore of the membrane subunit, in open configuration, should permit either inward flow of $Ca(H_2O)_6^+$ ions (diameter ~ 7.9 Å) or outward flow of $H_2PO_4^-$ ions (diameter ~ 5.2 Å) to depolarize the axon membrane. This analysis answers the objections of Watanabe, et. al., (36) to the Hodgkin-Huxley theory and permits the nerve impulse to be explained by the following ion chelation model of allosteric protein control of hexagonal subunits in the axon membrane.

The striking resemblance has been noted between the arrangement of the cholesterol molecules of the hexagonal subunit, Fig. 4, and that of the leaves of a between-the-lens shutter of a camera. In both cases some type of mechanical drive is required to turn the leaves cooperatively in a manner that opens and closes the central pore or aperture. An important clue to the nature of this drive in an axon plasma membrane is the previously cited discovery by Mueller and Rudin (25) that the antibiotic, alamethicin, a circular polypeptide with the composition $(Gln)_2(Glu)_1(Pro)_2(Gly)(Dimethyl\text{-}Ala)_8(Val)_2(Leu)_1$, induces nerve-like electrical behavior when added to one side of a black lipid membrane (e. g., lecithin-decane-squalene) separating two aqueous solutions. The alamethicin-treated membranes exhibit increased cation conductance and action potentials on electrical stimulation. The steady state conductance increases as the sixth power of both the alamethicin concentration and the cation concentration and also as the sixth power of the potential across the membrane (or the sixth power of the positive charge on the alamethicin side of the membrane). These data indicate that the reaction involved in development of the conductance is a highly cooperative phenomenon involving six alamethicin molecules and six cations. Mueller and Rudin (25) suggested that this phenomenon might be allosteric in nature, involving intra-molecular chelate bond configurations, but were unable to develop the concept much further. This concept will now be developed in terms of the hexagonal subunit model.

Fig. 7A is a schematic scale drawing showing how six alamethicin molecules might be adsorbed in symmetrical fashion on one side of an hexagonal subunit. (Not shown is protamine, which probably is also adsorbed on the lipid ring in some manner.) In the partially closed resting configuration, an oxygen atom, O, of the glutamic acid side chain and two nitrogen atoms, N, of the glutamine side chains of each alamethicin molecule are shown separated by surface tension (and, possibly, by electrical repulsion) forces that stretch the polypeptide ring into a flattened ovoid shape. Fig. 7B shows the allosteric interaction of six sodium ions with the alamethicin-hexagonal subunit assembly.

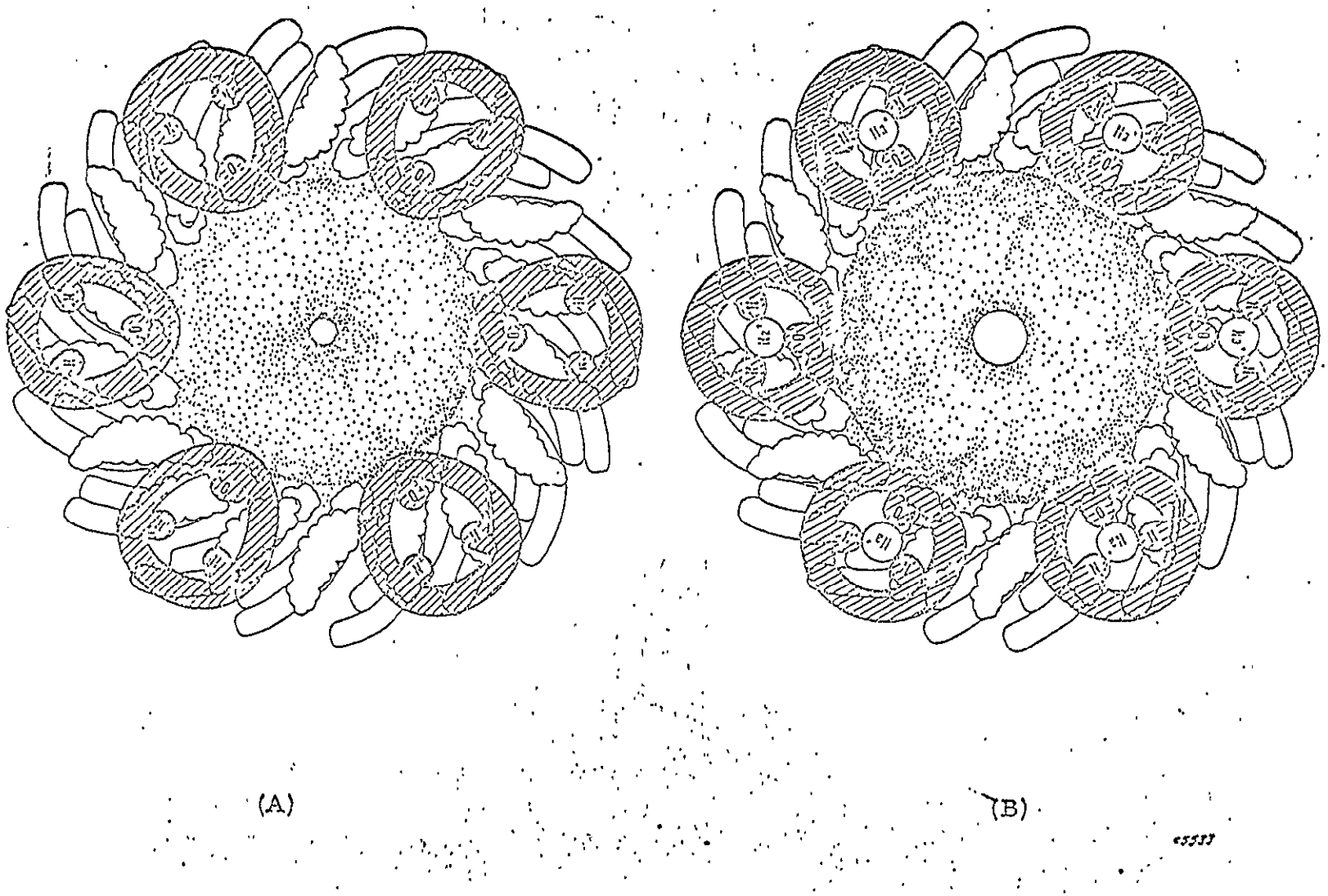


FIGURE 7. Schematic representation of (A) six alamethicin molecules adsorbed on hexagonal subunit in resting configuration (B) allosteric chelation interaction of six $Na(H_2O)_3^+$ ions with six alamethicin molecules to effect an open ion-gate configuration.

Each sodium ion enters into a chelation complex that draws the N, O, N atoms together and shortens the radial dimension of the alamethicin molecule. This dimensional change provides the mechanical drive that turns the cholesterol "shutter leaves" and opens the ion gate; each alamethicin molecule controls the angular position of three cholesterol leaves. It appears possible that other cations of comparable or even smaller size could substitute for sodium in forming a chelate complex with alamethicin. In Fig. 7B each sodium ion is depicted as a disk with a chelation or hydrogen bonding diameter of ~6.2 Å which is somewhat less than the ~7.9 Å van der Waals width of $Na(H_2O)_3^+$. (Note, however, that the hydration number of the sodium ion and hence its effective diameter may be altered by the chelation process.)

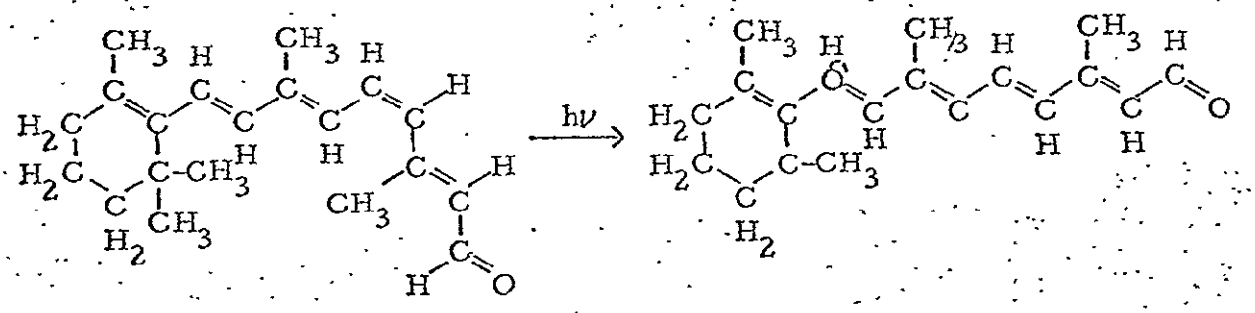
In the case of an actual cellular plasma membrane, the molecule that provides the drive for the ion gate is probably much larger than alamethicin. Mueller and colleagues (38, 39) have discovered a proteinaceous excitability inducing material (EIM) of cellular origin, that lowers the electrical resistance of black lipid membranes, develops resting membrane potentials in ionic gradients and produces nerve-like electrokinetic phenomena. EIM, which is present in a variety of tissues, is consistently liberated by certain bacteria into either egg-white or synthetic media. EIM, which has a nominal molecular weight under 70,000, apparently requires protamine to exert its action on black lipid membranes. The protamine probably helps stabilize the hexagonal phase in the membrane.

An electron micrograph of a black lipid membrane with adsorbed EIM is not yet available. However, a possible approximation of the arrangement of the EIM molecules in an ion-gate is provided by high-resolution electron micrograph by Fernandez-Moran and Ohtsuki (1) of bovine L-glutamate dehydrogenase (GDH). This enzyme has a molecular weight of 310,000 corresponding to an oligomer which may contain six identical subunits and six active sites (40). Figure 6C of reference 1 shows a circular aggregate of GDH containing more than six subunits but it appears that each GDH subunit might be ~30 Å in diameter and that about six of the GDH subunits surround the central void of the aggregate to form an oligomeric ring or rosette ~50 Å i.d. and ~105 Å o.d., that would just fit over the lipid ring of the

plasma membrane hexagonal subunit shown in Fig. 4. Recent experimental observations (40) indicate allosteric cooperative interaction between the subunits of the GDH oligomer. Such interaction would be consistent with a postulated role of the oligomer as one specialized type of mechanical drive of an hexagonal subunit of a nerve membrane. Interaction of the GDH oligomer with glutamate and NAD coenzyme substrate could allosterically open the hexagonal subunit causing a localized depolarization of the membrane; release of product α - ketoglutarate and NADH could close the subunit. Such a transient electrical behavior has been observed (41) for ox brain black lipid films treated with GDH; addition of substrates causes a transient decrease in membrane impedance, which effect can be demonstrated repeatedly. This effect is of great interest because of the role postulated (42) for glutamate as an excitatory transmitter substance in the mammalian nervous system and because of the relationship, by decarboxylation, of glutamate to gamma-aminobutyric acid, GABA, which appears to function as an inhibitory transmitter substance. A similar effect was obtained (40) with acetylcholine esterase and acetylcholine, a known excitatory transmitter substance.

Returning to EIM, then, it might be expected to form an oligomeric ring, (possibly with six subunits, and six active sites analogous to the GDH oligomer) which could act as an allosteric drive for an ion gate in a plasma membrane, in particular the nerve membrane. This action of EIM should be similar to that of alamethicin depicted in Fig. 7. A model of the axon membrane with large numbers of EIM oligomers adsorbed on the interior surface, over the lipid rings of hexagonal subunits, is consistent with the well known amplifying characteristics of the membrane. A stimulus that opens only a few hexagonal subunits to influx of $Na(H_2O)_3^+$ ions would be sufficient to trigger a change traversing the whole membrane. These $Na(H_2O)_3^+$ ions would immediately interact with EIM molecules of neighboring subunits, opening the latter to influx of more $Na(H_2O)_3^+$ ions, etc., thereby setting up the nerve impulse wave.

The extraordinarily high amplification characteristic of membranes of the nervous system is well illustrated by the fact that the dark adapted human eye will detect a brief flash of light when only five photons are adsorbed by five rod cells (43). Evidently, only a single photon is enough to trigger the discharge of a dark adapted rod cell in the retina. From the pioneering research of Wald (44), Hubbard and Kropf (45), it is known that the primary action of a light photon on the visual pigment, rhodopsin, is to change the configuration of the retinal chromophore from the bent and twisted 11-cis form to the straight all-trans form:



11-cis retinal all-trans retinal

This action triggers an allosteric configurational change in the opsin structure exposing two-SH groups and one proton-binding group, perhaps imidazole. Adsorption of a second photon by the all-trans retinal, while still attached to the opsin, can re-isomerize the chromophore to the 11-cis form, reversing the allosteric transformation and regenerating rhodopsin. This regeneration can also be accomplished by an isomerase that converts detached all-trans retinal to the 11-cis isomer which then recombines with the opsin according to the reaction scheme.

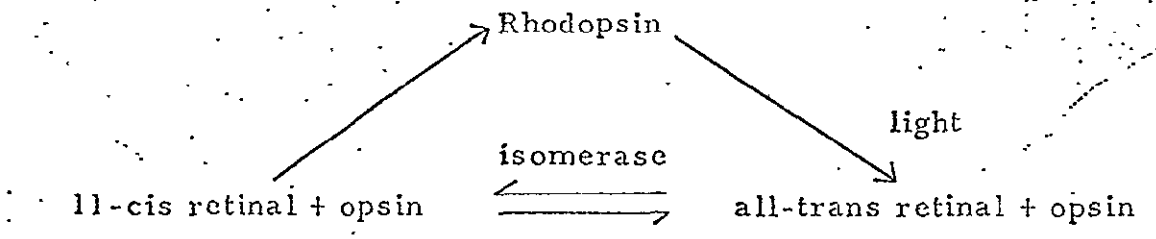


Fig. 8A shows how one rhodopsin molecule and about five EIM or EIM-like molecules, adsorbed on the lipid ring, might act as an allosteric shutter release and mechanical drive, respectively, for a hexagonal ion gate in the unit disk of a vertebrate photoreceptor membrane. The cross-hatched areas of the figure (i.e., highly schematic cross-sections of the control protein in the vicinity of the allosteric effector sites) suggest the size and relative position of these proteins with respect to the lipid ring. The EIM molecules are pictured as behaving like alamethicin molecules in their ability to form reversible chelate complexes with $Na(H_2O)_3^+$. In so doing, the EIM molecules undergo a cooperative allosteric transition that turns the underlying cholestero "shutter leaves" in an angular direction that increases the aperture of the ion-conducting unordered water pore. To effect this overall change, the rhodopsin molecule must also undergo an analogous allosteric transition in synchrony with the EIM molecules. However, this transition, and therefore the opening of the ion gate, is blocked by the bent and twisted form of the 11-cis retinal isomer. Adsorption of only one photon converts this chromophore to the straight all-trans isomer, thereby removing the steric hindrance to the overall configurational change that opens the ion gate and triggers the electrical activity of the retinal rod (Fig. 8B).

It is conceivable that the receptor proteins involved in the other senses might operate in a manner analogous to that of rhodopsin. Instead of retinal, for example, the touch receptor protein might contain a pressure-sensor molecule whose bent-twisted shape (or high energy state) is stabilized by a slight steric hindrance among intramolecular groups. A slight pressure impulse could disturb this delicate equilibrium, permitting the sensor molecule to straighten out, thereby unlocking the ion gate. A second pressure impulse or an enzyme could then restore the bent-twisted shape (or high energy state) of the pressure sensor molecule which would interact with the touch receptor protein to return it to its original configuration. The specific sound-, heat-, and cold-receptor proteins might also function in a similar manner. Sound, in essence, is a cyclical pressure impluse and temperature differences are easily translated into pressure differences by expansion-contraction. It is not implied that all these sensory receptor proteins are identical; however, they may have evolved from a common prototype and could have many similar structural features.

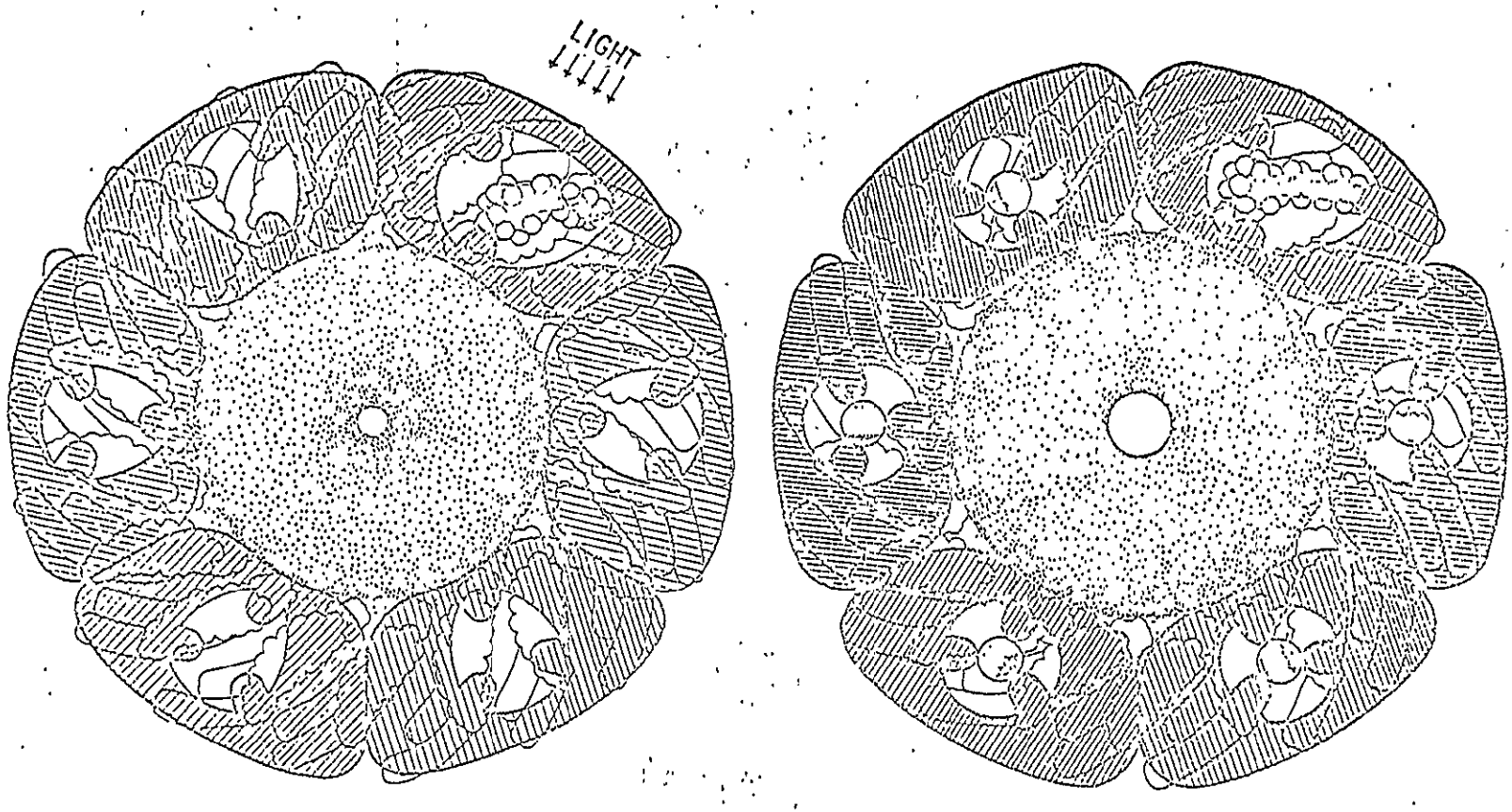


FIGURE 8. (A) Hypothetical arrangement of one rhodopsin molecule and several EIM (or EIM-like) molecules adsorbed on the interior surface of a lipid ring of a hexagonal subunit in the retinal rod membrane. (B) Representation of the ion-gating allosteric transition resulting from the adsorption of one photon by 11-cis retinal of a rhodopsin molecule and the chelation of sodium ions by the EIM (or EIM-like) molecules.

Even the olfactory sensory receptor protein might operate in a similar manner to open and close an ion gate. Adsorption interaction between an odor molecule and a receptor site could result in a preliminary allosteric transition that would trigger the main allosteric transition involving the pressure sensor molecule. Odor discrimination and identification seems to depend on an approximate stereochemical lock and key fit between the receptor site and the odor molecule. The approximate size and shape of the olfactory receptor sites for five "primary" odors have been characterized by Amoore, Johnston and Rubin (46). Two additional "primary" odors pungent (e.g., formic acid ⊕) and putrid (e.g., hydrogen sulfide ⊖) apparently interact with their specific receptor sites on the basis of charge, not shape. However, in these cases (and possibly also in the others) direct allosteric interaction between the odor molecule and the receptor protein may suffice to release the hexagonal ion gate, without mediation by the hypothetical pressure sensor molecule.

The Hexagonal Subunit in Active Transport

The enzyme system in cell membranes that transports $Na(H_2O)_3^+$ outwards and $K(H_2O)_2^+$ inwards is known as "$(Na^+ + K^+)$-activated ATPase," or more simply as the "sodium pump." In the nerve axon, this pump is required to hold the interior sodium ion concentration to about 10 per cent that of the exterior fluid; at the same time the interior potassium ion concentration is maintained at a level 30-times greater than in the exterior. In the human erythrocyte, the energy of one ATP molecule, when hydrolyzed by the membrane ATPase, is sufficient to actively transport some 2.4 ± 0.3 potassium ions inwardly and 3.2 ± 0.2 sodium ions outwardly (47). The following model of the sodium pump is consistent with this stoichiometry.

Consider an oligmeric group of four or five EIM-like molecules and one ATP-hydrolysis enzyme adsorbed on the interior of the axon membrane on a lipid hexagonal subunit in a manner analogous to that illustrated in Fig. 8 for the EIM-rhodopsin system. Fig. 9 indicates the sequential configurational changes in the unordered water pore as a result of the allosteric interaction of an ATP molecule with the hydrolysis enzyme. It will be recalled from the earlier discussion that the Hodgkin-Keynes radiotracer diffusion experiments (37) yielded a value of 2.5 for the average number of $K(H_2O)_2^+$ ions that could fit in the pore in resting configuration; in Fig. 9A two of these ions from the axon exterior are shown in the pore. There is an electrical repulsion force between these ions which, presumably, is counterbalanced by the adsorption-hydration interaction of the ions with the pore wall (i.e., the closely fitting ordered water sheath surrounding the pore). In Fig. 9B, the adsorption of an ATP molecule by the hydrolysis enzyme and the chelation of $Na(H_2O)_3^+$ ions by the EIM-like molecules has triggered an allosteric configurational change, at the inner layer of the lipid ring. This change progressively opens the pore in a manner that loosens the adsorption-hydration interaction of the pore wall with the two $K(H_2O)_2^+$ ions, thereby permitting them to diffuse into the axon interior. In 9C, the pore has opened sufficiently to admit three $Na(H_2O)_3^+$ ions from the axon interior. Here again there probably exists mutual electrostatic repulsion between these hydrated sodium ions that is counterbalanced by adsorption-interaction with the closely fitting pore wall.

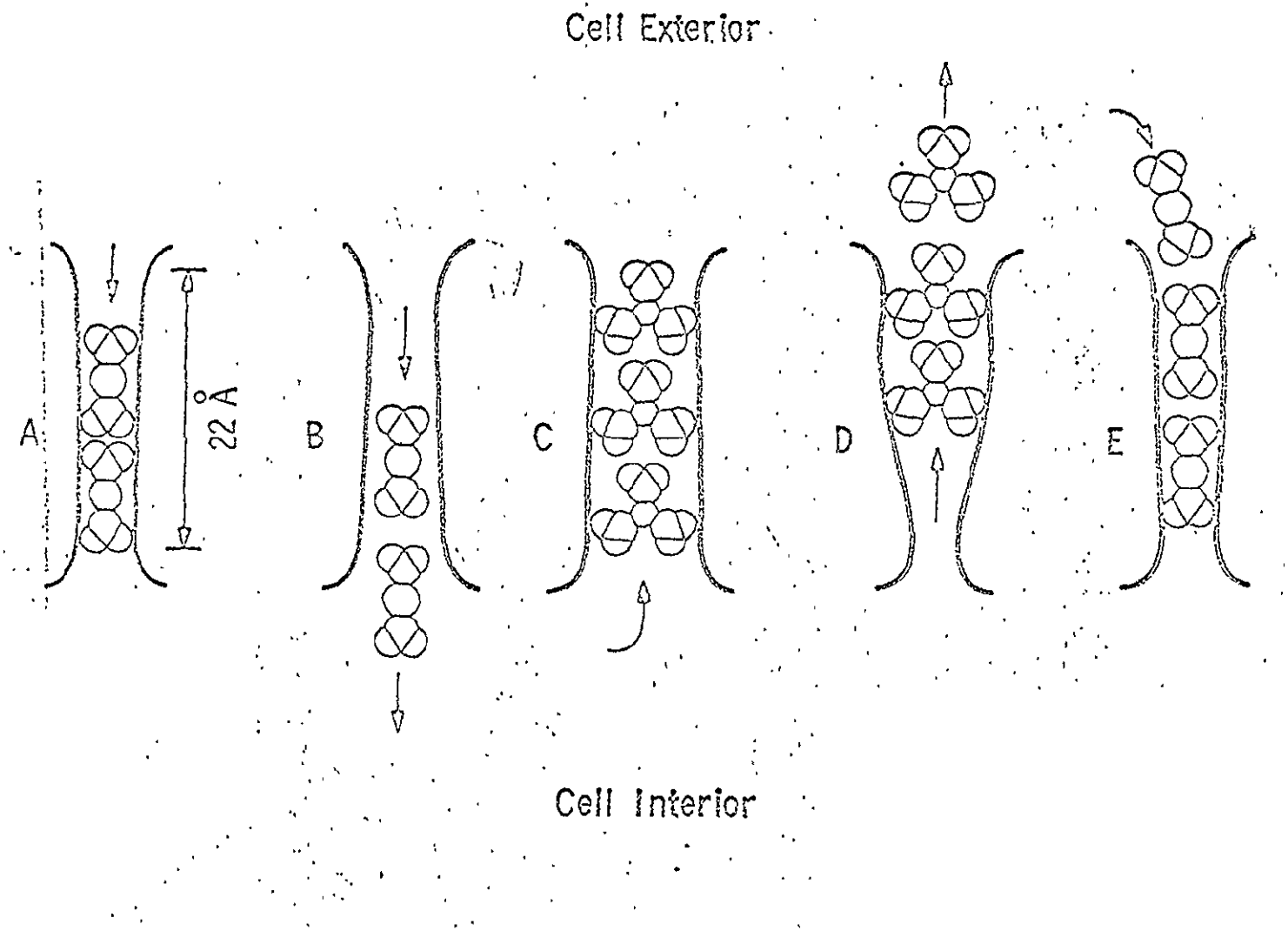


FIGURE 9. Sequential configurational changes of the pore of unordered water of an hexagonal subunit during the operation of the sodium pump.

Potassium ions entering the pore immediately before this stage no longer possess this close fit and would be repelled by any sodium ion already in the pore. This would account for the sodium ion selectivity of the pore as it approaches its open configuration. In 9D, hydrolysis of ATP by the enzyme has triggered an additional allosteric configurational change that progressively closes the pore. The closing process produces a peristaltic action by the pore wall that eventually ejects the $Na(H_2O)_3^+$ ions. In 9E, three $K(H_2O)_2^+$ ions from the axon exterior are entering the pore to restart the cycle.

The peristaltic action (and also the slight asymmetry) of the pore arises from the fact that the cholesterol "shutter leaves" at the inner surface of the hexagonal subunit are not directly geared to those at the outer surface. When, for example, the inner leaves move cooperatively to decrease pore aperture, there is a time delay before the forces of surface tension induce a similar motion in the outer leaves. There is also a time delay involved in reorganizing the ordered water sheath from the open to the closed configuration. These time delays are translated into peristaltic pumping action in the manner shown in Fig. 9. This type of pumping action by hexagonal subunits is probably involved not only in the sodium pump but also in active transport, in general, through plasma membranes. Specific membrane transport proteins, whose properties were recently reviewed by Pardee (48), may be constituents of oligomeric rings of allosteric control proteins adsorbed on lipid rings of hexagonal subunits. Each protein ring would probably contain an allosteric substrate-recognition subunit (similar to that postulated earlier for the olfactory receptor protein) and an hydrolysis enzyme that allosterically converts the energy of ATP (or other high energy molecule) to a peristaltic pumping action. Formation of a reversible complex between a substrate molecule and the recognition subunit would trigger this pumping action, which would continue until the substrate concentration in the vicinity of the pore decreases to a level that permitted dissociation of the recognition complex.

It was implied in the preceding discussion that the hexagonal subunit pattern is a basic characteristic of the entire cellular plasma membrane rather than a specific feature of tight intracellular junctions as suggested by previous investigators. (17) (19) Past failure to resolve the hexagonal subunit pattern in electron microscopic studies of non-junctional areas may be the result of a sensitivity of the membrane to the rather drastic preparative and fixation procedures employed (e.g. treatment with deoxycholate). Also, the staining characteristics of mucopolymer layers of the complex membrane may obscure the features of the layer comprised of hexagonal subunits.

A case in point is Vibrio fetus whose negatively stained preparations, when first studied by Ritchie, et. al., (49) (50) failed to show evidence of a regular geometric pattern in the cell envelope, viewed frontally. In cross-section, however, the centripetal border marking the innermost surface had a finely scalloped appearance, reminiscent of the Mauthner cell synaptic disk studied by Robertson (16). To resolve this apparent inconsistency, Ritchie and Bryner (51) studied V. fetus cells that had been infected with phage. As a consequence of this infection or of an interaction with enzymes released by phage-infected cells, part of the mucopolymer component of the cell wall was removed revealing a subunit structure. This is shown in the rather remarkable electron micrographs, Figures 10-11, reproduced by courtesy of Dr. A. E. Ritchie. Figure 10 reveals an overall subunit structure in what may be the "skelcon" of V. fetus. Figure 11 shows a phosphotungstate-stained cell wall fragment with a hexagonal subunit array (center-to-center spacing ~ 90 Å) comparable to the previously described "paste-up" models of the cellular membranes, Figure 6. The experimental technique of Ritchie and Bryner appears to have resolved the unordered water pore region in the hexagonal subunit as an electron-dense component in the center of a relatively unstained annular sheath of ordered water. The postulated lipid ring surrounding this sheath, however, is not resolved presumably because it is covered by a darkly-stained layer of allosteric control protein or mucopolymer. Fig. 12 demonstrates the close correspondence between the hexagonal pattern in the Vibrio fetus cell wall and the paste-up model of the cellular membrane (Fig. 6B) reduced to the same scale.

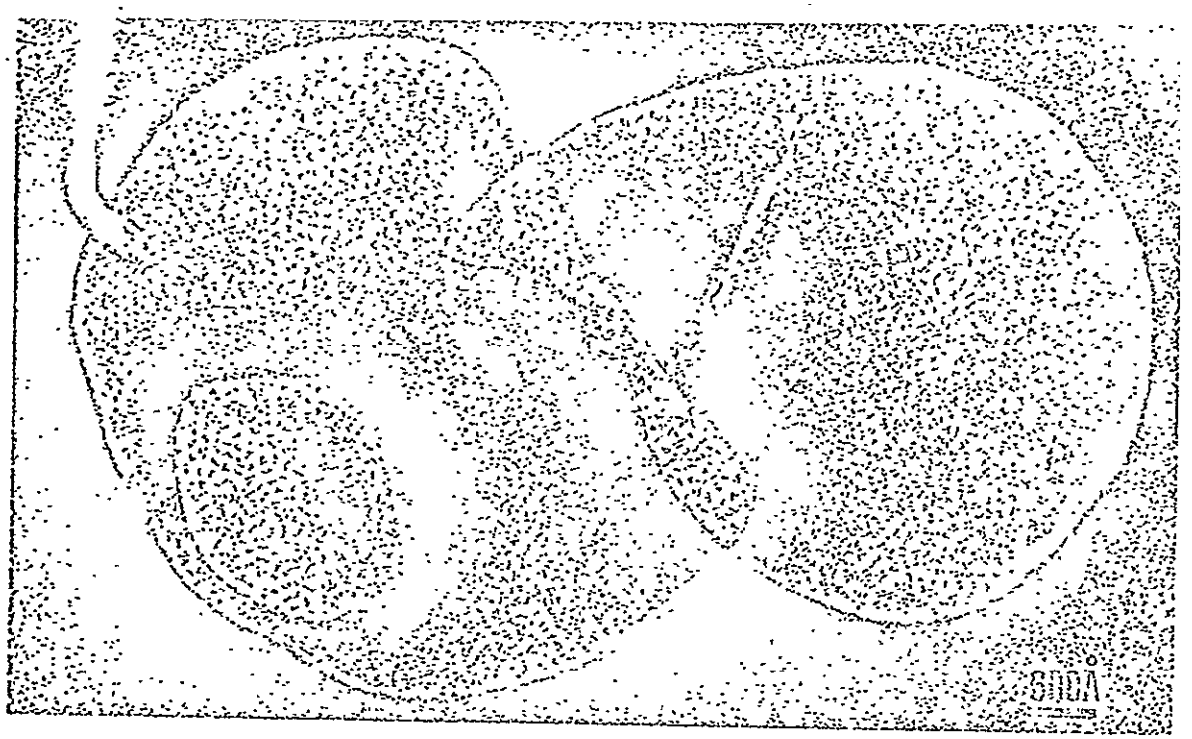


FIGURE 10. Partially degenerated wall of phage-infected Vibrio fetus cell (with portion of phage tail) suggesting an overall hexagonal subunit structure. Reproduced by courtesy of Dr. A. E. Ritchie.

FIGURE 11. Fragment of cell wall of phage-infected Vibrio fetus showing hexagonal subunit array. Reproduced by courtesy of Dr. A. E. Ritchie.

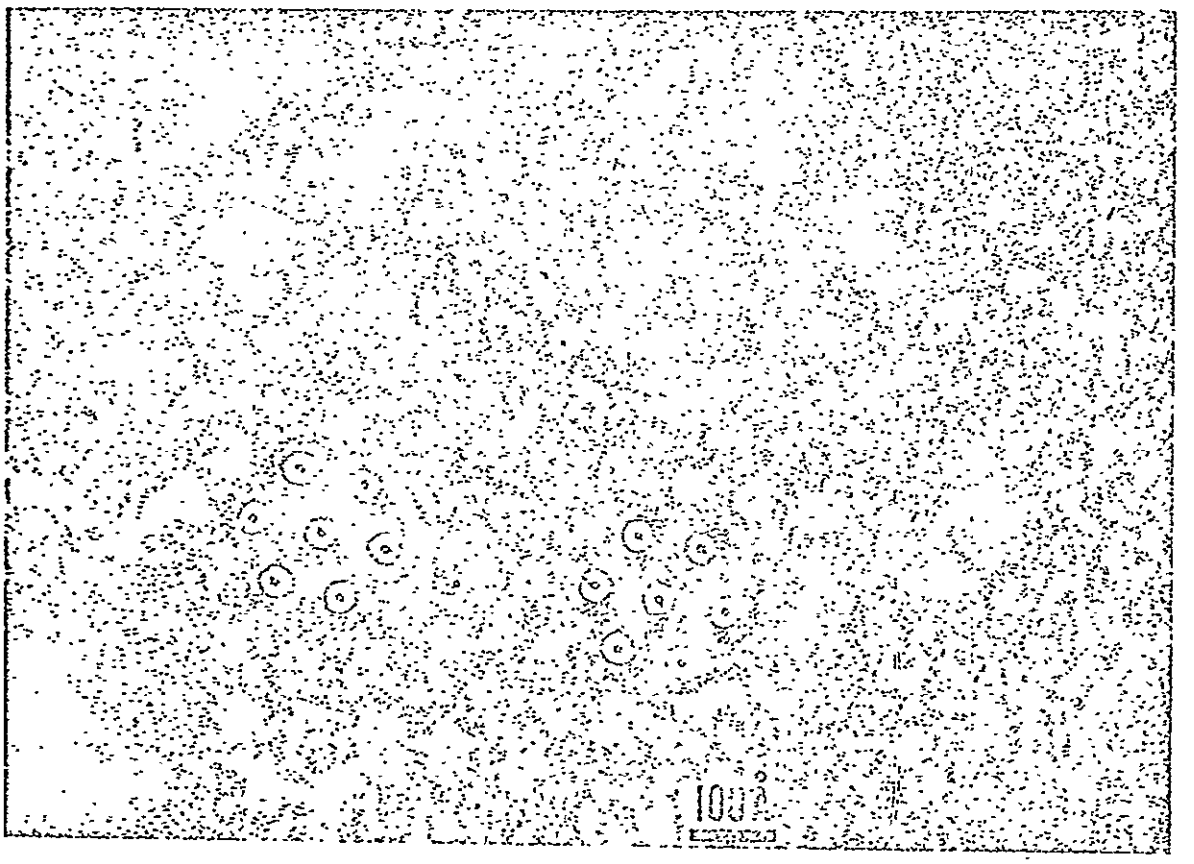


FIGURE 12. "Paste-up" approximation of closed packed array of hexagonal subunit models superimposed on previous figure of fragment of cell wall of Vibrio fetus.

In view of these results, serious consideration must be given to abandonment of the simple bimolecular leaflet "unit membrane" model in favor of a hexagonal subunit array concept of the cellular plasma membrane.

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DOUGLAS PAPER 10,247

CHARACTERIZATION OF ORDERED WATER IN HYDROPHILIC MEMBRANE PORES

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INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY
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SEPTEMBER 1–5, 1969

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CHARACTERIZATION OF ORDERED WATER IN HYDROPHILIC MEMBRANE PORES

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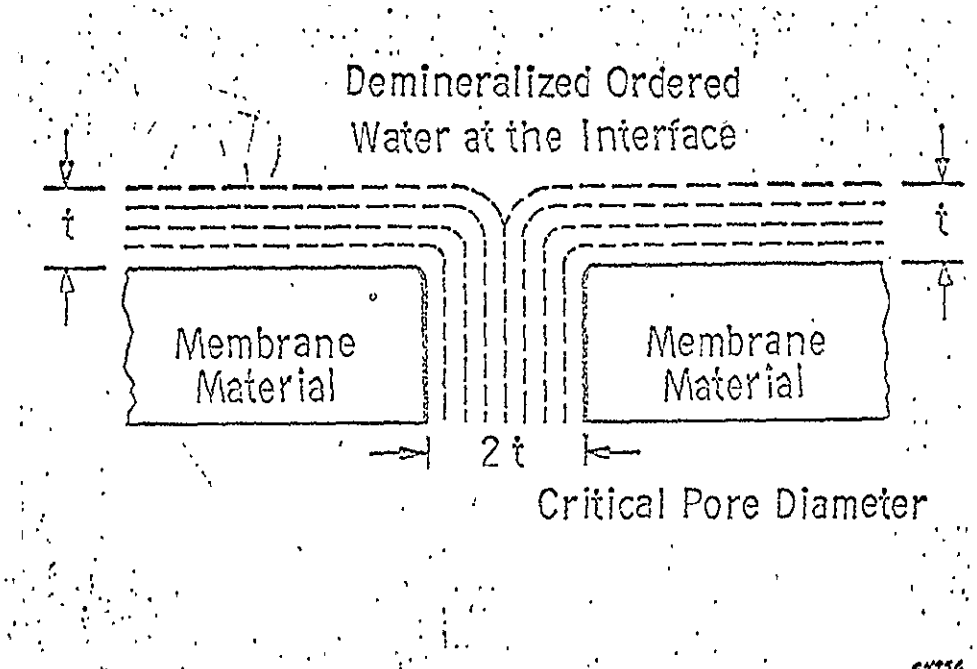
ABSTRACT Ordered water, whose presence in biological membranes is often postulated, has been characterized in cellulose acetate and porous glass desalination membranes. Above a certain critical diameter, each membrane pore is lined with a ~22 Å thick hydration sheath of highly hydrogen-bonded water in which salt is essentially insoluble. A pore with an ideal critical diameter of ~44 Å is almost entirely filled with ordered water whose average viscosity is 0.36 poise, almost 39 times greater than that of ordinary water at 23°C. The simple assumption of approximate equality between hydration sheath thickness and the average diameter of a "flickering cluster" permits a rate process calculation of the permeation of salt-free ordered water through an ideal desalination membrane in good agreement with experimental data. The aqueous cylinders in the Luzzati-Husson liquid-crystalline hexagonal phase of a brain phospholipid-water system are mainly ordered water stabilized by the polar ends of the phospholipid molecules. The insolubility of salt in the ordered water indicates a high electrical resistivity. A new model of the cellular plasma membrane, based on these concepts, is developed in a forthcoming paper from this laboratory.

INTRODUCTION

The importance of organized "ordered" water as an integral structural component of biological membranes has been emphasized by Fernández-Morán (1-5), repeatedly, and by Hector (6). Nevertheless, the development of the concept (1) of a cellular membrane with selectively permeable molecular pores lined with ordered water has, heretofore, been hindered by a lack of accurate knowledge of the ultrastructure and properties of such water. As early as 1955, Jacobson (7) proposed that macromolecular

surfaces stabilize surrounding water into "lattice-ordered hydration shells." This structural change was attributed to hydrogen bonding between the water lattice and certain hydrophilic atoms in the macromolecular surface. The shells or sheaths were considered to be ice-like in the sense that the thermal vibrations in the lattice are reduced and that the disorganization of the lattice is less than that in pure water. However, the density and other thermodynamic properties of the hydration sheaths were thought to resemble the properties of water more than ice. Unfortunately, Jacobson's theory was premature and ran into considerable opposition because his experimental method of estimating the thickness of the hydration "shell" or sheath in contact with a macromolecule (NaDNA) yielded erroneous results (8). However, from neutron scattering experiments (9) and studies of water structure by Frank and Wen (10) and Nemethy and Scheraga (11, 12) it is now possible to envisage ordinary bulk liquid water as being made up of "flickering clusters" of about 50 to 100 molecules with an average lifetime of 10^{-11} seconds. The stability of a cluster is the result of a resonance effect in the highly hydrogen-bonded structure. The adsorption-interaction of a cluster with a polar or hydrogen bonding group in a macromolecular surface should increase this resonance effect, thereby increasing the cluster size; also the average lifetime of a cluster should increase to some value between that of ice (10^{-4} sec) and that of water (10^{-11} sec), as measured by the reciprocal of the frequency for dielectric dispersion. If the surface density of the polar hydrogen groups is sufficiently great, these adsorbed clusters will interact with each other resulting in additional resonance, size, and stability until an almost continuously clustered hydration sheath of appreciable thickness is formed.

If this view is valid, it follows by analogy with the known properties of highly hydrogen-bonded water clathrates and ice, that the solubility of salt in the lattice-ordered hydration sheath at a macromolecular or solid interface should be very much lower than that in bulk water. [Supporting evidence on this point, for silica and alumina, has recently been reported (13).] The hydration sheath may, therefore, be identified with the layer of demineralized water (of thickness t) that Sourirajan (14) postulated to be present at the interface of a porous desalination membrane. From Fig. 1 it is readily seen that the thickness, t, of the hydration sheath is about half the



Figure, 1. Relationship between salt-free ordered water hydration sheath at hydrophilic membrane interface and critical pore diameter for desalination.

critical diameter (2t) of a pore that will reject 100% of salt from the pressurized feed water. Because Sourirajan (14) lacked the necessary experimental data on pore sizes in his cellulose acetate desalination membranes, he was unable to evaluate t, but it is now possible to do this on the basis of the cellulose acetate subunit structure revealed by the electron microscopic studies described in the following section.

ELECTRON MICROSCOPIC STUDIES OF CELLULOSE ACETATE MEMBRANES

An ultrathin (~600 Å) film of Eastman 398-10 cellulose acetate was prepared on a glass slide, by evaporation of a dilute acetone solution according to the method of Carnell (15) as modified by Riley et al (16). The fresh film, in place on the slide, was shadowed with 80:20 Pt-Pd alloy by vacuum evaporation at a low angle of 7° to emphasize small surface detail. After deposition of a thin (~200 Å) carbon layer over the shadow, the cellulose acetate was dissolved away with amyl acetate followed by acetone. A Hitachi HU-11 electron microscope was used to obtain electron micrographs of the preshadowed carbon replica at 19,000 to 30,000 X. A two-stage printing procedure was used to obtain reversed prints (165,000 X) in which the shadow areas without deposited metal appear as dark areas with high photographic density and the Pt-Pd impingement areas appear white to simulate surface illumination by light at a 7° angle. ("Up" on the final print is "up" on the sample.) This print (Fig. 2) reveals a surface consisting of rounded subunits linked together in an irregular closely packed array. Measurements of distance from shadow-edge to shadow-edge of adjacent subunits indicate an average subunit diameter of about 188 Å ± 3 Å. The rod-like features marked a and b may indicate microcrystals of unacetylated or partially acetylated cellulose of the type observed by Battista and Smith (17).

Fig. 3 is a similar electron micrograph of a carbon replica of the smooth dense air-dried surface of a modified Loeb-Sourirajan type cellulose acetate membrane, cathodically etched in an argon discharge to remove organic contaminants and possibly low molecular weight amorphous components. The membrane was prepared and annealed according to the method of Manjikian et al (18) from a casting solution consisting of 25% cellulose acetate (Eastman 398-10), 30% formamide and 45% acetone. The electron micrograph of the

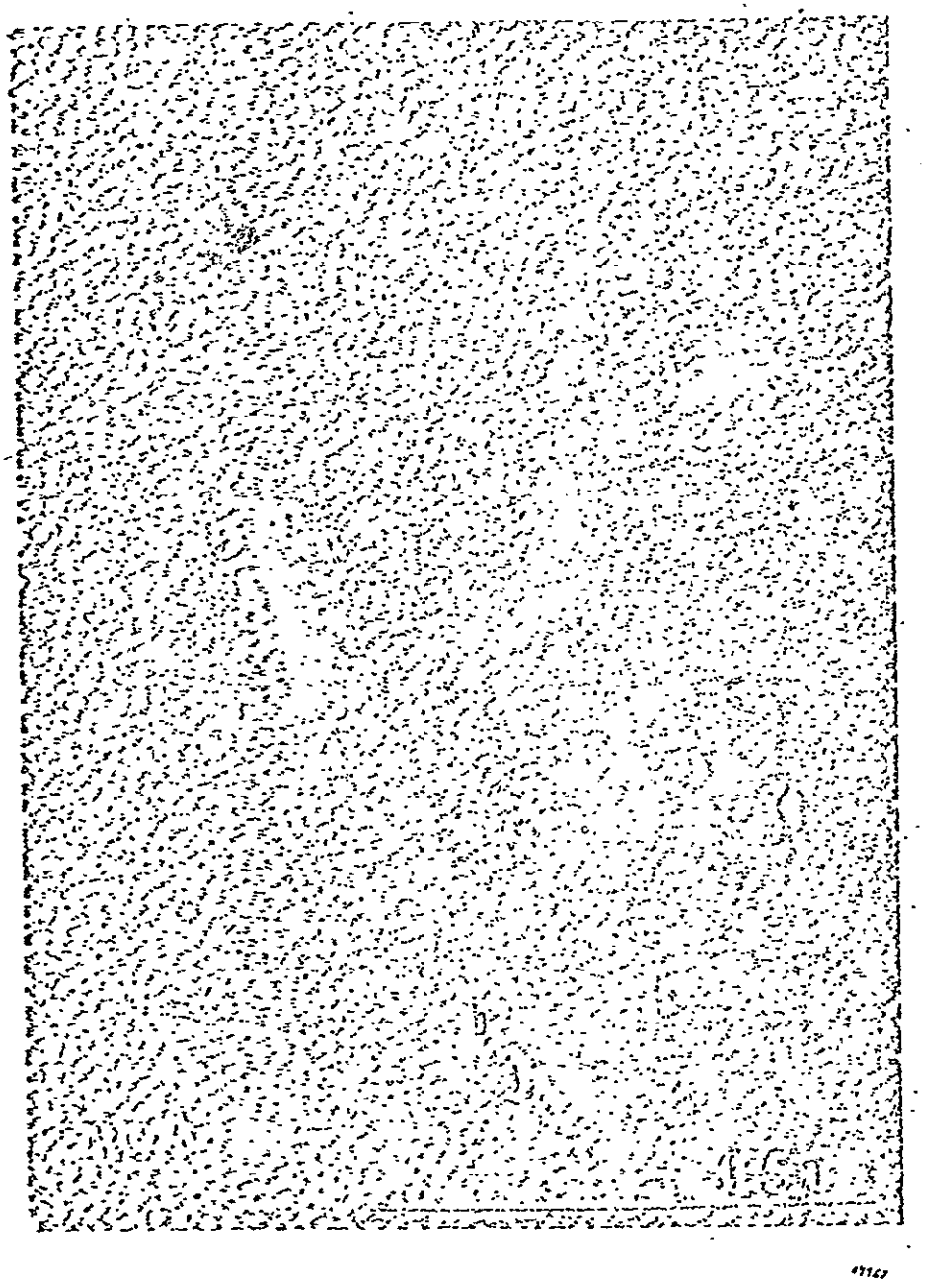


Figure 2. Electron micrograph of Pt-Pd preshadowed carbon replica of the surface of an ultrathin (~600 Å) cellulose acetate membrane.

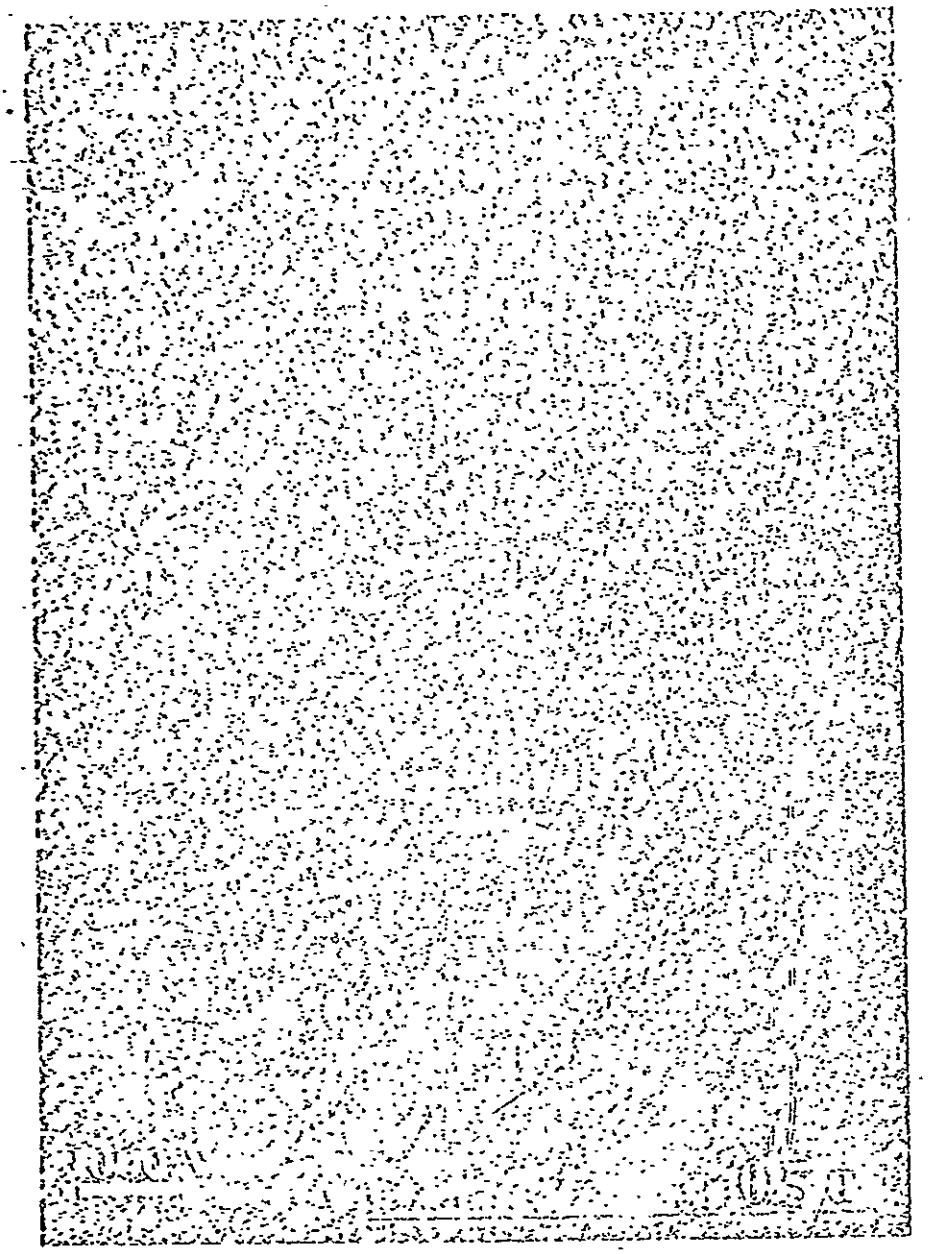


Figure 3. Electron micrograph of Pt-C preshadowed carbon replica of the surface of the active desalination layer of a modified Loeb-Sourirajan type cellulose acetate membrane.

replica, preshadowed with Pt-C at a 9° angle, resolves an array of randomly packed subunits which appear as hemispheres, 180-190 Å in diameter, similar to those observed in the ultrathin membrane (Fig. 2). Strikingly similar modes of random subunit packing have been shown by electron microscopy to be present in collodion film (19) and in a preparation (possibly artifactual) of innermost rigid layer of the cell wall of E. coli Strain B (20). Similar packing of folded-chain subunits in quenched crystallized and annealed PCTFE bulk polymer has been described (21).

RATE PROCESS CALCULATION OF PERMEATION OF ORDERED WATER THROUGH A CELLULOSE ACETATE DESALINATION MEMBRANE

Tortuosity Factor, Effective Pore Radius, and Surface Pore Density for Poiseuille Flow

The interstices between microcrystalline subunits in bulk polymer samples may contain amorphous or low molecular weight material (21). However, in the case of cellulose acetate desalination membranes prepared by evaporation or precipitation from solution, the amorphous or low molecular weight fractions should remain in the mother liquor. Therefore, as a temporary working hypothesis (to be resolved in a later section) it will be assumed that the interstices of these membranes are comparatively free of such material and form a system of interconnecting channels, permitting transport of ordered water by viscous (i.e., Poiseuille-type) flow. For the special case where the membrane pores have the ideal critical diameter for desalination (Fig. 1) this flow is given by the equation

$$J = \frac{n_p \pi P (r_p^*)^4}{8 \eta L \tau} \qquad (1)$$

where J is the ordered water permeation rate, ml/cm^2 sec; n_p is the surface pore density, pores/cm^2; P is the driving pressure, dynes/cm^2; r_p^* is the effective pore radius, cm; η is the ordered water average viscosity, dyne-sec/cm^2, (i.e., poise); L is the thickness of the active desalination layer of the membrane, cm; and τ is a tortuosity factor indicating that the

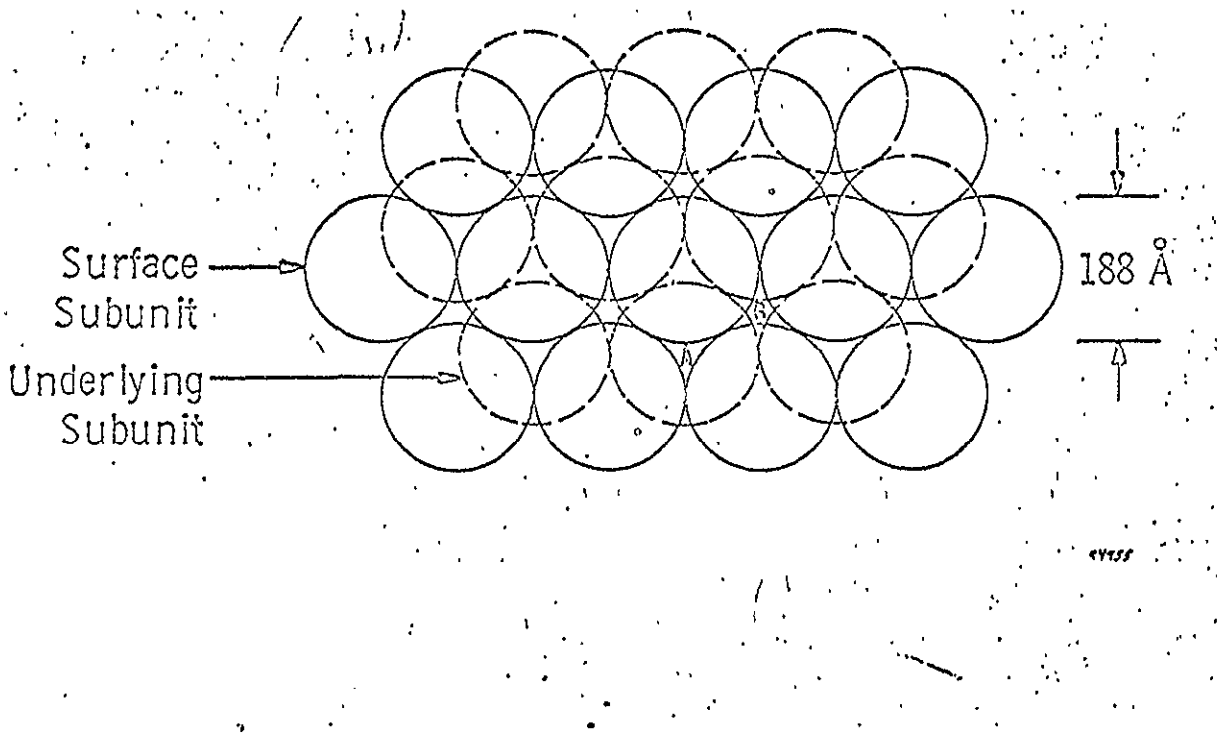


Figure 4. Active desalination layer of cellulose acetate membrane idealized as assembly of close packed 188 Å diameter spheres. Ordered water permeates through interstitial pores (A and B).

effective pore length is greater than the membrane thickness. The assumed pore system is nearly equivalent to the random mesh of noncircular, tortuous and interconnecting pores whose flow properties were analyzed by Carman (22). His analytical treatment is equivalent to assigning a value of 2.5 to the tortuosity factor τ in equation (1).

If a cellulose acetate membrane is approximated as an assembly of closely packed spheres 188 Å in diameter (Fig. 4), a simple calculation gives $94^2 (\sqrt{3} - \pi/2) = 1430$ Å^2 for the cross section of a triangular shaped pore. From this, a mean pore radius $r_p \backsimeq 21.3$ Å may be estimated. However, the effective pore radius r_p^*, to be used in equation (1), will be approximated as less than r_p by the thickness (2.8 Å) of a monomolecular layer of water, assumed to be immobilized by adsorption on the pore wall. Accordingly $r_p^* \backsimeq 1.85 \times 10^{-7}$ cm.

The number n_p of surface pores per cm^2 of a cellulose acetate membrane is estimated as the reciprocal of the area of an equilateral triangle of side equal to the diameter of a subunit. Accordingly, $n_p \backsimeq 1/0.433(1.88 \times 10^{-6})^2 \backsimeq 6.5 \times 10^{11}$ pores/cm^2.

Note that a Loeb-Sourirajan cellulose acetate membrane, as cast, has an extremely high water permeation rate and little tendency to reject salt. Heat treatment in water at 80-90°C, lowers the water permeation rate and drastically increases salt rejection. Lateral shrinkage of only 11% in the membrane plane, after 15 minutes at 87°C, increases salt rejection, from <10% for the "as cast" membrane to 95% (23). It follows that twice the average pore radius (r_p = 21.3 Å), previously estimated from Figs. 2-4, should be nearly equal to the ideal critical pore diameter, 2t, for desalination (Fig. 1) On this basis, the thickness of the lattice-ordered hydration layer at a cellulose acetate interface is estimated as $t \backsimeq r_p \backsimeq 21.3$ Å. Accordingly, the interstitial pores of the heat-treated membrane may be considered completely filled with ordered water and equation (1) should be valid for calculation of the flow rate.

Cluster Size and Viscous Flow Activation Energy of Ordered Water in a Cellulose Acetate Membrane Pore

Values of the number of molecules n_{cl} in an average-size "flickering" hydrogen-bonded cluster in bulk water, from the Nemethy and Scheraga (11) analysis, are plotted in Fig. 5 as a logarithmic function of a reciprocal reduced absolute temperature $(T-150)^{\circ}K$. (This reduced temperature has been shown by Miller (24) to be proportional to the volume fraction of unbonded water molecules in the Nemethy-Scheraga analysis.) The Jacobson (7) concept of lattice-ordered water at a hydrophilic macromolecular interface will now be updated by approximating the average cluster diameter of water in the hydration sheath as equal to the sheath thickness t. From the known volume of a water molecule (29.9 $Å^3$) and from the value $t \simeq r_p \simeq 21.3$ Å, it is readily calculated that the number of molecules n_{cl} in the average cluster of water in a cellulose acetate membrane pore is about 162 at room temperature (i.e., $T = 296^{\circ}K$). The linear extrapolation shown in Fig. 5 indicates that this cluster size is equivalent to that in supercooled water at a temperature $T' = 255^{\circ}K$. In a restricted sense, therefore, it might be imagined that the polar or hydrogen bonding groups in the membrane pore wall lower the effective temperature of water molecules in the hydration layer to T'. However, it will become evident from subsequent calculations that it is incorrect to assume that all of the viscous flow properties of the ordered pore-water (which after all, is at ambient temperature) are identical with that of water supercooled to $255^{\circ}K$. Nevertheless, the activation energy for viscous flow of such supercooled water, calculated by the empirical relationship of Miller (24),

$$\Delta H^{\ddagger} = 1020 \left[T'/(T'-150) \right]^2 \text{ cal/mole} \qquad (2)$$

turns out to be 6015 cal/mole in excellent agreement with the asymptotic experimental value 5950 cal/mole reported by Keilin et al (24) for permeation of water through a Loeb-Sourirajan type cellulose acetate desalination membrane of 53.5% water content and 99.5% salt rejection capability.

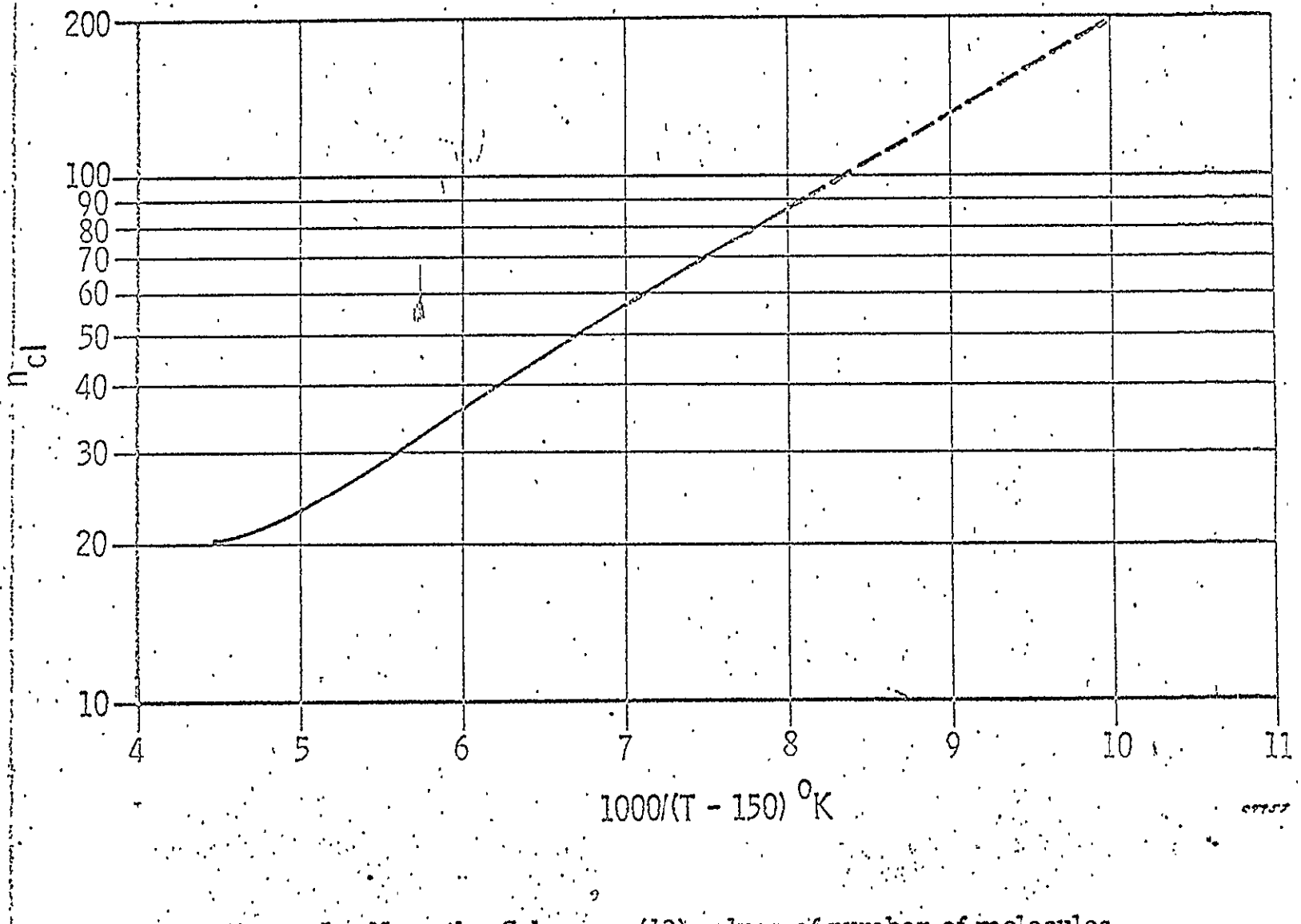


Figure 5. Nemethy-Scheraga (12) values of number of molecules in water cluster, as function of temperature.

The Glasstone-Laidler-Eyring (26) rate process treatment of viscous flow of liquids yields the equation

$$\eta = \frac{hN}{V} e^{\Delta F^{\ddagger}/RT} = \left[\frac{hN}{V} e^{-\Delta S^{\ddagger}/R}\right] e^{\Delta H^{\ddagger}/RT} \quad (3)$$

where η is the viscosity in poise; h is Planck's constant 6.62×10^{-27} erg-sec; N is Avogadro's number 6.02×10^{23} mole^{-1}; V is the molar volume of the liquid, cm^3/mole; $\Delta F^{\ddagger}$ is the free energy of activation for the viscous flow process, cal/mole; $\Delta S^{\ddagger}$ is the corresponding entropy of activation, cal/mole-$^{\circ}$K; R is the gas constant, 1.99 cal/mole $\cdot$ $^{\circ}$K; T is the absolute temperature, $^{\circ}$K; $\Delta H^{\ddagger}$ is the enthalpy of activation, cal/mole.

A striking achievement of the rate process theory of viscous flow is the important relationship

$$\left(E_{vap} / \Delta F^{\ddagger}\right) = 2.45 \quad (4)$$

which has been found to hold for nearly 100 nonmetallic substances, including water and other associated liquids, even glycerol, and nonpolar substances such as hydrocarbons. This relationship should, therefore, be valid for lattice-ordered pore water, for which E_{vap}, the molar energy for vaporization, will be approximated by the equation

$$E_{vap} \backsimeq E_w + C_w (T - T') \quad (5)$$

where E_w is the latent energy of vaporization of bulk water at ambient temperature, cal/mole; C_w is the molar heat capacity of liquid water, cal/mole $\cdot$ $^{\circ}$K; T' is the temperature (in $^{\circ}$K) at which the cluster size of bulk water is the same as that in pore water at the ambient temperature T. From (5) it is estimated that $E_{vap} \backsimeq 9900 + 18\,(296-255) \backsimeq 10,640$ cal/mole and from (4) that $\Delta F^{\ddagger} \backsimeq (10,640/2.45) \backsimeq 4350$ cal/mole. The relationship, $\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\,\Delta S^{\ddagger}$, gives $\Delta S^{\ddagger} \backsimeq 5.63$ cal/mole $\cdot$ $^{\circ}$K. Substitution of these values into equation (3) yields a value of 0.35 poise for the viscosity of the lattice-ordered pore water in a cellulose acetate membrane at 296°K. This viscosity is about 37 times greater than the value of 0.00936 poise for ordinary water at this temperature (i.e., 23°C).

Comparison of Theoretical and Experimental Water Permeation Rates Through Cellulose Acetate Desalination Membranes

The computed viscosity (0.35 poise at 296°K) of ordered water in a cellulose acetate membrane pore is now used to estimate the flux of water through the membrane. Substitution of this value, along with the other previously estimated parameters, into equation (1) yields a water permeation rate $\simeq 9.6 \times 10^{-4}$ ml/cm^2 · sec (i.e., 20 gallons/ft^2/day) through a 2400 Å thick effective desalination layer subjected to a driving pressure of 1000 psi. This theoretical value is in good agreement with the experimental value 22 gfd obtained in this laboratory with a modified heat treated Loeb-Sourirajan type membrane (No. 52), for which the surface structure is shown in the electron micrograph, Fig. 3. (Salt rejection was 95%, after 20 hours, for 1% NaCl feed water.) This type of membrane consists of a very thin (~2500 Å) dense layer of cellulose acetate over a porous sponge-like matrix that offers little resistance to salt and water flow (27, 28). The thickness of the dense layer, and hence the water permeation rate, appears to be independent of the overall membrane thickness (29). For this type membrane with sea water feed at 296°K, subjected to 1500 psi operating pressure (i.e., 1331 psi driving pressure after correcting for osmotic pressure) a permeation rate of 27 gfd is calculated. This rate is in fair agreement with a published value (29) of 35 gfd initial rate, dropping to 21 gfd in 24 hours, presumably because of matrix compaction.

For 1% NaCl feed at 296°K subjected to 1500 psi operating pressure (i.e., 1381 psi driving pressure), a rate of 28 gfd is calculated in reasonable agreement with a published value (16) of 19.7 gfd for an ultrathin, 2400 Å, cellulose acetate membrane similar in type to that shown in Fig. 2. This last rate corresponds to a 0.5 hour run in which 98% salt rejection was obtained and in which a "100 Å" millipore filter support (dull surface in contact with the membrane) may have reduced the water permeation to a value somewhat below theoretical.

RATE PROCESS CALCULATION OF WATER PERMEATION THROUGH A POROUS GLASS DESALINATION MEMBRANE

Porosity and Desalination Characteristics of Unfired Vycor-type Glass

In the previous theoretical analysis of water permeation through a cellulose acetate membrane, it was assumed that the ~40 Å diameter pores between the rounded polymeric subunits were wide open, not collapsed nor filled with short molecular chains of cellulose acetate or debris. In order to remove doubts about that assumption, it will be demonstrated that the same theoretical analysis is valid for permeation of water through a desalination membrane of known open-pore structure, namely unfired Vycor-type glass.

It is the experience of this laboratory that the salt rejecting capability of Corning No. 7930 porous glass membranes varies from lot to lot, suggesting that certain parameters in their manufacture are not well controlled. Kraus, et al. (30) worked with a lot in which the distribution of pore diameters peaked narrowly at 53 Å and in which 95% of the pores had diameters between 36 and 56 Å. This lot (which rejected only 5 to 10% of the salt from a 0.03M NaCl feed solution) had a surface area of only 133 m^2/g. This lot seems to be similar to the "early samples" of unfired Vycor glass (surface area 121 m^2/g) described by Nordberg (31) who noted, however, that this porous glass can also be obtained with a high surface area slightly above 200 m^2/g and a 40 Å average pore diameter.

About three years ago, the authors worked with Corning No. 7930 porous glass capillaries (presumably of intermediate porosity and surface area) whose walls rejected 50 to 65% of the salt from a 10,000 ppm NaCl feed solution. Fig. 6 is a transmission electron micrograph of fragments of one of these capillaries crushed between two microscope slides. (Light areas in the electron micrograph indicate pores.) A sponge-like structure with voids ranging in diameter from 30 to 100 Å is observed; however, some channels connecting voids seem to be about 35 to 45 Å in diameter. Salt rejection probably occurred at these channels, in accordance with the mechanism depicted in Fig. 1.

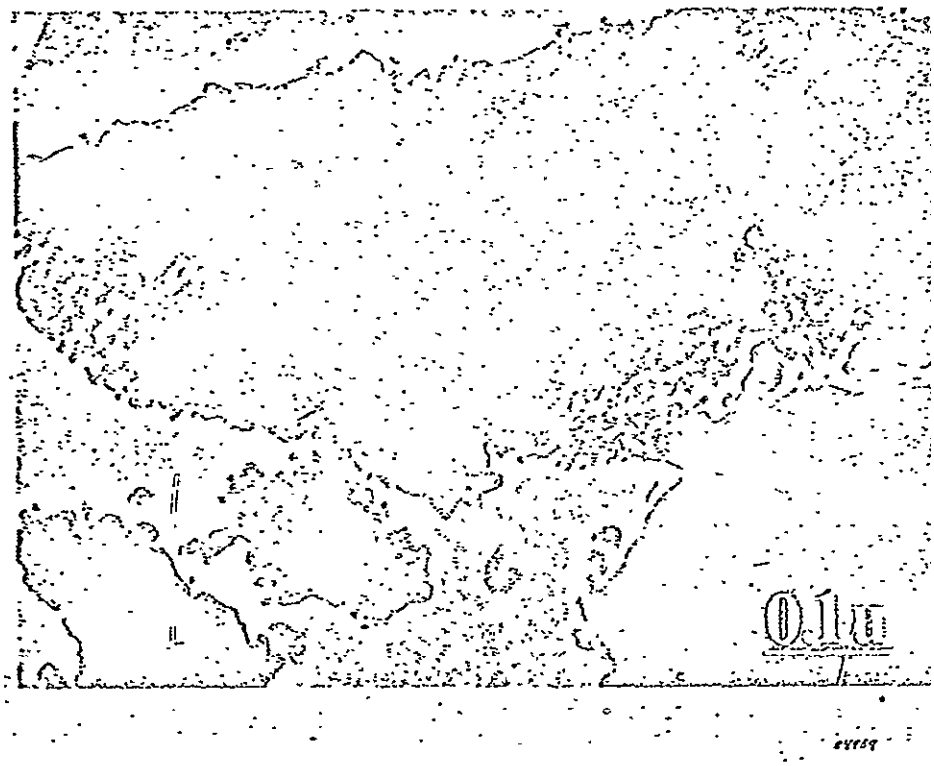


Figure 6. Transmission electron micrograph of fragments of Corning 7930 porous glass capillary (medium area lot).

More recently, the authors worked with unfired Vycor-type porous glass capillaries (some from Corning and some from laboratory sources) whose 0.010" diameter walls are able to reject 85 to 97% of the salt in 10,000 ppm feed water at a driving pressure of 1500 psi. Preliminary electron microscopic data indicate that the glass of these capillaries is similar to Corning porous glass No. 7930 with the high surface area (ca. 200 m^2/g) and narrow average pore diameter (ca. 45 Å). The extent of salt rejection indicates that this diameter is nearly the critical value for desalination (Fig. 1).

Parallel Cylindrical Pore Model of a Porous Glass Desalination Membrane

To simplify subsequent calculations, an unfired Vycor membrane will be approximated as a set of parallel cylindrical pores perpendicular to the membrane plane. The following set of physical constants for this idealized membrane is reasonably consistent with the properties given by the manufacturer (31, 32) for Corning porous glass No. 7930 (high area type).

ρ_m	=	membrane density (dry)	= 1.45 g/cm^3
ρ_s	=	density of solid portion	= 2.18 g/cm^3
n_p	=	area density of pores	= 2.15×10^{12} pores/cm^2
r_p	=	average pore radius	= 22.3×10^{-8} cm
S	=	specific surface area	= 208 m^2/g

This set of physical constants is also consistent with the equation for specific surface area:

$$S = 2\pi r_p n_p / \rho_m \tag{6}$$

and for the membrane density

$$\rho_m = (1 - n_p \pi r_p^2) \rho_s \tag{7}$$

The value listed above for the average pore radius r_p is somewhat greater than derived by the manufacturer (31, 32) from nitrogen adsorption data. [In that derivation, the 3.56 Å depth of an adsorbed N_2 monomolecular layer was ignored. It seems reasonable that some fraction of this thickness (e.g., slightly more than half) should have been added to the manufacturer's estimate of the average pore radius.]

As in the previous calculation, for cellulose acetate membranes, it will be assumed that a monomolecular layer of water is immobilized by adsorption on the pore wall, so that the effective pore radius r_p^*, for Poiseuille flow is less than r_p by the diameter of a water molecule (i. e., $r_p^* \simeq 22.3\text{-}2.8$ Å $\simeq 1.95 \times 10^{-7}$ cm). However, the average water cluster diameter, the critical pore radius for desalination, and the thickness of the ordered water hydration sheath will be estimated as being approximately equal to $r_p \simeq 22.3$ Å. On this basis, the permeation rate of lattice-ordered water through a high area type porous glass membrane can be calculated in a manner similar to that described previously for cellulose acetate. This calculation is summarized by the following parameters:

$r_p = t$	=	22.3×10^{-8} cm
n_p	=	2.15×10^{12} pores/cm
r_p^*	=	19.5×10^{-8} cm
P	=	$1500 \times 6.89 \times 10^4$ dynes/cm (= 1500 psi)
τ	=	1 (required by cylindrical parallel pore approximation)
L	=	2.54×10^{-2} cm
n_{cl}	=	191
T'	=	251°K
T	=	296°K
$\Delta H^{\ddagger}$	=	6,300 cal/mole
ΔE_{vap}	=	10,710 cal/mole
$\Delta F^{\ddagger}$	=	4,380 cal/mole
$\Delta S^{\ddagger}$	=	6.49 cal/mole · °K
η_{296K}	=	0.37 poise (dyne-sec/cm^2)
$J_{296°K}$	=	1.3×10^{-5} ml/cm^2/sec = 0.28 gallon/ft^2/day

For a narrow temperature range around 296°K, the theoretically calculated permeation rate of the ordered water, for 1500 psi driving pressure through a 0.010 inch thick high area porous Vycor-type glass membrane, is given by the Arrhenius type equation

$$J_{theory} = 1.25 \times 10^{4} e^{-6300/RT} \text{ gal/ft}^2\text{/day} \qquad (8)$$

This equation is plotted in logarithmic form in Fig. 7 along with experimental rate data (Table I) obtained in our laboratory. The agreement between theoretical and experimental values is within experimental accuracy, over a surprisingly broad temperature range. It seems, therefore, that the characteristics of ordered water in a porous glass desalination membrane are very similar to those in a cellulose acetate desalination membrane.

Ordered Water in Cellular Plasma Membranes

A biological membrane structure hypothesis that has gained wide acceptance is the concept of the "unit membrane", proposed by Robertson (33, 34), which is patterned after the earlier Gorter-Grendal (35) and Danielli-Davson (36) "paucimolecular" plasma membrane models. The postulated "unit membrane" structure consists of a bimolecular leaflet of phospholipids whose non-polar portions, mainly fatty acyl chains, are oriented inwardly perpendicular to the membrane plane and whose polar ends, covered by protein and carbohydrate layers, form the internal and external surfaces of the cell wall. This concept has recently been the subject of detailed criticism by Korn (37) who holds that the unit membrane theory is not supported by the data that led to its formulation. One of the bulwarks of the theory is its ability to account for the high electrical resistivity of the cellular membrane which according to Grundfest (38, 39) is about 10^9 ohm-cm in a post-synaptic region of a nerve cell. Such high resistivity has been attributed, in the past, to the essentially liquid hydrocarbon character of the opposed fatty acyl chains in the "unit membrane." However, the ultrafiltration data presented in the present paper show that salt is essentially insoluble in ordered water, presumably because its highly hydrogen-bonded structure cannot accommodate ions. (For the same reason, salt can be separated from brine by freezing.) This suggests that ordered water in a cellular membrane could have a high electrical resistivity approaching the value 10^8-10^9 ohm-cm proposed (40) for pure ice; accordingly,

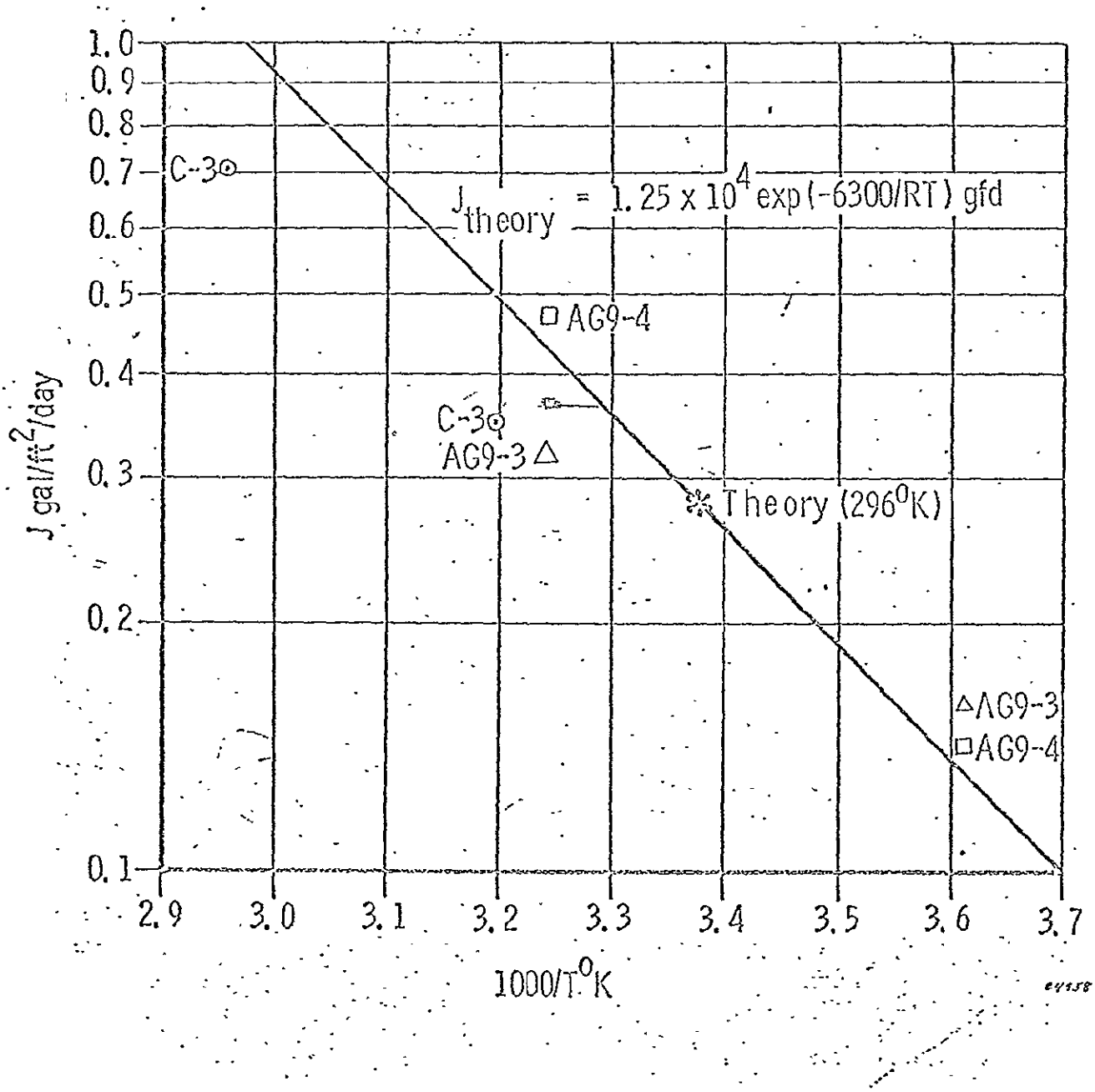


Figure 7. Permeation rates of water through 0.010 inch thick unfired Vycor-type porous glass membranes at 1500 psi.

TABLE I

EXPERIMENTAL PERMEATION RATES OF ORDERED WATER THROUGH 0.010" POROUS UNFIRED VYCOR-TYPE GLASS MEMBRANES

(Initial NaCl concentration 10,000 ppm, applied pressure 1500 psi)

Glass Lot	T (°K)	1000/T	Flow (gfd)	Salt Rejection (%)
C-3	313	3.195	0.35	85
	338	2.959	0.71	70
AG9-3	309	3.236	0.32	92
	277	3.610	0.16	92
AG9-4	309	3.236	0.47	92.5
	277	3.610	0.14	92.5
Theory *	296	3.378	0.28	100%

*For 1500 psi driving pressure, neglecting 119 psi osmotic pressure.

Note: The flow rates listed in this table were obtained with glass capillaries whose wall thickness is an appreciable fraction of the overall diameter. Accordingly, the average diameter used to calculate effective membrane area is the logarithmic mean $D_{av} = (D_o - D_i)/2.303 \log_{10} (D_o/D_i)$ where D_o and D_i are the outer and inner diameters, respectively. The wall thickness for Astropower glass lots AG9-3 and AG9-4 was 0.010". For lot C-3 from Corning Glass Works, the wall thickness was 0.015" and the actual experimental flow rate was increased by a factor of 1.5 to obtain the value listed in the table.

the bimolecular leaflet postulate may no longer be necessary to explain the resistivity of the membrane. This makes it possible to envisage a plasma membrane structure resembling the hexagonal liquid crystalline phase of the brain phospholipid-water system studied by Luzzati and Husson (41) and Stoeckenius (42). As depicted in Fig. 8, this phase consists of water cylinders of indefinite length and diameter d_w, in contact with the polar ends of the lipid molecules, which surround each cylinder. From the knowledge acquired in the present investigation, it is reasonable to consider the water in these cylinders to be in the ordered state, stabilized by the polar ends of the lipid molecules. Even if the diameter d_w of the water cylinders were as large as 44 Å in the hexagonal phase in a plasma membrane, the membrane resistivity could still be between 10^8 and 10^9 ohm-cm. A forthcoming paper (43) from this laboratory develops this membrane concept further and provides detailed models of the exquisite allosteric molecular machinery that opens and closes the membrane pores. These pores are considered to be channels of unordered water in the center of the ordered water cylinders of the hexagonal phase. The diameter (not to be confused with d_w) of these unordered water channels, in open configuration, would correspond to the "equivalent pore" diameter measured for various cells by the solute penetration techniques of Solomon (4) and his school.

The authors are indebted to Dr. George Moe for his encouragement of this work and to Mr. Gerald Croopnick for his assistance in obtaining the experimental data.

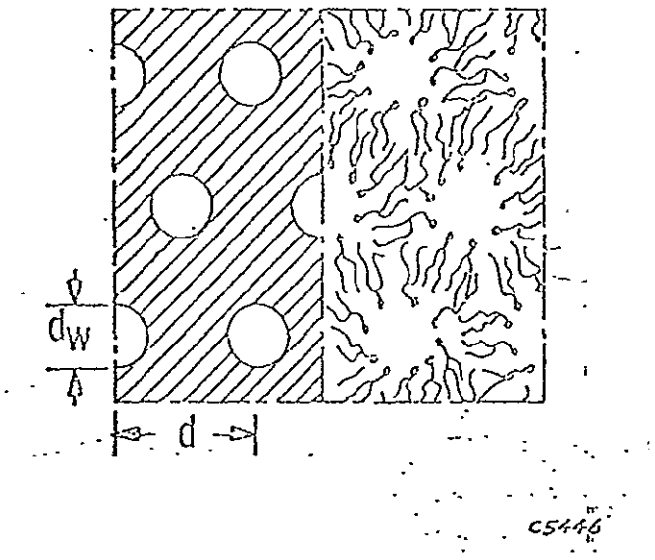


Figure 8. Rough schematic diagram of the hexagonal liquid crystalline phase of a phospholipid-water system, after Luzzati and Husson (41). The white areas represent cross-sections of water cylinders of indefinite length; the dots and wiggly lines represent polar ends and fatty acyl chains, respectively, of the lipid molecules.

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High Resolution Electron Microscopy Applied to the Study of Nerve Membranes, presented at the Neurosciences Research Program Work Session, February 9-11, 1969, M.I.T., Boston, Mass., by H. Fernández-Morán, M.D., Ph.D.

Elucidation of the molecular organization of cell membranes is a fundamental problem of biomedical research and a major challenge to further progress in molecular biology.

General characteristics of membrane organization have been formulated from correlated ultrastructural and biochemical studies. These features include coherent paucímolecular layers which extend laterally and indefinitely and which appear to consist of a periodic hydrated lipoprotein substrate, integrated with specific macromolecular repeating subunits. These subunits are organized within the plane of the layers in asymmetric "paracrystalline" arrays (1).

The elementary particle of the mitochondrion (2) is considered to be a prototype of these repeating subunits or macromolecular assemblies (3) found in association with membranes of all types.

Based on these ultrastructural and biochemical studies (2,4,5), Changeux and his colleagues have stated the following aspects of membrane organization (6): 1) membranes are made up by the association of repeating macromolecular lipoprotein units, 2) the conformation of these units differs when they are organized into a membrane structure or dispersed in solution, and 3) many biological or artificial lipoprotein membranes respond in vivo, as well as in vitro, to the binding of specific ligands by some modification of their properties which reflects rearrangement of the

membrane organization and presumably of the repeating units' conformation.

Specific enzymes or enzyme complexes are also associated in all membrane systems and are significant in determining their structural and functional organization (1).

The detection of DNA in mitochondria (7-11) and in chloroplasts (12) has provided important leads to the problem of membrane biosynthesis.

Our research program includes the following related problems of nerve membrane ultrastructure which are particularly suitable for correlated electron microscopic investigations: 1) elucidation of multienzyme and other macromolecular components closely associated with cell membranes for carrying out energy and information transduction functions, 2) study of the association of nucleic acids and the protein synthetic machinery with cell membranes to gain a better understanding of membrane biosynthesis, including (a) study of DNA and RNA conformations associated with membranes in chloroplasts, mitochondria, and in nerve cells, and (b) study of RNA polymerase and its participation in the differential RNA transcription upon DNA templates.

High resolution electron microscopy and electron optics have now progressed to a stage where they can contribute significantly to these studies. Several new approaches in instrumentation and preparation techniques are responsible for these advances which have resulted in the attainment of point-to-point resolutions of 2-3Å and which bring direct readout of molecular structures closer to reality.

1) Improved point cathode sources with single-crystal filaments and a new type of molybdenum gun are used to provide stable coherent microbeam illumination of high brightness, small spot-size, and low energy spread (13).

2) High resolution phase contrast imaging is carried out with zone plate apertures which are produced by special microengraving methods (Figure 1a). When these precise phase contrast apertures were used, together with short focal length objective lenses, structures of 5-10Å were resolved in unstained biological molecules (Figure 1b).

3) High voltage electron microscopy is particularly promising for examining biological specimens because of the increased penetration power, reduction in radiation damage, and improved resolution. We obtained resolutions of 3.6-6.0Å in crystalline lattices and 4Å point resolutions in 250-350Å-thick biological specimens with our 200 kV (Hitachi) microscope (Figures 5,6,7,8). High resolution electron diffraction carried out with the 200 kV microscope resulted in 50 to 100 diffractions for biological specimens (Figure 9), as compared with the typical 5 to 10 diffractions obtained from lower voltage microscopes (Figures 10,11).

4) The development of cryo-electron microscopes operating with high-field superconducting solenoid lenses at liquid helium temperatures represents one of the most significant advances (14a,b). The instrument provides superstable lenses, ultrahigh vacuum, minimized specimen damage, contamination, and thermal noise, and enhanced image contrast. In earlier experiments, biological

specimens were recorded at 4.2°K, revealing new electron optical phenomena (Figure 2).

5) So that we can continue this promising superconducting work on a systematic and more practical basis, we have had a closed cycle liquid helium refrigerator system specially designed for our laboratories in collaboration with Prof. Samuel Collins of A.D. Little, Inc. The unit is now adapted to our facilities and is the first installation of its kind in the world (Figures 12,13,14). Prior to shipment, the system was tested out successfully at 1.85°K with 10 watts refrigeration capacity. When the final tests are completed at our labs, we will be able to work continuously in the range of 1.85° to 2.0°K.

6) We have now a unique opportunity to apply simultaneously the advantages of superconducting microscopy and high voltage electron microscopy to biological investigations. The new installations have provided us with the only existing high voltage electron microscope with superconducting lenses operating at closed-cycle liquid helium temperatures (Figures 12,13,14). During preliminary experiments with this facility, we discovered an anomalous transparency effect for 200,000 volt electrons in thick (ca. 1000-2000Å) lead and niobium films at liquid helium temperatures (Figures 15,16). This remarkable effect extends previous observations by Boersch (15) and our own group at 30-75 kV. It poses interesting theoretical and experimental questions, particularly since it is coupled with a marked decrease in radiation damage in both organic and inorganic specimens.

7) With superconducting electron microscopy carried out on a regular practical basis, it should be possible to pursue Gabor's wavefront reconstruction microscopy and high resolution holography (16), enhanced by the superstability of superconducting lenses and coherent microbeam illumination.

8) We are planning, in collaboration with Dr. H. Träuble, correlated high voltage electron microscopic studies of ion permeation in cell membranes and model systems with cyclic antibiotics (17).. Special vacuum-tight microchambers for examining thin membranes in their natural liquid state will also be used.

These developments in electron microscopy and electron optics described above offer us unprecedented opportunity not only to tackle existing tangible problems, but also to envisage their practical application to more speculative problems.

For example, high resolution electron-optical information storage and retrieval, involving demagnification and recording on special thin film substrates, is progressing whereby, potentially, the contents of several million books could be recorded on a single page and then read out with a miniature electron microscope and television display. By related electron optical techniques, integrated circuits and computer elements of submicron sizes can also be produced (Figure 3).

In the optimal projection of these techniques, one can foresee integrated ultramicroelectric circuits, near the size of macromolecular assemblies, being incorporated

into key junctional sites of living nerve membranes without causing serious perturbation. These "submicroscopic prosthetic sensors," whose envelopes would be composed of biosynthetically produced protein coats so as to form integral components of the nervous system, could be of unique service. Because they could be produced in large number and inserted throughout the central nervous system, the sensors could effect a direct operational link at the macromolecular level between the central nervous system and man-made information processing systems, such as computers of commensurate complexity (1).

Our knowledge about nerve membranes is but a harbinger of many as yet unknown and unexpected properties of these basic cell constituents. Electron microscopy in its broadest sense will undoubtedly play an important role in this fascinating quest, since it represents the ultimate direct extension of eye and hand into the strange domain of molecules and atoms. ——

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List of Illustrations

List of Illustrations, Continued

Reprinted from *J. Mol. Biol.* (1968) 32, 467–469

Electron Microscopy of a Hemagglutinin from *Limulus polyphemus*

H. Fernández Morán, J. J. Marchalonis and G. M. Edelman

J. Mol. Biol. (1968) 32, 467–469

LETTERS TO THE EDITOR

Electron Microscopy of a Hemagglutinin from *Limulus polyphemus*

In a previous communication on the electron microscopy of hemocyanins, we noted that the hemolymph of *Limulus polyphemus* contained macromolecular components which could not be identified (Fernández-Morán, van Bruggen & Ohtsuki, 1966). The components had a ring structure with a diameter of about 100 Å, and the height of the molecules was tentatively estimated to be 65 Å. These features were sufficient to differentiate the ring-shaped molecules from hemocyanins, and the possibility was raised that the rings might represent another kind of protein in the hemolymph.

Biochemical studies (Marchalonis & Edelman, 1968) have shown that approximately 5% of the hemolymph protein is a hemagglutinin with a molecular weight of about 400,000 and a subunit structure. In this report we furnish electron microscopic evidence that the ring structures previously observed correspond to *Limulus* hemagglutinin, and we present additional details of its structure.

These studies were designed to provide optimum conditions for consistent attainment of high resolution. The microscopes were provided with a highly regulated power supply (50 kw motor generator set, equipped with a new solid-state regulator giving better than 0·1% voltage stability and very low harmonic distortion).

A Siemens Elmiskop I and Elmiskop IA, and a Hitachi 11B microscope were used, operating mainly at 75, 80 and 100-kv.

All microscopes were provided with improved pointed filaments (Fernández-Morán, 1960) of single-crystal tungsten with a tip radius of 1 to 10 μ, in special guns of the thin-film molybdenum cap type (Fernández-Morán, 1966).

For high-resolution studies, the measured astigmatism of the objective lens was 0·05 μ or less. One of the electron microscopes was provided with a separate, specially regulated power supply with reduced ripple for the short focal length objective lens, with improved controls permitting recording of through-focal series in steps of 30 to 100 Å.

All of the high-resolution micrographs were recorded using liquid-nitrogen cold stages and anticontamination devices. A combination of low-intensity microbeam illumination and controlled specimen cooling (−80 to −180°C) was found to be very useful for the study of delicate structures of the hemagglutinin molecules.

Electron micrographs were recorded on Ilford N40, and on Kodak High Definition 70 mm films on a thin polyester base. Calibration of the microscope was carried out using a diffraction grating replica (2160 lines/mm) and the crystalline lattice planes of the chrysotile filaments added to the specimen. Measurements were carried out directly on the plates and films using an optical comparator (Nikon Shadowgraph model 6C).

Specimens of hemagglutinin were purified as previously described (Marchalonis & Edelman, 1968). The protein was dissolved in Tris buffer, 0·05 M, pH 8·0, which was 0·15 M in NaCl and 0·01 M in $CaCl_2$. A droplet of the hemagglutinin solution, diluted to a protein concentration of 25 to 100 μg was placed on the specimen grid. For

negative and positive staining, 0·5 and 1% solutions of phosphotungstic acid, borotungstic acid (neutralized with KOH or NaOH to pH 7·2), and of unbuffered uranyl formate were used (Fernández-Morán *et al.*, 1966). In some cases, the sample was fixed in 1·5% glutaraldehyde before staining. Microdroplet cross-spraying techniques were also used to obtain controlled, brief interactions of specimen microdroplets with microdroplets of 1 to 2% potassium phosphotungstate (pH 7·2), uranyl formate, or other heavy-metal solutions (Fernández-Morán, 1962; Fernández-Morán, Reed, Koike & Wilms, 1964).

Ultrathin carbon films were prepared by evaporation on to freshly cleaved mica in an ultrahigh vacuum (Fernández-Morán *et al.*, 1966). In some cases, fenestrated films were used without any supporting film (Huxley & Zubay, 1960; Fernández-Morán, 1962). A useful variant of this procedure consists in bridging the holes of the fenestrated film with suitably dispersed ultrathin filaments of asbestos (chrysotile) prior to deposition of the negative-staining film. The asbestos fibers act as reinforcing structures to bridge the larger holes, and an extremely thin film of the negative stain is frequently found wedged between the hole contours and the asbestos fiber. The fibers exhibit a characteristic periodic lattice parallel to the long axis with periods of 7·3 and 3·65 Å and they serve as an internal calibration standard.

Very uniform ring-shaped structures with a diameter of about 100 Å were observed throughout all areas of the different types of preparations (Plates I and II). This type of structure was the only one encountered. Each particle had a well-defined central dense core which was 20 to 40 Å in diameter, and close examination of single molecules suggested that they had a hexagonal shape. Elongated structures of 450 Å × 100 Å which were occasionally seen (Plate III(a)) probably correspond to a side view of a stack of 100 Å rings. From a periodicity with a spacing of about 65 Å, the height of individual molecules may be tentatively estimated as 65 Å (Plate III(d), arrow).

A comparison of the electron micrographs of *Limulus* hemolymph and of purified hemagglutinin (Plates I and III) suggests that the ring structures previously observed in hemolymph are hemagglutinin molecules. About three to ten per 100 molecules in the hemolymph pictures were 100 Å rings. This proportion is consistent with the estimate that about 5% of the hemolymph protein is hemagglutinin (Marchalonis & Edelman, 1968).

Additional structural details could be resolved by means of the improved preparation techniques and instrumentation. Thus, as shown in Plates I to III, the individual flat, disc-shaped molecules about 65 Å thick can be stained and embedded without any further support in ultrathin (50 to 100 Å) layers of uranyl formate extending over the small holes of fenestrated carbon films. In contrast to spherical virus particles and related specimens which can be several hundred Ångström units thick, the hemagglutinin specimens have a total thickness in the order of 50 to 150 Å. This provides ideal conditions for high-resolution phase-contrast imaging, particularly when short focal length objective lenses are used to record through-focus series in focal steps of 10 to 100 Å.

In specimens stained without prior fixation (Plates I(a), (b) and III(b)) and after glutaraldehyde fixation (Plates II and III(d), (e)), the central dense core can be further resolved into an axial cavity, about 15 to 20 Å in diameter, lined by an electron-dense annular region, about 40 to 45 Å in diameter. This core is surrounded by a doughnut-shaped lighter shell which gives the particles their typical polygonal or hexagonal shape. Average diameters of 100 to 110 Å were measured, but rather wide

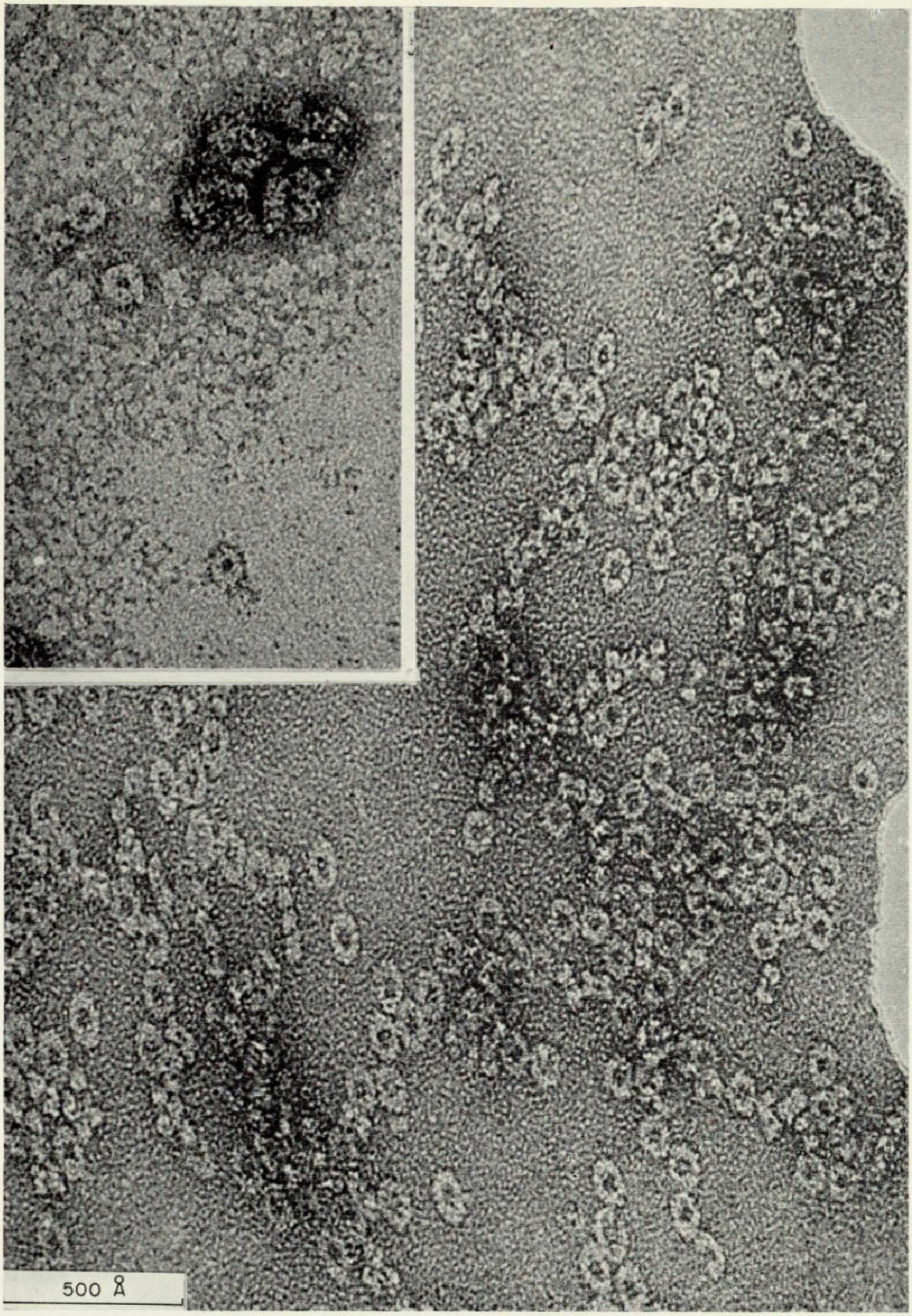

PLATE I. (a) Hemolymph of *Limulus polyphemus* stained with uranyl acetate showing, in addition to hemocyanin molecules, typical ring structures about 100 Å in diameter.

(b) Hemagglutinin isolated from *Limulus polyphemus* stained with uranyl formate showing uniform 100 Å particles with the same kind of ring structure. × 550,000.

[*facing p. 468*

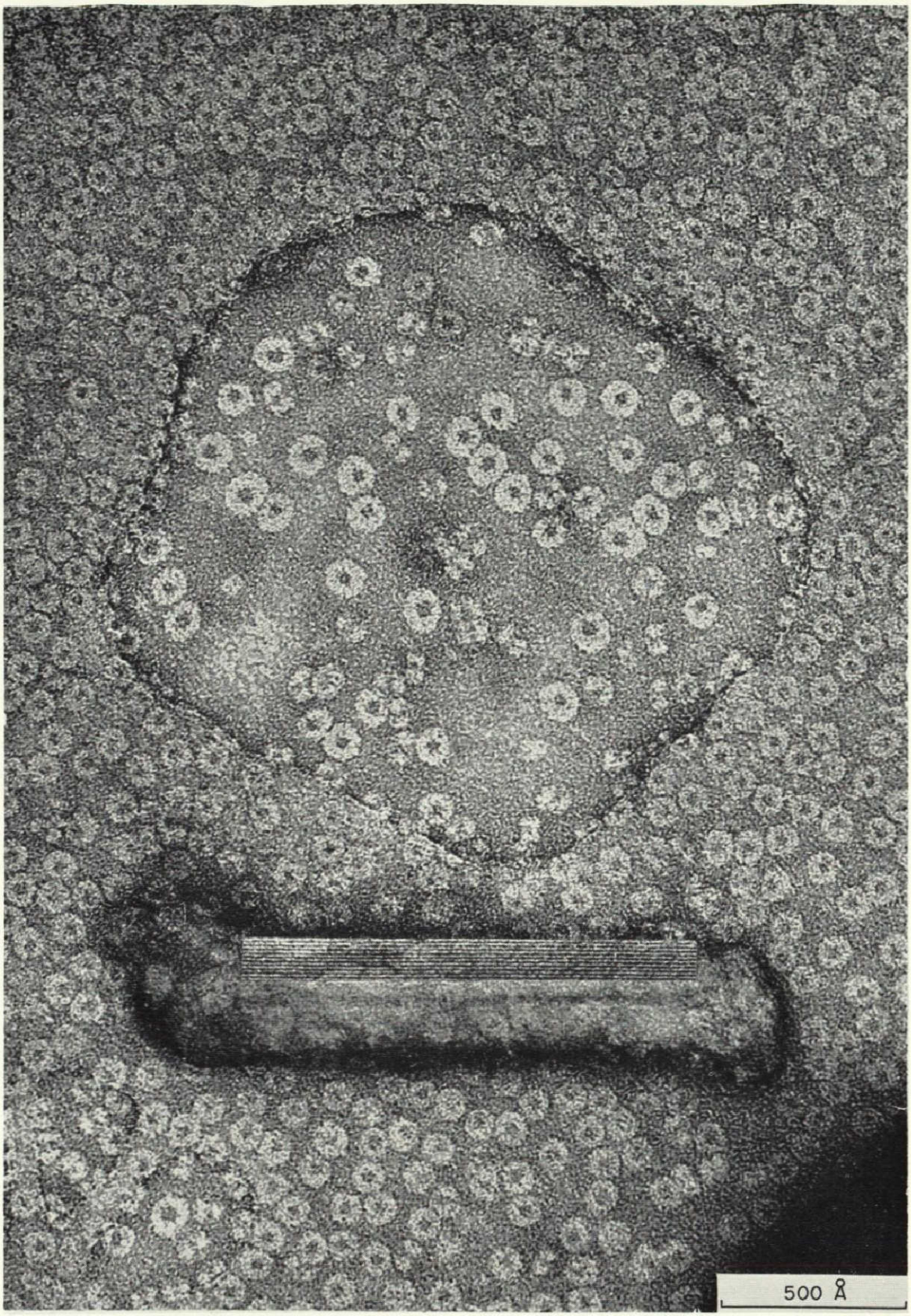

PLATE II. *Limulus* hemagglutinin fixed with glutaraldehyde, stained and embedded in ultrathin film of uranyl formate extending unsupported over hole of fenestrated carbon film. In central areas the ring-shaped molecules are often distended, displaying their fine structure in various orientations. × 550,000. Asbestos filament (insert: × 1,000,000) exhibits lattice period of 7·3 Å which serves as accurate calibration standard.

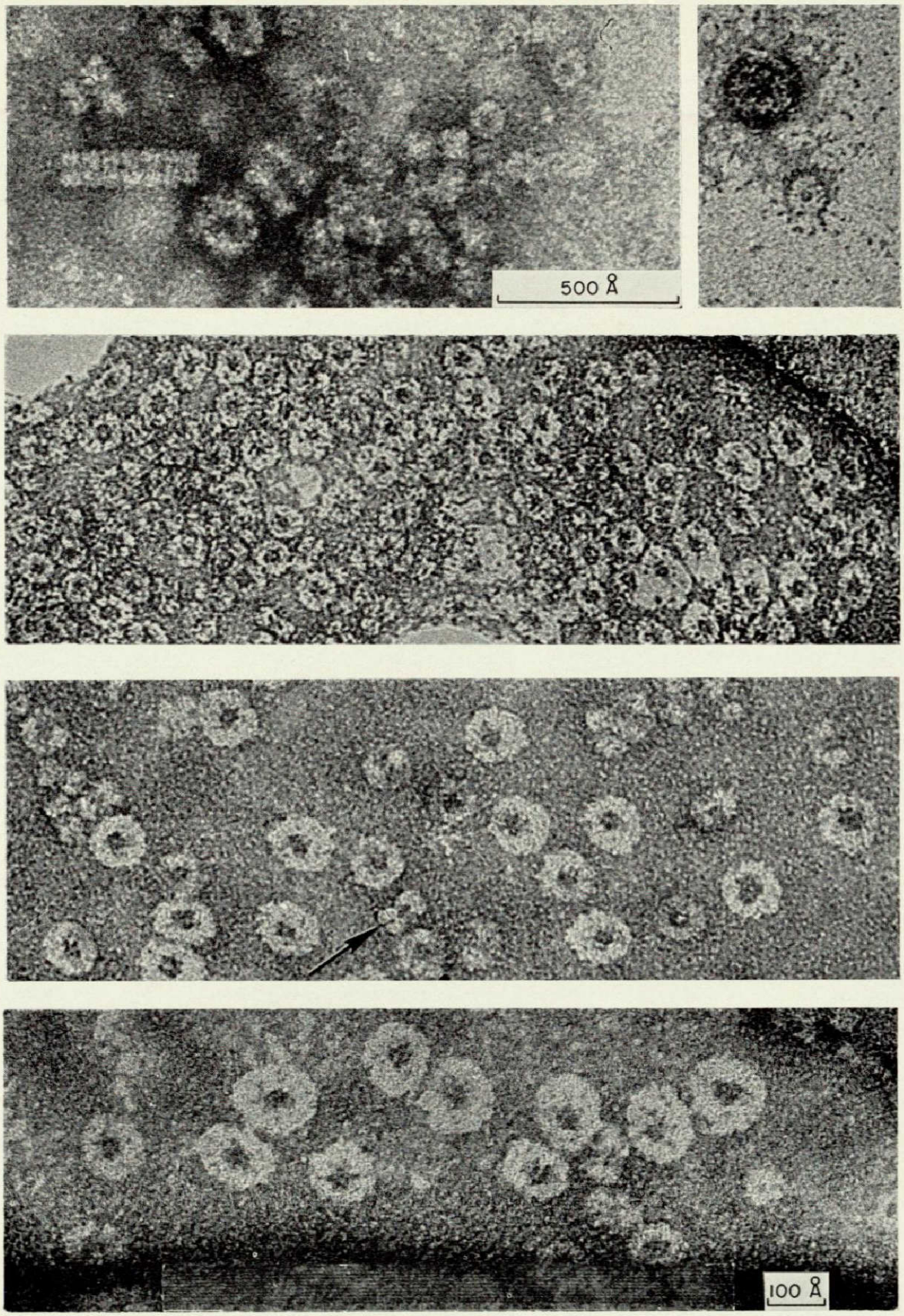

PLATE III. Structural details of ring-shaped molecules in (a) fresh *Limulus* hemolymph stained with phosphotungstate, and uranyl acetate (b); (c) *Limulus* hemagglutinin stained with uranyl formate without prior fixation, and after fixation with glutaraldehyde ((d)(e)). × 550,000; × 800,000.

variations in size and shape could be detected in certain regions. Although the size and shape of the dense core appear to be relatively constant, the shell can be considerably stretched out and distorted. This is particularly noticeable in central areas of the thin films (Plates II and III(c), (d)) where the molecules embedded in the tenuous layers may be subjected to varying degrees of stretching in different directions.

One of the most striking features of the electron micrographs is the uniformity of the preparations. This homogeneity and the internal standard provided by the periodic asbestos spacing give confidence in assigning the over-all dimensions of the hemagglutinin. Although the detailed shape of the rings is not completely certain, the 6-fold symmetry with a hexagonal outline seen in some cases is consistent with the proposed structure based on hydrodynamic studies, which consists of six units each composed of three subunits (Marchalonis & Edelman, 1968). In some of the pictures (Plates II and III) details of subunit structure may be seen, although at this stage of the investigation the exact number and shape of the subunits have not been determined from the electron micrographs.

Because of the uniformity of the hemagglutinin preparations and the availability of increased resolution, it is now feasible to attempt structural analysis of the subunits of this protein. In addition, the mode of attachment of the hemagglutinin to red cell membranes should be amenable to study by combined biochemical and electron microscopic techniques.

Further work along these lines is currently in progress, and the results will be described in later papers.

We are indebted to Miss R. Keane for technical assistance, to Messrs C. L. Hough, C. Weber, and Miss J. Hill for expert assistance with photographic reproductions, and to Miss J. Hopkins for help in preparing the manuscript. Sincere thanks are also due to Hengo Haljamäe for his valuable assistance and suggestions in the course of this work. We gratefully acknowledge stimulating discussions with Dr F. O. Schmitt and his colleagues of the Neurosciences Research Program, which provided a stimulating background for these correlative studies.

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The Electron Microscope—Part 1:

Toward The Center Of Life

Advances in instrumentation and technique are opening new pathways to the study of biological ultrastructure and future 'genetic surgery'

by Humberto Fernandez-Moran, M.D., Ph.D., Professor of Biophysics, University of Chicago

The electron microscopist, may soon begin to manipulate his magnified world of atoms and molecules. He may someday be able to alter the molecules that determine man's genetic makeup and correct molecular defects. He can also use the device like an inverted telescope to condense massive libraries onto a single page, or print electronic circuits the size of red blood cells to transmit information from within the human body.

The optical microscope, which extends the principle of sight, has been developed to a point where it can magnify an object only up to 2,000 times, and at the same time resolve features on it 2,000 angstroms (A) apart. (One A equals 1/100,000,000 centimeter, or about the diameter of an atom.) This limitation is due to the nature of light and distortions inherent in lenses. The problem is that it is impossible to resolve points on an object that are closer together than the length of the wave that is carrying the image.

Magnify one million times

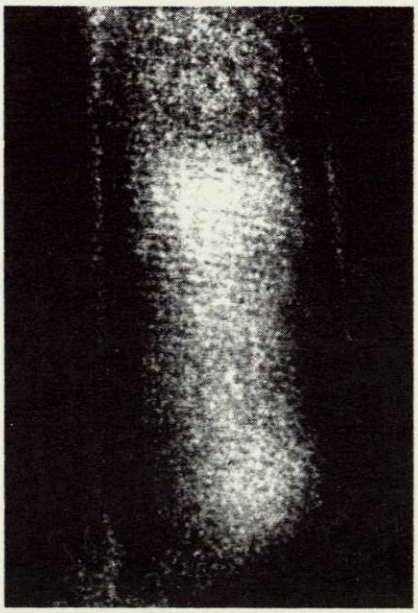
The unique properties of electrons (they are both electrically charged particles and waves) permit them to be focused like light. But, because electron waves are several hundred thousand times shorter than light waves, they can resolve much smaller objects. Today's electron microscopes can, in practice, resolve points on an object only 5A apart, while magnifying the object as much as 1 million times.

Furthermore, the illumination of the electron microscope can be varied from a few to several million electron volts, and the lenses can be focused over a wide range.

This versatility promises to let us look even further into the world of atoms and molecules. To push back the frontier, however, we must make improvements in three major areas: (1) The environment of operation, (2) our instruments, and (3) our preparation teehniques.

Because electron microscopes are extremely delicate, they are adversely affected by many external factors, such as electromagnetic interference, vibration and contamination. We have gone to great lengths to minimize these factors at our nine-microscope laboratory at the University of Chicago. For example, to reduce electro-magnetic disturbances, we use incandescent rather than fluorescent lights. Also, all the electrical wiring is shielded in grounded conduits behind the walls. Even the ventilator ducts are made of nonmagnetic stainless steel.

To eliminate vibration, we mount the microscopes on individual concrete blocks that sit on springs in the floor and are insulated by shock pads. The contamination problem is met by having as dirtfree a laboratory as possible.



THE CRYO-ELECTRON microscope, developed by the author, promises even further improvement in resolution as seen in this photomicrograph which shows the lattice structure of the enzyme, catalase.

Improving instrumentation

To improve the electron microscope itself, we must start with the elimination of lens fluctuation. All electronic systems are subject to thermal noise caused by hot electrons moving through the circuits. In an electron microscope, this noise causes variations in the focusing of the lenses.

One way to solve this problem is to place the windings of the electromagnetic lenses in a very cold environment: That of liquid helium. At about 4.2°C above absolute zero, the current goes into a state of superconduction; that is, the power may be turned off and the current will continue to flow without meeting electrical resistance. This sustains a very constant magnetic field.

Another avenue of instrument improvement is the development of high-voltage microscopes. Electrons are absorbed by very thin layers of matter. Therefore, specimen tissues must be sliced thin enough (from 50 to 100A) so that most electrons can pass through them. Living systems, such as bacteria, are much thicker than

*Adapted from Science Year, The World Science Annual. C 1968 Field Enterprises Educational Corp.

this, and viewing them requires the more energetic high-voltage electrons.

The primary advantage, however, lies in better resolution. As the voltage in increased, the wave length of the electrons becomes smaller. High voltage microscopes now being developed provide resolution approaching 1A.

Another goal in our technology is the improvement of the electron beam itself. We would prefer a beam that is coherent — all the electron waves traveling in step with each other, like an army on parade. This would not only improve the resolution, but used in conjunction with superconducting lenses, would also make possible the technology of high resolution holography.

With the invention of the laser, it became possible to make holograms using visible light. This device provides a coherent beam of light necessary for the successful imaging and reconstructing of realistic three dimensional scenes. In the electron microscope, holography will reveal interatomic distances and show us structures in three dimensions. This application, however, must await the development of laser-like devices that will provide a coherent electron beam.

Study biological ultrastructure

Our primary concern at the University of Chicago is the development of improved instrumentation and preparation techniques for high resolution electron microscopy and the study of biological ultrastructure. In addition to the successful application of superconducting electron microscopy, high voltage electron microscopy, which we now have access to, appears to be a very promising approach in this field.

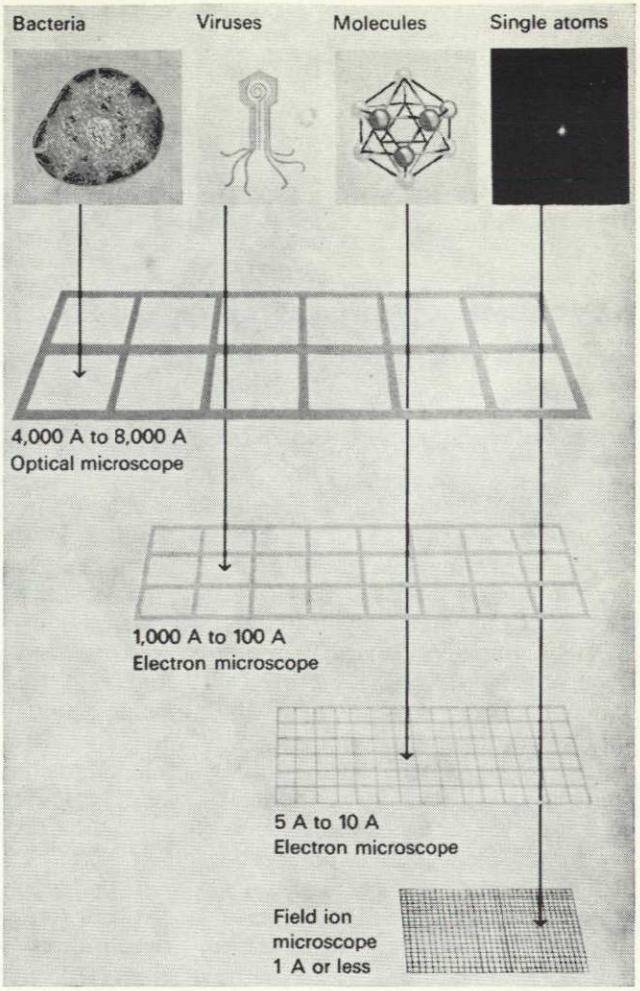
We have just installed a 200 kV electron microscope built to our specifications. This instrument, which we believe is the first of its kind to be used for biological work in this country, has been installed in a special room with minimum magnetic, electrical and mechanical perturbations and provided with a highly regulated main power supply.

Operating under these optimum conditions, we have succeeded during the past few weeks in consistently obtaining resolutions of 3.6°A to 6°A in crystalline lattices, and of about 4°A point-to-point resolutions in thick (about 250°A to 350°A) biological specimens. We believe that this represents the highest resolution yet achieved at 200 kV.

The combination of greater penetrating power, reduced radiation damage, and high resolution demonstrated in the 200 kV electron microscope should prove to be of key value in the study of biological specimens under conditions approaching the living state. Within a few weeks, we hope to be able to operate the 200 kV scope at liquid helium and liquid nitrogen temperatures.

An interesting variation on conventional electronic microscopes is being developed by Albert Crewe, working with another group here at the University of Chicago. In this microscope, a highly concentrated electron beam is focused before reaching the specimen, and is then scanned across it.

The electrons that pass through the specimen are collected and compared with those that are scattered. The energy lost by the traversing electrons can be used



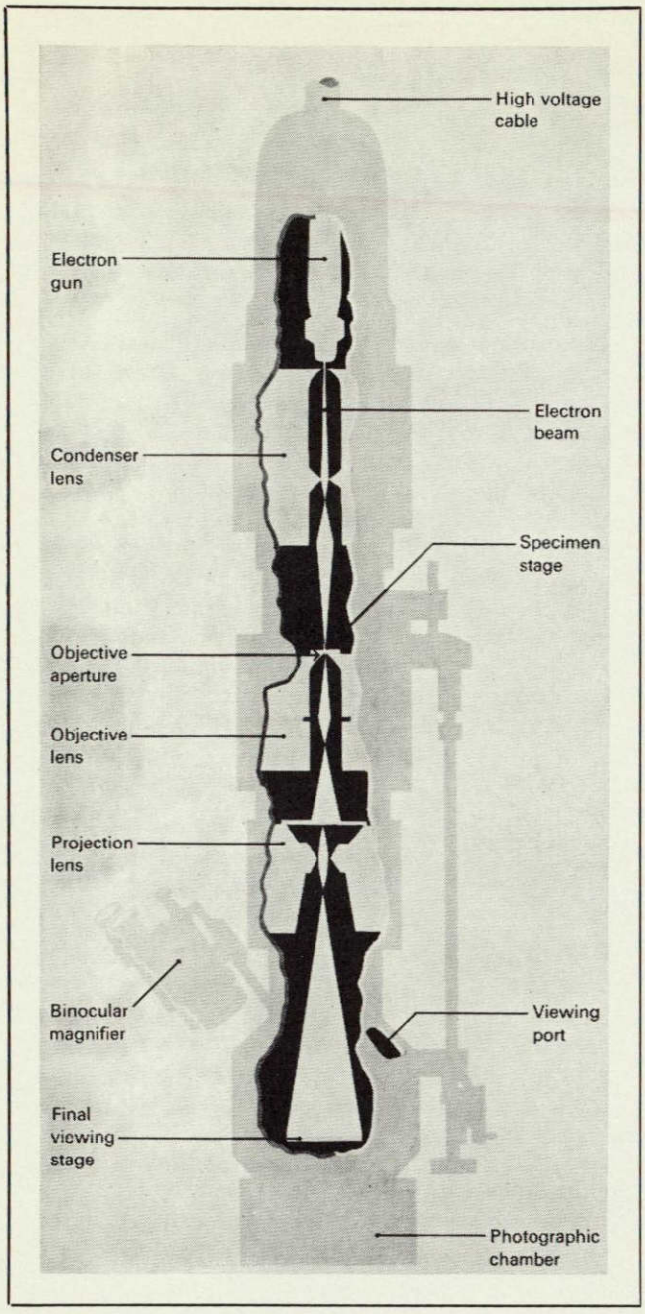
RESOLUTION OF POINTS on an object is proportional to the wave length of the illuminating source. The best optical microscopes, resolving points 4,000 to 8,000 angstroms (A) apart, permit the viewing of bacteria. Conventional electron microscopes can show viruses in the 100 to 1,000 A range or groups of molecules at from 5 to 10 A. The special features of field-ion microscopes may provide a resolution of 1 A, or the diameter of a single atom.

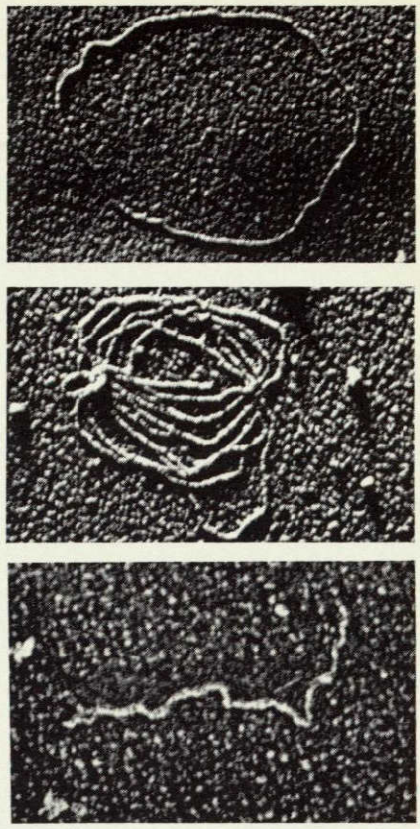
to identify the specimen. Thus the scanning electron microscope will be able to analyze a material while simultaneously observing its atoms and molecules. And because the electron beam examines a smaller portion of a specimen at a time, it has a potential for much improved resolution.

The diamond knife

Sharing importance with advances in the electron microscope are improvements in the techniques for preparing specimens. One of the most spectacular developments in this area has been a system for precisely slicing specimens into ultrathin sections. This system consists of a diamond knife operating in an evacuated microtome. It can be operated at very low temperatures in order to keep rearrangement of the molecules in biological specimens to a minimum.

Also needed in this preparation system was a way to hold the specimens after they were sliced. We devised a protective chamber in which the specimens are sealed between layers of ultra-thin graphite film through which electrons can freely pass.





CLOSED STRAND of virus DNA (top) can be seen in an electron microscope. After reaction with nucleotides and an RNA polymerase, varying width filaments are formed (middle). With the diamond knife, large biological molecules can be cut into viable segments, such as the section of virus DNA shown (bottom).

One result of the improvements is the ability to make quantitative predictions about the behavior of biological matter. The ability to predict in the biological world will have important consequences. Until recently, for example, we knew relatively little about the nature of deoxyribonucleic acid (DNA), the substance that contains all the data needed to program the construction of a man from his brain to his toenails.

Although it is one of the largest molecules, DNA still is extremely small. A human being contains approximately 50 trillion cells, each of which contains 46 chromosomes. These chromosomes, in turn, have more than 1,000 genes, each containing vast numbers of minute DNA ribbons. With the electron microscope we are beginning to be able to view this programming phenomenon, including the transfer of information from DNA to the building centers of the cells by ribonucleic acid (RNA).

Visible atoms

Our primary goal, however, is to view the structure of molecules directly. This means entering the domain of 1 to 2A. In some materials we can already see the atoms in the crystalline lattice arranged to make up the incredibly complex organic molecules. We have also been able to observe cell membranes. For example, in the subunit structure of myelin, a substance that forms part of the nerve fiber, we have actually watched cells in the process of rearranging their molecular structure.

Because the diamond knife can cut specimens as thin as 50A, we can now do chemistry by cutting. We can cut up a starch molecule in such a way that it becomes sugar. We can slice a virus in half. It may even be possible to correct genetic errors.

For example, we can examine the DNA ribbon in the gene causing hemophilia. It may be possible to edit this ribbon — actually cut into the nucleotides and rearrange them in proper order. The edited DNA could then be copied in great numbers and inserted in a female ovum to crowd out the faulty genes. Since the genetic likelihood of a disease such as hemophilia is relatively easy to predict, this technique could, in a few generations, eliminate the disease. □

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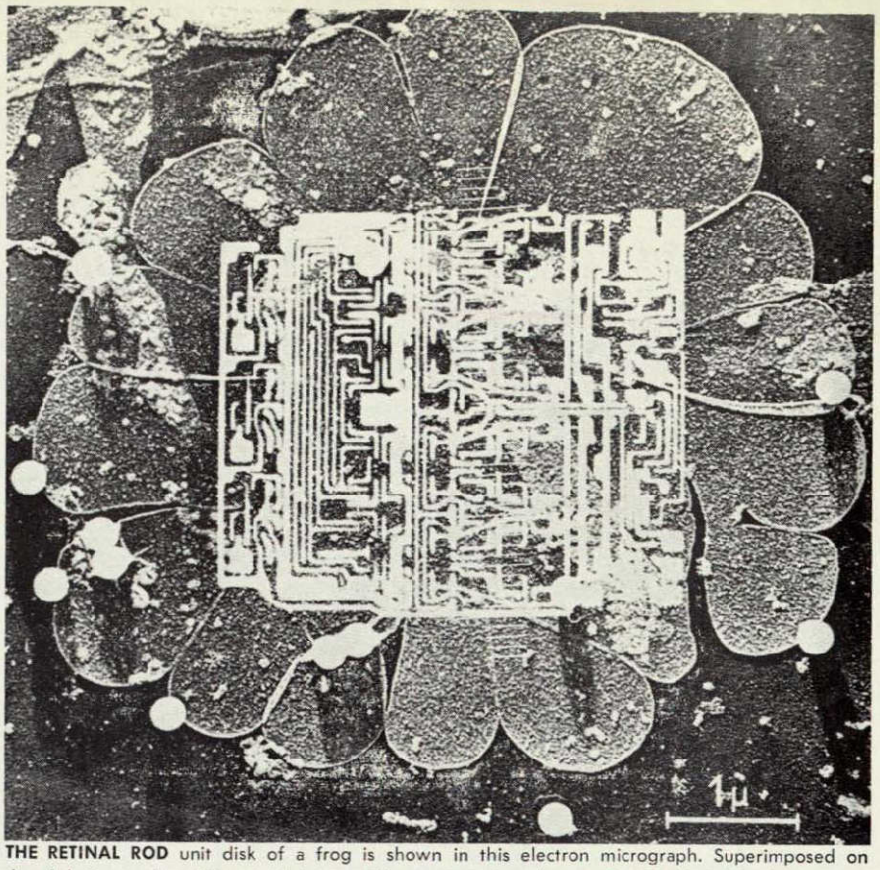
Electron Microscope, Part II*

Microscopists are working toward broad new applications in the study of human perception and development of prosthetic sensors



THE RETINAL ROD unit disk of a frog is shown in this electron micrograph. Superimposed on the disk is an ultraminiaturized integrated circuit mask and text, demagnified 10^{-4}x electron-optically on organometallic thin film substrate. It measures about 6 microns across. The above was prepared by the author in collaboration with C. Hough.

Big Future For Demagnification

By Humberto Fernandez-Moran, M.D., Ph.D., Professor of Biophysics, University of Chicago

The use of the electron microscope in reverse — to demagnify — offers many fascinating possibilities to the biomedical scientist.

One is the storage and retrieval of information. Using grainless film developed in our laboratory, we can photographically reduce pages, such as the one you are reading, to an almost invisible dot.

Letters on such a page are only about 100 atoms high. With the electron microscope, the entire collection of the Library of Congress could be reduced to a single sheet, 8 inches by 10 inches, transferred to micro-tape, and later displayed, page by page, on a television screen.

Further development of the electron microscope's potential in this direction could lead to its use in miniaturized computers. By printing electronic circuits on film and reducing them in the same way pages of type are reduced, we could provide more compact, and thus more efficient computers.

Unlocking the brain

The microscope may also teach us how to duplicate our own ultraminiaturized molecular information storage system — the memory portion of the brain.

The packing density of our brain — the number of working elements in a given volume — is 10 to 100 billion elements per cubic inch. Packing densities of present computer components range up to 1 million elements per cubic inch. By improving this density with electron microscopy techniques, we would, among other things, greatly enhance the speed of retrieving information.

Holography with the electron microscope may also help explain the memory apparatus of the human brain. Our ability to summon words, sentences, and other behavioral sequences from our experiences seems to be a random and nonlocalized process. We believe that human memory banks are highly repetitive — all the data being stored in every portion of the brain.

The retrieving mechanism may act somewhat like a reverse of holography in which the illuminating laser beam is matched to a hologram to produce the reference beam. One application for visible light holography envisions rapidly passing an enormous file of fingerprints, stored on microfilm, past an illuminating beam that is shining through a hologram of the fingerprint being sought. When the hologram matches the print on file, the reference beam flashes.

If we could demonstrate that the brain uses a similar system to summon our thoughts, we would begin to understand the phenomena of perception. Also, we could experiment with the cells or cell clusters responsible for storing sensory information.

A fascinating application for ultrareduced printed circuits could be

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as a prosthetic sensor. Placed on a red blood cell, it could then transmit information from within the body.

We have already successfully placed an amplifier circuit on a retinal rod — that portion of the eye that reacts to faint light. Such devices, only 6 microns across, could be produced in large quantities and incorporated at key sites of the body where, for example, they could monitor the operation of the nervous system. They would, of course, have to have biosynthetically produced protein coats so they would not be rejected by the immune responses of the body.

Neurological sensors

Using the natural electricity in the body for power, these sensors would transmit neurological electric impulses similar to those recorded by an electroencephalogram. But they would be transmitted by radio, eliminating the need for physical attachments to the body, and permitting the patient to be monitored as he goes about his affairs.

The electron microscope even has a potential for gaining information about the structure and organization of space, which like life itself, is written in the atoms.

Atoms throughout the universe obey the same laws, and interstellar dust is only a few hundred atoms in diameter. Thus, much of what we will someday find in space will make sense only if we can examine it at the submicroscopic level. The electron microscope may become the primary tool with which we will define matter far beyond our present concept.

The members of our laboratory range widely in disciplines, covering both the physical and biological sciences. Our ability to see things that no one has seen before and to think about them in a way no one has thought about them, will depend on extending this range of disciplines.

This marvelous instrument has begun to show us how intimately man is linked to the domain of atoms and how minute matter in the universe influences his destiny. New concepts in this technology will someday permit us not only to predict, but also to design life at the molecular level. □

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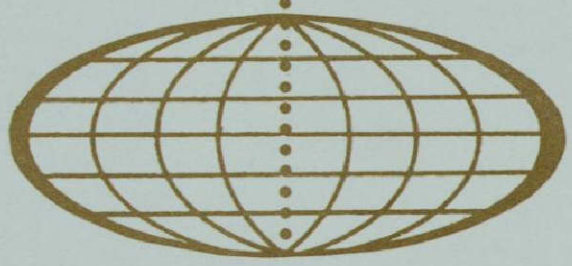


tearsheets from

The World Book

Encyclopedia

A Science Year Report

By Robert S. Hoffmann

Russian Science: A Personal View

The electron microscope even has a potential for gaining information about the structure and organization of space which, like life itself, is written in the atoms. Atoms throughout the universe obey the same laws, and interstellar dust is only a few hundred atoms in diameter. Thus, much of what we will someday find in space will make sense only if we can examine it at the submicroscopic level. The electron microscope may become the primary tool with which we will define matter far beyond our present concept.

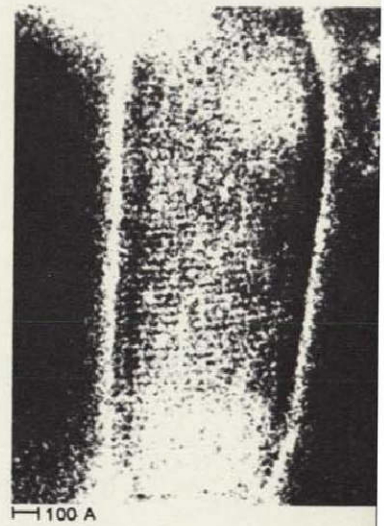
Beginning with Max Knoll and Ernest Ruska in Germany during the 1930s, the development of the electron microscope has been an international achievement. Major advances were made by Francis O. Schmidt and Cecil Hall at Massachusetts Institute of Technology and a Rockefeller University group under the direction of Keith Porter and George Palade. High-voltage instruments were pioneered in France and Japan, and Gabor's work in England, of course, resulted in holography. In our University of Chicago laboratory, we have scientists and technicians from throughout the world, including Cuba, Italy, Japan, South America, and Sweden. I feel strongly that these observatories should continue to have an international character. When a science acquires the unique capabilities we are approaching with the electron microscope, it should not be held the property of one or a group of nations.

The members of our laboratory also range widely in disciplines, covering both the physical and biological sciences. Our ability to see things that no one has seen before and to think about them in a way no one has thought about them, will depend on extending this range of disciplines. We will need scientists familiar with crystallography, modern mathematics, and quantum mechanics.

The greatest need will be for highly skilled operators. Operating a microscope is much like playing a fine musical instrument. The quality of the performance, requiring intuitive and interpretive abilities, depends on the talent of the performer. He must have a good ear, a good eye, a good hand—and patience.

This marvelous instrument has begun to show us how intimately man is linked to the domain of atoms and how minute matter in the universe influences his destiny. New concepts in this technology will someday permit us not only to predict, but also to design life at the molecular level. Scientists will have a power more awesome than any ever imagined. In turn, they will have the grave responsibility of using this power wisely.

The author created and developed the ultralow temperature electron microscope. Promised improvement can be seen in the photomicrograph, *below*, which shows the lattice structure of the enzyme, catalase.



100 A

For further reading:

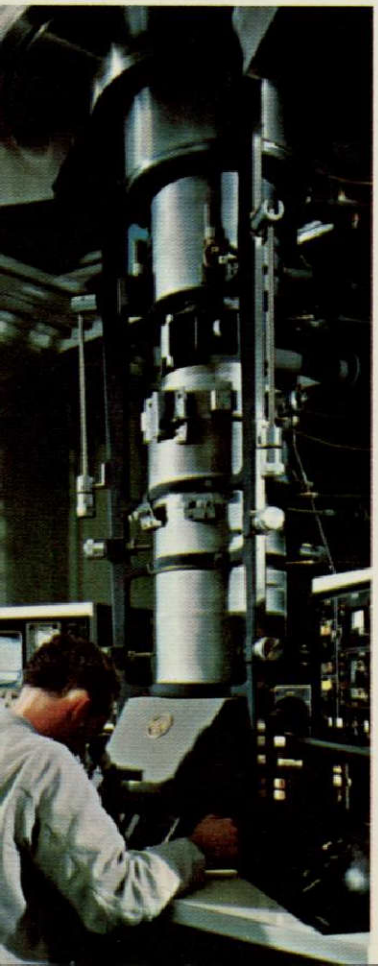
Bradbury, S., *The Evolution of the Microscope,* Pergamon Press, 1967.

Cosslett, V. E., *Modern Microscopy,* Cornell University Press, 1966.

Freundlich, Martin F., "Origin of the Electron Microscope," *Science,* Vol. 142, Oct. 11, 1963.

"One-Atom Microscope," *Scientific American* (Science and the Citizen), March, 1968.

Accelerators, *above*, generate electrons for a million-volt RCA electron microscope, *below*. These electrons travel at about 94 per cent the speed of light, with wave lengths to .009 A. High energy electrons can easily penetrate thicker specimens, and provide improved resolution.



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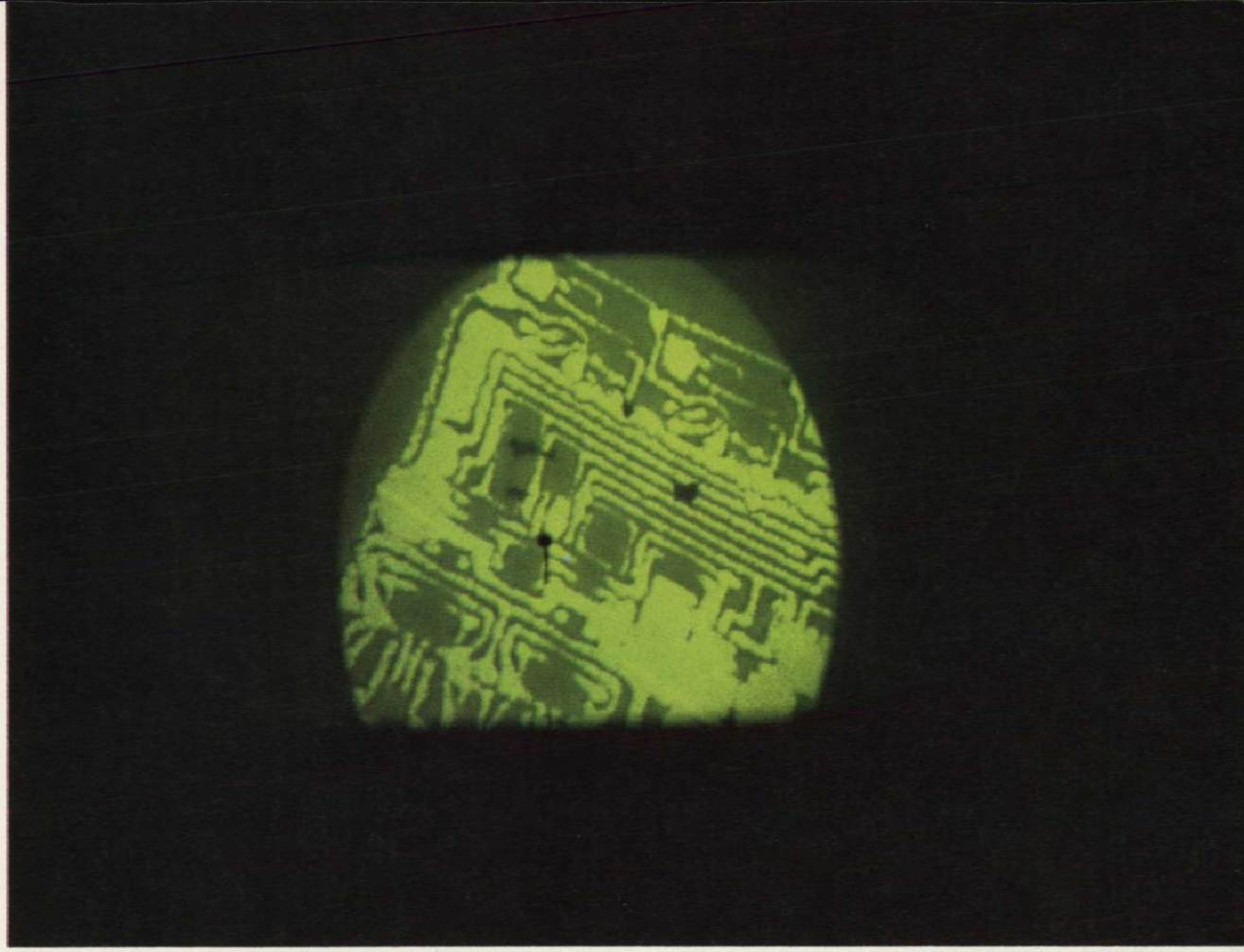
A fascinating application for ultrareduced printed circuits could be as a prosthetic sensor. Placed on a red blood cell, it could then transmit information from within the human body. We have already successfully placed an amplifier circuit on a retinal rod—that portion of the eye that reacts to faint light. Such devices, only 6 microns across, could be produced in large quantities and incorporated at key sites of the body where, for example, they could monitor the operation of the nervous system. They would, of course, have to have biosynthetically produced protein coats so they would not be rejected by the immune responses of the body. Using the natural electricity in the body for power, these sensors would transmit neurological electric impulses similar to those recorded by an electroencephalogram. But they would be transmitted by radio, eliminating the need for physical attachments to the body, and permitting the patient to be monitored as he goes about his affairs.



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Because the diamond knife can cut specimens as thin as 50 A, we can now do chemistry by cutting. We can cut up a starch molecule in such a way that it becomes sugar. We can slice a virus in half. It may even be possible to correct genetic errors. For example, we can examine the DNA ribbon in the gene causing hemophilia. It may be possible to edit this ribbon—actually cut into the nucleotides and rearrange them in proper order. The edited DNA could then be copied in great numbers and inserted in a female ovum to crowd out the faulty genes. Since the genetic likelihood of a disease such as hemophilia is relatively easy to predict, this technique could, in a few generations, eliminate the disease.

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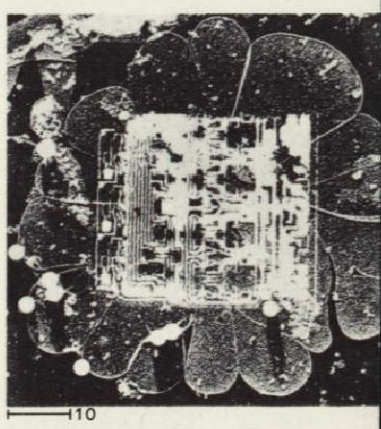
Demagnifying techniques also permit printing electronic circuits, seen in electron microscope viewing port, *above,* onto blood cells or retinal rods, *below.* These circuits can be implanted in the body. The circuit is only about 6 microns across.

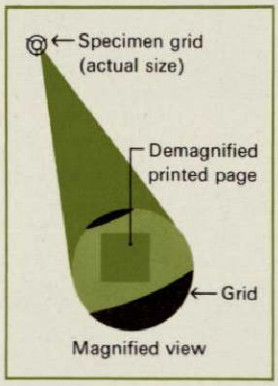


layers with atomically smooth knife edges. I began slowly and carefully to pare away the layers of a diamond with the only tool hard enough to do so—another diamond. After many hours of work, I was rewarded with the finest knife yet made.

Also needed in this preparation system was a way to hold the specimens after they were sliced. For this, we devised a protective chamber in which the specimens are carefully sealed between layers of ultrathin graphite film through which electrons can freely pass.

One result of the improvements is the ability to make quantitative predictions about the behavior of biological matter. Physicists can make predictions because they can observe or measure basic components. For example, knowing the length of a pipe in a pipe organ and the speed of sound in air, we can predict all the tones and overtones that can be produced. In biology, however, the basic components are molecules, which we have not been able to see. Thus, we have had to work with unpredictable groups of molecules.

The ability to predict in the biological world will have important consequences. Until recently, for example, we knew relatively little about the nature of deoxyribonucleic acid (DNA)—the substance that contains all the data needed to program the construction of a man from his brain to his toenails. Although it is one of the largest molecules, DNA still is extremely small. A human being contains approximately 50 trillion cells, each of which contain 46 chromosomes. These chromosomes, in turn, have more than 1,000 genes, each containing vast numbers of minute DNA ribbons. With the electron microscope we are beginning to be able to view this programming phenomenon, including the transfer of information from DNA to the building centers of the cells by ribonucleic acid (RNA).



Electron microscopes demagnify type, *below*, to a point where letters are only 100 atoms high. Size of page, similar to the one you are reading, is shown relative to specimen grid, *above*. When reduced to the actual size, it is an almost invisible dot.

light necessary for the successful imaging and reconstructing of realistic three dimensional scenes. In the electron microscope, holography will reveal interatomic distances and show us structures in three dimensions. This application, however, must await the development of laserlike devices that will provide a coherent electron beam.

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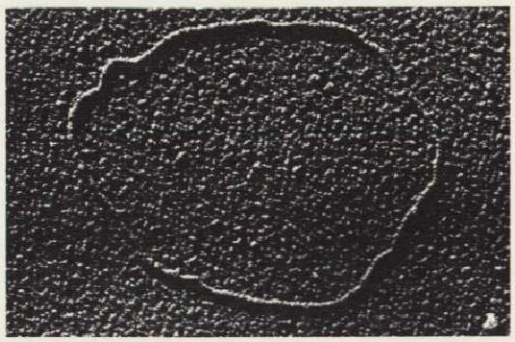
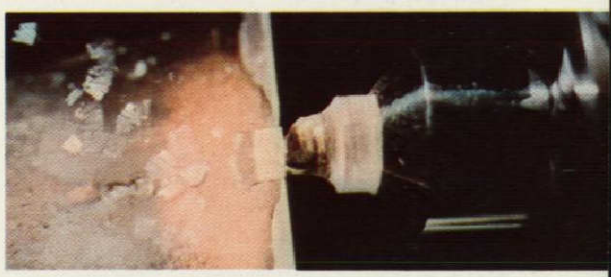
The idea for this system came to me while flying over Angel Falls in Venezuela. As I looked down on the beautiful cascading waters of the world's highest waterfall, I suddenly realized that our sectioning problem could be solved by a device that provided a precise, circular motion in a smoothly recurring flow system. The result was the invention of the ultramicrotome–a fine rotating slicing machine similar to a meat slicer.

Our precision machine needed an ultrasharp knife–sharper than anything available. I ruled out steel because of the limits to which it could be ground. Then I thought of diamonds. Because they are chemically inert, they would not contaminate organic specimens. But even better, I knew, from having studied diamonds under the electron microscope, that they are giant crystals composed of

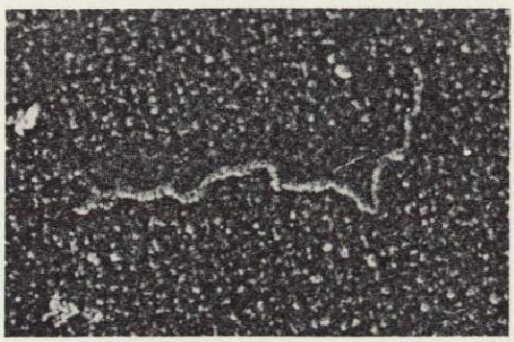
Editing the Code of Life



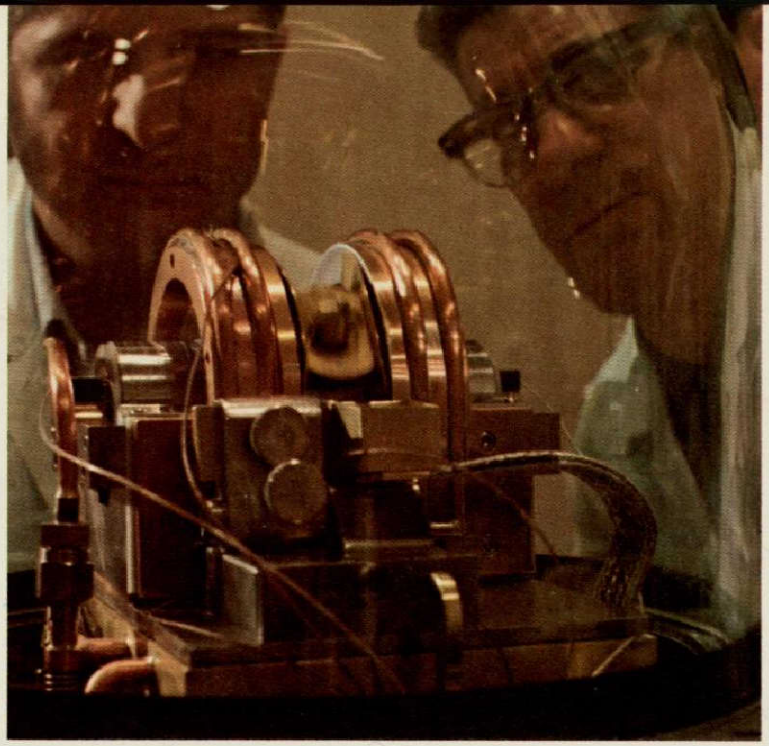
Closed strand of a virus DNA, *above,* can be seen in an electron microscope. After reaction with nucleotides and an RNA polymerase varying width filaments are formed, *below.*



The diamond knife, *above,* can cut large biological molecules into viable segments, such as a section of virus DNA, *below.* The technique may permit genetic defects to be corrected under the microscope.

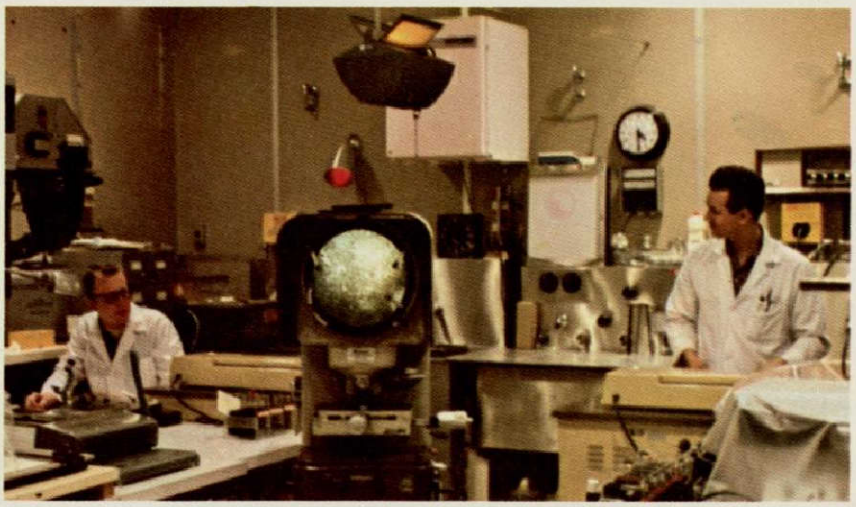


Scientists and technicians prepare specimens to be viewed through the electron microscope under conditions that approach operating room cleanliness.



A new cryogenic ultramicrotome is used to section frozen biological specimens.

Video recording system with image intensifier is a valuable tool for registering specimen behavior for large group demonstrations.



Thin layer of metal is deposited on specimens in ultrahigh vacuum, *left*. Photographic laboratory, *above*, includes shadowgraph on which projected image from the microscope can be studied in detail.

from 50 to 100 A–so that most electrons can pass through them. Living systems, such as bacteria, are much thicker than this, and viewing them requires the more energetic high-voltage electrons. The primary advantage, however, lies in better resolution. As the voltage is increased, the wave length of the electrons becomes smaller. High-voltage microscopes now being developed provide resolution approaching 1 A.

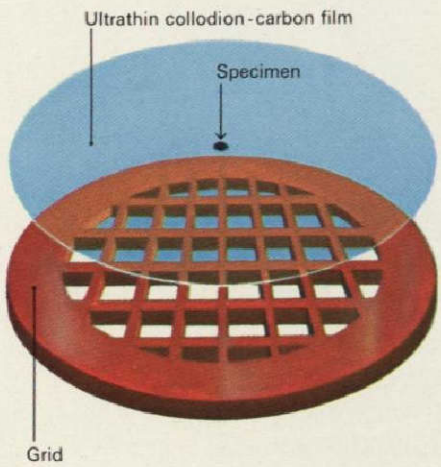
Another goal in our technology is the improvement of the electron beam itself. We would prefer a beam that is coherent–all the electron waves traveling in step with each other, like an army on parade. This not only would improve the resolution but, used in conjunction with superconducting lenses, would also make possible the technology of high resolution holography.

Holography is a method of recording images on film without a lens. It requires a coherent illuminating beam, divided so that one part lights the object, while the other, called the reference beam, goes directly to the film. At the film, the reference beam and the light from the object cause an interference pattern that contains all the information on the image in three dimensions. The scene can then be reconstructed by viewing the developed film with another coherent illumination source.

This imaging system was, in fact, first proposed by a scientist working with electron microscopes. In 1948, Dennis Gabor, a Hungarian physicist then at the University of London's Imperial College of Science and Technology, had come to believe that electromagnetic lenses could not be substantially improved. So he proposed taking magnified pictures with an electron microscope by exposing a photographic film to the electron waves from the specimen before they were focused, and reconstructing the jumbled image with visible light. Because light waves are much longer than electron waves, the reconstructed image would be greatly magnified.

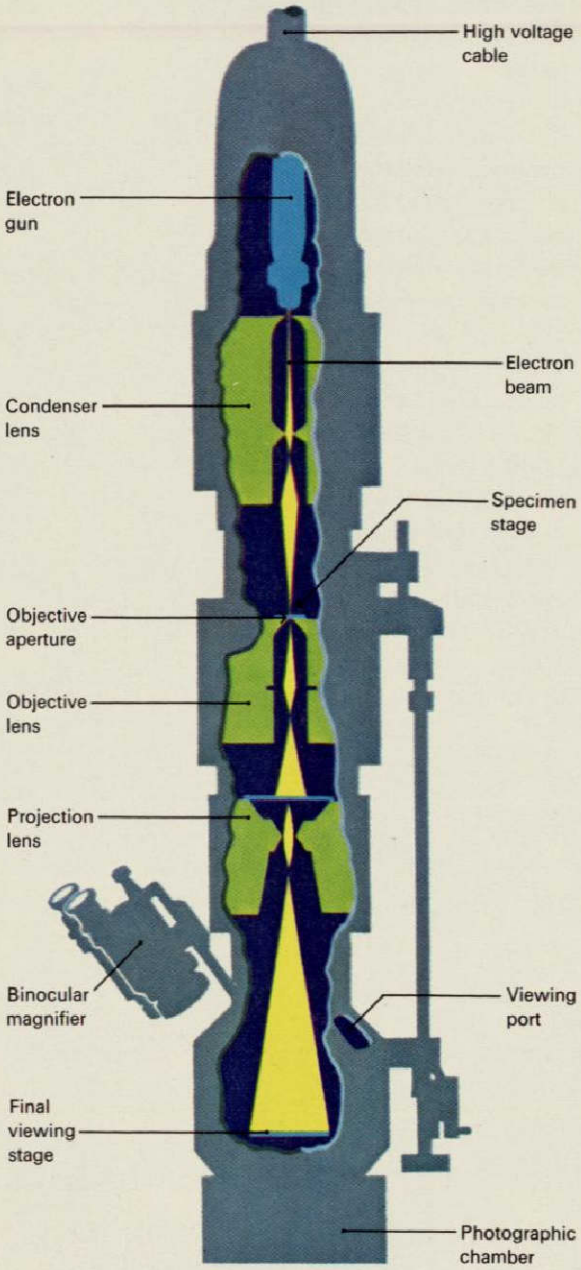
With the invention of the laser, it became possible to make holograms using visible light. This device provides the coherent beam of

Holding the Specimen

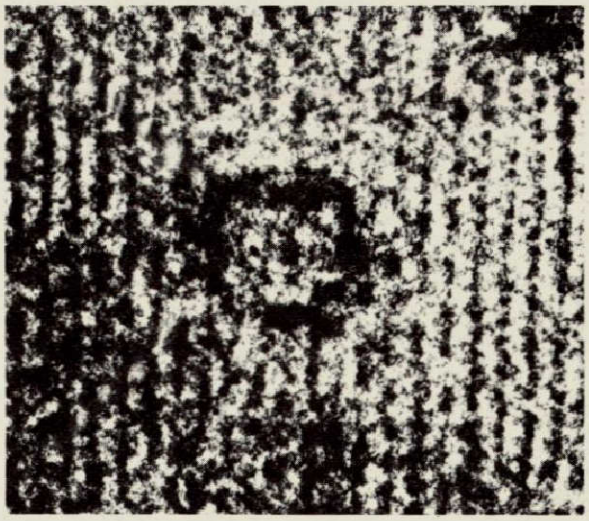


Specimens require special handling devices such as the grid, *left,* shown slightly enlarged, *above.* Laboratory technician, *right,* prepares ultrathin films used to support the specimens on the grids.

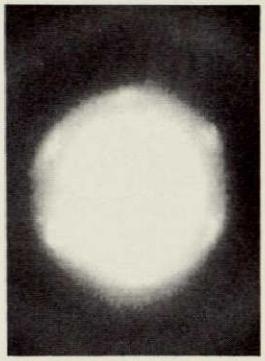
The Electron Microscope



In the electron microscope, specimens are illuminated by externally generated electrons that are beamed by an electron gun into an electromagnetic condenser lens. The concentrated beam passes through the specimen, which scatters the electrons. A series of lenses then focus the electrons into an image of the specimen and adjusts its magnification. A final lens projects the image on a fluorescent screen where it can be viewed directly or it can be photographed. The microscopes can be operated over a wide range of voltages and lens magnifications.

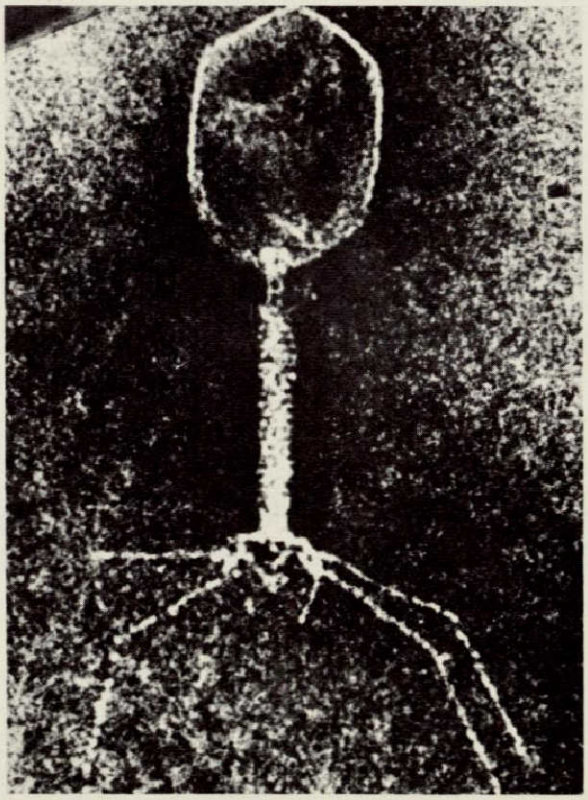


100 A

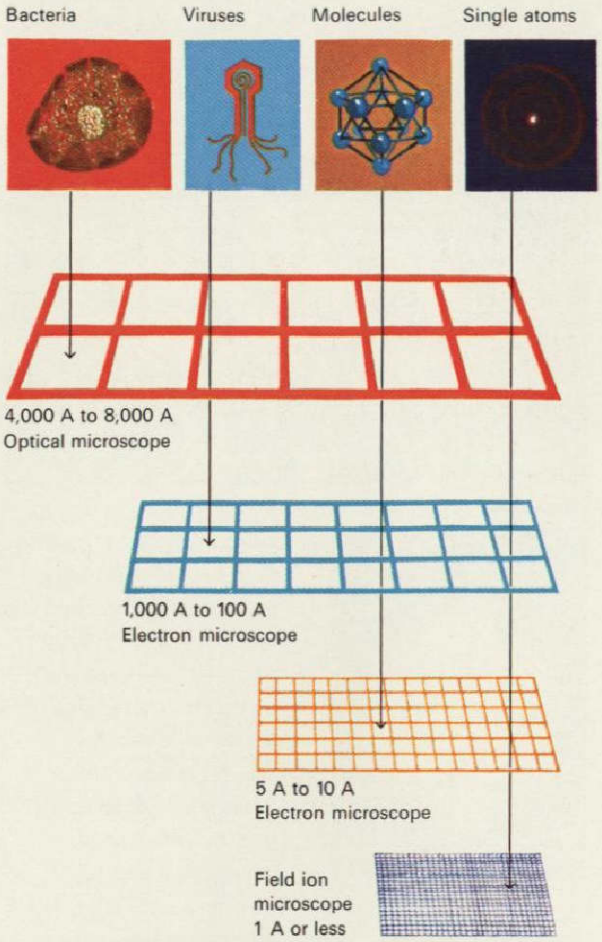


Special methods permit direct view of an organic molecule, ***above,*** coupled with electron diffraction of a selected area, ***left,*** to determine details between 2 and 3 A. The high resolution of a bacterial virus, ***below,*** portrays the structure of its head and tail.

100 A



What Microscopes Resolve



Resolution of points on an object is proportional to the wave length of the illuminating source. The best optical microscopes, resolving points 4,000 to 8,000 angstroms (A) apart, permit the viewing of bacteria. Depending on choice of magnification, conventional electron microscopes can show viruses in the 100 to 1,000 A range or groups of molecules at from 5 to 10 A. The special features of field-ion microscopes may provide a resolution of 1 A, or the diameter of a single atom.

To eliminate vibration, we mount the microscopes on individual concrete blocks that sit on springs in the floor and are insulated by shock pads. The contamination problem is met by having as dirt free a laboratory as possible. All who enter the air-conditioned laboratory must pass through a special anteroom where they stop to don white nylon coats. Employees wear special shoes and visitors are furnished plastic bags to wear over their shoes.

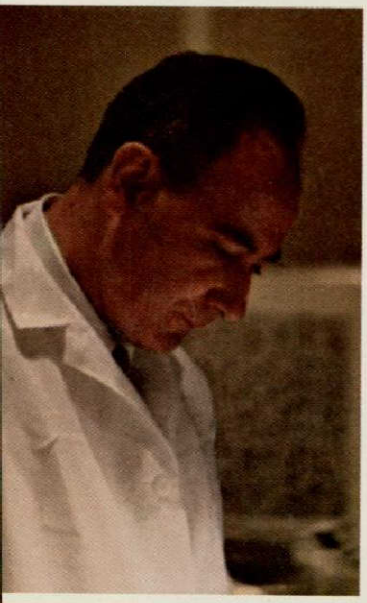
To improve the electron microscope itself, we must start with the elimination of lens fluctuation. All electronic systems are subject to thermal noise caused by hot electrons moving through the circuits. In an electron microscope, this noise causes variations in the focusing of the lenses. One way to solve this problem is to place the windings of the electromagnetic lenses in a very cold environment–that of liquid helium. At about 4.2°C. above absolute zero, the current goes into a state of superconduction; that is, the power may be turned off and the current will continue to flow without meeting electrical resistance. It thus sustains a very constant magnetic field.

Another avenue of instrument improvement is the development of high-voltage microscopes. Electrons are absorbed by very thin layers of matter. Therefore, specimen tissues must be sliced thin enough–

in detail, we bring it closer to our eyes to spread out its features and make it appear larger. However, we cannot focus on objects less than about 10 inches from our eyes. We must then use an external lens, such as in a magnifying glass, to further spread the features.

The optical microscope, which extends this principle, is basically a system of glass lenses used in conjunction with a source of concentrated light that illuminates the object to be viewed. Optical microscopes have been developed to a point where they can magnify an object up to 2,000 times, and at the same time resolve features on it only 2,000 angstroms (A) apart (one A equals 1/100,000,000 centimeter, or about the diameter of an atom). This is about the limit of optical microscopes because of the nature of light and distortions inherent in the lenses. The central problem is that it is impossible to resolve points on an object that are closer together than the length of the wave that is carrying the image.

This limit was breached with the development of the electron microscope a little over 30 years ago. The unique properties of electrons—they are both electrically charged particles and waves—permit them to be focused like light. Because electron waves are several hundred thousand times shorter than light waves, they can resolve much smaller objects. Today's electron microscopes can, in practice, resolve points on an object only 5 A apart, while magnifying the object as much as 1 million times.

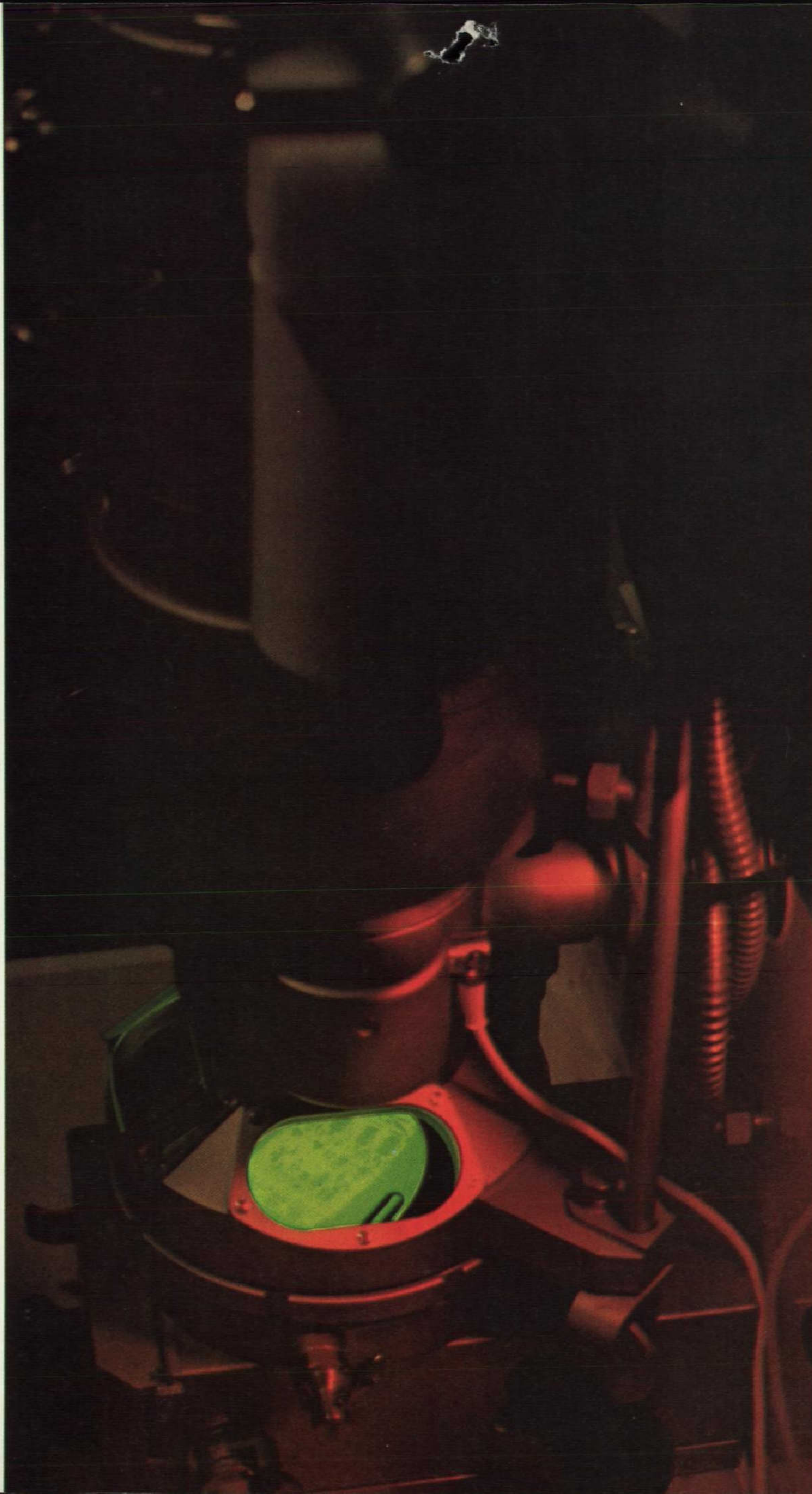


The author:
A native of Venezuela, Humberto Fernandez-Moran holds an M.D. degree from the University of Munich, an M.D. from the University of Caracas, an M.S. in cell biology and a Ph.D. in biophysics from the University of Stockholm. He is one of the leading inventors and developers of electron microscopes and microscopy techniques.

In the conventional electron microscope, the source of illumination is electrons, and the lenses are electromagnetic fields existing in a vacuum. A beam of externally generated high-voltage electrons enters the microscope through a tube at the top. After being concentrated by a condenser lens, it passes through the specimen to be viewed. This scatters the electrons, which are then formed into an image by passing through a series of focusing lenses. The beam finally arrives at a fluorescent screen near the bottom of the microscope where the electronic image is converted into a visual image. This greatly magnified image is viewed through an observation window.

The electron microscope is a highly versatile instrument. Its illumination can be varied from a few to several million electron volts, and the lenses can be focused over a wide range. This versatility promises to let us look even farther into the world of atoms and molecules. To push back the frontier, however, we must make improvements in three major areas: (1) in the environment of operation, (2) in our instruments, and (3) in our preparation techniques.

Because electron microscopes are extremely delicate, they are adversely affected by many external factors. Chief among these are electromagnetic interference, vibration, and contamination. We have gone to great lengths to minimize these factors at our nine-microscope laboratory at the University of Chicago. For example, to reduce electromagnetic disturbances, we use incandescent rather than fluorescent lights. Also, all the electrical wiring is shielded in grounded conduits behind the walls. Even the ventilator ducts are made of nonmagnetic stainless steel.



By Humberto Fernandez-Moran

The World of Inner Space

With the electron microscope, man is beginning to discover the secrets of atoms and molecules, and may soon be designing matter at this level

Man is exploring a new universe, inner space—through a powerful and promising instrument, the electron microscope. As does his counterpart, the astronomer, the electron microscopist is using his technology to overcome the limitations of his eyes. And, like the astronomer, he is attempting to find order and meaning in the world of the infinite he observes.

But the electron microscopist has one great potential advantage. An astronomer can only observe the planets, the stars, and the galaxies. The microscopist, however, not only can look into his magnified world of atoms and molecules, but may soon begin to manipulate it. For example, he may someday be able to alter the molecules that determine man's genetic makeup and correct molecular defects. He can also use the device like an inverted telescope, to condense massive libraries onto a single page, or print electronic circuits the size of red blood cells to transmit information from within the human body.

To understand the significance of the electron microscope, we need to understand the principles of magnification. We see an object because light waves carry its image to our eyes. In examining the object

In the eerie green glow of electron microscope viewing ports, scientists observe specimens with a spectacular degree of magnification and detail.

Will it someday be possible to build the brainlike machine that Warren McCulloch envisioned? Perhaps, even without the extreme miniaturization that Feynman suggests. Some experts, for instance, count on scientific and engineering genius to create a multitude of "functional circuits." According to Jack Morton, who directed the transistor into a practicable device at Bell Telephone Laboratories, designers are still thinking conventionally, in terms of individual circuit parts acting together. Functional circuits of the future would, however, have no recognizable parts that could be identified as, for example, a transistor. Instead, a special material would be processed to perform an elaborate function, such as the coding of spoken messages into dots and dashes.

The functional vanguard

A few functional devices do exist. A familiar example is the quartz crystal in a phonograph pickup that converts mechanical vibrations into a varying electric current. Another functional circuit is the laser. It does what no ordinary assemblage of electronic parts can accomplish in absorbing random energy and emitting light waves of a specific frequency that are precisely in step with one another.

Henry Zimmerman, director of M.I.T.'s Research Laboratory of Electronics, is optimistic about yet another approach. He points out that the Hungarian-American mathematician John von Neumann worked out a general theory for building highly reliable machines from unreliable parts. Such a machine would, in principle, perform much like a brain, which works remarkably well with its erratic nerve cells. Zimmerman explains that only up to a point does an increase in the complexity of a machine reduce its reliability. Beyond this point, with more circuits, machines can take advantage of a different principle. The brain seems to be a parallel machine; it works on a great many tasks at the same time. Up to now, computers have been sequential; they complete one task before starting the next. Using the speed and reliability of ICs that will be available a few years from now, it may be possible to design information processors that are organized in a parallel fashion.

Zimmerman, like most scientists, doubts that man will soon reproduce his brain in electronic hardware. But, using a multitude of ICs, ultraminiaturized, working in parallel, together with functional circuits, he may build electronic devices tomorrow that seem like impossible dreams even today.

For further reading:

"Gulliver-Size Need for Lilliputian Products," *Time*, Sept. 2, 1966.
Hittinger, William C., and Sparks, Morgan, "Microelectronics," *Scientific American*, November, 1965.
"Hookup to the Future," *Business Week*, Nov. 4, 1967.

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FIELD ENTERPRISES EDUCATIONAL CORPORATION
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I.R-100 AWARDS BANQUET
LECTURE

HUMANITY AND SCIENCE AT THE CROSSROADS

Dr. Humberto Fernandez-Moran
Professor of Biophysics
University of Chicago

October 4, 1968
New York Hilton

WELCOME to a unique exhibit and banquet—the annual **I·R 100!**

Here the best of the nation's innovators, those who have distinguished themselves by developing the 100 most significant new technical products of the year, are honored and their new products exhibited.

The **I·R 100** Awards, considered by many as the applied research equivalent of the Nobel Prize, are presented by Industrial Research Inc. to stimulate and recognize the scientific and technological achievements of the nation's $25.5-billion research industry. Since the **I·R 100** began in 1963, some 300 industrial, university, and government laboratories and more than 1,000 scientists and engineers have been cited.

These innovators of modern technology truly represent the best technical minds in the country today.

Their 100 products are selected (from about 10,000 new technical products developed each year) by the Editorial Advisory Board of Industrial Research Inc. This 30-man panel includes five Nobel laureates, heads of research institutes and laboratories, and the inventors or discoverers of radar, stroboscopic photography, radiocarbon dating, galactic astronomy, communications satellites, the laser, and many other scientific accomplishments of our time. (See inside back cover for a listing of the judges.)

A few facts about the 1968 winning products: This year's awards took an average of 29 months to develop—two months more than the average for last year's I·R 100 winners. The cost of developing a winning product was $422,000, on the average, compared with $250,000 last year. Only 17 of the products were developed with the aid of federal funds, compared with 26 a year ago. It would take $1.3-million to purchase all of the 100 winning products this year—or about $22,000 per product.

The entire **I·R 100** exhibit and formal banquet are part of National Industrial Research Week (Sept. 29 through Oct. 5) being observed by the nation's 15,000 industrial laboratories. Open houses, exhibits, and other activities have been scheduled during this Week to call attention to the contributions of the 350,000 scientists and engineers in this country engaged in applied research and development.

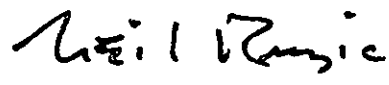
Among activities during the Week are an open house at Outboard Marine Corp.'s laboratory in Milwaukee, exhibits at Varian Associates in Palo Alto, a science career program by Xerox in Rochester, and a community salute to U.S. Steel Corp. and other companies with research facilities in Monroeville, Pa.

General Electric, Ford, Sperry Rand, Univac, Westinghouse, Thiokol Chemical, and many other companies also are scheduling special events during National Industrial Research Week.

The National Conference on Industrial Research is held during this Week too. Limited to about 500 corporate presidents, research directors, and senior scientists and engineers, the conference is concerned with making new product development more effective and profitable.

During the research conference two annual awards will be made. The "I·R Man of the Year" award and $1,000 check will go to Dr. Bernd T. Matthias, professor of physics, University of California at San Diego, and technical staff member of Bell Telephone Laboratories, for his achievements in increasing transition temperatures of superconductive materials. And the "I·R Laboratory of the Year" awards will be made to several new outstanding research laboratories.

All of these events, awards, and activities are sponsored by Industrial Research Inc., publishers of Industrial Research, Materials Applications, Oceanology International, and Electro-Technology magazines. Purpose of the **I·R 100** and associated events is to stimulate our country's vital "industry of innovation" and to honor outstanding applied research achievement.



Neil P. Ruzic
president
Industrial Research Inc.

THE I·R 100 COMPETITION AND NATIONAL CONFERENCE ON INDUSTRIAL RESEARCH

The *I·R 100* Competition and the National Conference on Industrial Research are sponsored annually by Industrial Research Inc. as a service to the nation's $25-billion research industry.

The 100 most significant new technical products of the year are selected in the *I·R 100* Competition. The products are displayed and their developers honored at the annual product exhibit and awards banquet.

The conference, which has "New Products — and Profits" as its theme this year, is sponsored by Industrial Research Inc. in cooperation with the Purdue University Krannert Graduate School of Industrial Administration, IIT Research Institute, and Battelle Memorial Institute.

I·R 100 SPECIAL SECTIONS

A complete report on the *I·R 100* winning products will be featured in the December issue of *Industrial Research.* Descriptions of the prize-winning electronic products will be published in the December issue of *Electro-Technology,* while a summary of oceanographic product results will appear in the December issue of *Oceanology International.*

Schedule of Events

NATIONAL CONFERENCE ON INDUSTRIAL RESEARCH and I·R 100 Awards Program

Oct. 3-4, 1968

THURSDAY, OCTOBER 3

8 to 9 a.m.	Conference registration	Ballroom Foyer
9 to 12 p.m.	Conference morning session	Trianon Ballroom
11 a.m. to 7 p.m.	"I·R 100" exhibit	Rhinelander Gallery
12·15 to 2 p.m.	Conference luncheon	Mercury Ballroom
2 to 5 p.m.	Conference afternoon session	Trianon Ballroom
Tour of	"I·R 100" exhibit	Rhinelander Gallery

FRIDAY, OCTOBER 4

9 to 12 p.m.	Conference morning session	Trianon Ballroom
11 a.m. to 7 p.m.	"I·R 100" exhibit	Rhinelander Gallery
12:15 to 2 p.m.	Conference luncheon "I·R Man of the Year" award presentation	Mercury Ballroom
2 to 5 p.m.	Conference afternoon session	Trianon Ballroom
6 to 7 p.m.	Reception	Rhinelander Gallery
7 to 9:30 p m.	Industrial Research awards banquet "I·R Laboratory of the Year" and "I·R 100" awards presentation	Grand Ballroom

KEYNOTE SPEAKER at the I·R 100 formal banquet Friday night, Oct. 4 is Dr Humberto Fernandez-Moran, often called a "renaissance man." Biophysicist, physician, neurologist, neuropathologist, radiation authority, inventor of cryogenic electron microscopes, philosopher, and former diplomat for Venezuela, Dr Fernandez-Moran will address the innovators of the nation's most significant new technical products on "Humanity and Science at the Crossroads."

I.R-100 LECTURE (Oct. 4, 1968)

H. Fernandez-Moran

INTRODUCTION

On rare occasions such as these, we are privileged to share and live through the high noon of an era which is rapidly moving towards a critical point of transition in space and time. Its thundering "leitmotiv" started reverberating about a quarter of a century ago with the controlled release of nuclear energy brought about through the concerted efforts of a group of dedicated men, many of whom are still among us, like Dr. Glenn Seaborg who addressed you last year, and Dr. Alexander Sachs, one of its inspired originators, who is here tonight. Since then, accelerated scientific and technological progress in all fields has enabled Man to visualize directly the complex structure of atoms and molecules in living matter, to venture out into space, and in general to achieve remarkable feats in control and communication on a planetary scale, vastly extending his intellectual powers--until now we confront a strange future laden with deep uncertainties and grave challenges, but still offering ever greater opportunities.

Resurrecting the conceptions of one of the boldest thinkers of all time, who worked and died in this city barely 13 years ago: Pierre Teilhard de Chardin, we must admit that Mankind has now reached a crucial stage, Mankind is perplexed and even bored, not knowing what to do with itself and yet paradoxically endowed with unprecedented potential for good or evil. It may not be the shallow "brave new world" once predicted, but it certainly is one of the most active and demanding, more attuned to the faith and vision of the optimist, than to the paralyzing confusion of the pessimist.

We are all keenly aware of the social turmoil affecting particularly the younger generation at the national and international levels; and the terrible "memento mori" has not ceased to haunt our era since 1938, dimming the full joy of a Promethean renaissance. In addition, the 'population explosion' is already so overwhelming that by the 1970's hundreds of millions of people are going to starve to death in spite of any foreseeable emergency programs which can now be implemented.

Yet, it is against this background of stark realism that I wish to invoke the spirit of individual innovation representative of this forum to state the central thesis that cooperative endeavors of human minds guided by science and technology will ultimately lead Mankind to higher levels of inventiveness and achievement.

This stems from our 'faith in Man' and his future which gives us the conviction that just as we appear to have reached the "critical mass for annihilation" under unrelenting biological and social pressures, so have we been likewise invested with an ample 'critical mass' capacity for survival and further evolution. As expressed by Faulkner in his memorable Nobel Prize speech: 'Man shall not merely endure, he shall prevail on this Earth', and all that Mankind stands for is inextricably linked to the progress of the Universe.

Man, the Thinker, the Believer, and the Dreamer, is at the very heart of the Cosmos. In this deeper sense, Man himself is the missing link.

Moreover, Man is a Trustee of Life, one of its many forms in the Universe. Therefore, he must fulfill the dual obligation of ensuring his own survival, while trying to preserve and understand all other forms of Life, no matter how alien. I understand Albert Schweitzer's 'Reverence for Life' primarily in this sense.

Perhaps the only permissible constraint that Man can invoke and exercise may be derived from an analogy to Pauli's exclusion principle. Since according to this principle (which I termed 'Vivethics'), no two forms of Life can 'occupy' or 'inhabit' the same time-space domain, Man could ultimately justify pre-empting other forms of Life inimical to his own existence, but he may never wantonly destroy or kill them. In general, from a 'rational point of view', the ethical principles may be literally a fundamental condition for survival and higher evolution of the human race.

So many great minds and hearts have already wrestled with these problems and pointed out possible solutions, that I can only aspire here to deal with certain salient approaches in the need for developing more effective methodological and conceptual tools for probing the future. I should like to set forth some of these more as a list of seminal problems which can be posed from a biophysicist's point of view to stimulate the interest and active participation of scientists and technologists focusing on key areas of the Neurosciences in exploring the human mind, of Molecular Biology, of Exobiology, and of Biomolecular Organization at all levels.

There is one specific 'case history' which I wish to dwell on--not only because it is eminently timely in light of recent events, but also because of its decisive historical and geopolitical significance.

Its urgency becomes manifest as we realize the serious threat to the whole future of our species inexorably building up when more than half of the world is hungry, many are starving to death, and we still keep on growing at such a rate that the present population of well over three billion people is expected to double within the next 30 years on a

sick planet already in the grip of environmental deterioration, pollution and declining natural resources. To counteract a vicious circle of such planetary proportions, the world population must rapidly be brought under control, through voluntary, conscious regulation of human reproduction coupled with an integrated program designed to increase food production greatly and achieve environmental restoration. This could mean such an abrupt reversal of a previously uncontrolled evolutionary trend that we would have to postulate the action of highly cooperative phenomena embracing basic affinities of the individual human minds, not unlike those involved with manifestation of quantum effects operating on a truly macroscopic scale, of which superconductivity is a remarkable example.

In any case, it is interesting to consider such cooperativity phenomena effective within certain collective domains on a social-geopolitical scale as being first localized and 'regional'. Ultimately, however, these 'Homo-cooperativity' processes could extend over the entire system and achieve international siginficance through purposeful, systematic interaction with technological communication media.

I submit that nowhere on this planet are conditions more favorable for attainment of true cooperativity phenomena at the present time than between the Americas. To begin with, the United States may be regarded as the typical and most successful example of such Homo-cooperativity phenomena. Thus, when Henry James defined Americans as "people of abysmal good nature and ultimate good sense," as Dr. Alexander Sachs recently told me, he was expressing the qualities which are essential for true cooperativity among human beings. Precisely this rare quality of being able to elicit the best efforts from individuals of all nationalities without distorting them in the process, accounts for the phenomenal

success of the American 'team effort' ranging from the economic to scientific and engineering cooperative projects. It explains why the U.S. now controls two-thirds of the world's capital market, using only about five per cent of the world's highly qualified labor force to do so. It also helps explain why the U.S.A., representing less than 1/15 of the world's population uses over half of all the raw material consumed each year.

In fact, this inherent capability for success has "activated" this enterprising nation to such a degree that it has unfortunately disposed or squandered its own resources with such prodigality, and it now finds itself lacking basic raw materials, and entangled in so many global commitments by virtue of its dual role of World banker and World philanthropist, that it is facing a generally estranged or hostile world.

And that in itself is a fundamental reason for turning to its other neighbor nations in Canada and Latin America at this crucial point of history.

Basic supporting factors for these cooperative projects between the Americas are the geopolitical proximity and the complementarity in filling each other's needs. U.S. primarily needs natural resources and markets; while Latin America essentially needs assistance in filling the technical, scientific, and managerial gap. Both inseparable halves of the great Continent most definitely require to know each other better, both as indiviiduals and as social entities, as well as to actively exchange the wealth of their cultural heritage at all levels.

Latin America as a whole now emerges as the only "viable" continent invested with the greatest untapped stores of prime resources. The entire continental United States can, for example, be emplaced in the gigantic Amazon valley, which

contains, among many other things, 25% of the world's timber, its greatest river and drainage system, etc. Despite manifest social ailments, Latin America turns out to have a remarkable bedrock strength in its closely related common languages, religion, unusually complex but vigorous genetic background. Blessed with great biological survival features, it enters the historical scene precisely at the time that our advanced technology (including nuclear engineering) permits us to cope effectively with the formidable barriers, now become instead welcome challenges and opportunities.

This would range from nuclear explosion and earth-displacement programs to form dikes and hydro-electric transportation networks interconnecting the Amazon, Orinoco, and Paraguay systems, to the use of integrated atomic power-reactors and petrochemical complexes, with "synergistic" minerals, agricultural, hydroponic, timber, and related development projects. Much of this has already been adumbrated by an increasing number of U.S., Latin American and European planners, including most recently the imaginative group of Panero and Kahn at the Hudson Institute (article in Fortune, December 1967, p. 148).

In order to be really successful on a long-term basis, these cooperative projects between the U.S.-Canada-Latin America should comprise a fully integrated program to fill the gaps primarily at the functional levels: general economic gap, foreign investment gap, research and development gap, a management gap and an education gap.

The educational gap is at the heart of the problem, encompassing secondary, technical and graduate schools, and particularly the modern American management education (e.g. Harvard's Business Management Institute), and the new technical graduate schools patterned after those two centers of excellence: M.I.T. and CalTech.

There will likewise be a great need for several "multiversity"-type international universities for key regions of Latin America. As we envision it, they would be an internationally-financed community with an international faculty, representing the professional excellence of a number of countries. They would have international classrooms connected by transcontinental electronic communications systems; international laboratories in which a U.S. and a Latin American student--each on his own campus--could cooperate on a scientific experiment; and international repositories of information coupled with computer retrieval facilities (Scientific Research, S. Winer, January 1968).

It is my firm conviction that America is an indivisible entity, which will either survive and fulfill its historical destiny as the backbone of this planet in a complete and voluntary cooperation between North and South, or disintegrate piecemeal. This complementarity is not hypothetical, but rests on solid historical background. Remember that Bolivar already conceived and laid the foundation for a "Pan American Union" nearly a century and a half ago. Following Bolivar's death, certain circumstances, notably the massive immigration from Europe in the 1840-50's, definitely favored the North; and it has since retained a historical preponderance while South America recovered from its hemorrhages and wrestled weakly as a sick giant with the titanic handicaps of its tropical domain. Now, the pendulum is gradually swinging the other way, and it takes intuition of rare order and great thoughtfulness and understanding to bring about a true cooperative "resonance" between the twin continents.

In closing, let me thank you for your hospitality and understanding. I can find no better way to convey the feeling of silent hope and reverence that we all cherish when we think of our common heritage and future destiny than

to quote those immortal words of Simon Bolivar, one of Mankind's greatest statesmen and seers, from his Address delivered at the inauguration of the Second National Congress of Venezuela in Angostura, February 15, 1819:

> "My imagination, taking flight to the ages to come, is captured by the vision of future centuries, and when, from that vantage point, I observe with admiration and amazement the prosperity, the splendor, the fullness of life which will then flourish in this vast region, I am overwhelmed. I seem to see my country at the very heart of the universe.....I behold her shipping to all corners of the earth the treasures.....which lie hidden in her mountains. I can see her dispensing....health and life to the ailing....I can see her confiding her precious secrets to the learned men who do not know that her store of knowledge is superior to the wealth with which Nature has prodigally endowed her."

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IMMEDIATE

Dr. Humberto Fernández-Morán has been named A. N. Pritzker Professor of Biophysics in the Division of the Biological Sciences and The Pritzker School of Medicine of The University of Chicago.

Dr. Fernández-Morán, 44, is internationally known for his work in electron microscopy.

The appointment was announced by John T. Wilson, Vice-President and Dean of Faculties of the University, who said:

"The Pritzker family has made many contributions to the University, and to medical science, that will have a lasting impact. In recognition of this contribution, we are pleased to designate one of our most distinguished faculty members as a Pritzker Professor."

In June, 1968, The University of Chicago School of Medicine was renamed The Pritzker School of Medicine of The University of Chicago in honor of the family and its support. Members of the Pritzker family of Chicago include the brothers A. N. Pritzker and Jack N. Pritzker and the former's sons, Jay, Robert, and Donald.

The family's contribution included a $12 million gift to support the medical school and create four endowed chairs to help strengthen the school's faculty.

Dr. Fernández-Morán commented on the professorship: "It is a privilege for me to accept this high honor for my colleagues and the University. I will do my utmost to continue to bring a contribution to the University in whatever capacity I may be called upon to serve."

-more-

A statement by Dr. Leon O. Jacobson, Dean of the Division of the Biological Sciences and The Pritzker School of Medicine, said:

"Dr. Fernández-Morán is an excellent choice to hold the first A. N. Pritzker Professorship. His achievements in science have been outstanding and have enabled countless other researchers to attack problems previously considered unsolvable. He is a brilliant and ingenious man and his work at the University has contributed much to our medical program."

Work in the field of electron microscopy has progressed rapidly since Dr. Fernández-Morán developed the diamond knife nearly 20 years ago, when he was a research fellow at the Nobel Institute in Stockholm, Sweden.

At that time, the only knives available for delicate microtomy work were of glass or steel, and neither was capable of cutting sections thin enough to take full advantage of the power of the electron microscope.

Disregarding professional diamond grinders who told him the job was impossible, Dr. Fernández-Morán selected a flawless industrial diamond and set to work. The final cutting edge was achieved by superpolishing in a special device using ultrafine diamond submicron powders.

When he completed his exacting work, Dr. Fernández-Morán had a mounted knife capable of cutting starch into its component sugars.

Then he began to use the electron microscope to examine structural details a thousand times smaller than bacteria. Research with the electron microscope has brought scientists to the borderline of life itself.

The "master molecule" now attracting primary attention is deoxyribonucleic acid--DNA; a tool by which it is being studied is the electron microscope.

Not content to dwell entirely on purely medical uses for the electron microscope, Dr. Fernández-Morán has been active in the field of subminiaturization.

"Its promise," he said, speaking of the electron microscope, "extends from producing radio transmitters which can be placed in human blood cells to imprinting the entire Library of Congress on a magazine-size sheet so books can be fed into home television."

-more-

Early this year, Dr. Fernández-Morán told an interviewer that he does not consider himself primarily a scientist, despite the fact that he is a biophysicist, neurologist, neuropathologist, authority on radiation and on cell biology, inventor of advanced electron microscopes and microscopy techniques, and founder of a laboratory for brain research.

"Science," he commented, "has been one of many areas for me, perhaps the unifying concept, but not the overwhelming one. It's a gift in its truest form. But it should not, must not, be the all-encompassing aspect of ones life."

And, indeed, his life has been varied. Born in Maracaibo, Venezuela, in 1924, he was educated in Germany and received his M.D. from the University of Munich, where he was one of the youngest students in its history. He then returned to Venezuela to become licensed to practice under tropical conditions. During this time, he received a second M.D. degree, from the University of Caracas. He interned in neurology and neuropathology at George Washington University, Washington, D.C., before going to the Nobel Institute in Stockholm.

In 1954, he again returned to Venezuela to establish a $50 million Venezuelan Institute for Neurology and Brain Research, working to develop it as a research center for all South America.

In 1955, Dr. Fernández-Morán headed his country's delegations to the first Inter-American Symposium on Nuclear Energy at Brookhaven National Laboratory. He also was active on the science commission of the Organization of American States and of UNESCO. In 1958, he was named minister of education for Venezuela.

His medical interests brought him to the United States, where he worked for four years at Massachusetts General Hospital in Boston and served on the faculties of Harvard University and the Massachusetts Institute of Technology.

Dr. Fernández-Morán joined The University of Chicago faculty in 1962 but he continues to list his official residence as Maracaibo, expressing his conviction that close personal ties are essential to further development of inter-American scientific research and education.

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Two Named Professorships Announced

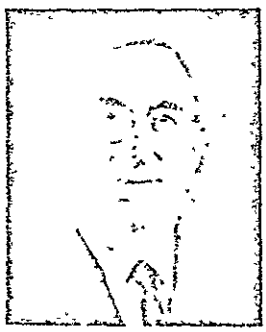
The University has announced appointments to two major named professorships in the Division of the Biological Sciences and The Pritzker School of Medicine.

Dr. Joseph B. Kirsner has been named Louis Block Professor of Medicine, a chair honoring the late Louis Block, who was president of the Blockson Chemical Company, Joliet, Illinois. He bequeathed funds for the Louis Block Fund for Basic Research and Advanced Study, established in 1956 to support the Divisions of Biological Sciences and Physical Sciences.

Dr. Humberto Fernández-Morán has been appointed A. N. Pritzker Professor of Biophysics. The Pritzker family of Chicago has contributed $12 million to support the medical school and to create four endowed chairs.

Dr. Joseph B. Kirsner

Dr. Joseph B. Kirsner joined the faculty of the University in 1935, after earning his M.D. from Tufts University School of Medicine, Medford, Massachusetts, in 1933. Since then, he has remained on the faculty in the Department of Medicine; in 1942, he received his Ph.D. in Biological Sciences from the University.



Dr. Joseph B Kirsner

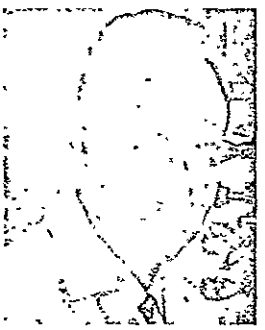
Author or co-author of approximately 400 publications dealing with various clinical and experimental problems in gastroenterology, Dr. Kirsner's major clinical and research interests include peptic ulcer and gastric secretion, regional enteritis and ulcerative colitis, and cancer of the gastrointestinal tract.

Over the years, the list of his professional memberships, and of his contributions to medical committees, from local to national levels, has grown to impressive proportions. Currently, Dr. Kirsner is a member of 15 professional societies and on the editorial boards of four medical publications.

He is past president of the American Gastroenterological Association, the American Gastroscopic Society, the Chicago Society of Internal Medicine, and the Jackson Park Branch of the Chicago Medical Society.

Dr. Humberto Fernández-Morán

Dr. Humberto Fernández-Morán, a native of Venezuela, came to The University of Chicago in 1962. Internationally known for his work in electron mircoscopy, he developed the diamond knife nearly 20 years ago when he was a research fellow at the Nobel Institute in Stockholm. His knife, capable of cutting starch into its component sugars, enabled scientists to study the tiniest organic details under an electron microscope, and opened up the field of electron microscopy.



Dr. Humberto Fernández-Morán

Recently, Dr. Fernández-Morán has conducted research in the field of subminiaturization, which he feels holds great promise.

Born in Maracaibo, Venezuela, in 1924, he was educated in Germany and received his M.D. from the University of Munich. He returned to Venezuela to obtain his license to practice, and, while there, earned a second M.D. degree from the University of Caracas.

Dr. Fernández-Morán has represented Venezuela in several international conferences, and in 1958, served as minister of education for his country.

Perspectives Journal Wins Award

An unconventional and highly respected medical quarterly published by the University has received the American Medical Writers Association's 1968 Honor Award for Distinguished Service in Medical Journalism.

In presenting the award, the Association described the quarterly *Perspectives in Biology and Medicine* as a "free-wheeling vehicle for speculative essays, sharp polemics, engaging biography, good poetry and philosophical reflection."

The quarterly was also honored with a citation from the Association in 1964.

Perspectives is edited by Dwight I. Ingle, eminent endocrinologist and physiologist and Professor of Physiology at the University.

Ingle is known primarily for establishing the fact that cortisone is biologically active. He was also the first to experimentally demonstrate that adrenal atrophy occurs in response to overdosage with cortisone or other steroids, and the first to experimentally demonstrate steroid diabetes. He also is known for his research on the role of the adrenal cortex in causing adaptation diseases.

He started *Perspectives* in 1957 in the belief that scientific writing should not be stilted, ponderous or dull.

"Authors," he said, "are encouraged to use an informal, humanistic style which preserves the warmth, excitement and color of the life and medical sciences."

The essays published in *Perspectives* are written primarily for medical scientists, practicing physicians and surgeons, biologists, and students of biology and medicine. However, its readers include intelligent laymen ranging from high school students to English teachers.

Published by The University of Chicago Press, *Perspectives* boasts an advisory board that includes Nobel Laureates Francis H. C. Crick, Sir John Eccles, Albert Szent-Gyorgyi, Arne Tiselius, and others.

'I would like to catch a fleeting glimpse of how the brain operates and functions'

Moran joined the Nobel Institute for Physics as a research fellow and began his work in electron microscopy. It was there he developed the diamond knife. He also studied and did research at the Institute for Cell Research & Genetics of the Karolinska Institute, was a resident at one Stockholm hospital and foreign assistant at another, and functioned as Venezuelan scientific and cultural attaché to the three Scandinavian countries. He still found time to earn an MS in cell biology (1951) and a PhD in biophysics (1952) at the Univ. of Stockholm.

"Sweden meant a great deal to me in those formative years," says Fernandez-Moran. "That is where I got my physics background. I learned methodology, and I learned about time. The Swedes are not out to do things on deadlines.

"I also had an ulterior motive. I wanted to learn how a country which is in essence a big power, but has the population of a little power, is run. In Sweden I had the inside track into the hospitals, as a doctor; into their science, through the Nobel Institute; into the politics, economics, the social life, as cultural attaché. I wanted to learn these things to take home to Venezuela with me."

RETURN TO VENEZUELA

In 1954 Fernandez-Moran returned to Venezuela to continue his studies. He considers this move one of the best he ever made: "The tropics are amazing. They opened my eyes to tropical medicine, the role of viruses and insects, and helped mold the pattern that had started in Europe with Karl von Frisch—who had helped me develop my direction in research with his study on bees' vision, and focused my interest on the insect brain.

"Also, my return renewed my love for Latin America and increased my sense of indebtedness to Venezuela," he recalls.

In 1954 the Venezuelan government gave Fernandez-Moran a mandate to establish a $50-million national biological research laboratory of his own conception. He used the free-wheeling commission to found the Instituto Venezolano de Neurologia y Investigaciones Cerebrales (IVNIC), whose aims went far beyond those of an institution limited to neurology and brain research. He conceived of it as a regional research center for all of South America that would eventually develop into an international training center as well.

IVNIC's founding charter, dated April 29, 1954, gave the institute autonomous status. Fernandez-Moran translated the charter into reality with prodigious energy. For a site, he flattened the top of a 5,000 ft. mountain seven miles from downtown Caracas. He took title to the site Jan. 1, 1955; by Dec. 2, only eleven months after the first bulldozer arrived on the scene, the President of Venezuela came to attend the opening of IVNIC's $3-million pilot unit. This included a building housing six institute departments, a workshop, three electron microscopes and a large library; staff residences; an 800-kW electric power station, and a water-supply system with a six-million-liter artificial lake.

PUBLIC SERVICE AND EDUCATION

In 1955 and 1957 Fernandez-Moran headed Venezuela's delegations to the first Geneva Conference on Peaceful Uses of Atomic Energy, and to the first Inter-American Symposium on Nuclear Energy at Brookhaven. He was also active on the science commissions of the Organization of American States and of UNESCO.

In 1958 he was named Minister for Education of Venezuela. Within 10 days the nation's president, dictator Gen. Marcos Perez-Jiménez, was ousted; Fernandez-Moran remained at his post throughout the coup—the only cabinet official to do so. In the eyes of the new regime he was convicted of guilt by association and given the opportunity to "take a sabbatical." He has not returned to Venezuela since.

He went to Boston on leaving his country and worked for four years at Massachusetts General Hospital as an associate biophysicist in neurosurgery while serving as visiting lecturer in biology at MIT and research associate in neuropathology at Harvard. In his spare time he organized the Mixter Laboratories for electron microscopy at Mass. General, building the lab, the microscope and the equipment.

In 1962 he accepted a full professorship of biophysics at the Univ. of Chicago, a post he still holds. (It is typical of the man, however, that he lists his address as: "Home: Apartado 362, Maracaibo, Venezuela; Office: Dept. Biophysics, Univ. of Chicago, Chicago.")

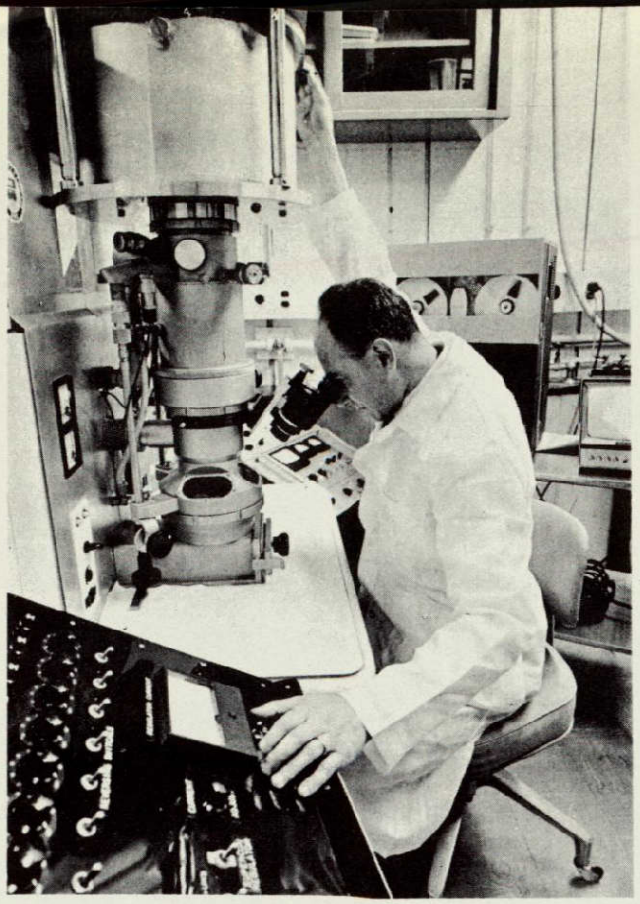
CONTRIBUTIONS TO MICROSCOPY

Fernandez-Moran has devoted much of his energy during the last six years to his electron-microscope laboratory, for he feels strongly that by improving the resolution of the electron microscope down to the 1-2 Å range (he has already penetrated as low as 3.5 Å) he will be able to read out molecular and atomic structure directly, and thus look into the essence of life itself.

"The electron microscope is the clue to the future," Fernandez-Moran declares. "I think, however, this country must get on the ball in this area. I see the U.S. getting into the exploration of inner space. Conditions are ripe for the high-voltage microscope, and the key to high-voltage microscopy, in my opinion, is the superconducting electron microscope to study the organization of membranes and key enzymes.

"But we need to develop the tools. What I would like to do some day is to catch a fleeting glimpse of how the brain operates and functions, to prove that the brain thinks with atoms. But more basically—and perhaps more within my grasp as a scientist—is to see the molecular structure of biological enzymes. Much has been accomplished—but we've miles to go before we sleep."

His interests and achievements in this area are wide, encompassing all aspects of electron-microscope development and use. For example, in sample preparation, Fernandez-Moran developed the diamond knife and the ultramicrotome, now widely



Photos by Michael Mauney, Black Star

philosopher . . .
experimenter . . .
teacher . . .
HUMBERTO FERNANDEZ-MORAN

Maracaibo to Chicago via Stockholm— journey of a protean scientist

by SUSAN WINER, *McGraw-Hill Chicago Bureau*

One of his generation's true renaissance men of science, Humberto Fernandez-Moran is so much the renaissance man that he does not even consider himself primarily a scientist.

Biophysicist and physician, neurologist and neuropathologist, authority on radiation and on cell biology, inventor of advanced electron microscopes and microscopy techniques, founder of a laboratory for brain research, and former Nobel Institute of Physics research fellow, he can yet say:

"Science has been one of many areas for me—perhaps the unifying concept, but not the overwhelming one. It's a gift, in its truest form. But it should not, must not, be the all-encompassing aspect of one's life."

A native of Venezuela now living in Chicago, Fernandez-Moran—who despite his accomplishments is only 43—has for the last six years been a member of the biophysics faculty at the University of Chicago. There he has, in the words of a colleague, "created one of the finest electron-microscopy laboratories in the world."

As a grace note to his busy career in science, Fernandez-Moran has also built a record of public service. A Venezuelan patriot before all else, he has been a diplomat, cultural attaché and Minister of Education. Living now in a foreign country, he still dreams of returning to his native land and making a contribution—by way of science and education—to its social and economic development and stability.

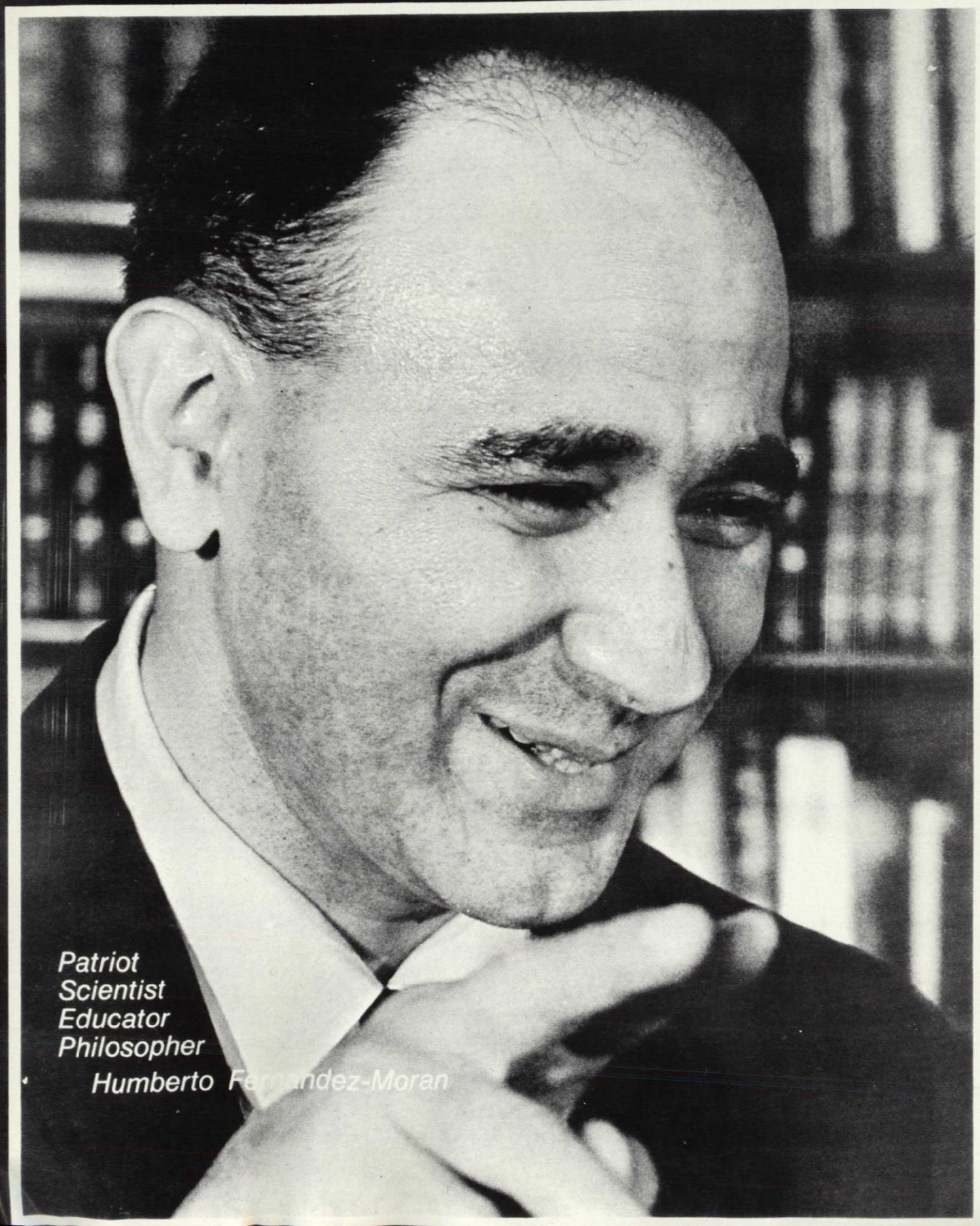
PERIPATETIC STUDENT

Born in Maracaibo in 1924, Fernandez-Moran was educated in Germany and entered the University of Munich as one of the youngest students in its history. He continued his studies during the war, and obtained his MD in 1944. In 1945 he returned to Venezuela to become licensed to practice under tropical conditions, and picked up a second MD from the Univ. of Caracas. Then, after a year as an intern in neurology and neuropathology at George Washington Univ., Washington, D.C., he moved on—to Stockholm. "Sweden at that time was one of the leading centers for the areas I was primarily interested in, namely neurosurgery and that part of molecular biology linked to physics."

Not only did Sweden represent for him one of the last truly free and independent countries, but it was also the home of the Nobel Institutes. In 1947 Fernandez-

SCIENTIFIC RESEARCH

McGRAW-HILL'S **NEWS** MAGAZINE FOR SCIENTISTS • JANUARY 8, 1968



Patriot
Scientist
Educator
Philosopher

Humberto Fernández-Morán

"It was with this in mind that I eagerly undertook the opportunity to provide Venezuela—and Latin America as a whole—with a research center, a school for the study of biophysics in the center of an area laden with all the elements for study, as the tropics are."

Although he was not able to follow through on his first attempt—IVNIC—Fernandez-Moran has not given up the idea. "I visualize a Venezuelan Institute of Technology," he declares, "modelled after MIT. And, perhaps, someday an association of universities in Latin America, too."

During his period of exile, Fernandez-Moran has nurtured in his imagination the concept of a "multiversity," an international university, to meet the needs of Latin America. Back in 1959, before there were communications satellites, he called for "continental classrooms" in the U.S. with communications satellites over the equator, to beam course lectures to all of South America and thus revolutionize teaching on that continent.

Fernandez-Moran's multiversity, as he envisions it today, would be an internationally-financed community with an international faculty, representing the professional excellence of a number of countries. It would have international classrooms connected by transcontinental electronic communications systems; international laboratories in which a U.S. and a Latin American student—each on his own campus—could cooperate on a scientific experiment; and international repositories of information available at electronic speed.

Fernandez-Moran foresees strong objections from conservative Latin America, on the grounds that his scheme is premature and too radical. "My answer is very simple," he says. "We have tried all approaches for over 400 years; they've failed. So let's try the Archimedean approach in this case. Let's put the substance of such a university outside of this entropic chamber which is called Latin America, until we have the proper catalyst to make it go into a critical mass. That's not difficult—in fact, it is the only way, because none of the Latin American countries has the requisite finance to do a good job alone. You need billions of dollars. So the money must come from outside.

"But the real reason why the impetus must come from the outside is that we do not have, at this time, a group of professors and people who are knowledgeable and capable of teaching. It would take us 20 years to develop this type of staff. But in 20 years the problem will be completely out of bounds. We are growing at the rate of 4% per year and within 20 years we will have some 700 or 800 million people."

CHICAGO AS SPRINGBOARD

Considered by his friends and peers as something of a rare bird in a white smock, Fernandez-Moran is popular with his faculty colleagues and is much admired by his students, with whom he maintains an easy camaraderie; in his laboratory all are equal without distinction of academic rank.

A natural linguist, Fernandez-Moran not only speaks fluent Spanish, Swedish, English (without a trace of accent), French, German and three or four other languages, but also has a smattering of several additional tongues. He is able to communicate with most of his students in their native languages.

An enthusiastic runner and boxer in his youth, his nose was smashed in an undergraduate bout in Germany. He still tries to run a mile every day. He is married to a tall, blonde Swede whom he met in his Stockholm days, has two daughters 13 and 11, no automobile, and lives in an apartment near the campus decorated with furniture and artifacts picked up on his travels around the world.

Although a busy speaker, Fernandez-Moran never accepts honorariums for talks, preferring to earmark the money to finance the attendance of university science students at distant conferences. As a result, his exceptional collection of rare books—the nucleus of which is a family inheritance—is periodically enhanced by many choice items of science literature received as gifts at speaking engagements.

"My dreams of an international university cannot come true unless I continue my work here in Chicago," says Fernandez-Moran. "While this may sound somewhat contradictory, it must be understood that I need the work I am doing here, the contacts I've made and the people I know as friends and peers in order to succeed."

"I would like to see," he says, "a truly international sharing of ideas, on a grand scale—an organization similar to the U.S. Associated Universities, something that would enlist and mobilize private funds, including the refugee funds in Switzerland, and using retired professors."

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January 1968

Another award for Fernandez-Moran

Newest accolade accorded Humberto Fernandez-Moran is the John Scott Medal, given by the City of Philadelphia under an 1816 bequest from an Edinburgh chemist of that name, "to ingenious men and women who make a useful invention that adds to the comfort, welfare and happiness of mankind."

Fernandez-Moran received the award for his invention in the early 1950's of the diamond knife, now an essential tool in biological and medical research. He is the first Latin-American to receive this honor. The presentation was scheduled for Jan. 10 in Boston at a meeting of the American Academy of Arts & Sciences.

used to cut ultrathin sections of biological tissue for examination. With these instruments he can cut sections only 50 Å thick—literally cutting up individual molecules; for instance, slicing starch into sugar!

In addition, he has developed thin-walled vacuum-tight chambers that make it possible to examine cellular materials in a condition approaching their natural hydrated state. This extends the potential of electron microscopy far beyond the study of dried specimens and brings the examination of solutions of DNA within range.

Turning to electron microscope design itself, Fernandez-Moran has worked extensively on: lens design —using superconducting solenoids to produce the magnetic fields that focus the electrons; the improvement of very high vacuums and high stability of fields—achieved with the superconducting magnets, among other techniques; and on highly coherent and stable electron sources. All of these improvements have led to electron microscopes that can probe down to the molecular level of 2-4 Å—the best resolution ever achieved, which Fernandez-Moran reached recently.

ULTRAMINIATURIZATION

Fernandez-Moran's intense curiosity and his ability to see across disciplinary lines is best exemplified by his most recent area of research. Turning from the life sciences, he has applied electron microscopy to information storage and retrieval, using the technique of ultraminiaturization.

"Precisely the same conditions that we have been striving for in high-resolution electron microscopy —very high vacuum, very high stability of fields, shaping of fields and high stability and coherence of the source—are prerequisites for being able to *demagnify* as well as magnify: to demagnify to the point which we have already achieved of reducing written pictures, at one stroke by direct imprinting, by a factor of 10,000 to 50,000," Fernandez-Moran said at a recent meeting of the Electron Microscope Society of America [SR, Nov '67, 21]. "In principle, this would permit us to put the Library of Congress on a sheet of typewriter paper."

A number of improvements make this possible. First, Fernandez-Moran has been able to develop new short-focal-length lenses that are necessary for demagnification. Second, new grainless films have been developed that permit the reduction of images to 100 Å or less. Third, improvements in electron sources—some of them made in Fernandez-Moran's laboratory—are improving the capability of electron microscopes; point cathode sources are essential, but are not yet developed to the degree needed.

"Some day we must find the equivalent of a laser for electrons," Fernandez-Moran says, "a highly coherent, spatially and temporally coherent source, just as we have for light."

THE SCIENTIST AS PHILOSOPHER

The notion that the practice of science is an art and that the true scientist is an artist is fundamental to Fernandez-Moran. "The electron microscope is the future's decoder of life," he has said. "But first the scientist must be able to understand life, and to do this he must live. For a scientist to lock himself in a laboratory is not to practice his art.

"Science is the most exciting avenue of knowledge open to man. It is the heights and depths of life and the study of life. But in science, as in anything else, one should not have too many 'musts' and 'don'ts.' It has to be a spontaneous phenomenon."

While immersed in the myriad detailed facets of electron microscope technology, Fernandez-Moran never loses sight of the larger patterns of things. For him, the electron microscope is not an end in itself but a tool that gives man entry into the molecular domain, and biophysics is the application of such tools as the electron microscope "to learn more about the organization of cells—about what makes us tick.

"The coherence of my scientific quest is really that I set out as a biologist and became interested in the brain as the ultimate in the liaison between body and mind. I soon found that this incredibly complex system is really built at the molecular level, below the microscopic level. So in order to enter into this area, I had to fashion the tools. This was a challenge that both fulfilled me from the point of view of what I wanted, and fulfilled my desires for gadgetry."

In the preface to his as-yet unpublished book, "Elements of Biomolecular Organization," Fernandez-Moran succinctly develops the premise for his interest. "Biological ultrastructure and molecular organization are considered to be the central unifying disciplines of the natural sciences. The structural orderliness, which in many cases is a crystalline or paracrystalline orderliness, has been shown by electron microscopy to extend throughout all hierachies of organization from the cell to the subcellular level, and beyond this into the domain of macromolecular assemblies.

"This new conception represents a genuine upheaval of biological thought. And it marks the disappearance of what may be called the colloidal conception of vital organization. By means of electron micrographs, the field of submicroscopic cell organization and molecular biology is suddenly gaining a life of its own."

THE SCIENTIST AS PATRIOT

Fernandez-Moran comes by his love of country naturally: his father was a state governor in Venezuela and, one of his ancestors (General Moran) fought with Bolivar. "I feel very proud of having been born in Venezuela," he says. "But, as a result, my life has been a sort of dialogue between the pride of having been born there and the tremendous obligation. If you are born as a scientist in a community that has already paid its debt to history, you can concentrate on doing modern science; but I have always felt a deep sense of responsibility to Venezuela, felt that I must be more than just a scientist—that it should be my task to contribute what little I could to the country of my birth. Not out of sentimentality, but out of love.

(continued)

Meet U. Of C.'s Renaissance Man

By Richard Lewis

Dr. Humberto Fernandez-Moran, physician, biologist, microscopist and inventor, is a scientist who works in two worlds and resides in two hemispheres.

In this quadruple mode, one of his worlds is the distant realm of the atom. He probes it with advanced electron microscopes at the University of Chicago.

He does not seek to split atoms but merely to see how they arrange themselves in molecules, particularly molecules of deoxyribonucleic acid (DNA) which form the thread of life.

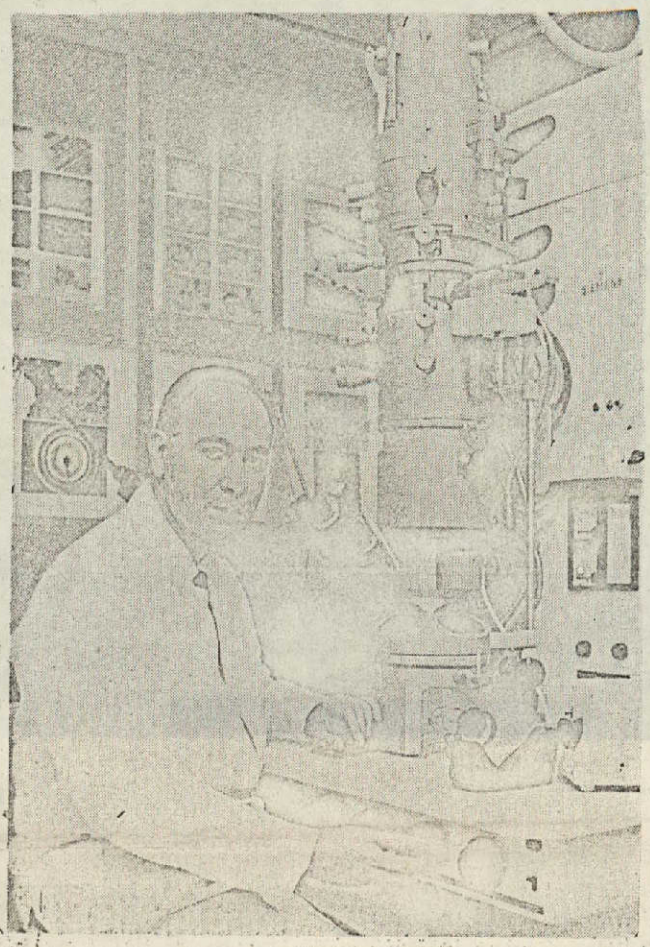
To this end, he has developed the electron microscope to the point of resolving an object only two atoms in diameter.

He hopes eventually to be able to bring into view for the first time a single atom.

With such enormous magnification, it becomes possible to read the genetic code in units of the actual chemical "letters" or nucleotides that make up the living gene.

The outer world of Fernandez-Moran covers a good deal of the globe and extends beyond into outer space.

He has been a pioneer in creating educational opportunities in his native Venezuela.



Dr. Humberto Fernandez-Moran works with electron microscope at the Fermi Institute at the University of Chicago. (Sun-Times Photo by Duane Hall)

CITYSCAPE

Under a space agency contract, he is developing a portable microscope for Project Apollo astronauts.

It is not designed to let them see microbes on the moon, but to read out miniaturized engineering documents and charts in an information storage and retrieval system he has designed.

Typewriter Period Size

By revising the microscope in his laboratory, the scientist can reduce 25 printed pages, each 8½ by 11 inches, to fit in a space no larger than a typewriter period.

In this manner, the Apollo spacecraft's voluminous engineering manual can be reduced to the size of a glass slide and read with a microscope. The system saves weight and space.

With such slides in his pocket and the portable microscope in his boot, where it is designed to be carried, the astronaut can take a whole library to the moon, including all the engineering and navigation documents that pertain to the mission.

In "Who's Who in America," Fernandez-Moran lists an interhemispheric address. His office is the Department of Biophysics, 5640 S. Ellis, Chicago, 60637. Home is Apartado 362, Maracaibo, Venezuela. He resides here at 5807 S. Dorchester.

At age 44, Fernandez-Moran comes as close to the image of the Renaissance Man as one might find on the Midway.

He is the product of three cultures — North and South American and European. Having served Venezuela as diplomat and public official, he bridges the gap between science and government. Some of his friends believe he should run for president of Venezuela but he brushes away the idea. His mission is science and education.

As a neurologist, biologist, biophysicist and, incidentally, a good mechanic, he displays a wide range of skills. Graduate students come to work in his laboratory from Europe and South America and he can speak to them in their own languages. He is fluent in Spanish, Portuguese, German, Swedish, Norwegian and French. His English is plain Chicagoese.

Generalist Among Specialists

Fernandez-Moran is a medium-sized, compact man with a somewhat flattened nose from student boxing in Germany. He likes to jog in the morning for exercise. He displays the energy of a fully charged battery, and his aides say he never runs down.

Born in Maracaibo, he received his elementary schooling there and in New York. In his twenties, he acquired two doctor of medicine degrees, from the University of Munich and the University of Caracas. He took a master of science degree in cell biology and a doctor of philosophy degree from the University of Stockholm.

A passionate booster of the future of South America, Fernandez-Moran has served Venezuela as diplomat, scientific representative abroad, administrator and educator. For 10 days in 1958, he was minister of education in the regime of Gen. Marcos Perez-Jiminez, who was ousted by a coup d'etat.

Fernandez-Moran then went to Boston where he worked at Massachusetts General Hospital in neurosurgery, lectured in biology at the Massachusetts Institute of Technology and did research at the same time at Harvard.

In 1962, he joined the faculty of the U. of C. where he is a generalist among many specialists, a man of all sciences, at the present engaged in developing electron microscopy.

Fernandez-Moran is also an inventor. He has developed a diamond knife so tiny and sharp that it can cut a molecule of starch into sugar. The blade can slice specimens as thin as 50 atoms.

Fernandez-Moran developed the technique for making the diamond knife at the Nobel Institute of Physics in Stockholm. It took him 10 years to discover how to put an edge on the blade that would cut starch or steel. He holds U.S. Patent No. 3,060,781 on it but gives the blades away to scientific institutions after he makes them.

Fernandez-Moran is intrigued with the possibilities of micro-miniaturization, the reduction to the very small. Aside from information storage and retrieval, the process has enormous potential, he believes, for electronics.

In his dustless, air-conditioned laboratory, Fernandez-Moran is administrator, researcher and mechanic. He switches from one to the other with almost electronic speed, just as he does from English to Spanish, or to German or Swedish.

Essentially, the scientist regards his laboratory as an observatory of the microcosm. He calls it a Pico Observatory.

In the language of the metric system, the term "micro" refers to one part in a million. Beyond this, the term "nano" refers to one part in a billion, and beyond this, "pico" to one part in a trillion.

"If you want to catch fish," said Fernandez-Moran, "you have to use a net with a mesh that is smaller than the fish. Light provides us with a net with mesh that is far too big for what we want to catch. We have to use another net."

The other net, of course, is the electron, the negatively charged component of the atom. An electron is 100,000 times shorter than the shortest wave length of visible light.

"With electrons," said the microscopist, "we have a net to catch the fish we want. The smallest fish."

While it is 30 years old, the electron microscope, which illuminates the target material with an electron beam instead of a beam of light, has a long way to go to reach its full potential.

That is the premise on which Fernandez-Moran's electron microscope laboratory is based. He is experimenting with ideas for improving resolution.

Wiggles Eliminated

By immersing the working parts of the electron microscope in a tank of liquid helium at 450 degrees below zero Fahrenheit, Fernandez-Moran said, he can create a lens that is absolutely rigid. In this super-cold medium, the infinitesimal "wiggles" of the magnification system are eliminated — and so is much of the distortion in the instrument.

The requirements are no less exacting than those for the huge radio telescopes, which must be shielded even from auto ignition.

There are nine electron microscopes in the laboratory. Each is fed by current that does not vary by as much as one part in one million. To achieve this, Fernandez-Moran has arranged to get electric power from a specially designed generator. Its voltage output is regulated and refined by a computer.

To eliminate vibration, the electron microscopes are mounted on concrete slabs which are suspended by springs in floor wells. There are other precautions:

The air conditioner controls temperature and humidity with high precision. Ventilation ducts are made of nonmagnetic, stainless steel. Incandescent rather than fluorescent lamps are used to reduce electromagnetic interference.

All electric wires, appliances and switches are shielded. Dust is minimal. Even the doors are dust-proofed. They swing on ball bearing hinges to prevent metal dust from being generated by the friction of standard hinges.

Under ideal conditions, Fernandez-Moran and his aides can achieve 2,500,000 magnifications with the cyrogenic (super cold) electron microscopes. He is confident he is shaping a powerful tool in man's quest to see the basic organization of living cells.

"It represents the ultimate, direct extension of the human eye and hand," he said. "It enables one to poke a finger into atomic dimensions. We may find new capabilities of seeing and doing in the microcosmic domains which still elude the mind's grasp."