N E U R O S C I E N C E S R E S E A R C H P R O G R A M
280 Newton Street, Brookline, Massachusetts, 02146

Bulletin

Volume I | October 1963 | Number 1

C O N T E N T S

This periodical was edited by Theodore Melnechuk and Delphine Tenney, with the technical assistance of the other members of the Neurosciences Research Program Center Staff: Francis O. Schmitt, Chairman, and Joan Morris, Millicent Taylor, Harriet Schwenk, and Janet Carew. The Neurosciences Research Program is supported by a National Institutes of Health Grant, No. GM 10211-02, and a National Aeronautics and Space Administration Grant, No. NsG 462. Funds from other sources listed elsewhere in this issue are administered by Massachusetts Institute of Technology and by the Neurosciences Research Foundation, Inc.

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280 Newton Street, Brookline, Massachusetts, 02146

Bulletin

A PERIODICAL CIRCULATED TO NRP ASSOCIATES AND STAFF MEMBERS AND TO A LIMITED NUMBER OF OTHER INVESTIGATORS, LIBRARIANS, EDITORS, AND WRITERS IN NEUROSCIENTIFIC FIELDS OF RESEARCH.

Volume II January-February 1964 Number 1

C O N T E N T S

This issue was edited by Theodore Melnechuk, Paul Wankowicz, and Delphine Tenney, with the technical assistance of the other members of the Neurosciences Research Program Center Staff: Francis O. Schmitt, Chairman, and L. Everett Johnson, Millicent Taylor, and Harriet Schwenk. The Neurosciences Research Program is supported in part by National Institutes of Health Grant, No. GM 10211-02, and a National Aeronautics and Space Administration Grant, No. NsG 462. Funds from other sources are administered by Massachusetts Institute of Technology, and by the Neurosciences Research Foundation, Inc.

Neurosciences Research Program

Bulletin

A PERIODICAL PRODUCED BY THE NRP COMMUNICATIONS STAFF AND CIRCULATED TO NRP ASSOCIATES, FELLOWS, AND CORRESPONDENTS AND TO A LIMITED NUMBER OF OTHER INVESTIGATORS, LIBRARIANS, EDITORS, AND WRITERS IN NEUROSCIENTIFIC FIELDS OF RESEARCH.

Volume II | March-April 1964 | Number 2

C O N T E N T S

E N C L O S U R E S

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SPONSORSHIP AND SUPPORT

The Neurosciences Research Program is sponsored by the Massachusetts Institute of Technology. It is supported in part by National Institutes of Health Grant No. GM 10211-02; National Aeronautics and Space Administration Grant No. NsG 462; The Rogosin Foundation, and Neurosciences Research Foundation, Inc.

NRP Brochure 63-4

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INFORMATION ABOUT THE CONCEPT, MEMBERSHIP, PROGRAM, AND SPONSORSHIP OF THE NEUROSCIENCES RESEARCH PROGRAM

THE CONCEPT OF THE NEUROSCIENCES RESEARCH PROGRAM

The approach of the Neurosciences Research Program to understanding the mechanisms and phenomena of the human mind applies and adapts the revolutionary advances in molecular biology achieved during the postwar period. The breakthrough to precise knowledge in molecular genetics and immunology -- "breaking the molecular code" -- resulted from the productive interaction of physical and chemical sciences with the life sciences. It now seems possible to achieve similar revolutionary advances in understanding the human mind. A wealth of research literature on the mind stems from the classical approaches of physiology and behavioral sciences. By making full use of these approaches and by coupling them with the conceptual and technical strengths of physics, chemistry, and molecular biology, great advances are foreseeable.

This concept led 27 eminent scientists in some 15 disciplines and from 23 universities and research institutions here and abroad to form a cooperative Neurosciences Research Program. Begun in early 1962, under the chairmanship of Francis O. Schmitt, Institute Professor at Massachusetts Institute of Technology, this program joins the talents and creative resources of 17 senior science professors and 14 directors of science departments and research institutions. All are distinguished and productive investigators in their fields of specialization; but more important, they share a goal in common -- to bring the insights of their special fields to bear on the multifaceted problem of human brain action.

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FUNCTIONS OF THE NEUROSCIENCES RESEARCH PROGRAM

From its inception early in 1962, the Neurosciences Research Program has enjoyed a minimum of formal organization. The basic concept, indeed, implies a maximum of decentralized research in the home institutions of the Associates. Yet, a centralizing framework is essential to maximize the opportunities for productive interaction among the Associates.

To assure a strong "drawing together" of the creative research potential of the widely dispersed group, and its enrichment by the most authoritative reviews of the state-of-the-art of research in their own and in other relevant disciplines, three interdependent measures are underway:

1. Neurosciences Research Center

An essential means of providing coordination within the Neurosciences Research Program is the Center established in Brookline, Massachusetts. With excellent facilities made available in the House of the American Academy of Arts and Sciences, the Neurosciences Research Center operates to serve the Program under the guidance of the Chairman, Dr. Francis O. Schmitt. Responsible for coordinating the development of plans and the production of publications is the Communications Director, Mr. Theodore Melnechuk, formerly senior Associate Editor of International Science and Technology.

The Center performs many necessary administrative and housekeeping functions for the Program, but its prime purpose is to serve the scientific activities of the Principal Investigator and the other Associates. This it does in three general ways:

a. As a facility, the Center provides a meeting place to which the Associates foregather in regular and special scientific meetings. The Center includes lecture and conference facilities, a specialized neurosciences library and information service, office spaces, and an "Associates' Lounge" for quiet study, contemplation, and relaxation. Associates are free to use the Center at any time, either singly or in groups.

b. The Center's information and publication services provide media for timely exchange of relevant information among the Associates and between this Program and its various deserving audiences.

c. The Principal Investigator and the Communications Director are recruiting a small, highly select staff to assist them in the most essential functions of the Program -- that of analyzing and integrating the scientific developments in the broad interdisciplinary fields of the neurosciences. This staff will maintain close contact with Associates and will help mediate the constant interactions that will allow the Program to achieve the most rapid and productive synthesis of results.

2. The Neurosciences Intensive Study Program

Scheduled for the summer of 1965, this program will entail extensive preparatory meetings and commitments leading to the convening of about 100 scientists for about a month of intensive lectures, discussion, and workshops. From this program will emerge a definitive volume intended to have widespread and enduring effect.

3. Neurosciences Research Meetings

During the first 18 months of the Program, six scientific meetings have been held with about three-fourths of the Associates present at each meeting. Many guest lecturers have given intensive coverage to subjects of great pertinence. The schedule allows extensive free discussions and creative interaction.

At the end of the first year, the First Convocation of the Neurosciences Research Program was held as a means of conveying to a broad assembly of friends and sponsors a synthesis of the scientific problems and challenges in the cooperative studies of the molecular and bio-systems bases of mind and memory.

In addition to the Stated Meetings of Associates and guests, several regional meetings, called Work Sessions, have been held on specialized topics to encourage more rapid progress in these component fields.

REPORTS DELIVERED AT NEUROSCIENCES RESEARCH PROGRAM MEETINGS

In the year and a half from its Second to its Sixth Stated Meetings (March 1962 -- July/August 1963), the Neurosciences Research Program occasioned 55 reports to its Associates.

Of these, five were reports of Work Sessions, which are open-ended seminars on subjects relevant to the Program, chaired by an Associate, but attended by 10 or so authorities who are not usually Associates. The remainder included two other kinds of reports: Tutorial Lectures and Research Lectures on relevant work by invited guests and Associates.

The 55 reports generally covered the background, materials and methods, results, and significance of the structure, functions, and/or interactions of components and/or systems at the following levels of organization:

SUBMOLECULAR

Structure

EIGEN, M. (1962, August)
"Physiochemical Considerations: Temporal Limitations on Chemical Information Storage"

KASHA, M. (1963, July)
"The Possibility of Molecular Ultrastructure Determination in Biological Lamellar Systems by the Exciton Model"

Function

PHILLIPS, W. D. (1963, March)
"Possible Mechanisms of Fast Readout of Information Stored in Neural Macromolecular Systems"

SCHMITT, O. H. (1963, July)
"Potential Interaction between Microstructure and Fields (Electric and Electromagnetic)"

MOLECULAR

Structure

AUGENSTEIN, L. G. (1962, August)
"Physiochemical Considerations: Role of Molecular Conformational Changes"

LEHNINGER, A. L. (1963, February)
"Molecular Assemblies as Units of Cell Structure and Function: Cellular 'Componentry' in Terms of Structured Complexes of Enzymes"

Function

OCHOA, S. (1962, March)
"Coding of Protein Synthesis"

ROBERTS, R. B. (1962, March)
"Original Theory of Macromolecular Memory Coding"

ROSENBERG, B. (1962, August)
"Physiochemical Considerations: Electronic Conductivity in Wet Proteins; Photoconductivity in Visual Pigments"

DAVISON, P. F. (1963, February)
"Molecular Coding in Biogenesis: Mechanisms of Inheritance and Control of Biosynthesis and Development Explained in terms of the Interaction of Molecules"

Interactions

DAVISON, P. F. and SCHMITT, F. O. (1963, March)
"Molecular Neurology; Its Origins in Molecular Biology and its Potential Role in the Neurosciences"

ORGANELLE

Structure

FERNANDEZ-MORAN, H. (1962, March)
"Membrane Ultrastructure"

LEHNINGER, A. L. (1962, March)
"Biochemistry of Mitochondria"

FERNANDEZ-MORAN, H. and LEHNINGER, A. L. (1962, August)
"Membranes and Solid State Assemblies of Protein (Enzyme) Molecules; Projection of Possible Situation in Neuronal and Glial Membranes"

SCHMITT, F. O. (1962, August)
"Neurofilaments and Myelin"

FERNANDEZ-MORAN, H. (1963, February)
"Cell Organization: The Molecular Organization of Cell Constituents as Revealed by Electron Microscopy"

BULLOCK, T. H. and GRUNDFEST, H. (1963, July)
"Graded Potentials on Cell and Synaptic Membranes"

LEHNINGER, A. L. (1963, July)
"Neuronal Membranes"

Function

HYDEN, H. V. (1962, August)
"Biosynthesis of RNA and Proteins in Neurons and Glia in Relation to Long-term Memory and Learning: As Determined from Micro-analyses of Cells Isolated from the Brain"

ROBERTS, R. B. (1962, August)
"Biosynthesis of RNA and Proteins in Neurons and Glia in Relation to Long-term Memory and Learning: As Determined by the Use of Metabolic Inhibitors and Analysis of Brain Samples"

Interactions

KATCHALSKY, A. (1963, July)
"Irreversible Thermodynamics of Membrane Processes"

NEURON (or Other Cell)

Structure

PALAY, S. L. (1962, August)
"Electron Microscopy of Neurons and Glia"

LOWRY, O. H. (1962, November)
"Chemical Study of Individual Nerve Cells"

PALAY, S. L. (1963, February)
"The Organization of Brain Cells: Internal Organization as Machinery for the Specific Mode of Operation of Brain Cells"

Function

BULLOCK, T. H. (1962, August)
"Electrical Transduction, Electrosensing, and the Possibility of Amplifier Action in Sensory Cells of Electric Fish; Infrared Sensing in Snakes; Sensing in the Far Infrared and Microwave Region"

SCHMITT, F. O. (1963, July)
"Bioelectric Phenomena in Nerve"

STRUMWASSER, F. (1963, July)
"Clock-Type Phenomena in the Aplysia Neuron and in other Neurons"

Interactions

CORNING, W. C. (1962, August)
"Invertebrates, Protozoa, and Bacteria as Material for Investigation of the Macromolecular Coding Theory: Critical Review of Planarian Experiments"

BULLOCK, T. H. (1962, August)
"Invertebrates, Protozoa, and Bacteria as Material for Investigation of the Macromolecular Coding Theory: Arthropod Possibilities"

HYDÉN, H. V. (1962, November)
"Glia RNA Changes in a Learning Experiment"

WEISS, P. A. (1962, November)
"Neuronal Dynamics; the Concept of Perpetual Neuronal Growth and Proximodistal Substance Convection"

HYDÉN, H. V. (1963, March)
"Glia-Neuron Response in a Learning Situation"

CHALAZONITIS, N. (1963, July)
"Photochemical Effects on Bioelectric Phenomena in Giant Neuron of Aplysia"

BRAIN (or Other Organ)

Structure

NAUTA, W. J. H. (1963, February)
"The Brain as a Network of Neuronal Components: Structural and Functional Hierarchies Within the Neuronal Net"

SCHMITT, F. O. (1963, February)
"The System-Component Dilemma; Operational Hierarchies Fundamental in the Functioning of Cells and of the Brain: Organism-Cell; Cell-Molecule -- Brain Neuron; Neuron-Molecule"

Function

ADEY, W. R. (1963, March)
"Studies of Cerebral Transaction Mechanisms by Impedance Measurements and Related Techniques"

GALAMBOS, R. (1963, February)
"Memory, Inherited and Acquired: What is Known and Inferred about Brain Processes Responsible for Memory"

GRUNDFEST, H. (1963, July)
"Structure and Function of the Electric Organ"

REICHARDT, W. (1963, March)
"The Transformation of Optical Information in the Limulus Compound Eye"

ROBERTS, R. B. (1962, August)
"Possible Use of Averaging Computer Techniques in Encephalography"

SHAW, T. (1963, July)
"Fundamental Bioelectric Phenomena"

Interactions

SCHMITT, F. O. (1963, February)
"Molecular Biology and Brain Function: The Role of Dynamic Molecular Organization of Brain Cells in Memory Processing and Learning"

CENTRAL NERVOUS SYSTEM (or Other System)

Structure

NAUTA, W. J. H. (1962, August)
"Organization of the Central Nervous System"

Interactions

WEISS, P. A. (1962, November)
"Characterization of Developmental Neurology"

ORGANISM (i.e., Behavioristically)

Function

DAVISON, P. F. (1963, March)
"Relationship between RNA Administration and Memory Deficit in Senile Patients"

ELKES, J. (1963, March)
"Behaviorally Active Amines; Some Problems and Approaches"

HOAGLAND, H. (1963, March)
"Studies of a Plasma-protein Complex in Relation to Schizophrenia"

KETY, S. S. (1963, February)
"Chemical Correlates of Behavior: Aspects of Behavior and Brain Function as Revealed by the Action of Metabolites and Psychotropic Drugs"

PERSON (i.e., Subjectively)

Interactions

KETY, S. S. (1962, November)
"Relationship between Brain Chemistry, Subjective State and Behavior"

INTERPERSONAL (e.g., Communication)

Interactions

JAKOBSON, R. (1962, August)
"Phonemes as Linguistic Code; Possible Relation to Molecular Informational Code"

MELNECHUK, T. (1963, July)
"NRP Communications"

SHANNON, C. E. (1963, March)
"Aspects of Information Theory"

SCHMITT, F. O. (1963, February)
"NRP Rationale"

SPONSORS OF THE PROGRAM

The Massachusetts Institute of Technology agreed to act as initial sponsor of the Neurosciences Research Program to facilitate its early organization and is quite willing to continue its present role until a sponsor, independent of any one of the Associates' institutions, can assume full responsibility.

The first step toward independent sponsorship was taken in 1962 with the incorporation of the Neurosciences Research Foundation in the Commonwealth of Massachusetts. This Corporation, wholly dedicated to the encouragement and support of the Neurosciences Research Program, is in process of establishing its tax-exempt status so that it can assume full sponsorship through receiving grants and donations for support of the Program.

The Academy of Arts and Sciences has contributed valuable sponsorship through granting a five-year lease for the Neurosciences Research Center. The Academy has also borne the costs of renovating the quarters now being used by the Center.

The Brandegee Charitable Foundation, as Trustees of the estate of Mrs. Edward (Mary B.) Brandegee, has made the estate home available to serve as the House of the American Academy of Arts and Sciences, in which the Neurosciences Research Center is located. The Foundation has also agreed to permit construction on the estate grounds of an additional building for the Neurosciences Research Program. This new building is conceived as a multipurpose structure to provide a large meeting room, offices, and residence facilities for the use of the Associates and guests who desire to stay and work at the Center for varying periods of time.

FINANCIAL SUPPORT

The Neurosciences Research Program gratefully acknowledges the financial support of the National Institutes of Health, through Grant No. GM 10211-02, and of the National Aeronautics and Space Administration, through Grant No. NsG 462.

Grateful acknowledgment is also made for the receipt of unrestricted funds in support of the general program from the following donors:

Cabot Foundation

David C. Crockett

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Albert and Mary Lasker Foundation

Louis and Eugenie Marron Foundation

Permanent Charity Fund

Rogosin Foundation

NRP Editorial 63-1

N E U R O S C I E N C E S R E S E A R C H P R O G R A M
280 Newton Street
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Area Code 617-522-6700

Here is the prototype issue of a new monthly publication intended primarily for the Associates and Staff of the NRP but available, within reasonable limits, to other people likely to find it useful, such as scientists, writers, editors, and librarians engaged in research and communication in the classical and molecular neurosciences. (See "NRP Communications Policy.")

Probably few issues will be as thick as this one, which contains a necessarily large amount of background material (e.g., "Information About ... the NRP") and of back-log material (e.g., the major part of the "Accessions List").

Future issues will merely update those features, concentrating rather on up-to-date Reports or on Discussion Papers like the two in this issue -- original work inspired by, or relevant to, the scientific research of the NRP. (See "On the Nature of Macromolecular Coding in Neuronal Memory" and "Immunologic and Psychic Memory.")

Your comments and inquiries are welcome. --T.M.

NRP Editorial 64-2

ON OUR NEW SERIES OF NRP WORK SESSION REPORTS

STARTING WITH THIS ISSUE, each NRP Bulletin is to contain at least one NRP Work Session Report.

The first of the series covers the NRP "Cell Membrane" Work Session held here last summer. Now in preparation are reports on such more recent Work Sessions as those on "Some Brain Structures and Functions Related to Memory," "Mathematical Concepts of Central Nervous System Function," and "Specificity in the Neurosciences."

Though these forthcoming reports will differ in structural detail from the prototype in this issue, they will have the same aim: to help the reader mine the gold in the ore represented by the literature of their subjects. NRP Work Session Reports will therefore differ from the familiar full transcripts of symposia in being more succinct, through stressing selection, summarization, interpretation, and evaluation. And checking the manuscript of each report with the Work Session participants just before its publication should ensure the accuracy and currency of its facts and ideas.

To work with Work Session participants, their chairmen, NRP Associates, and NRP Fellows on the task of gleaning a harvest of ideas from NRP Work Sessions, our new Communications Staff has grown to include two additional staff editors, Miss Anne S. Harris and Miss Catherine M. LeBlanc and a technical typist, Miss Jane Wilson. We welcome their enlistment in our interdisciplinary cause.

A word on the enclosures. In the envelope containing this issue you should find enclosed a reprint and a brochure. These contain respectively a rationale and a description of the NRP, the latter obsolescent only in its list of staff members. -- T.M.

NRP Guide 64-1

N E U R O S C I E N C E S R E S E A R C H P R O G R A M
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GUIDE TO RECENT SUMMARIES OF NEUROCHEMISTRY

An NRP Staff collation
of a table and list prepared by

Eugene Roberts and Claude F. Baxter

Department of Biochemistry
City of Hope Medical Center
Duarte, California

NOTE: This guide has been adapted from Table II, pp. 543-545, and the list of literature cited, pp. 546-552, of "Neurochemistry," a survey of the literature completed in September 1962 and published in Annual Review of Biochemistry, Vol. 32, 1963. We suggest that our readers obtain the original volume and review article, which is recommended by competent NRP Associates. It is hoped that forthcoming volumes will update and revise the material in "Table II," which was described in the original as "intended to serve as a guide to some of the areas of neurochemistry which have been summarized recently in books, symposia, or review articles. It is hoped that the reader will find the material suitable for orientation and as a starting point for obtaining references to the literature prior to 1962."

	Historical Background	Chemical Architecture. Histochemistry and Developmental Changes	Nerve Activity: Excitation Inhibition and Transmission	Barriers, Fluids, and Transport Phenomena	Ionic Movement, Electrolytes and Minerals	Mineral Metabolism	General Metabolism	Carbohydrates and Carbohydrate Metabolism	Nitrogen Metabolism Amino Acids, Peptides and Proteins	Nucleotides and Nucleic Acids	Lipids	Biogenic Amines, Indoles and Acetyl Choline	Vitamins and Cofactors	Hormones	Pharmacology and Toxicology	Diseases of the Nervous System	Biochemical Lesions (Hereditary Disorders)	Nutrition and Tissue Culture	General Chemistry, Surveys, and Methodology	Avant-garde Reading
1962 Elliott, K. A. C., Page, I. H., and Quastel, J. H., Eds., *Neurochemistry*, 2nd ed. (Charles C Thomas, Publisher, Springfield, Ill., 1035 pp. 1962)	3–9	10–84	212–225 522–557 657–727	376–430	226–237 558–577		113–225	150–211 238–266	276–375 636–656	331–375	276–287 870–896	431–521 578–635	267–275	954–976	728–869	694–727 897–929	977–1012	930–953			
Fazekas, J. F., and Alman, R. W., *Coma, American Lecture Series* (Charles C Thomas, Publisher, Springfield, Ill., 114 pp., 1962)																all					
Holden, J. T., Ed., *Amino Acid Pools* (Elsevier Publ. Co., Inc., Amsterdam, 815 pp., 1962)									449–511 545–563												
Jacob, H., Ed., *Histochemistry and Biochemistry of the Diseases of the Central and Peripheral Nervous System* (Georg Thieme Verlag, Stuttgart, 256 pp., 1962)		all							all		3–62					all					
Jacob, H., Ed., *Electronmicroscopy of the Central and Peripheral Nervous System: Biology and Culture of Nervous Tissue* (Georg Thieme Verlag, Stuttgart, 305 pp., 1962)		all														all					
Kasha, M., and Pullman, N., Eds., *Horizons in Biochemistry* (Academic Press, Inc., New York, 604 pp., 1962)																				all	

	Historical Background	Chemical Architecture, Histochemistry and Developmental Changes	Nerve Activity: Excitation Inhibition and Transmission	Barriers, Fluids, and Transport Phenomena	Ionic Movement, Electrolytes and Minerals	Mineral Metabolism	General Metabolism	Carbohydrates and Carbohydrate Metabolism	Nitrogen Metabolism Amino Acids, Peptides and Proteins	Nucleotides and Nucleic Acids	Lipids	Biogenic Amines, Indoles and Acetyl Choline	Vitamins and Cofactors	Hormones	Pharmacology and Toxicology	Diseases of the Nervous System	Biochemical Lesions (Hereditary Disorders)	Nutrition and Tissue Culture	General Chemistry, Surveys, and Methodology	Avant-garde Reading
McIlwain, H., and Rodnight, R., *Practical Neurochemistry* (J. & A. Churchill, Ltd., London, 296 pp., 1962)																			1–296	
Pope, A., and Hess, H. H., in *Metabolism of the Nervous System*, 72 (Pergamon Press, Ltd., London, 1957) [sic]			all																	
Schmitt, F. O., Ed., *Macromolecular Specificity and Biological Memory* (Mass. Inst. Technol. Press, Cambridge, Mass., 119 pp. 1962)																				all
Terzuolo, C. A., and Edwards, C. *Ann. Rev. Physiol.*, 24, 325 (1962)			all																	
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Abstr. Intern. Symp. Enzymic Activity of the Central Nervous System, Göteborg, Sweden, 1962, Acta Neurol. Scand., Suppl. 1, 38 (1962)		42–56	57–68						15–31		32–41					69–83				
1961 Brodie, B. B., Sulser, D. F., and Costa, E., *Ann. Rev. Med.*, 12, 349 (1961)															all					

	Historical Background	Chemical Architecture, Histochemistry and Developmental Changes	Nerve Activity: Excitation Inhibition and Transmission	Barriers, Fluids, and Transport Phenomena	Ionic Movement, Electrolytes and Minerals	Mineral Metabolism	General Metabolism	Carbohydrates and Carbohydrate Metabolism	Nitrogen Metabolism Amino Acids, Peptides and Proteins	Nucleotides and Nucleic Acids	Lipids	Biogenic Amines, Indoles and Acetyl Choline	Vitamins and Cofactors	Hormones	Pharmacology and Toxicology	Diseases of the Nervous System	Biochemical Lesions (Hereditary Disorders)	Nutrition and Tissue Culture	General Chemistry, Surveys, and Methodology	Avant-garde Reading
Dobbing, J., *Physiol. Rev.*, **41**, 130 (1961)				all																
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NRP News & Views 64-1

N E U R O S C I E N C E R E S E A R C H P R O G R A M
280 Newton Street
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N E W S A N D V I E W S

PHILIP ABELSON, in "Trends in Scientific Research," Science, Vol. 143, No. 3603, (January 17, 1964) p. 222:

"Perhaps the greatest research frontier of our times is investigation of the human mind and the way it functions. There is increasing evidence that very fine scientists from many fields are beginning to concentrate on this problem. The area seems difficult, but its importance guarantees first-class efforts. Related to the study of the mental process is work in the behavioral sciences. It would be bold to suggest that this field can be conquered by laboratory science, but major contributions will be made and tools such as electronic devices and computers will be helpful."

NORBERT WIENER, in "Dynamical Systems in Physics and Biology," New Scientist, No. 375, (January 23, 1964) p. 211:

"It is becoming abundantly clear that the nucleic acid complexes not only play a fundamental role in genetic memory, but that they probably play an analogous role in nervous memory ... and we shall have to consider the interplay of what Professor Francis Schmitt of MIT calls "dry" neurophysiology, dealing with the established nervous network, and "wet" physiology, which is going to center more and more about the nucleic acids."

WATSON DAVIS, in "Neglected Scientific Areas," Science Newsletter, Vol. 85, (January 11, 1964) p. 19:

"MECHANISMS OF MENTALITY -- The broad sweep of the training and remodeling of the human intellect, from the cure and prevention of mental illness to faster and more effective education at all levels, needs a major research effort. People are suspicious of such efforts because of

"their preconceptions and prejudices, but newer research in psychology and psychiatry points to bold and daring experiments that could revolutionize the operation of human intellect and emotion. We might even learn the ways of keeping peace in the world."

THE EDITORIAL OFFICES of Experimental Neurology, an Academic Press publication, were moved in January from the National Institutes of Health, Bethesda, Md., to New York. The change was necessitated by the transfer of the journal's editor, William F. Windle, formerly chief of the Laboratory of Perinatal Physiology, NIH, to the New York University Medical Center, where he has been appointed research professor in the Institute of Physical Medicine. Communications to the magazine should be addressed to Dr. Windle at the Medical Center, 400 East 34th Street, New York, 10016.

ACCORDING TO THE secretary-general of the International Union of Scientific Psychologists, the 2nd edition of the International Directory of Psychologists, which is being compiled under a National Science Foundation grant, will be published in September 1964.

THE NATIONAL LIBRARY of Medicine, which has awarded a grant to Fordham University to compile a Directory of Medical and Biological Research Institutions in the USSR, expects the manuscript to be completed in about six months. Publication is to occur shortly thereafter. The directory will include a description of the organizational structure of Soviet scientific activity in medical and allied fields, and a listing of schools, libraries, societies, and pertinent publications.

Readers are invited to use this column for announcements.

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NRP Editorial 64-1

N E U R O S C I E N C E R E S E A R C H P R O G R A M
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LET ME BEGIN by correcting an error of transcription made in the letter from Heinrich Waelsch printed in the November-December issue of this Bulletin. The fourth sentence of the first paragraph on the second page, following the question "How could such a change in threshhold for a stimulus be visualized?", should read: "One might suggest that the K and Na activated ATPase which appears to be involved in the transport of K and Na may affect conformational changes in the membrane proteins which depending on the presence of certain other proteins might provide intraprotein stabilization. (I favor the hypothesis of selection.)"

We regret this error of omission, and thank Dr. Waelsch, who is currently Honorary Executive Secretary of the International Brain Research Organization, for the gracious wit with which he pointed it out. We prefer to evoke from him a comment that is scientific rather than editorial.

Errors should be fewer, hits more frequent, with the expansion of our staff, now underway. Already we have been joined by Mr. Paul Wankowicz, a scientist-engineer turned writer-editor, whom we welcome to our multidisciplinary group. --T.M.

NRP News & Views 64-2

N E U R O S C I E N C E S R E S E A R C H P R O G R A M
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N E W S A N D V I E W S

On Meetings and Organizations

TWO POSTGRADUATE COURSES in brain research will be given in July in Amsterdam, Netherlands. Both courses are intended for physicists, chemists, biologists, physicians, and engineers. One course, on the neuron, is scheduled for July 6-10; the other, on sensory mechanisms, is scheduled for July 13-17. For more information, write Dr. J. P. Schade, Netherlands Central Institute for Brain Research, Mauritskade 59B, Amsterdam, Netherlands.

AN INSTITUTE ON molecular biophysics is scheduled for August 17-28 at Squaw Valley, California. Its co-sponsors, the U. S. Office of Naval Research and NATO, invite international participation, and grants for living or travel expenses are available to both U. S. and foreign applicants.

For more information, write Dr. B. Pullman, Institut de Biologie Physico-Chimique, 13, rue Pierre Curie, Paris 5, France.

A EUROPEAN MOLECULAR BIOLOGY ORGANIZATION was formed in Geneva early in February by a fifteen-member group representing ten nations. EMBO proposes to raise funds for the support of molecular biology in European colleges; for advanced training of scientists; and for sponsorship of joint research proposals among European scientists, possibly leading to the eventual formation of a European Research Institute of Molecular Biology. For more information on EMBO, write its chairman, Dr. M. F. Perutz, Medical Research Council Laboratory of Molecular Biology, Cambridge, England.

NRP ASSOCIATE RICHARD B. ROBERTS is the recently elected president of the Biophysical Society.

FACTS AND HYPOTHESES on the nervous system, and on embryology and differentiation, comprised the main subjects of the annual meeting of the Fellows of the Salk Institute for Biological Research at LaJolla, California, on February 22-26.

Dr. Donald Glaser and Dr. Robert Livingston were discussants, while Dr. Walle J. H. Nauta spoke on the general structure of the mammalian nervous system and Dr. Seymour Kety spoke on three aspects of behavioral neurochemistry: the energy metabolism of the brain and its relation to mental state; biogenic amines and behavior; and neurochemical theories of memory. Dr. Oscar Hechter spoke both on the nature of macromolecular coding in neuronal memory (which NRP Bulletin readers will recall as the title of his Discussion Paper in the October 1963 issue) and on a possible molecular basis for 'polypeptide coding' in the action of polypeptide hormones upon all membrane receptors.

Other speakers and discussants included Dr. Joel Elkes, who helped organize the sessions and who spoke on psychomimetic drugs; Dr. David Hubel, who spoke on central nervous processing of visual information, especially as regards form and color, in cat and monkey and on changes that can be induced in the CNS by altering the normal sensory inflow in immature animals; Dr. J. David Robertson, who spoke on studies of the fine structure of neural tissue; and Dr. Roger Sperry, who spoke on split-brain experiments and on neurospecificity in relation to various problems of behavior and embryonic development.

A copy of the program, which contains abstracts of the talks, may be obtained from Mr. William Glazier, Assistant to the Director, The Salk Institute for Biological Studies, P.O. Box 9499, San Diego, California.

From Publications and Letters

K. A. LANGE, in "Current Problems in the Physiology of the Nervous System," Vestnik Akademii Nauk SSSR (Herald of the Academy of Sciences USSR), No. 9, Moscow, Sept. 1963, pp. 92-94; available in translation for 50¢ as OTS 63-41067 from the Office of Technical Services:

"The Fourth All-Union Conference on Electrophysiology of the Nervous System convened in May at Rostov-on-the-Don. The program of this conference included problems such as the physico-chemical bases of electrical activity, physiology of the neuron, electrophysiological criteria of pharmacodynamics, electrophysiological study of the activity of various divisions of the nervous system, and receptor apparatuses under normal and pathological conditions. A great deal of attention was devoted to the methodology of electrophysiological investigations.

"Approximately 1,000 persons from 30 cities of the country participated in the meeting.

"The conference developed recommendations concerning further application of electrophysiological methods in modern physiological investigations, the development of complex investigation of nerve activity on the molecular, neuron and systemic levels, and the application of modern achievements of electrophysiology in medical practice. Many symposiums and conferences were planned for the next few years on the most pressing problems of electrophysiology, particularly in the investigation of the phenomenon of memory, coding of impulses and transmission of information, the electrophysiology of the neuron, etc."

REPRESENTATIVE EMILIO Q. DADDARIO of Connecticut, Chairman, and other members of the Subcommittee on Science, Research, and Development of the House Committee on Science and Astronautics, in "A Statement of Purpose," their first report on government and science, dated December 2, 1963:

"The product of science is a knowledge and understanding of the ways of the universe--ranging from the forces in the atomic nuclei to the motions of the stars in the galaxies; from the growth of a living cell to the functions of the brain; from the measured responses of man to the dynamics of social organization. The study of such subjects and all the rest of nature is the province of science.

"This Nation recognizes the power and influence of research and development. Research expands man's knowledge and outlook. Scientific knowledge may also be put to use to cure disease or to create new industries. That such knowledge is useful is a matter of faith, justified by history from the first use of celestial navigation to today's application of vaccines and nuclear power.

"Hopefully, these activities will bring benefits ranging from an insight into the most fundamental facts of nature to concrete applications in a variety of areas of national concern."

"In considering the terms of reference of the Science, Research, and Development Subcommittee, it was decided to request comment from the full committee's Panel on Science and Technology. Replies were thoughtful and stimulating, and, among other things, suggested the following:

> (1) More adequate and thorough dissemination and feedback of information derived from scientific research must be achieved to insure that new projects are considered with due awareness of the state of the art.
>
> .....
>
> (10) There should be greater use of cross-departmental groups at the working level in order to improve communication and effectiveness of research."

NRP ASSOCIATE SEVERO OCHOA and MIT Provost Charles Townes were two of the five eminent scientists who were interviewed on January 15 by Charles Collingwood on the CBS television program "Chronicle". They described in layman's language the scientific advances with which they have been intimately involved--respectively, the genetic code and coherent electromagnetic radiation. Single copies of the verbatim transcript, which is suitable for reading by the wives and children of NRP Bulletin readers, are available from Mr. Warren V. Bush, CBS News, 485 Madison Ave., N.Y. 22, N.Y.

PROFESSOR SIR AUBREY LEWIS, Institute of Psychiatry, University of London, in "Changes in Psychiatric Methods and Attitudes," New Scientist, No. 378,(February 13, 1964), p. 423:

"The concepts and terms of psychology will be closer towards fusion with those of neurophysiology, and there is ground for supposing that we shall by 1984 know a great deal about the chemical and electrical happenings in the nervous system which are responsible for our wakefulness, our awareness of hunger, our memory, our sexual behaviour, and many other psychological phenomena.

"Recent studies of drugs directly introduced into the ventricles of the brain suggest further possibilities of concentrated local action."

"Judging from surgery's place now in the treatment of selected cases of temporal lobe epilepsy, and of lesions of the central nervous system or the endocrine system which lead to abnormal behaviour, its future role will depend on the discovery of further such somatic causes of mental illness: these may not be macroscopic, and the techniques of implantation and neuronal stimulation may be called for."

AN AMERICAN STUDENT, in a letter dated February 26, 1964 to a British scientist:

"I am in the eighth grade and about to enter in a local science fair. I am basing my project on the connections between DNA, RNA, and memory. I was inspired to this project by the October 4, 1963 issue of Life magazine in which your photograph appeared. In this issue there was a statement saying RNA can be prepared from yeast and can be administered to an animal causing an improvement in its memory. Is this possible and if so do you or your laboratory know how to make RNA in the prescribed manner? If you can spare information on the production and administration of this substance, please do so and please aknowledge (sic) as soon as possible. Time is crucial. Thank you very much."

LORD BRAIN, in "Knowing Our Minds Better", New Scientist, No. 384, (March 26, 1964) p. 806:

"Considering the complexity of the human mind and brain, twenty years is not a long time for their further unravelment. By 1984, however, we should understand what the brain does when we think. Already the cyberneticists are constructing models of what it may do: one obstacle to knowing how it does work is the extreme complexity of the interrelationships of the nerve cells (10,000 million in number) and our present ignorance of some essential anatomical and physiological details. If, however, what is important is the behaviour of "cell assemblies" in the brain, we may expect to learn a great deal about that without necessarily knowing all we should like to know about the behaviour of individual nerve cells. Already the study of how brain function breaks down in disorders of perception and language is beginning to throw light upon how these functions are organised in the nervous system.

"We may expect, also, to understand the cerebral basis of memory. Already a certain amount is known about its anatomical organisation both in man and animals. We do not know whether, physiologically, it depends upon the organisation of nerve-impulses or reversible molecular changes, or possibly both. Animal experiment may well provide the answer to this, with help from the physicists on the mutual relationship between nerve-impulses and reversible molecular structure in RNA molecules. It would be naive, however, to suppose that "a memory" is stored in "a cell": remembering must involve a complex organisation of activity in space and time through many millions of cells."

C. M. POMERAT, Research Director, Pasadena Foundation for Medical Research, 99 North El Molino Avenue, Pasadena, California, in a letter dated April 2, 1964:

"....We have evolved a method of analyzing the rhythmic contractile activity of oligodendrocytes and Schwann cells, as we see them, with time-lapse, phase contrast cinematographic records using a cadmium sulfide scanner and a 'strip

chart recorder. A paper describing this technique has been accepted by the Journal of the Royal Microscopical Society and a report of rhythmic activity of cells in a culture of an oligodendroglioma has been submitted for publication in the Journal of the National Cancer Institute.

"Currently we are studying the effect of irradiation on nerve tissue following various exposure dosages with gamma, proton and alpha particle energies. We are astounded at the radioresistance of neurons. We have several cine records showing emigration of axons after exposure to 60,000 r from a gamma source."

NRP ASSOCIATE LEROY AUGENSTEIN, in a letter dated April 9, 1964:

"This is just a note to tell you how much I enjoyed the prepublication report (on a hysteresis effect in a polyelectrolyte) by Aharon Katchalsky at our last Stated Meeting of the Neurosciences Research Associates. His report saved us a lot of work and also the purchase of some relatively expensive equipment."

"Recently we observed some unusual anomalies in the emission from nucleotides and polynucleotides when we introduced different ions into the solution. We had almost convinced ourselves that this reflected an unusual solute-solute interaction. After hearing Katchalsky we now realize that the way in which we had treated our solutions prior to the emission studies may have introduced a hysteresis effect comparable to theirs. Before ordering all of the necessary, expensive equipment to track down our original hypothesis, we have now gone back to check if we do have primarily a pH effect. We hope to know fairly soon. In any event, he may have saved us a lot of time and the taxpayers quite a few dollars."

"I hope you will continue to have these kinds of reports, as I am sure they must help others the same way that we were helped in this case."

FOUR RECENT ARTICLES on the molecular basis of learning and memory have come to our attention. They cover a range in level from the popular to the professional.

The most popular is "The Worm Learns," by John Bird (who is clearly eo-ornithic), Saturday Evening Post, March 28, 1964, pp. 65 & 67; a birds' eye view of worm running.

The second, written for a reader of culture, is by NRP ASSOCIATE Aharon Katchalsky. Called "Molecules and the Psyche," it leads off the Winter 1963 issue of Rehovoth, a periodical published by the Weizmann Institute of Science, Rehovoth, Israel, and contains sections not only on the molecular basis of learning and memory but also on electrical tests, psychochemical substances, and hallucinogens.

A third, written for an engineering audience, is "Towards a Theory of Memory," by Dr. Steven Rose, currently at the Instituto Superiore di Sanita, Rome, Italy. It appears on pp. 38-43 of Discovery, April 1964. We quote its blurb:

"How does the brain store the vast amount of information it accumulates during its lifetime? Almost certainly RNA is involved--and scientists are now beginning to understand exactly how this chemical acts as a 'memory store'."

The fourth, by Dr. Wesley Dingman and Dr. Michael B. Sporn, is "Molecular Theories of Memory," Science, Vol. 144 (April 3, 1964) pp. 26-29. It is not a review but a critique of some theoretical approaches. We quote the authors' summary:

"If one establishes a rigorous set of criteria for defining a given type of molecule as a memory trace in the nervous system, then no one type of molecule may at present be regarded as the sole engram of a permanent memory trace. Much evidence already exists that RNA and protein metabolism are intimately involved in the process of memory storage, but the role of other molecules, such as lipids, must also be considered. Sophisticated techniques of molecular biology and enzymology will undoubtedly provide valuable data on biochemical processes involved in memory storage. However, a comprehensive theory of the structural basis of memory must also consider the function of the entire neuron,, with consequent emphasis on the reciprocal relationships between the cell body and the synapse, as well as the complex functional interrelationships between neurons."

* * *

NRP Staff Report 64-1

N E U R O S C I E N C E R E S E A R C H P R O G R A M
280 Newton Street
Brookline, Massachusetts, 02146
Area Code 617-522-6700

THE SEVENTH STATED MEETING OF NRP ASSOCIATES

MONDAY, FEBRUARY 10

Morning

* NRP Chairman Francis O. Schmitt opened the meeting by welcoming the newest Associate, Dr. Robert B. Livingston; the first resident Fellow, Dr. Robert G. Ojemann, MD; and the new Business Manager, Mr. L. Everett Johnson.

* T. Melnechuk then introduced new Communications Staff members Anne Harris, Catherine LeBlanc, and Paul Wankowicz.

* Associate Claude E. Shannon introduced Dr. Lawrence Stark, of MIT, Chairman of the Work Session on Mathematical Concepts of Central Nervous System Function (January 31-February 1, 1964), who presented an oral summary of that meeting. It will be reported in a forthcoming NRP Bulletin.

Afternoon

* Dr. Peter F. Davison of MIT, who was introduced by Chairman Schmitt, gave a Laboratory Progress Report on Immunochemical and Neurophysical Studies of Neurofilament Protein. This was in large part a prepublication report covering material since published by his colleague Francis Huneeus-Cox, "Electrophoretic and Immunological Studies of Squid Axoplasm Proteins," Science, 143, No. 3610 (1964), pp. 1036-1037.

file: telepathy

* The afternoon was devoted to a report and discussion of a NRP Work Session on Specificity in the Neurosciences (February 4-5, 1964) chaired and summarized by Associate Paul A. Weiss. It too will be the subject of an NRP Work Session report in a future issue of the NRP Bulletin.

TUESDAY, FEBRUARY 11

Morning

* The second day began with a Progress Report on RNA in Neurons and Glia and RNA's Relation to Memory and Learning by Associate Holger Hyden. Dr. Hyden was introduced by Associate Norman Davidson.

* Next, Chairman Schmitt reviewed his seven-week trip (Summer, 1963) to a number of Brain Research Institutes and other relevant research centers in Western Europe.

* In the same international vein, the next speaker, Dr. Herbert Jasper, gave an informal outline of the history and organization of the International Brain Research Organization. IBRO publishes a bulletin, is compiling a directory of world brain research centers, sponsors exchange study programs, and holds seminars and colloquia which are later published.

Afternoon

* A procedural departure opened the afternoon session. The Associates as a whole were invited to participate as panelists in a discussion, chaired by Associate Robert Livingston, of Technological and Social Implications of Neurosciences Research.

* The discussion was followed by a report by Associate Richard B. Roberts, who had chaired a two-day Symposium on Parapsychology (September 27-28, 1963). Dr. Roberts reviewed the kinds of evidence adduced for the reality of such parapsychological phenomena as telepathy, psychokinesis, precognition, and clairvoyance.

Article Relieved? Yes!

WEDNESDAY, FEBRUARY 12

Morning

* The third day began with a report by Associates Walle J. H. Nauta and Sanford L. Palay of the Work Session on Some Brain Structures and Functions Related to Memory (January 10-11, 1964) at which they were co-chairmen. Out of their evaluative summary grew a spirited multidisciplinary discussion that grappled with the basic issues of the mind-brain-cell-molecule problem. A report deriving from this NRP Work Session is also in progress and will appear in the NRP Bulletin soon.

Afternoon

* The afternoon session began with Dr. Eugene Roberts' presentation of "The Synapse as a Cybernetic Unit: A Biochemist's Phantasy," a version of which was printed in the January-February 1964 issue of the NRP Bulletin.

* Its discussion led to the presentation by Dr. Walter Moore of his biophysical work on biological membrane modeling.

Evening

* The NRP constituted the program of the concurrent Stated Meeting of the American Academy of Arts and Sciences, whose House the NRP shares. Three NRP Associates spoke to an unusually large audience:

* Chairman Francis O. Schmitt, pointing out that the scientific disciplines studying mind have for too long been disparate, described the NRP as a site for the integration of both the "systems" attitude typified by the social sciences, psychology, neurophysiology, computer science, etc., and the "components" approach expressed in biophysics, biochemistry, and molecular neurology.

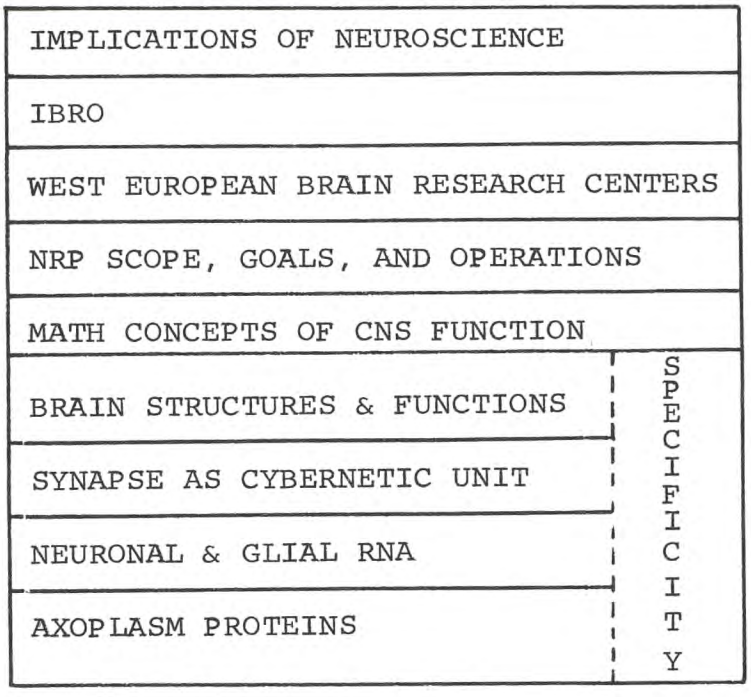
* Representing the "systems" point of view was Associate Seymour S. Kety, while Associate Manfred Eigen spoke for the "components" approach. Together, they outlined the necessity for the investigation of mental phenomena at every level of organization from molecule to brain to society.

* Finally, Theodore Melnechuk outlined the three essential functions of the NRP--investigation, synthesis, and communication--and described the means established to accomplish these--special and general meetings, publications, and programs of education and public information.

CONCLUSIONS

Rationale

The agenda of the Seventh Stated Meeting of NRP Associates can be represented by the following sketch, ranging from the molecular to the social levels of organization:



Decisions

The dates of the next Stated Meeting of NRP Associates will be August 10-12, 1964. The agenda is to be more open-ended. --T.M.

* * *

NRP Work Session Report 64-1

N E U R O S C I E N C E S R E S E A R C H P R O G R A M
280 Newton Street
Brookline, Massachusetts, 02146
Area Code 617-522-6700

C E L L M E M B R A N E S

REPORT OF AN N.R.P. WORK SESSION
HELD JULY 27-28, 1963

WORK SESSION PARTICIPANTS:

B. Chance	University of Pennsylvania, Philadelphia
H. Fernandez-Moran	University of Chicago, Chicago
D. E. Green	University of Wisconsin, Madison
J. L. Kavanau	University of California, Los Angeles
E. P. Kennedy	Harvard Medical School, Boston
A. L. Lehninger*	Johns Hopkins University School of Medicine, Baltimore
J. D. Robertson	Massachusetts General Hospital (McLean Division), Belmont
F. O. Schmitt	Massachusetts Institute of Technology, Cambridge
F. S. Sjöstrand	University of California, Los Angeles
D. S. Smith	University of Cambridge, Cambridge, England; currently, University of Virginia, Charlottesville
W. Stoeckenius	Rockefeller Institute, New York
T. E. Thompson	Johns Hopkins University School of Medicine, Baltimore

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* Dr. Lehninger, an Associate of the N.R.P., was Chairman of this Work Session.

ON NRP WORK SESSIONS

by

Theodore Melnechuk
Director of NRP Communications

The Neurosciences Research Program (NRP) is a voluntary, inter-university, international organization of scientists and scholars who are attempting to integrate the physical, biological, and behavioral sciences in a multidisciplinary attack on the problem of mental processes in man.

Subjects identified as being central to the Neurosciences Research Program are examined in depth by panels of experts at meetings called "Work Sessions." These concentrated discussions are carefully structured in advance in order to harvest in the time available (usually two days) a maximum of information and expert judgment, leading to the best evaluative summary of the current state of the science.

Chairmen, participants, and NRP Staff cooperate to report proceedings of Work Sessions to the NRP Associates, who subject the material to multidisciplinary critical examination.

Details of the production of this particular report are given on the back cover. Here, let me express the hope that its circulation may provide a feedback to Work Session participants that will partly repay them for their scientific contributions and subsequent editorial cooperation, and be of value to other investigators and communicators in the neurosciences.

TABLE OF CONTENTS

FOREWORD

by

Francis O. Schmitt, Chairman
Neurosciences Research Program

Dr. Lehninger's summary of the highly productive two-day NRP Work Session on Cell Membranes seems to me to be an excellent reflection of the consensus of the group.

I felt that the participants were eminently successful in depicting the highlights of membrane ultrastructure, particularly in mitochondria; the nature both of enzyme assemblies and of lipid bilayers, which may model cell membranes and axonal membranes.

The basic discussion of facts and artifacts deducible from positive and negative staining of structures viewed in the electron microscope at high resolutions was of great value in itself.

Admittedly little was said about neuronal and glial membranes, especially as they relate either to bioelectric processes or to the storage, transfer, or readout of information in synapses. These might well form the subject of a subsequent Work Session based on the groundwork laid on July 27 and 28, 1963.

On behalf of the Associates and Staff of NRP, I extend my deep personal appreciation to all participants for their willingness to take time to come here for the two-day meeting and to annotate and augment Al Lehninger's summary.

EVALUATIVE SUMMARY:

NRP WORK SESSION

ON CELL MEMBRANES

by

A. L. Lehninger

INTRODUCTION:

THE ISSUES AND THEIR IMPORTANCE

I would like to remark on the problem of reporting to a general group as concentrated a Work Session as was this one.

The Work Session covered three subjects: First, ultrastructure, as revealed by optical methods (mostly by electron microscope). Second, biochemical aspects of membrane structure and function. Finally, the physical chemistry of membrane structure.

Fig. 1 outlines the details of this program and the major speeches--if we can call them such. Actually, nearly each formal speech was limited to a rather short period.

FIG. 1. Subjects and Speakers.

ULTRASTRUCTURE	
Brief summary of the Davson-Danielli concept and "unit membrane" hypothesis	ROBERTSON
Differentiation of membranes at high resolution	SJÖSTRAND
What is stained? Dimensions?	STOECKENIUS
Negative contrast	SMITH; SJÖSTRAND; STOECKENIUS
Substructures	SMITH
Artifacts. "Open vs. "Closed" phases	KAVANAU
Summary	LEHNINGER
BIOCHEMICAL ASPECTS	
Comparative biochemistry of membrane lipids	KENNEDY
"Structure proteins"; sheath membrane	GREEN
Concept of "assemblies," respiratory and other	CHANCE
"Elementary particles"	GREEN
Summary	LEHNINGER
PHYSICAL CHEMISTRY	
Stable arrays of phospholipid-water systems	THOMPSON; STOECKENIUS
Summary	LEHNINGER

You are aware, I believe, that ideas and progress are achieved in the various fields of sciences, not by a nicely ordered, logical plan, but by the interplay of personalities and controversies, as well as the discovery of new facts. At this particular Work Session, we had both the personal interplay and the experimental data. The logical-looking outline given in Fig. 1, therefore, does not altogether represent how the subjects were developed. This report is not a stenographic chronicle but an interpretive summary. The coverage I give to special points is proportional neither to the time they actually occupied nor to the importance they no doubt have. I am sure the distinguished participants will understand that this report is shaped primarily to the need of my fellow N.R.P. Associates for the gist of things.

Before getting into any details, however, I want to map out the lay of the land. Accordingly, I'll first sketch a broad picture of why this Work Session on Cell Membrances was important to the Neurosciences Research Program at this particular time* and what the major issues were.

The Importance of Cell Membranes

The whole problem of membranes and membrane structure is very timely and significant for the Neurosciences Research Program, from a number of points of view. In the first place, I want to impress on N.R.P. Associates a fact that I've mentioned before to this group: Membranes comprise a very large fraction of the total cell substance of many different kinds of cells.

Fig. 2 is a diagram of a rather idealized cell, perhaps an epithelial cell. There are many membranous structures in it: not only the plasma membrane, but also the endoplasmic reticulum -- a very complicated network of membranes, mitochondria, Golgi complex, nuclear envelope, and the rest. In some kinds of cells 80 to 90 percent of the total cell substance is actually membrane material plus the enzymes and other special components that are affixed to membranes.

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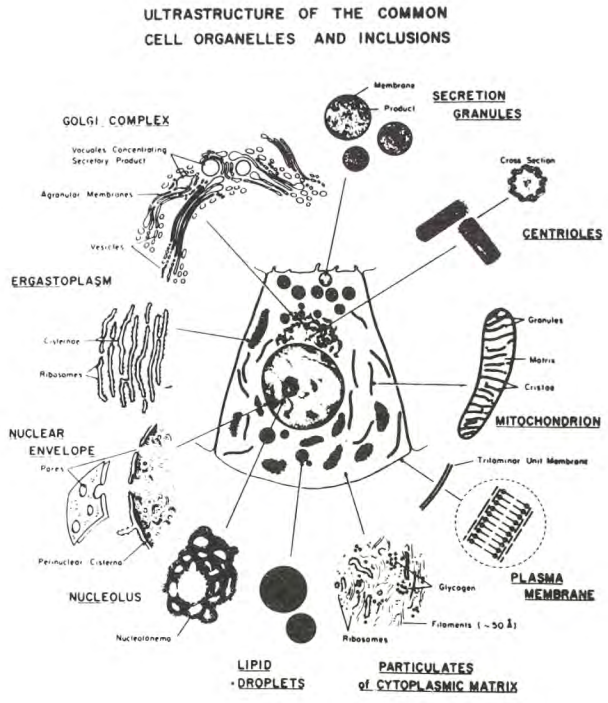
*Summer 1963.

Fig. 2. Ultrastructure of the common cell organelles and inclusions.



- Don W. Fawcett

Other types of cells, such as the liver cell, have relatively less, but even here the calculation has been made that membrane material makes up about 60 percent of the total cell content. This is rather a large figure, and it suggests that membranes are very important. They are not just bags, not just dividing walls. They have a lot of molecular componentry on them to carry out different kinds of cellular functions.

Neurons, by and large, are very rich in membranous material. You may recall that some of them are long, narrow cells, almost tubular, where a large part of the mass is membranous. Many of the functions of the neuronal cells are attributed to exchanges and activities at their membrane surfaces. Furthermore, there are membrane contacts at synapses.

Now let us consider the major biological properties of membranes -- properties of relevance to neuroscience. First, membranes usually contain enzyme assemblies for special functions; the respiratory assemblies are characteristic of the mitochondrial membrane. Next, the membrane also is responsible for ion fluxes which are related to potentials and impulses. We need also to account for the specific molecular switching devices that presumably exist at synapses. Then we come to the subject in which many of us are very interested: the molecular devices for bringing about memory coding. Possibly, coding of memory occurs at two levels to encompass innate memory and acquired memory. Thus each of these functions may conceivably be accounted for by the structure and properties of the membranous elements of the neuron.

Finally, discussion of membranes is very timely for another reason. The progress of the past ten or fifteen years on the structure of proteins and nucleic acids has revealed the major principles of their structural organization: the steric and physical factors that result in the thermodynamic stabilization of their characteristic secondary and tertiary structures, such as the alpha-helix, the double helix of DNA, etc.

With these discoveries behind us, the next big "sound barrier" to be cracked in molecular biology is the next higher order of molecular structure in cellular organization. This would appear to be the membrane,

in which we have the integration of lipid and protein structure to produce a film which is only a few molecules thick, although it may extend for thousands and thousands of molecules laterally.

There is good reason to feel that this may be the next barrier to be cracked. In the past two or three years, we have seen several very important developments in various areas of membrane science:

First, there has been a refinement of fixing, sectioning, and tissue-preparation techniques for electron microscopy, to give us new and different ideas on the structure of membranes.

Second, recent research on the enzymatic organization of membranes has revealed new ideas on how the molecular assemblies are arranged in the membrane for carrying out functions such as active transport, oxidative phosphorylation, and so on.

Third, lipid biochemistry has come of age in the last few years. Until recently, lipid chemistry was a messy field, but the refinement of new chromatographic methods for separating and identifying lipids on a small scale has really revolutionized lipid chemistry. For the first time we have a more or less detailed accounting of the various kinds of lipids in certain biological membranes.

Properties of Membranes

Below is a list of the properties of membranes which must be accounted for, ultimately, in any genuinely unifying theory of the molecular structure of membranes. These properties are not necessarily listed in the order of their importance. Furthermore, I suspect that some of the activities that are listed separately may turn out to be different facets of a single property.

To give some idea of the gamut of activities associated with membranes of different kinds, I list these properties:

Electron transport and oxidative phosphorylation, carried out by multi-enzyme assemblies in the mitochondrial membranes.

Active transport of sodium (Na^+), potassium (K^+), calcium (Ca^{++}), and magnesium (Mg^{++}) -- which are brought about by enzymatic reactions occurring in the membrane. These have very wide distribution among different kinds of membranes.

Permeability changes -- which may be determined by enzymatic activities of the membrane. Such permeability changes may be one of the control systems of the cells to regulate inflow and outflow.

Contractility -- Many membranes show "active" contractility which is apparently driven by ATP hydrolysis. This is well-known in the mitochondrial membrane, which is most easily studied, but this property also is seen in protoplast membranes and erythrocyte membranes.

The electrical properties of membrane systems have been known for a very long time, of course, and I won't dwell on them any longer at this point since they comprise a central interest of neurophysiology and will be the subject of other N.R.P. discussions.

Last, but not least, there is growing evidence that the biosynthesis of membranes, that is, the biosynthesis of the protein and lipid parts of membranes, proceeds via mechanisms which may not be identical with the mechanism of biosynthesis of free soluble proteins in the cytoplasm. The idea has come up that possibly membranes may furnish the template for their own biosynthesis. This is only an idea at present, yet I think that the problem of membrane biosynthesis will prove to be one of the most important aspects of future work in all biology, since it underlies nearly all of embryology and morphogenesis. Actually, the characteristic arrangement of cytomembranes is fundamental to the differentiation of cells.

So much, then, for a very general statement of a number of different properties and functions that are associated with membranes.

Focus on Mitochondrial Membranes

Our conference of a day and a half could not, of course, answer all the questions raised. We could focus our attention on only a very few items that were both central and relevant to many of these other considerations.

A good part of the discussion in this Work Session did not actually touch at all on the membranes of neurons, but really centered around the structure, properties and functions of mitochondrial membranes. This emphasis was not entirely due to the specific interests and background of the Chairman and some of the more visible and vocal members of the Work Session. The fact is, the mitochondrial membranes are the best understood today, from the standpoint of both structure and function.

Mitochondria are easy to isolate in some quantity, while -- as you know -- neuronal membranes are hard to get at, at least in the amount that enzymologists would like to have for the study of some of these properties. Also, for two generations we have had a large body of biochemists working on the mechanism of electron transport and oxidative phosphorylation, which take place in the membranes of mitochondria.

Electron microscopists have recently concentrated their efforts on mitochondrial structure, to try to relate all these complex enzymatic functions -- which have been explored with such success recently -- to features of the ultrastructure of the membranes.

Now let me review briefly the major features of mitochondrial structure. Mitochondria are present in all aerobic cells; they are the power plants, as I have noted, in which the ATP is manufactured at the expense of respiratory energy. The number of these varies from one cell type to another. In the liver cell, for example, there are perhaps one or two thousand mitochondria. These are roughly two or

three microns long and about half a micron wide. They have a double membrane that stains with osmic acid. The inner one infolds at points to form the so-called cristae, as shown in Fig. 3.

Other important points are illustrated in Fig. 4. During the biological oxidations in cells, the carbohydrate, fat and amino acids are oxidized by a process of dehydrogenation. The hydrogen atoms which are stripped off by dehydrogenase action become protons in the aqueous medium, and the equivalent electrons then flow down a chain of enzymes known as electron carriers; the chain is known as the respiratory chain.

These electrons come in first at the level of DPN, or diphosphopyridine nucleotide, which is the first carrier in a sort of cascade. Next, these electrons flow along a series of reversible oxidation-reduction systems, prosthetic groups attached to specific protein molecules, and ultimately come out at the end and reduce molecular oxygen to water. During this process there is a large free-energy drop, which is harnessed with the formation of ATP from inorganic phosphate and ADP.

In the cell, ATP is the energy-carrying molecule between energy-yielding reactions and energy-requiring reactions, whereas ADP may be looked on as the discharged or spent form of this energy carrier. In brief, ATP formed by mitochondria drives muscle contraction, ion transport, and biosynthetic reactions. During the latter processes ATP loses its energy and is dephosphorylated to form ADP and phosphate. These come back again to the mitochondria and become "recharged" at the expense of the energy yielded by electron transport. This process is called oxidative phosphorylation.

The molecular details of oxidative phosphorylation are not yet known: they constitute today one of the greatest challenges to enzymology. The entire process is believed to be carried out by an "assembly" of enzyme molecules

Fig. 3. Mitochondria of liver and kidney cells.

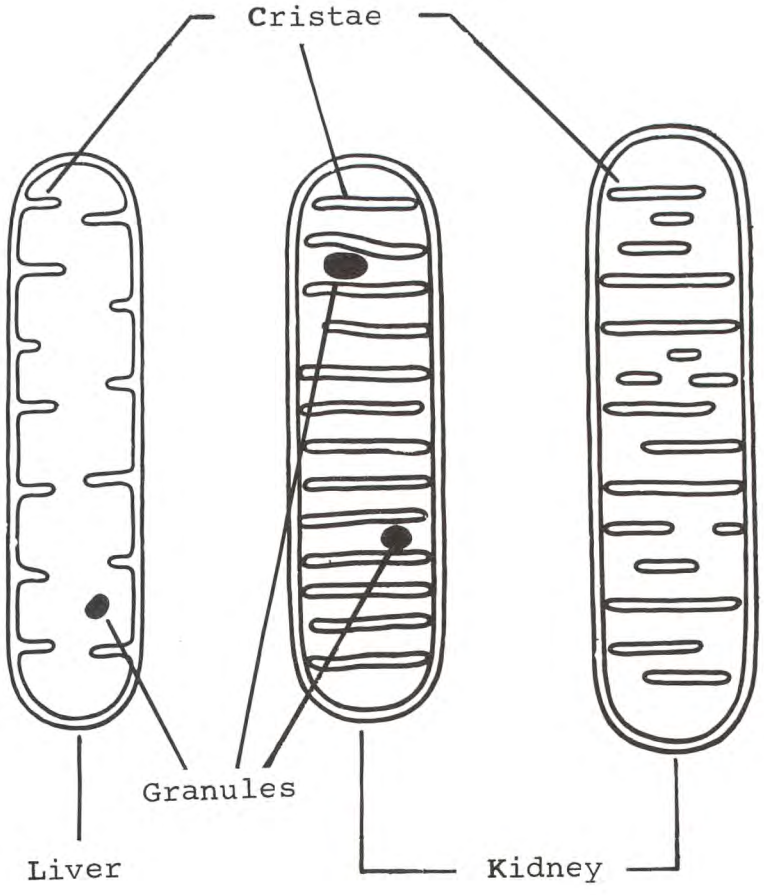
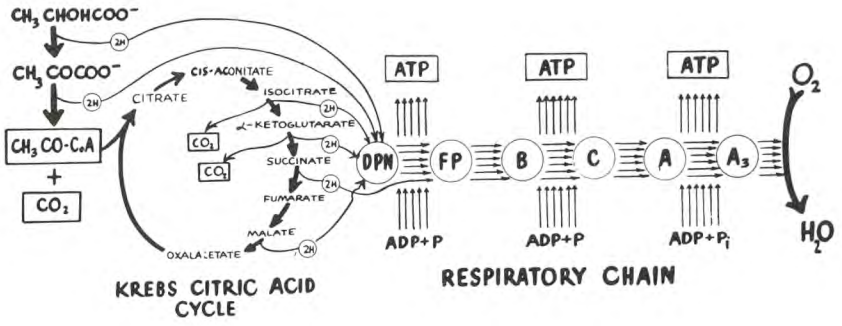


Fig. 4. Dehydrogenation and the respiratory chain.



comprised of one molecule of each of the electron carriers. This molecular machine is called a respiratory assembly. Now, in a liver mitochondrion like that shown in Fig. 3, there may be something like 15,000 to 20,000 of these respiratory assemblies, which are studded in the membrane, specifically in the inner membrane system of the mitochondrion. In fact, these respiratory assemblies constitute almost 25 percent of the total membrane substance. This means, then, that the membranes of the mitochondrion are not just inert skins, but rather sheets comprised of recurring multi-enzyme assemblies. This point will be elaborated later in greater detail.

As membranes go, the mitochondrial membrane is the most complex that we know today in regard to enzymatic organization, at least among animal tissues. In plant tissues its counterpart is the chloroplast, where the arrangement of photosynthesizing apparatus and chlorophyll has a complexity which is undoubtedly at least as great as the complexity of organization of the mitochondrion. Not only are mitochondrial membranes complex enzymatically speaking; they are perhaps the most complex morphologically speaking. This point will be developed later in discussing Sjöstrand's work.

Mitochondria do at least two other things that are of great significance in the cellular economy. One of these is to carry out active transport of ions of Ca^{++}, Mg^{++}, Na^{+} and K^{+}. Mitochondria may be looked upon as islands inside the cytoplasm which are capable of sequestering or segregating ions from the surrounding hyaloplasm, to maintain the internal ionic homeostasis of the cell.

The second activity of mitochondria that is also related to membrane structure is contractility. Mitochondria undergo pulsatile swelling-and-contraction cycles, some of which, in some cells, are of high frequency.

So first we see in mitochondrial membranes an extreme complexity since they are probably, morphologically speaking, the most complicated membranes. Secondly, we see an enzymatic organization that is the most complex of any

membrane known. Third, we find in the mitochondrion itself almost a microcosm, in that we can detect massive active transport mechanisms, permeability changes, and contractility changes.

And, although no one has really studied this in great detail, there is also evidence of electrical changes in mitochondrial membranes, which may be significant. It may be recalled that McIlwain and others some years ago found that respiration of isolated mitochondria could be modified by subjecting them to square-wave electrical stimulation. The relevance of this to neuronal function is not yet known, but I daresay that some serious study of the electrical properties of mitochondria would be valuable. This is a subject that really needs much more work.

So much, then, for the mitochondrion as a central study object in membranology today. Throughout the discussion on mitochondrial membranes, we tried to bring the discussion back to the three major points of relevance mentioned earlier in regard to the membrane of the neuron.

Controversial Issues

Before I begin on the substantive content of the Work Session I think it is important to note that there were some controversial issues under discussion. A few simple diagrams will illustrate the nature of these issues. One-half of such a controversy is illustrated in Fig. 5. Largely due to the work and ideas of Robertson, an idea known as the unit membrane concept has been promulgated over the past dozen years. This is based on the more classical picture of membrane structure first enunciated by Davson and Danielli some twenty-five years ago.*

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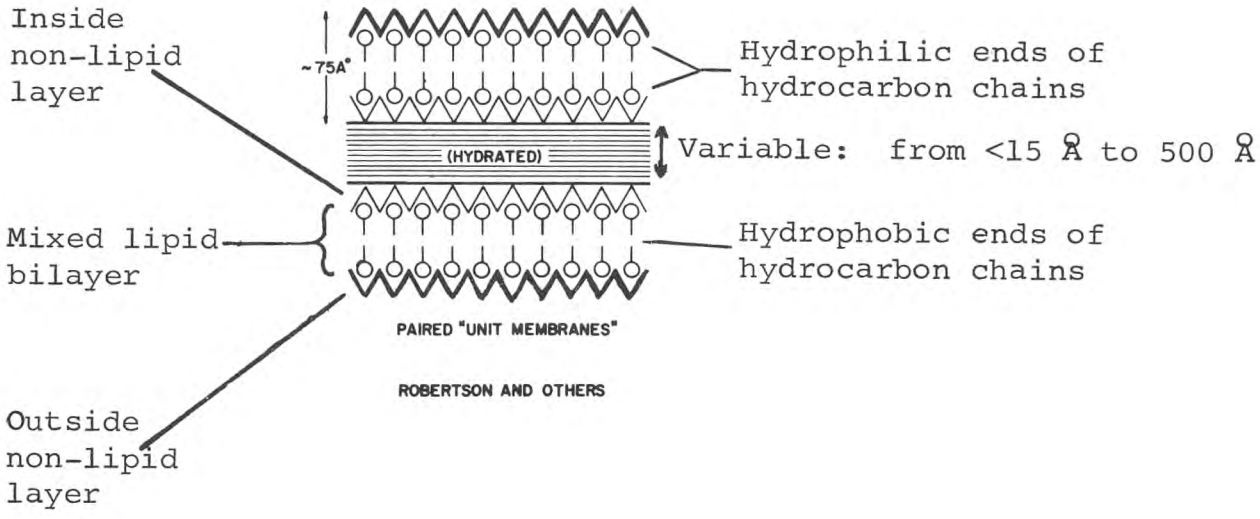
* Robertson: This statement is not quite correct historically. It is often said or implied that the unit membrane concept is based on the Davson-Danielli model. This is quite inaccurate and misleading. The Davson-Danielli model, advanced in the mid-nineteen-thirties, had its origins in physiological studies of membrane permeability by Overton and various biophysical studies of membranes during the early nineteen-thirties by Harvey and Shapiro, Cole, Danielli and Harvey and others. Information primarily about permeability and surface tension properties were used by Danielli and Davson in proposing their

Fig. 5. Unit membrane concept.



pauci-molecular theory. They had no basis on which to say that the core of the membrane was a single lipid bilayer, and, in fact, they said no such thing. Further, they could say nothing about asymmetry - an important feature of the unit membrane concept.

The unit membrane concept had a very different origin. It was based on direct observations of native and model membrane structures by electron microscopy correlated with X-ray diffraction and polarization optical studies. It established the lipid bilayer limitation and the notion of asymmetry in the non-lipid monolayers. It also established certain well defined restrictions on the patterns of organization common to all membranes. It has its own origins and did not evolve from the Danielli-Davson model.

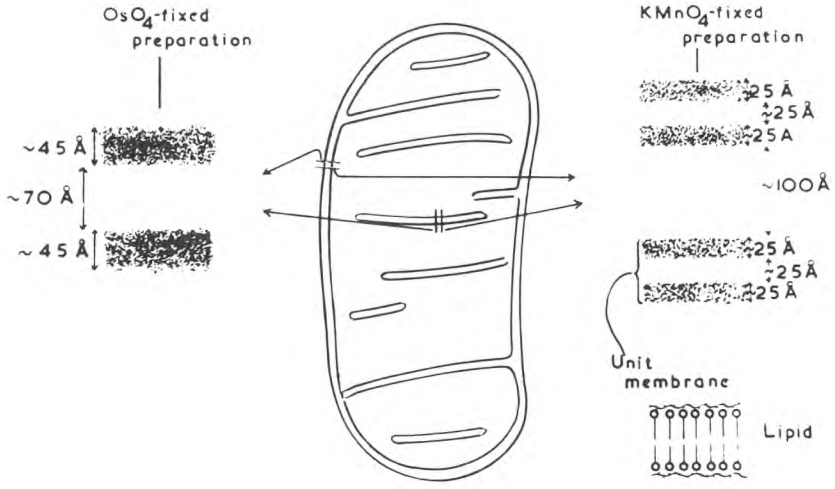
Robertson's theory holds that all biological membranes or membrane systems are comprised of so-called "unit membranes," Each unit membrane, in turn, is composed of a mixed lipid bilayer with the hydrocarbon chains in a smectic or "neat" array. On each side there is a non-lipid layer which is presumably protein.

A single unit membrane represents the structure of single membranes such as the plasma membrane. Robertson has suggested that a double or compound membrane system, such as is found in mitochondria, is comprised of two unit membranes which may be adjacent to each other.

Now for the past ten or fifteen years, most electron microscopists, in order to visualize membranes, have employed either osmium tetroxide or potassium permanganate (and certain other heavy metal reagents) to stain these membranes characteristically. It happens that osmium, the most widely used, sometimes stains membranes in a characteristic way: one dense line (see Fig. 6) for each unit membrane suggested by Robertson. The part stained is the hydrophilic end of the phospholipid molecule, and possibly part of the protein. Permanganate also stains membranes characteristically, but the spacing of the permanaganate lines is somewhat different from the spacing of the osmium lines, as is shown in Fig. 6. These images are called positive contrast images, because the osmium actually fixes on a specific chemical region of this membrane and gives us positive staining, or positive contrast.

On the other hand, Fernandez-Moran, one of our NRP Associates, applied a new way of looking at membranes some two years ago. He employed negative contrast, in which it is not the structure itself that is attacked by the stain. Rather, all the space around the structure is filled up with the material, which may be phosphotungstate, as a potassium salt, uranylacetate, or certain other heavy metal salts which are relatively inert under the conditions of the fixation (pH, $\Gamma/2$, etc.) and do not attack or combine with the membrane. The object to be examined is thus seen in silhouette.

Fig. 6. Characteristic positive stains.*



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* Robertson: A comparison of mesaxon-compact myelin junctions in $KMnO_4$ and OsO_4 fixed material led me to choose the permanganate picture as more representative. (Robertson, J. D., Cell membranes and the origin of mitochondria, Regional Neurochemistry, S. S. Kety and J. Elkes, ed., Pergamon Press, p. 497 (1961)). More recent work has borne out this interpretation by showing that osmium displays essentially the permanganate picture especially if aldehyde prefixation is used. (Robertson, J. D., Unit membranes: a review with recent new studies of experimental alterations and a new subunit structure in synaptic membranes, Cellular Membranes in Development, Michael Locke, ed., Academic Press, p. 24 (1964)).

The image of the mitochondrial membrane seen under these circumstances is represented in Fig. 7. There is a series of large particles, on each side of the membrane, having a diameter of 80 to 100 Å, although here again some variation has been noted. These seem to have some granularity or fine structure. There is a stalk connecting each one of these so-called elementary particles to a central core, and they are arranged more or less symmetrically on each side. The space between the particles is something like 100 to 120 Å. The total thickness of the structure is around 300 Å, in comparison with the unit membrane alone, which is about 75 Å.

One of the central issues under discussion, particularly by the electron microscopists, is the following: Is this an image of a true structure of the membrane, or not? Is it an artifact caused by the staining procedure? Of course, for many years the question has been raised, "Isn't the osmium line an artifact, too?" And it really has taken ten years of research to convince most people -- some remain unconvinced -- that osmium is actually staining certain specific chemical portions of such a membrane. Still, one must say that the characteristic osmium staining does not prove a molecular arrangement of this kind. It is consistent with it, but it does not prove it.*

By the same token, one is also entitled to raise such questions about this relatively new negative staining method as applied to membranes, for the negative contrast method was first successfully employed with viruses. This then is one of the central issues under discussion, though our Work Session revealed that some of the important workers in this field have been moving closer to unanimity on the occurrence of granular regularity in membranes. Thus,

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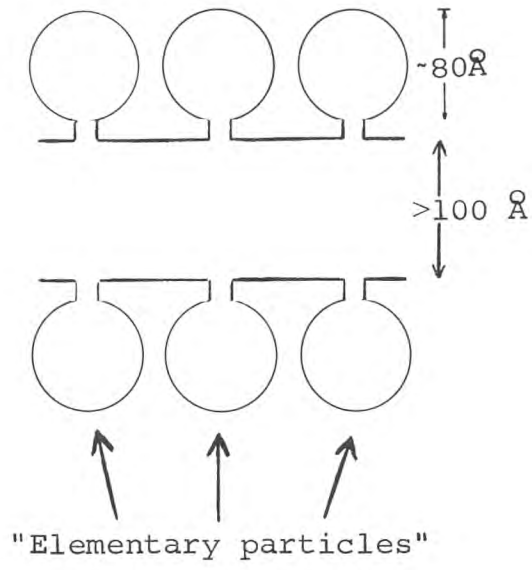
* Robertson: The correlation of X-ray diffraction results from studies of native material and of model systems with those of electron microscopy makes possible decisions about which pictures are artifacts and which are real. In the next paragraph mention is made of the application of negative staining techniques to studies of viruses. In this field decisions were made about artifacts by comparison with independent X-ray diffraction findings. For example, the hole in the middle of the TMV particle was first predicted by X-ray results and then demonstrated by negative staining. Working from such baselines structure seen first by electron microscopy can in turn be investigated by X-ray methods. Without a correlation between the two approaches it seems to me difficult to reach decisions about artifacts.

Fig. 7. Negatively stained mitochondrial membrane.



Sjöstrand is prepared to grant that there is a recurring regularity in the membrane, possibly associated with the respiratory assemblies; a regularity that is reflected by the phosphotungstic images, whether or not they are artifacts. If they are artifacts, they are very regular and indicate that the original had regularity.

A second major issue under consideration should also be discussed now, as it will make other parts of the discussion clearer. This concerns the nature of the geometric organization in the mitochondrial membrane of the enzymes responsible for electron transport and oxidative phosphorylation.

You will recall that these enzyme assemblies make up about 25 percent of the total membrane protein, and are characteristic of this very complex mitochondrial membrane.

Five years ago, I happened to propose an idea* which got fairly wide circulation; namely, that the respiratory assemblies may be arranged in a planar array embedded in or attached on the monolayer of protein of the "unit membrane" of the inner membrane system.

There may be fifteen enzyme molecules in this assembly, as shown schematically in Fig. 8. Filling out the space between the assemblies are so-called "structural protein" molecules, which represents the"floor space" for the machines. These molecules may be enzymatically inactive, whereas the assemblies are catalytically active.

There are other possible configurations of the assembly. It may be rolled up in a ball for example. However, a flat planar array appears to be most favorable to achieve asymmetry of organization.

On the other hand, the negative contrast work of Fernandez-Moran (which, by the way, has now been confirmed in a number of laboratories, including ours) has revealed the occurrence of so-called elementary particles. Green and

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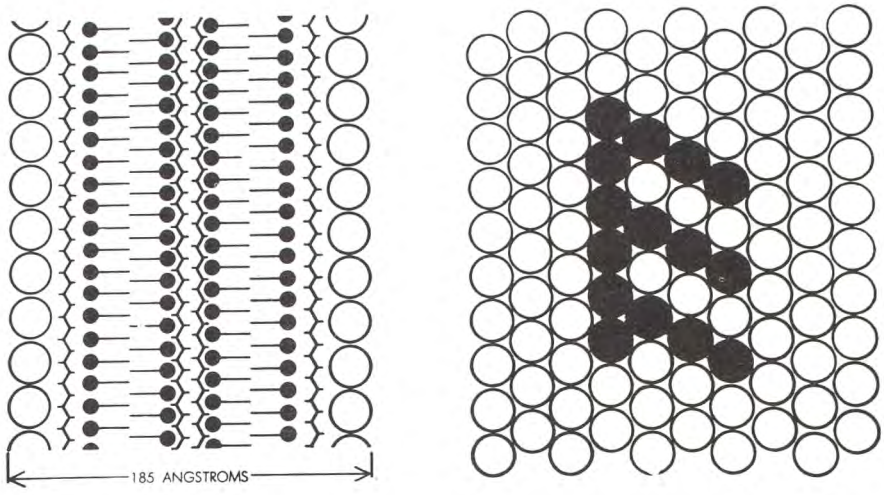
* Science, 128, 450 (1958).

Fig. 8. Hypothetical planar array of enzyme assemblies on protein monolayer of unit membrane.



- From Scientific American

Fernandez-Moran have collaborated on the suggestion that each one of these elementary particles contains the whole group of enzymes that constitutes a respiratory assembly. The enzyme molecules are believed to be arranged in a spherical cluster, which may endow these elementary particles with the fine granularity that is visible in the electron micrographs. The big question now is, and this has been raised by many members of the biochemical fraternity, "Are these particles big enough to accommodate all the known enzymes of the respiratory assembly?"

In a memorable Gordon Conference last year* there was quite a battle, which is still raging, as to whether one can squeeze all the known catalysts of electron transport and phosphorylation into this package of 75-90 Å diameter. This is the major bone of contention among the enzyme chemists, which I don't think is resolved yet. But at least, both sides of the story were presented.

There is at least one other point of view. Chance has felt that there is not enough room in these particles to accommodate all the enzymes, and he has proposed a radically different concept -- namely, that each one of these particles may contain only one specific enzyme of the assembly. Therefore, the enzymes of the assembly are not physically contiguous or adjacent to each other. These particles may however interact with each other by translational diffusion -- limited, of course, by the length and flexibility of the stalks which attach the particles to the membrane.

These are the major points of controversy. My opening remarks on them have been extensive, I know, but helpful, I hope, in interpreting the substantive contributions.

Other Membrane Enzyme Arrays

A final point, on the fact that in addition to this kind of solid-state array, others are known to exist that are also on membranes, but not necessarily for electron transport.

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* Structure and Function of Multilayer Systems in Cells, June 1962.

Although most of our discussion centered around the molecular and structural organization of the mitochondrial membrane and its very complex enzymatic machinery, the point was brought out that other cell membranes may contain some enzyme "gadgets" in them, which are asymmetric in organization, and are probably organized in very specific ways. For one thing, the elementary particles are seen only in mitochondrial membranes, not in the plasma membrane or certain other cellular membranes.

There is also other evidence, which Sjöstrand brought out, that the mitochondrial membrane just looks more complicated,no matter how you stain it and fix it, than other kinds of membranes.

Although all membranes contain some enzymes, I think that there is only one system that is characteristic of all membranes no matter where they are, and that is an ATP-ase activity: an enzyme complex that can split ATP and thus release its chemical energy, and use it to do certain things, such as active transport and active contractility. We know that ATP is ultimately the fuel for muscular exercise, but, by the same token, membranes appear to contain contractile elements which are also driven by ATP hydrolysis. In fact, it has been suggested that the muscle myofibril itself may be derived from a membrane which has lost its lipid.

Other membranes also contain oxidation-reduction enzymes; thus, the endoplasmic reticulum contains a vestigial respiratory chain. Green* also mentioned some evidence that several of the glycolytic enzymes which make lactic acid out of glucose may also be attached in packets to erythrocyte membranes. Thus, all membranes have specific kinds of enzyme components.

Now, to turn to the substantive text of the Work Session.

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* Unpublished studies of H. Hultin, S. Richardsen, G. Brierley, B. Salmon, E. Murer and D. E. Green.

I. MEMBRANE ULTRASTRUCTURE

The first leg of the program was devoted to ultrastructure, and I hope that as I cover the content of the discussion you'll keep in mind the major points of controversy I have brought out. Let's begin with the contributions of the electron microscopists.

Unit Membrane Hypothesis

I asked Dave Robertson to elaborate on the unit membrane hypothesis and its experimental foundations, and how he would now relate it to the work on negative contrast staining with phosphotungstate.

Robertson's discussion can be boiled down, first, to a statement of what the unit membrane hypothesis really is. I've already described this in terms of the diagram in Fig. 5. You will recall that there's a phospholipid bilayer and two non-lipid layers, one on either side. Robertson didn't want to specify these beyond saying that the outside layer differs in some important chemical way from the inside layer. For example, the inside could be largely protein and the outside largely muco-protein.

In other words, his theory is noncommittal as to the exact chemical nature of the substance on either side of the bilayer, though we all know it must be largely protein. It is also noncommittal as regards the exact nature of the lipid components. It deals only with the general pattern of organization.

The second point that Robertson developed with regard to the unit membrane hypothesis, and I think that he made a rather forceful statement on it, goes like this. (It was not, I believe, a major feature of the theory as first enunciated, but it is a corollary.*) Robertson pointed out that unit membranes don't have to have the same dimensions from one membrane to another, for these reasons: the phospholipid molecules present in different membranes vary in composition and structure. Some membranes are rich in phosphatidyl choline, some in cardiolipin, and so on. All of these compounds have different space-filling properties and different conformations.** Because of this, one might logically expect that the phospholipid bilayer may have different thicknesses in different membranes, depending on the lipid composition. In addition, he noted that different membranes have different enzymatic apparatus in their protein layers, and so for this reason, too, we can expect a certain amount of variation in the thickness of the non-lipid layers of the unit membrane.

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* Robertson: This statement is essentially correct, but I have been so often misunderstood on this important point that I would like to elaborate on it somewhat. In no case have I ever knowingly stated or implied that all membranes have the same dimensions. The "~" symbol, meaning "order of magnitude" was always very carefully placed before the 75 Å figure for membrane thickness I have generally used in all my papers. I thought its use would make clear that I only intended to imply an order of magnitude. The basis for this was spelled out on page 361 of my paper in 1958 (J Biophys Biochem Cytol 4(4), 349). All I have ever maintained is that all living membranes have an identical pattern of molecular organization and that certain restrictions apply to them. Obviously, this does not require that they be identical in terms of thickness or of features such as the particular molecular species organized in the unit.

** As discussed more fully on pages 45-48.

Robertson next suggested that the unit membrane hypothesis can take account of the many variations in thickness and appearance of cell membranes under the different fixing and staining conditions which have come to light in the past three or four years. With this extension, it seemed to me that he made a very solid point.

The final point that Robertson brought up was that after careful osmium fixation and high resolution microscopy, some membranes appear on close inspection to show not solid black lines, but a fine structure of a granular nature within the dense lines, which earlier had always been visualized as essentially continuous. Robertson, himself, has been able to identify, in synaptic discs, evidence of a significant granularity of structure, in which this dense black line may be visualized as broken down into granules which are not unlike the "elementary particles" seen in negative contrast images, although probably smaller.

In brief, Robertson has accepted the idea, because he has seen for himself, that membranes (even when stained with a classical osmic acid) will show a granular fine structure in the dense lines, which may be analogous to the elementary particles seen by Fernandez-Moran. I think that he has a very open mind on this possibility.

This summation* of Robertson's contribution does not do full justice to his pictures or to the very interesting comments Robertson made later on about other aspects of his recent work.

Variation in Membrane Structure

Next, I want to turn to Fritiof Sjöstrand's contribution. I asked him to speak on the general subject of the variation in structure among different natural membranes -- one of the directions of work in which he has been active -- as well as on the evidence for asymmetry in membranes, and also on evidence for granular fine structure. Indeed, he hit all three points, and his picture can be outlined as follows:

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* See statement by Robertson, p. 75.

Sjöstrand has not been a believer in the unit membrane hypothesis. He made this point very clear at the beginning, saying that the unit membrane hypothesis cannot possibly account for all the variations in structure and dimensions of natural membranes, even with the extension of Robertson's hypothesis mentioned above.

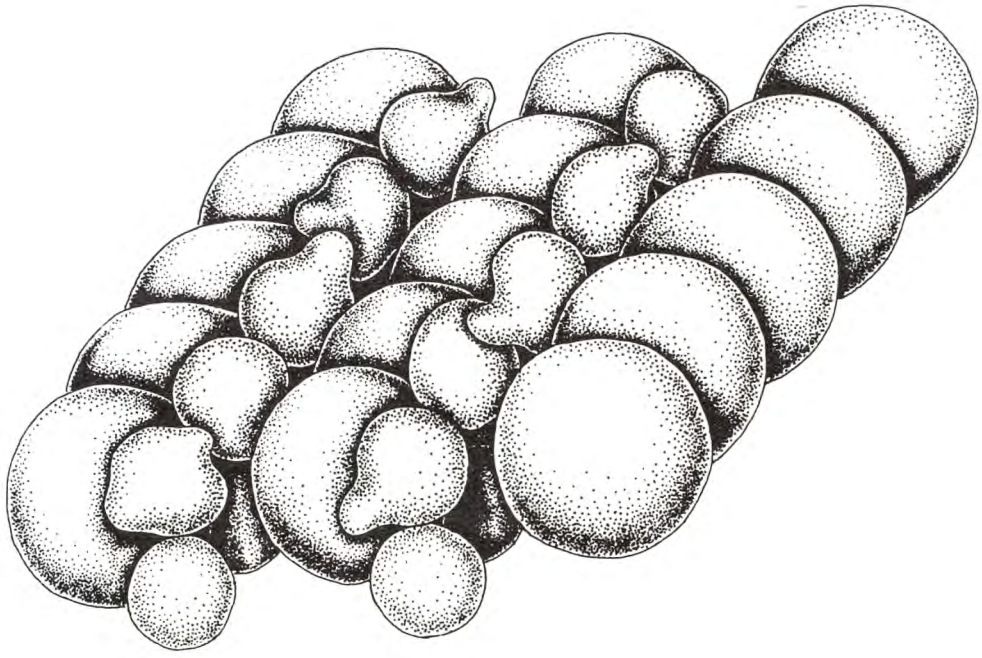
Sjöstrand says that in reality all membranes can be classified into three major groups, which differ characteristically in the spacing of dense lines with osmium: those which have a spacing of

50-60 Å (globular)
60-70 Å (globular membrane as in the Golgi)
90-100 Å (as in the plasma membrane)

Sjöstrand feels that these variations in thickness and the appearance of the images of different kinds of membranes, grouped in these three classes, are due not necessarily to variations of thickness in the phospholipid and protein layers, in the manner visualized by Robertson, but rather to the possibility that membranes of these three different classes might be characterized by having their lipids arranged in different types of arrays. Sjöstrand feels that in some membranes the lipids are arranged in a bilayer; in others, the lipids may be in a micellar arrangement, where separate micelles are arranged adjacent to each other.

Sjöstrand finds very definite evidence of granular structure after high-resolution osmium staining of mitochondrial membrane and smooth-surfaced cytoplasmic membranes. He feels that in this particular type of thick membrane the phospholipid is arranged, not as a bilayer but as a micelle, as shown in Fig. 9, and that the granules are accounted for by arrangement of phospholipid molecules in a series of successive globular micelles in this manner.

Fig. 9. Hypothetical micellar model of membranes.



Large spherical globules represent lipoprotein micelles. Smaller irregularly shaped bodies symbolize enzymes in the respiratory chain.

Sjöstrand also suggests that the protein part of the membrane is arranged around the micelle. So we have a series of globular micelles in the mitochondrial membrane, with other protein molecules arranged along channels formed by the adjacent micelles. In the case of the plasma membranes, on the other hand, he acknowledges the possibility that the lamellar bilayer may characterize the structure of the membrane. A phase transition between micellar and lamellar structure was pointed out as an important factor for dynamic functional changes.

As I'll point out later, Kavanau also feels that membranes in general are capable of phase transitions of the phospholipids between the micellar array and the lamellar array. I'm not sure that Sjöstrand agrees with this, but he feels, at least, that some membranes may be characterized by having a micellar array and others by a lamellar.

Sjöstrand has some beautiful micrographs -- I think he is a real artist -- showing in the same section, with the same staining method, a mitochondrial membrane, a cristae membrane, and a plasma membrane showing the structural differentiation between a mitochondrial membrane and a plasma membrane.

Sjöstrand then challenged the Robertson unit membrane hypothesis on another score. The Robertson hypothesis says that membranes are probably symmetrical. Sjöstrand feels that the plasma membrane is asymmetrical, and under certain staining and fixing conditions, he can demonstrate very nicely (he had some good pictures) that the plasma membrane has one rather dense line, and another line that is much lighter and much less dense. Sjöstrand also had some very good electron micrographs of the surfaces of membranes in frozen-dried material, again showing a globular arrangement.

Structure and Staining

Stoeckenius spoke next on "What stains what?" Stoeckenius is not only a microscopist but also a physical chemist. He has done a great deal of work in the physical chemistry of lipid and protein layers, and of so-called myelin figures. He has been one of the people who

have most effectively correlated physical-chemical study in model systems with electron microscopy. He has also been in the forefront of workers who have attempted to decide what chemical structures are actually stained by osmic and permanganate.

Briefly, Stoeckenius' discussion concerned correlation of X-ray data and electron microscope data on artificial phospholipid bilayers. The point was that X-ray diffraction data can be used as a yardstick for assessing the validity of different fixing methods and staining methods with the electron microscope. The basic issue is the extent to which the normal procedures for fixing such material distort the structure. He showed that these procedures cause a shrinkage of phospholipid layers. The X-ray data indicate for the phospholipid mixture a shrinkage of 10 Å after fixation with OsO_4. This may be partly due to removal of water from between the bimolecular lipid layers and partly to changes in the fatty acid chain structure. Further shrinkage can occur upon exposure of sections to the electron beam in the microscope. The total shrinkage in the thickness of lipid bimolecular layers may vary between 15 to 40 percent.

There was general agreement with Stoeckenius' explanation for the shrinkage of these films through the collapse of the chains. Also, he stated briefly that there is now general agreement that osmium tetroxide stains the hydrophilic portion of the phospholipids -- specifically, the hydrophilic portion of those lipids containing serine, choline, and ethanolamine. These are found in most membranes, in greater or lesser quantities.

Stoeckenius also made the point that myelin figures could be produced containing cytochrome c, which is a member of the respiratory chain. Cytochrome c is a small molecule of 12,000 molecular weight, but it is characterized by having a very high isoelectric pH -- which means that there are many positively charged residues on it. Cytochrome c is known to make complexes of fairly definite composition with certain phospholipids like phosphatidyl ethanolamine, where about 30 molecules of the phospholipid combine with one molecule of cytochrome c.

Stoeckenius showed that when cytochrome c was added to phospholipid systems he got a new structure with a period of 68 Å. Then, when this was hydrated (cytochrome c plus water), it went to 90-100 Å. This type of experiment is a necessary first step in reconstituting an ordered array of protein and lipid in vitro.

Another question that came up concerned the mechanism of the attack by osmium tetroxide on double bonds of the fatty acid chains. Osmium tetroxide is known to add across double bonds, to yield a number of structures that have been isolated and studied. However, Stoeckenius said that under the conditions of normal fixation, the first reaction product of OsO_4 with the double bond is not stable and undergoes further reactions which cannot be specified at present but which involve the hydrophilic groups. Also some of the double bonds may not be accessible to OsO_4.

I supplemented his statement on this by pointing out that a large body of work now exists on the auto-oxidation of double bonds in the phospholipids of natural membranes in their native state. This work indicates that auto-oxidation does not occur very rapidly. Oxygen cannot attack such double bonds unless the bilayers are first disrupted in some manner.

Negative Contrast Staining

Next, I'd like to cover the three talks that were devoted to negative contrast staining -- largely of mitochondrial membranes. I asked David Smith -- who came all the way over from Cambridge, England -- to lead off the discussion on this, because he has taken some beautiful pictures of phosphotungstate-stained images of the membranes of the mitochondria in the flight muscles of insects.

At this point let me sketch in some history. Phosphotungstate negative staining has been employed with great success, in England particularly, in studies by Horne and others* on the structure of viruses. Here it was possible

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* Brenner, S., and Horne, R. W., Biochim Biophys Acta 34, 103 (1959).

to correlate very effectively the dimensions of phosphotungstate images with X-ray data, since these viruses could be obtained in fairly pure form. It turned out that phosphotungstate staining provided a pretty accurate replica of the structure of the simpler viruses -- particularly of those not containing lipids.

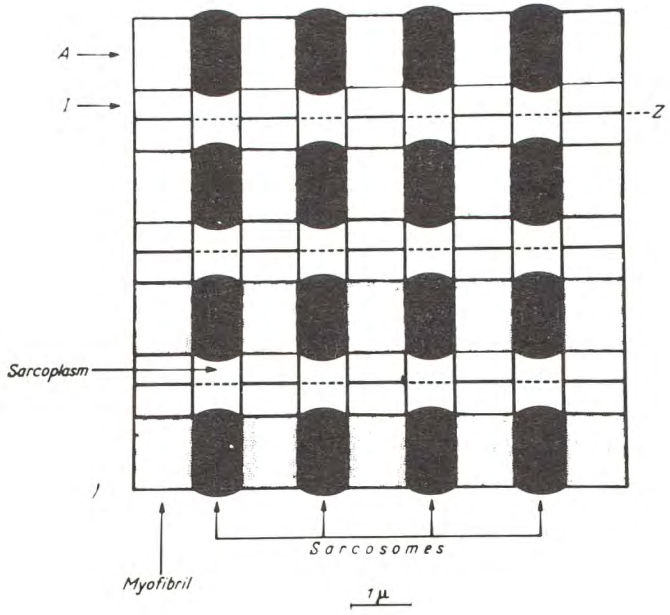
As I said before, the great success of phosphotungstate negative staining of viruses led to the efforts by Fernandez-Moran (and since then in many other laboratories) to apply this method to other biological materials. In general, this sort of staining employs unfixed material. Sectioning is not used, as a rule. The point is to get the material down on a grid in its native form, if that is possible, and to surround it with phosphotungstate, in order to take maximum advantage of the electron density of the phosphotungstate, vis-a-vis the electron lucidity of the biological structure.

By way of introduction to the PTA (phosphotungstic acid) staining of mitochondrial particles, which Dr. Smith recounted, Fig. 10 represents a muscle fiber; at the points where contractile events are supposedly taking place we have the mitochondria. At least, in aerobic active muscles, the mitochondria are located in a rather regular way near the bands of these muscles. In some types of muscles in which there are high rates of respiration and activity, we have such a close and regular juxtaposition that it is reminiscent of a crystalline array. If we cut the muscle transversely, as Dr. Smith showed in some actual pictures of the flight muscles of insects like the blowfly, the dragonfly, and so forth, there is a regular hexagonal array of the mitochondria and the myofibrils.

The flight muscles of insects, I suppose, have the most intensely respiring mitochondria of any known. These mitochondria have an activity that may be ten times as great as those of heart muscle or kidney, which represent the most active mitochondria in mammals.

The higher the respiratory rate, the greater the number of respiratory assemblies in the mitochondrial structure. The assemblies occur very largely in the cristae,

Fig. 10. Muscle fiber



since the cristae offer much more surface than the outer membranes. In fact, the cristae comprise a device to provide maximum surface to accommodate the respiratory assemblies.

Fig. 11 is a diagrammatic reconstruction of the cristae inside a single flight-muscle mitochondrion; Dr. Smith prepared it from his electron micrographs. In the mitochondria here we have an enormous number of cristae stacked in flat fashion. Actually, the spacing between these cristae is exaggerated here, for they are really packed close together, about 70-100 Å apart.

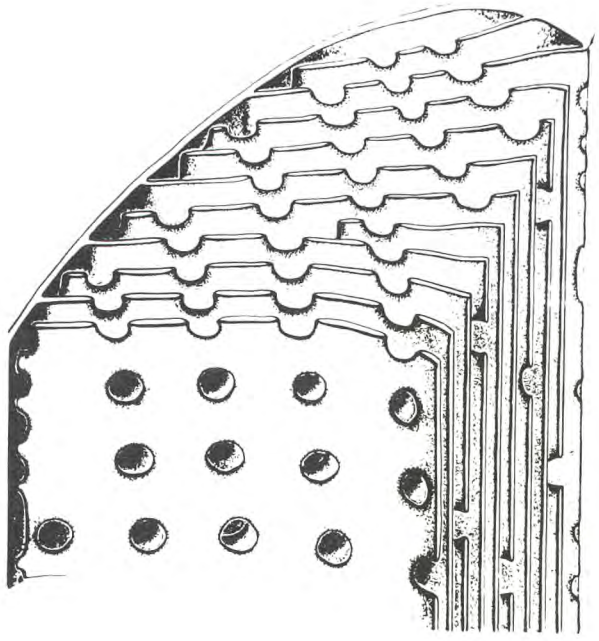
Another interesting thing is that, in addition to all the surface presented by these cristae, there are regular fenestrations going through the stacks of these cristae. I suppose you could pass a needle right through them, if you had a needle that small. These openings are, presumably, devices to facilitate the diffusion of ATP, ADP, oxygen and the other things that have to get in and out of mitochondria in order to take care of the intense rate of respiration.

In preparing such material for observation, it is usually necessary to damage the mitochondria somewhat, in order to open the outer envelope to allow the phosphotungstic acid (which is a large molecule) to get inside. There it surrounds the membrane structure in a glassy matrix.

The cristae themselves are flat, as you saw in Fig. 11, but on exposure to water they apparently degenerate and come apart into tubular structures (Fig. 12). Here you see such a structure embedded in phosphotungstate. You can see the "elementary particles" shown on either side of the membrane and also a central core corresponding to the central space in Fig. 7.

I asked Dr. Smith to show these pictures because they show much more of the fine structure of this inner core than most other pictures that have been published of negatively stained mitochondrial cristae. Dr. Smith's images seem to show that each of the elementary particles appears to be attached by a stalk to the other half of a dumbbell arrangement, the latter being embedded in the core.

Fig. 11. Cristae within an insect flight muscle mitochondrion.

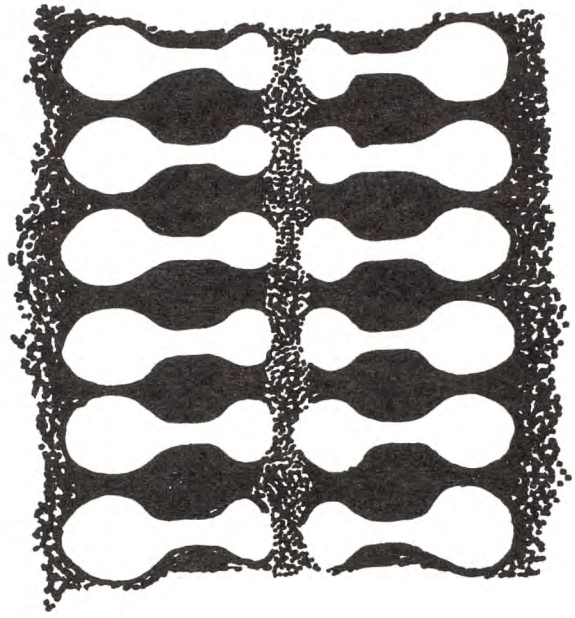


A semi-diagrammatic reconstruction of the three-dimensional organization of a flight muscle sarcosome of the blowfly Calliphora, based on electron micrographs of sectioned material. Note that no attempt has been made to incorporate into this figure the evidence on membrane structure, revealed by negative staining of fragmented mitochondria.

The subparallel cristae are fenestrated, and the fenestrations are aligned to define cylindrical channels within the sarcosome matrix: these channels lie within the plane of section in the upper portion of this "block." As is indicated here, the cristae are linked, at intervals, with the inner limiting mitochondrial membrane, while the outer membrane follows an uninterrupted course around the structure.

N.B. For simplification in the diagram the space between successive cristae and the regularity of the fenestrations have been exaggerated: the actual appearance of these features may be seen in the accompanying electron micrographs in the article by Smith in J. Cell. Biol., 19, 115 (1963).

Fig. 12. Diagram based on an E. M. of a tubule derived from a crista.



- After Smith, D. S., J. Cell Biol. 19, 115 (1963).

The central core, then, has a fine granular structure of its own, made up of globules which are complementary both in size and appearance to the spherical particles outside. These pictures and some more recent ones of Fernandez-Moran's show this fine structure in the core very nicely.

It turns out that the granules are arranged in clusters or rosettes which are more or less regularly spaced, occurring at intervals along the membrane. There are five to a dozen in each cluster, as shown in Fig. 13.

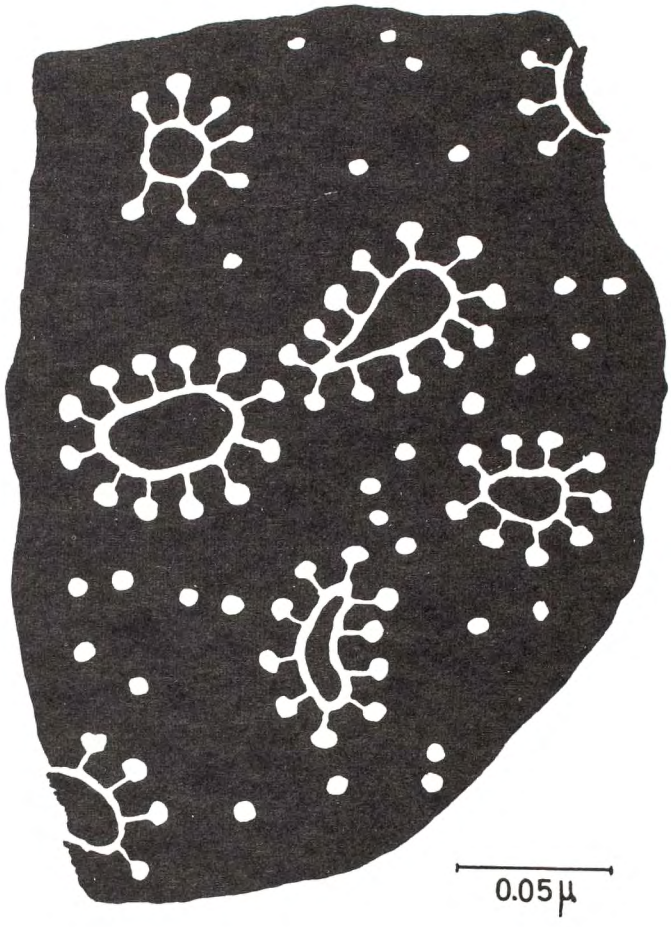
Let me return to Sjöstrand, who was also asked to talk on the subject of negative contrast staining. He was very critical of these negative contrast images when they were first shown by Fernandez-Moran, especially about a year ago at the Gordon Conference. Since that time he has done a great deal of work on his own, and he has been able, of course, to reproduce most of these observations. I visited his lab earlier this year, and he had very beautiful pictures indeed.

However, Sjöstrand flatly announced that the elementary particles "are all artifacts." Fritiof's point is that these ribbons or tubules with these elementary particles, which I've been showing, appear only after drastic damage to the mitochondrial structure. He points out that one has to lyse mitochondria in water to get these images. Furthermore, the elementary particles do not appear immediately after lysis but require some time to develop.

It is the feeling of the other workers also that you need to lyse them in order to get the phosphotungstate into the mitochondria. Sjöstrand showed that the appearance of the particles is time-dependent, and that when mitochondria are exposed to agents such as ascorbic acid and glutathione, which cause the mitochondria to swell, he could take out samples of these and show the gradual appearance of the elementary particles.

We must remember that Sjöstrand has shown with osmium staining that there are big globules in the membrane, so he is not opposed to the occurrence of particles in the membrane. What he challenges is the equating of the granules seen by osmic staining with those seen by PTA staining. I think that this is the central point at issue.

Fig. 13. Surface views of crista showing "rosettes."



- After Smith, D. S., J. Cell Biol. 19, 115 (1963).

Lamellae and Micelles as Phases

Kavanau was the last speaker in this particular session: a mathematician who then went into embryology. He has also worked on animal behavior, and he is now writing a book on membrane structure. Recently he wrote a rather interesting speculative article in Nature.*

One aspect of Kavanau's theory is shown in Figure 14. I've already pointed out the classical evidence for the continuous lipid bilayer in membranes, and on the other hand, I have noted the more recent idea of Sjöstrand and others that possibly lipids might be arranged in micelles.

Kavanau postulates that the lipid phase of biological membranes is parcelled up into micellar subunits. It is proposed that these subunits undergo transformations between forms of complementary geometrical asymmetry during the course of some membrane functions. The form corresponding roughly to the familiar bilayer consists of hexagonally-packed disc-like micelles primarily in the liquid-condensed state, the "closed configuration." Another form, the "open configuration," consists of hexagonally arrayed cylindrical micelles primarily in the liquid state. The pumping of cytoplasmic matrix by membranes, and the contraction of membranes, for example, are achieved by means of a complex series of transitions of the lipid phase and of the protein envelopes during which the lipid phase transforms back and forth between the disc-like and cylindrical micellar forms. In the contracted configuration the cylindrical micelles become closely packed.

The method of pumping of the cytoplasmic matrix by a tubular membranous element illustrated in Figure 14 involves a propagated transformation from the metastable open configuration to the stable closed configuration. During this transformation the matrix between the cylindrical micelles becomes propelled by adhesive drag with the collapsing micelle boundaries. By this means it is force-filtered through one of the membrane envelopes, an action which begins at the point of initiation of the transformation and progresses thence to adjacent regions.

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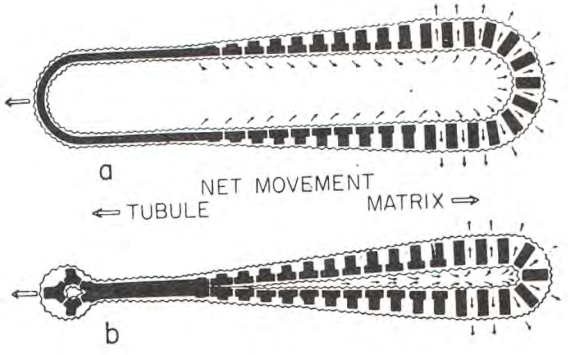
* Structure and Functions of Biological Membranes, Nature 198(4880), 525-530 (1963).

Fig. 14. Kavanau's membrane model.



Cross-sectional diagrammatic representation of membrane transformations and movements accompanying the jet propulsion of two idealized, discrete, tubular, membranous elements. Zig-zag lines depict the protein envelopes, solid black areas the lipid micelles. Small arrows show movement of matrix. (a) Large-diameter tubule (lumen = 550-600 Å) being propelled by intramembranous transformations only. The membrane in regions to the left is in the closed configuration. Matrix 'pumped' into the lumen from between the envelopes egresses through regions between cylindrical micelles of portions of the membrane not yet transforming to the closed configuration. (b) Small-diameter tubule (lumen = 180-200 Å) being propelled by both intra-membranous transformations and the expulsion of luminal matrix on lumen obliteration. The region at the far left has begun the recovery transformation to the open configuration, beginning the intake phase of the next propulsion cycle, while the regions to the right are completing the previous cycle.

- From Nature

Kavanau feels that his hypothetical transition is a biologically occurring phenomenon, and I thought that it would be interesting to have him present this idea, in view of the points I made earlier. You will recall that Sjöstrand thinks that some of the particles in membranes may be micelles; whereas other membranes may contain the lamellar or bilayer arrangement of lipid.

II. BIOCHEMICAL ASPECTS OF MEMBRANE STRUCTURE

The crux of the matter (and the part of the discussion which took the longest!) was the basic question: What is the nature of the respiratory assembly?

I asked Gene Kennedy, a biochemist and specialist in lipid biosynthesis and structure, to summarize very briefly the new developments in lipid biochemistry that are relevant to membrane structure. He made the general point that in the last few years chromatographic methods have been developed for lipid analysis, isolation and identification. These have permitted a more thorough and more complete cataloging of the different kinds of lipids found in membrane structures. We now have available a complete listing of the lipid content of about six or seven different membranes. And, now that we know their organic structure, the preparation of models -- space-filling models -- of phospholipids is possible.

One basic point is that the lipids found in membranes differ a great deal in their structure, hence in their space-filling properties and their charge location, density, and so on. Kennedy didn't actually show molecular models, but I think I can make his point clear by representing them schematically.

Fig. 15 diagrams a series of packing models representing the space-filling properties in fully extended conformation of the different kinds of phospholipids which are normally found in different membranes. It also clearly shows that the phospholipid molecules found in membranes are not all identical in structure.

In phosphatidyl ethanolamine we have a negatively charged phosphate group and a positively charged amino group on the ethanolamine moiety, and, of course, the extended hydrocarbon chains. In phosphatidyl serine we have a

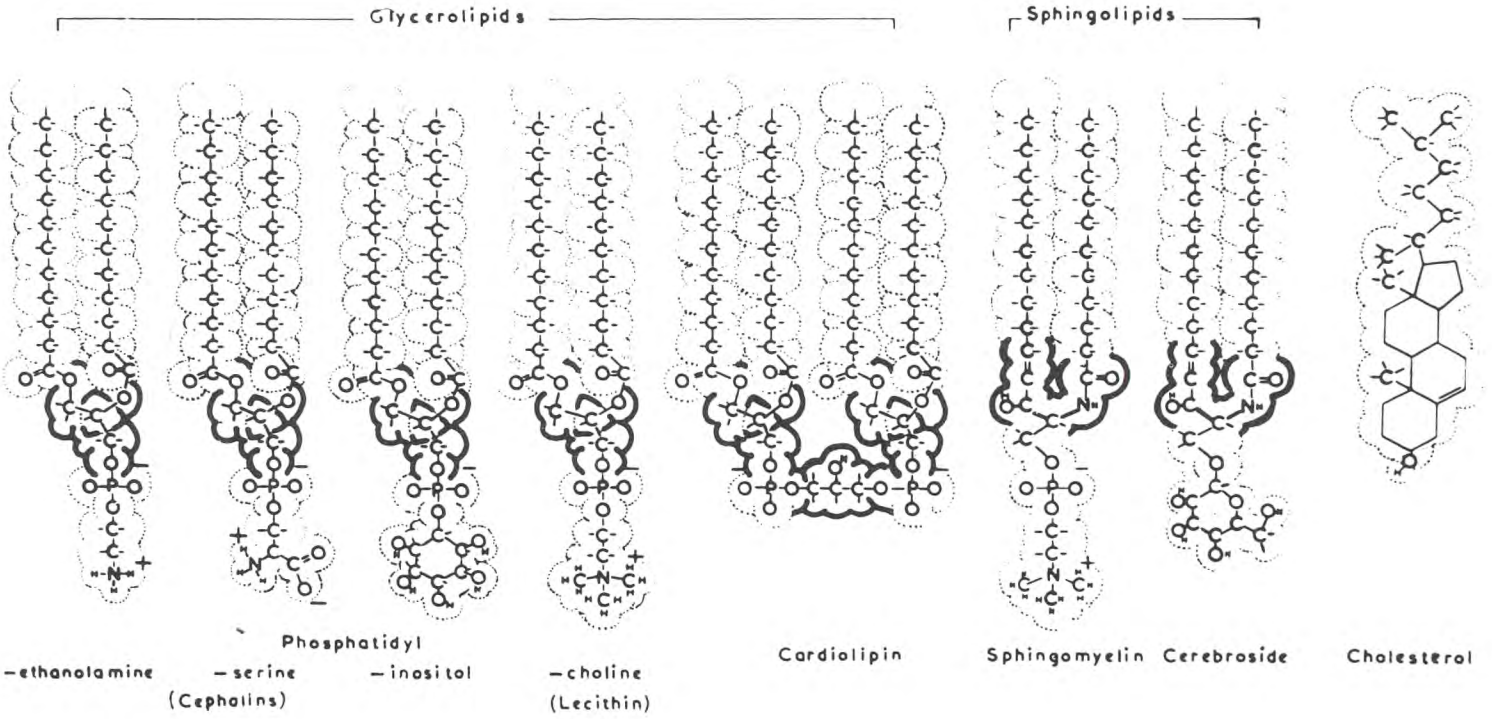


Fig. 15. Lipid models.

serine moiety with a dipolar ion or 'zwitterion' form and a negative charge at the phosphate.

Now with phosphatidyl inositol we have a different thing: we have no charge at all on the inositol moiety, but a large, very hydrophilic molecule--which is rather different in its behavior in an aqueous solution from the ethanolamine or serine or choline building blocks.

The model of the distearoyl lecithin molecule shows the fully extended hydrocarbon chains and a fully extended hydrophilic position. You can see the glycerol molecule and the two fatty acids esterified to two hydroxyl groups of glycerol. There is a phosphate group esterified on the third hydroxyl of glycerol, and then at the end we have choline. There's no net charge on the molecule, since it has a single negative charge at one place and a single positive charge at another.

The fully extended representation in Fig. 15 does no violence to bond angles, but it is a less probable conformation, as has been shown by X-ray data and other kinds of evidence which indicate that the choline folds back on the phosphate.

On the other hand, we have a whole class of molecules called the phosphatidyl glycerol lipids. Cardiolipin is a representative of this class. It is composed of two glycerol molecules, each of which bears two unsaturated fatty acids separated by a third glycerol, which furnishes a "bridge"; they are bonded through two phospho-diester linkages. Obviously, cardiolipin has different space-filling properties from the other lipids. It has a net negative charge, whereas in phosphatidyl choline, of course, the two charges cancel out and we have no net charge. So, specific lipid molecules not only occupy specific geometries in space, characteristic for each type of lipid, but can also differ in net charge.

The less-well-understood lipids are the sphingomyelins and cerebrosides. Fig. 15 contains a representation of their structure and that of a neutral lipid, namely cholesterol.

Kennedy makes the point that the phospholipid composition of different membranes varies considerably. That is, mitochondrial membranes, erythrocyte membranes, and protoplast membranes of bacteria vary considerably in lipid composition. In the latter we have an extraordinary situation in some species where cardiolipin or its derivatives may make up 50 percent or more of the phospholipids in the membrane.

Kennedy stressed the point that we might look on the lipids as having two major types of functions:

The first would be a structural one, in which the space-filling properties, the charge location, and so on, are the primary elements in determining the stability of the lipid bilayer or micelle. The second contribution made by the phospholipid portion of the membrane would be that one or another phospholipid may have a very specific enzymatic function. There are one or two cases in point. In mitochondrial swelling and contraction it is known that phosphatidyl inositol is a specific component of the machinery that brings about the swelling and contraction phenomena.

Structural Protein

The next part of the discussion dealt with the "structural protein" of the mitochondrial membrane. David Green and his colleagues have done most of the work on this. What they have done, in short, is to find that 50 percent or more of the total protein of the mitochondrial membrane is apparently made up of a single kind of molecule, which recurs in identical fashion in the membrane in a manner similar to the recurring protein unit we see in the sheath of the tobacco mosaic virus.

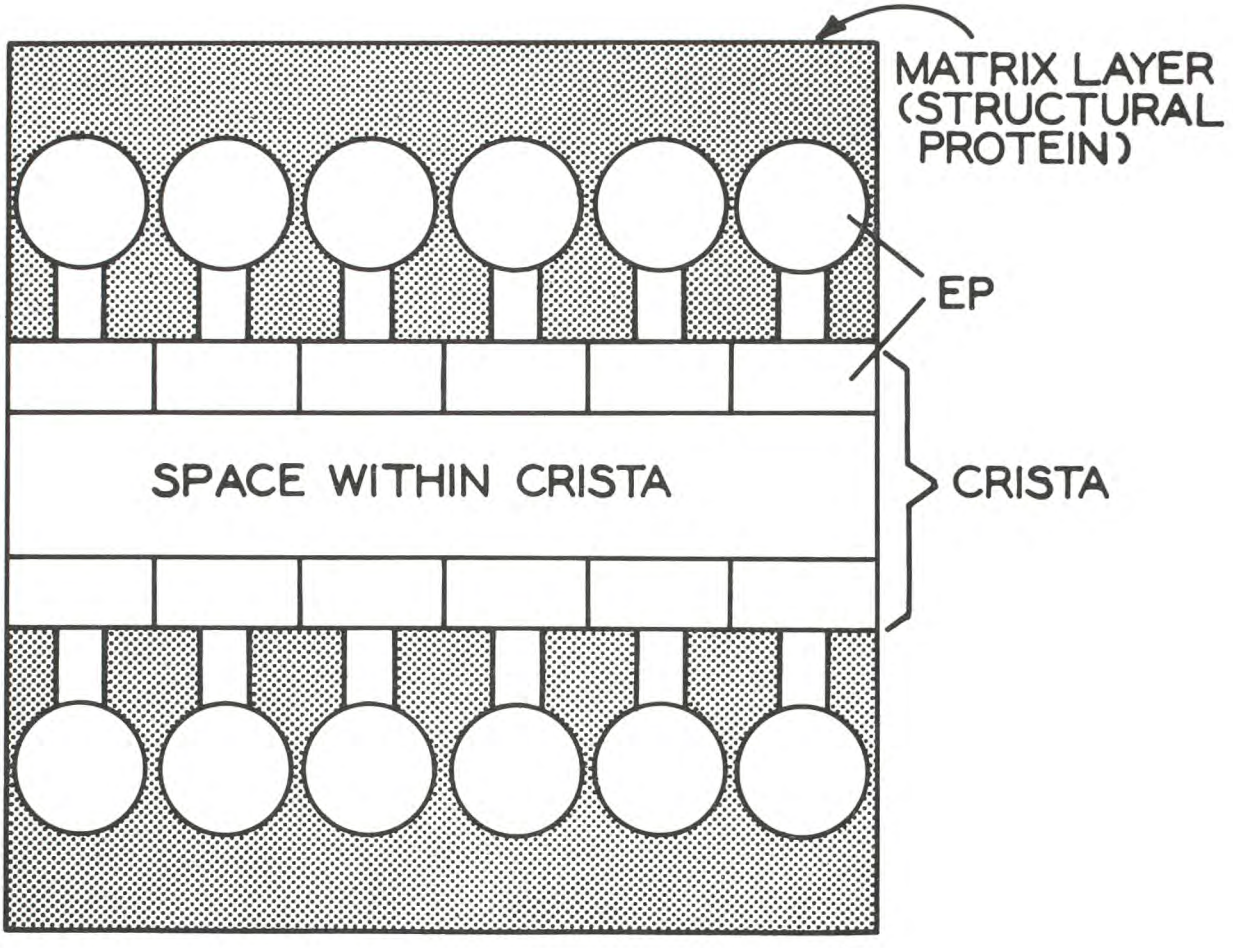
This so-called "structural protein" of mitochondria was described briefly by Green. It has a recurring monomer, with a molecular weight of about 22,000. They have worked out the amino acid content of this, and there is nothing terribly remarkable about it. It looks like almost any other protein. Structural protein is very insoluble. The monomer of this protein is not soluble at pH 7, but it can be gotten into solution at a high pH, or in the presence of detergents.

The chemical evidence for identity of these recurring units is not yet satisfactory, but the chances are very good indeed that a single protein will be found which repeats, and forms the major protein fabric of the membrane from this monomeric unit. A question arises as to the bonding between these monomers. Green has some evidence that the monomeric units are bound together by hydrophobic bonding. His basic line of evidence is that structural protein cannot be brought into solution by raising the ionic strength. On this point he was severely challenged by a number of people in the group. This point is still unsettled, but it is very likely that hydrophobic bonding will be found to provide the major force that is holding these monomers together.

Structural protein -- we may abbreviate it as SP -- is capable of combining with two other components of the membrane. It can form complexes with phospholipids such as lecithin, which are fairly specific in their stoichiometry. The structural protein can also form complexes with components of the respiratory chain, like cytochrome $\underline{c}_1$ or cytochrome $\underline{b}$. Green concluded that structural protein is central to the holding together of the whole mitochondrion through hydrophobic bonding forces according to the scheme in Fig. 16.

Recently, a new idea has been developed about membrane protein. Work by Ohnishi and Ohnishi has shown that there is a contractile protein in mitochondria with properties

Fig. 16. Green's representation of membrane structure.*



* CML (Ed.): This Figure was provided by Green, March 1964.

very similar to actomyosin. Their work seems to suggest that the structural protein has actomyosin-like properties, in that there can be changes of conformation produced in such complexes in the presence of ATP. So, structure protein can conceivably be equated with the so-called contractile protein of mitochondria.

The amount of contractile protein that can be separated from the mitochondria is rather large; it is of the same order of magnitude as the amount of structure protein.*

Green gave some figures for the ratios of some of these SP complexes. In beef heart mitochondria, there is a relationship between the total number of phospholipid molecules and the structure protein molecules of eight to one. There is a relationship of structure protein molecules and "elementary particles", such as we find by negative staining, of 120 to one.

This summarizes very briefly, I think, the gist of David Green's discussion of structure protein.

Arrays of Phospholipids

Now let's turn to the subject of lipid arrays. Thompson spoke very briefly here to the general point that phospholipid molecules in aqueous systems will tend to stabilize in certain specific molecular arrays. These have been under considerable study in the last few years, particularly with highly purified lipids that have not undergone autoxidation.

In recent years a number of workers, particularly Luzzati in France, have made some rather significant studies of the different phases of phospholipid-water systems. Two of these systems are illustrated diagrammatically in Fig. 17.

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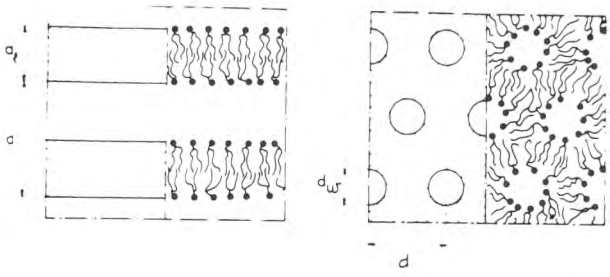
* Schmitt: This is quite important in the neuronal case, because we know very well, from a variety of forms of evidence, that there is a protein -- a structure protein, if you like -- associated with the membrane, as has been mentioned here.

Fig. 17. Phospholipid-water systems.



LAMELLAR
$d_l \tilde{=} 46$ Å
$\%H_2O \tilde{=} 30$

HEXAGONAL
$d_w = f(c) = 8\text{-}40$ Å
$\%H_2O \tilde{=} 3$

LIQUID CRYSTALLINE PHASES OF PHOSPHOLIPID-WATER SYSTEM (LUZZATI & HUSSON, 1962)

These were not shown at this meeting, but I thought they might be helpful in making clear what I mean by the different phases, which are simply the arrangement of the molecules that are most stable under a given set of conditions. To be brief, Luzzati showed that there are about three or four relatively stable arrays in which phospholipids and water molecules can be arranged, depending on the degree of hydration. There is a lamellar array, a phospholipid bilayer system. There is also a so-called hexagonal array, in which we have the polar parts arranged in the form of a tube. But there are still other ways to pack these molecules to make stable arrays, depending on the water content. In the "hexagonal"array there is a very low water content of only three percent, and for this reason this type of array is not a favored one in biological systems, which are much more highly hydrated. There is evidence that membranes are highly hydrated indeed, so that the lamellar or micellar arrays are probably the preferred conformations of phospholipid-water systems in biological materials.

This summarizes what Thompson contributed here, though he also had new experimental information on this that I will describe later.* Other workers active in this field are Bungenberg de Jong, Overbeek and, of course, Stoeckenius. A picture is emerging, revealing that there are several different kinds of phospholipid-water phases, some of which are relevant to biological membranes.

So much for what is perhaps too brief a condensation of what is a very important and central piece of physical chemistry relating to membrane structure.

Enzyme Organization

The big argument that occupied us most of the time in this second part of the Work Session was the question of the geometric organization of the respiratory enzymes in the mitochondrial membrane. We were able to consider this question in the light of the new information on membrane structure revealed by negative staining methods.

Here then are the arguments regarding the conformation of the enzymes in the respiratory assemblies, and how they are attached to or embedded in the membrane. Some years

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* See pages 61-66

ago, as I mentioned earlier,* I postulated a flat, planar array of these enzyme molecules on the surface of the unit membrane, as visualized in terms of Robertson's hypothesis, either in the surface or on the surface of the unit membrane. More recently, with the availability of the information on the phosphotungstate images of mitochondrial membranes, some different conformation of the respiratory assemblies can be visualized. If it is accepted that the elementary particles are real, then there are two different views that have been expressed as to their identity.

Thus, Green and Fernandez-Moran have postulated that all the enzymes comprising the respiratory assembly are packaged into the elementary particle and that each one of these EP's then is a functional molecular machine.

On the other hand, Chance favors the view that each of these particles contains not a whole assembly, but only a single carrier molecule, so that several adjacent EP's comprise an assembly. He suggests that the EP's knock against each other, or collide, while still attached to the stalks. They are like pingpong balls attached by short strings, knocking back and forth in thermal motion. Actually, such a swinging arm has been postulated in certain enzyme systems, and has a reasonable molecular basis.

What is the experimental evidence that bears on the validity of one or the other view? One question is, "Can a single elementary particle of known dimensions contain all the known molecular components of the respiratory chain?" In summary, Green says that the particles are just barely large enough to accommodate all known members of the respiratory chain. On the other hand, Chance says that they are not large enough to accommodate the whole assembly.**

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* See page 23.

** TM, CML (Eds.): That a semantic ambiguity may have existed at the Work Session seems implied by the following quotation from a later paper coauthored by Green about publications (abstracted on page 84) coauthored by Chance: "When Chance, Parsons, and Williams submitted their manuscripts for publication, they were unaware of our evidence for the tripartite structure of the particle as seen *in vitro*. They assumed that the head piece, alone, was being equated with the electron transfer unit. As mentioned before, the discrepancy in size no longer applies to the tripartite elementary particle."

Green made an accounting of the molecular weights and ratios of the components at the Work Session. I have his actual numbers here -- and it's on these numbers that some controversy exists. Before I go further, I must make a few remarks about the dimensions and content of lipid and protein in these elementary particles, as deduced by Green from particles isolated from detergent and sonic-treated mitochondria. By such treatments, Green has been able to isolate respiratory particles which are somewhat larger in diameter than the elementary particles seen on the intact membrane. It is this apparent similarity in size which is their main criterion for identifying respiratory particles with "elementary particles."

The isolated particle has a molecular weight, calculated on the basis of the lipid and protein content, about the same as the particle's weight extrapolated from electron micrographs of the intact cristae, namely about 1.4×10^6.

Let's have a summation here, as Green presented it, of the molecular weights of the different components. In the first place, Green has suggested that the elementary particles contain only the respiratory chain enzymes that carry electrons from substrate to oxygen, and nothing else. This is a minimal statement of what a respiratory assembly is.

There are other workers in the field who feel that the respiratory assembly may be bigger and more complicated, containing coupling factors and the other enzymatic machinery necessary to make ATP. Now, if we look at a catalog in Fig. 18 of the molecular weights and the number of components, we have 80,000 for succinic dehydrogenase, 37,000 for DPNH dehydrogenase, and other molecular weights as listed.

Now, there are two basic bones of contention in this accounting. In the first place, other workers would not agree with these figures for succinic dehydrogenase and DPNH dehydrogenase. In fact, these figures are from unpublished work of Green's; we don't know the details. But suffice it to say that succinic dehydrogenase, in the hands of Singer and other investigators, has been found to have a molecular weight of 200,000, rather than 80,000. The figures for the molecular weight of DPNH dehydrogenase range from 1,000,000 for Singer's enzyme to 100,000 for King's enzyme, whereas Green's figure is 37,500.

Fig. 18. Molecular weights of the components of respiratory particles, as given by Green.****

Protein Component (bound components only)	Molecular Weight	Number of Molecules	Total Contribution to Particle Weight
Succinic flavoprotein*	80,000	1	80,000
DPNH flavoprotein*	37,500	1	37,500
Cytochrome a	70,000 (40,000)**	6	420,000 (240,000)
b	28,000	3	84,000
c_1	37,000	1	37,000
Nonheme iron protein	25,000***	9	225,000
			883,500 (703,500)

* Extrapolated value from composition data of molecular complexes in which the flavoprotein is linked to other proteins.

** Lower value in parenthesis is based on an unpublished estimate of Richard Criddle of the University of California in Davis.

*** There are 18 atoms of nonheme iron per chain - all protein bound. One protein has been isolated with 2 atoms of iron per molecular weight of 25,000. It has been assumed that the other 16 atoms are associated with proteins of the same molecular weight and with the same Fe:protein stoichiometry.

**** CML (Ed.): Including corrections by Green, March 1964.

For the other enzymes, cytochromes b, c_1, c and a, the molecular weights given by Green are traditional values.

However, a related bone of contention is involved. Green has defined a new component, "non-heme iron-protein." He suggests that succinic and DPNH dehydrogenase contain non-heme iron in the more native forms of these enzymes, at least the forms isolated by Singer and by King. Green suggests the non-heme iron is a part of specific enzyme molecules which can be separated from the dehydrogenases. He suggests they can act as electron carriers; however, the way in which they interact is not clear because none of this work has been published.

You may wonder, "Can't physical chemists and biochemists measure molecular weights accurately?" The problem is something like this. When you tear these enzymes out of the membrane you may really wreck them rather badly without destroying catalytic activity. The isolated enzyme may contain some molecular moieties not necessary for catalytic activity, for example, but necessary for binding to the assembly. On the other hand, the form of the enzyme with the lowest molecular weight is not necessarily the most native form. These are the major discrepancies in succinic and DPNH dehydrogenase.*

Now, for the major bone of contention. Britton Chance, by spectroscopy of the respiratory pigments in different kinds of intact mitochondria, has found that the cytochromes exist in a simple molar ratio with each other: approximately 1:1:1:1:1. This has been confirmed in many different kinds of tissues by Kingenberg and his associates in Marburg, Germany.

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* Green: Singer is talking about f_S or f_D linked to other proteins. I am talking about f_S or f_D freed from these other proteins. In fact some of our estimates of the molecular weight of f_S can be made directly from the data of Singer by assuming a molecular weight of 25,000 for the protein associated with each two iron atoms in the complex. The activity of the pure f_S or f_D has no relevance to the question of molecular size.

However, the data Green assembled for the purpose of computing the particle weight of the respiratory assembly showed rather different molar ratios of cytochromes. He said that there are three cytochrome b molecules to one of c_1* and six of cytochrome a to one of cytochrome c_1.

Chance's measurements have some error because it is a problem to determine true light absorption in systems that scatter light heavily. Chance has not convinced everyone that his molar absorption indexes for the carrier molecules are really correct for their native state in the intact mitochondrion. However, they are the best approximations that can be made. It seems unlikely that the errors will be as great as six-to-one.** I think there are enough pieces of evidence -- internally consistent pieces of evidence, in the case of Chance's work, which has been supplemented by direct chemical analysis -- to indicate that the one-to-one ratio is essentially correct. Another consideration follows:

Last year, Gibson and Greenwood and also Green and his colleagues arrived at the conclusion that cytochrome a is not a simple monomer: it is probably a hexamer in its active state. Thus, there are six subunits, each with a molecular weight of 70,000. Four of these are cytochrome a; two are cytochrome a_3. However, the monomer weight of 40,000 given now is much smaller than the 60-70,000 figure arrived at by earlier work.

Now, if cytochrome a is a hexamer, then all the other cytochromes have to be hexamers, if Chance's spectroscopic data are right. Of course, if they are all hexamers, then you can't possibly fit all of these cytochromes into one particle.

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* Green: Only cytochrome c_1 should be used as the basis of comparison since cytochrome c is not a fixed component of the chain and it can be absent on a particle with an otherwise complete electron transfer chain.

** Green: The 6:1 versus 1:1 discrepancy is a consequence not of experimental errors but of the validity of assumptions with respect to absorption coefficients and what is being measured.

The figures Green has chosen for the molecular weights of the different carriers and for their ratios are, therefore, under question. If we add them up the way Green presented them they come to 883,500; and if we now add in this 30 percent lipid, which he says is in these particles, it will add up to something like 1.1×10^6, which can fit into the volume of a single elementary particle.

As I said, these figures are not accepted by everyone. Most investigators will feel that they are actually quite a bit larger. We will simply have to wait for Green's evidence.

There is still at least one other position that can be taken, and it is a position that I prefer to take myself: I think that neither representation is correct. There are all kinds of artifacts; they range from 100 percent artifact to 2 percent artifact. There is no way of fixing tissue that is going to produce a system that is 100 percent free of artifacts. There will always be some artifact along the line.

I would like to suggest that maybe there has been a disruption of membrane structure here, and that the conformation of these respiratory assemblies may, in fact, be different. It could be planar, or tetrahedral, or it could be anything else. They don't necessarily have to be packaged like this.

It is important to remember that there is an important and basic reason for believing that assemblies of enzyme molecules exist; namely, to facilitate collisions. Electron transport proceeds through collisions of large and slowly-diffusing protein molecules, and not through small-molecular-weight substrates and particles. In free, true solution, they are not going to move fast enough to collide frequently enough to give us the known rates of respiration. Thus, we must have the molecules packaged close together to facilitate fast reaction.

The only alternative is that there are electron or energy transfers in these assemblies through non-classical, non-collision mechanisms, such as those taking place in solid-state semiconductors. This has, in part, been considered,

but Chance has pointed out that the temperature dependence of both respiratory rate and ATP production has the Q_{10} of about 2.0. Furthermore, the kinetics of each individual step of electron transfer, which have been worked out so beautifully by Chance, show that every parameter agrees with classical collision kinetics. There is, therefore, no necessity to invoke solid-state, temperature-independent reactions.

It seems to me that this summarizes Chance's arguments, perhaps too tersely, but I want to add one more point which I can reconstruct very briefly. The two representations of Chance and of Green, as they are now presented, in my opinion do not provide enough asymmetry of organization to account for the asymmetrical reactions known to take place in mitochondria. In the first place, it is known that there is a directionality about the formation of ATP and the utilization of ADP. Second, the active transport phenomena are polarized across the membrane. You need to have asymmetric organization for the membrane enzymes in order to utilize their active sites as pumps for ions. Further, we have a directional contractility in the mitochondrial membrane. Again, this requires an asymmetric arrangement of the respiratory assembly which is providing the energy for the contractility. So in my opinion, the respiratory assembly must be ultimately organized so that the active sites of some of the enzymes are oriented specifically, to account for the asymmetrical reactions.

III. PHYSICAL CHEMISTRY OF LIPID BILAYERS

I can finish up with Thompson's contribution to our discussion of the physical chemistry of membrane systems, and especially of lipid bilayers.

I had developed the notion, in constructing the Work Session, that at this stage of the game in molecular biology it might be useful to consider membranes in the same way Pauling and others have considered protein structure. If you know the structure of the amino acid building blocks, as well as that of the peptide bond, then you are able to make deductions on the structure of polypeptides and proteins. This is actually what happened in the history of protein chemistry.

X-ray diffraction analysis was first done on simple things, and then we worked our way up.

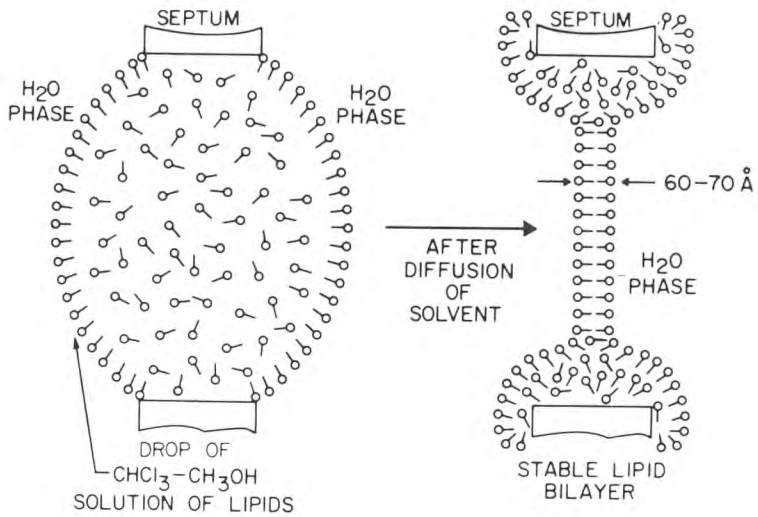
Secondly, a lot is being learned about protein structure by the study of polyamino acids. Now Ephraim Katchalsky has done important work in making polymers of single amino acids. The physical properties of these polymers have been very revealing in working out the forces which stabilize the true proteins. In the same way, if we had information on how a single phospholipid building-block unit can arrange itself in an aqueous system, and study such stable arrangements thoroughly, we might be able to deduce the basic physical principles which stablize more complex phospholipid-water systems. Then we could utilize this information to reconstruct the more complicated systems that arise when we have 5, 10, or 20 different types of phospholipid molecules in a membrane, tógether with many different proteins.

Now I'll use another analogy: The many different types of phospholipids which are found in different kinds of biological membranes may be analogous to the twenty different amino acids from which all proteins are constructed.

With a series of common building blocks, an extraordinary variety of protein molecules can be constructed; similarly, the phospholipids may be the alphabet of membrane structure. Thompson has studied a reconstructed lipid bilayer membrane prepared by the method first described by Rudin and Mueller. The system consists quite simply of a two-compartment vessel arranged in a thermostated bath, with a septum of polyethylene which separates the two compartments. There is an aperture of one to two millimeters in this septum. Each side has an 0.1 M solution of sodium chloride stirred with magnetic "fleas." The lipid bilayer is constructed in the aperture. Fig. 19 indicates the procedure used. He takes a chloroform-methanol solution of highly purified egg lecithin and tetradecane, or some other neutral fatty molecules, the nature of which I will specify later. This dilute solution of the two lipids in chloroform-methanol is brushed across the aperture in the septum with a camel's hair brush, which leaves behind a drop of chloroform-methanol containing these lipids. This drop now has on each side an aqueous phase of 0.1 M sodium chloride. At the interface, we have the hydrophilic parts of the phospholipid molecules oriented toward the aqueous phases on each side, and the hydrophobic portions inward. Inside the droplet, the phospholipid molecules are randomly oriented.

Now as the chloroform and the methanol diffuse out of this organic phase into the aqueous phase on each side, the droplet becomes thinner. This diffusion process which results in the formation of a thin membrane is followed by observing the optical interference produced by light passing through the two surfaces; the color depends on the thickness. After all the solvent has diffused out, the two interface surfaces come together to form a stable bilayer system, the thickness of which is measurable by suitable optical methods. In this membrane, the hydrocarbon moleculés face inside to form a smectic or neat phase. There is a torus of the lipid solution around the rim of the aperture; it contains lipid molecules that have been in solution in chloroform-methanol. The bilayer that is formed here is stable for an hour or two so that optical and electrical

Fig. 19. Construction of a smectic lipid bilayer.



measurements may be made on it with some accuracy. It is believed that this is a true, smectic phospholipid bilayer, since it would be expected that the hydrophilic ends of the lipid molecules would be exposed to the aqueous sides of the system, while the hydrocarbons would be inside.

What kinds of measurements can be made on these membranes? Fig. 20 summarizes the data.

The thickness of the lipid bilayer has been determined by a complex optical method which took a long time to work out. The latest figure is 61 plus or minus 10 Angstrom units. This is a little thicker than one would expect for a phospholipid bilayer system, and there was some discussion as to why it should be thicker. Possibly there are still some stray lipid molecules within the bilayer. Or perhaps there is some chloroform and methanol solvent still left in the layer.

Electrical resistance and capacitance were measured, and a comparison was made between these measurements and those on natural membrane, with findings of surprising consistency. Capacitance is in the range of natural mitochondrial membranes. The surface tension is also similar to that of natural membranes, but perhaps at the lower end of the range. He suggested that the surface tension might be altered if a protein were on that layer. The water permeability has been measured with tritiated water. It is about the same as that of natural membranes.

Thompson points out that high water permeability coupled with a very high electrical resistance means that this membrane is freely permeable to water, but probably not freely permeable to charged molecules. Another significant point is the high breakdown voltage of this synthetic bilayer.

It has been known for a long time that natural membranes have breakdown voltages, or can sustain potentials, of this order of magnitude. Thompson made the specific point that these phospholipid bilayers have a high breakdown voltage in relation to their thickness. This is a much greater

Fig. 20. Thompson's measurement of smectic phospholipid bilayers.

	Natural membranes (20-25°C)	Bilayer membranes (36°C)
Thickness of lipid bilayer, Å	40-45 (1)	61 ± 10
Resistance, Ω cm^2	10^2-10^5 (2)	$(0.2\text{-}4.0) \times 10^6$
Capacitance, μf cm^{-2}	1.1-1.3 (3)	0.75
Surface tension, dyne cm^{-1}	0.03-3.0 (4)	0.5
Water permeability, micron sec^{-1}	0.25-58 (5)	3.5
Dielectric breakdown, mV	100 (6)	200 ± 20

(1) W. Stoeckenius (1962), "The Interpretation of Ultrastructure," Ed. R. J. C. Harris, pp. 349-367, Academic Press, Inc., New York.
(2) K. Cole (1940), Cold Spring Harbor Symp. Quant. Biol. 8, 110-122.
(3) H. Pauly and L. Packer (1960), J. Biophys. Biochem. Cyto. 7, 603-612.
(4) E. Ackerman (1962), "Biophysical Science," pp. 236-238, Prentice-Hall, Englewood Clifts, New Jersey.
(5) H. Davson and J. Danielli (1952), "The Permeability of Natural Membranes," 2nd Edition, p. 111, Cambridge Univ. Press, London.
(6) A. M. Shanes (1958), Pharmacol. Rev. 10, 59-164.

figure than it would be for a porcelain film of the same thickness, so that, as an insulator, the phospholipid bilayer is truly an extraordinary structure.

We invited Rudin and Mueller to come to the Work Session, but they were unable to do so. They originally devised this system for making the membranes; however, their membranes were not made from single, well-defined components, but rather from a mixture of total brain lipids. They were able to show that it had a limiting thickness of the same order of magnitude as natural membranes. Now, if they added protein factors (of unidentified nature) to such membranes, it conferred electrical excitability. They showed some very startling records of potentials developed in these membranes on application of these materials of stimulation, and so on. They have had great difficulties in reproducing this work because they are not able to find the source of egg powder which contains the factor.

To come back to Thompson's experiments again: he has found that if he measured the electrical resistance of the bilayer as a function of temperature, he got extraordinary effects. On lowering the temperature to 30° from 37°, the resistance suddenly dropped in a discontinuous fashion to a point at 28. And then, on lowering the temperature more, it went back up again in a regular fashion. When it hit 20 degrees, there was again a sudden discontinuous drop, and then it started to rise. To explain these phenomena, Thompson suggested that there are phase transitions occurring in this membrane as a function of temperature. He hasn't done this yet, but he is going to get to work on the optical properties as well, and on the thickness, as a function of temperature. It seems to me that this is a very promising system, then, for studying slight perturbations in the phases of the phospholipid bilayers.

Finally, I must say that we never really got down to ways and means of coding memory, or of switching devices at synapses in this discussion, except for a few minutes at the end. In brief, the two-dimensional surface of a membrane, with its many different phospholipids arranged in a mosaic and containing also different kinds of proteins, offers extraordinary possibilities for two-dimensional coding of memory.

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SUPPLEMENTARY STATEMENTS

I. ON QUESTIONS OF MITOCHONDRIAL ORGANIZATION AND THE ELECTRON TRANSFER CHAIN*

by
David E. Green

I think the purposes of the work session would be defeated if I did not register my dissent with the summary of the chairman insofar as it deals with the presentation of our own contributions. The chairman has marshalled his reasons for preferring his own representation of the electron transfer chain and of mitochondrial organization. To my recollection these speculations of the chairman were hardly discussed at the work session. As everyone present will recall there was an animated controversy between Britton Chance and myself with respect to some fundamental issues, and I fail to find a clear statement in the summary as to what these issues were. I would like, therefore, to complete the record by spelling out the fundamental areas of disagreement and the issues at stake.

Structural Protein

The fact of the existence of a structural protein accounting for a high proportion of the total protein of the mitochondrion is now generally accepted. In our definitive publication on this protein (Criddle *et al.*, 1962) we have presented evidence that the protein is homogeneous by all criteria tested and that the various interactions (polymerization, complex formation with myoglobin and with cytochromes *a*, *b* and c_1, interaction with lipid) can be accounted for predominantly if not exclusively in terms of hydrophobic bonding. The chairman has indicated that he is not convinced that our evidence for homogeneity of the protein or for hydrophobic bonding of the units in the polymer is complete. We will accept this reservation but it should be noted that the chairman had no specific reasons for challenging our conclusion of homogeneity other than the fact that we were unable to find an N-terminal amino acid in the mitochondrial structural protein (a property widely shared by proteins of the same class).

The possible identity of the structural protein with the contractile protein discovered by Ohnishi and Ohnishi in mitochondria has been suggested by the chairman. We now consider this to be highly unlikely. The contractile protein can

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* Title supplied by editors.

be isolated from mitochondria under conditions in which structural protein is not extracted. Moreover, the yield of contractile protein extractable from the mitochondria of beef heart muscle is considerably less than that of structural protein (one-tenth to one-fifth).

Localization of Structural Protein

The localization of the structural protein-lipid network in the mitochondrion has proved to be a baffling problem. We were aware that the electron transfer chain can be chemically dissected from the matrix but precisely where the matrix is localized was not readily deducible from the electron micrographs of mitochondria. The electron micrographs of F. Sjöstrand obtained by $KMnO_4$ staining of mitochondrial sections appear to provide a clear answer to this dilemma. These micrographs point to the localization of the structural protein matrix layer on the interior side of the cristae. It is as if the cristae were fingerlike protrusions into the matrix layer. The mitochondrion could then be visualized as a matrix material around which were wrapped a double membrane envelope with the inner membrane layer periodically invaginating into the matrix in the form of cristae.

The Elementary Particle

When Fernandez-Moran and I first described the elementary particle in mitochondria we were aware of a spherical repeating unit, 80-100 Å in diameter, but were uncertain of the connection of this spherical unit to the membrane layer. Since this first description we and others (D. Smith, W. Stoeckenius, D. Parsons) have recognized that the repeating unit is tripartite, consisting of the 80-100 Å sphere noted above, an ovoid base piece, 115 Å by 45 Å, and a cylindrical stalk connecting the sphere and base piece, 55 Å by 30 Å. The combined volume of these three parts of the elementary particle would correspond to a unit of 1.4×10^6 molecular weight if one assumes a density of 1.25 (calculated for some 30% lipid by weight) and an underestimation factor of 12% (from the studies of Bangham and Horne). The tripartite structure we call the elementary particle is of the right size to accommodate a complete electron transfer chain. This correspondence of size admittedly does not prove that the particle <u>is</u> the housing for the chain. But in our opinion the issue is no longer one of size but of evidence for assignment. The issue to be resolved is whether

the elementary particle as visualized by electron microscopy has anything to do with the electron transfer chain. Our evidence for the identification is still only indirect. But as yet no conclusive evidence to the contrary has been presented.

Separation of Headpiece from Elementary Particles

There have been a series of reports from the laboratories of Chance and Parsons and from that of Crane that the spherical headpieces or knobs of the elementary particles can be detached from the base pieces by sonication and that these detached knobs do not contain the components of the electron transfer chain. We have examined very carefully the experiments reported by these laboratories and carried out an extended investigation designed to evaluate these experiments. We have already reported in Information Exchange Group memo no. 96 on our findings. We have failed to find any evidence that sonication of ETP under the conditions of Chance et al. (1963) or Crane and Stasny (1964) can fragment the electron transfer chain or that a colorless component can be separated from the chain under these conditions. We have been able to show that the fraction with the so-called detached knobs is rich in primary dehydrogenases which may have their origin in the outer mitochondrial membrane. We have concluded that the question whether the knobs do or do not contain components of the electron transfer chain is still open since a positive identification of the particles isolated by Crane and Stasny as the knobs seen on the cristae has not been made.

The Relation of the Elementary Particle to the Electron Transfer Chain

While the evidence is highly suggestive that the elementary particles contain the electron transfer chain, it cannot be excluded that other elements are present in the elementary particle. We know, for example, that there is an intimate connection between the electron transfer chain and the coupling mechanism of oxidative phosphorylation and between the coupling mechanism and ion translocation. One further possibility of arrangement of these various elements has to be entertained. The electron transfer chain could be localized in the base pieces of the elementary particles; the stalk and headpiece could be concerned with the coupling function and translocation. Such a disposition of parts would necessitate equating each base piece not with a complete chain but with one or more complexes of the chain.

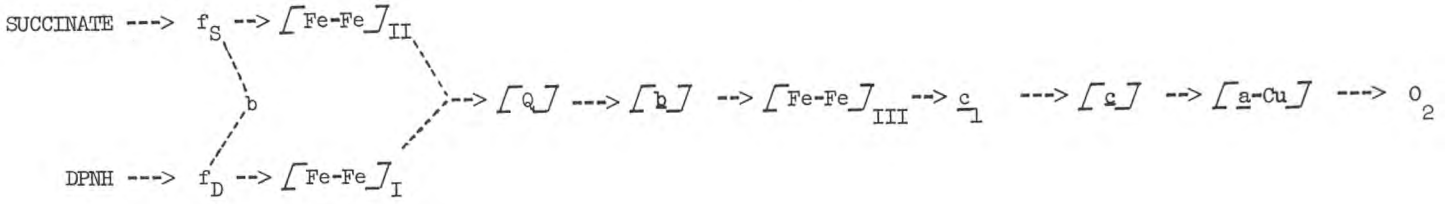
The Electron-Transfer Chain

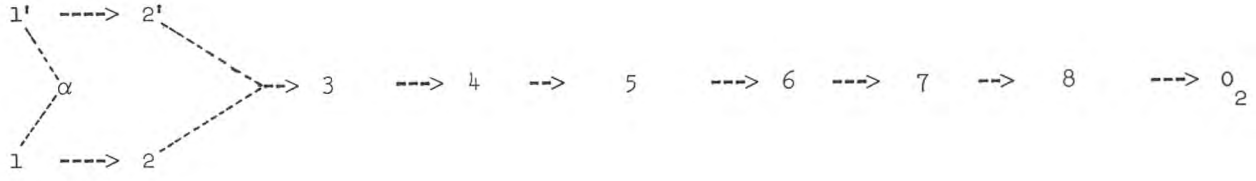
It may be difficult for non-experts to believe that there is no unanimity in the field with respect to the fundamental question of what constitutes the electron transfer chain. Basically there are two opposed interpretations. Our own formulation may be summarized as follows. The electron transfer chain consists of a set of protein molecules linked together with phospholipid in the form of an integrated unit. The chain is built up of four subunits or complexes (cf. Figure a) in 1:1:1:1 molecular proportions. Each chain consists of one molecule each of the two flavoproteins (f_S and f_D) and of cytochrome c_1; three molecules of cytochrome b; six molecules of cytochrome a; and an undetermined number of nonheme iron protein molecules (18 iron atoms per chain). The molecular proportions of the chain have been deduced a) by isolation of the electron transfer chain in the form of submitochondrial particles, b) by resolution of the chain into the four component complexes, c) by reconstitution of the chain from the four isolated complexes, d) by analytical studies on the individual proteins of the chain, and e) by analysis of the composition of the particles containing the complete chain as well as of particles containing the individual complexes. Our formulation rests on this body of evidence.

The Britton Chance formulation of the chain may be summarized as follows. There are eight proteins in the chain (f_D, f_S, cytochromes a, b, c_1, c and a_3). These proteins are all in equimolar concentrations (except for cytochrome c). A diagrammatic representation is shown in Figure b. It is to be noted that Chance in effect denies the existence of complexes, the role of nonheme iron proteins as obligate electron carriers, and the presence of more than one molecule of each protein species in a given chain. According to his formulation the eight oxidation-reduction proteins are arranged in linear sequence and there is direct transfer of electrons from one protein molecule to the next. In our formulation the complexes are the units of electron transfer and small molecules such as coenzyme Q and cytochrome c shuttle electrons between complexes.

In our opinion the evidence for the existence of four complexes as the units of electron transfer is convincing and we hold that the formulation of Chance and his stoichiometry for the components of the chain are invalid. We refer the interested reader to two recent publications in which the technical aspects are fully aired (Green and Wharton, 1963, and

Figure a


SUCCINATE ---> f_S --> [Fe-Fe]$_{II}$
b
DPNH ---> f_D --> [Fe-Fe]$_I$
--> [Q] ---> [b] --> [Fe-Fe]$_{III}$ --> $\underline{c}_1$ ---> [$\underline{c}$] --> [$\underline{a}$-Cu] ---> O_2


1' ----> 2'
α
1 ----> 2
--> 3 ---> 4 --> 5 ---> 6 ---> 7 --> 8 ---> O_2

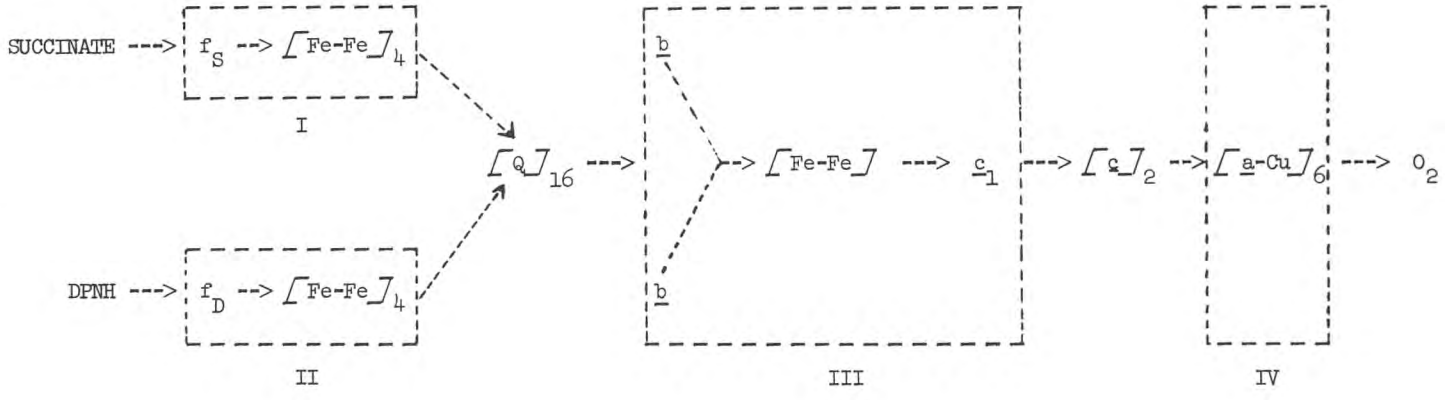
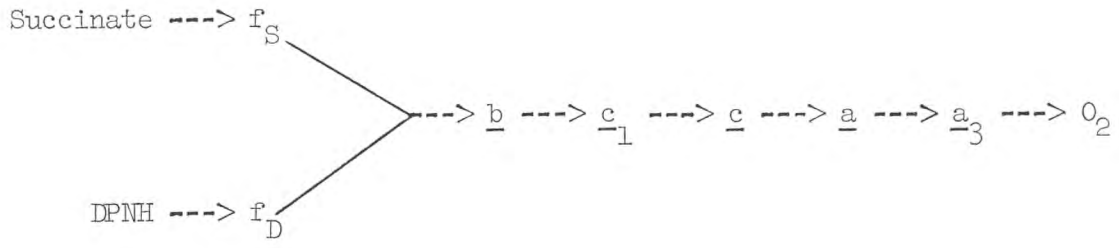

SUCCINATE ---> f_S --> [Fe-Fe]$_4$
I
DPNH ---> f_D --> [Fe-Fe]$_4$
II
[Q]$_{16}$ --->
$\underline{b}$
--> [Fe-Fe] ---> $\underline{c}_1$
$\underline{b}$
III
---> [$\underline{c}$]$_2$ --> [$\underline{a}$-Cu]$_6$ ---> O_2
IV

Figure b



Formulation of the electron transfer chain by B. Chance according to Figure 12 in Biological Structure and Function, ed. by T. W. Goodwin and O. Lindberg, Volume 2, 119 (1961), Academic Press, London-New York.

Green, Tisdale, and Fernandez-Moran, 1964). The formulations which Lehninger has represented in Figure 4 are variations on those of Chance. These representations are based on a minimum of experimental evidence and for that reason they are, in my opinion, highly misleading. The chairman has rightfully questioned our formulation pending the introduction of additional evidence but is inconsistent when he fails to apply the same criteria to his own formulations.

The Ultimate Protein Components of the Chain

The chairman did not succeed in making clear in his summary how we arrived at values for the molecular weight of some of the components of the electron transfer chain. In any of the four complexes we have a chemical association of a set of proteins and lipid. For example, complex III contains four protein molecules in combination (one molecule each of cytochrome $\underline{c}_1$ and nonheme iron protein and two molecules of cytochrome $\underline{b}$). This complex can be stepwise resolved into its component proteins but until very recently the resolution was only partial. A nonheme iron protein has now been separated from complex III and its molecular weight determined to be 25,000 from ultracentrifugation data. On the basis of chemical analysis there are two atoms of iron per 25,000 molecular weight.

It is becoming increasingly clear that many of the individual proteins of the electron transfer chain have relatively low molecular weights (cytochrome $\underline{b}$, 28,000; cytochrome $\underline{c}_1$, 37,000; cytochrome $\underline{c}$, 12,500; nonheme iron protein of complex III, 25,000). Some of the proteins of the chain have yet to be brought to the same stage of purity but with improvements in methods of isolation the molecular weights of all the proteins in the chain may approach 30,000. If we consider 30,000 as the average molecular weight of all proteins of the chain and 21 as the total number of fixed proteins in the four complexes, then the ultimate molecular weight of the electron transfer chain would be of the order of about 600,000 on a protein basis. Adjusted for 30% lipid the molecular weight would be about 860,000.

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Chance, B. and D. F. Parsons, Science 42, 1176 (1963).

Chance, B. and D. F. Parsons, Information Exchange Group No. 1, Scientific Memo 85 (1963).

Chance, B., D. F. Parsons, and G. R. Williams, Information Exchange Group No. 1, Scientific Memo 86 (1963).

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SUPPLEMENTARY STATEMENTS

II. ADDENDUM TO SUMMARY OF N.R.P. WORK SESSION ON CELL MEMBRANES INCLUDING CERTAIN NEW X-RAY DIFFRACTION FINDINGS ON RETINAL ROD OUTER SEGMENT MEMBRANES

by

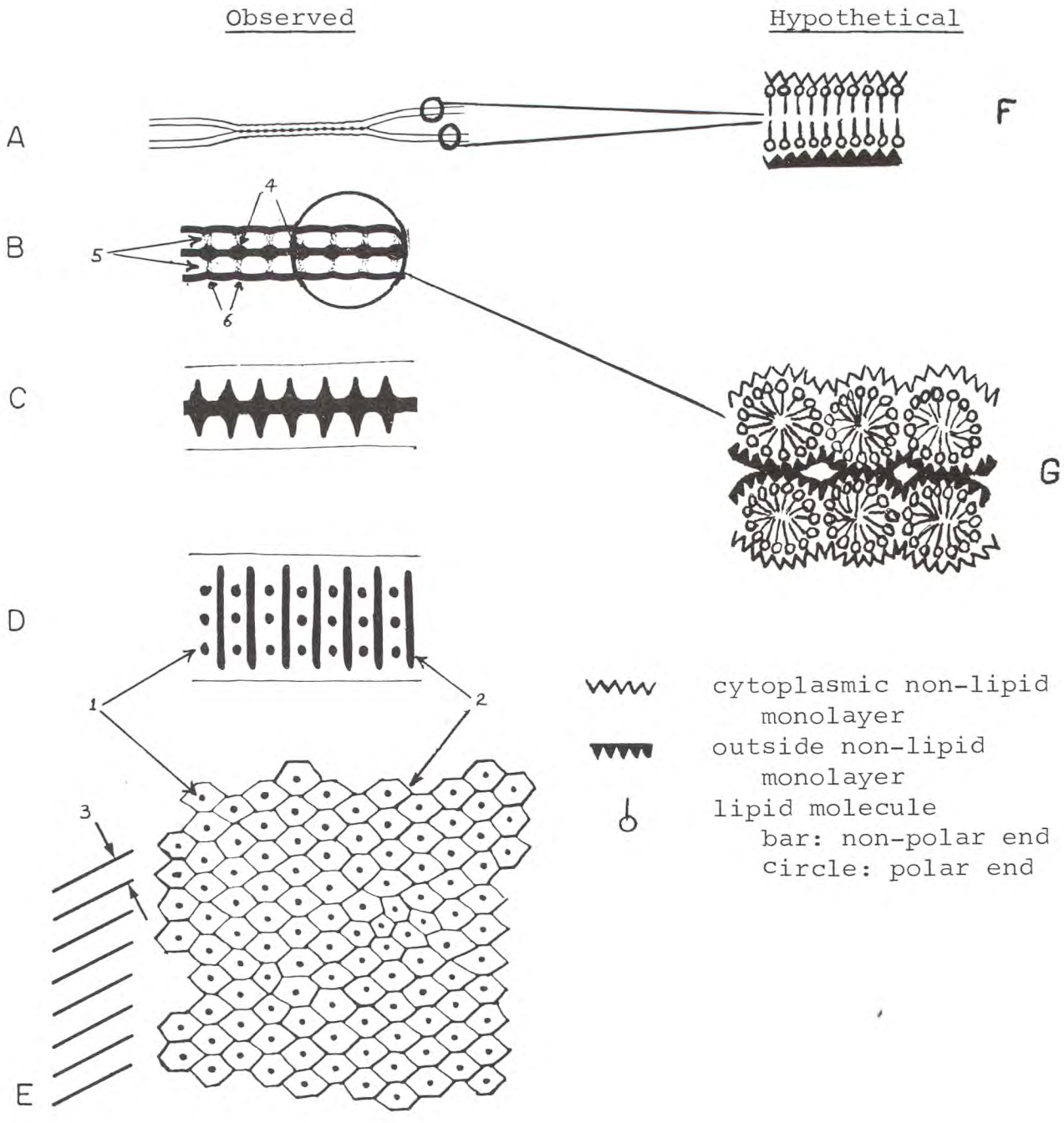
J. David Robertson

It is evident from the second two paragraphs on page 29 of Dr. Lehninger's summation of my presentation that I did not make myself quite clear on the subject of "granularity" in membranes, particularly as related to the synaptic disc structures. I shall attempt to clarify this by some additions to Fig. 19 of my article in J. Cell. Biol 19(1), 214 (1963) in which my findings on synaptic discs are shown in diagrammatic form. I am writing this on April 8, 1964 and inevitably, I shall draw upon some more recent findings (unpublished) in formulating this comment. I now believe that there is very serious reason to regard the "elementary particles" or "electron transport particles" as seen in PTA negatively stained mitochondrial fragments as well as certain of the features of my findings in synaptic discs as representing artifacts, albeit very interesting ones.

Synaptic Disc Granular Fine Structure

I believe Dr. Lehninger has used the term "granular fine structure" for what I would call "globular fine structure". In my view these are very different things in which dimensions become crucial. There are two kinds of "granular" fine structures in the figure. First, there are the dense granules in the center of each subunit facet in "E" designated by arrow 1. These measure under 25 Å in diameter and are also visible in the tilted disc in "D". Second, there is the border of the subunit facets designated by arrow 2. These are more rodlet than granular, but I shall not quibble over the term. However, it is important to note that the rodlets are each well under 20 Å in diameter. Now these rodlet borders are aligned to make the repeating unit of ~90-95 Å in the hexagonal network designated by arrow 3 in "E". In the tilted disc in "D" these borders overlap to appear as a line of thickness greater than the individual rodlets. In the less tilted disc in "C" the overlap is greater and the densities are broader. In the vertically sectioned disc in "B" each of the beads or grains designated by arrows 4 is produced,

Diagrams of synaptic discs



A shows a synaptic disc with the adjacent SMC segments at medium power in vertical section. B shows a higher power view with more detail. Note the beading of the central dense stratum. C shows the disc tilted slightly. D shows a greater degree of tilt. E shows a disc in frontal view. The main repeat period of 95 A is designated by the aligned arrows. (Robertson, J. Cell. Biol., 19, 201 (1963).)

I believe, by the overlap of the aligned dense borders of the subunit facets seen in frontal view in "E". In "B" the net effect is a grain that measures about 40 Å in diameter. This dimension, however, is, in a sense, an image artifact since the underlying structures making up the image are individually well under 20 Å in diameter. It is useful here to remember that the thickness of the sections studied is ~1,000 Å, and the discs are ~2,000-5,000 Å in diameter. It is for this reason that the frontal view measurements are the more meaningful ones for here the image is formed by a beam passing through only the thickness of the flat disc (~130 Å). The grain designated by arrows 1 evidently contribute to the dense line between the beads at arrows 4 in "B", but since there is much less substance overlapping, there is less electron scatter and an apparently thinner line.

All of these interpretations were made before the work session and are included in my paper in the Journal of Cell Biology last year. I believe that this pattern of structure may represent a direct derivative of some native molecular pattern, conceivably a mixture of a structural protein and a polysaccharide. This, however, is only a guess, since I have as yet no real evidence beyond the regularity of the directly observed structures and the fact that the pattern appears in both $KMnO_4$ fixed and formalin-OsO_4 fixed material.

Synaptic Disc Globular Fine Structure

In my opinion none of the above described structural features are directly related to the "elementary or electron transport particle" story. However, there is another feature of the synaptic discs shown in "A" and "B" that I believe might be related. In order to make my meaning clear I have drawn highly schematic molecular diagrams for cases "A" and "B". In "A" I have made a sketch of the usual unit membrane molecular configuration for each of the unit membranes united in a different region to make the synaptic disc. Lipid molecules are designated as "  " with the circles representing polar groups and the bars the hydrophobic carbon chains. The non-lipid monolayers are shown as zig-zag lines with the outer one filled in to suggest a chemical difference. The lipid here is shown as a continuous "smectic" bilayer. In the synaptic disc, I believe the lipid may have undergone a phase transition from the smectic state such as the ones shown to occur in some lipids by Luzatti and by Stoeckenius. Here the lipids might be

arranged in three dimensions, either as micro-cylinders or planar arrays of closely packed micro-spheres as indicated in the diagram. The polar groups would then be expected to produce some densities in the micrographs accounting for the transverse dense lines in "B", arrow 5, and the cytoplasmic surface would be expected to be slightly deformed to produce the scalloped effect shown in both "A" and "B", arrow 6. The circles made by the lipid polar groups would be expected to appear in the micrographs as a globular substructure with each globule measuring ~90 Å in diameter (center-to-center spacing of the central beads or grains in "B"). Indeed one can see the outlines of such globules ~90 Å in diameter in "B" by tracing out the postulated positions of the lipid polar groups which are assumed to produce densities.

Relation to other Globular Substructures

The size (~90 Å) of the synaptic disc globules is not incompatible with that of the "elementary particle" or "electron transport particle" (~80 Å) and may well be related. In some of Fernandez-Moran's published micrographs, e.g. Figs. 4 and 5 in the 1962 ARNMD paper,* a rather similar but less regular globular fine structure is pointed to in mitochondrial membranes and, very importantly for my purposes here, in the membranes of retinal rod outer segments. All these globules are labeled EP for "elementary particle". I believe that this kind of structure is similar to the globular (not granular) fine structure seen in the synaptic discs. In this same paper Fernandez-Moran shows, in his Figs. 10 and 11 of a negatively stained mitochondrial fragment preparation, globular structures of about the same dimensions, again labeled EP. These are either free or attached to a matrix ~100 Å thick in Fig. 10 and ~140 Å thick in Fig. 11. Stoeckenius in his Fig. 6 (J. Cell Biol 17, 447 (1962)) shows the same kind of structures, but here attached to a matrix ~180 Å thick. Here the matrix shows two zones of decreased density that could reasonably be interpreted as lipid bilayer negative images. In my view the spherical structures in both these sets of micrographs and in the ones shown at the work session by Smith, and in those published by

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* Fernandez-Moran, H., Cell Membrane Ultrastructure: Low-Temperature Electron Microscopy and X-ray Diffraction Studies of Lipoprotein Components in Lamellar Systems. Ultrastructure and Metabolism of the Nervous System, Vol. XL, Chapter XII, 1962.

Parsons, may reasonably be interpreted as globular micro-spheres of lipid derived from the lipid core of unit membranes. In the case of the sectioned material I would say that the globular substructure is due to a phase transition in the lipid of the unit membranes altering the usual smectic bilayer arrangement.

If these interpretations are correct, we must consider whether or not any such lipid organization exists in native undegraded unit membranes or whether they represent simply artifacts of preparation. In order to test this, I believe strongly that we must do more than merely look at electron micrographs of material that we know is very likely to have been greatly altered by our preparatory procedures. Ideally, we need to look at a membrane known to display this kind of structure in electron micrographs in the unfixed, undegraded native state. Of course, we cannot do this now by electron microscopy. It can be done, however, indirectly by X-ray diffraction methods. If such a membrane system can be oriented properly in an X-ray beam, we should be able to obtain diffractions from any such spherical or cylindrical component repeating in a direction parallel to the planes of the aligned and oriented membranes. This diffraction should be a relatively easy one to detect by any technique capable of detecting the myelin ~170 Å layer spacing, for the spacing would be smaller and, because of the reciprocal Bragg relationships, the diffractions should appear further away from the central beam.

The retinal rod outer segment (ROS) is an almost ideal structure in which to test this proposition. Its membranes are known to display the EP globular substructure in fixed material. I might mention that we have confirmed Fernandez-Moran's results on this point, not only in sections of fixed material but in direct frontal views of ROS lamellae after $KMnO_4$ fixation and ultrasonic fragmentation. We have succeeded in aligning ROS's in intact retinal fragments in a micro (100 μ) X-ray beam and obtaining a diffraction pattern from intact unfixed oriented ROS's. The pattern shows the expected lamellar spacing in a direction parallel to the rod axes. This appears as the first and second order of a 320 Å spacing. Detached ROS's spun down in a quartz capillary tube have also shown a similar pattern with a 319 Å spacing only in the direction in which the rod axes would be expected to become

aligned during sedimentation. If the substructure in question were present, some diffractions should have been seen in the opposite direction parallel to the planes of the rod lamellae. No diffractions were seen in this direction, either in situ or in isolation.

The pattern of ROS's in whole retina referred to has been obtained after many trials and failures due, I believe, to difficulty in hitting the ~40 μ thick retinal rod layer with the ~100 μ diameter X-ray beam, sufficiently parallel to the layer plane. So far I have only two clear-cut patterns. The equipment is now being reworked to improve the performance. However, the two patterns obtained so far are very clear and definitely come from the ROS's, since they are the only structures present that reasonably could have produced the 320 Å diffraction. Admittedly, we must obtain more such patterns before this result can be regarded as more than preliminary and for this reason the results are still unpublished. However, since I have been asked to write this statement to make clear my views and, since the X-ray findings on this are so important in determining them, I have decided to present these very tentative findings. Mainly because of them, I am inclined at this point to regard the evidence of globular substructure in electron micrographs of unit membranes with great caution if extrapolation to native membranes is intended. It will be important to define more precisely the conditions under which such alterations can be made to occur. Meanwhile, I would consider very detailed biochemical and structural correlations to be premature.

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FURTHER READING

A. SOME RELEVANT PAPERS BY WORK SESSION PARTICIPANTS PUBLISHED SINCE THE WORK SESSION

I. MEMBRANE ULTRASTRUCTURE

ROBERTSON, J. D.

Robertson, J. D., The occurrence of a subunit pattern in the unit membranes of club endings in Mauthner cell synapses in goldfish brains, J. Cell Biol. 19, 201 (1963)

Abstract: Observations additional to those previously reported (34) on boutons terminaux and club endings on Mauthner cell lateral dendrites, primarily as seen in sections of permanganate-fixed material, are described. Certain new findings on OsO_4-fixed endings are also included. The boutons terminaux are closely packed in the synaptic bed with ~ 100 to 150 A gaps between their contiguous unit membranes and a few interspersed glial extensions. Their synaptic membrane complexes (SMC) appear as pairs of unit membranes separated by ~ 100 to 150 A clefts. They contain many vesicles and unoriented mitochondria, but no neurofilaments. The club endings after $KMnO_4$ fixation are, as after OsO_4 fixation (34), again seen surrounded by a layer of extracellular matrix material. These endings contain relatively few synaptic vesicles, a few unit membrane limited tubules ~ 300 A in diameter, and mitochondria oriented perpendicular to the SMC. Neurotubules and neurofilaments are not clearly seen. These components are also virtually absent in the Mauthner cytoplasm. No ribosomes are seen in the $KMnO_4$-fixed material. The unit membranes of the SMC of club endings show up clearly in essentially the same junctional relations described after formalin-OsO_4 fixation (34). In addition, the synaptic discs in transverse section show a central beading repeating at a period of ~85 A associated with scalloping of the cytoplasmic surfaces. In oblique views, dense lines are seen repeating at a period of ~ 90 A. In frontal views a hexagonal array of close-packed polygonal facets is seen. These repeat at a period of ~ 95 A. Each has a central dense spot < 25 A in diameter. Similar subunits are seen in the unit membranes of synaptic vesicles.

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1. The ultrastructure of the plasma membrane of columnar epithelium cells of the mouse intestine, J. Ultrastructure Res. 8, 517 (1963)

2. A new repeat structural element of mitochondrial and certain cytoplasmic membranes, Nature 199, 1262 (1963)

3. A new ultrastructural element of the membranes in mitochondria and of some cytoplasmic membranes, J. Ultrastructure Res. 9, 340 (1963)

4. A comparison of plasma membrane, cytomembranes, and mitochondrial membrane elements with respect to ultrastructural features, J. Ultrastructure Res. 9, 561 (1963)

5. The fine structure of the columnar epithelium of the mouse intestine with special reference to fat absorption, Proc. Intern. Conf. Biochemical Problems of Lipids, Birmingham, 1962. In Biochemical Problems of Lipids, A. C. Frazer, Ed., pp. 91-115, Elsevier Pub. Co., Amsterdam, 1963

6. Ultrastructural changes in skeletal muscle myofibrils in connexion with contraction, Nature 201, 47 (1964)

7. The granular structure of mitochondrial membranes and of cytomembranes as demonstrated in frozen-dried tissue (with L.-G. Elfvin), J. Ultrastructure Res., in press

SMITH, D. S.

Smith, D. S., The structure of flight muscle sarcosomes in the blowfly Calliphora erythrocephala (Diptera), J. Cell Biol. 19, 115 (1963)

Abstract: The electron microscopic structure of sectioned indirect flight muscle fibers of the blowfly Calliphora is described. Particular attention is paid to the organization of the sarcosomes (mitochondria) of this tissue, and this description is accompanied by an account of the appearance of these bodies in negatively stained preparations. In sectioned material, it has been shown that these sarcosomes are similar to other mitochondria in the disposition of the outer and inner limiting membranes, but that the cristae, confluent with the latter, are unusually regular, and form parallel plates, containing circular fenestration forming cylindrical channels within the matrix. Negatively stained preparations of disrupted sarcosomes reveal that both the outer limiting membrane and the cristae membranes bear large numbers of small particles, similar in appearance to those described by Fernandez-Moran and others in various mitochondria. In Calliphora, these particles consist of a subspherical "head" and a cylindrical "stalk," and appear to be arranged on the mitochondrial membranes either randomly distributed, or collected into circular or elongated groups. Recent suggestions concerning the nature of these submitochondrial particles are discussed, and an attempt is made to correlate the aspects of organization of Calliphora sarcosomes, revealed by conventional sectioning of the "intact" structures, and by negative staining of sarcosomal derivatives.

II. BIOCHEMICAL ASPECTS OF MEMBRANE STRUCTURE

CHANCE, B.

1. Chance, B., and Parsons, D. F., Cytochrome function in relation to inner membrane structure of mitochondria, Science 142(3596), 1176 (1964)

Abstract: Projecting subunits of an average diameter of 80Å are found on the cristae of mitochondria prepared from the muscle of Ascaris lumbricoides. A spectroscopic examination of the cytochrome content of these mitochondria shows no detectable cytochrome c_1, a, or a_3 and does reveal cytochromes of types c and b. Subunits in the same size range are found in cytochrome c deficient mitochondria of the emergent bee, while the frequency of their occurrence along the cristae is decreased relative to the adult bee. Apparently, the cytochrome content of the respiratory chain is not related to the size of the subunits, but may be related to the frequency of occurrence of the subunits.

2. Chance, B.; Parsons, D. F.,and G. R. Williams, Cytochrome content of mitochondria stripped of inner membrane structure, Science 143(3602), 136 (1964)

Abstract: The cytochrome composition of mitochondrial fractions which have been stripped of inner membrane subunits by exposure to high-frequency sound have been examined by low-temperature spectroscopy. The ratio of cytochrome c to cytochrome a is not changed by the treatment, but the concentration of cytochrome per milligram of protein is increased and the concentrations of cytochromes c_1 and b change slightly. These cytochromes (c_1 and b) may be at least partly located in the subunits of the inner membrane, but the idea that all the respiratory components are located in single subunits of the mitochondrial cristae may be considered to be disproved.

FERNANDEZ-MORAN, H.
GREEN, D. E.

Fernandez-Moran, H., and Oda, T., Blair, P. V., and Green, D. E., A macromolecular repeating unit of mitochondrial structure and function, J. Cell Biol., in press

Abstract: A repeating particle associated with the cristae and the inner membrane of the external envelope has been recognized and characterized in beef heart mitochondria by correlated electron microscopic and biochemical studies. Many thousands (ca. 10^4 to 10^5) of these particles, disposed in regular arrays, are present in a single mitochondrion. The repeating particle, called the elementary particle (EP), consists of three parts: (1) a spherical or polyhedral head piece (80 to 100 Å in diameter); (2) a cylindrical stalk (about 50 Å long and 30-40 Å wide); and (3) a base piece (40 x 110 Å). The base pieces of the elementary particles form an integral part of the outer dense layers of the cristae. The elementary particles can be seen in electron micrographs of mitochondria in situ, of isolated mitochondria, and of submitochondrial particles with a complete electron transfer chain. Negative-staining with phosphotungstate is only one of several techniques that can be used for reproducible demonstration of the repeating particles and underlying subunit organization of mitochondrial membranes. A particulate unit containing a complete electron transfer chain can be isolated from beef heart mitochondria. The isolated unit approximates in size that of the elementary particle in situ. The molecular weight of the particle in situ is calculated to be 1.3×10^6. Evidence is presented for identifying the isolated unit with the elementary particle visualized in situ. The elementary particle of the mitochondrion is believed to be a prototype of a class of functional particles or macromolecular assemblies of similar size found in association with membranes generally.

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2. Green, D. E., The mitochondrion, Sci Amer 210(1), 63 (1964)

III. PHYSICAL CHEMISTRY OF LIPID BILAYERS

THOMPSON, T. E.

1. Huang, C., Wheeldon, L., and Thompson, T. E., The properties of lipid bilayer membranes separating two aqueous phases; formation of a membrane of simple composition, J. Mol. Biol. 8, 148 (1964)

2. Thompson, T. E., The properties of bimolecular phospholipid membranes, Cellular Membranes in Development, ed. M. Locke, Academic Press, 83-94 (1964)

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67	Criddle, R. S., Bock, R. M., Green, D. E., and Tisdale, H., Biochemistry 1, 827 (1962)
68	Bangham, A. D. and Horne, R. W., Nature 196, 952 (1962)
69	Chance, B. and Parsons, D. F., Information Exchange Group No. 1, Scientific Memo 85 (1963)
69	Chance, B., Parsons, D. F., and Williams, G. R., Information Exhange Group No. 1, Scientific Memo 86 (1963)
69	Crane, F. L. and Stasny, J. T., Information Exchange Group No. 1, Scientific Memo 94 (1964)
69	Chance, B. and Parsons, D. F., Science 42, 1176 (1963)
70	Green, D. E. and Wharton, D. C., Biochem. Z. 338, 335 (1963)
71	Green, D. E., Tisdale, H., and Fernandez-Moran, H., Information Exhange Group No. 1, Scientific Memo 96 (1964)
75	Robertson, J. D., J. Cell. Biol 19(1), 214 (1963)
78	Stoeckenius, W., J. Cell Biol 17, 447 (1962)
78	Fernandez-Moran, H., Cell Membrane Ultrastructure: Low-Temperature Electron Microscopy and X-ray Diffraction Studies of Lipoprotein Components in Lamellar Systems. Ultrastructure and Metabolism of the Nervous System, Vol. XL, Chapter XII (1962)

CHRONOLOGICAL POSTSCRIPT

Readers may be interested in the history of this report.

At the end of each half-day of the "Cell Membrane" Work Session in July 1963, its Chairman, Dr. Lehninger, led the participants in developing a summary of the material covered during that session. These summaries formed the basis of an evaluative summary that he delivered orally in August 1963 at the Sixth Stated Meeting of NRP Associates.

Dr. Lehninger's oral report was tape recorded by Wardwell Holman; then transcribed by Margaret Little and Delphine Tenney, and edited by Theodore Melnechuk, in September 1963.

In October 1963, the initiation of the NRP Bulletin provided a vehicle for the wider distribution of such NRP Work Session reports, and Dr. Lehninger, before departing for Europe, revised the transcription of his oral report.

His redaction was then circulated to the original participants, all of whom cooperated with editor Catherine M. LeBlanc in up-dating the report with annotations and augmentations; particularly Drs. Smith and Green, in March 1964, and Dr. Robertson in April 1964.

--T.M.

NRP Accessions List 63-1

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ACCESSIONS LIST

Compiled by

Joan Morris
Director, Information Center

PREFACE

"Where there is much desire to learn, there of necessity will be much arguing, much writing, many opinions; for opinion in good men is but knowledge in the making." --John Milton, 1644

The Accessions List is in effect a key to the information housed in the Neurosciences Information Center. Except in format, it is to be the equivalent of a card catalog to the Center's holdings.

I think it appropriate that this first NRP Accessions List be published one year after our Information Center was established in the building of the American Academy of Arts and Sciences. Succeeding issues will not require year-long intervals, but are intended to appear monthly in our NRP Bulletin, to augment this first issue serially, both by updating it and by adding Sections to those being distributed now.

The envisaged Sections include:

I. Journal Abstracts -- This Section will collate titles, and as far as possible abstracts, of all journal articles relevant to the NRP, including reprints of all articles by our Associates. This first issue covers the Associates' reprints for 1963 (mostly) and the last six months of appropriate journal articles. See the cover page of Section I for an explanation of the asterisk code denoting the origin of the starred entries.

II. Book Collection

III. Bibliography Collection -- This section will list the published works of our Associates, Work Session panelists, and other guests and contributors.

IV. Journal Subscriptions for 1964 -- As with the book collection, our periodical collection tries to be, not exhaustive, but selective. This selection was worked out in cooperation with the Chairman and the Associates.

V. Russian Translations -- Abstracts in English of articles originally in Russian.

Other Sections will be announced in the near future. Your comments and inquiries are welcome. --J.M.

NRP Accessions List 63-1 (S-I)

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Section I

JOURNAL ABSTRACTS

* Associates' Reprints
** Associates' Recommended Reading List
*** Dr. F. O. Schmitt's Recommended Reading List

4. Liu, Chung Laung. SOME MEMORY ASPECTS OF FINITE AUTOMATA. MIT-TR-411, 75p. 5/31/63.

The most important characteristic of a finite automaton is that it has a "memory". By this, it is meant that the behavior of an automaton is dependent upon its past history. Several special cases are studies in which the unique determination of a behavior of an automaton is possible, even when a portion of its past history is unknown. (Author)

*5. Reichardt, W., and Fermi, G. OPTOMOTORISCHE REAKTIONEN DER FLIEGE MUSCA DOMESTICA (OPTOMOTOR REACTIONS OF THE HOUSEFLY MUSCA DOMESTICA). Kybernetik 2(1) 15, 1963. (Eng. Tr.)

The torque exerted by the housefly Musca domestica during fixed flight was used as a measure of the optomotor reaction of the insect elicited by the rotation of cylindrical patterns with periodic distributions of surface brightness. Measurements were made of the dependence of the reaction on the wave length, speed of rotation, contrast, and mean brightness of the stimulus patterns. The effect on the reaction of modulation of the light illuminating the stimulus pattern was examined. Further experiments indicated that stimulation of only one of the two complex eyes is sufficient to elicit an optomotor reaction, and that there is overlap between the visual fields of neighboring photoreceptor units in the complex eye. Estimates of the rates of absorption of light quanta by individual ommatidia in the complex eye indicated that these rates are low enough that the Poisson statistics of the light quanta results in a significant level of noise in the light signals received by the photoreceptors, when the brightness of the stimulus pattern is low but still sufficient to elicit a measurable reaction. The contrast that is required of a rotating stimulus pattern in order to elicit a just-measurable reaction was found to depend upon the mean brightness of the pattern in a manner that is consistent with the hypothesis that the noise due to the statistics of the light quanta absorbed by the photoreceptors in the complex eye is a principle cause of the breakdown of the optomotor reaction at low values of the contrast and mean brightness of the stimulus pattern. (Author)

**6. Spreng, M., and Keidel, W. D. NEUE MOGLICHKEITEN DER UNTERSUCHUNG MENSCHLICHER INFORMATIONSVERARBEITUNG (NEW POSSIBILITIES FOR RESEARCH IN HUMAN INFORMATION PROCESSING). Kybernetik 1(6) 243, 1963.

By means of an electronic averaging technique it is possible to measure the evoked responses to acoustic stimuli on the outside of the human scalp, in spite of a signal-to-noise ratio of about 1:20.

BIOCHEMISTRY

*1. Blair, P. V., Oda, T., and Green, D. E. (with the technical assistance of Donald R. Silver), and Fernandez-Moran, H. (with the technical assistance of Frederick B. Merk). STUDIES OF THE ELECTRON TRANSFER SYSTEM. LIV. ISOLATION OF THE UNIT OF ELECTRON TRANSFER. Biochemistry 2, 756-764, 1963.

The prccedure described for the fractionation of mitochondria leads to the isolation of a particle, which uniquely contains all of the fixed components of electron transfer; the enzymic activity increases concomitantly with the purification of the particle. The rates of oxidation of DPNH and of succinate have been increased by a factor of 2.5 and the concentration of cytochrome a by a factor of 2.2. The specific concentrations of the other components have been increased to about the same degree. The isolated particle (designated the elementary particle) appears to be a physical and functional aggregate of the four complexes know collectively to constitute the electron transfer system: the theoretical molecular weight (on a protein basis) is 1.4×10^6; that of the particle described was calculated to be 2.1×10^6. Further purification (by removal of structural prctein, etc.) led to an estimated minimal molecular weight of 1.4×10^6 but also decreased the enzymic activity. (Author)

CYBERNETICS

2. Barlow, H. G. THE INFORMATION CAPACITY OF NERVOUS TRANSMISSION. Kybernetik 2(1) 1, 1963.

3. Fukutome, T. H., and Sugata, K. AN ELECTRIC ANALOGUE OF THE NEURON. Kybernetik 2(1) 28, 1963.

An electric analogue model of the neuron is described. The model neuron is composed of the active units which simulate the electric behavior of the active loci of the membrane of a neuron. The active units include the model axon and the model synapses of six different types (the ordinary, incremental and decremental ones each having the excitatory and inhibitory types). The properties of the models are described. (Author)

The normal information flow gathered in the periphery by all sense organs, is reduced nearly for 10^{-7} on its way to higher storage centers. This diminution is caused partially by the so called "optimizing function" of the central nervous system, which is supposed to contribute most of the limited capacity of the higher parts of the information channel to that sensory system, which just contains the most (essential for the living organism) information. Based probably on the "unspecific pathway" the "optimizing function" is common to all sensory channels. Results of our experiments on the influence of the optimizing function and on multisensory stimulation give some view in information processing in man. (Authors) (Contributed by M. Eigen)

ELECTROPHYSIOLOGY

7. Crain, S. M., and Peterson, E. R. BIOELECTRIC ACTIVITY IN LONG TERM CULTURES OF SPINAL CORD TISSUES. Science 141, 427-429, 1963.

Fragments of embryonic spinal cord (human, rat, and chick) can regenerate and differentiate in tissue culture. Complex bioelectric activity evoked by electric stimuli indicates that nerve cells in cultures may maintain for months in vitro not only the capacity to propagate impulses along their neurites but also a remarkable degree of functional organization resembling the activity of synaptic networks of the central nervous system.

8. Dudel, J., Gryder, R., Kaji, A., Kuffler, S. W., and Potter, D. D. GAMMA-AMINOBUTYRIC ACID AND OTHER BLOCKING COMPOUNDS IN CRUSTACEA. I. CENTRAL NERVOUS SYSTEM. J. of Neurophysiology 26(5) 722-728, 1963.

The three principal blocking substances in a water extract of the lobster central nervous system were gamma-aminobutyric acid, taurine, and betaine. Three other blocking substances, alanine, beta-alanine, and an unidentified compound were also found. Of these gamma-amino-butyric acid is the most active physiologically.

9. Hagiwara, S., and Morita, H. CODING MECHANISMS OF ELECTRO-RECEPTOR FIBERS IN SOME ELECTRIC FISH. J. of Neurophysiology 26(4) 551-567, 1963.

Properties of electroreceptors were analyzed by recording impulse discharge of single lateral line nerve fibers of Staetogenes (electric organ discharge of 40-60 cyc./sec.), Gymnotus (40-60 cyc./sec.), Eigenmannia (250-400 cyc./sec.), and Sternopygus (60-100 cyc./sec.). The intensity of the potential field produced by the electric organ pulse is coded by a single lateral line nerve fiber principally by the gradation of number of impulses in a train produced by each pulse in fishes having a low frequency discharge of the electric organ such as in Staetogenes

and Gymnotus. However in fishes of a high frequency electric organ discharge such as in Eigenmannia the coding is performed by grading the probability of impulse initiation after each pulse of the electric organ. In Sternopygus both mechanisms are operative. Small conducting or dielectric objects in the water just over the receptor respectively suppress or enhance the nerve response and anterior or posterior to the receptor area gives rise to the opposite type of responses. The threshold for an imposed electric pulse to produce one nerve impulse is about 10 mV/cm along the long axis of the fish and is the same for a pulse of any duration above 5 msec. in Staetogenes and Gymnotus. The threshold for an imposed a.c. field is about mVsec. in Eigenmannia is higher when the frequency of a.c. departs far from that of the electric organ discharge. (Authors)

10. John, E. R., Ruchkin, D. S., and Villegas, J. SIGNAL ANALYSIS OR EVOKED POTENTIALS RECORDED FROM CATS DURING CONDITIONING. Science 141, 429-431, 1963.

Correlation coefficients were computed between average response waveforms recorded from different brain regions of trained cats, before and after a specific stimulus acquired cue value. Application of signal-analysis techniques to the correlation matrix shows marked increase in the similarity between wave shapes evoked by that stimulus in sensory-specific and nonsensory-specific regions.

11. Lissmann, H. W. ELECTRIC LOCATION BY FISHES. Scientific American, 208 (3) 50-59, 1963.

**12. Sokolov, E. N. HIGHER NERVOUS FUNCTIONS: THE ORIENTING REFLEX. Ann. Rev. Physiol. 25: 545-580, 1963.

The orienting reflex as a complex functional system includes the integrative activies of different brain areas. Its distinguishing characteristic is that it arises in response to novelty. It depends upon elaboration of a nervous model of stimulus and the mismatch between the model and a new stimulus. The elaboration of the neuron model consists in fixation by the nervous system of stimulus traces. The origin of the orienting reflex apparently lies in a mismatch of extrapolatory impulses and afferent signals reaching common efferent neurons. The modifications of the EEG, the shift in steady potential of the cortex, and the increase in functional state represent components of the orienting reflex arising in response to any change of a repetitive stimulus. A special component of this reflex is a local intracortical activation, arising as a result of cortical stimulation by meaningful specific sensory information in the absence of reticular formation activation. To explain both the appearance and the extinction of local

cortical activation, a hypothesis involving interaction of afferent, extrapolatory, and comparator neurons with glial cells is offered. The molecular mechanism of the memory trace is discussed. The orienting reflex at the neuronal level converts nonresponsive into responsive neurons, produces facilitation and enhancement of unit responses, and increases critical flicker fusion frequency. Analysis of this reflex indicates that it facilitates the transmission of information at all levels of the analyzers. (Author) (Contributed by F. O. Schmitt)

INSTRUMENTATION

*13. Kjellberg, R. N., Koehler, A. M., Preston, W. M., and Sweet, W. H. STEREOTAXIC INSTRUMENT FOR USE WITH THE BRAGG PEAK OF A PROTON BEAM. Confinia Neurol. 22 (3/5): 183-189, 1962.

LEARNING, MEMORY

14. Adey, W. Ross. PHYSICOCHEMICAL CHANGES IN BRAIN TISSUE IN LEARNING: POSSIBLE RELATIONSHIPS OF RNA TO MEMORY. Neurology 13 (4) 359, 1963

15. Buschke, H. RETENTION IN IMMEDIATE MEMORY ESTIMATED WITHOUT RETRIEVAL, Science 140, 56-7, 1963

16. Chamberlain, T. J., Rothschild, G. H., and Gerard, R. W. DRUGS AFFECTING RNA AND LEARNING. Proceedings National Academy of Sciences 49(6) 918-25, 1963

The fixation or consolidation of experience upon which learning is based has been postulated to result from a dynamic process causing a permanent structural change in the neurons or neural networks of the CNS. Intraneuronal macromolecules, in particular ribonucleic acid (RNA), have been suggested as critical sites of these structural changes. Several investigators have studied this hypothesis and have offered empirical evidence in its support. Experiments were undertaken in light of this attractive hypothesis to establish whether learning phenomena can be affected by drugs which have a profound effect on RNA metabolism.

17. Cook, L., Davidson, A. B., Davis, D. J., Green, H., and Fellows, E. J. BODY CHEMICAL IMPROVES LEARNING AND MEMORY. Science 141, 268, 1963.

Acquisition of a behavioral response motivated by shock was enhanced in rats chronically treated with yeast ribonucleic acid, and resistance to extinction was greater in rats so treated than in controls. This extends the role of ribonucleic acid to include a behavioral effect in laboratory mammals treated with a purified preparation from yeast.

18. Ditchburn, R. W. INFORMATION AND CONTROL IN THE VISUAL SYSTEM. Nature, 198, 630-632, 1963.

19. Gerard, R. W., Chamberlain, T. J., and Rothschild, G. G. RNA IN LEARNING AND MEMORY. Science 140(3565) 381, 1963.

20. HEADS ARE BETTER THAN TAILS AT TELLING BLACK FROM WHITE. New Scientist 360, 103, 1963.

K. Roe describes series of trials of flatworms in an ingenious hexagonal maze. The maze consisted of a central white hexagonal track with six black radial arms; choice in one of the black arms resulted in a worm receiving an electric shock. The worms underwent a daily series of trials in the maze. 21 of 30 original worms reached a criterion of 12 consecutive choices of a white arm on two consecutive days in less than 900 trials. These were then cut in half and left to regenerate. Regenerated worms were trained again and it was found that those growing from a head relearned the maze to the original standard faster than the original worms. The result differs from that of another recent experiment in which worms regenerated from tails relearned a conditioned response to light as fast as those regenerated from heads. The difference probably indicates that maze learning, involving color discrimination and decision making, needs the worms cerebral ganglia which are in the front half for its establishment and storage.

21. Rahmann, H. INFLUENCE OF CAFFEINE ON MEMORY AND BEHAVIOR IN GOLDEN HAMSTERS. Pflueger Arch. Ges. Physiol. 276, 384-397, 1963. (Ger.)

22. Russell, I. S., and Ochs, S. LOCALIZATION OF A MEMORY TRACE IN ONE CORTICAL HEMISPHERE AND TRANSFER TO THE OTHER HEMISPHERE. Brain 86, 37-54, 1963.

***23. Young, J. Z. SOME ESSENTIALS OF NEURAL MEMORY SYSTEMS, PAIRED CENTERS THAT REGULATE AND ADDRESS THE SIGNALS OF THE RESULTS OF ACTION. Nature 198, 626-630, 1963.

MEMBRANES

24. Duncan, C. J. EXCITATORY MECHANISMS IN CHEMO- AND MECHANO-RECEPTORS. Journal of Thecretical Biology 5, 114-126, 1963.

Certain difficulties concerning Beidler's theory of taste stimulation are presented and similarities between the properties of mechano- and chemo-receptors are emphasized. It is suggested that the response of a sense organ may not be proportional to the logarithm of the stimulus intensity, but rather that a hyperbolic relationship exists between these two variables. Evidence is therefore presented which suggests that in

these sensory receptors the change in membrane permeability, which is recorded as the receptor potential, is dependent on the rate of an enzyme reaction.

*25. Fernandez-Moran, H. MEMBRANE, BIOLOGICAL. McGraw-Hill Yearbook Science and Technology, 323-329, 1963.

The ultrastructure of the components of mitochondrial membranes namely the EP (elementary particle) and the ETP (electron transport chain) are revealed in electron microscope studies of the author. The "lamellar systems", such as the nerve myelin sheath, photoreceptors (Figs. 1 and 2), chloroplasts and mitochondriaare revealed as derivatives of multiply folded cell membranes, disposed in highly ordered "paracrystalline" arrays. Work of D. E. Green, A. L. Lehninger, G. Palade, J. F. Danielli, and H. Dawson and W. Arnold is placed in perspective with respect to ultrastructure analyses, correlation of structure and function, organization at the molecular level, and semiconductors.

*26. Fernandez-Moran, H. PARTICIPANTS OF AN INTERMEDIATE OF OXIDATIVE PHOSPHORYLATION IN ION ACCUMULATION BY MITOCHONDRIA. Science 140, 60-2, 1963. (Abs.)

27. Furukawa, T., Fukami, Y., and Asada, Y. A THIRD TYPE OF INHIBITION IN THE MAUTHNER CELL OF GOLDFISH. J. of Neurophysiology 26 (5) 759-774, 1963.

The present communication is concerned with late collateral inhibition (LCI) in the Mauthner cell (M-cell) of goldfish. This late collateral inhibition, as distinct from the earlier occurring one which is transmitted electrically, has been thought to be accounted for by the familiar mechanism of chemically transmitted inhibitory postsynaptic action which, by rendering the membrane more permeable to certain ion species such as Cl-, shunts the excitatory postsynaptic potential (EPSP) as well as the antidromic spike. A detailed inspection of the effect of LCI upon the EPSP suggested, however, that there might be an additional factor other than the inhibitory postsynaptic action. It has now become clear that there exists a distinct type of inhibition which might well be called a third type of inhibition in the M-cell. The evidence which led to the above conclusion is described in this paper.

28. Grundfest, H., and Gainer, H. RELATION OF MEMBRANE POTENTIAL TO PERMEABILITY FOR ALKALI METAL IONS OF LOBSTER MUSCLE FIBERS. Biophysical Society, Seventh Annual Meeting, 1963 (Abstract)

29. Grundfest, H., Reuben, J. P., Lopez, E., and Brandt, P. W. VOLUME REGULATION BY NA IN FROG MUSCLE FIBERS. Fed. Proc. 22 (565) 1963.

*30. Katchalsky, A. and Kedem, O. PERMEABILITY OF COMPOSITE MEMBRANES. Trans. Faraday Soc., 1918-1930, 1963. Part 1, Electric Current, Volume Flow and Flow of Solute through Membranes.

In crder to treat composite membranes arranged in a parallel or series array of elements, a set of phenomenological equations for the transport of electrolytes through membranes was derived. The phenomenological equations are based on practical, straight and cross coefficients readily tested experimentally. A series of relations was developed for the coupling coefficients which allows a ready transition from one system of coefficients to another. (Authors)

*31. Part 2, Parallel Elements, Ibid; 1931-1940, 1963

The transport behaviour of membranes composed of a parallel array of elements was studied on the basis of two assumptions--the equality of force for all elements and addivity of flow. It was shown that under certain conditions the practical phenomenological coefficients exhibit marked deviations from additivity indicating strong circulation of flow among the membrane elements.
The behaviour of such membranes was calculated for a special case based on the Teorell and Meyer and Sievers model. It was found that in a parallel combination of positive and negative membranes there may develop a marked increase in salt permeability which will also be expressed by a pronounced negative anomalous osmosis. (Authors)

*32. Part 3, Series Array of Elements, Ibid; 1941-1953, 1963

The permeability coefficients of membranes composed of a series-array of elements are evaluated from the coefficients of the membrane elements. The calculations are carried out for stationary flows and assume continuity of chemical potentials across the interfaces between elements. Extensive use is made of "corresponding quantities", representing concentrations, pressures and electromotive forces in the membrane in terms of those of aqueous solutions equilibrated with the examined volume element of the membrane phase.
It was found that the overall resistances (reciprocal permeability coefficients) are not generally composed by addition of those of the elements. Moreover, the system is not described by the linear phenomenological equations, unless the flows are very slow. Polarity of flow is to be expected in many cases. A few numerical examples demonstrate the combination of the elementary coefficients and the polarity of flows. These examples are based on the model of Teorell and Meyer-Sievers. (Authors)

*33. Katchalsky, A., and Kedem, O. THERMODYNAMICS OF FLOW PROCESSES IN BIOLOGICAL SYSTEMS. Biophysics 2(2) Pt. 2, 53-78, 1962.

Showing the need for a thermodynamic theory of flow in open systems as a prerequisite for applications of thermodynamics to biological systems the author traces progress from Onsager to date with respect to transport processes in membranes.

34. Lakshminarayanaiah, N., and Shanes, A. M. THIN MEMBRANES OF PARLODION. Science 141, 43-44, 1963.

Brief description of a method of preparing parlodion membranes 50-1000A thick by depositing isoamyl solutions on the air-water interface under conditions of controlled evaporation. Capacitance and resistance measurements are used to check the integrity of the membranes.

35. Mauro, A., and Finkelstein, A. EQUIVALENT CIRCUITS AS RELATED TO IONIC SYSTEMS. Biophysical Journal 3(3)215-237, 1963.

36. Revel, J. P., Fawcett, D. W., and Philpott, C. W. OBSERVATIONS ON MITOCHONDRIAL STRUCTURE. J. of Cell Biology 16, 187-194, 1963.

37. Sjostrand, F. S. A NEW REPEAT STRUCTURAL ELEMENT OF MITOCHONDRIAL AND CERTAIN CYTOPLASMIC MEMBRANES. Nature 199(4900) 1262-1264, 1963.

The mitochondrial outer surface membrane as well as inner membranes are paired structures with two membrane elements forming the outer membrane as well as each inner membrane. Each constituent membrane element appears triple-layered after potassium permanganate fixation, as shown by Robertson, who has included the mitochondrial membrane elements as part of his unit membrane system.
The same triple-layered unit membrane structural pattern has been observed in the ℰ-cytomembranes ("rough-surfaced membranes"), and the Golgi membranes ("smooth-surfaced membranes").
The triple-layered structure of the unit membranes has been assumed to reflect their basic structural feature, a bimolecular leaflet of lipids sandwiched between two thin layers of protein (or of non-lipid material), according to the membrane model by Danielli and Davson, Robertson, and myself. (Author)

38. Stämpfli, R. DIE DOPPELTE SACCHAROSETRENNWANDMETHODE ZUR MESSUNG VON ELEKTRISCHEN MEMBRANEIGENSCHAFTEN MIT EXTRACELLULAREN ELEKTRODEN (THE DOUBLE SUCROSE GAP METHOD FOR THE MEASUREMENT OF ELECTRICAL MEMBRANE PROPERTIES WITH EXTRACELLULAR ELECTRODES.) Helv. Physiol. Acta 21, 189-204, 1963. (Ger.)

With the double sucrose gap method it is possible to measure membrane properties of nerve and muscle fibers with external electrodes. Details of the method are presented and examples of its utilization with single striated frog muscle fibers are given.

39. Stoeckenius, Walther. SOME OBSERVATIONS ON NEGATIVELY STAINED MITOCHONDRIA. J. of Cell Biology 17(2) 443-454, 1963.

40. Tria, E., and Barnabei, O. PRESENCE OF A PHOSPHATIDO-PEPTIDE FRACTION IN LIVER CELL MEMBRANE AND ITS POSSIBLE ROLE IN THE ACTIVE TRANSPORT OF AMINO-ACIDS. Nature 197, 598-599, 1963

MOLECULAR BIOLOGY

41. Asakura, S., Taniguchi, M., and Oosawa, F. MECHANO-CHEMICAL BEHAVIOR OF F-ACTIN. J. of Molecular Biology 7, 55-69, 1963.

Even at very low ionic strengths, actin assumes a polymerized form of moderate size under sonic vibration. The short actin polymer formed by the vibration splits added ATP enzymically. After stopping the vibration a large amount of ATP is split. The initial velocity of this ATP splitting is proportional to the actin concentration. This finding was explained by assuming that each short actin polymer contains an appreciable proportion of partially interrupted structures of the F-form and their reformation is coupled with the ATP splitting.
From the re-examination of the facts obtained from the sonic experiments the following conclusions were drawn: (1) The interruption-reformation process is essentially reversible; (2) interruption is accelerated by mechanical forces; (3) reformation is accelerated by the dephosphorylation of added ATP. Taking into account these results, various dynamic properties of an F-actin filament which theoretically follow from the helical polymer model are presented.

42. Brodie, B. B., and Costa, E. CONCEPT OF THE NEUROCHEMICAL TRANSDUCER AS AN ORGANIZED MOLECULAR UNIT AT SYMPATHETIC NERVE ENDINGS. Activitas Nervosa Superior 5(3) 264-279, 1963.

The structure of the biophysical unit which stores norepinephrine (NE) at nerve endings and releases the amine to sympathetic receptors is gradually unfolding. NE is formed continually inside a lipoid compartment regardless of sympathetic tone and is stored in two pools, a readily mobile pool and a reserve granular pool. The NE is maintained in the storage compartment by an energy-requiring active transport or "pump".

Monoamine oxidase, outisde of storage compartment, controls the level of NE so that it does not diffuse onto receptor sites. In this scheme the level of NE in the neurochemical transducer reaches a steady state when the rates of efflux and formation are equal. The amine can leave the storage compartment by discharge onto the receptor site or by diffusion through the membrane of the storage compartment. Nerve impulses presumably antagonize the pump vis-a-vis the receptor for after stimulation of the nerve, the amine appears in blood stream undeaminated. Present studies indicate, that the serotonergic and adrenergic neurochemical transducer function in a similar manner.

43. Bondy, S. C., and Perry, S. V. INCORPORATION OF LABELLED AMINO ACIDDS IN THE SOLUBLE PROTEIN FRACTION OF RABBIT BRAIN. J. of Neurochemistry 10, 603-9., 1963.

The metabolism of brain protein, insofar as it can be assessed by the incorporation of isotopically labelled amino acids, has recently been shown to be more active than was previously supposed (Gaitone and Richter, 1956; Clouet and Richter, 1957; Lajtha, Furst and Waelsch, 1957; Palladin, 1957). Indications of specialized protein metabolism in brain arise from investigations relating protein turnover with physiological activity (Hyden, 1943: Clouet and Richter, 1959) and evidence which suggests that the protein requirements of the axon are supplied from the cell body (Hyden, 1947). The work described here was carried out with the aim of further investigating the protein synthesizing system of brain and characterizing those components of the soluble fraction which have high levels of incorporation and may be of special significance for brain function. Some aspects of it have already been briefly described (Bondy and Perry, 1963).

44. Davidson, N., Vinograd, J., Morris, J., and Dove, W. F., Jr. THE BUOYANT BEHAVIOR OF VIRAL AND BACTERIAL DNA IN ALKALINE CsCl. Proceedings, National Academy of Sciences, 49, 12-17, 1963.

45. Kay, R. E. EXPERIMENTAL STUDIES FOR THE DETECTION OF PROTEIN IN TRACE AMOUNTS. Aeronutronic, NASA-CR-50385.

A method of detecting biological macromolecules, based upon the observation of spectral changes due to aggregation of a dibenzo-thiacarbocyanine dye when it is adsorbed on the macromolecular structure, was investigated. The reactions of the dye with inorganic salts, microorganisms, pollen, polypeptides, simple proteins, conjugated protens, synthetic polypeptides, polynuclectides, carbohydrates, amino acids, pyrimidine and purine bases, nucleosides and nucleotides were investigated. In trace amounts (less than 0.002%),

only proteins, synthetic polypeptides, nucleic acids, microorganisms, pollen, and substituted polysaccharides cause changes in the absorption spectrum of the dye. The influence of such variables as pH, temperature, and dye-macromolecule stoichiometry on the stability and formation of the dye-macromolecule complexes and dye aggregates was also determined. The optimum conditions for dye-macromolecule formation and stability appear to be a pH of 7 to 9, dye concentration of 4×10^{-5}M, and a temperature of 20 to 40 C. The relationship of macromolecule structure to the absorption spectrum of the macro-molecule-dye complex was examined; it was found, especially in the case of DNA, that the nature of the absorption spectrum of the dye-macromolecule complex could give significant information about the structure of the macromolecule. The macromolecule-dye reaction was investigated for its applicability to detection of macromolecules in heterogeneous materials by observing its behavior with soil extracts and suspensions of microorganisms. (Author)

46. Kendrew, J. C. THE FUTURE OF MOLECULAR BIOLOGY. New Scientist 19(356), 545-546, 1963.

The study of large biological molecules and their vital functions in living cells is expanding very rapidly, but there are signs that research in Europe is lagging behind. Apart from almost unlimited opportunities in fundamental research, medical and industrial applications may soon begin to appear.

47. Lea, E. J. A. PERMEATION THROUGH LONG NARROW PORES. J. Theoret. Biol. 5, 102-107, 1963.

The passage of molecules through long narrow pores is discussed. An expression is derived relating the ratio between tracer flux and total flux to the number of sites or places which can accommodate the molecules in the pore. On this basis values are calculated for the number of sites in the pore and compared with those calculated using previous treatments of the same effect.

48. Medvedev, Zh. A. REPRODUCTION AND TRANSFER OF INFORMATION DURING THE SYNTHESIS OF BIOLOGICAL MACROMOLECULES. OTS 62-13533.

This is concerned with the application of principles of information theory to the analysis of laws governing biochemical processes.

49. Ozaki, Masa-aki, Tanaka, Masahiro, Teramoto, Ei. DEPENDENCE OF THE TRANSITION TEMPERATURES OF DNA MOLECULES UPON THEIR BASE COMPOSITIONS. Journal of the Physical Society of Japan, 18(4), 551-557, 1963.

Thermal denaturation and renaturation processes of DNA-type molecules are studied, using two models of DNA-type double-chain which were previously treated by Zimm and Hill. In these two models, the difference between the bond energies of the two kinds of base pairs, guanine-cytosine and adenine-thymine, is taken into account, assuming the random sequences of four bases in the double-chain. Then it can be shown that the two models have essentially different characters for the dependence of transition temperature upon the guanine + cytosine content. The first model gives an almost linear relationship between the transition temperature and guanine + cytosine content, but the second gives an almost quadratic relationship. These results are discussed, using the experimental data given by Marmur and Doty, and Eigner.

*50. Schmitt, Frances O. THE MACROMOLECULAR ASSEMBLY, A HIERARCHICAL ENTITY IN CELLULAR ORGANIZATION(review). Develop. Biol. 7, 546-559, 1963.

In this paper the author (1) indicates the biochemical and evolutionary significance of MMA as a hierarchical entity (2) emphasizes the desirability of applying the methods of solid state physics to the study of MMA and (3) suggests that an active search be made for MMA's as mediators of certain vital processes not presently associable with definite componentry at the molecular or colloidal levels.

51. SORTING OUT THE RNA MOLECULES. New Scientist 360, 103, 1963.

Transfer or soluble ribonucleic acid (s-RNA) is the RNA with relatively small molecules which react with amino acids in the living cell to form complexes that are important as intermediates in protein synthesis. One of the main difficulties in the study of s-RNA is to separate its 20 or more kinds, each of which combines amino acid. Various fractionation and purification methods have been devised but none so far has proved completely satisfactory.
A. H. Mehler and A. Bank of the Laboratory of Biochemistry, National Institutes of Health, Bethesda, have recently described a new fractionation technique which may prove to be of great value (Journal of Biological Chemistry, Vol. 238, Preliminary Communications, p. 2888). The method is based on the formation

of an insoluble precipitate when an amino acid-s-RNA compound reacts with the N-carboxy anhydride of epsilon-trifluoroacetyllysine.
In practice, a sample of natural s-RNA, containing many species, is charged with one amino acid only - such as valine - and then submitted to the reaction. The precipitate is separated and the insoluble trifluoro-acetyllysine polymer which builds up on the free amino group of the valine can then be removed, leaving a solution highly enriched in valine-specific s-RNA. The process can be repeated by recharging the remaining unprecipitated s-RNA with all the different amino acids in turn and thus theoretically should permit the isolation of large amounts of purified, amino acid-specific s-RNA from any organism.

**52. Westphal, O., and Luderite, O. DIE BIOLOGISCHE BEDEUTUNG DER CHEMISCHEN FEINSTRUKTUR BAKTERIELLER ZELL-GRENZFLACHEN. Die Naturwissenschaften 12, 413-426, 1963. (Ger.)

"Quite pertinent to a discussion of the structural features of surface membranes and does give some insight into the chemical constitution of these membranes which, in turn, gives a clue not only to the way in which they are constructed but to the way in which they might function as well." - M. Calvin.

NEUROANATOMY

*53. Palay, S. L., and Grillo, M. A. CILIATED SCHWANN CELLS IN THE AUTONOMIC NERVOUS SYSTEM OF THE ADULT RAT. Journal of Cell Biology 16, 430-436, 1963.

NEUROCHEMISTRY

*54. Davidson, N. et al. THE BUOYANT BEHAVIOR OF VIRAL AND BACTERIAL DNA IN ALKALINE CsCl. Proceedings of the National Academy of Sciences 49(1), 12-17, 1963.

55. Larrabee, Martin G., Klingman, Jack D., and Leicht, William S. EFFECTS OF TEMPERATURE CALCIUM AND ACTIVITY ON PHOSPHOLIPID METABOLISM IN A SYMPATHETIC GANGLION. Journal of Neurochemistry 10, 549-570, 1963.

The major purpose of these experiments was to investigate the changes in phospholipid metabolism which occur when nerve cells are activated by naturally conducted impulses. For this purpose impulses were initiated under experimental control at a point effectively remote from that at which the metabolic effects of conduction and synaptic transmission were measured. Observations such as those of Hokin, Hokin and Shelp who used acetylcholine as a stimulant of sympathetic

ganglia and other nervous tissues, have thus been extended to effect caused by conducted nerve impulses, with partly conflicting results. Moreover, additional information has been obtained by varying the frequency of stimulation, the environmental temperature, and the calcium concentration and also by labeling the lipids with 14C from glucose as well as with 32P from inorganic phosphate.

*56. Ochoa, S., Koivusalo, M., Elorriaga, C., and Kaziro, Y., BACTERIAL BIOTINIDASE. Journal Biological Chemistry 328(3) 1038-1042, 1963.

Biotinidase hydrolyzed all of biotin derivatives tested in order of decreasing reactivity: biotin amide, N-biotinyl-p-aminobenzoic acid, biotin methyl ester, biocytin (ϵ-N-biotinyl-L-lysine) ÷ N biotinyl-β-alanine and N-biotinyl-L-aspartic acid. ϵ-N-Acetyl-L-lysine was not hydrolyzed. Biotinidase is specific for biotin moiety of simple biotin esters and amides. Enzyme released equimolar amounts of biotin and lysine from biocytin. Biotinidase activity was present in liver, blood plasma, kidney, heart, brain, spleen and intestine among animal tissues; in yeast, Propionibacterium shermanii and Streptococcus faecalis. There was little or no activity in Lactobacillus arabinosus 17-5 which can utilize free biotin only. (Authors)

*57. Ochoa, S., Weissmann, C., and Simon, L., INDUCTION BY AN RNA PHAGE OF AN ENZYME SYNTHESIZING RIBONUCLEIC ACID. In: 47th Annual meeting of the Federation of American Societies for Experimental Biology, 1963. Fed. Proc. 22(2 Pt. 1), 463, 1963. (Abstract)

58. Pakkenberg, H. CYTOPLASMIC BASOPHILIA IN THE NERVE CELLS OF THE CEREBRAL CORTEX. IV. RNA CONTENT IN THE NERVE CELLS OF THE RABBIT CORTEX. Journal of Comparative Neurology 121(1), 1-4, 1963.

A rabbit brain is fixed by perfusion with Carnoy's fluid through the carotids, and the brain removed from the skull after four hours. Sections of controlled thickness are stained with gallocyanin and the extinction values determined at four points in the cytoplasm of each nerve cell by means of a microspectrophotometer. The measurements are made on the large cells of the third and fifth layers, 50 cells from each of eight different sites in the cortex symmetrically for both hemispheres. The histograms show that the extinction values vary considerably in the cell population of all the cortical regions measured, but that there is no characteristic distribution of chromophobic, chromoneutral and chromophilic cells. No difference in extinction values is observed between the two hemispheres. The differences

in extinction-values were nearly the same in a Susa-perfused rabbit-cortex. Optimum histological preparation thus shows quite considerable differences in the cyto-plasmic concentration of RNA in the nerve cells. This may be taken to indicate that differences exist in the firing activity of the cells at the moment of fixation.

59. Tanaka, Ryo, and Abood, L. G. ISOLATION FROM RAT BRAIN OF MITOCHONDRIA DEVOID OF GLYCOLYTIC ACTIVITY. Journal of Neurochemistry 10, 571-576, 1963.

The conventional mitochondrial fraction obtained from rat brain by differential centrifugation in a sucrose-ethylenediamine tetraacetate (EDTA) medium was further subjected to subfractionation by employing the technique of gradient centrifugation in a medium of Ficoll-sucrose-EDTA. Electron microscopy revealed that the lightest subfraction was composed of fragments of axonal processes, fragments of myelin sheaths, synaptic vesicles, and other vesicles of various sizes. The second subfraction contained intact nerve endings and membrane fragments of probably synaptic origin, while the two heaviest subfractions contained largely mitochondria. The pattern of distribution of phosphorylative and hexokinase activity in the four subfractions was parallel and the reverse of the pattern for glycolytic activity. It would be concluded that glycolytic activity was not associated with mitochondria from rat brain as hitherto believed.

NEUROCYBERNETICS

60. A MODEL BRAIN IN KENSINGTON GORE. New Scientist 20(360) 70, 1963.

The Upjohn model of the brain will be on show at the Royal College of Art in Kensington Gore. It forms part of an exhibition called "Visual Aspects of Science". It is large - it contains concave aluminum discs 9 ft. 3 in. across to represent the memory cortices and, among other things, 38 miles of electrical wiring and 30,000 lights and its works. Hearing and vision are employed in perceiving a singer in a concert hall and the model brain shows with travelling chains of lights how impulses adjust the iris of the eye to the concert hall lights and produce conscious perception of the singer via the visual cortices. (An image appears on a large central screen). Similar impulses travel from the ears and the brain becomes conscious of the song (at which point it is heard in the earphones). The brain now recalls memories of events and other singers and finally the motor cortex is stimulated to applaud.

61. Napalkov, A. V. THE EXAMINATION OF THE PRINCIPLES OF THE ASSIMILATION OF INFORMATION BY THE BRAIN. English Absts. Soviet Bloc and Mainland China Tech. Jour. Ser. VI: Bio Sci. 61-11, 145(22) n. p., 1962.

Cybernetics gives new possibilities for the examination of the principles and the rules of information assimilation by human and animal brains; (2) the examination of the structure of the nervous system. The author characterizes both research directions, as well as their prospects and concrete results. The author particularly emphasizes the development of a new approach to the working of the brain based on the examination of the principles of coding and information assimilation; the designation "information physiology" rather than "information psychology" is suggested for this direction.

NEUROGLIA

62. Cammermeyer, Jan. SIMILARITIES BETWEEN OLIGODENDROCYTE AND CEREBELLAR GRANULE CELL NUCLEI IN MAMMALIA AND AVES. Amer. J. of Anat. 112(1) 1963.

63. Glees, P. NEUERE ERGEBNISSE AUF DEM GEBIET DER NEUROHISTOLOGIE: NISS'L-SUBSTANZ, CORTICALE SYNAPSEN, NEUROGLIA UND INTERCELLULARER RAUM. Deutsche Zeitschrift fur Nervenheilkunde 184, 607-631, 1963. (Ger.)

The first part of this paper reviews the progress made on the chemistry and electronmicroscopical structure of interneuronal connections, the synapses. The last section of the paper is dedicated to the structure and function of the neuroglia, work which has been accumulated since the author published his monograph in 1955. (Glees, P., Neuroglia: Morphology and Function. Oxford, Blackwell, 1955). This section also discusses the construction of cerebral capillaries, the problems of intercellular space and the blood brain barrier. (Author)

*64. Hamberger, Anders, and Hyden, H. INVERSE ENZYMATIC CHANGES IN NEURONS AND GLIA DURING INCREASED FUNCTION AND HYPOXIA. J. of Cell Biol. 16(3) 521-525, 1963

65. Nakai, J. TRANSFORMATION AND MULTIPLICATION OF NEUROGLIA IN TISSUE CULTURE. In Proceedings IV.International Congress of Neuropathology, 4-8 Sept 1961, Stuttgart, Georg Thieme Verlag, 1962.

The present report is concerned with transformation and multinucleation of neuroglia cells as they appeared in the cultures of the central nervous tissues, since it is very characteristic of glia cells in cultures that there appeared various "transitional" forms especially of astrocytes and multinucleate cells

similar to those in the malignant tumors. Various regions of the cerebrum and cerebellum of two to three months old human fetuses, new-born kitten and rabbit and adult guinea pig were cultivated for a few weeks to more than a year by roller tube method with flying cover slips. The problems of transformation and multinucleation are conclusively solved only by continual observations and recording of the behavior of one particular cell. Thus the data reported here was based on the records by the phase contrast time-lapse cinematography.

NEUROPHYSIOLOGY

66. Buser, P., Bruner, J., and Sindberg, R. INFLUENCES OF THE VISUAL CORTEX UPON POSTEROMEDIAL THALAMUS IN THE CAT. J. of Neurophysiology 26(5) 677-691, 1963.

Two groups of related experiments were carried out to study the control by the visual cortex over visual responses in a posteromedial thalamic region comprised of the centrum medianum and adjacent structures. In the first series of experiments, the primary visual cortex was stimulated electrically, while this area of the thalamus was mapped with concentric recording electrodes. A short-latency response field was found in this region, approximately coinciding with the area reported by several other investigators to respond to light, sound, and somesthetic stimulation. These data were taken to indicate the presence of rather short latency pathways through which cortical control could be exerted.

Another group of experiments was devoted to studying the effects of modifying cortical excitability by drugs and by local cooling. Strychinization of the visual cortex, while producing an augmentation of responses in the cortex, also increased the response in the posteromedial thalamus. On the other hand, application of KCl or of a local cooling agent to the cortex decreased or eliminated such responses. These actions were here specific, for no such augmentations or depressions were observed at the same time for somesthetic or auditory responses. Thus the state of excitability of the visual cortex has specific influence on the amplitude of visual responses in this posteromedial thalamic region. From the data obtained, it is suggested that this cortical influence takes place by determing the amount of descending corticifugal outflow which is triggered by the incoming specific corticipetal volley.

67. Flickinger, R. A. ACTINOMYCIN D EFFECTS IN FROG EMBRYOS: EVIDENCE FOR SEQUENTIAL SYNTHESIS OF DNA-DEPENDENT RNA Science 141, 1063-1064, 1963.

Early gastrulae of Rana pipiens exposed to actinomycin D for 2 days were then cultured in saline. The resulting larvae were immotile; histological examination revealed impaired development of axial nerve and muscle tissues, but normal differentiation of sensory, pronephric, heart, and digestive tissues; this suggests sequential synthesis of the different tissue-specific molecules of DNA-dependent RNA.

*68. Galambos, R., Rupert, A., and Moushegian, G. UNIT RESPONSES TO SOUND FROM AUDITORY NERVE OF THE CAT. J. Neurophysiol. 26(3) 449-465, 1963.

Tungsten microelectrodes were place in the auditory nerve of both anesthetized and unanesthetized cats and the sound- induced activity of 45 units was recorded and intensively studied. Three criteria (anatomical, slow-wave morphology, and spike latency) were used to establish unequivocally the location of the electrode tip and every eighth nerve unit described in this paper met all three criteria. Our results show that the spontaneous discharges of auditory nerve units can be inhibited by sounds, but since some are inhibited promptly and others considerably after tone onset, two mechanisms of inhibition are inferred. Some units discharge with interspike intervals related primarily to stimulus period while others do not. These latter units seem related to the stimulus intensity and presumably may mediate intensity information. Finally, some spontaneously active units respond to certain continuous tones by grouping their discharges with no increase in discharge rate. These results of the behavior of auditory nerve units suggest that highly complex processing occurs at the cochlear level where the impulses are generated. (Authors)

69. Green, John D. THE FUNCTION OF THE HIPPOCAMPUS. Endeavour 22(86) 80-88, 1963.

70. Kravitz, E. A., Kuffler, S. W., Potter, D. D., and Van Gelder, N. M. GAMMA-AMINOBUTYRIC ACID AND OTHER BLOCKING COMPOUNDS IN CRUSTACEA. II. PERIPHERAL NERVOUS SYSTEM. J. of Neurophysiology 26(5) 729-738, 1963.

Peripheral nervous systems of lobsters and crabs were surveyed for compounds that block transmission at the crayfish neuromuscular junction. Ten such substances were isolated. Gamma-aminobutyric acid (GABA), taurine, and betaine contributed most of the blocking activity of the extracts. Other compounds were: β-alanine, alanine, homarine, glutamine, aspartic acid, and two unidentified substances. These materials were separated by electrophoretic and paper chromatographic techniques and GABA was further identified by a specific enzymic assay procedure. GABA had the strongest blocking activity. The only excitatory substance in the extracts was

glutamic acid.
GABA does not appear to be produced by destruction of a larger molecule during extraction.
The GABA content of peripheral nervous tissues increased together with the proportion of known inhibitory axons.

71. Kravitz, E. A., Kuffler, S. W., and Potter, D. D. GAMMA-AMINOBUTYRIC ACID AND OTHER BLOCKING COMPOUNDS IN CRUSTACEA. III. THEIR RELATIVE CONCENTRATIONS IN SEPARATED MOTOR AND INHIBITORY AXONS. J. of Neurophysiology 26(5) 739-751, 1963.

Two efferent axons of similar diameter, one inhibitory, the other excitatory, run side by side in the leg of the lobster (Homarus americanus). Long unbranched stretches of these neurons were removed and separated; the isolated axons were analyzed for their content of gamma-aminobutyric acid (GABA) and nine other synaptic blocking compounds that had been previously found in the crustacean nervous system. The GABA contents were compared enzymically; the contents of the other blocking substances were compared chromatographically or by physiological assay.
The GABA content along the course of the inhibitory axon was about 0.5% of its wet wt., while no GABA was detected in the accompanying excitatory fiber. GABA may therefore be confined to inhibitory nerves. The other blocking compounds, with the possible exception of β-alanine, were found in both neuron types. The distribution of β-alanine (a much weaker blocking substance than GABA) cannot be stated with confidence. The findings indicate that GABA has a function specifically related to inhibitory neurons.

72. Lebedev, V. P. STUDY OF SPONTANEOUS DISCHARGES FROM INDIVIDUAL INTERNUNCIAL NEURONS OF THE SPINAL CORD AS A METHOD OF DIFFERENTIATING THEM. Federation Proceedings, Translation Supplement, 22(4)Pt. II, T732-736, 1963.

Most internuncial neurons of the spinal cord generate a continuously detectable spontaneous discharge even when impulses from afferent and descending pathways are rather completely eliminated. The character of this spontaneous activity is exceedingly varied.
It may be assumed that the variety in the spontaneous activity of the individual internuncial neurons reflects aspects of their structure and physiological properties and may be distinct for these most numerous nerve cells of the spinal cord.
The present work was undertaken to find the most typical forms of this spontaneous activity. An attempt was made to establish a correlation between the form of spontaneous activity and the location and afferent links of the nerve elements generating it, as well as the changes in each type of discharge induced by certain drugs.

73. Mountcastle, Vernon B., Poggio, Gian F., and Werner, Gerhard. THE RELATION OF THALAMIC CELL RESPONSE TO PERIPHERAL STIMULI VARIED OVER AN INTENSIVE CONTINUUM. J. of Neurophysiology 26(5) 807-834, 1963.

The aim of our present studies is to quantitate neural events in successively more centrally located projection regions of the somatic afferent system, events which begin with the activation of peripheral receptors by physical stimuli. It is expected that such investigations will yield results permitting one to recognize laws governing the transformations intervening between peripheral sensory and central neural events. Studies of this kind deal with two quantifiable variables: a stimulus continuum measured in physical units, and a continuum of neural activity measured in some units deemed appropriate to the nature of the neural response. The task is, then, to assign numbers to observations in such a way that one can perform numerical operations which establish the quantitative relation between the two continua of measurement.

74. Poggio, Gian F., and Mountcastle, Vernon B. THE FUNCTIONAL PROPERTIES OF VENTROBASAL THALAMIC NEURONS STUDIED IN UNANESTHETIZED MONKEYS. J. of Neurophysiology 26(5) 775-806, 1963.

It was the purpose of the studies described in the present series of papers to investigate certain neural mechanisms essential for sensation and perception. The field of interest includes a study of first-order input, the transforms imposed upon that input at successive synaptic relays of afferent systems, and those complex intracortical phenomena interposed between the transformed input and the output it evokes. These neural events are thought to be mechanisms essential to the subjective experience of sensation. Dealing with but a small part of the subject: the nature of the central reflection of sensory stimuli at the thalamocortical stage of an afferent system. Making a further limitation, and emphasizing concern with the neural mechanisms for what might be termed the immediate aspects of sensation: the recognition that a stimulus has been delivered, the rating of and discrimination between stimuli ordered along intensive continua, the localization of stimuli in space, the identification of stimulus quality, the signaling of stimulus rhythm. Neural phenomena less directly concerned with sensation, e. g., those regulating the degree of attention, have not been the subject of study, important as they are in a general sense for a complete understanding, for so far they cannot be brought under direct experimental control.

75. Scharlock, Donald P., Tucker, Thomas J., and Strominger, Norman L. AUDITORY DISCRIMINATION BY THE CAT AFTER NEONATAL ABLATION OF TEMPORAL CORTEX. Science 141 (3586) 1197-1198, 1963.

Some auditory discriminations cannot be acquired by the cat after large bilateral ablations of auditory cortex at maturity. However, if such ablations are sustained during infancy these discriminations are readily learned. The function of the cortex in auditory discrimination depends on the age of the nervous system at the time of injury.

76. Teuber, Hans-Lukas, and Rudel, R. G. BEHAVIOUR AFTER CEREBRAL LESIONS IN CHILDREN AND ADULTS. Developmental Medicine and Child Neurology 4, 3-20, 1963.

77. White, R. J., Albin, M. S., and Verdura, J. ISOLATION OF THE MONKEY BRAIN: IN VITRO PREPARATION AND MAINTENANCE. Science 141;1060-1061, 1963.

Sustained viability of the primate brain, as a totally isolated organ preparation, was achieved by utilizing an extracorporeal (compatible donor) circulation. Five rhesus monkey brains, completely isolated neurogenically and vascularly, were perfused in vitro for 30 to 180 minutes. Retention of biological activity was evidenced by: (i) persistent electrocortical activity, and (ii) significant mean A-V_{O2} (5.8 volumes percent) and V-A_{CO2} (5.0 volumes percent) differences across the isolated brain.

PHYSICAL CHEMISTRY

*78. Davidson, N., and Stewart, R. F. USE OF AN ULTRAMICROTOME FOR THE PREPARATION OF THIN SECTIONS OF SINGLE CRYSTALS FOR ABSORPTION SPECTROSCOPY. Proceedings of the National Academy of Sciences 49(2) 146-150, 1963.

"Summary: Sections of single crystals of organic substances with thicknesses down to 0.1 can be prepared by using a diamond knife and an ultramicrotome as a polishing tool. It is believed that this technique will be generally useful for preparing specimens for single crystal absorption spectroscopy." (Authors)

*79. Eigen, M. WASSERSTOFFBRUCKEN SYSTEME ALS MEDIEN CHEMISCHEN STOFFTRANSPORTS (HYDROGEN BOND SYSTEM AS THE MEDIUM OF CHEMICAL ELEMENT TRANSPORT.) Die Naturwissenschaften 12, 426-437, 1963. (Ger.)

"it will be found that the significance of the hydrogen bond for physiology is greater than that of any other structural feature." L. Pauling.
In this article the analogy between biological regulatory prccesses and engineering electrical control systems is often drawn. The fundamental elements of technical control systems are the electron tube, the transistor or other non-inertial working "switches". We know that the natures of these switching elements make little use of information transfer, though indeed, they regulate in time intervals of milliseconds, working and responding through reflexes. The biological regulatory processes make use of proteins, nucleic acids and other macromolecules and to serve this purpose are organized in contact with solutions of substances of low molecular weight. If one wishes to understand the overall mechanism of such processes then one must know the function of the individual elements just as you trace the "curve" of an electron tube before you use it in a switch. The "curves" of biological switching elements are defined through the constants of chemical element step reaction. Hydrogen bond systems are the model building materials for such switching elements because of their potential for building specific structures and capacity for fast reactions. (Author - Tr. by J. M.)

*80. Eigen, M. PROTONENUBERTRAGUNG, SAURE-BASE-KATALYSE UND ENZYMATISCHE HYDROLYSE. TEIL I: ELEMENTARVORGANGE (PROTON TRANSFER, ACID-BASE-CATALYSIS AND ENZYMATIC HYDROLYSIS. PART I: ELEMENTARY PROCESSES) Angewandte Chemie 12, 489-588, 1963. (Ger.)

The proton acts as a carrier and mediator of the chemical reaction in the solvent phase, a distinguishable arrangement. Many reactions of organic chemistry are catalyzed by acids or bases, particularly including most enzyme work groups which mediate acid-base-catalysis. An understanding of the reaction mechanism presupposes a knowledge of elementary steps and its periodic expiration. (?) The systematic investigation of the elementary steps is first reported in the development of new methods for the investigation of very fast reactions. The results of such investigations are summarized in this paper. It shows a relatively complete model of the elementary mechanism of proton transport and leads to a comprehensive description of the laws of acid-base-enzyme catalysis. (Author - Tr. by J. M.)

81. Lundqvist, S., and Brandt, W. POSSIBLE NEW ATOMIC RESONANCES. Phys. Letters 4(1) 47-48, 196? (NASA CR50345)

Equations are presented to support the theoretical evidence for the possibility of the occurrences of new resonances in atoms, that are different in kind from single-particle excitations. These resonances mark the coherent response of an atom as a whole. If this preliminary calculation which is made for a simple statistical model applies to real atoms, it can be concluded that new atomic resonances may appear over a more or less extended intermediate range of atomic frequencies.

*82. Onsager, L., and Fuoss, R. M. THE CONDUCTANCE OF SYMMETRICAL ELECTROLYTES. I. POTENTIAL OF TOTAL FORCE. Journal of Physical Chemistry 66, 1722, 1962.

By means of a multiplicative expansion of the distribution functions which describe local ionic concentrations, the 1932 Onsager-Fuoss equation of continuity can be integrated with explicit retention of the Boltzmann factor, instead of approximating the latter by a truncated power series. The result is expressed in terms of the potential μ_{ji} of total force acting on a given ion: the present approximation to $\nabla\mu_{ji}$ includes the external field, the forces due to neighboring ions and to the asymmetry of the ionic atmospheres, and the virtual forces due to local concentration gradients. The differential equations which will lead to the forces from the velocity field also have been derived. (Authors)

*83. Onsager, L., and Fuoss, R. M., THE CONDUCTANCE OF SYMMETRICAL ELECTROLYTES. II. THE RELAXATION FIELD. Journal of Physical Chemistry 67, 621, 1963.

The Poisson equation for the asymmetry potentials of a symmetrical electrolyte in the conductance process has been integrated by the use of the corresponding Green's function in order to obtain the purely electrostatic terms of the relaxation field. The Boltzmann factor in the distribution function was retained explicitly as an exponential throughout the calculation, instead of approximating it as a truncated series as has been customary in previous derivations. The consequence of this refinement in mathematical methods is the appearance in the relaxation field of a term which will lead to a decrease in conductance with increasing concentration or decreasing dielectric constant. The decrease is proportional to the product of concentration and the square of the mean activity coefficient. It depends on dielectric constant through a function which has as its asymptotic limit e^6/b^3 ($b = \epsilon^2/aDkT$), which is the form of the theoretical association constant for contact pairs. (Authors)

*84. Onsager, Lars, and Fuoss, Raymond M. THE CONDUCTANCE OF SYMMETRICAL ELECTROLYTES. III. ELECTROPHORESIS. Journal of Physical Chemistry 67, 628, 1963.

The electrophoretic velocity in a dilute solution of a symmetrical electrolyte has been computed, with the following improvements over earlier treatments of the problem: (1) the volume force is calculated as the gradient of the potential of the total force acting on an ion instead of being approximated merely by the force due to the external field; (2) the Boltzmann factor is retained explicitly, instead of being approximated by a truncated series; and (3) the Oseen equations of motion (rather than the Stokes) are used. The result gives the Onsager 1926 limiting value ($-\epsilon j\kappa X/6\pi\eta$) as the leading term; to next approximation, this is opposed by a term proportional to concentration, which depends on $b = \epsilon^2/aDkT$ in a non-exponential fashion. For example, for $b = 1.5$ ($D \approx 100$), $F(b) = 2.31$ and for $b = 15$ ($D \approx 10$), $F(b) = -0.77$. The coefficient goes through zero near $b = 5$.

85. Nemethy, G., Steinberg, I. Z., and Scheraga, H. A. INFLUENCE OF WATER STRUCTURE AND OF HYDROPHOBIC INTERACTIONS ON THE STRENGTH OF SIDE-CHAIN HYDROGEN BONDS IN PROTEINS. Biopolymers 1:1, 43-69, 1963.

PSYCHOLOGY

85. Fletcher, J. L. (Ed.) MIDDLE EAR FUNCTION SEMINAR. (AD 405755)

Presented are the papers given at a seminar held at US Army Medical Research Laboratory, 7-8 May 1962, representing the work of those in the many scientific disciplines aimed at a better understanding of the structure, functioning and implications for research and diagnosis of middle ear. Papers were delivered by Drs. Charles E. Blevins, O'Dell W. Henson, F. Blair Simmons, J. R. Mundie, Jozef Zwislocki, Mr. Emanuel Mendelson, Drs. Howard Weiss, Michel Loeb, Scott N. Reger, W. Dixon Ward, and Mr. Robert Fleer. The seminar summary was written by Dr. William D. Neff.

QUANTUM CHEMISTRY

*86. Kasha, Michael, QUANTUM CHEMISTRY IN MOLECULAR BIOLOGY. In: M. Kasha, and B. Pullman, Editors. Horizons in Biochemistry. Albert Szent-Gyorgyi Dedicatory Volume. New York, Academic Press, 1962. pp 583-599.

In this essay a number of topics in quantum chemistry have been singled out for evaluation and comment as an indication of the potential which these topics offer for detailed molecular electronic mechanisms in biological phenomena. A number of important topics, such as ligand-field theory of metal ions, the intricacies of intra-molecular electronic excitation, and numerous others have been omitted from discussion here. In fact, the limits of space on such an article have prevented more than a passing commentary. Nevertheless, the author hopes some illumination of the field will result. (Author)

PSYCHOPHARMACOLOGY

87. Friedman, Alexander H., Aylesworth, Robert J., Friedman, Gertrud. TREMORINE: ITS EFFECT ON AMINES OF THE CENTRAL NERVOUS SYSTEM. Science 141, 1188-90, 1963

The administration of tremogenic doses of Tremorine, 1,4-dipyrrolidino-2-butyne, is followed by a significant decrease in the concentration of norepinephrine in the brain stem of three common laboratory species. The change in the concentration proceeds at a rate which coincides with the occurrence of the tremor in each of these species. In the rat, the change in norepinephrine is followed by a progressive increase in the concentration of 5-hydroxy-tryptamine in the brain stem. Bilateral adrenalectomy in the rat enhances the Tremorine-induced changes in the concentration of norepinephrine and antagonizes the increase in the concentration of 5-hydroxytryptamine.

SLEEP

88. Adey, W. Ross, Kado, Raymond T., and Rhodes, John M. SLEEP: CORTICAL AND SUBCORTICAL RECORDINGS IN THE CHIMPANZEE. Science 141, 932-933, 1963.

Electroencephalographic sleep patterns of chimpanzees reveal greater similarities to human records than those of lower mammals. Flash-evoked responses in the midbrain reticular formation remain during "paradoxical" sleep, which does not appear to necessarily involve deep unconsciousness. Characteristic spindling occurs in the amygdala during a "paradoxical" type sleep while other areas were desynchronized. Telencephalic sleep-control mechanisms in higher primates are considered.

89. UCLA SCIENTISTS INDUCE SLEEP WITH BELL. Science News Letter 84, 197, 1963

Although an alarm clock's ringing is supposed to awaken, scientists at the University of California, Los Angeles, can put animals to sleep with a bell.

It is all part of a study of sleep systems of the brain being carried out by Drs. C. D. Clemente, M. B. Sterman, and Wanda Wyricka of the UCLA Brain Research Institute.
It has been shown that by electrically stimulating a region known as the basal forebrain, sleep could be induced in animals within 30 seconds.
Borrowing a method used by Pavlov, the famous Russian expert on behavioral conditioning, the UCLA scientists introduced a bell tone of a specific frequency each time the forebrain received electrical stimulation.
By the time this procedure had been repeated 20 times, it was found that the animal would go to sleep in response to the bell tone-alone, without electrical stimulation.
The electrical stimulation and/or the bell tone initially produced the synchronous brain wave patterns characteristic of sleep.
These results suggest that just as there is a wakefulness system, known to be centered in the brain stem, there is a sleep system of which the forebrain appears to be an important part.
The sleep system probably acts by inhibiting the wakefulness system.
Early conditioning is perhaps most responsible for our sleep-wakefulness patterns, Dr. Clemente says.

THERMODYNAMICS

90. Dainty, J., and Ginzburg, B. Z. IRREVERSIBLE THERMODYNAMICS AND FRICTIONAL MODELS OF MEMBRANE PROCESSES WITH PARTICULAR REFERENCE TO CELL MEMBRANE. J. of Theoretical Biology 5(2) 256-265, 1963.

The transport of solute and water through the conventional lipid-pore model of the cell membrane is treated by the method of irreversible thermodynamics, resulting in simple formulae for the hydraulic conductivity and reflection coefficient of the membrane.

*91. Katchalsky, Aharon. NONEQUILIBRIUM THERMODYNAMICS. International Science and Technology, 42-49, 1963.

Classical thermodynamics dealt with closed systems in equilibrium, and it could not describe the irreversible transport processes of open systems. Modern insights about the components of entropy production and its relationship to time permit expressing the dissipation of a system's free energy as a function of forces and flows. A flow is seen to be drivable not only by the force to which it is conjugated-current by emf, for example-but also, through coupling, by the forces driving other kinds of simultaneous flows. Onsager's law reduces the coupling coefficients to the number necessary and sufficient to describe process behavior. Workers are still trying to give physical meaning to the coefficients of complicated

systems involved many or rapid flows, but the understanding so far achieved of slow and simple systems has already explained old anomalies and predicted new data. (T. Melnechuk)