Scanning Electron Microscopy/1981

Part 2

SCANNING ELECTRON MICROSCOPY/1981/II

An

INTERNATIONAL JOURNAL

of

SCANNING ELECTRON MICROSCOPY,

RELATED TECHNIQUES,

and

APPLICATIONS

PART II

Guest Editors

J. D. Shelburne	Duke Univ. Med. Ctr, Durham, NC	(Microanalysis)
A. Reith	Norwegian Radium Hosp., Oslo	
R.M. Albrecht	Univ. Wisconsin Sch. Med., Madison	

Published by

SCANNING ELECTRON MICROSCOPY, Inc.
P.O. Box 66507
AMF O'Hare (Chicago), IL 60666, USA

For all additional information or inquiries contact:

Dr. Om Johari, SEM, Inc. (Phone: 312-529-6677)
P.O. Box 66507
AMF O'Hare, (Chicago), IL 60666, U.S.A.

LIBRARY OF CONGRESS CATALOGUE NO. 72-626068

ISBN:	SEM/1978/Set	0-931288-00-2	SEM/1980/III	0-931288-13-4
	SEM/1978/I	0-931288-01-0	SEM/1980/IV	0-931288-14-2
	SEM/1978/II	0-931288-02-9	SEM/1980/Set	0-931288-15-0
	SEM/1979/I	0-931288-04-5	SEM/1981/I	0-931288-17-7
	SEM/1979/II	0-931288-05-3	SEM/1981/II	0-931288-18-5
	SEM/1979/III	0-931288-06-1	SEM/1981/III	0-931288-19-3
	SEM/1979/Set	0-931288-10-X	SEM/1981/IV	0-931288-20-7
	SEM/1980/I	0-931288-11-8	SEM/1981/Set	0-931288-21-5
	SEM/1980/II	0-931288-12-6		

LIBRARIANS AND OTHER USERS OF THIS PUBLICATION PLEASE NOTE:

For this publication the volume number and the year are the same. 1981 volume will have 4 parts, SEM/1980 had 4 parts, SEM/1979 had 3 parts and SEM/1978 had 2 parts.

The correct way to refer to papers in this journal is:

Scanning Electron Microsc. year; part: range of pages.

DIVISION OF PAPERS IN DIFFERENT PARTS:

Part I contains papers of general interest, techniques, and physical applications.
Part II contains general papers of biological interest, including cell biology, cell culture, biological microanalysis, and related topics.
Part III contains papers on biological applications, including cancer, clinical applications, biomedical applications, mineralized tissue, plants, and food microstructures.
Part IV will contain papers on all topics which were processed after above parts were prepared.

This division of papers is same as in 1980 volume

DATES OF FUTURE SEM MEETINGS:

Scanning Electron Microscopy/1982 - April 25-30, Anaheim, CA, See p. xvi.
Scanning Electron Microscopy/1983 - April 18-22, Dearborn (Detroit), MI.

PRINTED IN THE UNITED STATES OF AMERICA

For a description of the aims and programs of SEM Inc. (a non-profit organization), see p. 510.

REVIEWING PROCEDURE
AND
DISCUSSION WITH REVIEWERS

Each paper in this volume contains a Discussion with Reviewers. This discussion follows the text and should be read with the paper. Each paper submitted to SEM, Inc. for publication is reviewed by at least three, up to an average of five, reviewers. The reviewers are asked to separate their comments from their questions. The comments are useful in determining the acceptability of the papers as submitted. Although the comments require no written response, in several cases, the authors have included responses to comments, or to questions phrased from, or based on, comments (either as a result of editorial suggestions or on the author's own initiative). Based on these comments approximately 15% of the submitted papers were not accepted for publication; while almost all of the others were asked to make changes involving from minor to major revisions.

The questions, for the most part, originate as a result of statements included in our cover letter accompanying each paper sent to the reviewers. The reviewers are asked to suppose they are attendees at a conference where this paper, as written, is being presented, and then ask relevant questions which would occur to them resulting from the presentation. From the questions so asked, some are not included with the published paper because the authors attended to them by text revisions. In some cases, editorial and/or space considerations may exclude inclusion of all questions asked by reviewers. The authors are asked to prepare their Discussion with Reviewers section in a camera-ready format in accordance with detailed instructions which they are sent by SEM, Inc. In some instances the authors edit the questions and/or combine several similar questions from different reviewers to provide one answer. While all efforts are made to check that the questions in the printed version faithfully follow the views of the specific reviewer, the editors apologize, if in some instances, the actual meaning and/or emphasis may have been changed by the author.

The cover letter to the reviewers states:

"1. Your name will be conveyed to the author with your review UNLESS YOU ASK US NOT TO.

2. The questions published in the Journal will be identified as originating from you UNLESS YOU ADVISE OTHERWISE..."

In all cases sincere efforts are made to respect the reviewer's wishes to remain anonymous; however, in nearly 95% of the cases, the reviewers have given permission to be identified; so their names are conveyed to the authors and are included with the questions printed with each paper. An overall list of reviewers is provided in the opening pages of each SEM part. We apologize for any error/omissions which may occur.

Finally, readers are urged to be cautious regarding the weight they attach to the authors' replies, since the answers to the questions represent the authors' unchallenged views--except for minor editorial changes--the authors generally have the last word. Also, please consider that the questions were, in most all cases, relevant to the originally submitted paper, and they may not have the same significance for the revised paper published in this volume.

If you disagree with the results, conclusions or approaches in a paper, please send your comments, as a Letter to Editor, typed in a column format (each column is 4-1/8 inches wide and 11-1/2 inches long; i.e., 10.5 by 29.3 cm.). Your comments along with author's response will be published in a subsequent issue.

The editor gratefully thanks the authors and reviewers (see p. ix-xii) for their contributions, invites your comments on ways to improve this procedure, and seeks qualified volunteers to assist with reviewing of papers in the future (see p. xii).

ERRATA: Despite the best efforts of authors, reviewers and editors, errors may remain. Please help by pointing out errors that you notice. Please provide enough information to locate each error (volume, part, page, column, line, etc.) and indicate suitable correction.

REVIEWERS LIST

The editors gratefully acknowledge the help of the following reviewers for papers included in this part, and for other papers on similar topics which are either not being published or will be published in Scanning Electron Microscopy/1981/IV.

Abel, J.H.	Univ Tennessee, Knoxville
Abraham, J.L.	Univ California, La Jolla
Ackerman, L.D.	VA Med Ctr, Iowa City, IA
Adler, K.B.	Univ Vermont, Burlington
Albrecht, R.M.	Univ Wisconsin, Madison
Ali, S.Y.	Royal National Orthopedic Hosp, Stanmore, U.K.
Allen, N.S.	Dartmouth College, Hanover, NH
Allen, R.D.	Dartmouth College, Hanover, NH
Allen, T.D.	Patterson Labs, Manchester, U.K.
Andres, G.A.	SUNY, Buffalo, NY
Andrews, P.M.	Georgetown Med Sch, Washington, D.C.
Antakly, T.	Coll of Physicians & Surgeons, New York, NY
Ashraf, M.	Univ Cincinnati Med Sch, OH
Bank, H.	Med Univ South Carolina, Charleston
Barbi, N.C.	Princeton Gamma-Tech, NJ
Barnard, T.	Univ Oslo, Norway
Barrnett, R.J.	Yale Univ Sch Med, New Haven, CT
Becker, R.P.	Univ Illinois Med Ctr, Chicago
Bell, P.B.	Univ Oklahoma, Norman
Bellairs, R.	University College, London, U.K.
Berezesky, I.	Univ Maryland Sch Med, Baltimore
Bernaert, D.	Univ Brussels, Belgium
Bernanke, D.H.	Massachusetts General Hosp, Boston
Billings-Gagliardi, S.	Univ Massachusetts Med Sch, Worcester
Bloom, S.	GWU Med Ctr, Washington, DC
Boekestein, A.	Univ Nijmegen, The Netherlands
Bolender, D.L.	Texas Tech Univ Health Sci Ctr, Lubbock
Boyde, A.	University College, London, U.K.
Breese Jr., S.S.	Univ Virginia, Charlottesville
Brinkley, B.R.	Baylor Coll Med, Houston, TX
Broadwell, R.	Univ Maryland Sch Med, Baltimore
Brody, A.R.	Natl Inst Environmental Health Sci, RTP, NC
Brunk, U.	Uppsala Univ, Sweden
Buja, L.M.	Univ Texas Health Sci Ctr, Dallas
Bulger, R.E.	Univ Texas, Houston
Burger, E.H.	Leiden Univ, The Netherlands
Buss, H.	Reinhard-Nieter Hosp, Wilhelmshaven, W. Germany
Cameron, I.L.	Univ Texas Health Sci Ctr, San Antonio
Carlson, B.	Indiana Univ, Bloomington
Carr, K.E.	Univ Glasgow, U.K.
Carraway, C.A.	Univ Miami Sch Med, FL
Carter, D.P.	Bucknell Univ, Lewisburg, PA
Carter, H.W.	St. Vincent Charity Hosp, Cleveland, OH
Challice, C.E.	Univ Calgary, Alberta, Canada
Chandler, J.A.	Welsh National Univ, Cardiff, U.K.
Chen, S.	Wayne State Univ, Detroit, MI
Clark, E.B.	Univ Iowa, Iowa City
Cohen, A.L.	Washington State Univ, Pullman
Cohn, P.D.	Univ Wisconsin, Madison
Colby, J.W.	Kevex Corp, Foster City, CA
Collins, V.P.	Karolinska Inst, Stockholm, Sweden
Davidson, D.L.	Southwest Res Inst, San Antonio, TX
De Harven, E.	Sloan-Kettering Inst Cancer Res, New York, NY
DeHaan, R.L.	Univ Paris, Orsay, France
Deamer, D.W.	Univ California, Davis
Dedman, J.R.	Univ Texas Health Sci Ctr, Houston
Dichiara, J.F.	Amerada Hess Corp., Tulsa, OK
Douglas, W.H.J.	Tufts Univ Med Sch, Boston, MA
Draenert, K.	Orthopedic Klinik Tech Univ, Munchen, W. Germany
Draenert, Y.	Orthopedic Klinik Tech Univ, Munchen, W. Germany
Echlin, P.	Cambridge Univ, U.K.

Edie, J.W.	Univ Iowa, Iowa City
Eisenberg, B.	Rush Presbyterian Med Sch, Chicago, IL
Engel, W.	Fritz-Haber Inst, Berlin, W. Germany
Erlandson, R.A.	Memorial Sloan-Kettering Cancer Ctr, New York, NY
Estable-Puig de, R.F.	Laval Univ, Quebec, Canada
Evan, A.P.	Indiana Univ Sch Med, Indianapolis
Fagerberg, W.R.	Southern Methodist Univ, Dallas, TX
Fairing, J.D.	Monsanto, St. Louis, MO
Falk, R.H.	Univ California, Davis
Fallon, J.	Univ Wisconsin Med Sch, Madison
Fiori, C.E.	National Institutes Health, Bethesda, MD
Fiskin, A.M.	Kansas Univ Med Ctr, Kansas City, MO
Fitzharris, T.P.	Med Univ South Carolina, Charleston
Flickinger, R.A.	SUNY, Buffalo, NY
Fonte, G.	Univ Colorado, Boulder
Franks, F.	Univ Cambridge, U.K.
Fujimoto, T.	Kumamoto Univ Med Sch, Japan
Funkhouser, J.W.	Miami Valley Hospital, Dayton, OH
Gabridge, M.G.	W. Alton Jones Cell Sci Ctr, Lake Placid, NY
Geissinger, H.D.	Univ Guelph, Ontario, Canada
Gennaro, Jr., J.F.	New York Univ, NY
Geoghegan, W.D.	Med Coll Wisconsin, Milwaukee
Gonda, M.A.	Frederick Cancer Res Ctr, MD
Gupta, B.L.	Univ Cambridge, U.K.
Haggis, G.H.	Canada Dept Agriculture, Ottawa
Hagler, H.K.	Univ Texas Health Sci Ctr, Dallas
Hanker, J.S.	Univ North Carolina, Chapel Hill
Harvey, D.M.R.	Univ Sussex, Brighton, U.K.
Hay, D.A.	Univ Florida, Gainesville
Hay, E.D.	Harvard Med Sch, Boston, MA
Hayes, T.L.	Univ California, Berkeley
Heatfield, B.M.	Univ Maryland Sch Med, Baltimore
Hodges, G.M.	Imperial Cancer Res Fund, London, U.K.
Holtrop, M.E.	Children's Hosp Med Ctr, Boston, MA
Horowitz, S.B.	Michigan Cancer Foundation, Detroit, MI
Hoyer, L.C.	Frederick Cancer Res Ctr, MD
Hsu, K.C.	College of Physicians & Surgeons, New \ork, NY
Humphreys, W.J.	Univ Georgia, Athens
Hutchinson, T.E.	Univ Washington, Seattle
Hynes, R.O.	MIT, Cambridge, MA
Ingram, D.	Baylor Coll Med, Houston, TX
Ingram, P.	Research Triangle Inst, RTP, NC
Ip, W.	Washington Univ, St. Louis, MO
Jacobson, A.G.	Univ Texas, Austin
Jones, S.J.	University College, London, U.K.
Joy, D.C.	Bell Laboratories, Murray Hill, NJ
Kalnins, V.I.	Univ Toronto, Canada
Kelley, R.O.	Univ New Mexico, Albuquerque
Kessel, R.G.	Univ Iowa, Iowa City
Kirschner, R.H.	Cook County Coroner's Lab, Chicago, IL
Koestler, R.J.	American Museum of Natural History, New York, NY
Kuhn, C.	Washington Univ Sch Med, St. Louis, MO
Kuijpers, G.A.J.	Catholic Univ Nijmegen, The Netherlands
Lauchli, A.	Univ California, Davis
Lee, R.M.K.W.	McMaster Univ, Hamilton, Ontario, Canada
Leif, R.C.	Univ Miami, FL
Lemanski, L.F.	Univ Wisconsin Med Sch, Madison
Linton, R.W.	Univ North Carolina, Chapel Hill
Litke, L.L.	Med Coll Ohio, Toledo
Lofberg, J.	Uppsala Univ, Sweden
Low, F.N.	LSU Med Ctr, New Orleans, LA
Luchtel, D.L.	Univ Washington, Seattle
MacKenzie, A.P.	Univ Washington, Seattle
Magiore, C.J.	Los Alamos Sci Lab, Los Alamos, NM
Manasek, F.J.	Univ Chicago, IL
Marchevsky, A.M.	Mount Sinai Med Ctr, New York, NY
Markwald, R.R.	Texas Tech Univ, Lubbock, TX
Marshall, A.T.	La Trobe Univ, Bundoora, Australia
Maser, M.D.	Marine Biological Lab, Woods Hole, MA
Mathieson, A.C.	Univ New Hampshire, Durham
Mayer Jr., B.W.	New York Med Coll, Valhalla
McCallister, L.P.	Pennsylvania State Univ, Hershey

McClain, D.A.	Rockefeller Univ, New York, NY
McDowell, E.M.	Univ Maryland Med Sch, Baltimore
McKee, A.E.	Naval Med Res Inst, Bethesda, MD
McNutt. N.S.	VA Hospital, San Francisco, CA
Meier, S.P.	Univ Texas, Austin
Mergner, W.J.	Univ Maryland Sch Med, Baltimore
Mestres, P.	Ruhr Univ, Bochum, W. Germany
Miller, M.M.	City of Hope National Med Ctr, Duarte, CA
Miller, S.C.	Univ Utah Coll Med, Salt Lake City
Molday, R.S.	Univ British Columbia, Vancouver, Canada
Moor, H.	Univ Zurich, Switzerland
Morse, D.E.	Med Coll Ohio, Toledo
Murakami, T.	Okayama Univ Med Sch, Japan
Murphy, J.A.	Southern Illinois Univ, Carbondale, IL
Nemanic, M.K.	VA Hospital, San Francisco, CA
Nicholson, W.A.P.	Univ Glasgow, U.K.
Nowell, J.	Univ California, Santa Cruz
Ogilvie, R.W.	Oral Roberts Univ, Tulsa, OK
Osborn, M.	Max-Planck Inst Biophy Chem, Goettingen, W. Germany
Osornio-Vargas, A.R.	Univ Maryland Sch Med, Baltimore
Ostrand-Rosenberg, S.	Univ Maryland Baltimore County, Catonsville
Overton, J.	Univ Chicago, IL
Pallaghy, C.K.	La Trobe Univ, Bundoora, Australia
Panessa-Warren, B.J.	SUNY, Stony Brook, NY
Pawley, J.B.	Univ Wisconsin, Madison
Peach, R.	Univ North Carolina, Chapel Hill
Peachey, L.D.	Univ Pennsylvania, Philadelphia
Pedersen, M.	Univ Uppsala, Sweden
Penasse, W.	Univ Brussels, Belgium
Peters, K.-R.	Yale Univ Sch Med, New Haven, CT
Pexieder, T.	Univ Lausanne, Switzerland
Phelps, P.C.	Univ Maryland Sch Med, Baltimore
Phillips, S.J.	Temple Univ Sch Medicine, Philadelphia, PA
Pooley, A.S.	Yale Univ, Peabody Museum, New Haven, CT
Porter, K.R.	Univ Colorado, Boulder
Pullen, A.C.	Univ California, Santa Cruz
Ragan, M.A.	National Res Council, Halifax, Canada
Reith, A.	Univ Wisconsin, Madison
Rempfer, G.F.	Portland State Univ, Oregon
Riddle, J.M.	Henry Ford Hospital, Detroit,MI
Robards, A.W.	Univ York, Heslington, U.K.
Robinson, K.M.	Univ Natal, Congella, S. Africa
Rodman, N.F.	West Virginia Univ, Morgantown
Roomans, G.M.	Univ Stockholm, Sweden
Rosen, S.	Beth Israel Hospital, Boston, MA
Russ, J.C.	North Carolina State Univ, Raleigh
Sandborg, A.O.	EDAX International Inc., Prairie View, IL
Sanfilippo, F.	Duke Univ Med Ctr, Durham, NC
Sawada, H.	Univ Tokyo, Japan
Schamber, F.H.	Tracor Northern Inc., Middleton, WI
Schneider, G.B.	Univ Massachusetts Med Sch, Worcester
Schoenwolf, G.C.	Univ Utah Coll Med, Salt Lake City
Schwarzer, R.	Univ Tubingen, W. Germany
Shapiro, I.M.	Univ Pennsylvania, Philadelphia
Shelburne, J.D.	Duke Univ Med Ctr, Durham, NC
Sheppard, C.J.R.	Oxford Univ, U.K.
Simson, J.A.	Med Univ South Carolina, Charleston
Singer, I.I.	Inst for Med Res of Bennington, VT
Solursh, M.	Univ Iowa, Iowa City
Sommer, J.R.	VA Hospital, Durham, NC
Spargo, B. H.	Univ Chicago, IL
Sparrow, G.R.	Advanced R & D Inc., St. Paul, MN
Spurlock, B.O.	Med Coll Georgia, Augusta
Spurr, A.R.	Univ California, Davis
Staehelin, L.A.	Univ Colorado, Boulder
Statham, P.J.	Link Systems, High Wycombe, Bucks, U.K.
Steere, R.L.	Agriculture Res Ctr, Beltsville, MD
Steinberg, M.S.	Princeton Univ, NJ
Stocum, D.L.	Univ Illinois, Urbana
Strauli, P.	Univ Zurich, Switzerland
Sutherland, R.M.	Univ Rochester Cancer Ctr, NY
Sybers, S.D.	Baylor Coll Med, Houston, TX

Thurston, E.L.	Texas A & M University, College Station
Tompa, A.	Univ Michigan, Ann Arbor
Toner, P.G.	Royal Infirmary, Glasgow, U.K.
Tosney, K.	Yale Univ, New Haven, CT
Trinkaus, J.P.	Yale Univ, New Haven, CT
Trotter, J.A.	Univ New Mexico, Albuquerque
Valdre, U.	Univ Inst Fisica a Righi, Bologna, Italy
Vallyathan, N.V.	DHW/NIOSH/ALOSH, Morgantown, WV
Van Praagh, R.	Children's Hosp Med Ctr, Boston, MA
Venables, J.	Univ Sussex, Brighton, U.K.
Vertel, B.M.	Syracuse Univ, NY
Vesely, P.	Inst Exp Biology, Praha, Czechoslovakia
Vocci, M.	Univ Maryland Sch Med, Baltimore
Wallace, N.	Duke Univ Med Ctr, Durham, NC
Waterman, R.E.	Univ New Mexico Sch Med, Albuquerque
Weavers, B.A.	Hercules Incorp Res Ctr, Wilmington, DE
Webster, R.E.	Duke Univ Med Ctr, Durham, NC
Wegmann, L.	Balzers Ltd., Liechtenstein
Welling, L.W.	VA Hospital, Kansas City, MO
Wendt-Gallitelli, M.F.	Univ Tubingen, W. Germany
Wergin, W.P.	Agriculture Res Ctr, Beltsville, MD
Wetzel, B.	National Inst Health, Bethesda, MD
Williams, G.M.	American Health Foundation, Valhalla, NY
Wisse, E.	Free Univ of Brussels, Belgium
Zierold, K.	Max-Planck Inst, Dortmund, W. Germany

CALL FOR REVIEWERS

The contribution of reviewers to the quality of this publication and our meetings is tremendous. We find suitable reviewers from the suggestions we receive from the authors, our advisors, and from our past contacts. We will welcome your suggesting your own name or other's names (along with full mailing address) as reviewers.

Important Note: The time restrictions we work under require that each reviewer returns his review (along with the manuscript sent) within a set time from its receipt. *Please do not commit yourself if you feel that you cannot respond within this time frame; while we are grateful for your desire and efforts to help us, the reviewers who do not respond in time, in fact, seriously hamper our efforts.*

CONTACT: Om Johari, SEM Inc., P.O. Box 66507, AMF O'Hare, IL 60666, USA
Phone - 312-529-6677

TABLE OF CONTENTS

*See page viii

---

*EXPLANATION OF THE TYPES OF PAPERS IN THIS VOLUME:

TUTORIAL: Presentation of established material in teaching format emphasizing techniques.

REVIEW: A review of the chosen subject with emphasis on author's own work, placing it in context with relevant literature and putting the topic in perspective.

Volunteers to prepare tutorial, review papers or bibliographies should contact Om Johari (see page ii).

SCANNING ELECTRON MICROSCOPY/1981/II (Pages 1-8)
SEM Inc., AMF O'Hare (Chicago), IL 60666, USA

DEMONSTRATION OF INTRACELLULAR STRUCTURES BY HIGH RESOLUTION SCANNING ELECTRON MICROSCOPY

K. TANAKA

Department of Anatomy, Tottori University School of Medicine
Yonago, 683 Japan

Abstract

Three dimensional structure of intracellular elements were observed by the Osmium-DMSO-Osmium method and by a field emission SEM equipped with a high resolution device.
(1) Rough endoplasmic reticulum: Their cisternae were closely opposed to form a lamellar system of flat cavities and they were supported at regular interval by fine thread-like structures. They were also connected to the cell membrane with similar threads.
(2) Golgi complex: The Golgi cisternae of the serous cell of rat submandibular gland showed a bell-shape fitting one above another. The most characteristic structure of this Golgi complex was its foot-like tubuli which projected to all sides from the periphery of the cisternae.
(3) Synaptic vesicles: The synaptic vesicles of rat retina were observed at the direct magnification from 100,000 to 200,000 fold with the field emission SEM. The vesicles ranged from 30 to 50nm in diameter, and their shapes were also various, such as spherical, cocoon-like and kidney-shape. The synaptic vesicles were studded with several granules, about 10 nm in diameter.
In addition, the fine structure of mitochondria and centrioles were examined.

KEY WORDS: Osmium-DMSO-Osmium method, rough ER Golgi complex, mitochondria, synaptic vesicles, centrioles, high resolution device.

Introduction

Generally speaking, it has been considered that the scanning electron microscope(SEM) is unsuitable for researching cellular fine structure, because it has lower resolving power than that of the transmission electron microscope(TEM). The SEM with high resolving power whose spot size is strongly decreased but has a strong enough probe current to provide contrast in the picture, is useful for researching the intracellular structures. As techniques to achieve high resolution in SEM, the low-loss method[1-3] and SEM in TEM method[4] have been reported. Nagatani and Okura[5] also devised a method to obtain high resolution by reducing the working distance and adding an electrically biased electrode next to the specimen in a field emission SEM.

As to specimen preparation techniques, on the other hand, two effective methods for revealing intracellular structures have been developed in recent years. The first method is "the freeze-fracture, thaw fix technique" devised by Haggis and Phipps-Todd.[6] Haggis and Bond[7] could successfully observe the chromatin fibers of chicken erythrocytes by this method. The second method is "the Osmium-DMSO-Osmium method"(O-D-O method) devised by us.[8] This method intends to remove the excess cytoplasmic matrices, which obscure cell organelles, from the frozen-cracked surfaces of previously fixed cells by a dilute osmic acid solution. With this method we could clearly disclose many kinds of intracellular structures such as Golgi complex, endoplasmic reticulum, mitochondria and others.

In this paper, three dimensional structure of intracellular elements, studied by the O-D-O method and by a field emission SEM equipped with a high resolution devise, are described.

Materials and Methods

As materials the rat pancreas, submandibular glands, retina and hamster liver were used. Immediately after excision, the small tissue fragments (1x1x5mm) were treated by the O-D-O method.
1) The specimens were fixed at 4°C for 60-90 minutes with 1% osmium tetroxide in M/15 phosphate buffer solution at pH 7,4.
2) After having been rinsed with the buffer solution, the specimens were successively immersed in

15, 30, 50% dimethyl sulfoxide (DMSO) for 30 minutes each.
3) The specimens were frozen on a metal plate chilled with liquid nitrogen and cracked into two by a razor blade and a hammer. We have used a freeze cracking apparatus(TF-1, EIKO Engineering Co. Ltd., Japan) for this procedure which has been very useful for our purpose.
4) The cracked pieces were immediately placed in 50% DMSO solution for thawing.
5) After having been rinsed in the buffer solution. they were transferred to 0.1% osmium tetroxide solution buffered at pH 7.4 with M/15 phosphate buffer solution and left standing at 4°C for 24-72 hours. This procedure was very effective for removing the cytoplasmic matrices.
6) The specimens were fixed again for 1 hour in 1% osmium tetroxide solution. They were treated with 2% tannic acid solution about 12 hours and then 1% osmium tetroxide solution for 1-2 hours. This procedure is necessary for enhancing the electric conductivity of specimens.
7) They were dehydrated through graded alcohol series. After treatment with isoamyl acetate, they were dried in a critical point dryer(HCP-2, Hitachi Koki Co. Ltd., Japan) with dry ice.
8) Dried specimens were coated with platinum in an ion-coater with rotating stage(VX-10R, EIKO Engineering Co. Ltd., Japan).
9) The metal coated specimens were observed with a field emission SEM.

Scanning Electron Microscope Used

The field emission SEM(Hitachi HFS-2ST) with a high resolution devise constructed by Nagatani and Okura[5] was used. The sample holder is equipped with a 45° pre-tilted pole 2mm in diameter, on which a sample is attached, and a cone-shape electrode which effectively guides the secondary electrons. The electrode can be supplied with a positive potential from 0 to 400V, when the sample holder is set on the stage in the specimen chamber. The sample holder itself can be independently biased negatively through the inlet terminal, which is normally used for absorbed current measurement. In the present study, positive 200V potential was supplied to the electrode and negative 10V potential to the sample holder. With this device, the working distance was reduced from 5 to 0 mm and consequently the spot size of the electron beam decreased (on calculation) from 2 to 1.45nm.

In addition, the SEM has an objective lens with a lower pole piece with an enlarged bore diameter of 7mm for keeping the movement of the specimen holder on X and Y axis (2mm each) at the working distance of 0 mm.

Results

Rough endoplasmic reticulum

Cisternae of the rough endoplasmic reticulum were closely opposed to form a lamellar system of flat cavities. These cisternae were linked to each other at regular interval with fine thread-like structures (Fig. 1). They were fixed also to the cell membrane with similar threads (Fig. 2). The thread-like structures are about 10-15nm in diameter. The feature of these thread-like structures remain to be examined.

The outer surface of rough endoplasmic reticulum was studded with numerous ribosomes. The ribosomes were observed as a combined body of two subunits of unequal size, and often formed a polysome (Fig. 3). The size of the ribosomes were 20-25nm in diameter. In general, ribosomes observed by SEM are considerably larger than those reported by TEM studies (15nm). Kirschner et al.[9] reported ribosomes to be 20-25nm in diameter in their SEM study on isolated mouse liver nuclear envelope. The difference in size between SEM and TEM observations probably depends upon the metal coat around the ribosomes of the SEM specimens.

Golgi complex

We previously reported that the Golgi complex of the rat epididymal cell consisted of three elements. These are the Golgi stacks with numerous small vesicles, the plexus of anastomosing tubuli with associated granules of various sizes, and large vacuoles which might be secondary lysosomes.[10] In the present study, the Golgi complex of the serous cell in rat submandibular gland was observed. Its construction differed considerably from that of epididymal cells.

The Golgi cisternae of the serous cell appeared to be formed from a number of bell-shapes fitting one above another. The number of the cisternae was 4-5, less than that of the epididymal cell. The most characteristic structure of this Golgi complex was its foot-like tubuli which projected to all sides from the periphery of the cisternae (Fig. 4). Some of the feet from the cisternae were gathered at the lower part of the Golgi complex as shown in Figure 6. They rarely anastomosed and did not form a plexus. Vacuoles which were found in the plexus of anastomotic tubuli of epididymal cells (probably lysosomes) were rarely ever observed in this gathering tubuli, but sometimes the end of a cisterna was expanded to form a vacuole (Fig. 5). On the other hand, some feet expanded from the cisterna and joined to the endoplasmic reticulum closely located around the Golgi complex (Fig. 6). The surface of the Golgi stack was studded with Golgi vacuoles, about 50nm in diameter, but their number was considerably less than that of the epididymal cell (Fig. 5). No secondary lysosome and multivesicular body were observed around the Golgi complex.

As described above, the constitution of the Golgi complex in the serous cell of submandibular glands is much different from that of the epididymal cell.

Mitochondria

For identification of mitochondria from other intracellular structures, the mitochondrial cristae must be clearly disclosed.[11,12] The O-D-O method is very useful for this purpose. The mitochondrial cristae or tubuli are easily observed *in situ* in the specimens prepared by this method.

Recently Masunaga[13] in our department studied the mitochondria of rabbit heart muscle cells by our method and reported the three dimensional structure of them. He showed that the outer and inner membranes that enclose the mitochondria were sometimes separately recognized. The outer membrane was a smooth contoured with a continuous limiting membrane, and the inner one generally ran

Demonstration of intracellular structures by SEM.

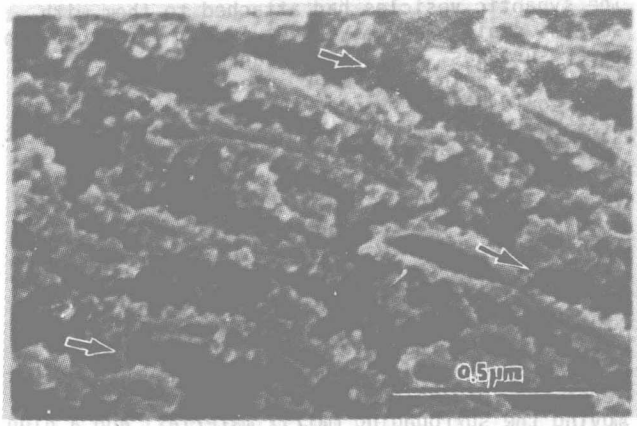

Fig. 1 Rough endoplasmic reticulum. The cisternae are supported at regular intervals with fine thread-like structures (arrow).

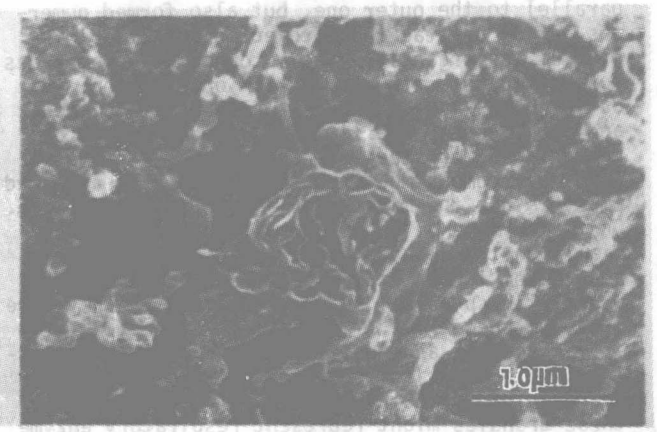

Fig. 4 Golgi complex of a serous cell in rat submandibular gland.

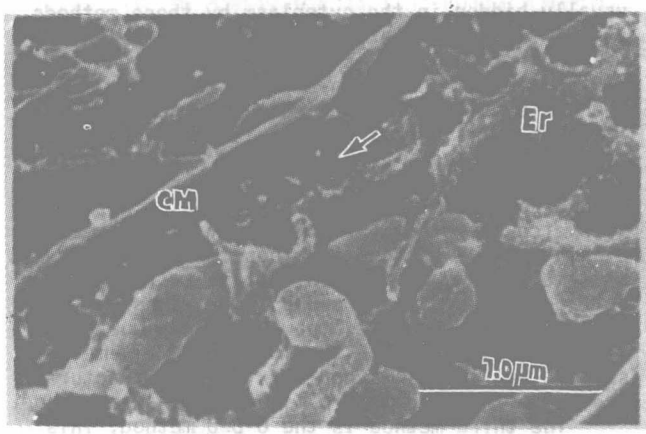

Fig. 2 Rough endoplasmic reticulum (Er). They are connected to the cell membrane (CM) with fine thread-like structures (arrows).

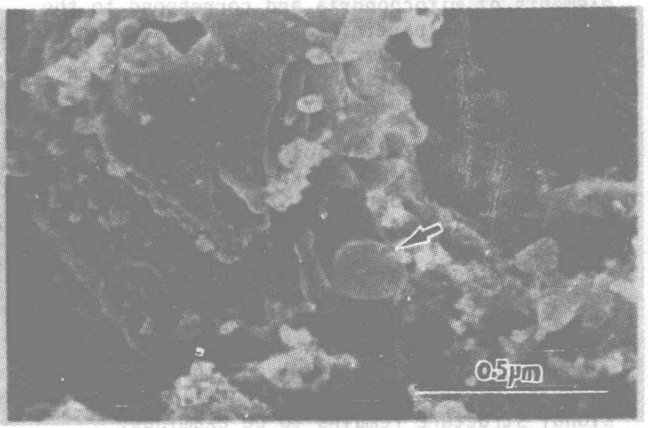

Fig. 5 Golgi complex of a serous cell. On the surface of the Golgi stack, many vesicles are seen (asterisk) and the end of a cisterna expanded to form a vacuole (arrow).

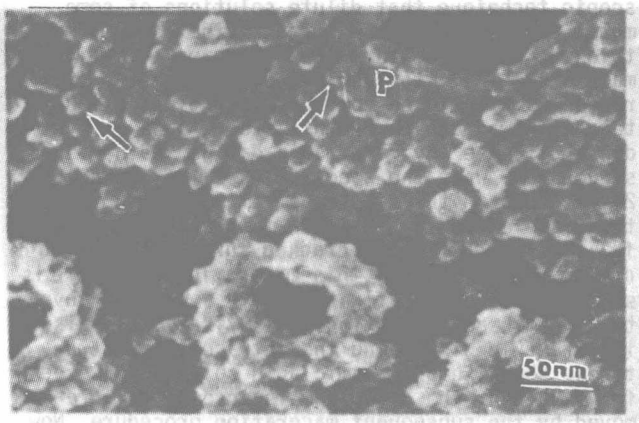

Fig. 3 A polysome observed on a rough endoplasmic reticulum (P). A ribosome consists of two subunits (arrow).

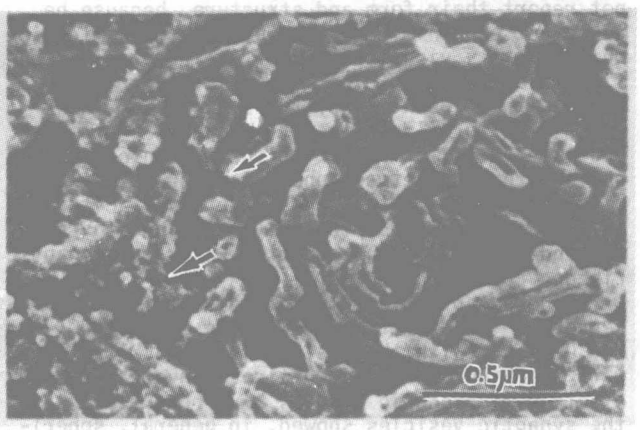

Fig. 6 The lower part of the Golgi complex in a serous cell. Many tubuli are gathering. Some of them lead to the endoplasmic reticulum (arrow).

parallel to the outer one, but also formed numerous narrow cristae that projected into the interior of the organelle. The mitochondrial membranes formed two compartments. By SEM observation, the outer compartment was not usually distinguished and consequently the limiting membrane of mitochondria and cristae were seen as thick plates. The inner compartment, on the other hand, appeared empty, since the mitochondrial matrix was removed. On the surface of the inner compartment, especially on the cristae, numerous small particles, about 20nm in diameter, were observed. They were usually in spherical shape but sometimes they were mushroom-shape.

Kirschner and Rusli,[14] in their study of isolated mitochondria, reported that the inner mitochondrial membrane had a granular surface and these granules might represent respiratory enzyme complexes perhaps the ATPase particles. We could also see mitochondrial cristae or tubuli clearly by the O-D-O method (Fig. 7) and could observe the small particles on the cristae or tubuli (Fig. 8). Thus we assume that such particles are common elements of mitochondria and correspond to the inner membrane particles seen in negative contrast preparations. However, it is necessary to confirm in the future by histochemical methods whether the particles are respiratory enzyme complexes or not.

Centrioles

Detection of centrioles under SEM was very difficult because nobody knew the shape the centrioles showed under SEM. Recently Mitsushima and Inoue[15] in our department found them in a rabbit plasma cell by the O-D-O method. According to their findings, the centrioles were situated at the center of the Golgi region beside the nucleus and appeared as a cylindrical body with small granules in its cavity. We could also find the same structure at the Golgi region of a rat lymphocyte. Unfortunately their detailed three dimensional structure remains to be examined.

Synaptic vesicles

As materials for higher resolution SEM, synaptic vesicles were used. In 1978, Furusawa[16] studied the synaptic vesicles of cat purkinje and spinal motor nerve cells with SEM. However, he did not report their form and structure, because he could not obtain micrographs of high magnification which permits visualization of their detailed configuration.

In the present study the synapses of the outer plexiform layer in rat retina were examined. The outer plexiform layer is the region of synaptic interplay between photoreceptor cells, bipolar and horizontal cells. The synaptic terminals of the photoreceptor cells are large pyramidal endings containing synaptic vesicles, synaptic ribbons and mitochondria.

The synaptic vesicles of this region were observed at the direct magnification of 100,000 - 200,000 fold with the field emission SEM equipped with the high resolution device. Many transmission electron microscopic studies have reported that the synaptic vesicles showed, in general, spherical shape and their size was 40-65nm in diameter. By our SEM observation, the vesicles had various sizes, from 30 to 50nm in diameter, and their shapes were also various, such as spherical, cocoon-like and kidney-shape (Fig. 9).

The synaptic vesicles had attached to them with several small granules, about 10nm in diameter, on their surfaces. With these granules, the vesicles were often joined together. Granules of similar size could be observed on the cytoplasmic surface of the synaptic membrane also (Fig. 10). Though it is uncertain yet whether both granules have the same features or not, we assume that the synaptic vesicles are probably attached to the synaptic membrane with these granules. In the present paper, details of the synaptic ribbon are omitted.

Discussion

For observing intracellular structures by SEM, it is indispensable to have a good method for revealing intracellular structures (i.e. for removing the surrounding matrix material) and a high resolution SEM to permit their adequate visualization. As to such preparation techniques, many kinds of cryofracture and cracking methods have been devised,[17-23] but it was practically impossible to expose the intracellular structures usually hidden in the cytoplasm by these methods alone. In recent years, three methods for revealing intracellular structures have been reported. The first is ion-etching method.[24-28] When this method was applied to the cracked surfaces of cells, the intracellular structures were clearly disclosed,[29] because moderate ion-etching was quite effective in exposing membranous structures from the cytoplasmic matrix which was much less etch resistant. Unfortunately, the ion-etching method has shortcomings. It often produces artificial cone figures which fail to reflect the intrinsic structures properly. The second method is the freeze-fracture, thaw fix technique devised by Haggis and Phipps-Todd.[6] In this method soluble protein is washed out from the fractured surface of unfixed materials at the thawing stage.

The third method is the O-D-O method. This method is characterized by the use of fixation with a slightly hypotonic osmium tetroxide solution and a maceration procedure for eliminating cytoplasmic matrices with a dilute osmium tetroxide solution. It is well known in light microscopic technique that dilute solutions of some metal salts such as osmium tetroxide, chromium trioxide and potassium dichromate are effective in macerating the cytoplasm. Though any one of these reagents may be used for the maceration, osmium tetroxide was chosen in this study, because it does not cause any remarkable damage to the fine structures. When the specimens are left standing 24-72 hours in the dilute osmium tetroxide solution, the excess cytoplasmic matrices are successfully removed from the cracked surfaces of fixed cells. The effect of this maceration procedure is strengthened by the fixation with a slightly hypotonic osmic acid solution. This fixation is also important in disclosing the intracellular structures. When isotonic osmic acid solution is used for fixation, the cytoplasm is incompletely removed by the subsequent maceration procedure. Now it becomes a question whether the treatment with the hypotonic fixative produces some artifacts or not. Fortunately we did not find any significant artifacts in the specimens prepared by this method. Thus we think that the O-D-O method is very