

Hydra: Research Methods

Edited by

Howard M. Lenhoff

*University of California
Irvine, California*

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

Hydra: research methods.

Includes bibliographical references and index.

1. Hydra. I. Lenhoff, Howard M.

QL377.H9H93 1982

593.7'1

82-24648

ISBN 0-306-41086-9



Cover photo courtesy of
Regula Bänninger and Prof. Pierre Tardent,
Zoological Institute, University of Zurich, Switzerland.

© 1983 Plenum Press, New York
A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted
in any form or by any means, electronic, mechanical, photocopying, microfilming,
recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

Contents

Introduction	1
Howard M. Lenhoff	
1. Terminology for Morphology and Cell Types.....	5
Richard D. Campbell and Hans R. Bode	
<i>I. Culture and Handling</i>	
2. Collecting Hydra	17
Richard D. Campbell	
3. Identifying Hydra Species	19
Richard D. Campbell	
4. Water, Culture Solutions, and Buffers	29
Howard M. Lenhoff	
5. Visual Monitoring of pH in Solutions with Phenol Red	35
M. Rahat and Vanda Reich	
6. Hatching Brine Shrimp Larvae Axenically and/or in a Range of Quantities.....	39
Howard M. Lenhoff	

7. Determining Growth Rates of Groups of Hydra and Budding Rates of Individual Hydra	47
Howard M. Lenhoff	
8. Culturing Large Numbers of Hydra	53
Howard M. Lenhoff	
9. Turbidimetric and Pipetimetric Measurements of Number of Hydra	63
Howard M. Lenhoff	
10. Culturing Hydra of the Same Species but of Different Sizes	67
Richard D. Campbell and Joann J. Otto	
11. Culturing Sexually Differentiated Hydra	71
Charles L. Rutherford, David Hessinger, and Howard M. Lenhoff	
12. Preparing Axenic Hydra.....	79
M. Rahat and Vanda Reich	
 <i>II. Histology</i>	
13. Preparing Hydra for Transmission Electron Microscopy	87
Richard L. Wood	
14. Preparing Hydra for Scanning Electron Microscopy.....	95
Richard L. Wood	
15. Preparing Hydra for Freeze-Fracture and Freeze-Etching	105
Richard L. Wood	
16. Whole Mounts for Light Microscopy	117
Richard D. Campbell	
17. Preparing Histological Sections for Light Microscopy.....	121
Richard D. Campbell	

18. Vital Staining: Fluorescent and Immunofluorescent,
and Review of Nonfluorescent Dyes..... 131
John F. Dunne and C. Lynne Littlefield

III. Macrophotography

19. Macrophotography..... 143
Richard D. Campbell

IV. Quantitative Cytology

20. Dissociating Hydra Tissue into Single Cells by the
Maceration Technique..... 153
Charles N. David
21. Cell Cycle Analysis of Hydra Cells..... 157
Charles N. David
22. Mitotic Index 165
Richard D. Campbell
23. Measuring Numbers of Nematoblasts, Nematocytes,
and Nematocysts 169
Hans R. Bode, G. Scott Smith, and Patricia M. Bode
24. Marking Epithelial Cells in Living Hydra
with Indian Ink..... 183
Joann J. Otto and Richard D. Campbell

V. Techniques Using Isotopes

25. Incorporating [³H]Thymidine into Hydra by
Microinjection..... 189
Charles N. David
26. Labeling with Gaseous ¹⁴CO₂ or by Feeding Hydra on

Radioactive Tissues.....	193
Howard M. Lenhoff	
27. Fractionating Small Amounts of Radioactive Tissue.....	197
Howard M. Lenhoff	
28. Rapid Whole-Mount Radioautography.....	205
Howard M. Lenhoff	
 <i>VI. Isolating Hydra Mutants by Sexual Inbreeding</i>	
29. Isolating Hydra Mutants by Sexual Inbreeding.....	211
Tsutomu Sugiyama	
 <i>VII. Manipulating Tissue Organization</i>	
30. Grafting: A Rapid Method for Transplanting Tissue.....	225
Harry K. MacWilliams	
31. Quantitative Interpretation of Transplantation Phenomena.....	233
Harry K. MacWilliams	
32. Dissociated Tissues into Cells and the Development of Hydra from Aggregated Cells.....	251
Kristine M. Flick and Hans R. Bode	
33. Culturing Interstitial Stem Cells in Hydra Aggregates.....	261
Charles N. David	
34. Separating Viable Tissue Layers.....	267
Georgia E. Lesh-Laurie	
35. Preparing Ectoderm/Endoderm Chimeras.....	273
Nancy Wanek	

VIII. Manipulating Cellular Composition in Vivo

- 36. Eliminating All Nonepithelial Cells Using Colchicine. 281
 Beverly A. Marcum and Richard D. Campbell
- 37. Culturing Epithelial Hydra. 287
 Beverly A. Marcum
- 38. Reducing Populations of Interstitial Cells and
 Nematoblasts with Hydroxyurea 291
 Hans R. Bode
- 39. Preparing *Hydra viridis* with Nerve Cells and No
 Interstitial Cells, or with Neither of These
 Cell Types. 295
 Patricia Novak
- 40. Eliminating Interstitial Cells with Nitrogen Mustard 299
 Charles N. David
- 41. Altering Cell Population Levels by Gamma Irradiation 303
 Cheng-Mei Fradkin
- 42. Reducing Number of Nematocytes in the Tentacles 305
 G. Scott Smith and Hans R. Bode

*IX. Assay and Isolation of Substances Controlling
 Morphogenesis in Hydra*

- 43. Assay and Isolation of Substances Controlling
 Morphogenesis in Hydra. 311
 H. Chica Schaller, Cornelis J. P. Grimmelikhuijzen,
 and Tobias Schmidt

*X. Isolation and/or Properties of Acellular Mesoglea
 and Nematocysts*

44. Isolating Mesolamellae 327
Robert M. Day and Howard M. Lenhoff
45. Isolating Undischarged and Discharged Nematocysts
from Acontiate Sea Anemones 331
Richard S. Blanquet
46. Dissolving the Nematocyst Capsule Wall and Identifying
Its Protein Component(s) 335
Richard S. Blanquet
47. Purifying an Inhibitor of Succinoxidase Activity from
Hydra littoralis 341
Edward S. Kline and Vaman S. Waravdekar
48. Assays for Activities of Nematocyst Venoms and
Their Components 347
David A. Hessinger

XI. Analytical Procedures

49. Special Techniques for Weighing Microgram Quantities
of Tissue and Assaying Them for Enzyme Activities 361
Charles L. Rutherford
50. Extracting and Characterizing Hydra RNA: Modifications
to Allow Extraction of Undegraded Material in the
Presence of High Levels of Degradative Enzymes 373
Georgia E. Lesh-Laurie, Joseph R. Volland, and
Stephen S. Macintyre
51. Colorimetric Analysis for Protein of Hydra 379
Howard M. Lenhoff
52. Determining Respiration and Oxygen Evolution of Green
Hydra with the Rank Brothers Oxygen Electrode. 383
Donald W. Phipps, Jr.

XII. Symbiotic Relationships

53. Isolating Endosymbiotic Algae from *Hydra viridis* 391
 L. Muscatine

54. Preparing Aposymbiotic Hydra 393
 R. L. Pardy

55. Introducing Symbiotic Algae into Aposymbiotic Hydra 399
 R. L. Pardy

56. Measuring Number of Algal Symbionts in *Hydra viridis* 401
 R. L. Pardy

57. Measuring *in Vivo* Translocation of Reduced Organic
 Carbon Compounds from Endosymbiotic Algae to
 Hydra. 407
 L. Muscatine

58. Spectrophotometric Assay for Maltose 411
 That T. Ngo, Jeanne Ivy, and Howard M. Lenhoff

XIII. Methods for Epizootiological Research with Hydra

59. Methods for Epizootiological Research with Hydra 417
 Alan E. Stiven

XIV. Electrophysiology and Behavior

60. Recording Electrical Activity. 429
 Robert K. Josephson and Norman B. Rushforth

61. Bioassay for, and Characterization of, Activators and
 Inhibitors of the Feeding Response. 443
 Howard M. Lenhoff, Wyrta Heagy, and Jean Danner

Index 453

Contributors

Richard S. Blanquet, Department of Biology, Georgetown University,
Washington, D.C. 20057

Hans R. Bode, Developmental Biology Center and Department of
Developmental and Cell Biology, University of California, Irvine,
California 92717

Patricia M. Bode, Developmental Biology Center and Department of
Developmental and Cell Biology, University of California, Irvine,
California 92717

Richard D. Campbell, Department of Developmental and Cell Biology,
University of California, Irvine, California 92717

Jean Danner, Biochemistry Section, NIOSH, Morgantown, West Virginia
26505

Charles N. David, Department of Molecular Biology, Albert Einstein
College of Medicine, Bronx, New York 10461

Robert M. Day, Department of Developmental and Cell Biology, University
of California, Irvine, California 92717

John F. Dunne, Developmental Biology Center, University of California,
Irvine, California 92717

Kristine M. Flick, Developmental Biology Center and Department of
Developmental and Cell Biology, University of California, Irvine,
California 92717

Cheng-Mei Fradkin, Department of Developmental and Cell Biology,
University of California, Irvine, California 92717

Cornelius J. P. Grimmelikhuijzen, Max-Planck-Institut für medizinische
Forschung, Abteilung Biophysik, 6900 Heidelberg, Federal Republic of
Germany

Wyrta Heagy, Department of Microbiology, University of Massachusetts,
Amherst, Massachusetts 01003

David A. Hessinger, Department of Physiology and Pharmacology, School
of Medicine, Loma Linda University, Loma Linda, California 92350

Jeanne Ivy, Department of Developmental and Cell Biology, University of
California, Irvine California 92717

Robert K. Josephson, School of Biological Science, University of California,
Irvine, California 92717

Edward S. Kline, Department of Biochemistry, Medical College of Virginia,
Virginia Commonwealth University, Richmond, Virginia 23298

Howard M. Lenhoff, Department of Developmental and Cell Biology,
University of California, Irvine, California 92717

Georgia E. Lesh-Laurie, Department of Biology, Cleveland State
University, Cleveland, Ohio 44115

C. Lynne Littlefield, Developmental Biology Center, University of
California, Irvine, California 92717

Stephen S. Macintyre, Department of Anatomy, Case Western Reserve
University, Cleveland, Ohio 44106

Harry K. MacWilliams, Department of Anatomy, University of
Massachusetts Medical Center, Worcester, Massachusetts 01605

Beverly A. Marcum, Department of Biological Sciences, California State
University, Chico, California, 95929

L. Muscatine, Department of Biology, University of California, Los
Angeles, California 90024

That T. Ngo, Department of Developmental and Cell Biology, University of California, Irvine, California 92717

Patricia Novak, Department of Developmental and Cell Biology, University of California, Irvine, California 92717

Joann J. Otto, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

R. L. Pardy, School of Life Sciences, University of Nebraska, Lincoln, Nebraska 68588

Donald W. Phipps, Jr., School of Life Sciences, University of Nebraska, Lincoln, Nebraska 68588

M. Rahat, Department of Zoology, The Hebrew University of Jerusalem, Israel

Vanda Reich, Department of Zoology, The Hebrew University of Jerusalem, Israel

Norman Rushforth, Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106

Charles L. Rutherford, Biology Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

H. Chica Schaller, Max-Planck-Institut für medizinische Forschung, Abteilung Biophysik, 6900 Heidelberg, Federal Republic of Germany

Tobias Schmidt, Max-Planck-Institut für medizinische Forschung, Abteilung Biophysik, 6900 Heidelberg, Federal Republic of Germany

G. Scott Smith, Developmental Biology Center and Department of Developmental and Cell Biology, University of California, Irvine, California 92717

Alan E. Stiven, Department of Biology 046A, University of North Carolina, Chapel Hill, North Carolina 27514

Tsutomu Sugiyama, National Institute of Genetics, Mishima, Shizuoka-ken 411, Japan

Joseph R. Voland, Department of Pathology, University of California, San Diego, La Jolla, California

Nancy Wanek, Developmental Biology Center and Department of Developmental and Cell Biology, University of California, Irvine, California 92717. Current address: Department of Biology and Health Science, Chapman College, Orange, California 92666.

Vaman S. Waravdekar, Microbiological Associates, Bethesda, Maryland 20016

Richard L. Wood, Department of Anatomy, University of Southern California, School of Medicine, Los Angeles, California 90007

Incorporating [³H]Thymidine into Hydra by Microinjection

Charles N. David

PURPOSE

To label hydra by injecting [³H]thymidine into the gastric cavity (Campbell, 1965; David and Campbell, 1972).

INTRODUCTION

Hydra, because of their low permeability, cannot be effectively labeled with radioactive materials by the usual method of soaking animals in a solution containing the isotope. Injection of [³H]thymidine into the gastric cavity offers several advantages over soaking hydra in medium containing isotope. (1) [³H]Thymidine is taken up 10 to 20 times more effectively from the gastric cavity than from the external medium, and only small amounts of isotope are required for injection. (2) The injected [³H]thymidine labels primarily hydra cells and not bacteria, which exist in large numbers on the outside of hydra. By comparison, >95% of the label is incorporated into bacterial DNA when hydra are soaked in [³H]thymidine. (3) Injection of [³H]thymidine yields a natural pulse label. A single injection is taken up within 45 min in unfed animals and within 90 min in fed animals.

Charles N. David • Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York.

MATERIALS

Hamilton syringe (10 or 25 μl ; Hamilton Co., Reno, NV), polyethylene tubing (Intramedic PE 50; Clay-Adams, Parsippany, NJ).

PROCEDURES

Preparing Polyethylene Needles

Polyethylene needles are superior to glass needles for injecting hydra because they are slightly flexible. Prepare the needles by warming polyethylene tubing (PE 50) near a small flame such as the pilot light of a Bunsen burner. When the tubing starts to melt, remove it from the flame and draw it out. The short melted section pulls out into a thin tube which hardens almost immediately. Cut the tubing with a razor blade on an angle at the point where its diameter is about 0.1 mm. Attach the needle to the end of a Hamilton syringe.

Filling the Needle

Remove an appropriate small amount (20–100 μl) of isotope from the stock solution and place it on the bottom of a 20-mm plastic Petri dish. The solution forms a round drop since the plastic surface is hydrophobic. Keep the dish covered to avoid evaporation. Draw up the isotope solution into the polyethylene needle but not into the syringe itself. Discard the needle at the end of the experiment.

Injection Procedure

Place 5–10 hydra in 10 ml medium in a 50-mm plastic Petri dish. Move the hydra to the center of the dish, loosen them from the bottom, and wait for them to stretch out a bit. During injection, view the hydra under low power in a dissecting microscope. With tweezers in one hand, position a hydra and hold it lightly behind the tentacles. With the other hand bring the Hamilton syringe into position and insert the needle into the mouth of the animal. The insertion is generally easy to do with thin needles and does not normally cause the animal to contract much or to open its mouth. Once the needle is inserted, gently press the plunger of the syringe to expel a small volume of solution into the hydra's gastric cavity. Estimate the amount injected by watching the movement of the meniscus of the solution in the needle; usually 0.25 μl occupies a length of 1 mm.

After the hydra swells up, wait a few second before withdrawing the needle. The mouth usually closes tightly as the needle is withdrawn and the hydra remain somewhat swollen. If individual animals contract immediately upon withdrawal of the needle, reinject them a minute or two later. With practice one can inject two to three hydra per minute.

For individuals with unsteady hand, the Hamilton syringe may be mounted in a motor-driven syringe pump activated with a foot pedal. If the injection volume does not need to be controlled, the injection needle may be connected directly to a mouth pipet and the isotope expelled by blowing gently.

REFERENCES

- Campbell, R. D. 1965. Cell proliferation in *Hydra*: An autoradiographic approach. *Science* **148**:1231–1232.
- David, C. N., and Campbell, R. D. 1972. Cell cycle kinetics and development of *Hydra attenuata*. I. Epithelial cells. *J. Cell Sci.* **11**:557–568.

Index

- Acid fuchsin, 129
- Acontia thread, 332
- Acrolein, 89, 90
- Acrylamide gel, 337–338
 - separation gel, 337
 - stacking gel, 337
- Activation of head, defined, 313
- Activation-inhibition model, 246–249
 - chi-square distribution, 247
 - gradient, 248
 - level, 247–248
 - log-linear model, 246
 - optimization method, 248
 - profile, shape of, 247–249
 - test, statistical, 246–247
- Activator, 311
 - of feeding response, 443–451
 - of head, 313
 - potency, 447–449
- Activity, electrical, 429–441
 - conduction velocity, 436–439
 - potential
 - spontaneous, 429–435
 - transepithelial, 435–436
 - recording, intracellular, 436
- Advantage of hydra in experiments, 3
- Aggregate, 261–268
 - development from, 251–259
 - from nitrogen mustard treatment, 261–268
 - into pellet, 254–257
- Aiptasia pallida*, see Sea anemone
- Aldehyde, fixation by, 88–91
- Alga, endosymbiotic, 388–411
 - introducing into hydra, 399
 - isolating
 - maltose secretion by, 411
 - measuring numbers by
 - fluorometric estimation, 403–404
 - growth kinetics, 402–403
 - maceration technique, 401–402
 - removing from hydra, 393
- Ammonium alum, 128
- Amputation and regeneration, 218
- Analytical procedures, 361–387
- Anchistropus* sp., 18
- Anesthesia, 97, 122
- Aniline blue, 129
- Antibiotics, 79–81, 212
- Araldite for embedding specimen, 88–92
- Artemia salina*, 39–46
 - cyst, 39–46
 - and bacteria, 41
 - decontamination, 41
 - and fungi, 41
 - food for hydra, 39–46
 - hatching
 - apparatus, 43
 - method, 42–44
 - by bubbling, 42, 44
 - by floating, 42
 - hatching solution, 41–42
 - incubation, 44
 - larva, axenic, 39–46
 - nauplii, 2, 39–46, 54, 213, 306, 393, 419

- Artemia salina*, nauplii (*cont.*)
 harvesting method, 44–46
- Autofluorescence, 134, 136
- Autoradiography, 155, 162
 of cells, 155
 of ectoderm, 119
 of whole mounts, 205
- Autotomy, 349
- Axenic hydra, 79–83
 and antibiotics, 79–81
- Bacteria as contaminants of hydra, 41, 189,
 257, 366
 growth, excessive, 287
see also Axenic
- Basal cell, 14
- Basal disk, *see* Foot
- Basement membrane, mammalian, 327
- Battery cell, 9
- Behavior, 429–451
- Bellows of camera, 144
 calculation for length, 144
- Bentonite, 374
- Biebrich scarlet, 128
- Bioassay, 349, 445–447
- 2,5-Bis(4'-aminophenyl-1')-1,3,4-oxiazole,
 158
- Bis-benzimide trihydrochloride pentahydrate
 (Hoescht 33258), 132
- BOA, *see* 2,5-Bis(4'-aminophenyl-1')-1,3,4-
 oxiazole
- Body
 column, 7
 morphogenic gradient of, 234
- Bouin fixative, 122, 124, 126, 129
- Brine shrimp, *see Artemia salina*
- Budding, 1, 7, 10, 299
 inhibition, 314
 rate, 52, 68, 70
- Buffers, listed, 374–375
- n-Butyl alcohol, 342, 344
- Capsule, 11
 of nematocyst, 2, 177, 335–339
- Carbon
 colloidal, 132
 compound, organic, and translocation into
 hydra, 407–409
- Carbon dioxide, radioactive, 193–195
- 6-Carboxyfluorescein, 133
 method, 137–138
- 6-Carboxyfluorescein (*cont.*)
 photobleaching with, 139
- Cell, 9–14
 basal, 14
 cloning, 262, 264
 composition, 290–291
 counting chamber, 178, 251
 cycle
 analysis, 157–163
 doubling time, 158
 G2-phase, 160–161
 kinetics, 157
 S-phase, 159–160
 time, 158–159
 dissociation, 153, 263
 ectodermal, 9, 10
 endodermal, 9, 10, 14
 interstitial, 11–14, 291–294
 absence of, 295–302
 eliminating, 299–304
 lineage, 11–14
 and nitrogen mustard, 299–302
 glandular, 14
 lineage
 epitheliomuscular, 9–11
 interstitial, 11–14
 loss, monitoring of, 285–286
 mucous, 14
 of muscle mat, 9
 of nerve, 295–297
 types, 2, 5–14
 zymogen in, 14
see also individual cell types
- Chimera formation, 273–277
- Chi-square distribution, 247
 test for, 312
- Chlorella*, endosymbiotic, 411
see Alga, endosymbiotic
- Chloroform, 374, 375
- Chlorohydra* sp., 22–24
- Cinclide, 332
- Cleaning hydra, 81–82, 253
- Clone-forming cell, assay for, 264
- Clorox, 106
- Cnidoblast, *see* Nematoblast
- Cnidocil, *see* Nematocyte
- Cnidocyte, 12
- Coelentron, 5
- Colchicine, 167, 258, 281–287, 295–303
 and cell disappearance, 285
 double treatment, 284–286

- Colchicine (*cont.*)
 single treatment, 282, 284
- Collecting specimens, 17–18
- Column, 6
 twisted mutant, 214, 216
- Computer algorithm, hill-climbing, 246
- Conduction velocity
 of axon, 437
 of propagated wave, 436
 recorded, 437
- Contamination, *see* Bacteria, Parasites
- Copper, as cell poison, 450
- Counting cells, *see* Quantification
- Crab, *see* Fiddler crab
- Crayfish, 348, 356–357
 desheathing, 357
 dissection, 357
 nerve failure, irreversible, 357
- Culturing hydra, 17–83
 large numbers, 53–62
 medium, 29–34
 contaminants, 29
 ingredients, 31–33
 phenol red, 35–37
 pure water, 30–31
 tray method, 54–60
 vertical plate method, 54–56, 60–62
- Cytology, quantitative, 153–186
 cell cycle analysis, 157–163
 maceration technique, 153–156
 mitotic index, 165–168
 numbers of
 nematoblasts, 169–182
 nematocysts, 169–182
 nematocytes, 169–182
- DAPI, *see* 4.6-Diamidino-2-phenylindole-2-hydrochloride
- Dehydration, 98
- Desmosome, 1, 9, 12, 21, 172, 173, 217
- Detergent
 Nonidet P-40, 328
 Sarkosyl NL-97, 328
- Development from aggregated cells, 251–259
- 4.6-Diamidine-2-phenylindole-2-hydrochloride, 132
 carcinogenic, 135
 method, 135–136
- Diadumene*, *see* Sea anemone
- 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 394
- Diethylpyrocarbonate, 374, 375
- Differentiation, sexual in hydra, 71–77
 by carbon dioxide tension, 73–74
 factors affecting, 71–72
 by feeding schedule, 75–76
 by temperature drop, 77
 by temperature rise, 72–73
- 3-Dimethylamino acid, 412
- Dimethylsulfoxide, 132
- Disadvantage of using hydra in experiments, 2
- Disk, basal, *see* Foot
- Disk electrophoresis, 336
 apparatus, 336
 stock solutions, 336
- Dissociating into cells, 153, 263
 medium for, 153, 257
- Dithioerythritol, 335
- Dithiothreitol, 338, 335
- DMSO, *see* Dimethylsulfoxide
- DNA
 bacterial, 189
 content of hydra, 162
 and hydroxyurea, 292
- DNase, 374, 376
- Doubling time of cells, 32, 158 *see* Growth
- Dye, *see* Stain
- Ecology dish, 263
- Ectoderm, 5, 108, 138, 267–271
 autoradiography, 119
 and carbon particle, 183
 -endoderm chimera, 273–277
 formation, 268
 isolation, 267–271
 by mechanical method, 269–270
 by perfusion method, 269
- Egg, 8
 hatching time, 214
 stimulating formation, 71
- Ehrlich's hematoxylin stain, 124, 125, 128
- Electrode, 429–433
- Electron microscopy
 scanning, *see* Scanning electron microscopy
 transmission, 87–94
 preparation for, 87–90
- Electrophoresis on acrylamide gel, 338
- Electrophysiology, 429–451
- Embedding specimen, 88, 89, 92, 123–124
- Endoderm, 5, 138, 267–271
 -ectoderm chimera, 273–277

- Endoderm (*cont.*)
 formation, 268
 isolating, 267–271
 peeling off, 270
- Endosymbiont, *see* Alga, endosymbiotic
 autotrophic, 407
- Enteron, 101
see Coelenteron
 surface, 109
- Enzyme assay, 361–371
 oil-well method, 367–377
- Eosin, 123, 128
- Epidermis, 5
- Ethanol, 117, 118, 122, 123, 128, 174, 198,
 199
- Epizootological research, 417–425
- Evans blue, 132, 133, 136
 carcinogenic, perhaps, 137
- Everting whole hydra, 270
- Extrusion mechanism for nematocyst, 331
- Fast green stain, 127
- Feeder-layer technique, 261
- Feeding
 forced, 304
 pipet, 288
 radioactive tissue, 194–195
 response, 443–451
 activator, 443–451
 potency, 447–449
 bioassay for, 443–451
 inhibitor, 443–451
 competitive, 448–450
 noncompetitive, 451
 and potassium ion, 450
 quantification, 447
- Ferric alum, 127
- Ferritin, 105
- Feulgen stain, 124, 125, 127, 162, 166
 method described, 127
 and mitotic cell, 118–119
 reagent, 162
- Fiddler crab, 342, 345, 348
- Film, photographic, 146, 147
- Filter, micropore, 197–198, 205–206
- Fixative, 87–90, 107, 121–129, 166, 174
- Fluorescent microscopy, 133
- Fluorometer, 404
- Foot (basal disk), 6–9, 231
 activator
 assay, 316–318
- Foot (basal disk), activator (*cont.*)
 defined, 318
 purification, 323
 formation
 activation, 238–241, 243
 frequency of, 232, 234, 236
 inhibition, 238–241, 243
 gradient, 244–245
 optimization of activation–inhibition
 level, 244–251
 uncertainty, statistical, 241–247
 inhibitor, 318
 assay, 319
 defined, 318
 purification, 323
 sectioning, 124
- Formalin, 402
- Forced feeding, 304
- Fractionation of tissue, 197–203
- Freeze-drying, 362–363
- Freeze-etching, 105–115
- Freeze-fracture, 105–115
- Fructose-1,6-diphosphate, 305–307
- Fungus, 41
- G2-Phase, 160–161, 291
- Gastric cavity, 5
- Gastrodermis, 5
- Gentamicin, 257
- Glass needle, 225–227
- Glass suction electrode, 431–432, 439
- Gluconic acid, 412
- Glucose oxidase, 411
- Glucose oxidase and peroxidase, 412
- Glucosidase, 412
- Glutaraldehyde, 89, 90, 96, 106
- Glutathione, 195, 269, 446
 and mouth-opening, 269
- Glutinant
 stereoline, 12
 streptoline, 12
- Glycerol, 128, 332, 393–394
- Gonad development, 213
- Gonocyte, meiotic, 14
- Gradient, morphogenic, 234
- Grafting, *see* Transplantation
 dish, 225–226
 tissue, 1, 2, 225–233, 274, 299, 301
- Granule, intramembranous, 110, 111, 114
- Green hydra, *see* Hydra
- Growth

- Growth (*cont.*)
count, 49–50
curve, 158
rate, 32, 47–52
 calculation, 51
 clonal, 48–52
 doubling time, 32, 158
 precautions, 51–52
- Gut, 5
 see Coelenteron, Enteron
- Hair point, 362
- Handling hydra, 17–83
- Hatching time of egg, 214
- Head, 6, 7, 231, 311
 activation–inhibition model, 238
 defined, 313
 gradient, 233, 235
 optimization level, 244–251
 uncertainty, statistical, 241–247
- activator
 assay
 fast, 313–314
 standard, 321–313
 purification, 321, 323
- formation, frequency of, 232, 234, 236, 238
- inhibitor
 assay
 alternative, 315–316
 standard, 314–315
 defined, 315
 purification, 323
 reduced, 231
- Heidenhain Susa fixative, 125
- Hemocytometer, 404
- Hematoxylin, 128, 166
 Ehrlich's, 124, 125
 iron, 124–127
- Hemolysis
 assay, 349–353
 curve, 352
 data, plotted, 352–353
 microtitration, 353
 percentage of, 352
 plotting data, 352–353
 spectrophotometry of, 351
 treatment, mathematical, 351–353
 see Rat red blood cells
- Hexadecane, 368
- Histology, 87–140
- Hoechst 33258, 133–135
 carcinogenic, 134
- Hydra, epithelial, 281
 cloning, 286
 culturing, 287–290
 definition, 287
 feeding technique, 288, 289
 by force, 288–290
 formation, 287
 by colchicine, 287
 by gamma-irradiation, 287
 by inbreeding, 287
 inserting shrimp into, 288
 maintaining, 290
 mouth-opening, 288
- Hydra, green
 oxygen evolution, 383–387
 photosynthesis, 386
 respiration, 383–387
- Hydra species, 19–28
 H. americana, 23, 25
 H. attenuata, 22, 26, 154, 175, 235, 281–286, 292, 295–297, 299, 304, 312, 450
 H. braueri, 23, 25
 H. canadiensis, 25
 H. carnea, 23, 26, 27
 H. cauliculata, 23, 26, 27
 H. circuminata, 25
 H. fusca, 25
 H. hadleyi, 22–24
 H. hymanae, 22–25
 H. littoralis, 23, 27, 31, 32, 235–237, 336–337, 341–346, 419, 445
 H. magnipapillata, 26, 211, 213, 281
 H. minima, 23, 25
 H. oligactis, 23–26, 267, 373
 H. ovata, 25
 H. parva, 25
 H. pirardi, 26, 299, 450
 H. pseudoligactis, 23–26, 267, 299, 329, 418, 419
 H. robusta, 25, 26
 H. rutgerensis, 23, 26, 27
 H. stellata, 25
 H. utahensis, 23, 25
 H. viridis, 22, 154, 213, 244, 247, 267, 281, 295–297, 329, 394–396, 411, 412, 418, 419, 450
 H. viridissima, 22–24
 H. vulgaris, 23, 26, 27

- Hydra* species (*cont.*)
 criteria for identifying, 19
 literature on systematics, 20
see specific aspects of hydra
- Hydramoeba hydroxena*, 18, 417–418
 growth, 420–421
 host mortality, 421
 hydra system, 417
 infection rate, 423
 stock culture, 418–419
 survivorship curve method, 422–423
- Hydranth, 5, 6
 count of, 49–50
- Hydroxyurea, 281, 291–294
 and cell population, 293
 and DNA, 292
- Hypostome, 5, 6, 92, 100, 102, 112, 113, 124, 231
- Immunofluorescence, indirect, 138–139
- Immunoglobulin (goat anti-mouse), 133
- Inbreeding, 287
 depression, 2, 3
 sexual, 211–221
see Mutant
- India ink marker, 183–186, 274
- Infection, bacterial, *see Bacteria*
- Inhibition, *see Foot, Head*
- Injecting hydra, 189, 400
- Interference microscopy, 119
- Iron hematoxylin stain, 124, 125, 127
- Irradiation
 gamma-ray, 287, 303–304, 396
- Isocitrate dehydrogenase, 368
 microassay for, 368
- Isorhiza, 217
 atrichous, 12, 173
 holotrichous, 12, 173
- Isotope technique, 189–203
- Kerona pediculus*, 18, 424
- Labeling
 double, 160
 by feeding, 193
 by injection, 193–196
 radioactive, 158–159, 189–207
 with $^{14}\text{CO}_2$, 196
- N-Lauroyl sarcosine, 328
- Lavdowsky's fixative, 118, 119, 122, 124, 166
- Lead nitrate stain, 171–176
 preparation, 172, 174
- L- α -Lecithin, 354
- Leeuwenhoek, Anthony van, 1
- Lethality
 bioassay, crustacean, 348–349
 LD₅₀, 349
- Light microscopy, 117–130
- Log-linear model, 246
- Lyophilization, *see Freeze*
- M solution, 31
- Maceration technique, 153–156, 166, 401
 autoradiography, 155
 counting cells, 154–155
 fixative, 153
 for single cells, 153–156
 solution for, 153, 402
 staining, 156
- Macrophotography, 143–149
- Maertín's solution, 412
- Magnesium uranyl acetate, 89
- Mallory's triple stain, 124, 125, 129
 one-step method, 129
- Maltose
 of alga, endosymbiotic, 411
 assay, spectrophotometric, 411–413
- McIlwaine's buffer, 412
- Medium, *see Culture solution*
- Membrane, 110, 111
- Mercaptoethanol, 335, 338
- Mesolamella, 2, 5, 102, 103, 112–114, 186, 281
 isolation, 327–329
 properties, 327–329
 sticky, 328
- Mesoglea, *see Mesolamella*
- Methanol, 318, 321, 404
- 3-Methyl-2-benzothiazolinone hydrazone hydrochloride, 412
- Methylene blue staining, 131
 of nematocyst, 119
- Methylphenyldiazene-carboxylate, 450
- Methylsalicylate, 123
- Metridium* sp., *see Sea anemone*
- Microassay, 367
 precautions, 370
- Microelectrode, glass capillary as, 429–432, 435
- Microinjection pipet, 399–400
- Micropipet, 184

- Microscalpel, 362
- Microscopy
 bright field, 181
 electron, 87–104
 fluorescent, 133
 interference, 119
 light
 phase, 169, 251
 polarization, 119
 scanning electron, 95–104
 transmission electron, 87–94
- Microtitration
 dilution, 353
 plate, 353
- Microtubule, 1, 2
- Mineral oil, 368
- Mitosis
 duration, 161
 index, 161, 165–168
 labeled as a technique, 160–161
- Mitotic index, 161, 165–168
 defined, 165
 for growth estimation, 165
 and growth rhythm, 167
- Morphogen, 311–324
 assay, biological, 312–318
 for activator, 312–314, 316–318
 for inhibitor, 314–316, 318
 purification on column, 318–323
- Morphogenesis, 311–324
 control, chemical, 311–324
- Morphology, 5–14
- Mount, whole, 117–120, 166–167, 174–175
- Mouse, 345
 CAF strain, 342
 lethality bioassay, 349
 LD₅₀, 349
 liver, radioactive, 195
 tissue, radioactive, 195
- Mouth, 7
 -opening, 269, 288
- Muscle mat, 9
- Mutant, 211–221
 cell types in altered proportions, 215, 219
 developmental, 211
 inbred, 211–221
 male, sterile, 270
 maxi, 214
 mini, 214
 multiheaded, 215–216
 nematocyst-deficient, 215, 217
- Mutant (*cont.*)
 regeneration-deficient, 215
 twisted column, 214, 216
- Mycostatin, 212
- Myoneme, 102, 103, 112, 114
- Narcosis, *see* Anesthesia
- Nauplii, *see* *Artemia salina*
- Neck, 6, 7
- Nematoblast, 11, 160, 219, 291–294, 299, 300
 lead nitrate-thioacetic acid stain, 171–176
 and microtubule, 2
 numbers of, 169–182
- Nematocyst, 24, 90, 91, 96, 98, 108, 304, 305, 327–387
 assay for
 hemolysis, 349–353
 lethality, 348–349
 phospholipase, 353–355
 capsule, 2, 177, 335–339
 composition, 337–338
 discharged, 331–333
 mechanism for, 331
 dissolution, 337
 examination, 20–21
 extrusion, 331
 medium for, 331
 isolation, 327–328
 isorhiza, holotrichous, 21
 number, 169–182
 properties, 327–387
 in sea anemone, 331–333
 suspension, 176–178
 in tentacle, 217
 thread protein, 335
 toxin, 331
 type
 desmoneme, 21
 isorhiza
 atrichous, 21
 holotrichous, 21
 photomicrograph, 170
 stenotele, 21
 undischarged, 331–333
 venom, 341, 347–358
- Nematocyte, 12, 162, 219, 301, 305–307
 on body column, 179–181
 and interference microscopy, 119
 and microscopy, bright-field, 181
 numbers, 169–182

- Nematocyte, numbers (*cont.*)
 reduced, 305–307
 suspension, 176–178
 in tentacle, 176–178
 and toluidine-blue stain, 179
- Nembutal, 122
- Nerve
 cell, 13, 295–297
 net, 14
- Neubauer cell counting chamber, 178, 251
- Neuron, sensory, 13
- Neutral red, 131
- Nile blue sulfate, 131, 132
- Nipple, 7
- Nitrogen mustard, 258, 261–268, 275–276, 299–302
 and cell cycle, 299–302
 precautions, 277
 treatment, 276
- Oöcyte, 8, 14
- Oögonium, 14
- Operculum, 108
- Optimization method, 244
- Orange G, 129
- Osmium tetroxide, 344
 dangers of, 345
- Ovum, 14
- Ovary, 7
- Oxygen
 consumption measured, 383–386
 electrode, 383
 calibration, 384
 chamber, 387
 instability, 386
 evolution, 386–387
 of green hydra, 383–387
 and photosynthesis, 386
- Paraffin, 121, 133
- Paraformaldehyde, 89, 90, 97, 106
- Parasites of hydra, 18
see also Anchistropus, Hydramoeba, Kerona, Trichodina
- Peduncle, 6, 7
- Pelmatohydra oligactis*, 32
P. pseudoligactis, 32
- Penicillin G, 212
- Phagocytosis, 183, 300
- Phase microscopy, 169, 251
- Phenol, 374
- Phenol (*cont.*)
 -chloroform extraction of RNA, 373–377
- Phenol red, 35–37
- Phospholipase
 assay
 manometric, 353–355
 titrimetric, 355
 carbon dioxide release, 354
 thin-layer chromatography, 355–356
 in venom, 353
- Phosphorus method, 356
- Phosphotungstic acid, 129
- Photobleaching, 394–395
- Photography, *see* Macrophotography
- Photoöxidation, 338
- Photosynthesis of green hydra, 386
- Phototaxis, 18
 in an eyeless animal, 1
- Physalia* sp. venom, 347
- Poisson distribution, 264
- Polarization microscopy, of muscle process, 119
- Polyethylene needle, 190–191
- Polyp, 5, 68, 69
- Pore, aboral, 7
- Portugese man-of-war, *see Physalia* sp.
- Potassium ion, and feeding response, 450
- Potassium acetate, 374–376
- Potential
 spontaneous, 429–435
 transepithelial, 435–436
- Procamborus clarkii*, *see* Crayfish
- Procedures, analytical, 361–387
- Pronematocyst, 11
- Protein
 analysis, colorimetric (Lowry *et al.*), 379–381
- Protozoön, 257, 263
- Pyridine nucleotide fluorescence, 367
- Quantification (numbers), 175
 and feeding response, 447
 pipetimetric, 64–65
 turbidimetric, 64
- Quartz fiber ultramicrobalance, 363–367
 calibration, 366
 precaution, 367
 use, 366
- Quartz filter, 365
- Radioactive hydra, 193–196

- Radioactivity
 assay, 199, 202
 calculation, 200, 202
 percentage, 202
- Radioautography, *see* autoradiography
- Rat red blood cell, 349
- Razor blade fragment as knife, 226, 228
 holder for, 228
- Reaggregation, 299, 301
- Red blood cell
 absorbancy unit, 351
 hemolysis, 349–353 *see* Hemolysis
 preparation, 349, 350
 standardizing, 350–351
 washing, 350
- Reflex camera, 144
- Regeneration, 1, 299
 after amputation, 218
- Relaxant, 122
- Reproduction, asexual, 1, 53
- Respiration of green hydra, 383–387
- Rifampicin, 212, 257, 282, 288
- RNA, 373–377
 buffer, 374
 electrophoresis, 376
 extraction by phenol-chloroform, 373–377
 fractionation, 87, 88, 376
 purification, 375–376
 ribosomal, 374
- RNase, 375
- Scanning electron microscope, 95–104
 resolution, 95
 x-ray microanalysis, 95
- Schiff reagent, 119, 127
- Sea anemone, aconitiate, 331–333
Aiptasia pallida, 332, 333, 347, 353
Diadumene sp., 332
Metridium sp., 332
 nematocyst venom, 347
 neurotoxin, 356
 sodium current, 356
- Sectioning hydra, 123–124
- SEM, *see* Scanning electron microscopy
- Separation gel of acrylamide, 337
- Sesame oil, 344
- Sexually differentiated hydra, 71–77
 methods for, 73–76
- Shock, electric, 306
 chamber for, 307
- Size of hydra, 67–70
- Sodium cacodylate, 89, 90, 106, 171
- Sodium citrate, 332
- Sodium dodecyl sulfate, 374, 403
- Sodium pentobarbital, 106
- Sodium thiosulfate, 300
- Solutions, culture, 30–31
- Species of *Hydra*, *see* *Hydra* species
- Specimen chamber, 147–148
 for photography, 148
 for shock, electric, 307
- Spermatid, postmitotic, 14
- Spermatocyte, 14
- Spermatogonium, 14
- Spermatozoa, 14
- S-phase of cell cycle, 159–160, 291
- Spurr's embedding medium of low viscosity,
 88
- Square wave
 pulse, 357
 stimulator, 306
- Squash preparation, 336–337
- Stacking gel of acrylamide, 337
- Staining, 88, 124–125
 maceration for, 156
 procedures, 118
 of tissue, 174
 vital, 1, 131–140
 by feeding
 colloidal carbon, 132
 colored food, 131
 fluorescent dye, 132
- Stains
 acid fuchsin, 129
 aniline blue, 129
 Biebrich scarlet, 128
 eosin, 123, 128
 Evans blue, 132, 133, 136, 137
 fast green, 127
 Feulgen, 118, 162
 application, 118–119, 124–125, 127,
 166
 fluorescent, 132
 hematoxylin, 124, 125, 128, 166
 India ink, 274
 iron hematoxylin, 124–127
 lead nitrate, 171–176
 Mallory's triple, 124, 125, 129
 methylene blue, 119, 131
 neutral red, 131
 Nile blue sulfate, 131, 132
 orange G, 129

- Stains (*cont.*)
 phenol red, 35–37
 thiolactic acid lead, 119, 171–176
 toluidine blue, 118, 124, 125, 129, 179, 180
see also Dye
- Stalk, 6
see Peduncle
- Stenotele, 12, 21, 172, 173, 178, 217
 dissolution, 337
 reduction in number, 306
- Stereoline glutinant, 12
- Streptoline glutinant, 12
- Streptomycin, 265
- Succinoxidase inhibitor, 341–346
- Susa fixative, 122
- Symbiont, green alga, 394, 401–405
- Symbiosis, 388–413
- Taeniola, 9
- Tannic acid, 92
- Teflon block, 368
- Tentacle, 7, 231
 isolation, 176–177
 whorl, 6
- Testis, 7, 81
 induce formation, 71
- Theca, 8
- Thioglycolate, 335, 338
- Thiolactic acid lead staining, 171–176
 of nematoblast, 119
- Thiol solution, 336, 337
- Thymidine, labeled, 132, 158–162
 injected into hydra, 189
- Tissue
 chimera, preparation of, 275–279
 culturing stem cells, 261–266
 dissociation, 154, 251–259
 ectoderm, 267–271
 endoderm, 267–271
 enzyme assay, 361–271
 grafting, 1, 2, 225–233, 274, 299, 301
 layer, viable
 separating, 267–271
 maceration, 153–156
 manipulation, 225–279
 microgram quantities, weighed out, 361–371
 organization, 225–279
 pieces of, 155
 radioactive, 193–196
- Tissue - radioactive (*cont.*)
 fractionation, 197–203
 regeneration, 1
 transplanting, 225–249
- Toluidine blue stain, 118, 124, 125, 129, 179, 180
 and interstitial cell, 118
- Track plate, nuclear, 205, 206
- Transection, 81–82
- Translocation, of reduced organic carbon compounds, 407–409
- Transplantation
 axial, 231
 experiments, 235–236
 of foot, 231
 of head, 231
 lateral, 230, 231
 property, intrinsic, 234
 quantitative interpretation, 233–249
- Tray method for culturing hydra, 54, 56–60
 cleaning, 56–58
 feeding, 56
 precaution, 59–60
- Treatment, chemical, 258
- Trembley, Abraham, 1
Mémoires (1744), 1
- Trichloroacetic acid, 198–199
- Trichodina pediculus*, 424
- Trimethoprim, 395
- t-Test, 312
- TX, *see* 6-Carboxyfluorescein
- Uca pugilator*, *see* Fiddler crab
- Uncertainty, statistical, 241–248
- Urethane, 122, 174
- Vital staining, 131–140
 review of, 131–132
see also Dye, Stain
- Van Harreveld solution, 356
- Variable, redundant, 245
- Vertical plate method for culturing hydra, 54–56, 60–62
 care of tank, 61
 feeding, 61
 precaution, 61–62
 seeding, 60
- Villus, endodermal, 11
- Volvent, 12
- Warburg flask, 194

X-ray microanalysis, 95
Xylene, 117, 118

Zenker fixative, 122
Zymogen, 14

