

# **Hydra: Research Methods**

**Edited by**

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# 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 Pipetometric 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 [<sup>3</sup>H]Thymidine into Hydra by  
Microinjection..... 189  
Charles N. David
26. Labeling with Gaseous <sup>14</sup>CO<sub>2</sub> 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

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Chapter 40

# Eliminating Interstitial Cells with Nitrogen Mustard

Charles N. David

## PURPOSE

To remove interstitial cells and differentiating nematoblasts from hydra leaving a shell of epithelial and gland cells.

## INTRODUCTION

Treatment of hydra with nitrogen mustard (NM) causes the rapid elimination of interstitial cells and differentiating nematoblasts from the tissue (Diehl and Burnett, 1964). Such hydra have been used to investigate the role of interstitial cells in budding and regeneration (Diehl and Burnett, 1965*a, b*). In addition, NM-treated hydra have been used as feeder layers for the culture of interstitial cells added to them by grafting (Diehl and Burnett, 1966) or reaggregation techniques (David and Murphy, 1977). The method, described here for *Hydra attenuata*, is essentially that of Diehl and Burnett (1964), who used *Hydra pseudoligactis* and *Hydra pirardi*.

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## COMMENTS

NM is a potent alkylating agent capable of adding ethyl residues to nucleic acids including DNA (see Goodman and Gilman, 1974, for review). It is strongly cytotoxic to proliferating cells; nonproliferating or slowly proliferating cells appear to be less affected.

NM acts on cells at any stage of the cell cycle. Progression through the cycle, however, is usually blocked in the  $G_2$  (premitotic) phase. Cells blocked in  $G_2$  continue synthesizing RNA and protein and often become enlarged due to unbalanced growth.

Treatment of hydra with 0.01% NM for 10 min leads to the disappearance from hydra tissue of interstitial cells and differentiating nematoblasts over the next 4–8 days. This process can be easily monitored by observing interstitial cells in whole mounts of hydra stained with Toluidine Blue (Diehl and Burnett, 1964) or by counting interstitial cells in macerations of treated animals (David, 1973). Subsequently the number of differentiated nerves and nematocytes also declines. By comparison, the epithelial and gland cell populations appear to be less affected. Five days after NM treatment, hydra consist of a shell of epithelial and gland cells, and in this condition they survive for 4 weeks or longer.

The effects of NM on hydra cells are best explained with reference to the proliferation kinetics of hydra cell types. Interstitial cells are rapidly proliferating cells with generation times of 18–27 hr (Campbell and David, 1974). They are rapidly killed by NM and eliminated from hydra tissue, probably by phagocytosis by epithelial cells. Epithelial cells have cell generation times of 3 days in well-fed hydra and more than 6 days in starving hydra (David and Campbell, 1972). They are also killed by NM, but because of their longer cell cycle they survive longer than interstitial cells and thus give rise to animals consisting only of epithelial cells. Gland cells have a similar cell cycle to that of epithelial cells and also survive for long periods in NM-treated hydra.

## MATERIALS

Two percent (w/v) sodium thiosulfate and 0.01% NM (Sigma Chemical Co.,). *Special precaution:* NM is toxic and should be handled with care. Carry out as much of the procedure as possible in a fume hood. In addition, have 1–2 liters of 2% sodium thiosulfate solution available to detoxify any spilled NM solution as well as any unused reagent.

## PROCEDURES

Because NM is unstable in water, prepare the reagent from the dry powder immediately before use. Add NM to hydra at a final concentration of 0.01%. Mix the solution thoroughly by stirring with a pipet, taking care that all hydra are in suspension and not stuck to the sides of the dish. After 10 min allow the hydra to settle, decant the solution and fill the dish with fresh medium. Repeat the washing procedure four times to stop the action of NM on the hydra. Thereafter wash the hydra at least once each day in order to remove dead hydra and debris from the dish.

After NM treatment hydra can be fed for about a week until their nematocytes are depleted (Diehl and Burnett, 1964). However, feeding stimulates cell cycling and accelerates the death of treated animals. If the purpose of NM treatment is to prepare hydra free of interstitial cells, then one feeding after NM treatment appears to optimally stimulate the disappearance of interstitial cells without adversely affecting the survival of the host animal.

A final concentration of 0.01% NM is usually effective in completely eliminating interstitial cells from tissue without immediately destroying the animals. However, the precise concentration of NM required to eliminate interstitial cells varies slightly between batches of NM. Thus, it is useful to test several concentrations when using a freshly opened bottle of NM. If, after prolonged use, the NM in a bottle loses some of its potency, increase the concentration of NM used in experiments. However, do not use more NM than necessary because it does destroy epithelial cells and thus may noticeably shorten the survival of treated animals.

## SPECIAL APPLICATIONS

NM-treated hydra free of interstitial cells are useful hosts in which to study the fate of interstitial cells added to them by grafting or reaggregation techniques. When normal hydra tissue is grafted into NM-treated tissue, interstitial cells migrate from the normal tissue into the NM tissue, where they proliferate and differentiate normally (Diehl and Burnett, 1966). Interstitial cells can also be introduced into NM tissue by preparing aggregates from dissociated NM-treated hydra (David and Murphy, 1977). Such aggregates regenerate normal hydra structures. When small amounts of normal tissue are dissociated and added to such NM aggregates, the added interstitial cells proliferate and differentiate normally. The number of cells



seeded in such aggregates can be easily controlled, and the aggregates function effectively as tiny tissue culture dishes (see Chapter 33).

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# 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- $\alpha$ -Lecithin, 354
- Leeuwenhoek, Anthony van, 1
- Lethality  
 bioassay, crustacean, 348–349  
 LD<sub>50</sub>, 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  
 LD<sub>50</sub>, 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

