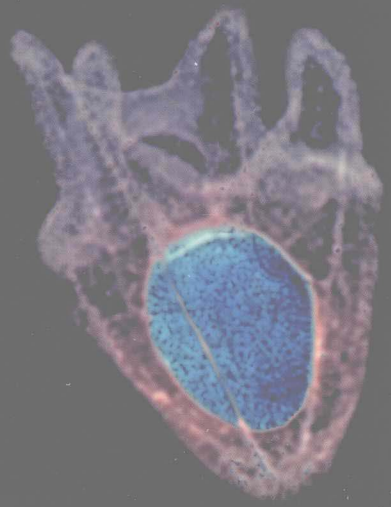
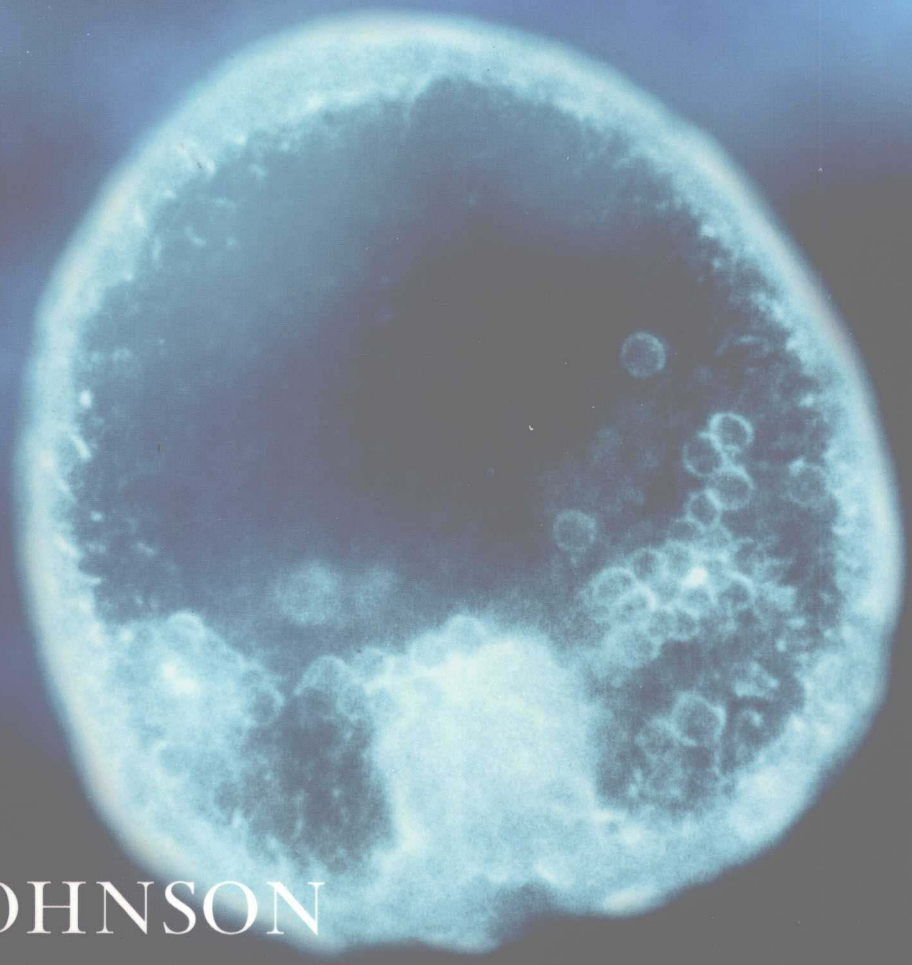
Johnson & Volpe's

THIRD EDITION

PATTERNS & EXPERIMENTS in Developmental Biology





LELAND G. JOHNSON

JOHNSON & VOLPE'S PATTERNS & EXPERIMENTS IN DEVELOPMENTAL BIOLOGY

Third Edition

Leland G. Johnson
Augustana College

Boston Burr Ridge, IL Dubuque, IA Madison, WI New York San Francisco St. Louis
Bangkok Bogotá Caracas Kuala Lumpur Lisbon London Madrid Mexico City
Milan Montreal New Delhi Santiago Seoul Singapore Sydney Taipei Toronto

McGraw-Hill Higher Education
*A Division of The **McGraw-Hill** Companies*

PATTERNS & EXPERIMENTS IN DEVELOPMENTAL BIOLOGY
THIRD EDITION

Published by McGraw-Hill, a business unit of The McGraw-Hill Companies, Inc., 1221 Avenue of the Americas, New York, NY 10020. Copyright © 2001, 1995, 1973 by The McGraw-Hill Companies, Inc. All rights reserved. No part of this publication may be reproduced or distributed in any form or by any means, or stored in a database or retrieval system, without the prior written consent of The McGraw-Hill Companies, Inc., including, but not limited to, in any network or other electronic storage or transmission, or broadcast for distance learning.

Some ancillaries, including electronic and print components, may not be available to customers outside the United States.

This book is printed on recycled, acid-free paper containing 10% postconsumer waste.

1 2 3 4 5 6 7 8 9 0 QPD/QPD 0 9 8 7 6 5 4 3 2 1 0

ISBN 0–07–237965–0

Publisher: ***James M. Smith***
Editorial coordinator: ***Brian S. Loehr***
Marketing manager: ***Thomas Timp***
Marketing coordinator: ***Tami Petsche***
Project manager: ***Christine Walker***
Media technology producer: ***Lori A. Welsh***
Production supervisor: ***Sherry L. Kane***
Freelance design coordinator: ***Michelle M. Meerdink***
Cover art provided by: ***Leland G. Johnson***
Compositor: ***York Graphic Services, Inc.***
Typeface: ***11/13 Garamond***
Printer: ***Quebecor World Dubuque, IA***

Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

www.mhhe.com

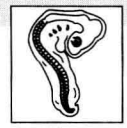
JOHNSON & VOLPE'S

PATTERNS & EXPERIMENTS IN DEVELOPMENTAL BIOLOGY

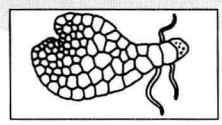
Contents

此为试读,需要完整PDF请访问: www.ertongbook.com

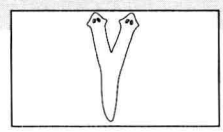
CHICK EMBRYO DEVELOPMENT



FERN GAMETOPHYTE DEVELOPMENT



EMERGING AND REGENERATING DEVELOPMENTAL PATTERNS



Preface

As with the earlier editions, the goal of this edition of *Patterns and Experiments* is to facilitate and encourage developmental biology and embryology laboratory experiences that bring students together with fascinating and dynamic developing systems. Professional biologists and nonbiologists both often relate that the study of some aspect of development of a living organism has been a memorable highlight in their educational experience. How fascinating it is to watch those tiny clusters of cells as one makes that first marathon set of observations of a batch of developing sea urchin embryos. How exciting it is to return to the lab to find a vigorously beating heart in an in vitro cultured chick embryo where there had been no visible heart and a much simpler form only twenty-four hours earlier.

My own view of biology and my career plans changed when I had that experience. I want to say to students who will use this manual that I envy you the excitement that comes with those first opportunities to experiment with living, developing organisms. I hope that a few of you might be inspired to go on to careers researching developmental processes and sharing the fascination of development with your own students. This is a truly exciting time in developmental biology because we are now able to investigate directly many of the genetic mechanisms underlying various developmental processes. However, as you begin your study of developmental biology, whether you pursue that study only in this course or study development for many years to come, I would like to offer one bit of advice from the perspective of many years in developmental biology. As intently as you may study certain individual developmental processes, please try not to lose sight of the whole developing organism and the still broader picture of the role of development in the perpetuation of species. Much of the fascination and beauty of development is to be found at those levels.

This third edition of *Patterns and Experiments* includes a number of additions and new features. Several of the additions are to the considerably expanded section on echinoderm development. There are much more detailed directions for caring for sea urchin and sand dollar embryos and larvae (Laboratory 1 and Appendix A). Several colleagues have reported that their students have been frustrated with their inability to observe development beyond the earliest stages, and I think that these directions will make it much easier for students to observe additional parts of development. The simpler and more effective procedure for blastomere separation that has been incorporated into Laboratory 2 should make it easier for students to conduct "twinning" experiments like those that have such a rich history in developmental biology's past. Laboratory 2 also includes a fascinating new experiment on the somewhat surprising, but very adaptive, capacity of echinoderm embryos and larvae to regenerate lost cilia. Also, reorganization of the echinoderm portion of the manual led to creation of a new part (Laboratory 3) that includes investigation of differentiation of an enzyme system. This investigation provides students a chance to study specific localized genetic activation in differentiation. Also, "Suggestions for Further Investigation of Echinoderm Development" was reorganized and substantially rewritten.

There is an important addition to the chick embryo section as well. In Laboratory 11, an earlier brief suggestion about investigating heart duplication has been expanded to a full experiment on heart rudiment separation and heart tube duplication that includes informative new illustrations.

Numerous other updates and additions, including several added illustrations, have been made throughout the manual. Well over one hundred new references have been incorporated into the "Suggestions for Further Investigation" that appear at the ends of the portions of the manual. Each set of references has been updated, and the majority of the new references are to works that have been added to the very dynamic literature of developmental biology since publication of the second edition of *Patterns and Experiments* in 1995.

I've also added citations to a number of the useful websites, many of which have come into being since 1995 as well. I've tried for a modest mix of specialized websites as well as general ones that provide links to many more of the valuable resources now available on the World Wide Web and which are likely to incorporate additional links to important sites that surely will be developed in the coming years.

Developmental biology is not a discipline isolated from other aspects of biology. This is particularly evident, for example, in regard to the worldwide ecological problem of declining populations of numerous amphibian species recognized during the 1980s and 1990s. Appendix G contains some suggestions concerning responsible use of amphibians in teaching that are relevant to this problem. That appendix also contains suggestions of strategies for teaching developmental biology without sacrificing adult vertebrate animals, which is an option that a number of biologists, including me, prefer to choose.

I thank the colleagues and students who have used the earlier editions of this manual and have taken the time to share some of their experiences in developmental biology. They have made insightful comments about the manual and have offered helpful suggestions and criticisms. A number of those suggestions led to additions to the second edition, and others have influenced the development of this third edition. I also warmly thank the many colleagues from colleges and universities across the United States and Canada who have participated over the years in my summer workshops on the Developmental Biology Teaching Laboratory at the University of Maine's Darling Marine Center. Those developmental biologists have brought their own individual perspectives and expertise to the workshop sessions, and we've shared some remarkable learning experiences in that beautiful setting. I owe them and my Darling Center colleagues a great deal.

Finally, I wish once again to offer my thanks to Peter Volpe who was my colleague and mentor in preparation of the first edition of this manual. Several of the amphibian development labs, especially Laboratories 4, 5, 6, and parts of Laboratory 8 have been only slightly updated and have remained largely as Peter conceived them, as has Appendix B. Some years ago, Peter's main interests moved into the areas of human development, medical genetics, and biomedical ethics, and he turned his full energy and attention to those pursuits. Thus, his direct involvement with this manual ended with the first edition, but his influence remains evident in a number of places. The manual's current title stands as a recognition of his original contributions.

Leland G. Johnson

Acknowledgements

The illustrations for the first edition, most of which have been carried over into this edition, were the work of the late **Carolyn Thorne Volpe. Rebecca L. Johnson** prepared the many new illustrations added to the second edition. York Graphic Services prepared several new illustrations in this edition. I am grateful for their creative efforts and the patience and persistence with which they worked.

I also am indebted to a number of colleagues and several publishers for their generosity and cooperation in providing photographs. Specific credit for these photographs is given in the respective legends.

I would also like to thank the colleagues who took the time to review this manual and make suggestions for its improvement.

Third Edition

Ken Belanger
University of Scranton

John Landolt
Shepherd College

David Lennartz
Mt. St. Marys College

John Sternfeld
State University of New York–Cortland

James Wetzel
Presbyterian College

First and Second Editions

Vicki J. Martin
University of Notre Dame

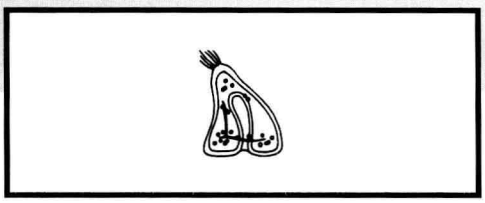
Kathryn W. Tosney
University of Michigan

William A. Thomas
Colby-Sawyer College

LABORATORY

1

Fertilization and Early Development of Sea Urchins and Sand Dollars



Echinoid echinoderms (sea urchins and sand dollars, which are also known as irregular urchins) have been the subjects of many investigations of fertilization and early development, and much of our understanding of developmental processes in animals has come from this research. Sea urchin and sand dollar gametes are readily obtained just before, and during, the breeding season and their developing embryos can be cultured in seawater or salt solutions that approximate the osmotic and ionic properties of seawater. Eggs and embryos of many species are quite translucent, so it is possible to observe a number of cell activities during early development, using a light microscope.

In this laboratory, you will have opportunity to observe development from fertilization through assembly of the pluteus larva, which is the swimming, feeding larval form that is characteristic of many of the echinoid echinoderms.

Techniques

Please read and understand the techniques for obtaining gametes for fertilization and for the observation of embryos before you begin this laboratory.

Obtaining Gametes

As in other echinoderms, the sexes are separate in sea urchins and sand dollars. In nature, gametes are discharged into the water, and the sperm swim freely until they reach an egg.

Since the sexes are difficult or impossible to distinguish by external features, sex of an individual animal must be determined by observing the gametes that it sheds. Injection of a small amount of potassium chloride into the coelom will induce an urchin to shed its gametes. The sex of the animal can then be determined by observing the color of gametes that are extruded from gonopores of the aboral (dorsal) surface of the animal within a few minutes after injection. The eggs of most sea urchins and sand dollars range in color from translucent yellow to pale orange, but eggs of some species are darker and may have a reddish cast. Sperm, when shed in mass, appear white or very light gray.

You should be very careful about conditions under which gametes and embryos are maintained. Temperature control is especially important, and your instructor will provide information concerning temperatures that are appropriate for the species you are studying.

1. Gently blot excess water off an adult urchin and place it on a clean surface with its aboral (opposite to, or away from, the mouth) surface down. Induce shedding of gametes by injecting 1 or 2 ml of 0.5 M KCl through the membrane surrounding the mouth opening (perioral membrane). Sand dollars should be injected with a fine-gauge needle inserted at a very shallow angle. To enhance effectiveness of the KCl injection, it is advisable to divide the injected dose of KCl among two or three sites in the perioral membrane. Several websites demonstrate these techniques (see Materials, p. 8).

It is very important to avoid possible contamination of eggs with sperm. This can be accomplished by using a separate syringe and needle for each animal, but that usually isn't practical. An alternative technique is to retain enough KCl solution in the syringe so that some can be expelled after each injection to flush the needle. Then rinse the needle surface with distilled water and dry it with a clean Kimwipe before refilling the syringe and injecting the next animal.

2. Collect eggs by inverting a female over a beaker or a finger bowl containing seawater. The water level in the beaker should be such that the female's gonopores are in the seawater. The eggs will flow out of the gonopores and settle to the bottom of the beaker. After the eggs have been shed, they should be washed by decanting the supernatant water and replacing it with clean seawater. This washing removes coelomic fluid, broken spines, and body surface debris from the water. Eggs should be washed twice if time permits. Alternatively, it is possible to collect shed gametes directly from the body surface with a pipette, which helps avoid contamination by debris and extraneous fluids. Direct collection by pipette often is the best technique to use when only a few gametes are shed, as is sometimes the case with sand dollars.

It is best to proceed with fertilization immediately, but if necessary, the eggs of some species can be refrigerated at 5° C for several hours and still respond fairly well in fertilization.

3. Active sperm, unlike eggs, are viable for only a few minutes in seawater. Thus, it is necessary to keep the sperm quiescent by collecting them under "dry" conditions (that is, in an undiluted suspension). A small portion of the "dry" suspension can be diluted in seawater each time active sperm are needed.

When an animal has been identified as a male, wipe away excess moisture from among the spines on the aboral surface. Invert the male over a clean, dry petri dish or Syracuse dish. After several large drops of the white sperm suspension are in the dish, remove the animal and snugly cover the dish with parafilm or aluminum foil. The sperm should be kept concentrated until just prior to use, when they are activated by dilution in seawater. Collected sperm may be stored "dry" at a cool room temperature for an hour or so, but they should be stored in a 5° C refrigerator if longer storage is required. Sperm of some species can be stored in a refrigerator for up to a day.

4. Observe suspensions of eggs and sperm microscopically and record your observations. To observe active sperm, add 1 drop of "dry" sperm to about 100 ml of seawater in a small container. Sperm are best observed using phase contrast, a dark-field technique, or some other type of microscopy that increases contrast or otherwise enhances visibility of very small objects. If you don't have available phase-contrast optics or a dark-field arrangement on the microscope that you are using, close down the iris diaphragm of the microscope's condenser. This will add some artificial contrast that will facilitate these observations.

The newly shed echinoid egg is surrounded by a transparent jelly coat that has a refractive index similar to that of seawater. If you wish to observe the eggs' jelly coats, mix a drop of India ink with a small quantity of seawater and observe eggs in the suspension. Since the India ink particles do not penetrate the jelly coat, each egg should appear to be surrounded by a clear area (the jelly coat) containing no ink particles.

Fertilization

1. The fertilization procedure involves mixing drops of a diluted sperm suspension with eggs in seawater. A dilute sperm suspension is prepared by placing 1 drop of the undiluted ("dry") sperm in a beaker containing 100 ml of seawater. Mix with a clean pipette to obtain a uniform, faintly cloudy suspension. The "dry" sperm suspension is quite viscous so it is sometimes difficult to control the amount transferred to the beaker of seawater. The final diluted sperm suspension should be only slightly cloudy, not milky, in appearance, because use of an excessively dense sperm suspension can lead to polyspermy. Polyspermy, the entry of more than one sperm into an egg, results in abnormal, arrested development. Since sperm activation requires several minutes, the dilute sperm suspension should be allowed to stand for 5 to 8 minutes before use.

此为试读,需要完整PDF请访问: www.ertongbook.com

TABLE 1.1 Timing of Some Fertilization Events

0 seconds	Insemination
30–40 seconds	Exocytosis of cortical granules
35–50 seconds	Initiation of fertilization membrane elevation (5–10 seconds following cortical granule exocytosis)
60–70 seconds	Completion of cortical granule exocytosis
65–80 seconds	Completion of fertilization membrane elevation
2 minutes	Hyaline layer formed
5 minutes	Fertilization membrane hardened

Transfer several drops of washed eggs to a container with about 100 ml of clean seawater. A thinly scattered layer of eggs on the bottom of a beaker or finger bowl is an appropriate egg density. Eggs, when they have settled, should cover no more than about one-third of the area of the bottom of the container. Then, add 2 or 3 drops of the dilute sperm suspension to the beaker or dish containing the eggs. Mix the sperm and eggs by stirring very gently with a clean pipette.

2. Transfer a sample of the suspension of eggs and sperm to a slide and observe it with a compound microscope. The most conspicuously observable event is the formation of the *fertilization membrane* (fertilization envelope), which is a visible indication that the union of sperm and egg has occurred. If fewer than two-thirds of the eggs display fertilization membranes after 2 or 3 minutes, add several more drops of dilute sperm suspension and stir gently. Repeat if necessary.

The fertilization membrane gradually rises away from the surface of the egg, beginning in the area of sperm penetration and spreading outward around the entire egg. Fertilization membrane elevation is usually complete within 1 to 2 minutes. Elevation of the fertilization membrane is associated with the exocytosis of the contents of cortical granules that are located just below the surface of the egg. The fertilization membrane, which initially is thin and soft, hardens within a few minutes after its elevation. The translucent *hyaline layer* that forms just over the surface of the egg also develops within a few minutes.

These processes are quite temperature dependent, and their timing varies among species, but table 1.1 shows the approximate sequence of events following the addition of sperm to eggs. Note that several of the listed processes cannot be observed with a light microscope.

3. Since the fertilization membrane is elevated rather quickly, you may miss its formation and wish to use another technique to observe the process directly. One means of direct observation consists of placing a drop of eggs and a drop of sperm side by side on a slide. With the microscope focused on the eggs, the 2 drops can be connected by pushing them together with a needle. This technique will permit you to observe sperm swarming around the eggs.

Another method that facilitates direct observation involves placing a pair of 1-mm thick threads of modeling clay parallel to one another on a microscope slide. After laying down the clay, place a drop of unfertilized eggs on the slide between the two strips of modeling clay and add a coverslip. Focus on a group of eggs and, without moving the slide, add a drop of sperm at one edge of the coverslip. You should be able to observe the arrival of sperm in your field of view and the elevation of fertilization membranes on the eggs as you watch them. You may also be lucky enough to observe an egg in which you can see the fertilization cone form at the point where sperm entry is occurring.

If you can produce dark-field or Rheinberg illumination on your microscope, you will find these dark-field techniques especially helpful in making these observations.

Caring for Embryos and Larvae

1. Once you are satisfied that most of the eggs have fertilization membranes, leave the beaker undisturbed until the zygotes have largely finished settling to the bottom. Then pour off the supernatant water and add clean seawater. This step eliminates many of the extra sperm that can degenerate later and foul the water around the developing embryos.

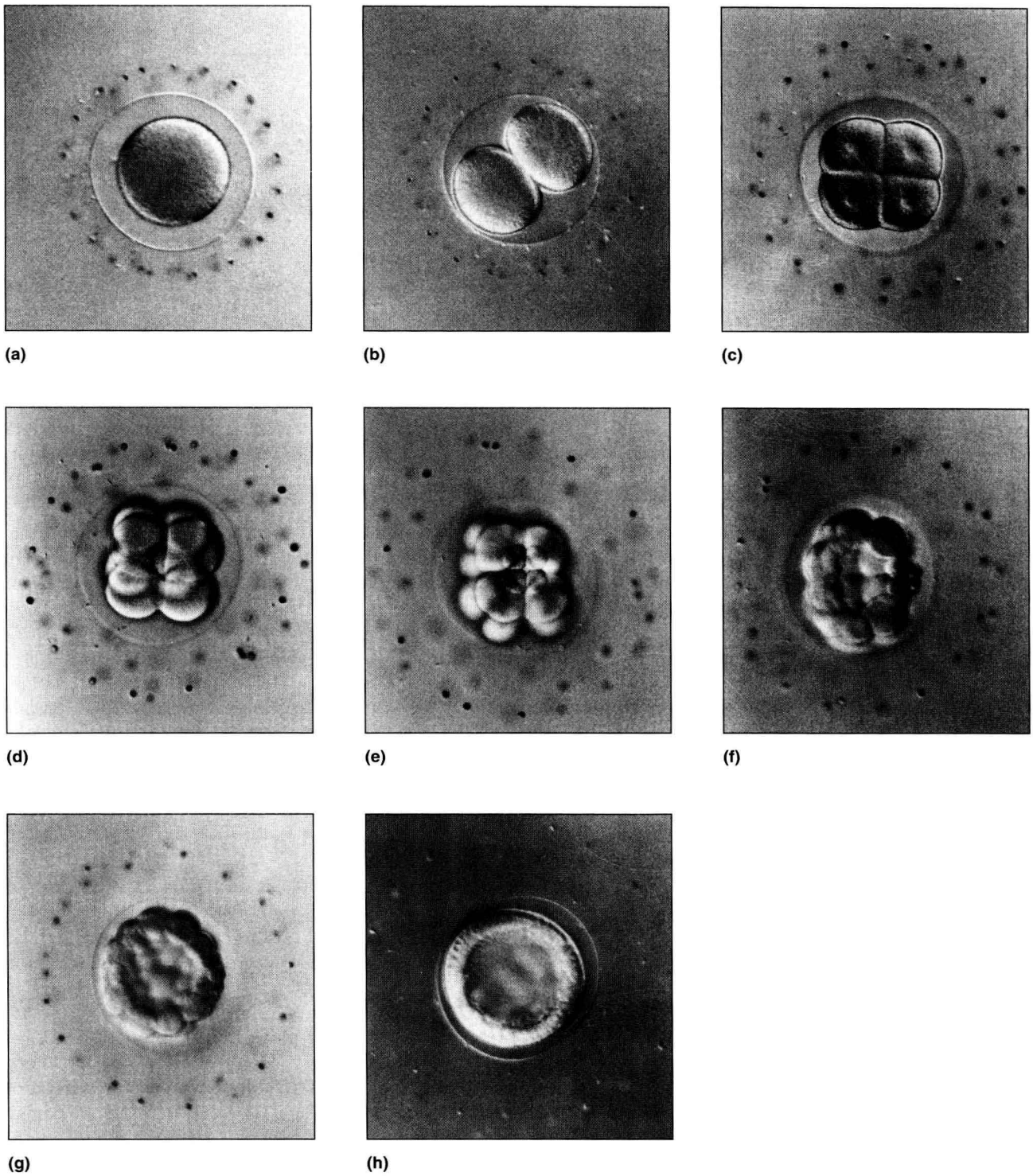


FIGURE 1.1 Cleavage stages in the sand dollar, *Dendraster excentricus*. (*a*) Zygote shortly after fertilization. The fertilization membrane (fertilization envelope) has been elevated. Pigment granules in the jelly coat are visible outside the fertilization membrane. (*b*) 2-cell stage. (*c*) 4-cell stage. (*d*) 8-cell stage, lateral view. (*e*) 16-cell stage, vegetal view. Note the presence of four micromeres that were produced during the fourth cleavage. (*f*) 32-cell stage, lateral view. Note the cluster of micromeres in the vegetal region at the right of this photo. (*g*) 64-cell stage, lateral view. (*h*) Blastula shortly before hatching. (The egg cell in *a* is approximately 120 μm in diameter, and all other photos are printed at the same magnification.)
Photos by R. B. Emlet.

2. Put a loose-fitting aluminum-foil cover over the beaker and set it aside or put it in an appropriate constant temperature chamber until you are ready to make the observations described in the following. Developing embryos and larvae must be maintained at a temperature appropriate for the particular species (See Appendix A, p. 201).

3. When blastulae eventually hatch, you will be able to see them swimming near the surface of the water in the upper part of the beaker. Sometimes shining a flashlight or microscope illuminator through the culture can help you to spot the swimmers. Once a substantial number of blastulae have hatched, pour the swimmers from the upper part of the culture into a clean beaker. Avoid pouring over the unhatched or nonswimming embryos that remain at the bottom of the original culture. These should be discarded so that they do not foul the water in the culture when they die and degenerate. While 100-ml beakers are very useful for some lab manipulations, longer-term cultures should be maintained in 250-ml or larger beakers.

4. Aerate the cultures twice daily by repeatedly and gently pipetting air to the bottom of the cultures. However, you need to be careful to avoid sucking embryos in and out the pipette. This means that once you have expelled air from the pipette into the culture, you should keep the pipette bulb compressed until you have lifted the tip of the pipette above the water's surface. Cultures also may be aerated with a very slow stream of air bubbles from an air line or an air pump, but this needs to be done very cautiously because vigorous bubbling can damage swimming embryos and larvae.

5. If you wish to extend the time that you maintain cultures and feed the developing larvae, you will eventually need to exchange the water in the cultures. A convenient technique for water exchange is described on p. 13 in Laboratory 2. For some hints on feeding larvae, see Appendix A (p. 208).

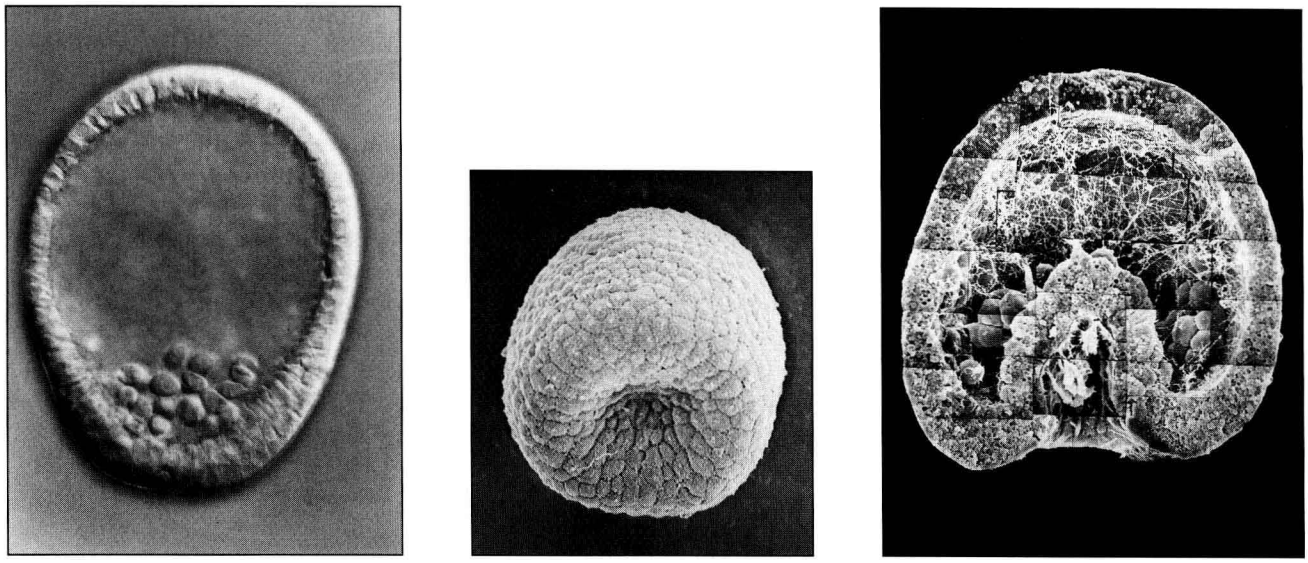
Embryonic Development

1. Cleavage of echinoid echinoderm embryos (fig. 1.1) is holoblastic; that is, the entire cell is divided at cytokinesis during each cleavage division. The first cleavage, which is meridional, produces a two-cell embryo. The second cleavage division is also meridional and yields a four-cell embryo. In the third cleavage, the plane of division is at right angles to the first two cleavages and the product of the division is an eight-cell embryo with upper and lower quartets of cells. During the fourth cleavage, the four blastomeres of the upper (animal) quartet divide equally to form a single tier of eight medium-sized cells called *mesomeres*. However, the divisions of the other four (vegetal) blastomeres are very unequal, producing a middle tier of four larger *macromeres* and a lower tier of four much smaller *micromeres* that lie at the vegetal pole of the embryo. As cleavage proceeds, the embryo becomes organized as a single-layered, hollow ball of cells surrounding a cavity that is known as the *blastocoel*. The embryo at this stage of development is called a *blastula*.

2. An embryo will hatch as a blastula, and just before hatching, the blastula begins to rotate within its fertilization membrane as a result of ciliary activity. Hatching involves enzymatic digestion of the membrane, which becomes fainter in appearance as it thins. Eventually, the membrane opens at one side, allowing the blastula to roll out. The time from fertilization to hatching varies among species and also is strongly influenced by the temperature at which development takes place. Once embryos have hatched, they are harder to observe because they swim more or less continuously. Some individuals eventually become "beached" near the edge of drops on slides, but it is also possible to take active steps to slow or stop them. If Poly-L-Lysine-coated coverslips or slides are available, embryos will settle out of a drop onto the coated surface and be held still while you observe them. Alternatively, embryos and larvae can be anesthetized. To do this, mix about 8 drops from the culture with 1 drop of saturated $MgCl_2$ solution in a small container before transferring the embryos or larvae to a slide for observation.

3. Gastrulation is a set of processes by which embryonic cells are repositioned as the basic body organization of the larva is established. It is somewhat difficult to observe details of gastrulation because embryos swim actively during these stages of development, but patient viewing of several embryos will permit you to see at least some of the interesting cellular activities that are involved. Review a description of gastrulation in your textbook or in references provided in the lab before observing various gastrulation processes for yourself.

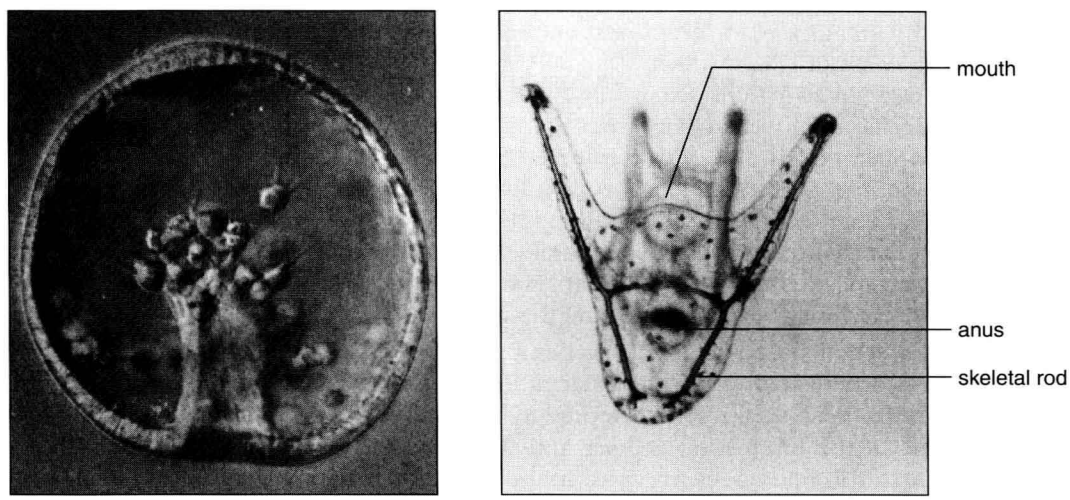
Just before gastrulation begins, one side of the blastula wall flattens and thickens to form a prominent *vegetal plate*. Cells of the vegetal plate play major roles in gastrulation. Gastrulation begins with the separation of the *primary mesenchyme cells* (fig. 1.2*a*) from the vegetal plate portion of the blastula wall and their subsequent inward movement (*ingression*). These cells are among the descendants of the micromeres that originally were established during the fourth cleavage. The primary mesenchyme cells move over the inner surface of the blastula wall to new positions where they form clusters of cells. Cells in these clusters will begin to assemble the crystalline spicules of the larval skeleton.



(a) (b) (c)

FIGURE 1.2 Gastrulation in sea urchin embryos. (*a*) Mesenchyme blastula of a sea urchin embryo. Primary mesenchyme cells have entered the blastocoel and are beginning to migrate over the blastocoel's inner surface. Note the vegetal plate made up of relatively taller cells. (*b*) Scanning electron micrograph (SEM) of an external view of a gastrulating sea urchin embryo showing the invagination of the vegetal plate at the beginning of archenteron formation. Cilia have been removed from the surface of this embryo. (*c*) Composite of SEM photos showing the interior of an embryo during archenteron invagination. Note the primary mesenchyme cells migrating over the surface of the blastocoel wall.

(*a*) is a differential interference contrast photo by R. B. Emlet; (*b* and *c*) are SEM photos by John B. Morrill. (*c*) is from Morrill and Santos, 1985, in R. H. Showman and R. M. Sawyer, eds., *The Cellular and Molecular Biology of Invertebrate Development,* Univ. S.C. Press.



(a) (b)

FIGURE 1.3 (*a*) Midgastrula stage of sea urchin development. As the archenteron lengthens, its wall thins. Some of the secondary mesenchyme cells at the tip of the archenteron have filopodia extending to the blastocoel wall. (This gastrula is approximately 120 μm long.) (*b*) A sea urchin pluteus larva. Propelled by cilia, a pluteus swims with its mouth and arms directed upward. This pluteus measures approximately 200 μm from apex to arm tip.

(*a*) is a differential interference photo by Jeff Hardin, Dept. of Zoology, University of Wisconsin; (*b*) is a light micrograph by John B. Morrill.

Movements of the primary mesenchyme cells involve rather complex individual cell behavior, but the shape changes that initiate development of the *archenteron* (primitive gut) depend upon the collective activity of a number of cells. Archenteron development begins with the invagination (inward sinking or "in-pocketing") of the vegetal plate (fig. 1.2*b* and *c*). You should be able to observe some parts of this invagination process. Once it has been established by invagination, the archenteron lengthens, and its wall thins appreciably. The final phase of extension involves activity of the *secondary mesenchyme cells,* a group of cells that become evident at the archenteron's tip when it has extended about halfway across the blastocoel. Some secondary mesenchyme cells extend long, thin projections known as *filopodia* that reach out to touch various sites on the inside of the blastocoel wall (fig. 1.3*a*). It is often possible to observe extended filopodia. Some of the contacts made by filopodia are temporary, and the cells retract these filopodia, but other filopodia remain attached if they have contacted the region of the *oral ectoderm* where the mouth will form. These attached filopodia contract, pulling the tip of the archenteron over into contact with the oral ectoderm, and the larval mouth develops in the contact area.

The oral surface becomes flattened, giving the embryo an angular appearance that characterizes the *prism* stage of development. The angularity of prism-stage embryos contrasts distinctly with the spherical shape of the blastula and gastrula stages. Skeletal spicules are clearly evident in prism-stage embryos.

Over the next day or two, you will be able to observe the differentiation of the pyramid-shaped, four-armed pluteus larva. First, two arms (the postoral arms), and slightly later, two more (the anterolateral arms) are extended (fig. 1.3*b*). These arms, along with the main portion of the body of the pluteus, are supported by skeletal rods whose development began with formation of the skeletal spicules. If you have a microscope with polarizing optics, it would be interesting for you to examine the developing skeleton using polarized light.

A pluteus larva swims with its arms directed upward and its beating cilia set up currents that sweep small food particles into its mouth. Observe as much detail of gut structure, skeleton organization, and other features of the pluteus larva as time permits. For example, differentiation of esophagus, stomach, and intestine can easily be seen. Watch for muscular contractions in the digestive tract.

Pluteus larvae will swim for some time but usually will not develop beyond the four-armed stage unless they are fed. Unfed larvae eventually starve, fall to the bottom of the culture container, and degenerate. Further development can be observed only if larvae are fed, and feeding usually requires availability of cultures of appropriate marine algae. (See comments on feeding pluteus larvae in Appendix A.)

Techniques

1. The jelly coat surrounding sea urchin eggs will slowly dissolve in seawater, so seawater in which eggs have been stored for a few hours can be used as "egg water" in making these observations. To more quickly prepare an "egg water" solution, vigorously shake a sample of unfertilized eggs in about 30 ml of seawater in a covered test tube. (Shaking helps to dissolve the jelly coats.) Filter the test tube's contents and collect the filtrate ("egg water"). Place a drop of "egg water" on a slide or in a watch glass. Prepare a milky, diluted sperm suspension and add one drop of it to the "egg water." Within 1 minute, the sperm suspension will take on a granular, or flocculent, appearance. Microscopically, it can be seen that the sperm are clustered.

2. You can test the functional capacity of the previously clumped sperm by tipping the suspension off the slide into a sample of fresh eggs on a depression slide. Check for fertilization membrane formation and, if you do observe any, determine the fertilization percentage.

3. If the fertilization percentage is very low, what conclusions might you draw? What are some alternative explanations of the results? What additional experimental step could you take to provide control results that would help to clarify interpretation of the results? Do the additional test. What are the results? What conclusions might you draw?

Materials

Equipment

Basic equipment and supplies for sea urchin and sand dollar experiments as listed in Laboratory 1
Test tube and stopper or cover for the tube during shaking
Filter paper and funnel

Solutions and Chemicals

Seawater or appropriate salt mixture (artificial seawater—see Appendix A)
0.5 M KCl solution

Living Material

Sea urchins or sand dollars

ARTIFICIAL PARTHENOGENESIS

The process of fertilization involves a complex set of cellular responses and interactions, and many aspects of the egg cell's physiology change as a result of the activation occurring during and after sperm contact and entry. Some of these changes can be initiated artificially by various treatments. Egg activation without sperm contact is called *parthenogenesis*, and artificial parthenogenesis in sea urchin eggs has been investigated by many biologists since it was first studied by Oscar and Richard Hertwig during the 1880s. Many experimental treatments have been found to cause some, or even many, of the activation responses to occur in sea urchin eggs.

In this experiment, you will have opportunity to investigate the effects of one of these treatments—immersion in seawater made hypertonic to egg cells by addition of 30 grams of sodium chloride per liter. This procedure is one recommended by the great early twentieth-century American developmental biologist Ethel Browne Harvey, who, near the end of her career, summarized and reinvestigated many of the experimental procedures used to investigate sea urchin development up to the 1950s.

Techniques

1. Transfer a sample of freshly shed eggs to hypertonic seawater (HSW) by pipette and allow them to settle to the bottom of the beaker or dish.

2. After 20 minutes, wash the eggs by pouring off as much of the HSW as you can and resuspending them in normal strength seawater. (Since eggs of different species, and even different batches of eggs of a single species, respond differently, you might consider trying several additional treatment times.)

此为试读,需要完整PDF请访问: www.ertongbook.com