THE AMERICAN ARBACIA

AND

OTHER SEA URCHINS

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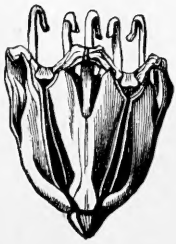
BY

ETHEL BROWNE HARVEY



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To the Memory of

EDMUND B. WILSON

PREFACE

The sea urchin, *Arbacia punctulata*, which occurs along the Eastern coast of North America, has for many years provided material for experimental work on cells, done mostly at the Marine Biological Laboratory at Woods Hole, Mass. The animals are readily obtained and the eggs are produced in large quantities throughout the summer. This species is the American *Arbacia* and is not found in Europe, where another species, *Arbacia lixula* occurs. The latter species and many other genera of sea urchins have been used for experimental work at Naples, on the Swedish coast, in the British Isles, France, Japan, and elsewhere. The eggs are fundamentally similar but differ in details.

The *Arbacia* egg is an ideal cell. It is spherical, thus rendering changes in size easy to determine. It is fairly simple in comparison with most cells. It is quite hardy and can be subjected, without damage, to moderate changes in the sea water, produced by the addition of water, or salts, or anaesthetics, or other chemicals, and to changes in temperature, pressure, light, and other physical factors. Harmful effects and recovery can be readily detected by fertilizing the egg and watching its development. The granules in the egg can be moved by centrifugal force, and the egg can be broken into halves and quarters containing different kinds of materials in definite amounts. The experimental work on sea urchin eggs has included every line of approach, cytology, embryology, physiology, and biochemistry, and has been concerned with the solution of many fundamental problems.

The earliest experiments on *Arbacia punctulata* eggs were those of Jacques Loeb at Woods Hole in 1892, who wrote a paper entitled "Investigations in Physiological Morphology. III. Experiments on Cleavage", published in the *Journal of Morphology*. It dealt with division of the nucleus without cleavage of the cell, caused by hypertonic sea water. The next paper was that of T.H. Morgan in 1893, published in the *Anatomische Anzeiger*, part of which was on the same subject, taking exception to some of Loeb's results.

Loeb and Morgan were succeeded by many well-known biologists. We have a fine heritage of experimental research on the *Arbacia* egg, and it is partly in an endeavor to gather together this work and make it more readily available to later investigators that this book has been written.

The book is divided into four parts. The first part deals with sea urchins in general—their history, which begins before Aristotle and

Pliny, their use as food, interesting facts concerning their mode of life and their habits, and other matters of general interest, including a classification of the *Echinoidea* mentioned in this monograph. The second part is devoted to the normal development of the *Arbacia punctulata* egg from fertilization through metamorphosis, with original photographs of the different stages. The third part is concerned with centrifuging, a subject which has particularly interested the author. The fourth part is a compilation of all other experimental work in which *Arbacia* eggs and sperm have been used since the pioneer work of Jacques Loeb in 1892 until 1954. This is arranged alphabetically according to subjects. The results of all investigators are given without any attempt to evaluate them. Together with the references to *Arbacia* there are given with each topic a few important references to other species. There is an extensive bibliography with complete titles. The illustrations are mostly original photographs by the author.

Many subjects have been treated in the compilation, and many of my co-workers at Woods Hole and Princeton and others elsewhere have been consulted on subjects most familiar to them. I take this opportunity to thank most heartily all who have helped me by supplying information and by reading parts of the manuscript. Among these are: E. G. Ball, E. S. G. Barron, H. F. Blum, E. G. Butler, A. D. Chiquoine, the late H. L. Clark, G. H. A. Clowes, K. S. Cole, K. Dan, L. V. Heilbrunn, B. F. Howell, the late A. C. Johnson, L. H. Kleinholz, M. E. Krahl, G. G. Lower, the late B. Lucké, D. A. Marsland, D. Mazia, A. Monroy, A. R. Moore, I. Motomura, A. K. Parpart, J. S. Rankin Jr., S. Ranzi, J. Runnström, the late D'Arcy Thompson, A. Tyler, and C. A. Villee. My sister, Dr. Mary N. Browne, a classical scholar, has been most helpful with the section on history.

I wish especially to thank my husband, E. Newton Harvey, who has been frequently consulted and has rendered invaluable assistance concerning topics of which he has expert knowledge.

May 1955

Ethel Browne Harvey
Princeton, N. J.

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PLATE II.

Maturation of the egg; polar body formation. Living eggs except 17, 18 which are stained sections. 1 to 6. Growth of the oocyte. 7, 8. Breaking of the germinal vesicle. 9, 10. First polar body. 11, 12. Second polar body; note flattening of the cell in 12. Photographs 13, 14, 15. Centrifuged oocytes. 16. Formation of blebs where spermatozoa hit the surface. 17. Stained section, showing a bleb and spermatozoa on the surface and inside the egg. 18. Section showing mitochondria (dark material) around the germinal vesicle. Magnified about 270 × except 17, 18, about 600 ×.

PLATE III.

Normal development of living eggs, from one cell to the gastrula. Times after fertilization at 23° C are given under each photograph. Magnification about 270 ×, the same throughout as nearly as possible. Photograph 1. Unfertilized egg. 2. Spermatozoa around the egg. 3. Fertilization cone above, center. 4. Fertilized egg. 5. Monaster. 6. "Streak" stage, narrow streak. 7. Broad streak fading out and enlarged nucleus. 8. Amphiaster. 9 to 11. First cleavage; note cells are separate in 10, close together in 11. Photograph 12. Four cells. 13. Eight cells. 14. Twelve cell stage, four colorless micromeres below. 15. Sixteen cells. 16. About thirty-two cells. 17 to 20. Blastulae. 21. Hatching from the fertilization membrane. 22. Late blastula. 23. Early gastrula. 24. Late gastrula, spicules forming.

PLATE IV.

Normal development, continued; gastrula to pluteus. Times after fertilization at 23° C are given under each photograph. Magnification (except 9) the same as Plate III, about 270 ×, as nearly as possible the same throughout; Photograph 9 is approximately 180 ×. Photograph 1. Late gastrula. 2. Same stage in dark field. 3. "Prism" stage. 4 to 6. Early

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Stained sections of developing eggs, fixed in Bouin, stained with Heidenhain's haematoxylin. Photograph 1. Unfertilized egg. 2. Sperm and sperm aster. 3. Sperm nucleus fused with female nucleus. 4. "Streak" stage, with enlarged nucleus. 5. Prophase. 6. Metaphase. 7. Anaphase. 8. Telophase. 9, 10. Second cleavage. 11. Four cells. 12. Early blastula; note that the spindles have no asters. Magnified about 450 ×.

PLATE VI.

Metamorphosis. All photographs (except 12) are to the same scale magnified about 24 ×. Photograph 1 is of eggs at this magnification. 2. Three day plutei. 3. A new pair of arms with red tips forms after 11 days if the plutei are fed. 4. Another pair of arms, median, forms when about 3 weeks old. 5. Arms longer; four weeks old. 6. Arms begin to degenerate; four tubular processes, the auricles, are present; 5 weeks old; the body of the adult grows inside the pluteus. 7. Five tube feet are formed in a sort of pocket; 2 months old. 8 to 10. Fifteen primitive spines, three adjacent to each tube foot; 2½ to 3 months. 11. More tube feet have formed; 3½ months, the oldest raised in the laboratory; diameter 1 mm. including spines. 12. Smallest young *Arbacia* brought from the sea, about 6 mm. including spines. (Biol. Bull. 97:292, 1949, modified.)

PLATE VII.

Breaking of the unfertilized egg by centrifugal force, 10,000 × g. Photograph 1. Stratified egg showing oil, clear layer, mitochondria, yolk and pigment. The nucleus is always in the clear layer under the oil cap. 2. White half, containing oil, clear layer, mitochondria, a little yolk and the nucleus. 3. White half, with further centrifuging. 4. Clear quarter, containing oil, clear layer and nucleus. 5. Mitochondrial or granular quarter, containing all the mitochondria and a little yolk. 6. Red half, containing yolk and pigment. 7. Red half with further centrifuging. 8. Yolk quarter, containing yolk. 9. Pigment quarter, containing a little yolk and all the pigment. 10. Eggs in three layers in sugar solution after centrifuging; white halves (top), unbroken whole eggs, and red halves (bottom). 11. Unfertilized eggs stratifying and breaking apart in the centrifuge microscope; photographed while rotating about 6,000 × g. 12. White halves, taken from the centrifuge

tubes. 13. Red halves. Magnification of Photographs 1-9, 270 ×. (Photographs 1-9 Biol. Bull. 79:167, 1940c. Photographs 10-13 Biol. Bull. 71:105, 1936, modified.)

PLATE VIII.

Development of centrifuged eggs (at 23°). Photographs 1 to 8. Elongate eggs. 1. Unfertilized elongate egg stratified as in Plate VII; note cortical granules at periphery of the clear layer. 2. Usual two cell stage, 1¼ hours after fertilization at 23°. 3. Usual four cell stage, 1½ hours. 4. Occasional four cell stage, 1½ hours. 5. About 16 cells, 2½ hours; note red cells are larger than the white ones. 6. About 32 cells, micromeres at left, 3 hours. 7. Slipper blastula, 5½ hours. 8. Hatching from the fertilization membrane, 7 hours. Photographs 9 to 20. Spherical eggs. 9. Unfertilized egg; same layers as in Photograph 1. Photograph 10. Monaster. Note pigment being redistributed as the aster forms. 11. First cleavage, usually at right angles to the stratification. 12. First cleavage parallel with the stratification. 13. Four cell stage, 1½ hours. 14. Eight cells, 2 hours. 15. Micromeres (without pigment) at the oil cap. 16. Micromeres (without pigment) at the centrifugal pole. 17. Blastula. 18. Late blastula, pigment still concentrated. 19, 20. Plutei with pigment still concentrated; in 19, near the mouth, in 20 in one arm, 24 hours. Magnification about 270 ×.

PLATE IX.

Development of white half-egg. Times (at 23°) after fertilization given under each photograph. Photographs 1 to 4. Elongate egg. 5 to 21. Spherical egg. 1 and 5. Immediately after removal from the centrifuge. 6. Monaster (not well marked). 7. "Streak" stage, enlarged nucleus. 8. Breaking of nuclear membrane. 9. Amphiaster. 10. Just before cleavage. 11, 12. First cleavage in any plane. 13 to 17. Cleavages to form blastula; blurring in 17 caused by blastula swimming inside the fertilization membrane. 18. Hatching from the fertilization membrane. 19. Free-swimming blastula. 20. Gastrula. 21. Pluteus; note lattice-like skeleton in the arms. Magnification about 270 ×. (Biol. Bull. 79:171 and 173, 1940c, modified.)

PLATE X.

Development of red half-egg, fertilized; fertilized merogone. Times after fertilization at 23° are given under each photograph; 1 to 4, elongate eggs; 5 to 16, spherical eggs. Photographs 1 and 5. Unfertilized eggs immediately after removal from the centrifuge. 2 to 4. Early

cleavages. 6. Soon after fertilization, showing fertilization membrane and hyaline layer. 7. Male nucleus. 8. Monaster. 9. Amphiaster and cleavage furrow. 10 to 13. Cleavages. 14. Hatching from the fertilization membrane. 15. Free-swimming blastula. 16. Pluteus, well formed. 17 to 20. Less regular development with fertilization membranes ruptured, so that cells are more scattered. 21 to 24. Division planes have failed to come in; nuclear division without cell division. 24. Egg peppered with small nuclei without cell division. Magnification about 270 ×. (Biol. Bull. 79:177, 1940c. Photograph 16 has been replaced by a later photograph.)

PLATE XI.

Development of red half-egg, parthenogenetic; parthenogenetic merogone. Times (at 23°) after activation are given under each photograph. Note the similarity of these photographs and those of the fertilized merogones on Plate X, facing this. Compare picture for picture. Photographs 1 to 4. Elongate eggs. 5 to 16. Spherical eggs. 1 and 5. Unactivated eggs immediately after removal from the centrifuge. 2 to 4. Early cleavages. 6. Soon after activation, showing fertilization membrane and hyaline layer. 7. Clear sphere, simulating a nucleus. 8. Monaster. 9. Amphiaster and cleavage furrow. 10 to 13. Cleavages; note especially 13, quite regular many celled organism, similar to the same stage of a fertilized merogone on preceding plate. 14. Hatching from the fertilization membrane. 15. Blastula. 16. Noncellular but healthy-looking parthenogenetic merogone of four weeks; there has been no differentiation. 17 to 20. Cleavages without fertilization membranes; cells are scattered. In Photograph 20, right, cell divisions have taken place in the light but not in the pigmented portion. 21 to 23. Multi-astral eggs with small spheres associated with the asters. 24. Many small spheres resembling nuclei. Magnification about 270 × (Biol. Bull. 79:179, 1940c.)

PLATE XII.

Development (at 23°) of clear quarter-eggs and of eggs centrifuged after fertilization. Magnification about 270 ×.

Photographs 1 to 13. Development of clear quarter.

Photograph 1. Fertilized clear quarter, showing fertilization membrane. 2. Monaster stage, 20 minutes after fertilization. 3. Two cells, two hours after fertilization. 4. Two cell stage of the clear quarter and about a 32-cell stage of the white half, two hours after fertilization. 5 and 6. Four cells, three hours after fertilization. 7. Eight cells, four hours after fertilization. 8 and 9. Sixteen cells, 5 hours after fertiliza-

tion. 10. Clear quarter, about 32 cells, and white half photographed at the same time, further advanced, 5 hours after fertilization. 11. Early blastulae of clear quarter and white half from the same culture, about 6½ hours after fertilization. 12. Free-swimming blastula of clear quarter, 20 hours after fertilization. 13. Pluteus of clear quarter, 10 days old.

Photographs 14 to 17. Eggs centrifuged *after* fertilization.

Photograph 14. Eggs centrifuged one minute after fertilization for six minutes at 10,000 × g; they break up into small pieces. 15. Egg centrifuged four minutes after fertilization for six minutes at 10,000 × g; it stratifies like the unfertilized eggs, but not nearly so well (Cf. with Plate VII, Photograph 1). 16. Egg broken apart by centrifuging for six minutes, thirty minutes after fertilization, the fertilization membrane having been removed previously; picture was taken 2½ hours after fertilization. Note that the white half has cleaved, and red half has not. 17. Eggs form long streamers when centrifuged 6 to 20 minutes after fertilization, the membranes having been removed just after fertilization. This photograph was taken while the eggs were rotating (about 6,000 × g). (Photographs 1-13 J. Exp. Zool. 102:269, 1946a.)

PLATE XIII.

Stratification of unfertilized eggs and size of half-eggs with varying centrifugal forces. The first vertical column shows stratification. The second vertical column shows size of half-eggs. With low forces, the eggs are well stratified and the white half is much larger than the red half. With high forces, the eggs are not well stratified and the white half is small while the red half is very large. Photograph 5. Red half obtained with 10,000 × g and recentrifuged at 10,000 × g. Control to Photograph 12; note clear layer at top, due to further settling of granules. Photograph 12. Red half obtained with 100,000 × g and recentrifuged at 10,000 × g. The granules had not been segregated in the first centrifuging with very high forces, and when recentrifuged, they stratify as in the original egg. The mitochondrial layer has been stained with methyl green. (Biol. Bull. 80:357, 1941a.)

PLATE XIV.

Stratification of unfertilized eggs and size of half-eggs when centrifuged in hypo- and hypertonic sea water (10,000 × g). Magnification about 270 ×. In hypotonic sea water (Photographs 1 to 3 in 60% and 4 to 6 in 80%), the clear layer is large, with few granules; the heavy granules are well packed as compared with control eggs in sea water (Photographs 7 to 9). The white half-eggs (Photographs 2 and 5) are

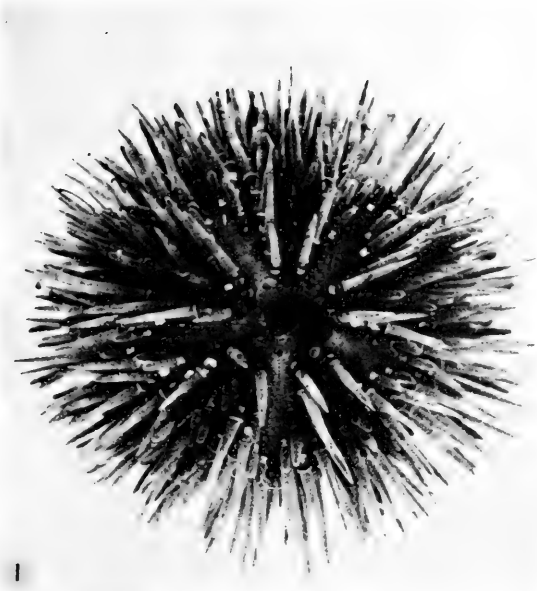
large, having the excess water. When centrifuged in hypertonic sea water (Photographs 10 to 12) the clear layer is small, the mitochondrial layer very thick and the heavy granules, yolk and pigment not well separated. The white half is small, containing less water as compared with the red half (Photograph 12). The red half remains about the same size in hypo- and hypertonic sea water, the change in water content taking place in the white halves. The change in size of the nucleus is easily observed, since it lies in the clear layer in these centrifuged eggs. Compare Photographs 1, 2, with 10, 12. (Biol. Bull. 85:143, 1943.)

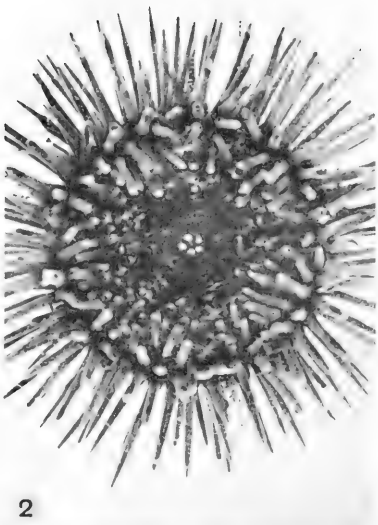
PLATE XV.

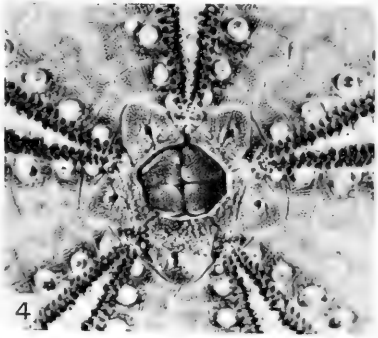
Stratification and breaking of unfertilized eggs when centrifuged in single salt solutions. The left column shows stratification of eggs centrifuged at 3,000 × g for two minutes in (1) NaCl or KCl; (3) sea water; (5) $MgCl_2$ or $CaCl_2$. The right column shows amount of breaking apart at 10,000 × g for four minutes in (2) NaCl or KCl; (4) sea water; (6) $MgCl_2$ or $CaCl_2$. The eggs stratify best in calcium, and least in potassium and sodium salts (Photographs 5 and 1). The order of decreasing stratification (increasing viscosity) is $CaCl_2 > MgCl_2 >$ S. W. $>$ NaCl $>$ KCl. The ease with which they break into halves is in the reverse order, those in NaCl break most readily, those in $CaCl_2$ least readily (Photographs 2, 4, 6). The ease of breaking must be determined by the effect of the salts on the surface layers and not on the interior viscosity of the protoplasm. (Biol. Bull. 89:73, 1945.)

PLATE XVI.

Miscellaneous. Photographs 1, 2. Electrical method of determining sex, showing sperm and eggs shed from genital pores. 1. Male. 2. Female. 3. Egg showing jelly layer. 4. Jelly layer centrifuged off. 5. Amoebocytes, red and white. 6. Hyaline layer centrifuged off as a crescent near lower surface of egg. 7. Development without fertilization membrane. 8. Twins. 9. Cleavage in sea water lacking calcium, showing clear micromeres. 10. Bloated pluteus caused by not being fed. Photographs 11 to 13 of stained sections of developing eggs. 11. Mitotic figure of first cleavage of whole cell, metaphase. 14. Anaphase. 12. Mitotic figure of fertilized red half, metaphase. 15. Anaphase. 13. Mitotic figures of parthenogenetic merogone; note absence of spindle but well formed asters. In 11 and 14 there are two nuclei; in 12 and 15 one nucleus and in 13 no nucleus. (Photograph 6 Biol. Bull. 66:233, 1934 no. 2; Photograph 8 Biol. Bull. 78:205, 1940a no. 9; Photograph 10 Biol. Bull. 97:290, 1949 no. 14; Photographs 11-15 Biol. Bull. 79:185, 1940c nos. 136-140.)







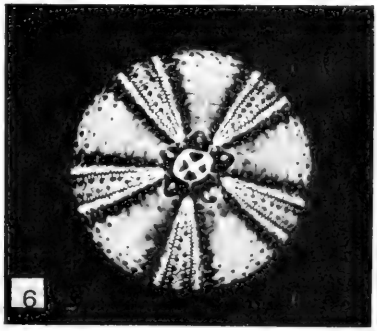


Plate 1. Tests

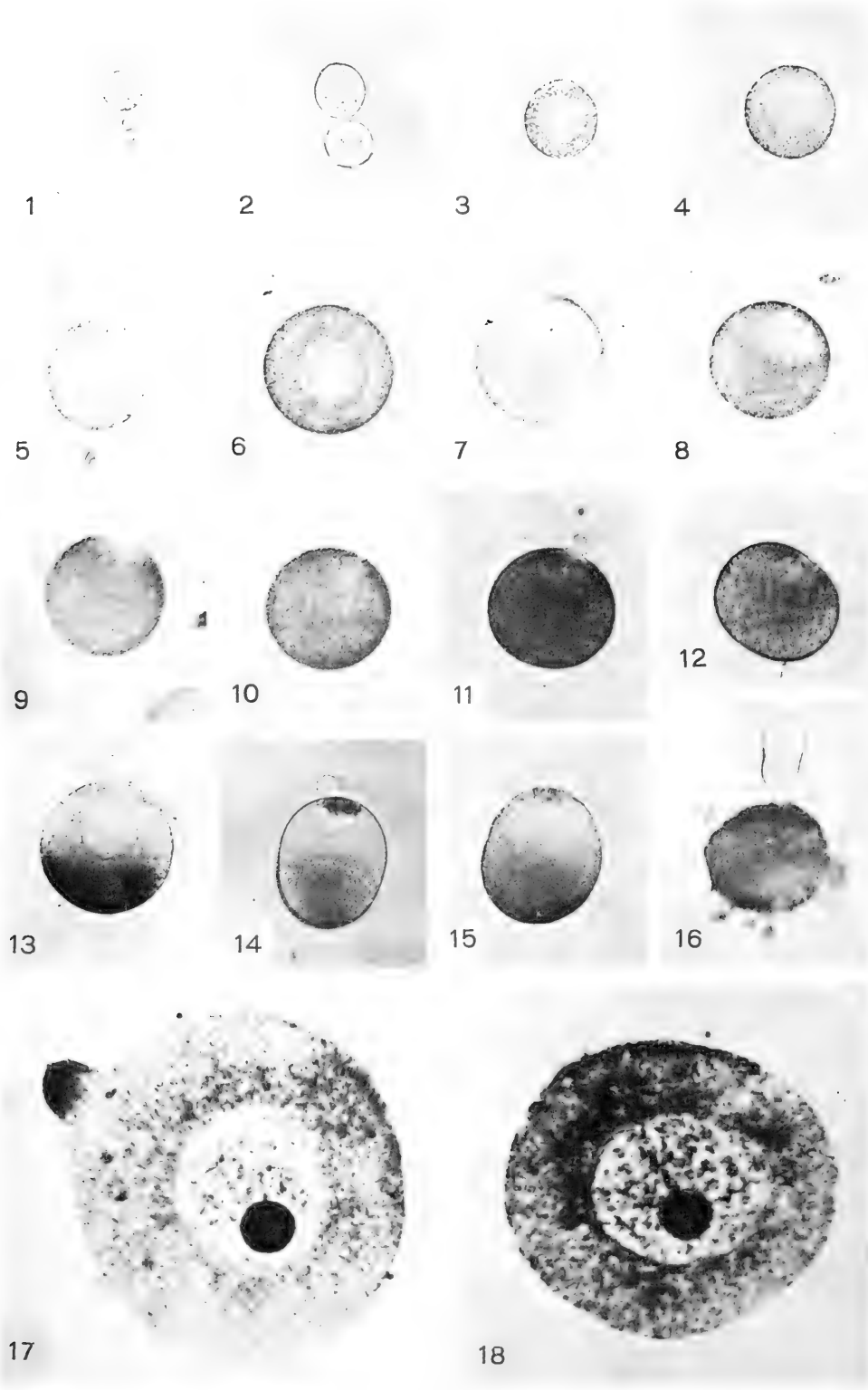
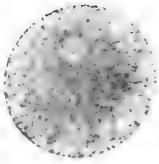
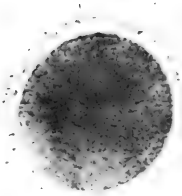


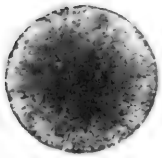
Plate II. Maturation of egg



1. Unfertilized

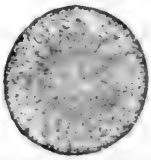


2. Fertilization

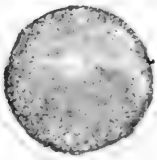


3. 3 min

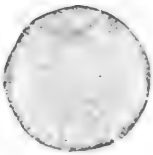
4. 3½ min



5. 10 min

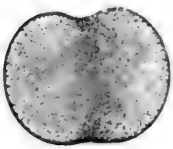


6. 25 min



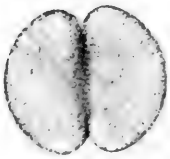
7. 35 min

8. 40 min



9. 45 min

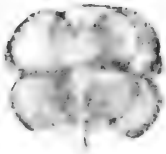
10. 50 min



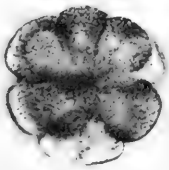
11. 70 min

12. 80 min

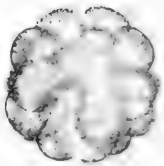
13. 1¾ hrs



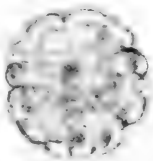
14. 2 hrs, 10 min



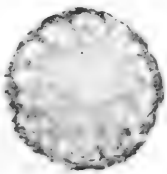
15. 2¼ hrs



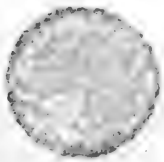
16. 2 hrs, 50 min



17. 4 hrs

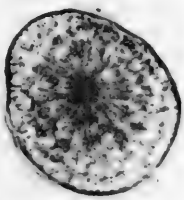


18. 4½ hrs

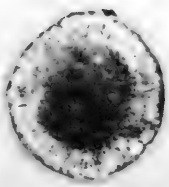


19. 6 hrs

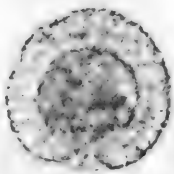
20. 7 hrs



21. 8 hrs



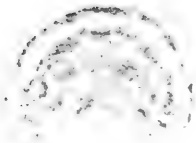
22. 12 hrs



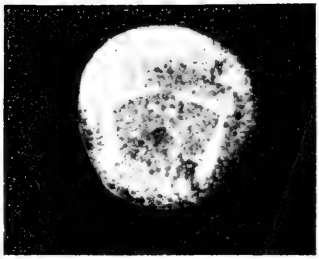
23. 15 hrs

24. 19 hrs

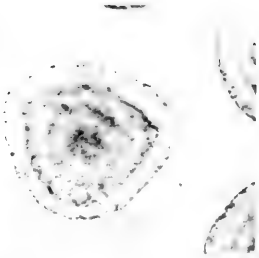
Plate III. Normal development (living)



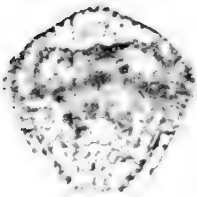
1. 17 hrs



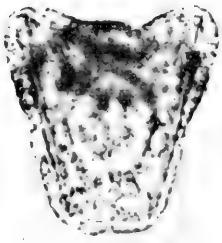
2. 17 hrs



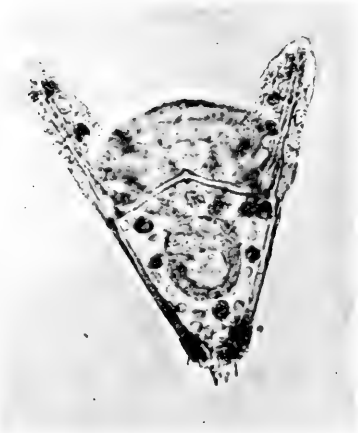
3. 18 hrs



4. 20 hrs



5. 22 hrs



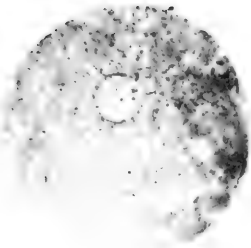
6. 1 day

7. 2 days

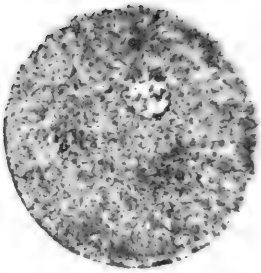
8. 2 days

9. 2 days

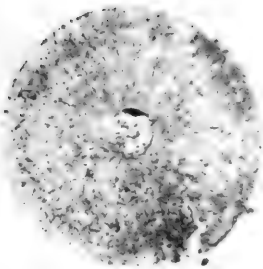
Plate IV. Normal development (living), continued



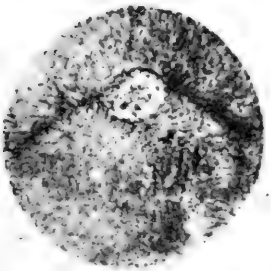
1. Unfertilized



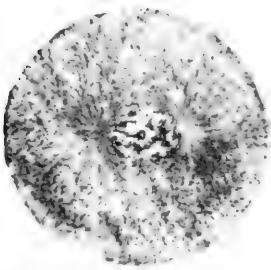
2. 8 min



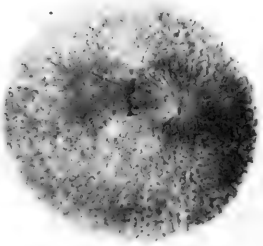
3. 10 min



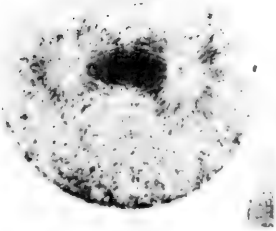
4. 25 min



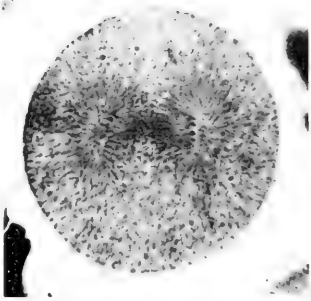
5. 35 min



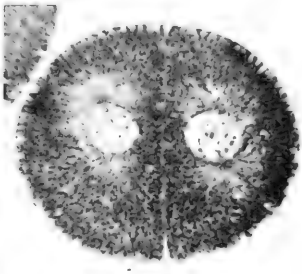
6. 40 min



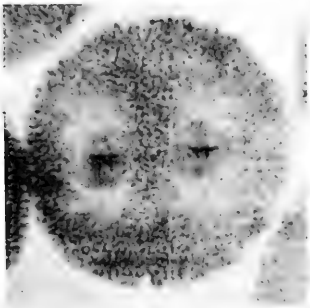
7. 42 min



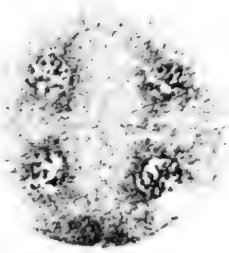
8. 45 min



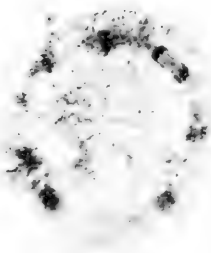
9. 70 min



10. 1¼ hrs



11. 1½ hrs



12. 4 hrs

Plate V. Normal development (sections)

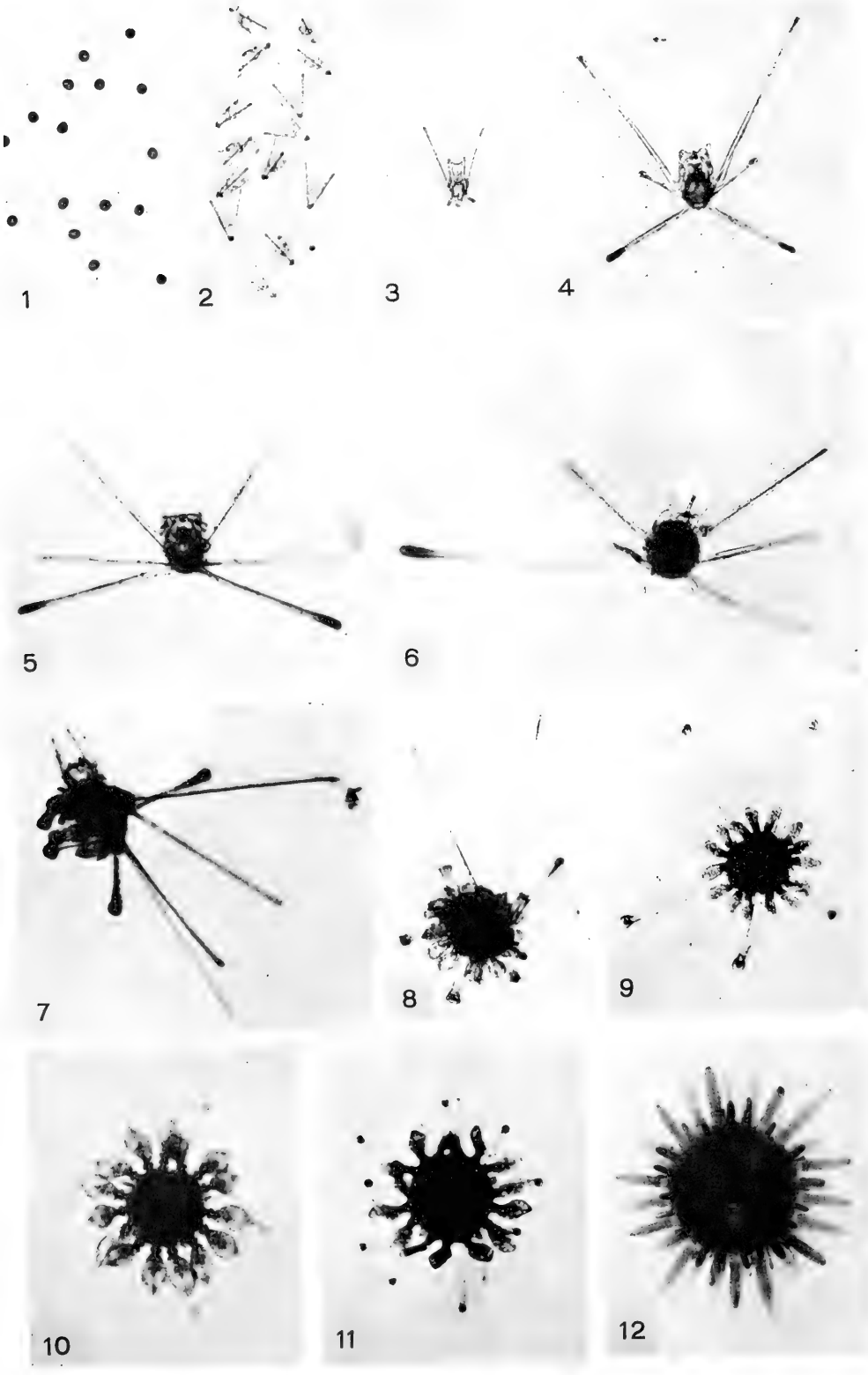
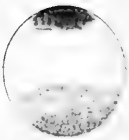


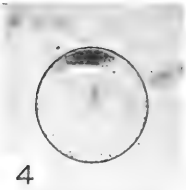
Plate VI. Metamorphosis

1



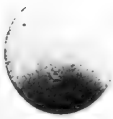
2

3



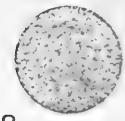
4

5



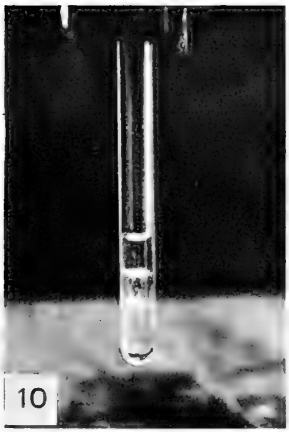
6

7



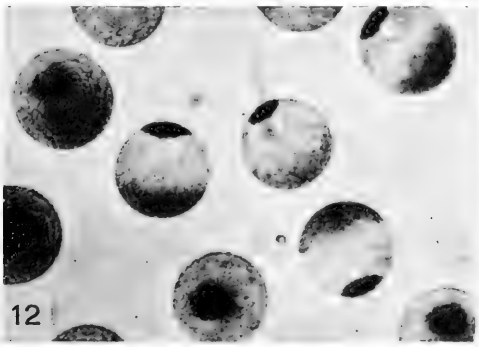
8

9

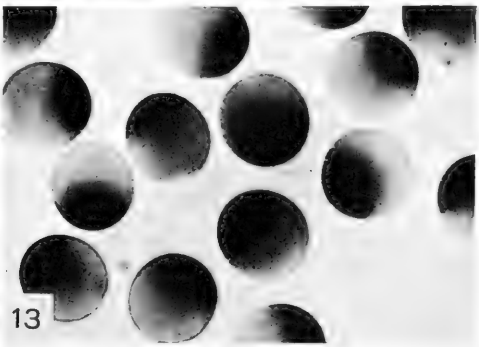


10

11



12



13

Plate VII. Centrifuged eggs

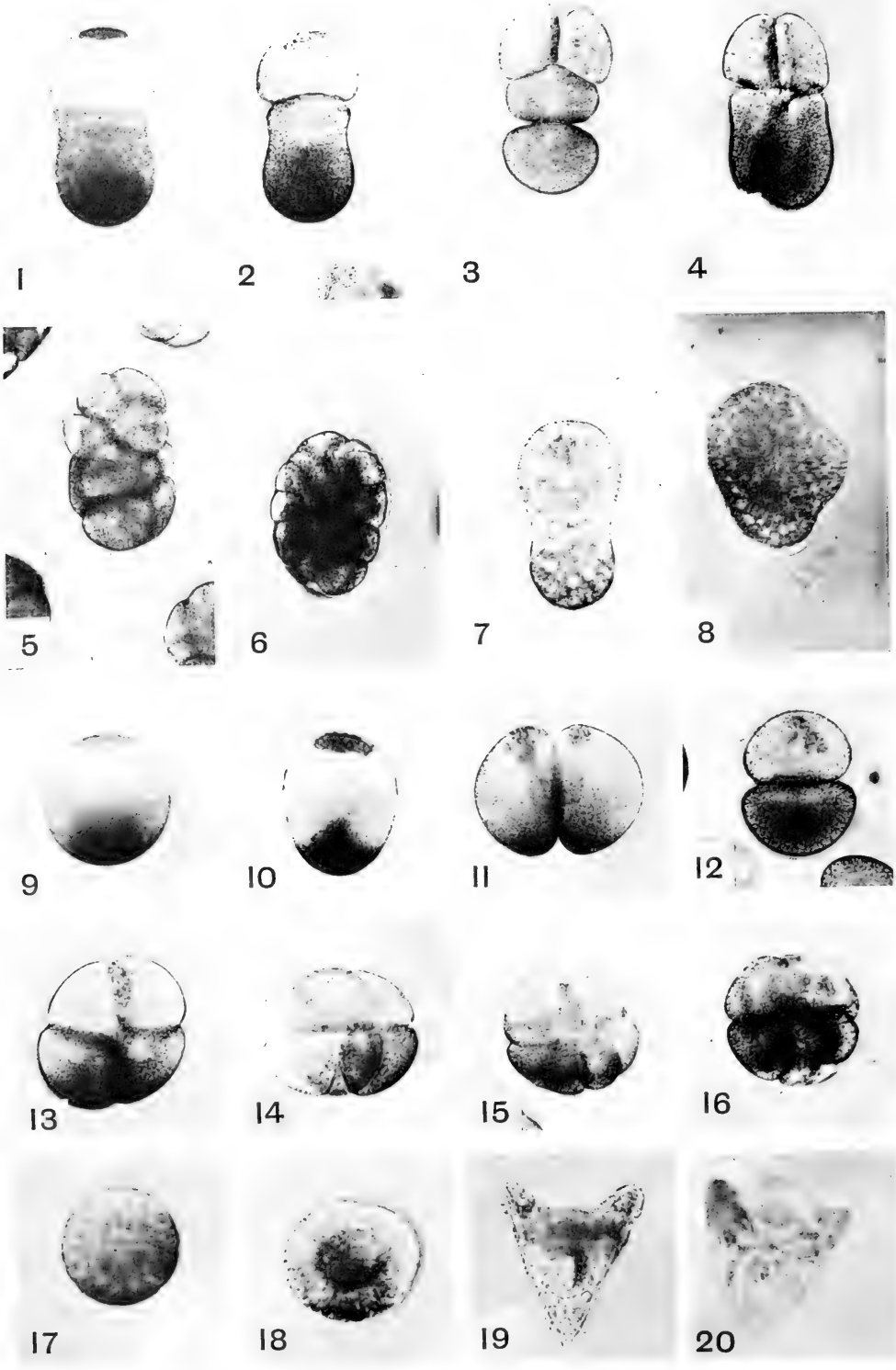
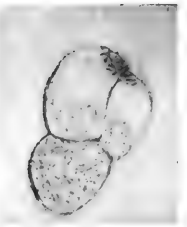


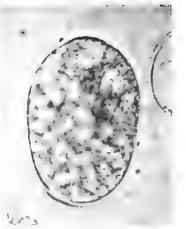
Plate VIII. Centrifuged eggs, development

1. Unfertilized

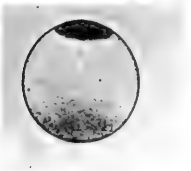
2. 1¼ hrs



3. 1½ hrs

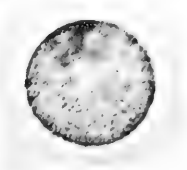


4. 4 hrs

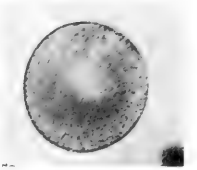


5. Unfertilized

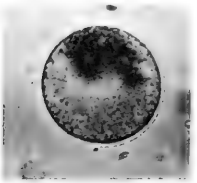
6. 10 min



7. 30 min

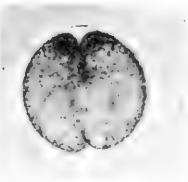


8. 35 min

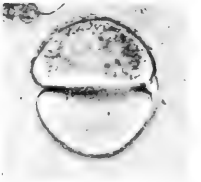


9. 40 min

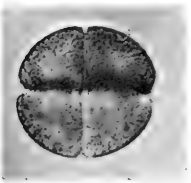
10. 50 min



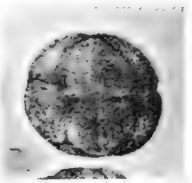
11. 56 min



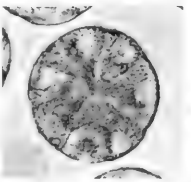
12. 56 min



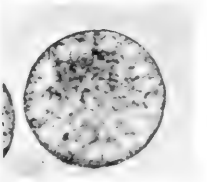
13. 1½ hrs



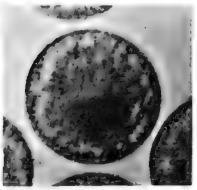
14. 2 hrs



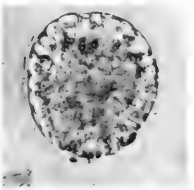
15. 2½ hrs



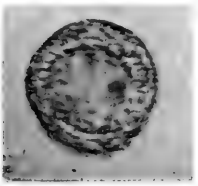
16. 5 hrs



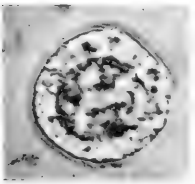
17. 9 hrs



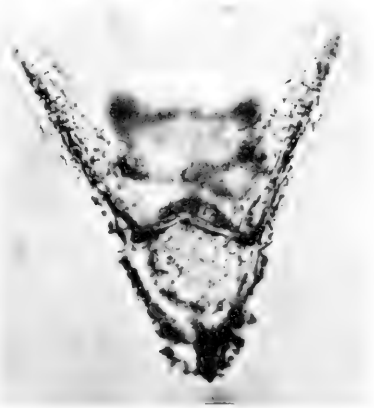
18. 9½ hrs



19. 16 hrs



20. 1 day



21. 4 days

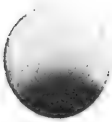
Plate IX. White half, development

1. Unfertilized

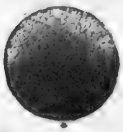
2. 2 hrs

3. 3 hrs

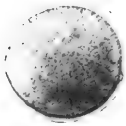
4. 4 hrs



5. Unfertilized



6. 10 min

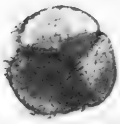


7. 20 min

8. 30 min

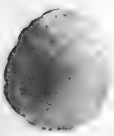
9. 1½ hrs

10. 2 hrs

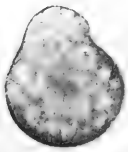


11. 3 hrs

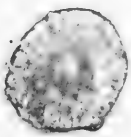
12. 4 hrs



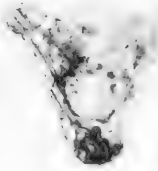
13. 8 hrs.



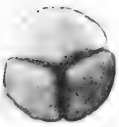
14. 11 hrs



15. 20 hrs



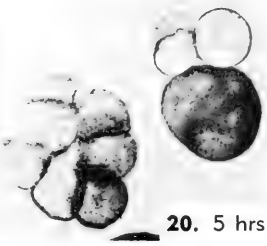
16. 3 days



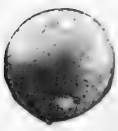
17. 3 hrs

18. 3 hrs

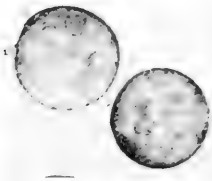
19. 4 hrs



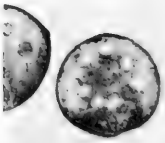
20. 5 hrs



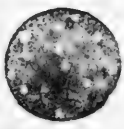
21. 4 hrs



22. 6 hrs



23. 7 hrs



24. 9 hrs

Plate X. Red half, fertilized, development (Fertilized merogone)

1. Unfertilized

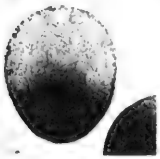
2. 3 hrs

3. 4 hrs

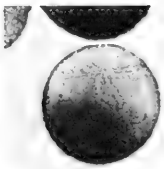
4. 18 hrs

5. Unfertilized

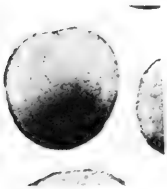
6. 10 min



7. 1 hr

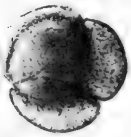


8. 1¼ hrs



9. 2½ hrs

10. 3 hrs

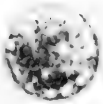


11. 4½ hrs

12. 5 hrs

13. 27 hrs

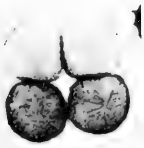
14. 28 hrs



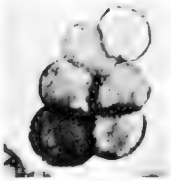
15. 30 hrs

16. 4 wks

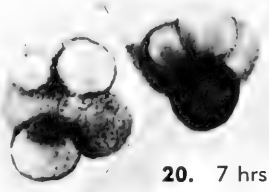
17. 4 hrs



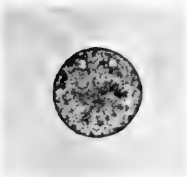
18. 4½ hrs



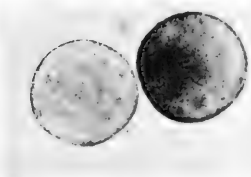
19. 6 hrs



20. 7 hrs

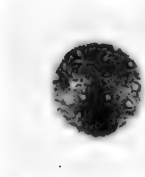


21. 3 hrs



22. 6 hrs

23. 10 hrs



24. 10 hrs

Plate XI. Red half, parthenogenetic, development (Parthenogenetic merogone)

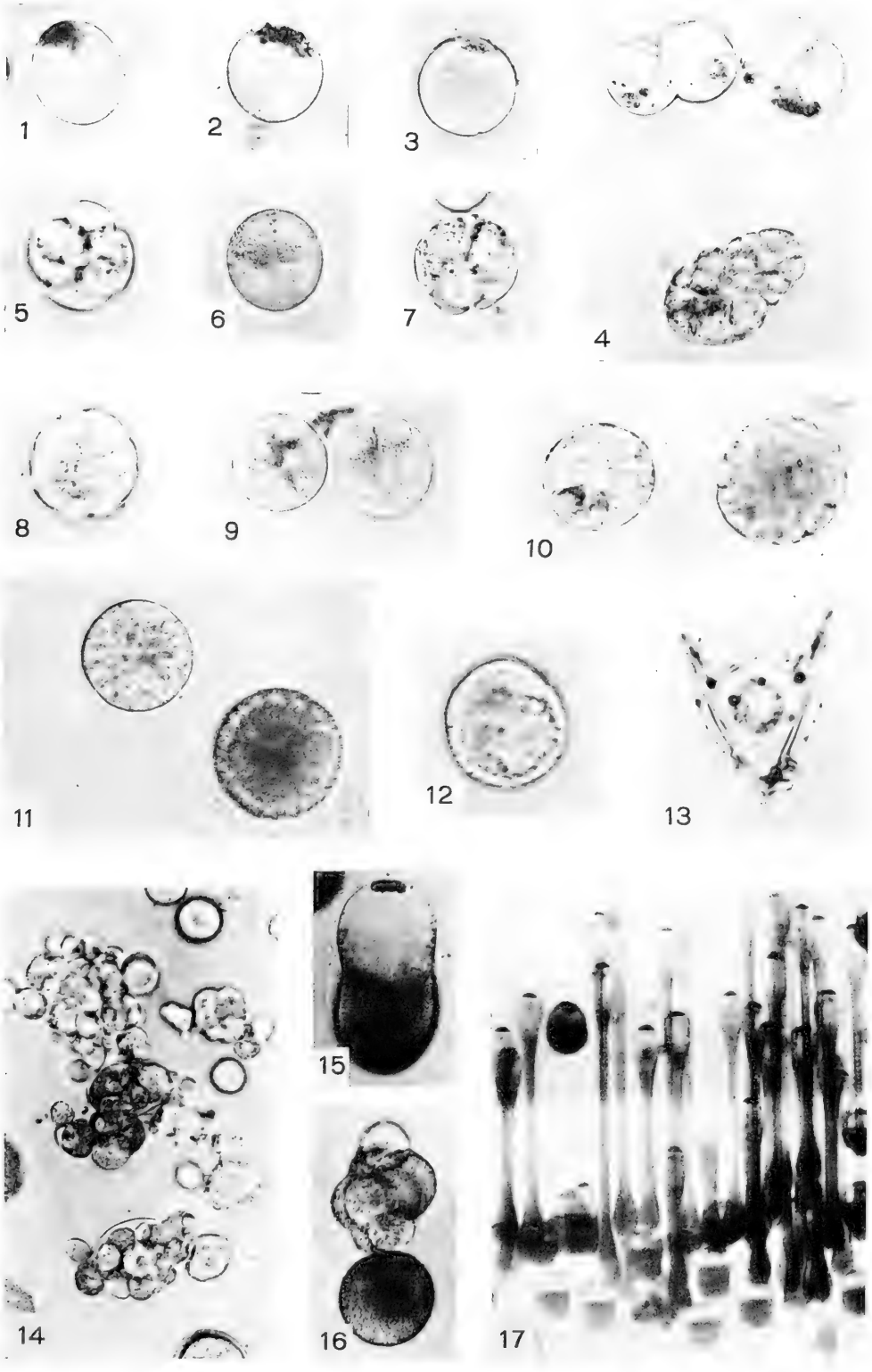


Plate XII. Clear quarter, and eggs centrifuged after fertilization

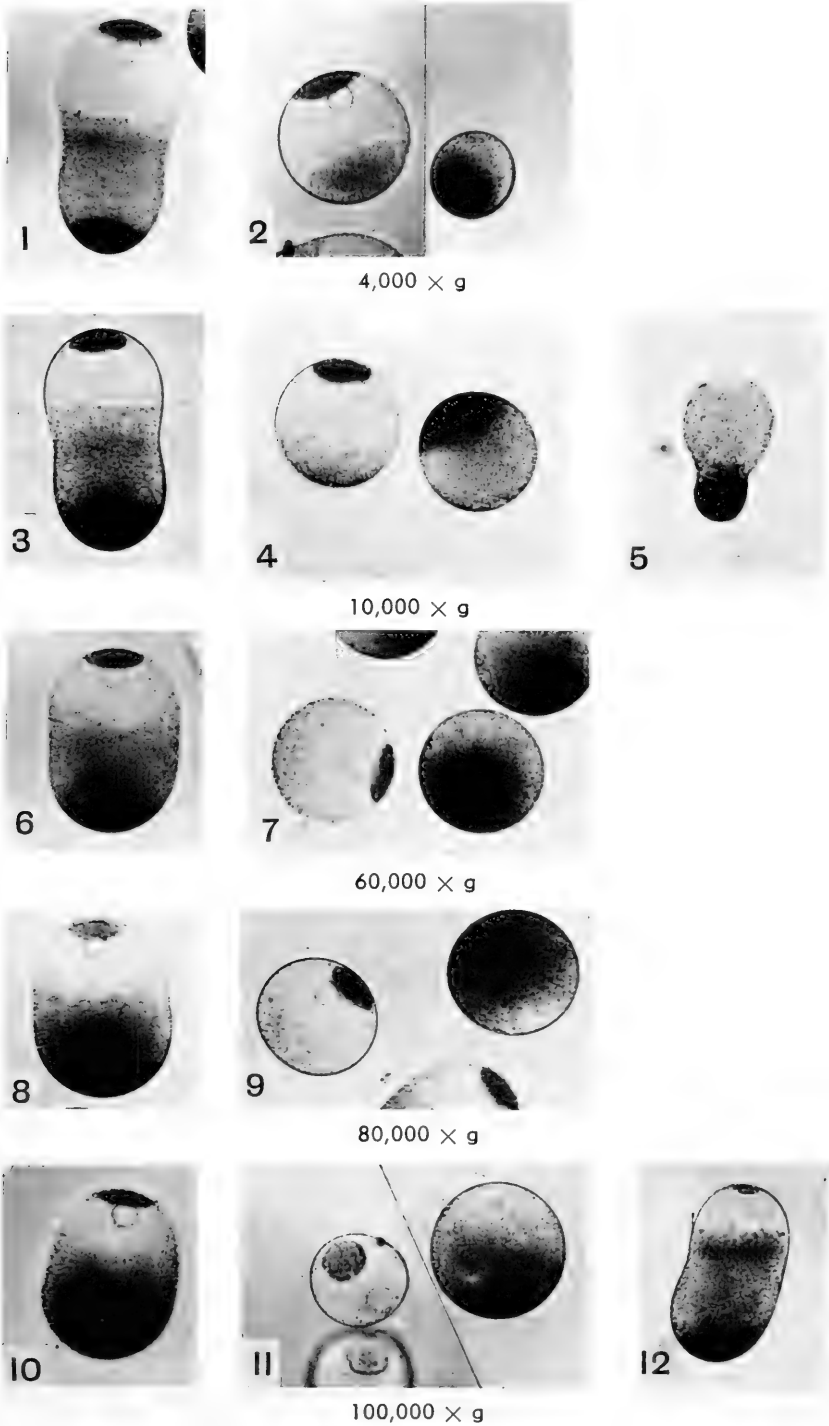


Plate XIII. Varying centrifugal force

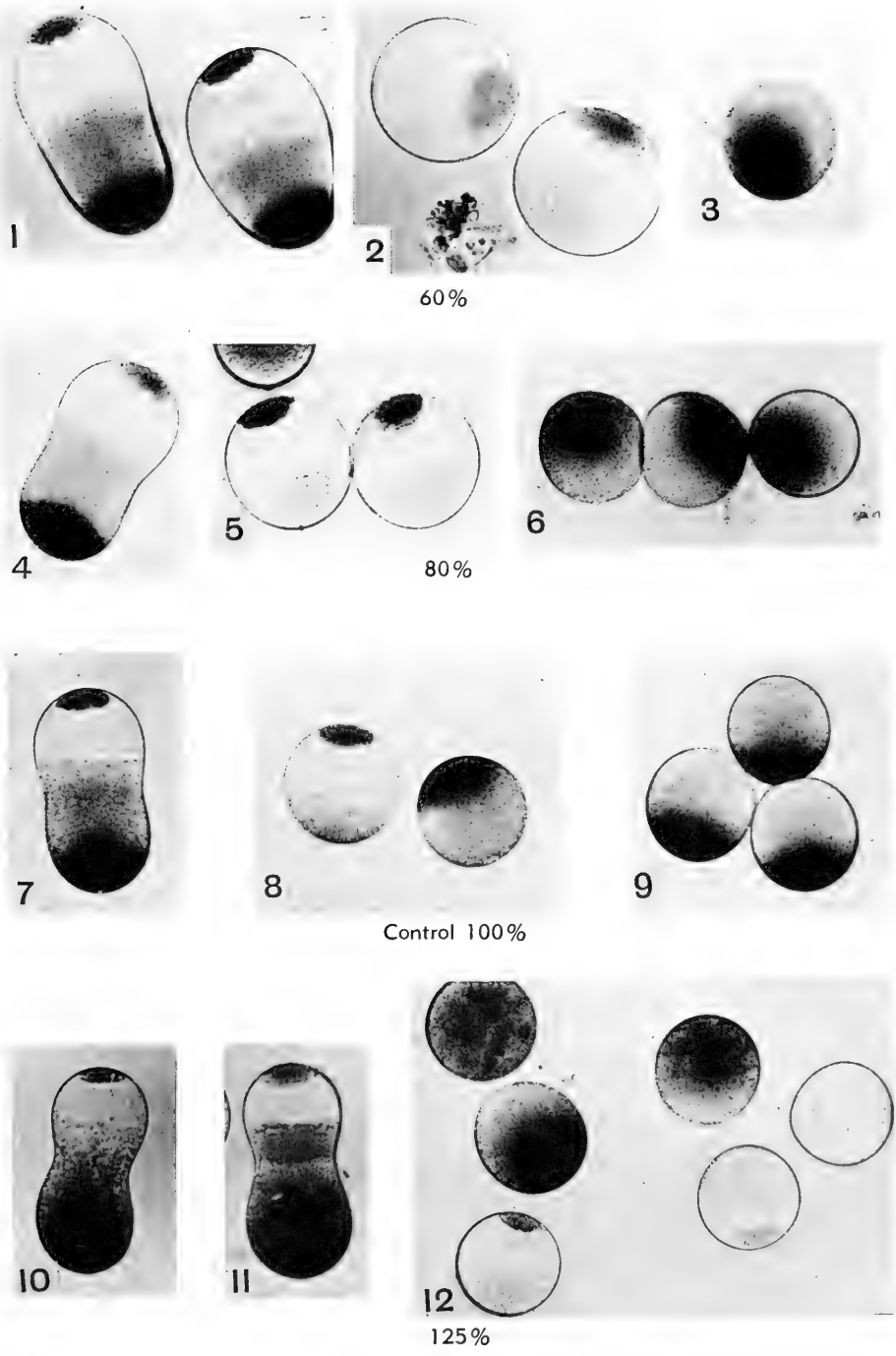


Plate XIV. Centrifuged in hypo- and hypertonic sea water

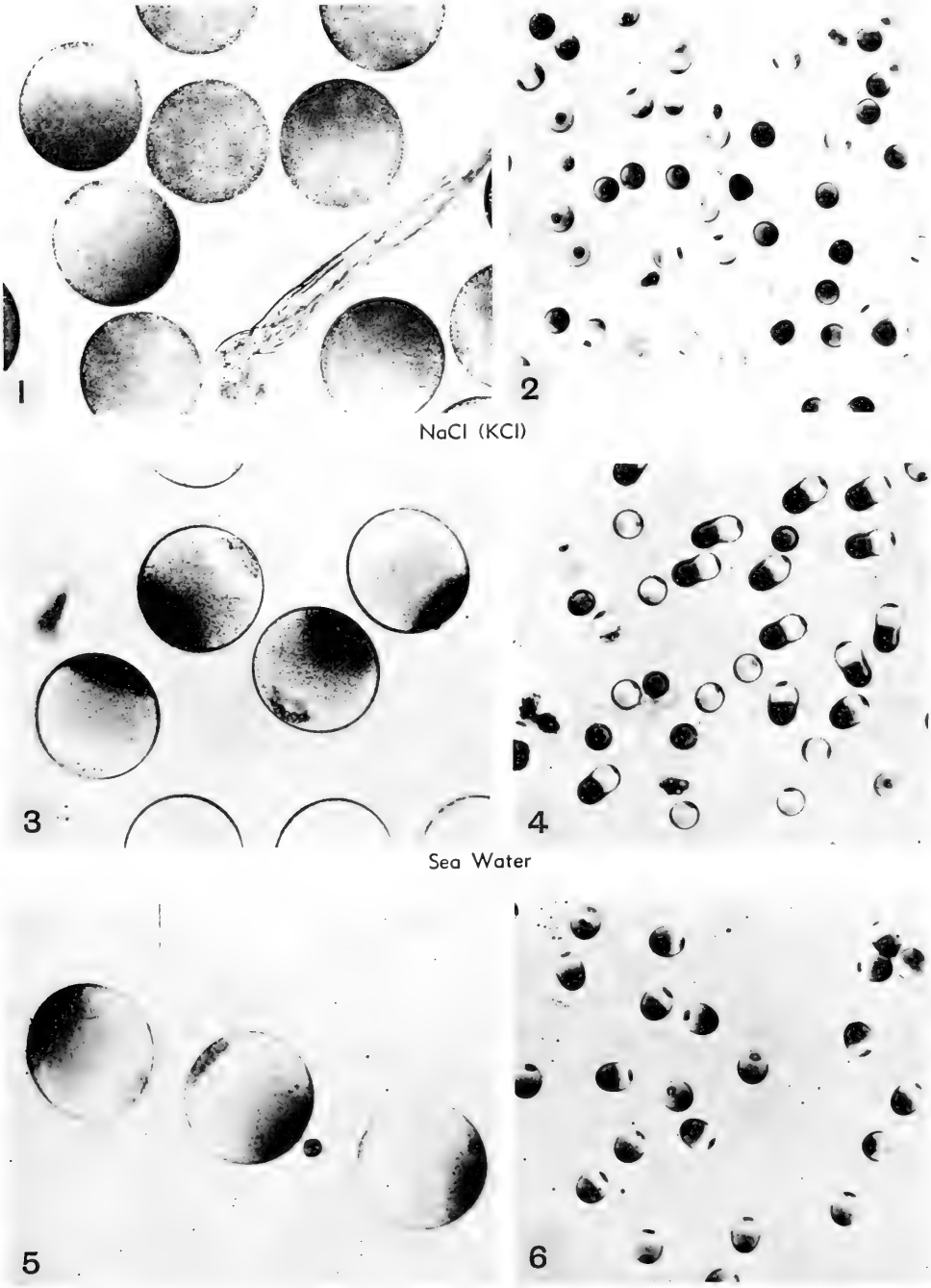


Plate XV. Centrifuged in single salt solutions

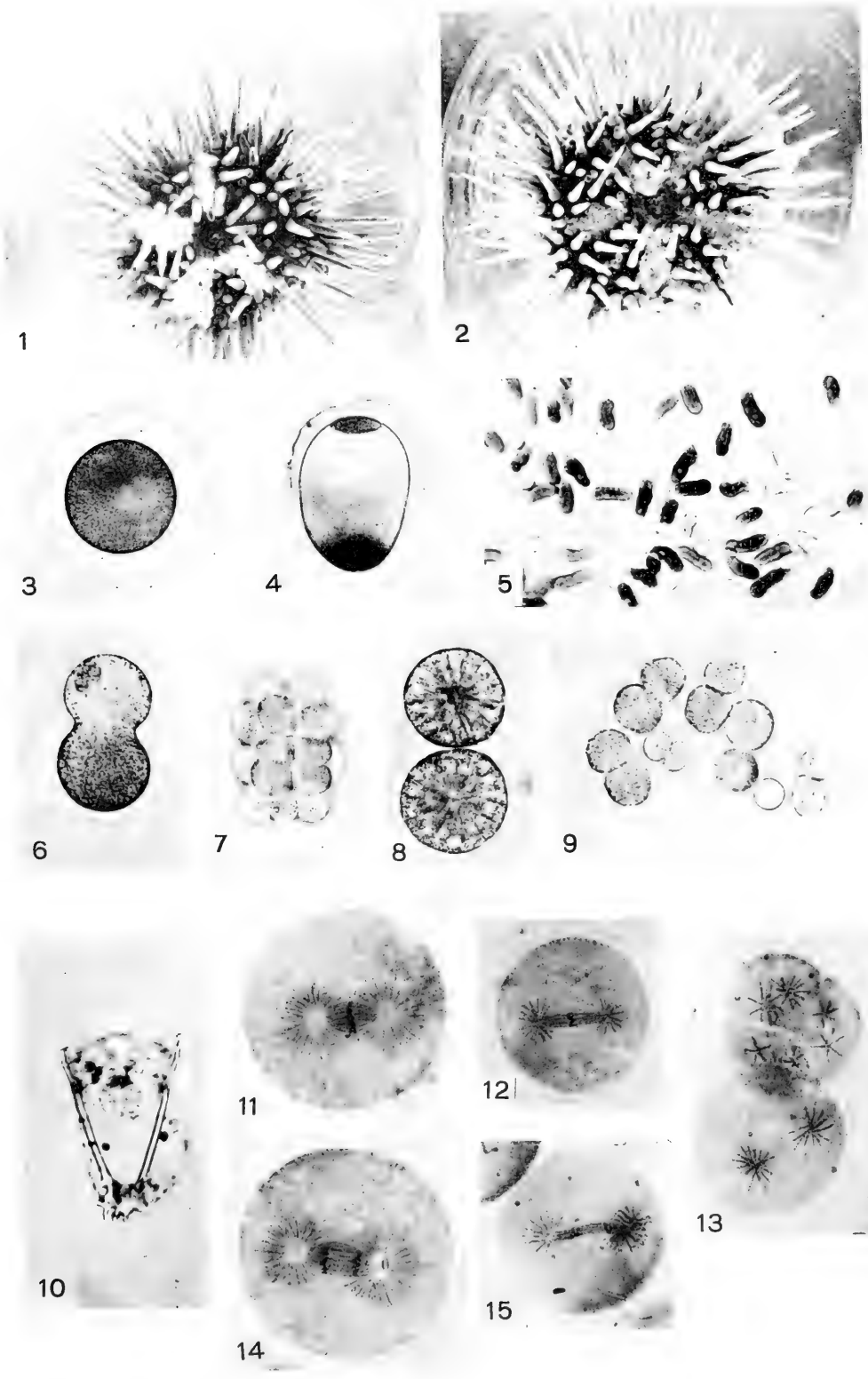


Plate XVI. Miscellaneous

PART I
GENERAL SUBJECTS

CHAPTER 1

Etymology, Nomenclature

a. Echinus and Sea Urchin

The sea urchin, *Arbacia punctulata*, belongs in the class of *Echinoidea*, this name coming from the Greek word ἐχῖνος, meaning hedgehog. A sea urchin is a hedgehog of the sea, ποντιος ἐχῖνος, as distinguished from a land urchin or hedgehog of the land, χερσαῖος ἐχῖνος, the word ἐχῖνος referring to the spiny nature of the animals. The name *Echinus* was used for the sea urchin by Aristotle and has been retained for the most common genus.

The English name "urchin" comes from the Middle English, *urchon*, which comes from the Old French, *irechon* or *herichon* (the modern French for hedgehog is *hérisson*), and this is derived from the Latin, *ericius*, meaning hedgehog. The scientific name for the hedgehog[1] which is common throughout Europe is *Erinacius europaeus*. Thus the words "urchin" and "echinoid" are derived, one from the Latin and the other from the Greek word for hedgehog. The Latin *ericius* is related to an older Greek word χήρ, also meaning hedgehog (cf. English "hirsute").

The term urchin or street urchin for a small boy comes through the application of the term to a fairy or elf which often took the form of a hedgehog playing pranks on children; later it was applied to the small child himself. Urchin is used in the sense of elf or goblin by Shakespeare:

> "For this, be sure, tonight thou shalt have cramps,
> Side-stitches that shall pen thy breath up; urchins
> Shall, for that vast of night that they may work,
> All exercise on thee." *The Tempest* I, ii, 325.
>
> (The notes say that urchins are hedgehogs or hobgoblins.)
>
> "But they'll nor pinch,
> Fright me with urchin-shows, pitch me i' the mire,

[1] The hedgehog is not found in America; the common Canadian and American porcupine *(Erithizon dorsatum)* is quite a different animal.

Nor lead me, like a firebrand, in the dark
Out of my way, unless he bid 'em."
The Tempest II, ii, 4.

"Like urchins, ouphes and fairies, green and white".
The Merry Wives of Windsor IV, iv, 50.

The country boys around Cambridge always call a hedgehog an urchin (D'Arcy Thompson, personal communication, February 1948).

The French, *oursin*[1], for sea urchin, has the same derivation (from the Latin *ericius*) as the English urchin, and is said to be a corruption of *hérisson* (hedgehog). It probably does not mean a little bear, as sometimes stated. The Italian name for sea urchin, *riccio di mare or riccio marino*, is from the same Latin root *ericius*, and means literally a hedgehog of the sea. The Spanish word is similar, *erizo de mar*. The German word for sea urchin, *Seeigel*, also means hedgehog (*Igel*); the older German word was *Meerigel*. The modern Greek word for sea urchin is *achinos*, similar to the ancient Greek.

In the earlier British literature, sea urchins were often referred to as sea hedgehogs, as for instance by Sir Thomas Browne (1658) in his *Garden of Cyrus*, where he philosophizes on the arrangement of the plates and organs in the sea hedgehog in series of five: "By the same number doth nature divide the circle of the sea star, and in that order and number disposeth these elegant semicircles, or dental sockets and eggs in the sea hedgehog." Sir John Hill (1752) also refers to the sea hedgehog. Sea urchins are still called sea hedgehogs in certain parts of England and Scotland. In *A History of British Starfishes* by the celebrated British naturalist, Edward Forbes (1841, p. 141) there is a most interesting woodcut, showing two boys at the seashore with a sea urchin and a hedgehog, apparently greatly amused at their resemblance (Fig. 1). Forbes calls them egg-urchins and sea-eggs, and they are still called sea-eggs in some regions of England and Scotland; in Jamaica, they are *usually* called sea-eggs. The term sea-egg probably does not refer to the eggs or to their use as food, but is rather based on the appearance and texture of the bleached, bare test as found on the beaches (H. L. Clark, 1933, p. 82). Sir Hans Sloane (1725) describes the fossil shells in the chalk pits of Kent as becoming filled with a fine chalk and used as a medicine for digestive troubles, whence they are called chalk eggs. The fossil spines of a large species are known as Jewstones, or *Judeo de mer* (see Oxford Dictionary). In an interesting

[1] The French *ours* (bear) comes from the Latin *ursus* and this from a different root, ἄρκτος.

letter from Governor Winthrop of Connecticut in 1670 to the Royal Society of London concerning some strange animals found there, he refers to the sea urchin as an egg-fish or buttonfish. They are also known as sea chestnuts by the English because of their resemblance to the spiny burrs of the chestnut; they are also called sea thistles, needle shells and porcupine stones and, at Plymouth, whore's eggs. The fishermen at Mousehole, in Cornwall, call them *zarts*, doubtless an old

FIG. 1. Sea urchin and hedgehog, from a woodcut of Edward Forbes, *A History of British Starfishes and other Animals of the Class Echinodermata* (London, 1841).

Cornish word (Trewavas, 1922). Sea urchins are called porcupines, chestnuts, burrs, spikes and whore's eggs by the collectors and fishermen at Woods Hole, Mass. and at Beaufort, N. C. The French call them *châtaignes de mer* (sea chestnuts) and the shells without spines *oranges de mer* and *pommes de mer*. The Germans, similarly call them *See-Kastanien* and *See-Äpfel*. The Genoise call them *zincin* (Rondelet, 1554). In his *Natural History of Chile*, Molina (1787, p. 165) describes a black sea urchin, *Echinus niger*, later called *Arbacia nigra* or *Tetrapygus niger* (see Mortensen *Monograph* II, p. 582) which he says has black eggs and is called the devil's hedgehog, and is *never* eaten.

b. Arbacia punctulata

The name of the genus, *Arbacia*, was given in 1835 by John Edward Gray, who removed it from the genus *Echinus* in which Linneus (1758) had included all the 17 species of sea urchins. The name, according to L. Agassiz in his *Nomenclator Zoologicus* (1842–1846) has no special derivation but is a "vox euphon". Bell (1889) and Mortensen (1935) call it a "nonsense" name, having no significance. The name was probably derived from Arbaces (reigned 876–848 B.C.) who was, according to some historians (e.g., Ctesias[1]), the founder and first king of the Medean Empire which was started by the rebellion of the Medean general, Arbaces, against Sardanapalus, the last of the Assyrian kings. The Medean dynasty lasted from 876 B.C. until its overthrow by Cyrus in 559 B.C. It seems likely that the name was suggested to Gray by the historical poem *Sardanapalus* by Lord Byron, in which Arbaces is a Medean satrap aspiring to the throne of Sardanapalus; this poem was published in 1821, a few years before Gray used the name. Another genus taken from *Echinus* at the same time by Gray was *Salenia*, possibly suggested by another character, Salemenes, in the same poem. An Egyptian character named Arbaces also occurs in Bulwer Lytton's *The Last Days of Pompeii*, but this book was published Sept. 1834, after Agassiz (Feb. 1834) had used Gray's name, so that this was probably not the source of the name *Arbacia*.

Arbacia just missed being called *Echinocidaris*, a name given by Des Moulins independently and at almost the same time as Gray's *Arbacia*. Gray's paper *On the genera distinguishable in Echinus* was read April 28, 1835, and published July 17, 1835 in the *Proceedings of the Zoological Society of London* (see Bell, 1889). The name had been adopted by L. Agassiz in his *Prodrome* which was read in February 1834 and published in July 1836; Agassiz had known of Gray's nomenclature through correspondence with him in 1834 before its publication. Des Moulins, not aware of Gray's work, published his *Études sur les Échinides* August 15, 1835, (dated July 1835), and gave the name *Echinocidaris* to what Gray called *Arbacia*. There seems no doubt that Gray's *Arbacia* has precedence over Des Moulins' *Echinocidaris*, and will not be changed as have so many other names of sea urchins. The altercation as to priority may be followed by reading L. Agassiz's *Monographies d'Échinodermes*, 1838, p. 17, and Des Moulins' *Études*, 1835–1837, p. 207, and the short note of Bell (1889).

[1] Herodotus (484–424 B.C.) does not mention Arbaces, and according to some modern historians Arbaces was purely legendary.

The specific name *punctulata* was given by Lamarck in 1816, and retained by Gray (1835) when he named the genus *Arbacia*, removing it from *Echinus*. The word *punctulata* doubtless refers to the shagreen-like surface of the dried test, though this is characteristic also of other species (personal communication from H. L. Clark, 1947).

CHAPTER 2

Historical

a. Greeks and Romans

Sea urchins were well known to the ancient Greeks and Romans, and have been frequently mentioned in their writings as a food, together with oysters, snails, and other sea food. Even before Aristotle, the *Echini* were well recognized as a food, e.g., by Epicharmus (born ca. 540 B.C.) in his comic poem *The Marriage of Hebe*, and Archippus in his comic play *The Fishes*, written ca. 415 B.C. Hippocrates (ca. 460–377 B.C.) also mentions them in his *De diaeta*. (See D'Arcy Thompson's *Greek Fishes*, p. 72).

But we owe to Aristotle (384–322 B.C.) the first very detailed description of the sea urchin, some of which is quite correct. Aristotle writes in his *Historia Animalium* iv. 5 (translation by D'Arcy Thompson, 1910, p. 530^a–531^a): "The urchins are devoid of flesh, and this is a character peculiar to them... There are several species (γένη) of the urchin, and one of these is that which is made use of for food; this is the kind in which are found the so-called eggs, large and edible, in the larger and smaller specimens alike; for even when as yet very small they are provided with them. There are two other species, the *spatangus*, and the so-called *bryssus;* these animals are pelagic and scarce. Further, there are the *echinometrae*, or 'mother urchins', the largest in size of all the species. In addition to these there is another species, small in size, but furnished with large hard spines; it lives in the sea at a depth of several fathoms; and it is used by some people as a specific for cases of strangury. In the neighborhood of Torone[1] there are sea

[1] Torone (now Toron) was a prominent ancient town near the tip of Sithonia (or Langos), the middle of the three peninsulas projecting from Chalcidice, the southern part of Macedonia, into the Aegean Sea, southeast of Salonika. Aristotle was born (384 B.C.) not far from here, at Stagira (or Stavros), also in Chalcidice, his father being physician to the king of Macedonia, the father of Philip. After studying with Plato in Athens (367–347), Aristotle returned to Macedonia to instruct the son of Philip, Alexander the Great, who was later of great assistance in providing him with money and collections of animals. Pliny says (viii. 17; Bostock and Riley, vol. 2, p. 265) that Alexander employed some thousands of men in every region of Asia and Greece to collect animals for Aristotle.

urchins of a white color[1], shells, spines, eggs and all, and that are longer than the ordinary sea urchin...

"All urchins are supplied with eggs, but in some of the species the eggs are exceedingly small and unfit for food, singularly enough, the urchin has what we may call its head and its mouth down below, and a place for the issue of the residuum up above. For the food on which the creature lives lies down below; consequently the mouth has a position well adapted for getting at the food, and the excretion is above near to the back of the shell. The urchin also has five hollow teeth inside, and in the middle of these teeth a fleshy substance serving the office of a tongue. Next to this comes the oesophagus and then the stomach, divided into five parts and filled with excretion, all the five parts uniting at the anal vent, where the shell is perforated for an outlet. Underneath the stomach, in another membrane are the so-called eggs [ovaries] identical in number in all cases, and that number is always an odd number, to wit five... The urchin uses its spines as feet; for it rests its weight on these, and then moving shifts from place to place."

It is on account of this description that the dental apparatus has been known as "Aristotle's lantern." The passage however, is somewhat confused in the original Greek, some manuscripts reading τὸ σῶμα (the body), and others τὸ στομα (the mouth). In the former case, the whole shell is compared to a lantern. D'Arcy Thompson prefers the latter, and gives as a translation, together with the original Greek and a discussion, in his *Greek Fishes* (1947, p. 71): "The sea urchin's mouth (or oral apparatus) is from beginning to end a continuous structure; but in surface view it is not continuous, but looks like a lantern with the horn-panes[2] left out all round." His drawing showing the comparison of the sea urchin's lantern of Aristotle, with an antique lantern is reproduced in Figure 2. Rondelet (1554), Gesner (1558), and Aldrovandi (1606) speak of Aristotle's comparison with a lantern, but do not call it "Aristotle's lantern." It seems to have been first called "Aristotle's lantern," *laternam Aristotelis*, by Klein in 1734 (p. 41-42) and Plate 31, Figs. a, b, c). Several standard works on zoology state that Aristotle's lantern was so named by Pliny (e.g., Bronn's *Thier-Reich*, Bd. II, Abt. 3, Buch IV, p. 1068), but I have found no reference to the lantern in Pliny.

[1] There are several species of white sea urchins, one of which is *Lytechinus variegatus*, the former *Toxopneustes variegatus* which occurs at Beaufort, N. C. and was used by E. B. Wilson in his classical studies published in the *Atlas of Fertilization*. The same species in Bermuda is usually brown, occasionally white. In Bermuda, *Tripneustes (Hipponoë) esculentus* is known as the white urchin.

[2] Horn was used in the place of glass.

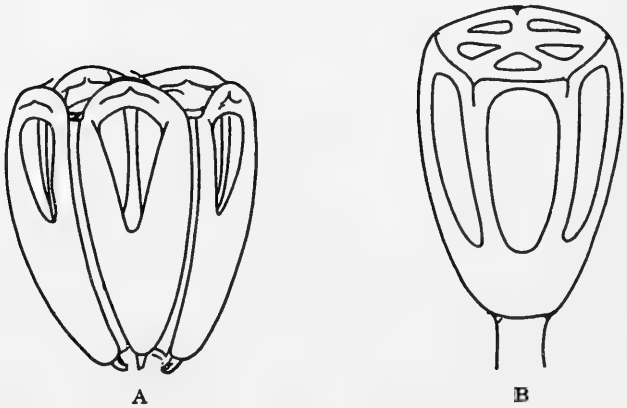


FIG. 2. (A.) The lantern of Aristotle. (B.) A Greek lantern, from D'Arcy Thompson, *A Glossary of Greek Fishes* (Oxford University Press, London, 1947).

In his *De Partibus Animalium* (translation by Wm. Ogle, 1911, p. 680[a]) Aristotle repeats much of this description of the sea urchin, noting especially its spherical shape and the radial symmetry of its organs and its edible eggs, and adds: "Though the ova are to be found in these animals even directly they are born, yet they acquire a greater size than usual at the time of the full moon; not as some think, because sea urchins eat more at that season, but because the nights are then warmer, owing to the moonlight. For these creatures are bloodless, and so are unable to stand cold and require warmth."

In his *De Generatione Animalium* (v. 3, translation by A. Platt, 1910, p. 783[a]) Aristotle's speculations are interesting but completely unscientific. The sea urchins "have large and hard spines because the sea in which they live is cold on account of its depth (for they are found in sixty fathoms and even more). The spines are large because the growth of the body is diverted to them, since having little heat in them, they do not concoct their nutriment and so have much residual matter and it is from this that spines, hairs and such things are formed; they are hard and petrified through the congealing effect of the cold."

Pliny (23–79 A.D.) has repeated in his *Natural History* (ix. 51, translation by Bostock and Riley, 1890–1900, vol. 2, p. 427) some of the description of Aristotle. Pliny says that the sea urchin has spines instead of feet, has a mouth in the middle of the body on the under side, and has five ovaries, and notes that the eggs are bitter. He also, like

Aristotle, refers to the white urchins at Torone; then follows this curious passage which has been repeated by many of the older writers: "It is said that these creatures foreknow the approach of a storm at sea, and that they take up little stones with which they cover themselves, and so provide a sort of ballast..., for they are very unwilling by rolling along to wear away their prickles (Fig. 3). As soon as seafaring persons observe this, they at once moor their ship with several anchors." This passage, no doubt, refers to those species like *Lytechinus variegatus* and *Psammechinus miliaris* which normally cover themselves with shells, algae, etc., probably for concealment or as protection against light (see under Phototaxis and Light Reaction, Chapter 6). Pliny also, like Aristotle, refers to the eggs occurring at full moon[1] (Pliny, ix. 74; Bostock and Riley, vol. 2, p. 465).

According to Pliny (xxxii. various chapters; Bostock and Riley, vol. 6) sea urchins were used as an antidote for certain poisonous plants (*dorycnium* and *carpathum*) and as a remedy for many ills, ulcers, inflammatory tumors, scrofula, eye troubles, etc., and the spines for strangury (cf. Aristotle). In some cases the shells were ground up in wine or vinegar, sometimes burnt; in other cases the eggs were used. Kidney stones were treated "by drinking sea urchins, pounded, spines and all, in wine; the due proportion being one semi-sextarius" (about a cup) "of wine for each urchin, and the treatment being continued till its good effects are visible. The flesh, too, of the sea urchin, taken as a food is very useful as a remedy for the same malady." "They should be burnt with vipers' skins and frogs, and the ashes sprinkled in the drink (vinegar); a great improvement of the eyesight being guaranteed as the sure result." For methods of cooking see *Poissons et Animaux Aquatiques au temps du Pline* by Cotte (1944, p. 237).

It is of interest that several places in Greece derive their names from *Echinus*. Pliny mentions an island named Echinussa (now Kimolos), one of the Cyclades in the Aegean Sea, near Melos, where fossil specimens are still abundant on the coast (iv. 23; Bostock and Riley, vol. 1. p. 322, and footnote). He also mentions a group of islands called Echinades, also called Kurtzolari, in the Ionian Sea off Acarnania on the west coast of Greece, at the mouth of the Achelous River (iv. 19; Bostock and Riley, vol. 1, p. 310 and footnote). There was also a town in Acarnania named Echinos, probably now Ai Vasili (iv. 2; Bostock and Riley, vol. 1, p. 273 and footnote).

There are many references to sea urchins by other Greek and Roman

[1] The lunar periodicity has been especially studied by Fox (1924a) and is taken up in Chapter 7.

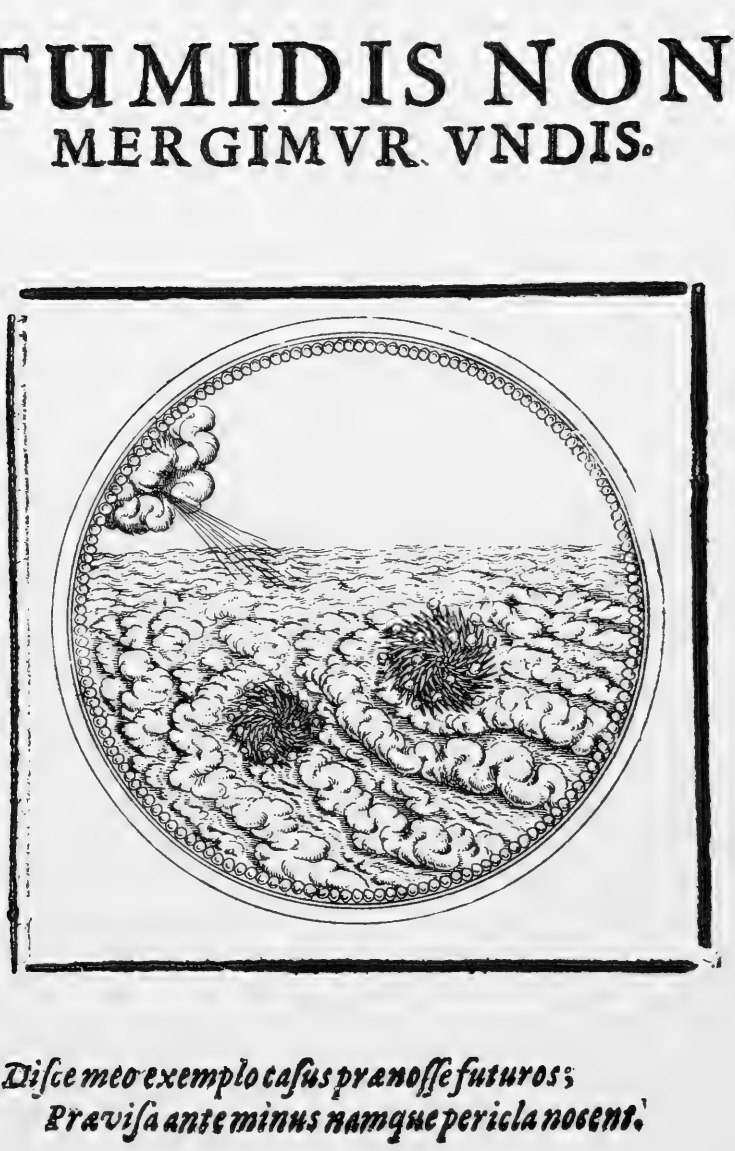


Fig. 3. Sea urchins covered with pebbles during a storm. From Joachim Camerarius, *Symbolorum et Emblematum Cent. IV*, Francofurti, 1654. Above the figure: "We are not submerged by the swollen waves". Below: "Learn by my example to become acquainted beforehand with the chances of the future; for dangers foreseen do less harm".

writers, both before and after Pliny. These are usually concerned with their use as a food or medicine or in relation to phases of the moon (see Fox 1924a, b; Zirpolo 1929; D'Arcy Thompson 1947) or to the storm. Among these writers may be mentioned: Ennius (239–169 B.C.) who spoke in his *Hedyphagetica* (ed. Vahlen, p. 220) of the "dulces echini"; Lucilius (ca. 148–103 B.C.) who said in his *Satirae* "luna alit ostrea et implet echinos"; Horace (65–8 B.C.) who said in his *Epodes* (v. 28) "horret capillis ut marinus asperis *echinus*"; Dioscorides (first century A.D., contemporary of Pliny), a botanist who said of *Echinus marinus* that "it is good for ye stomach, good for ye belly and ureticall; the raw shell of which, being roasted does well to be mixed amongst detergentia medicamenta made for ye psorae (itch). Being burnt it cleanseth foule ulcers and doth repress excrecencies of ye flesh." Galen (130–200 A.D.) spoke of sea urchins both as food and medicine. Athanaeus wrote (after 228 A.D.) in his *Deipnosophistae* or *Banquet of the Learned* (iii. 91) that echini are "tender, juicy, easily digested, and when eaten with honey, vinegar, parsley and mint they are wholesome, sweet and good-tasting. In some places they are rather bitter, and those in Sicily act as a laxative." St. Augustine (354–431 A.D.) wrote in his *De Civitate Dei* (v. 6) "Et lunaribus incrementis atque decrementis augeri et minui quaedam genera rerum, sicut echinos et conchas."

b. Middle Ages

Michael Glycas, in the twelfth century, in his annals of events from the beginning of the world speaks of the forecast of a storm by the sea urchins loading themselves with stones as described by Pliny. The poet Manuel Phile (ca. 1275–1340) also refers to this belief in one of his natural history poems, *De Echino Aquatili*, and Joachim Camerarius (1534–1598) speaks of it in his *Symbolorum et Emblematum* (Centuria quarta, p. 51) and shows a fine woodcut of a sea urchin with the pebbles on its back (Fig. 3).

In his encyclopaedia, *De Proprietatibus Rerum*, Bartholomaeus (Anglicus) (ca. 1240) wrote concerning hedgehogs and sea urchins: "The Urchin is a beast heled with pricks, hard and sharp, and his skin is closed about with pikes and pricks, and he closeth himself therewith. And he is a beast of purveyance" (i.e. the hedgehog). "And there is a manner kind of Urchins with a white shell and white pikes, and layeth many eggs... In Urchins is wit and knowing of coming of winds north or south... Also the Urchin breedeth five eggs better than other, and the eggs of some be much and great, and some be less; for some be

better to seething and to defying (i.e., digesting) than other... Also Urchins have a little body and many pikes" (this is, of course, the sea urchin). This is from the English translation of J. Trevisa (1398) from Seager's *Natural History in Shakespeare's Time* (1896).

Chaucer (1340?–1400) refers to sea urchins in his *Boethius:* "Sharpe fishes that highten echines" (Skeat ed., 1897, Bk. III, Meter VIII).

c. Rondelet to Louis Agassiz

Rondelet (1507–1566) is probably the first writer after Pliny to give a description of the sea urchin all of which was not taken directly from Aristotle and Pliny. It is said that his figure (1554) of a sea urchin cut across "so that it may be observed better" is the earliest figure of a dissected invertebrate (see Singer, 1931, p. 95) (Fig. 4). He also describes the dental structure which, he says, was compared to a lantern by Aristotle; and he remarks that "there is nothing in the whole sea more elegant and pleasing to look at."

Belon (1553) added nothing to Aristotle's account. Gesner (1558, 1563) and Aldrovandi (1606) have for the most part repeated Rondelet's description and reproduced his figure of a dissected sea urchin.

Descriptions and classification of sea urchins made some progress through Rumphius (1705), Breynius (1732), and Klein (1734). The accepted binomial classification dates from Linneus (1758), though he grouped all the Echinoids under one genus, *Echinus*, in which he included 17 species; Klein had divided them into 24 genera and 60 species. Leske (1778) reintroduced Klein's classification, using the binomial nomenclature. Lamarck in 1816 described the species *Echinus punctulatus*, which Gray in 1835 changed to a new genus *Arbacia punctulata.* Both Lamarck (1816) and Cuvier (1817) and many later writers included the Echinoderms among the *Radiata* together with Coelenterates, Infusoria, etc. The term *Echinodermata* originated with Klein (1734) to include only the *Echinoidea;* then the *Asteroidea* were added (by Bruguières 1789) and then the *Holothuroidea* and *Crinoidea* (by Lamarck 1816); see Cuvier (1834, p. 537). The *Echinodermata* were established as a primary division of the animal kingdom by Leuckart in 1848. But, though this classification was generally accepted, L. Agassiz in his *Essay on Classification* (1857, p. 71) still maintained that "the undivided type of *Radiata* appears to me as one of the most natural branches of the animal kingdom, and I consider its subdivision into *Coelenterata* and *Echinodermata* as an exaggeration of the anatomical differences between them."

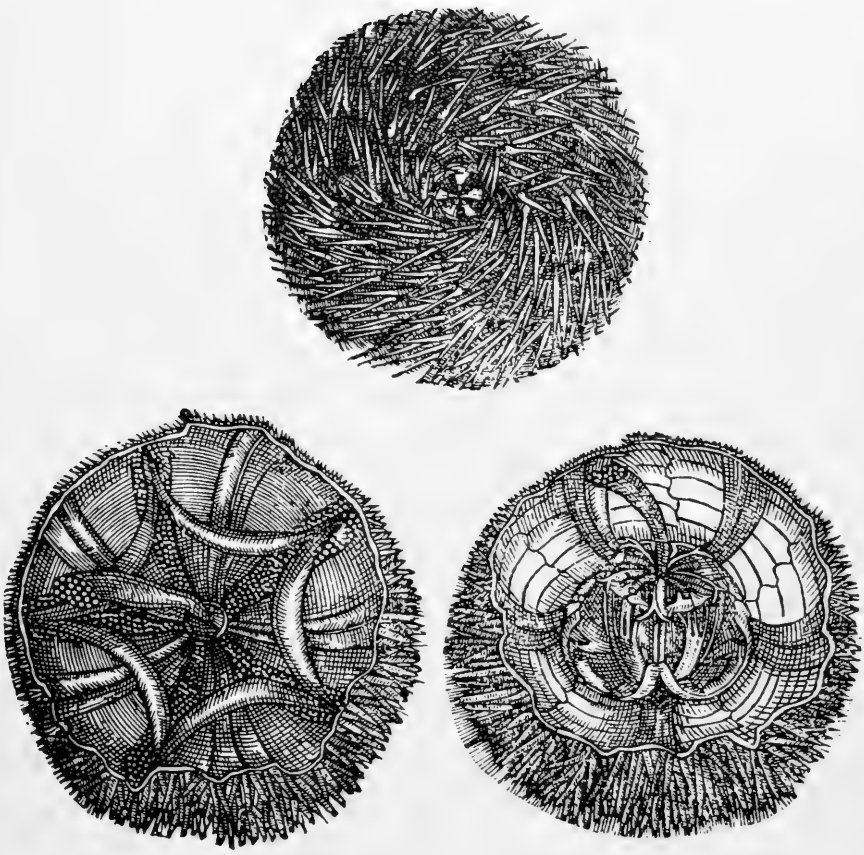


FIG. 4. Rondelet's figure of a sea urchin, from *De Piscibus Marinis*, Lugduni, 1554. This is said to be the earliest figure of a dissected invertebrate.

The history of the classification of the sea urchins, including *Arbacia*, and the chronology of the nomenclature (and a comprehensive bibliography) are given in A. Agassiz's classic monograph *Revision of the Echini* (1872–1874). A good historical review is given in Bronn's *Thier-Reich* (1904, Bd. II, Abt. 3, B. IV, p. 1002, 1007, 1321–1338), and in Lambert and Thiéry (1909, p. 9–21); a short account is given in Lankester's *Treatise on Zoology* (1900, Part 3, p. 282–285).

Tiedemann (1816) added to our knowledge of the structure of the sea urchin. A very detailed description of the anatomy of *Echinus lividus*

has been given by Valentin (1841), in a monograph included in L. Agassiz's *Monographies d'Échinodermes;* another of these monographs is that by L. Agassiz on *Des Salénies* (1838), in which he mentions the genus *Arbacia* as having been established by Gray. A most interesting book of this period is one referred to previously, by the celebrated marine naturalist, Edward Forbes, entitled *A History of the British Starfishes, and other Animals of the Class Echinodermata* (1841), and dedicated to Louis Agassiz. It is a somewhat popular but scientific account of animals belonging to the five main classes of Echinoderms, including several different kinds of British sea urchins. He reckoned that in a moderate sized common British sea urchin, which he calls *Echinus sphera* (*E. esculentus*), there are 1,860 suckers, and twice that number of pores (3,720) and about 600 plates "all dove-tailing together with the greatest nicety and regularity, bearing on their surfaces above 4,000 spines, each spine perfect in itself, and of a complicated structure, and having a free movement on its socket. Truly the skill of the Great Architect of Nature is not less displayed in the construction of a sea-urchin than in the building up of a world!" (p. 153). Especially interesting are the many woodcuts, some of anatomical features, some of scenes, and some imaginative; one of these has been reproduced in the present Monograph as Fig. 1.

The great Swiss naturalist, Louis Agassiz (1807–1873) made important contributions to our knowledge of the Echinoids, both living and fossil, his work being followed by that of his son Alexander Agassiz (1835–1910). The late R. T. Jackson (1861–1948) published in 1912 an important volume on the *Phylogeny of the Echini* and in 1927 *Studies of Arbacia punctulata and Allies*, a detailed study of the variation of the skeletal parts.

d. Modern

The two recent authorities on living sea urchins are the late Danish investigator, Th. Mortensen (1868–1951) and the late American investigator, Hubert Lyman Clark (1870–1947). The large *Monograph of the Echinoidea* by Mortensen (1928–1951), consisting of 16 quarto volumes of text and plates and an index, was completed just before his death. It is a monumental piece of work devoted to the *Echinoidea* and a masterpiece of completeness and accuracy. It contains an excellent and very comprehensive account of *Arbacia punctulata* and its relatives (referred to here as M II: 529–580, Mortensen, vol. II, p. 529–580). These two eminent authorities are in general harmonious, but disagree on a few points of classification and relationships, e.g., *Diadema* and

Centrechinus. Mortensen holds to the old name of *Diadema*, which Clark abandoned for the "proper" name of *Centrechinus* (Clark, 1925, p. 41; 1946, p. 278; Mortensen, 1940, M III, 1: 243).[1]

c. History of Embryology

Fertilization (by artificial insemination) of the living sea urchin egg was first described by Derbès in 1847, in *Echinus esculentus;* he also described and figured the cleavage and development to the well-developed pluteus of about two weeks; stages described as later than that are probably degenerative and not developmental forms. A little earlier in the same year, 1847, cleavage was described, probably for the first time, from observations made in 1845 on the same species, by von Baer of St. Petersburg; he described the development only to the free-swimming blastula just after hatching. Dufossé (1847), also before Derbès, described cleavage, hatching and early development of *Echinus esculentus*, but this he believed to be entirely radial, and his description is confused and difficult to follow without illustrations. Some years later, a detailed study was made of fertilization and early cleavage of the eggs of the starfish and the sea urchin, *Toxopneustes lividus (Paracentrotus?)* independently and almost simultaneously by O. Hertwig (1876) and Fol (1877, 1879), and of *Toxopneustes variegatus* by Selenka (1878).

f. The Pluteus

The name of *pluteus* for the larval form of some Echinoderms was originated by Johannes Müller in 1846. He gave the name *Pluteus paradoxus* (1846a) to what he thought was a new animal which came from the North Sea near Helgoland. Later in the same year (1846b), he found this to be the larval form of an Ophiuran, later identified as *Ophiura albida* (Mortensen, 1921, p. 14). The Latin word pluteus he translated into German as *Staffelei* or *Gestell*, and the English have translated the German into easel. The word was chosen because of the resemblance of the larva, turned upside down (as Müller always drew them) to an easel. "Wir sehen ein Gestell vor uns aus 2 Seitenleisten, die nach oben convergieren... nach unten divergieren und von

[1] The long controversy between Mortensen and H. L. Clark (and others) concerning Diadema vs. Centrechinus was finally settled in November 1953 in favor of Diadema (International Commission on Zoological Nomenclature, Opinions and Declaration vol. 3, Opinion 206). The controversy began with R. T. Jackson in 1912.

denen jede noch ein Fußgestell von 2 Stäben abgiebt... Da einmal alles einen Namen haben muß, so mag dieser Körper Pluteus heißen, was so viel als Staffelei oder Gestell bedeutet" (1846a, p. 108, 110). The Latin word, *pluteus*, however, is according to both Latin-German and Latin-English dictionaries usually applied to a military structure, shed or shelter (from *pluvia*, rain), or to a stand or bookcase. But, of course, the shape of a shelter might have suggested an easel to Müller. The English word, easel, comes from the Latin *asellus*, or little ass, which carries things.

Johannes Müller published many papers on the plutei of different species of Echinoderms and their metamorphosis between 1846 and 1855, including a series of nine in the *Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin;* these are classics and the illustrations are particularly fine. A very good list of studies on larval forms arranged by species, up to 1921 is given by Mortensen (1921, p. 12, and a list arranged phylogenetically by Fell, 1948). Among the most complete studies are those of *Arbacia punctulata* by Brooks (1882) and E. B. Harvey (1949); *A. lixula* and *Paracentrotus lividus* by von Ubisch (1913a, b); *Echinocyamus pusillus* by Théel (1892); *Echinus esculentus* by MacBride (1903, 1914a. *Text-book of Embryology* vol. 1, p. 504, and by Shearer, De Morgan and Fuchs (1914); *Enchinocardium cordatum*, by MacBride (1914b); *Heliocidaris crassispina*, *Mespilia globulus*, *Strongylocentrotus pulcherrimus* and other Japanese forms by Onoda (1931, 1936). Especially notable are the *Studies of the Development and Larval Forms of Echinoderms* by Mortensen (1921; 1931, I and II; 1937, III; 1938, IV). This last series (I–IV) comprises studies made during expeditions to Kei Islands (Amboina), Java-S. Africa (Onrust, Mauritius) and Egypt (Ghardaqa). See p. 117.

The pluteus of Arbacia is somewhat different from that of other sea urchins. The development of the three or four day pluteus of the European species, *A. lixula* was described by Busch in 1849, and later stages by Johannes Müller in 1854 under the name of *Echinocidaris aequituberculatus;* he figures the six-armed stage, which has been copied in several textbooks, and the later stages and metamorphosis. A more complete study of *A. lixula* has been made by von Ubisch (1913a, b, c, 1927, 1932). The late pluteus of *A. stellata* (*incisa*) from the Gulf of Panama, which is similar to that of *A. punctulata* and *A. lixula*, has been described and figured by Mortensen (1921 p. 29, Plate VII, Fig. 3).

The development of *Arbacia punctulata*, the pluteus and metamorphosis, is quite similar to that of *A. lixula* (see Chapter 15 and Plate VI of this Monograph). The pluteus was first raised by A. Agassiz at his

laboratory at Newport, R. I. Several stages were described and figured by him in his *Revision* of 1872–1874, the six-armed stage (p. 729, Fig. 66), and the very young metamorphosed form three weeks older, with primitive spines and tube feet (p. 734, 735, Figs. 68, 69, and Plate V, Fig. 9). A more complete account has been given by Fewkes (1881), working in Agassiz's laboratory, and a more detailed description of the late stages by Agassiz himself (1904, p. 53, and Plates 53, 54). Another group of investigators studied the development and metamorphosis of *Arbacia punctulata* at Beaufort, N. C. W. K. Brooks has published these observations together with excellent drawings in his *Handbook of Invertebrate Zoology* (1882), a laboratory manual written for his students at Beaufort. He has included observations and drawings of his students, Garman and Colton, published in 1883. A. Agassiz has selected the best figures of *A. punctulata* (Figs. 38–45) for Plate IX of his monograph of 1883a from both groups of investigators, and figures of *A. lixula* (Figs. 20–25; 28–37) from Busch and J. Müller.

CHAPTER 3

Fossils

The oldest fossil sea urchin is probably *Bothriocidaris*[1] from the Ordovician of Russia (Esthonia). This fossil was accepted as the ancestor of the *Echinoidea* until Mortensen (1928, 1930), the eminent Danish authority, considered it a cystoid and not an echinoid. Mortensen's views were opposed by equally good authorities, Hawkins (1929, 1931), Jackson (1929), and H. L. Clark (1932, 1946), but Mortensen in his comprehensive monograph of 1928–1951 (1935, M II, p. 15) still holds to his own view. However, Jackson (1912, p. 208) has said: "The most primitive type of *Echini*, I believe emphatically, is *Bothriocidaris*."

Though rare in the Ordovician, and few in the Silurian and Devonian, fossil sea urchins are abundant in all periods after the Devonian, and have been excellent material for the palaeontologist. There are about 6,000 fossil species (Grassé, 1948, p. 162; Clark, 1946, p. 277).

Fossils of sea urchins were scattered over northern Europe during the glacial period, and these have played a part in folklore. They were regarded as thunder stones, fallen from heaven with the thunder, and thus supposed to protect against thunder. They were also used in prehistoric times as amulets (Mortensen, 1927a, p. 9).

Fossil spines of some large sea urchins are known as *Jewstones* or *Lapides Judaici*. These were especially well known in Syria and Palestine, where they were used in medicine as a diuretic (Sir John Hill, 1751; Oxford Dictionary). They have been found also, together with the tests filled with chalk in the Chalk Pits of Kent. These "chalk-eggs" were used to cure digestive troubles and were therefore saved by the workmen (Sir Hans Sloane, 1725).

Fossil sea urchins are used by oil companies to determine the age of the beds at certain depths. This is especially true of the Cretaceous of Texas.

The family of *Arbaciidae* is known from the Tertiary to Recent (Jackson, 1912, p. 209) and there are many fossil genera in the family (Mortensen, 1935, M II, p. 548). Fossil tests and spines of *Arbacia*

[1] The statement by Jackson (1912, p. 244) that Aldrovandi figured *Bothriocidaris* in 1606 is apparently a mistake (Mortensen, 1913a, 1928; Jackson, 1929).

punctulata (called *Anapesus carolinus* or *Echinus punctulatus*) and also of *Lytechinus variegatus* have been found well preserved in the post-Pliocene beds of South Carolina (Holmes, 1860). They were taken from excavations made for tidal drains in the upper part of the city of Charleston. A little earlier figure (without description) similar to the one of Holmes under the name "*Echino-cidaris*, species doubtful," from the Pliocene of South Carolina is given by Tuomey and Holmes (1857). Mortensen (1935, M II, p. 566 and 1943, M III 2, p. 446) considers this fossil as possibly an ancestor of the present *Arbacia punctulata*, and the *Anapesus carolinus* of Holmes as identical with the present form. (See also A. Agassiz, 1883b, p. 85).

Lytechinus fossils have been found in Bermuda when dredging during the construction of the air base at Castle Harbor in 1941–1943 (H. B. Moore and D. M. Moore, 1946).

CHAPTER 4

Uses of Sea Urchins

a. As Food

Sea urchins have been used by man as food since ancient times, especially around the Mediterranean and in tropical countries. Only certain species are edible and only the gonads; both sexes are usually used, but in some places only one sex, usually the female. In general they are eaten raw, often with a little lemon, but in some places they are cooked.

The ancient Greeks and Romans used sea urchins (*echini*) as food together with oysters and clams; they are mentioned by Epicharmus (b. ca. 540 B.C.), Archippus (ca. 415 B.C.), Aristotle (384–322 B.C.), Ennius (239–169 B.C.), Horace (68–8 B.C.), Pliny 23–79 A.D.), Athenaeus (ca. 228 A.D.) and many others. Sea urchin shells have been found in the kitchens of Pompeii (Kellar, 1913). Sea urchins usually preceded the main course of a dinner, and were often highly seasoned "with honey, vinegar, parsley and mint" (Athenaeus, *Deipnosophistae* iii. 91). Several authors have written of the famous supper of Lentulus when he was made priest of Mars, at which *echini* were the first dish (see Pennant, 1777, p. 68). They were also cooked, and several recipes have been handed down by the gourmet of ancient times, Apicius (80 B.C.–40 A.D.). Among them is the following: "Put the urchins singly in boiling water, cook. To the meat thus cooked, add a sauce made of bay leaves, pepper, honey broth, a little oil, bind with eggs, sprinkle with pepper and serve."

In Italy, sea urchins are known as *ricci di mare*, and together with oysters and other sea food as *frutta di mare*. In Naples they are sold in the markets and by the fishermen along the water front. *Paracentrotus lividus* is the form usually eaten; *Sphaerechinus granularis* is also eaten but mostly by the fishermen; it is difficult to get because it lives in deep water and occurs mostly only around Gajola. *Psammechinus microtuberculatus* is too small and *Arbacia lixula* is not eaten. The native fishermen have the curious idea that *Arbacia* are the males and not edible, and *Paracentrotus* are the females and edible, so that one asks for

females in the market. If the animals are in water too deep to be taken by hand, the fishermen dive and grasp the urchin with a "canna", a reed pole with a split end. *Paracentrotus* is eaten in other places in Italy and also in Sicily. In modern Greece, sea urchins are eaten raw or cooked with rice. They are also eaten in Portugal and in South America, *Loxechinus albus* in southern Chili (Bernasconi, 1947).

In France, *Paracentrotus lividus* is commonly used as food along the Mediterranean, and this species but not the large *Echinus esculentus* is eaten on the north coast (Roscoff) and is also sent to Paris, where one obtains them at oyster bars. Over a million sea urchins a year are brought into the fish markets of Marseilles, and sold (before 1904) for 20,000 francs (Bronn's Thier-Reich, p. 1307). One also dips bread into them "à la manière des oeufs—à la mouillette" (Dujardin et Hupé, 1862, p. 458); this is called *une oursinade*. When cooked they are said to taste like crayfish (écrevisse). The early French writers, Belon (1553) and Rondelet (1554) called the edible sea urchins *doulcins* or *doussins* and the non-edible ones *rascasses* (a very bony fish, *Scorpaena*, used chiefly in making bouillabaisse).

In England to-day sea urchins are not commonly eaten. Pennant in 1777, p. 68, said they "are eaten by the poor in many parts of England and by the better sort abroad." According to several present-day British biologists, they are not eaten at the marine stations.

They are not usually eaten by Americans. On the Pacific Coast, at Pacific Grove, both *Strongylocentrotus franciscanus* and *S. purpuratus* are eaten, mostly by the Italian and other immigrants. However, some Americans have found "the gonads of *S. franciscanus*, eaten à l'Italienne (raw) with French bread, very good—extremely rich, and possibly more subtle than caviar. If it were not for the fact that the race is already being depleted by the appreciative Italians, urchins could be highly recommended as a table delicacy" (Ricketts and Calvin, 1948, p. 58). Some years ago, the Chinese at Pacific Grove sent quantities of sea urchins to China to be used as food (Kellogg, 1899). *S. franciscanus* is eaten by the Indians and Greeks in British Columbia. *S. dröbachiensis* is eaten by the inhabitants of the Aleutian Islands and Alaska. Even in New York City, one finds sea urchins for sale in the markets and on the streets of Greenwich Village, where they are bought and eaten mainly by Italians; they cost about five cents apiece. They are also served in a few restaurants in New York. These are *S. dröbachiensis*, and come from the northern waters around Rhode Island, Boston, and Maine. *Arbacia punctulata* and *A. lixula* are not usually eaten.

In many tropical countries, sea urchin gonads are commonly eaten,

both raw and cooked. In the West Indies, the natives take the gonads of several individuals of the large *Tripneustes* and bake them in the half shell of one (H. L. Clark, 1933, p. 82). When fresh and properly cooked they are said to be as good as any fish roe. In the Barbadoes, it has been necessary to pass laws regulating the gathering and sale of *Tripneustes*, for the persistent demand threatened their extinction. The eggs of this form, called *chardron blanc*, are made into an omelet in Martinique (Cotte, 1944, p. 238). They are eaten by the Portuguese in Bermuda. The Malays near Singapore use *Diadema saxatile* (*Centrechinus setosus*) as food (Bedford, 1900).

In Japan at least six species are edible, in the north (Asamushi), south (Kyushu), and at Misaki, and some have commercial value. They are expensive, costing about fifty cents apiece (Motomura, personal communication). The gonads are eaten raw, sometimes with a little lemon, and also cooked. They are boiled and eaten with a little lemon, or used as a soup. They are often placed on a shell and heated in a fire, and this dish is said to be very good (Motomura). A customary way of preparing the gonads is to mix them with about three times as much salt and make a paste. This is then stored in very attractive special jars and used, about a half teaspoonful at a time, as a relish. It is said to taste like caviar (Inoué, personal communication).

b. As Medicine

As a medicine Pliny recommended sea urchins, both raw and cooked for many ills, ulcers, tumors, kidney troubles, etc. (see under *Historical*). Galen, Athenaeus, and other writers also speak of their value as a medicine. In more modern times, it is said that in certain parts of the Midi of France, one drinks the perivisceral fluid to help digestion; the dose is half a glass a day (Mourson and Schlagdenhauffen, 1882). The use of "chalk eggs" and Jewstones has been mentioned under *Fossils*. In Dalmatia also the shells are used as an astringent (Faber, 1883).

For their use as food and medicine, see D'Arcy Thompson's *Greek Fishes* and Tortonesi (1939).

c. As Food for Other Animals

Besides serving as food for man, sea urchins are eaten by many fish (Bronn's *Thier-Reich*, p. 1302), and in Cape Cod waters *Arbacia* and *Strongylocentrotus* are devoured by the cod and haddock (A. Agassiz, 1872–1874, p. 707). *Arbacia* are consumed by starfish, spider crabs

and also by other *Arbacia*, even while still living. In northern regions, *Strongylocentrotus dröbachiensis* is eaten by the arctic fox, sharks, sea otters, and sea birds (Mortensen, 1943, M III 3, p. 209). A picture of sea otters eating sea urchins while swimming is reproduced as Figure 5.

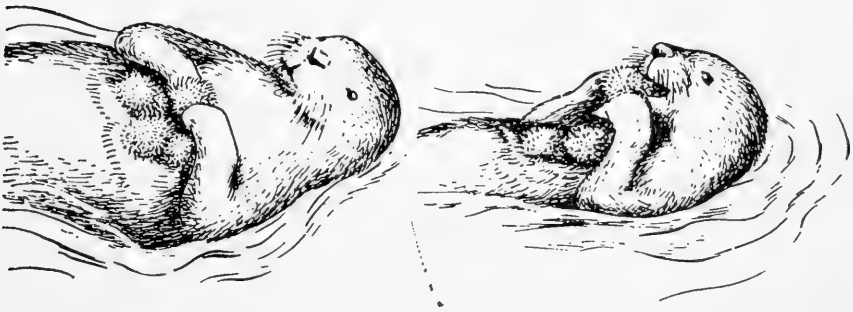


FIG. 5. Sea otter eating sea urchins, after Barabash-Nikiforov, *The Sea-Otter*, in Russian, 1933. From Mertens 1935.

d. USE OF SHELLS AS CUPS, LAMPS, INK

Shells of sea urchins were used in ancient times by apothecaries to hold drugs, as mentioned by Hippocrates, Lucilius, and others. In modern times shells of the large species are used as lamps with an electric bulb inside. In Italy, *Sphaerechinus* is so used and in Sweden and England, *Echinus esculentus*. In Monaco, the shells of *Paracentrotus lividus* are beautifully prepared at the Musée Océanographique (for sale) and are most striking with a small bulb inside making all the little holes for the tube feet light up in rows, as shown in Plate I, Photograph 6. In Japan the shells are filled with a fish oil and provided with wicks (Robins, 1939, p. 126). In some places in England (Robin Hood's Bay), *Echinus esculentus* shells are used as flower pots, especially for cactus. In Maine, sand dollar shells (*Echinarachnius parma*) are ground up and used as an indelible ink (Verrill and Smith, 1874, p. 69).

e. IN ART, ON COINS AND AS JEWELRY

Figures of sea urchins are to be found in ancient mosaics and on vases (Keller, 1913). A reproduction of a mosaic in the baths of Medeina showing various sea animals including (probably) a sea urchin, is to

be found in Dahremberg, Saglio, and Pottier (1904, t. III, part 2, p. 2116). Imprints of sea urchins were stamped on ancient coins, e.g., from Segesta and Teos (see Plate VIII, Figs. 42, 43 of Imhoof-Blumer und Keller, 1889).

In architecture, a part of the capital of a Corinthian or other column is known as Echinus (see Oxford Dictionary).

Necklaces of sea urchin shells were apparently used in olden times, as pictured by Klein (1734).

Use of fossil sea urchins by oil companies and as amulets has been described under *Fossils*.

f. Use as Experimental Material

Perhaps the most important use of sea urchins is as experimental material for biological work. The extensive use of the eggs and sperm is made evident by the large compendium which follows as Part IV of this Monograph; this deals mostly with only one species, *Arbacia punctulata*, and with investigations carried out almost exclusively at Woods Hole. Experiments on other species done in Naples, Sweden, Denmark, England and other places are equally if not more extensive.

CHAPTER 5

Description

a. Size and Shape. Plate I

The shells (without spines) of *Arbacia punctulata*, collected at random at Woods Hole during many summers measure 0.6–5.6 cm. in diameter and 0.3–2.7 cm. in height. They are roughly twice as broad as high. The spines measure 0.35–2.5 cm. in length. The overall diameter (including spines) is 1.3–10 cm. The average animal has a diameter of about 4 cm. without spines and about 8 cm. with spines, and a height of about 2 cm. The spines vary in length and in shape in different regions of the body. The spines on the oral surface are flattened and short, those on the aboral surface are long and pointed. Jackson (1927) measured 14,100 specimens of *Arbacia* at Woods Hole during the summers of 1913, 1914, and 1915 and his figures are quite similar to mine; his smallest one measured 0.5 cm. in diameter (without spines), and the largest ones 5.3 cm. The most numerous were 3–4 cm. in diameter.

There is considerable variation in the shape of the shell, some shells being more flattened than others, and some quite conical. D'Arcy Thompson (1942, p. 944) in his *Growth and Form* has an interesting discussion on the shape of sea urchin shells, suggesting that they are similar to drops of liquid and flatten by their own weight, the small ones remaining spherical. The conical shells, he thinks, may be due to accumulation of lighter substances, such as oil in the eggs, in the upper part of the shell. Lowndes (1944b) has pointed out some of the fallacies in these arguments; he found that the shells will withstand heavy weights (4 kilos) placed on them, without injury, and calls attention to the fact that there is very little oil in the eggs. Measurements have been made of the diameter and height of a group of 26 dried shells of *Arbacia* of varying sizes and it was found that the ratio diameter/height in the largest shells (4.2 to 4.7 cm. diameter) was 1.96/1; in the medium sized shells (2.0 to 2.7 cm.) it was 1.956/1; in the smallest shells (1.53 to 1.95 cm.) it was 2.01/1. There is no flattening with age. Even in a newly metamorphosed animal, the body is not spherical, but somewhat flattened.

The *Arbacia punctulata* collected by me in 1942 at Beaufort, N. C. are considerably larger than those at Woods Hole, averaging 5 cm. in diameter (without spines) and 3 cm. in height; their spines are more slender and longer, measuring up to 3.5 cm. The eggs of the Beaufort form are also larger, 80 μ in diameter as against 74 μ for the the Woods Hole form. Jackson (1927) thinks that "the Florida material differs enough from the Woods Hole type so that it might well be considered a distinct local form" (p. 449).

b. Color

The color of *Arbacia punctulata* is quite variable, from reddish gray to reddish to purplish to brownish to almost black. Young individuals are inclined to be lighter. There is no change in color when kept in the light or dark for short periods, as shown by Parker (1931) for a period of 10 hours. I have confirmed this several times, and Kleinholz (unpub.) has also found this. But they are affected by light if kept for longer periods. A group of six animals of medium coloration which I kept in the dark room at Woods Hole (in 1948) were decidedly lighter than a similar group kept in bright light in a laboratory room after a period of two months; there was a slight difference even after a week. I have carried out similar long-time experiments for several summers. Dark animals kept in the dark become darker (black); dark animals kept in the light become lighter (brownish). Light animals kept in the dark become greyish; light animals kept in the light become reddish or purplish. The results are complicated by the fact that there are at least two different kinds of pigment in the test and spines, a melanoid and an echinochrome, and that the test and spines are different in color. For pigments in *A. lixula* and *P. lividus* see Glaser and Lederer (1939).

Another experiment was carried out (summer of 1954), using the same animals for light and dark exposure. A group of six *Arbacia* of different sizes were kept in an aquarium in a very bright room with sunlight (in the mornings) for a month, and kodachrome pictures were then taken. This same group was then kept in a dark room under exactly similar conditions (not fed), and completely shielded from any light, for a month. Kodachrome pictures were taken on the same film with exactly the same conditions of lighting, exposure, etc. When exposed to the light the animals were decidedly more red, and there was also more black pigment.

Ultraviolet light causes the animals to become reddish (E. B. H.

unpub.). H. L. Clark (1933, p. 80) found a difference in color in northern and southern forms. "While northern specimens have the test commonly a deep brown, with a reddish cast, and the spines lighter and often a dull brownish red, in the south there are two extremes; on the one hand, the test is a light wood-brown, with spines very light (a dingy cream color) at the base, becoming dull reddish purple at the tip, and on the other, test and spines are deep reddish purple or almost black in certain individuals. Both these extremes are observed in specimens from Florida. Specimens from Cuba, Yucatan, and Tobago, are very dark and have slender, relatively long, spines."

The color change in *A. lixula* was studied many years ago in Naples by von Uexküll (1896), who found that they became black in the light and brown in the dark. I confirmed this in Naples in 1934 (unpub.) finding that light specimens of *A. lixula* became darker in the light and dark specimens became lighter in the dark over a period of a month; in some individuals which were isolated, the change was noticeable within a day. Kleinholz (1938) found that from a group of 12 dark specimens of *A. lixula* at Naples the ones kept in the light for 6 hours remained dark, while those kept in the dark became brownish. Some dark individuals turned brownish in the dark in 90 minutes, and when brought into the sunlight became dark again in an hour. The two species of *Arbacia* react to light by change in color in much the same way, but *A. lixula* reacts more rapidly. In nature *A. lixula* is much darker than *A. punctulata.*

Centrostephanus longispinus (at Naples) reacts in much the same way, a dark animal turning gray in the dark within two hours (von Uexküll, 1896; Kleinholz, 1938). Von Uexküll found that the change in body color was due to changes in the chromatophores of the body skin which were expanded in the light and concentrated to small points in the dark. Kleinholz found that the tube feet isolated from an illuminated animal were brownish red while those from an animal kept in the dark were pinkish white, owing to the dispersed or contracted phase of the chromatophores.

A still more rapid change in color occurs in *Diadema (Centrechinus) antillarum* in Jamaica, especially in the young animals; they become lighter, almost white in the dark (Millot, 1950). The change is due to changes in the large skin chromatophores, expanded in the light and contracted in the dark. Even a spot of light will cause the change.

No albino specimens of *Arbacia* have been reported, but Prince (1913) has described an albino of *Strongylocentrotus dröbachiensis* from

St. Andrews in New Brunswick, Canada; and H. L. Clark (1933, p. 79) states that albino or partially albino individuals of *Centrechinus antillarum* are occasionally seen in the West Indies. Cuénot (1912) found entirely white specimens of *Psammechinus miliaris* at Arcachon "comparable to albinos," but he also found transitions to the usual color. Deep sea forms are often, according to Koehler (1927, p. 9) extremely brilliant.

c. Morphology. Plate I

At the aboral end of *A. punctulata*, surrounding the anus, there are (usually) four anal and five genital plates including the large madreporic plate (Plate I and Figs. 6, 7). There may be only two or as many as fourteen anal plates; there are often three or five (Jackson, 1927, p. 464). Each of the five genital plates has typically one genital pore through which the eggs and sperm exude; there are frequently two genital pores in one plate, sometimes more, and in one case seven (Jackson, 1927, p. 458). Between and below the genital plates are five small ocular plates. There are considerable variations in the number and arrangement of plates (Osborn, 1898, 1901; Jackson, 1927). At the oral end project the five teeth of Aristotle's lantern. The lantern is composed of 40 calcareous pieces controlled by 60 muscles (Jackson, 1927, p. 483; Reid 1950).

The test is made up of small pentagonal plates arranged in double rows, five narrow (ambulacral) and five broad (interambulacral) rows. These plates are studded with tubercles which bear the spines and pedicellariae. The pedicellariae[1] have been described by Agassiz and Clark (1908, p. 71). Tube feet project through a double row of pores arranged in ten lines, one on each side of the narrow (ambulacral) row of plates. As growth occurs both by the increase in number of plates and by increase in size of each plate, the total number of plates depends on the size of the animal. It has been estimated that a large *Arbacia* (4.5 cm. diameter) has about 1,000 plates and a small *Arbacia* (2.5 cm. diameter) has about 700 plates. These figures are based on measurements made by the students in the invertebrate course at the Marine Biological Laboratory at Woods Hole in 1951,

[1] The pedicellariae of *Toxopneustes pileolus* are poisonous (Fujiwara, 1935, *Annot. Zool. Jap.* 15 : 62–69; Mortensen, *Monograph* III 2, p. 469). The long pointed spines of the Diademas are poisonous (Mortensen, *Monograph* III 1 p. 249). See C. J. Fish and M. C. Cobb (Thayer), 1954, *Noxious marine animals of the central and western Pacific Ocean.* Research Report 36 of U. S. Fish and Wildlife Service.

under the direction of Dr. Ralph I. Smith of the Univ. of California. The plates of 72 animals were counted by members of the class, and there seems no doubt that the number of plates varies with the size of the animal. Jackson (1927, p. 471) states that the increase in size in *Arbacia* is attained more by the increase in size of individual plates than by increase in number; an animal 4.7 cm. in diameter had 16 plates in an interambulacrum, and one 1.4 cm. had 10 plates. A careful study of three Chinese (and Japanese) species, *Temnopleurus toreumaticus*, *T. hardwickii* and *Strongylocentrotus pulcherrimus*, by Hsai (1948) collected from Kiao-chow Bay, Tsingtao, showed that in young animals the number of plates is a function of the diameter of the test, but that as the animals approach maximal size, they stop forming new plates and growth takes place only by the enlargement of the plates. Many years ago, the celebrated naturalist Forbes (1841) estimated that in *Echinus esculentus*, a much larger form than *Arbacia*, there are in an animal of moderate size 600 plates, 3,720 pores, 1,860 suckers and 4,000 spines.

There is a simple coiled alimentary canal, a water vascular system and a nervous system, but the main body of the animal (in season) is filled with the five ovaries or testes (Fig. 7). The ovaries are red, or brownish late in the season, and the testes are brownish white, the oozing sperm being white. The histological structure of the gonads and gonad walls of *A. punctulata* is described with drawings and photographs by L. Palmer (1937) and L. Palmer Wilson (1940). A photograph of the ovary is given by Liebman (1950). In other species, the genital glands have been described by R. Koehler (1883) for *A. lixula* and many other species; by Hamman (1887) for many species; by Tennent, Gardiner, and Smith (1931) and Miller and Smith (1931) for *Echinometra lucunter;* by Lindahl (1932b) for *Paracentrotus lividus;* by Aiyar (1938) for the Indian species, *Salmacis bicolor;* and by Tennent and Ito (1941) for the Japanese species, *Mespilia globulus*.

An excellent account of the structure of *A. punctulata* is given by Reid in Brown's *Selected Invertebrate Types* (1950), written for the Invertebrate Zoology course at Woods Hole (Figs. 6, 7). Other accounts of *A. punctulata* are those of: A. Agassiz (1872–1874) *Revision of the Echini* (pp. 263–266); H. L. Clark (1902) *Echinoderms of the Woods Hole Region*, p. 563; Coe (1912) *Echinoderms of Connecticut*, pp. 85–108; Jackson (1912) *Phylogeny of the Echini*, (1927) *Studies of Arbacia punctulata and Allies* (external structure); Petrunkewitch (1916) *Morphology of Invertebrate Types*, pp. 191–201. W. K. Brooks (1882) in his *Handbook of Invertebrate Zoology* describes and gives good drawings of the external and

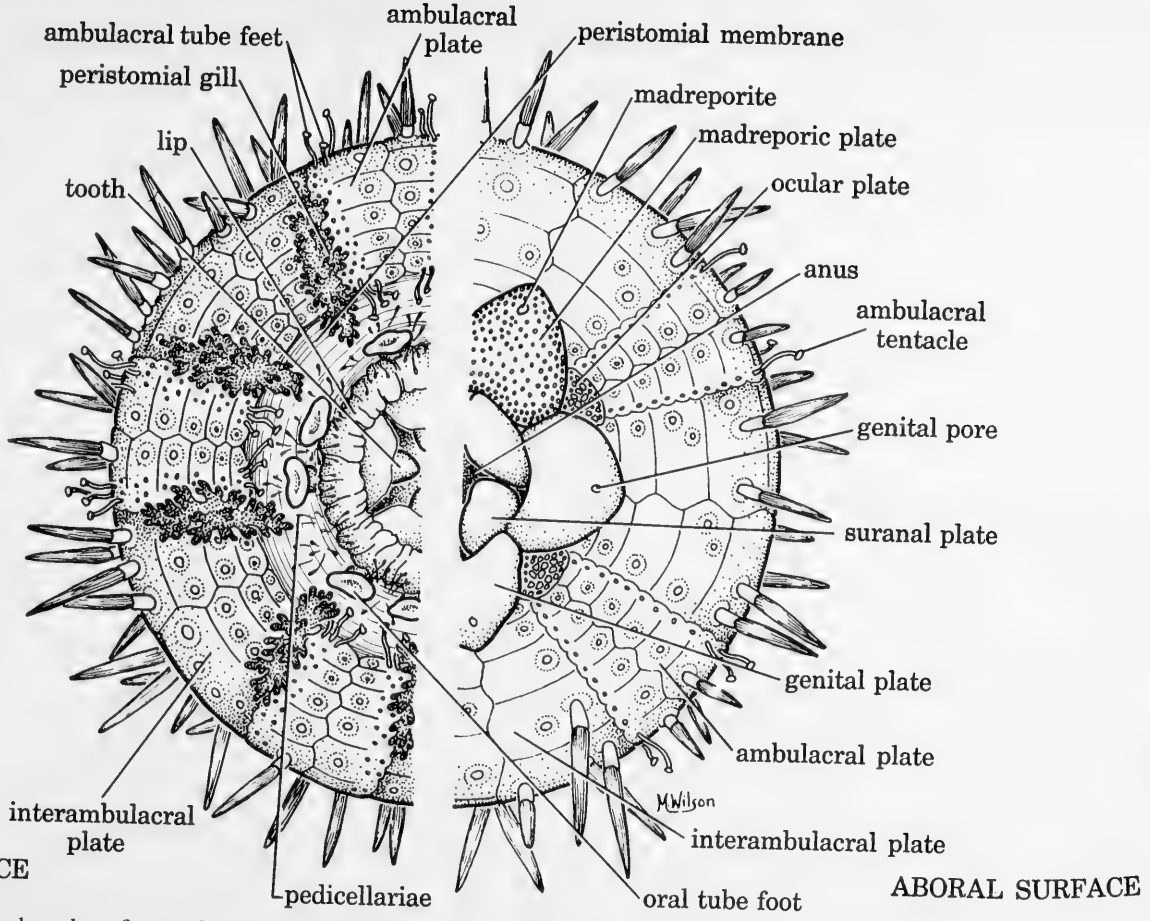


FIG. 6. Oral and aboral surfaces of the test of *Arbacia punctulata*, with spines partly removed, to show detailed structure. From W. M. Reid in *Selected Invertebrate Types*, edited by F. A. Brown, Jr. (John Wiley and Sons, New York, 1950). Reproduced by courtesy of F. A. Brown, Jr. and the publisher.

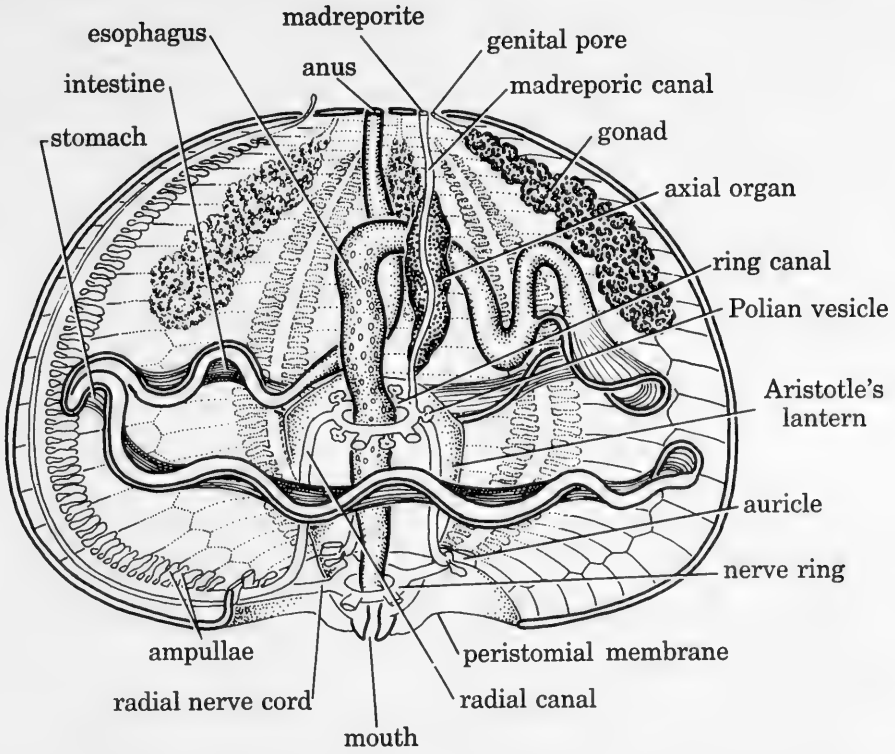


FIG. 7. Diagrammatic vertical section of *Arbacia punctulata*, to show internal organs. From W. M. Reid in *Selected Invertebrate Types*, edited by F. A. Brown, Jr. (John Wiley and Sons, New York, 1950). Reproduced by courtesy of F. A. Brown, Jr. and the publisher.

internal anatomy and embryology of *A. punctulata*, having used this book as a laboratory guide for his students at Beaufort, before the Marine Biological Laboratory at Woods Hole was started. An exhaustive monograph on sea urchins including the *Arbaciidae* has just been completed by the authority on the subject, Mortensen (1928–1951); *A. punctulata* is in vol. II, pp. 573–575, 1935.

For a good general description of the morphology of a sea urchin *(Echinus esculentus)* reference may be had to MacBride's account in the *Cambridge Natural History*, vol. I (1906), or to Lankester's *Treatise on Zoology*, part 3 (1900), or to the more recent volume of Grassé, *Traité de Zoologie*, t. II (1948). A more complete comparative account with experimental data and comprehensive bibliography is given in Bronn's *Thier-Reich* Bd. II, Abt. 3, Buch IV (1904). The excellent volume on *Echinodermata* in the series of *Invertebrates* by Libbie H. Hyman (1955) was published after this book was sent to press.

CHAPTER 6

Natural History

a. Feeding Habits

Arbacia punctulata eats almost anything in an aquarium, and probably does so in its native habitat. They have been observed eating *Fucus*, *Laminaria*, *Ulva*, polyps of coral, sponges, mussels, sand dollars, and other *Arbacia*, both the soft parts and the shell. Parker (1932) observed and photographed two *Arbacia* eating a *Fundulus* which "may be caught and eaten by the sea urchins. The capture usually takes place at night, and the prey is almost always a partly spent fish. I doubt if a fully vigorous *Fundulus* is ever taken by a sea urchin. I have never witnessed the first steps in the capture" (Parker, 1932 p. 95; see also Gudger, 1933). *Arbacia* certainly cannot capture live fish, but eat them when moribund or dead (E. B. H. observations for many years). For feeding habits of A. punctulata and other sea urchins see van der Heyde (1922).

Arbacia live well in aquaria supplied with *Fucus* and *Laminaria* and shells peppered with holes containing the sulphur boring-sponge, *Cliona celata*, and of course with running sea water; some animals and plants decay and pollute the water. Although they will eat almost anything that is at hand, they can get along without food, except for the small organisms brought in with the sea water, for several months. Two sets of six *Arbacia* were kept in aquaria (capacity about four gallons), one set in the dark and one set in the light, without being fed, for two months. All individuals of both sets were perfectly healthy after that period.

The food for the developing pluteus is diatoms, especially *Nitzschia closterium* which can be cultured with Miquel's solution (E. B. Harvey, 1949) or more simply by adding 0.002% $Na_2HPO_4 12H_2O$ + 0.01% KNO_3 to sea water (see Part II). When the shell is developing, the calcareous material can be supplied by the calcareous protozoon *Trichosphaerium* and later by the red alga *Corallina*, according to Shearer, de Morgan, and Fuchs (1914).

b. Growth and Age

Very little is known of the growth and age of sea urchins, especially of *A. punctulata*. From fertilization through metamorphosis of *A. punctulata* takes (under laboratory conditions and about 23° C.) three or four months, and the animal is then about 1 mm. in diameter (Plate VI, Photo 11). The smallest sea urchin brought in from the outside by the collectors July 22, 1952, was 6 mm. in diameter with spines, 3.6 mm. without spines, and was 1.6 mm. high. (Plate VI, Photo 12). Smaller specimens, just metamorphosed, have not been found, though a careful search has been made on the stones and shells brought up from the beds and in the plankton above the beds. The newly metamorphosed young of most species do not seem to be common, but Mortensen (1927a, p. 333) says the larvae and newly metamorphosed young of *Echinocardium cordatum* are sometimes found in enormous numbers in some places. *Arbacia* mature when very young; several about 1 cm. in diameter (without spines; 1.7 cm. with spines) had ripe functional eggs or sperm, and the eggs when fertilized developed into fine plutei. One that was 0.7 cm. (without spines) had ripe functional sperm, and one that was only 0.6 cm. had well developed ovaries containing immature eggs of all sizes, many of maximum size, and a few mature eggs, which, however, did not fertilize. Several small urchins, 2 cm. in diameter (without spines) brought in Aug. 1, 1951, contained all mature and no immature eggs. This early maturity has been observed in other forms. *Psammechinus miliaris* has been found with ripe eggs and sperm when 1.2 and 1.3 cm. and even when less than 1 cm., when they are less than a year old; and *Echinus esculentus* and *E. acutus* "when very small" (Shearer, de Morgan, and Fuchs, 1914, p. 270; Orton, 1920, p. 352 footnote). *Sphaerechinus granularis* is sexually mature when 0.8 cm. in diameter (Mortensen, 1927 a, p. 311). It is of interest that Aristotle noticed this in his sea urchins, for in the Historia Animalium he says "the eggs (ovaries) are found in the larger and smaller specimens alike; for even when as yet very small they are provided with them" (see under Historical).

A. punctulata of all sizes have been brought in by the collectors, ranging from 3.6 mm. to 56 mm. in diameter (without spines), but until recently the small ones were found very rarely. Nothing is known as to the age of the large ones formerly brought in and used for experimental work. It seems probable that these came from old beds in which the young ones could not establish themselves, or perhaps were carried by currents, as plutei, elsewhere. The small *Arbacia* probably

come from newer beds recently discovered. A similar situation was found by H. B. Moore (1934) for *Echinus esculentus* near Port Erin. One bed, at Breakwater, had no small ones, none less than 7 cm. while two other beds, at Brest and Chickens had mostly small ones, under 4 cm.

The best figures for the growth of sea urchins over a period of time are those of Elmhirst (1922) for the large urchin *Echinus esculentus* and of Bull (1939) for the smaller *Psammechinus miliaris*, given in the following table:

Diameter of test (without spines) in cms.

Echinus esculentus. (Elmhirst, 1922 at Millport, Scotland; Mortensen, 1927 a, p. 299)

Just metamorphosed	6 mos	1 yr	2 yrs	3 yrs	4 yrs	5 yrs	6 yrs	7–8 yrs (probably)
2		4 mature	4–7	7–9	9–11	—	—	15–16

Psammechinus miliaris. At Cullercoats, Northumberland (Bull, 1939)

0.1	0.33	2.0 mature	2.62	2.92	3.03	3.7	3.87

Figures for the size of *Psammechinus miliaris* in different months and years are given by Lindahl and Runnström (1929). The Indian form *Salmacis bicolor* at Madras was found to measure 4–5 mm. at 3 months, 13 mm. at 6 months, and 16 mm. at 12 months (Aiyar, 1935).

There are no figures published for *Arbacia punctulata* owing to the difficulty in keeping the animals over winter. The growth during the three month season (June to September) at Woods Hole was found to be only about 1 mm. even when well fed with Fucus and Cliona (E. B. H.) A group of ten, of approximately the same size, had an average diameter (test) of 22.4 mm. on June 23, 1951 and 23.5 mm. on Sept. 24, an increase of 1.1 mm. in three months.[1] These were kept at Woods Hole over the winter, but owing to lack of running sea water for several days only one survived. It measured 25 mm. on May 29, 1952, an increase of 1.5 mm. during the eight winter months. Judging from meager data, a medium sized *Arbacia* test grows about 3 mm. a year, and continuously through the year. A very young *Arbacia*, the smallest one yet collected, was 3.6 mm. (test) on July 23, 1952 and 5.2 mm. on Sept. 28, a growth of 1.6 mm. in two months. The percentage increase in size is much greater in the small one, as one would expect.

According to H. B. Moore (1934, 1935a, 1937), the growing period of *Echinus esculentus* is limited to the spring months, when spawning also takes place. The duration of life is 4–8 years.

[1] Milligan (1916, Zoologist 20 : 399) found that Echinus (Psammechinus) miliaris (27 mm. diameter without spines) increased in the laboratory about 1 mm. in 5 months.

The age of an individual can be told in some cases by the rings of growth on each genital plate, these being marked by an annual deposit of pigment (Deutler, 1926; H. B. Moore, 1935a, 1937; Yonge, 1949, p. 166). Increase in size of the test is brought about both by accretion to the old plates and addition of new ones.

c. Regeneration

Though regeneration is not so marked in sea urchins as in starfish, they can regenerate spines, tube feet, pedicellariae and spheridia, and even large fractures of the shell are healed (Mortensen, 1927a, p. 262; Grassé, 1948, p. 149). Chadwick (1929) reports a case of *Echinus esculentus* in the aquarium at Port Erin, which had lost all its spines, fallen to the bottom and seemed dead, but then regenerated its spines. I have observed many *Arbacia* with apparently regenerated spines, and have observed the process in a few cases; they regenerate fairly rapidly. A medium-sized animal measured 5.2 cm. with spines, the test without spines 2.8 cm.; the spines removed measured 1.2 cm. Three rows of spines were removed on June 18, 1952. They regenerated at the rate of a little over 1 mm. a week; in a month they measured 0.5 cm. and in two months (Aug. 17) they were almost completely regenerated. H. W. Jackson (1939), in a similar experiment with *A. punctulata* obtained similar results; the spines (10–12 mm.) regenerated 5 to 6 mm. in a month.

For regeneration of spines in *Strongylocentrotus*, and references in the literature, see E. F. Swan, 1952.

The regenerative power of the pluteus of *Arbacia* is great; an arm is regenerated in a few days (E. B. Harvey, 1949).

d. Habitat, Burrowing Forms and Collecting

1. – Rock-Boring Forms

Arbacia punctulata, like most sea urchins, which are all sea forms, live in aggregations which are often widely separated. They live on rocky or shelly bottoms, or adhere to rocks, but do not make excavations in rocks as do several other species, especially those exposed to rough seas. One of the rock-borers is *Echinometra lucunter* in Bermuda, where it is known as the "rock" or "reef" urchin (H. L. Clark, 1899; Verrill, 1907; E. B. Harvey, 1947). *Paracentrotus lividus* is found in holes in the rocks in exposed places as observed and pictured long ago by Rondelet (1554). It is found especially on the west coast of France (Cailliaud,

1856; Fischer, 1864) and of Ireland as described and photographed by Southern (1915) in the rock pools of Clare Island (see also Forbes, 1841, p. 169 for its occurrence in Ireland). *P. lividus* is not found in holes around Naples nor is any other species, probably because there is little tide there. *Psammechinus miliaris* has been reported as rock-boring on the French coast (Cailliaud, 1856, and other older investigators), but Runnström says (personal communication) that it is not rock-boring on the Swedish coast. The California form, *Strongylocentrotus purpuratus* is rock-boring (A. Agassiz, 1872–1874, p. 706; Ricketts and Calvin, 1948, p. 129, and Plate XXVII). It has recently been reported that this form bores into steel piles and has caused damage to the piles driven by oil companies for their oil-well piers near Santa Barbara (Irwin, 1953). Fewkes (1890a, b) has reported that *S. dröbachiensis* makes excavations in the rocks at Grand Manan in Canada, as well as two species, *Eucidaris thouarsii* and *Echinometra vanbrunti*, at Guaymas Harbor in Mexico. According to John (1889), *Arbacia lixula* in the Azores makes holes in the lava rocks. It is generally agreed that the boring is done with their spines and teeth, aided by waves and currents. As the animals grow, they often become imprisoned in their holes. For reviews of rock-burrowing Echinoids, see John (1889) and Otter (1932); they also discuss the methods of boring. *Echinocardium cordatum* is an interesting sand-burrowing form (Grassé, 1948, p. 198; Yonge, 1949, p. 238).

2. – Distribution

Arbacia punctulata occurs along the North American east coast from the southern coast of Cape Cod to Florida; Woods Hole is probably its northern limit. It also occurs on the north coast of Cuba, in Yucatan, Curaçao, Trinidad, and Tobago, but not in the Lesser Antilles, Jamaica, Puerto Rico, or Bermuda. (H. L. Clark, 1902, 1923, 1933; Mortensen, 1935, M II, p. 575). The species is supposed to have arisen together with the closely related *A. spatuligera* on the west coast of tropical America and to have reached its present home on the east coast while there was still open water between the Caribbean Sea and the Pacific Ocean; the original species then diverged into the present two species, *A. punctulata* and *A. spatuligera*. The origin of the Mediterranean form, *A. lixula*, cannot be traced.

Around Woods Hole, many years ago (1874–1919) *Arbacia* were taken in many places in Buzzard's Bay and Vineyard Sound, near Quisset, North Falmouth, North of Nashawena, Kettle Cove, off West Chop (Martha's Vineyard), Sankaty Head (Nantucket), and around

the Fish Commission wharf (Verrill and Smith, 1874; Clark, 1902; Sumner, Osburn, and Cole, 1911; Allee, 1919, 1923a, b, c).

In more recent years, they have been obtained off Naushon Island, Hadley Harbor, Lackey's Bay, Tarpaulin Cove, and off Nobska. They disappeared from Nobska about 1937, and later from other beds, until in 1948–1950 they were obtained from only two beds, Lackey's Bay and Tarpaulin Cove, and here too they were becoming scarce. The scarcity of the urchins became so serious that a survey of the whole Woods Hole area was made in the summer of 1950 in an effort to discover new beds, under the direction of J. S. Rankin Jr., the Summer Naturalist at the M. B. L. For a period of six weeks, only one *Arbacia* was found, at Cotuit. Then in September, 1950, a good bed was found on Martha's Vineyard between Menemsha Bight and Gay Head. Unlike the other beds, this bed had small as well as large urchins, and has subsequently become a source of supply, though they are difficult to collect and many are too small for experimental work.

In 1951, while exploring further afield, many beds were found on the south-east coast of Fisher's Island, off the south coast of Connecticut. These urchins were of all sizes, young ones as well as large ones. Unfortunately the distance from Woods Hole (62 miles) necessitates a two-day trip, often hampered by bad weather, but it is another source of supply of *Arbacia*. Also, in 1954, *Arbacia* were found near the "old Nobska" bed, having returned there after an absence of 17 years.

Some of the old beds which have been unproductive for some years have now been stocked with urchins, young and old from the newer beds, in the hope that they may again furnish many urchins in the future.

3. – Quantities Used

Arbacia punctulata are found in shallow water and at depths down to 700 ft. They are obtained by dredging in water 20–90 ft. deep, usually with a tangle. The associations in which they are found around Woods Hole have been studied by Allee (1923a, b).

In an ordinary summer, ten or twenty years ago, 20,000–25,000 animals were used at the Marine Biological Laboratory. The greatest number used in one summer (1933) was approximately 70,000. The table below shows the decrease in numbers used, in the last twenty years. In 1947, 45,000 were delivered to 18 investigators. In 1948, 15,000 were delivered to 20 investigators; 50,000 were ordered. In 1949, only 4,000 were delivered and in 1950 only 3,000. The following table is taken mostly from the report for 1950 of J. S. Rankin, Jr.

YEAR	NUMBER ORDERED	NUMBER COLLECTED
1933 and 1934	—	70,000
1939	21,000	20,000
1940	36,000	31,000
1941	31,000	28,000
1942–1946 (war years)	15,000 per year	13,000 per year
1947	54,000	45,000 (600 delivered daily to 18 investigators)
1948	50,000	15,000 (250 daily to 20 investigators)
1949	70,000	4,000 (45 daily)
1950	?	3,000 (3–7 daily)

In 1950, orders had to be kept over until they could be filled, owing to scarcity of animals. In 1951, 7,000 were brought in, from Menemsha Bight; 3,000 were returned to the sea as too small for laboratory use. In 1952, 11,000 were brought in from Fisher's Island on 7 trips. The supply in 1953, 1954 and 1955 was adequate.

The value of the *Arbacia* supplied may be judged by the fact that they sell for $ 13 a hundred at the Biological Supply House. As an interesting comparison, about 63,700 lbs. of lobster were brought in by local fishermen from the Woods Hole area in 1949; this is approximately 56,000 lobsters. In 1950 there were 44,500 lbs. or 39,000 lobsters. One lobster averages 1 1/8 lbs. (Data kindly supplied by Homer P. Smith, business manager of the M.B.L.). A lobster sells for $ .80 at Woods Hole, or $ 80 a hundred.

The cause of the decreasing quantities of *Arbacia* around Woods Hole is not known. Depth bombing during the war years has been suggested, and also the increase in numbers of starfish, their especial enemy. The scarcity may be due to climatic conditions, such as hard winters and the two recent hurricanes (1938, 1944). But it seems probable that it has been caused by over-collecting; most animals which are systematically taken for food or wearing apparel, e.g., lobsters, birds, seals, are protected by law in some way to prevent their extinction. *Arbacia* are not protected. Also the quantities of *Arbacia* needed for the newer chemical investigations of eggs and sperm are enormous in comparison with the few needed in the past, for morphological or even for physiological work.

Arbacia have been recorded as scarce several times previously. A survey was made by the U. S. Bureau of Fisheries in 1874 (Verrill and Smith), and again in 1902 (H. L. Clark) of the region around Woods

Hole and localities noted in which *Arbacia* and other Echinoderms occurred. It was found in a later report (Sumner, Osburn, and Cole, 1911) that *Arbacia* which had been abundant in the summer of 1903 were scarce after the severe winter of 1903–1904, in the summers of 1904 and 1905, and were not abundant for several years; in the summers of 1908 and 1909 large quantities were again obtained in Vineyard Sound. The following table is taken from the more complete table of Sumner, Osburn and Cole (1911, p. 114). The numbers in the first column are station designations (l.c., p. 201).

	1903	1904
7522 Nobska Light	Many	None
7523 ,, ,,	Several	1 spine
7524 ,, ,,	Very abundant	None
7530 ,, ,, , West Chop	Abundant	None
7532 West Chop, Tarpaulin Cove	Many	Few spines
7549 Tarpaulin Cove, Nobska	Many	Few fragments & spines
7563 Gay Head, West Chop	Many	Fragments & spines

In 1905, throughout Vineyard Sound as a whole, living *Arbacia* were taken only five times during the summer and never more than two at one time. *Strongylocentrotus dröbachiensis* and *Echinarachnius parma* were not similarly affected at this time (l.c., p. 115).

In the summer of 1911, there was again a dearth of *Arbacia* (Morse, 1912). They were particularly abundant in the summer of 1917, being taken at Kettle Cove, Quisset, North Falmouth, and off Nobska (Allee, 1919, 1923c). But in 1918, following the severe winter of 1917–1918, only a few were taken. They had recovered by 1920 (Allee, 1923a, c). The scarcity in 1949, however, followed a very mild winter, but the previous winter had been severe.

The earliest indication of any decrease in the numbers of *Arbacia* was made by Loeb as far back as 1900 when he says "The sea urchins have practically died out in the immediate neighborhood of the Woods Hole laboratory, and we have to send out the steam launch to collect them. For this reason even at the height of the spawning season there is little danger of the sea water containing spermatozoa in such quantities as to interfere with experiments with unfertilized eggs (Loeb 1900a, p. 450)."

A similar disappearance of the British sea urchin, *Echinus esculentus*, was noted in the deep water of Plymouth Sound (120 ft.) in 1899 after a south-west gale (MacBride, 1906, p. 504). *Psammechinus miliaris* dis-

appeared from Whitstable after an exceptionally cold winter of 1928–1929; it had been abundant there (Yonge, 1949, p. 278).

Temporary migrations have been reported for *Echinus esculentus* at Millport (Elmhirst, 1922; Stott, 1931). An inshore migration during the spawning season has been noted and a seaward movement caused by frosts and heavy weather. It has been suggested that migrations are due to food conditions (Gemmill, 1900). See also Deutler (1926, p.193). Nothing is known about any migrations of *Arbacia.*

There seems no doubt that the abundance of sea urchins, the localities in which they are found and the general condition of the animals vary from year to year; this applies especially to *Arbacia punctulata.*

e. Phototaxis and Light Reaction

Arbacia punctulata move away from the light and go to a shaded or darker region, by the combined action of spines and tube feet; certain individuals, however, go to the light (S. J. Holmes, 1912). Removal of the so-called eyes on the ocular plates does not change the reaction. Strong light on the tube feet causes them to be withdrawn. Many other experiments are described by Holmes.

The very young animals are often found in empty bivalve shells, probably to get away from the light. The smallest one yet brought in by the collectors at Woods Hole (diameter 6 mm. with spines) was found in an empty *Venus* shell. When taken out it was observed always to move to a shaded area, often under the empty shell, sometimes at the rate of 1 cm. in 5 minutes (E. B. H.).

Other species, *Arbacia lixula*, *Sphaerechinus granularis*, *Centrostephanus longispinus*, and *Paracentrotus lividus*, have also been found to move away from the light and seek the dark (von Uexküll, 1896; Mangold, 1909); and also *Psammechinus miliaris* (Bolin, 1926; Lindahl and Runnström, 1929). However Romanes (1885) says that "the *Echini* manifest a strong disposition to crawl toward and remain in the light" (p. 319).

Reactions to changes in light intensity have been studied in *A. lixula*, *Centrostephanus longispinus* and *Lytechinus variegatus*, especially with regard to the spines and pedicellariae, by von Uexküll (1896, 1899, 1900, 1909), Mangold (1909), and Cowles (1911), and more recently *Diadema antillarum* has been studied by Millot (1950, 1954, Phil. Trans. Roy. Soc. London B238: 187-220).

The habit of "decorating" or "masking" themselves with bits of shell and seaweed, characteristic of certain littoral species of sea urchin, has been attributed by some observers to protection against light (H. L.

Clark, 1921; Lindahl and Runnström, 1929, p. 407; Mortensen, 1943, M III, 3 p. 134).

"A number of specimens *(Psammechinus miliaris)* were held some time in a basin with running water, alternately kept in light or in total darkness. When in the light, the specimens covered themselves with pieces of algae, etc., when in the darkness they dropped these covering pieces" (Mortensen, 1943, M III, 3 p. 135). Several explanations for the habit other than light have been offered: concealment against enemies, camouflage, protection against drying in low water (Orton, 1929). Boone (1928) thought it must be to prevent detection by enemies or potential victims rather than protection against light, for he found in the case of *Lytechinus* that specimens kept in the laboratory at Miami, Fla. in relatively dark aquaria "for weeks at a time camouflaged themselves quite as thoroughly and industriously as their relatives on the open reefs" (p. 21). This has been criticised by Mortensen (1943, M III 2, p. 443).

The best known species having the habit of decorating themselves are *Lytechinus variegatus* (H. L. Clark, 1921; Boone, 1928; Mortensen, 1943 M III 2, p. 442); *Psammechinus miliaris* (Lindahl and Runnström, 1929; Orton, 1929; Mortensen, 1943 M III 3, p. 134); *Psammechinus microtuberculatus* (Noll, 1881); *Paracentrotus lividus* (Mortensen, 1927, p. 308; 1943, M III 3, p. 164); *Sphaerechinus granularis (Toxopneustes brevispinosus)* (Dohrn, 1875; Mortensen, 1943 M III 2, p. 522); *Tripneustes esculentus* (H. L. Clark, 1921), and the Japanese species *Strongylocentrotus pulcherrimus* (Dan, personal communication).

The ancients had a curious explanation for this habit, given by Pliny and referred to by many of the older writers, even as late as Camerarius in 1654 in his book on symbols which has an interesting picture of an urchin covered with stones (Fig. 3). They thought that at the approach of a storm the sea urchins cover themselves with small stones to provide a sort of ballast to prevent them rolling around and wearing away their spines. When the urchins were covered with stones it was an indication to sailors that a storm was approaching and they should anchor their ships (see under Historical).

f. Geotropism

No data have been found for geotropism in *Arbacia*, *punctulata* or *lixula*, but *Diadema* and *Lytechinus* are negatively geotropic, climbing upwards in an aquarium, irrespective of dark and light and also of access to oxygen (Parker, 1922, 1936). Other urchins found to be negatively

geotropic are: *Psammechinus microtuberculatus* (Baglioni, 1905) and *Psammechinus miliaris* (Bolin, 1926; Lindahl and Runnström, 1929).

The blastulae and gastrulae of *Arbacia punctulata* swim at the surface of the water; the plutei are scattered (Lyon, 1906b, 1907). The surface swimming is not due to light for it occurs in total darkness, nor is it due to oxygen supply for it takes place when the tube containing them is inverted. This is a true negative geotropism since the larvae are heavier than sea water; their specific gravity is about 1.06 (Lyon, 1906b, 1907). The blastulae from centrifuged eggs also come to the top, though the heavy pigment makes them heavier on one side (Lyon).

g. Locomotion

Sea urchins move in any direction by means of their spines and tube feet (Holmes, 1912). According to A. Agassiz (1872–1874, p. 264) "The mode of moving of *Arbacia* is quite different from that of our common *Strongylocentrotus;* instead of dragging itself along by means of the suckers of the actinal surface, it makes free use of its spines, and by a sort of tilting motion advances quite rapidly. The spathiform shape of the spines around the actinosome in species of this genus is undoubtedly due to the wear and tear produced by this means of locomotion."

Specimens of *Arbacia punctulata* have been observed to move over the glass of an aquarium at the rate of 35–40 mm. per minute after being disturbed; they average 22.2 mm. per minute when undisturbed (H. W. Jackson, 1939). I observed a very small *Arbacia*, 6 mm. in diameter (with spines) move at the rate of 3 mm. per minute.

Echinus sphaera (esculentus) and *lividus* (probably *Paracentrotus lividus*) moved along a horizontal surface 6 in. per minute, and up on a vertical surface 1 in. in 4 minutes (Romanes and Ewart, 1881; Romanes, 1885). Gemmill (1912) found that *Echinus esculentus* and *miliaris* usually moved, out of water, 1 in. in 5 minutes, the fastest being 3–4 in. in 5 minutes.

Lytechinus variegatus ascended a glass plate with an average speed of 1.8 mm. per minute, and a maximum speed of 12 mm.; on a vertical surface it progressed by means of its tube feet exclusively. On a horizontal surface of sand it progressed by means of its spines exclusively at the rate of 82 mm. per minute, average, with a maximum of 137 mm. (Parker, 1936).

The sand dollar, *Echinarachnius parma*, averaged 13.7 mm. per minute, with a maximum of 18 mm., when progressing in the sand (Parker, 1927).

As early as 1712, the locomotion of sea urchins aroused the attention

of Réaumur, who noticed that they used their spines as feet and marvelled at the number of muscles it must take to move 2,100 spines.

Arbacia punctulata can "right" themselves, i.e., turn over to the ventral side when placed on the dorsal side, easily and quickly, both large and small ones. In a flat-bottom glass dish, they raise themselves to their side sometimes within two minutes and then fall to the ventral side quickly by gravity (E. B. H.). Von Uexküll (1909, p. 106) says *A. lixula* cannot turn over on a flat surface, only on a slanting surface, but *A. punctulata* seems to have no difficulty, if in water. It cannot turn over when out of water.

The righting movements for *Echinus esculentus* and *P. lividus* have been described in detail with illustrations by Romanes and Ewart (1881) and Romanes (1885), and for *Arbacia lixula* by von Uexküll (1909, p. 106). Parker (1927) and Parker and Van Alstyne (1932) have described locomotion and righting movements in *Echinarachnius*.

Though locomotion is usually accomplished by the tube feet and spines, it can also be effected by the lantern as described by Romanes and Ewart (1881) and by Gemmill (1912) in *Echinus*. The lantern may be used both for progression and rotation when the animal is out of water.

CHAPTER 7

Sex and Breeding

a. Sex Ratio

To investigators working on *Arbacia* eggs, it always seems as though there were many more males than females. An accurate count of the animals shows that this is not so, but they are approximately equal in number. Shapiro (1935a) found that throughout one season the ratio was 1 male to 1.03 females (2,358 animals). Ikeda (1931) found the ratio in *Temnopleurus toreumaticus* was 1 female to 1.018 males (2,093 animals).

b. Sex dimorphism (Other Species)

No external morphological difference between the sexes has been detected in *Arbacia punctulata*, and this is true of most sea urchins. There are, however, a few species in which the males and females differ or have been reported to differ in size or shape of the test, in the genital pores or genital papillae, and in the color of the tube feet.

In *Arbacia lixula*, Cerami (1924, as quoted by La Cascia, 1930) thought that the male has a taller and rounder shell and a larger peristome and lantern, but La Cascia (1930), on measuring a greater number of specimens found no sex difference.

There is some disagreement about *Paracentrotus lividus*. O. Schmidt (1878 in Brehm's *Thierleben* Abt. 4, Bd. 2, Wirbellose Thiere) says that his boatman, in Lesina, could tell the males and females apart from the boat; the males were smaller, darker, and more spherical, the females flatter and more reddish violet. He says that his boatman was never deceived, but he had difficulty himself in distinguishing them. Camerano (1890) found no difference in color in the two sexes, but agreed with Schmidt that the males were smaller and the females flatter. However, La Cascia (1930), on making a more thorough study, concluded that one could not distinguish the males and females by shape or size. In the Japanese form, *Temnopleurus toreumaticus*, Ikeda (1931) found from a careful biometric study that the males are slightly

taller than the females; but this has been denied by Mortensen (1943, M. III, 2 p. 79 footnote).

In *Cidaris membranipora (nutrix)*, according to Studer (1880b) the shell of the female is flatter, while in *Hemiaster (Abatus) cavernosus*, the shell of the male is flatter. In *Goniocidaris canaliculata* there is no difference in external form, but there is a difference in the genital plates. The brood-pouches of these and several other forms also indicate the sex (Studer, 1880a) (Fig. 8).

In some forms, genital papillae are sometimes found at the opening of the genital ducts (see Bronn's *Thier-Reich*, 1904, Bd. II, Abt. 3, Buch IV, p. 1135), and are sometimes different in the two sexes. In *Echinocardium mediterraneum* the genital papilla of the female is thicker and shorter than that of the male (Hamann, 1887 and Plate XVIII, no. 7). In *Echinocyamus pusillus* it is much longer in the male, while in *Psammechinus miliaris* it is present in the male, often with red pigment, and is lacking in the female (Marx, 1929; see Grassé, vol. 11, p. 160). In this species, the difference between the males and females is sufficient for the sexes to be separated into different aquaria, as is done in Runnström's laboratory in Kristineberg, though sometimes there is a mistake and the female tank becomes clouded with sperm (personal communication).

M. M. Swann recently (1954) has found a slight difference in the sexes in *Echinus esculentus*, *Paracentrotus lividus*, *Psammechinus microtuberculatus*, *Ps. miliaris*, and *Sphaerechinus granularis*. "In the male the five genital pores are borne on short papillae... their edges are glistening white... In the female the genital apertures are not borne on papillae but are more or less sunk below the level of the surroundings... They are usually somewhat smaller than in the male and often oval in shape. The glistening white edges characteristic of the male papillae are never visible... The descriptions given do not apply to *Arbacia lixula*. Here the genital apertures are sunk in circular pits, and papillae may be present or absent in either sex."

In *Lytechinus anamesus* and *L. pictus*, the gonopores of the female are larger than those of the male (Tyler, 1944 and verified by C. B. Metz in 1953).

A rather striking sex dimorphism has been found by Motomura (1941a) in the Japanese species, *Strongylocentrotus pulcherrimus;* the tube feet on the oral side are yellow in the female and white in the male.

c. Sex Determination in Arbacia Punctulata

Though there is no morphological difference in the sexes in *Arbacia* there are several ways of distinguishing males and females. One method is by forced extrusion of gametes from a gonopore (E. B. Harvey, 1940d).

With a Luer syringe and very fine (no. 27) hypodermic needle, insert a drop of sea water saturated with KCl, into one genital pore. The operation is most easily done with an electric light shining from above on the aboral surface of the animal so as to show up the genital pores, and under a binocular microscope. A fine glass pipette, of slightly smaller bore than the genital pore can be used but is liable to break. A few eggs or a little sperm will almost immediately begin to ooze out from this pore alone, and the sex can be distinguished by the color (red ♀, white ♂). The shedding is stopped at once by placing the animal in a jar of still, not running, sea water; leave for a few hours before returning to running sea water. The readiness with which the Arbacia may be thus "sexed" depends somewhat on the maturity of the animal and the time of year.

In a second method a small amount of material is drawn out with a Luer syringe (and no. 27 needle), or fine glass pipette, applied at the opening of one of the genital ducts; it is examined in a drop of sea water under the microscope. As there is a sharp bend in the gonoduct just beneath the surface (see Fig. 7 from Brown's *Invertebrate Types*), care must be taken not to insert the needle into the coelomic cavity instead of the gonoduct. Also the red color may be due not to eggs but to red amoebocytes present in both sexes.

The electrical method of determining sex (E. B. Harvey, 1952, 1953, 1954) is by far the best for distinguishing male and female in *Arbacia* and other sea urchins. An alternating current of 10 volts is passed through the animal, which will at once shed its sperm or eggs. Ordinary 60-cycle alternating current can be used, with the 110-voltage, reduced to 10 volts by a transformer. Lead electrodes have been found best, as they are non-toxic and are easily made from lead tubing or from heavy lead wire. The electrodes are placed at any two points on the shell of the animal which lies, aboral side up, covered with sea water. Almost immediately after the current is passed, the eggs or sperm will exude from each of the five gonopores, the sperm in a thin white thread, and the eggs in a thicker red (in *Arbacia*) thread, later tending to clump (Plate XVI, Photographs 1, 2). When the current is stopped, the shedding ceases; but it begins again when the current is allowed to pass. In this way, the sex of the animal can be quickly determined, and a few eggs or a little sperm obtained without harming the animal; and the same animal can be used repeatedly. The eggs, if removed at once, fertilize perfectly and develop normally. The method is of great value in places where sea urchins have become scarce.

The rapid response of the sea urchin is due to the presence in the walls of the ovary and testis of a layer of smooth muscle cells which are stimulated by the electric current, causing the walls to contract and force out the eggs or sperm.

A much more elaborate electrical method was described several years ago by Iwata (1950) for *Mytilus* and for a Japanese species of sea urchin, *Heliocidaris crassispina*. The simple method described above has also been used successfully on the sand dollar and on several species of annelids.

Sea urchins containing only immature eggs do not respond. In some species a higher voltage may be required.

d. Hermaphroditism

Hermaphroditism is quite rare in *Arbacia*, as also in other sea urchins, but it does occur. Among the many thousands of *Arbacia* opened in the course of twenty-five summers at Woods Hole, I have found only two cases of well marked hermaphroditism. One was fully described by me in 1939a. This animal appeared to have four red ovaries and one whitish testis with oozing sperm. On careful examination it was found that each of these gonads had a very slight amount of tissue of the opposite sex. The eggs from this animal were readily self-fertilized and developed normally into normal plutei. One summer (July 10, 1946, unpub.) an animal was opened which was to all appearances a female, but some of the eggs became fertilized without applying sperm, and no male had been opened, so that contamination was unlikely. On a careful examination, it was found that there were small bits of testis tissue in two of the ovaries, and sperm from this tissue, after becoming motile in sea water, would fertilize the eggs. It seems likely from this experience, that most cases of supposed contamination resulting in accidental (and provoking) fertilization of eggs, such as many competent investigators have occasionally observed, are due not to carelessness in technique but to a very slight hermaphroditism of the animal, the testis tissue being too small to be detected.

Three other cases of hermaphroditism in *Arbacia punctulata* have been reported, two by Heilbrunn (1929). In one of these, there were four ovaries and one ovotestis, and in the other, two ovaries, two testes, and one ovotestis. Both of these gave rise to normal larvae when self-fertilized. Shapiro (1935a) described an animal with four testes and one ovary, but the development of the eggs when self-fertilized was abnormal and only a few developed to gastrulae. K. C. Fisher found a case of hermaphroditism (July 10, 1942 unpub.) among the *Arbacia* used in his physiology class at Woods Hole; this animal had three ovaries with some testis tissue and two testes. These eggs when self-fertilized developed normally into plutei. In 1954, Mrs. Cornman observed an *Arbacia* spawning spontaneously when the collector brought in the animals. Eggs came from four gonopores, sperm from one. The spawning could be controlled at will with the electric current applied to the shell (see under c. Sex Determination).

References to hermaphroditism in other species of sea urchins and above data for *Arbacia punctulata* are given below.

Arbacia punctulata

Heilbrunn (1929); 4 ♀, 1 ☿; 2 ♀, 2 ♂, 1 ☿.
Shapiro (1935a); 1 ♀, 4 ♂.
E. B. Harvey (1939a); 5 ☿.
(1946 unpub.); 3 ♀, 2 ☿.
K. C. Fisher (1942, unpub.); 2 ♂, 3 ☿.
M. E. Cornman (1954, unpub.); 4 ♀, 1 ♂.

Arbacia lixula (pustulosa)

J. Gray (1921); questionable.
Reverberi (1940); 4 ♀, 1 ♂; 3 ♀, 2 ♂.
(1947); 3 ♀, 1 ♂, 1 ☿; 1♀, 4 ♂; 1 ♀, 4 ♂; 1 ♀, 1 ♂, 3 ☿.
Neefs (1953); ☿.

Dendraster excentricus

Needham and A. R. Moore (1929); 2½ ♀, 2½ ♂.

Echinocardium cordatum

Giard (1900); protandrous.
H. B. Moore (1935b); sex not stated.

Echinus esculentus

H. B. Moore (1932); 4 ♀, 1 ♂.

Paracentrotus lividus

Fuchs (1914a); 3 ♀, 2 ☿.
Herlant (1918); 4 ♂, 1 ☿.
J. Gray (1921); same as Fuchs (1914a).
Drzewina and Bohn (1924); 4 ♀. 1 ♂.
Neefs (1937); ☿.

Psammechinus microtuberculatus

Herbst (1925 unpub. See Tab. Biol. VI, p. 501, 507); 3 ♀, 2 ♂.
Reverberi (1947); 1 ♂, 4 ☿.

Sphaerechinus granularis

Viguier (1900); sex not stated.
Neefs (1952); ☿.

Strongylocentrotus dröbachiensis

Gadd (1907); 4 ♀, 1 ♂.

e. Breeding Season; Shedding of Eggs and Sperm

With few exceptions, the unfertilized eggs and the sperm of sea urchins are shed in the sea water; the eggs are there fertilized and develop into free-swimming larvae or plutei which pass through a complicated metamorphosis before acquiring the adult form. This is true of *Arbacia punctulata*. However, a few viviparous species of *Echinoidea* have been described, mostly from the southern hemisphere. The eggs which are usually very large, 1–2 mm. (Hesse u. Doflein, 1914, II p. 619; Tab. Biol. VI, p. 503), develop in a sort of brood pouch formed by the long spines, either dorsally or ventrally. The larval stage is usually imperfectly developed or omitted entirely and the animals come out as young adults. Among these may be mentioned: *Anochanus sinensis* from the China Sea, as described by Grube (1868); *Hemiaster (Abatus) cordatus*, collected on the Transit of Venus expedition (1874–1875) by Dr. Jerome H. Kidder (1875–1876), near the Kerguelen Islands and described by A. Agassiz (1876); the same species and *Cidaris nutrix* and *Goniocidaris canaliculata*, collected on the Challenger expedition (1873–1876) near the Kerguelen Islands and the Falkland Islands and described by Wyville Thomson (1877, 1878). One of his interesting woodcuts is reproduced as Fig. 8 in this monograph; several of them are reproduced in MacBride's text-book (1906, p. 535, 555, 603). An account of these and other *Echinoidea* collected on the Challenger Expedition may be found in A. Agassiz's report (1881). *Hypsiechinus coronatus* and *Goniocidaris umbraculum* and other species are described by Mortensen (1927a, p. 293; 1927b; 1931, p. 7).

The breeding season for *Arbacia punctulata* at Woods Hole is from the middle of June till the middle of August, though the season varies from year to year according to temperature. During May and early June, the gonads are small and it is often difficult to tell whether they are ovaries or testes. The males ripen earlier and remain in good condition later than the females. The eggs mature in the ovary, so that when they are shed or procured from the ovary during the season most of them have already given off (and lost) their polar bodies, and the eggs are ready for fertilization and immediate development. For about a week before the middle of June, the eggs are ripening and all sizes of eggs in the germinal vesicle stage and all stages of polar body formation are then found. Some immature eggs are often found among the ripe eggs later on until towards the end of the season. There are usually many immature eggs late in the season in animals from the new beds at Menemsha Bight and Fisher's Island.

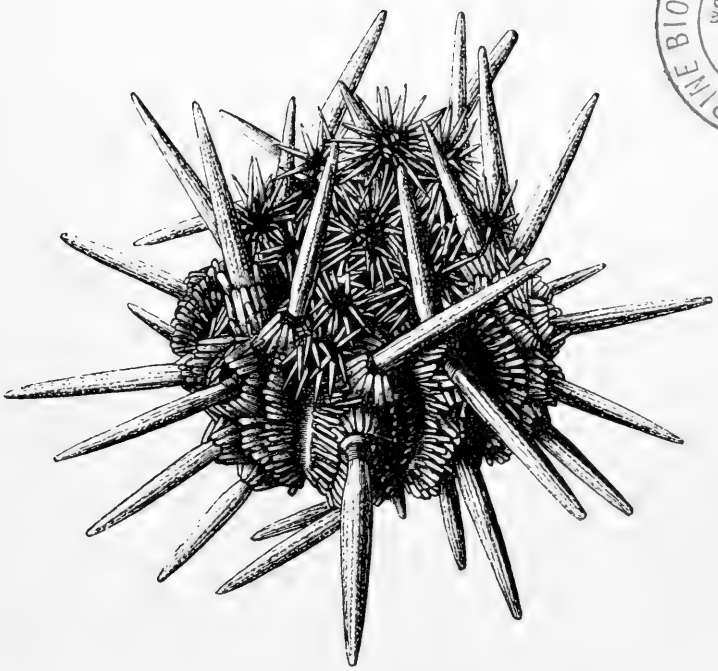


FIG. 8. Young sea urchins in brood pouch of the Falkland Islands sea urchin, *Goniocidaris canaliculata*. After Sir C. Wyville Thomson in *The Voyage of the "Challenger" The Atlantic*. Vol. II, p. 224, 1877.

Arbacia is different from the starfish, *Asterias forbesi*, which sheds its eggs in the germinal vesicle stage, and maturation of the eggs takes place after they are laid. These (starfish) eggs should be fertilized during or just after the first maturation division, one to one and a half hours after removal from the animal.

When *Arbacia* are brought in from the sea after the middle of August, many of them have already shed. By September first, most of the animals have shed, although one finds a few with eggs and many with sperm. If, however, the animals are brought into the laboratory earlier in the season before they have shed, and kept in aquaria (capacity 16 gallons or more) with running sea water, they retain their eggs and sperm and remain in good condition through October (E. B. Harvey, 1939b). The animals require no special food, but apparently they eat

each other. It is quite essential that they are not overcrowded (150–200 in an average sized aquarium, 16 gallons), that there is a steady flow of salt water, and that the water is kept unpolluted. Any unfavorable condition will cause the animals to shed, and when one sheds the others are likely to shed also. Two lots of six animals each were kept, one set in the light and one set in the dark from July 1 to Sept. 16, 1948, and all were in perfect condition at the end of that time.

The eggs from animals kept for some time in the aquaria are slightly different late in the season, taking longer to cleave, irrespective of temperature, and they are more viscous, taking longer to stratify and break apart with centrifugal force; also some of the red pigment granules remain in the white half. The rate of oxygen consumption is decreased (Shapiro, 1935c). The amount of carbohydrate and of phosphorus is greater and of nitrogen is less in the stored eggs (Hutchens, Keltch, Krahl, and Clowes, 1942; Crane, 1947). It seems probable that there are other differences of a physiological or chemical nature, but for many purposes the eggs are quite suitable to use as they develop normally and give normal plutei. A difference between summer and winter eggs has been observed for *Paracentrotus lividus* at Naples (Hörstadius, 1925). Some eggs obtained from *A. punctulata* which had been kept at Woods Hole until the middle of January appeared normal except that they were pale. These, when fertilized, developed to blastulae (A. E. Navez, personal communication, 1936). Good sperm has been obtained from animals kept over winter until March; the females from this lot had full ovaries, but only degenerate and abnormal eggs which had apparently not been shed the previous season (E. B. Harvey, 1942).

It would seem that in general at Woods Hole under natural conditions, *A. punctulata* shed their eggs, when fully ripe, the latter part of the summer, then do not have ripe eggs again until the following summer. It is quite possible, however, under laboratory conditions for a ripe animal to shed some of its eggs at one time and more later on.

The breeding season of *A. punctulata* at Beaufort, N. C., is considerably in advance of that at Woods Hole. They are fully ripe and in fine condition during May (E. B. H.). Some animals have ripe eggs and sperm in January (C. B. Metz, personal communication, Jan. 1951). In Florida, at Alligator Harbor, they are ripe from September until June and in fine condition in February, March, and April (C. B. Metz, personal communication, Jan. 1955).

The closely related species, *A. lixula*, which is common at Naples, is ripe all the year according to Lo Bianco (1909), but the eggs are bad

at certain periods, especially in September (R. D. Allen, personal communication, Jan. 1955). It has been stated by Orton (1920) that in parts of the sea where conditions such as temperature do not change much, marine animals will breed continuously. This would apply to the tropics. He bases his conclusion on the statement of Semper (1881, p. 136) in *Animal Life* that in the Philippines he "could not detect a single species of which he could not at all seasons find fully grown specimens, young ones and freshly deposited eggs." Mortensen (1921, p. 245; 1938, p. 12), however, has found this not to be true of several species in the tropics which had no ripe eggs when he examined them, e.g., *Diadema antillarum* and *Echinometra vanbrunti*. He thinks it likely that some species in the tropics have more than one breeding season.

O. Koehler (1916, p. 258) made a special study of *Paracentrotus lividus* at Naples, examining a group of 20 animals every five days for two months (November, February), and found no regular variation in size or content of the gonads. He had an ingenious method of cutting a window in the test to obtain a piece of gonad, then sealing it over with wax, using the same gonad for successive observations (p. 127). He found that during the summer in aquaria at Naples it took one and a half to two months to form ripe genital products (p. 257). Fox (1924a), however, using Koehler's window technique, found that at Roscoff during the summer (water temperature 17–19 °C.), *Paracentrotus lividus* which just after spawning contained no ripe eggs, gave ripe eggs in abundance after nine days. In the one form, *Diadema* at Suez, that has been shown to have a lunar periodicity (see below), it must take about four weeks (temperature of sea water 26–29 °C.) to form new genital products (Fox 1924b). Monroy (personal communication) finds that at Naples the sea urchins usually shed after a storm or the scirocco, and it takes a week before one can obtain new eggs. Jacques Loeb (1915a) observed that at Pacific Grove, California, *Strongylocentrotus purpuratus* in a certain region (temperature 12–15 °C.) shed their eggs and sperm one day in March, and immature eggs began to appear during the next week and ripe eggs in about ten days. Similarly Tennent (1910) observed that *Lytechinus variegatus* at Beaufort, N. C. again had ripe eggs a week after he had found them empty. Tyler (1949) obtains new batches of eggs from *Lytechinus* and *Strongylocentrotus* of the west coast at two week intervals after forced shedding, during the breeding seasons. Whether the eggs obtained two weeks after forced shedding in *Arbacia* at Woods Hole are from a regenerated ovary as Tyler thinks, or are the eggs left from an incomplete previous shedding, it is difficult to say, though this could be determined by Koehler's

window technique (1915, p. 127). I have found no evidence of immature or maturing eggs, indicative of a regenerating ovary, either when examined fresh or in sectioned material a week or ten days after forced shedding from animals fully ripe; the ovaries are full of ripe mature eggs which were obviously not shed when the others were.

f. Lunar Periodicity (Other Species)

The idea that sea urchins and other sea animals are full of eggs at the period of full moon was quite generally held by the ancients. Both Aristotle and Pliny expressed this belief, and other classical writers, e.g., Lucilius and St. Augustine (see under Historical). The belief prevailed through the middle ages and is still held by the fishermen around the Mediterranean. No periodicity has been verified by investigators at the Naples Station. However, one of the earliest workers on fertilization of sea urchin eggs, Fol (1879, p. 86), states that in *Toxopneustes lividus (Paracentrotus lividus)* and *Sphaerechinus brevispinosus (S. granularis)* at Messina, the sexual products are liberated each month at full moon, and the animals are then empty for a few days; there are more of the sexual products in spring and summer than in fall and winter; he says that the fishermen are aware of these facts, since sea urchins are used for the table. There is some evidence that *Lytechinus (Toxopneustes) variegatus* at Beaufort is ripe at full moon and empty just after (Tennent, 1910a, p. 658 footnote) but the evidence of lunar periodicity is not conclusive (Tennent, Gardiner, and Smith, 1931, p. 7). It is said that in Greece the gonads of sea urchins used for food appear on the market only during certain phases of the moon and at other times the animals are spent (Just, 1919, II, p. 17 footnote, quoting Tennent).

A special study of lunar periodicity was made by Fox (1924a, b) who found that there *is* a lunar periodicity in the Red Sea form *Centrechinus (Diadema) setosus* at Suez. At full moon during the breeding season, an animal spawns, becomes again full of ripe eggs or sperm at the next full moon. But at nearby Alexandria, there was no periodicity in the Mediterranean form *Paracentrotus lividus* and none in this species at Naples, Marseilles, or Roscoff. Fox is of the opinion that the belief in periodicity spread in ancient times from Suez, where it does occur, to Greece and the Mediterranean countries where it does not occur, and the belief persists to the present day (see also Zirpolo, 1929). Mortensen (1937, III) could not confirm Fox's observations on *Diadema setosum*, finding that ripe specimens could be obtained irre-

spective of the full moon near Suez. In a later paper, however (1938, IV), he agrees with Fox with regard to *Diadema*, but for other echinoderms studied "there is not much support for a lunar periodicity," and he considers lunar periodicity in the Echinoderms near Suez a rare exception (p. 12).

There is no evidence of periodicity in *Arbacia punctulata*, but the lunar periodicity of the worm *Nereis limbata* at Woods Hole is well established (Lillie and Just, 1913), also of the "fireworm" in Bermuda, of the palolo worm and of many other animals. A good evaluation of lunar periodicity in sea urchins and other animals is given by Korringa, 1947, *Ecological Monographs*, 17 : 349–381.

g. Forced Shedding

Arbacia punctulata may be induced to shed its eggs and sperm by cutting the animal or injuring it in other ways. Miss Palmer (1935, 1937) discovered that spawning may be induced by injecting tissue extract, KCl, or $CaCl_2$ into the perivisceral cavity through a slit in the test. I found that one has merely to inject with a pipette some KCl or NaCl solutions into the mouth, through the teeth to induce shedding (E. B. Harvey, 1939c). Tyler (1949a) advocates an injection of 0.5 cc. of 0.5 M KCl which is practically isosmotic, into the body cavity of an average sized Arbacia. The animal, after injection, is held over a dish of sea water into which the eggs or sperm are shed. Although eggs may be collected in this way and the animal remain uninjured, it has been my experience that sometimes the eggs are not fertilizable, even after repeated washings with sea water (E. B. Harvey, 1939b). This has also been the experience of many other investigators, especially during the summer of 1950 when the scarcity of *Arbacia* necessitated conservation of the animals. It would seem that there is some substance on the outside of the shell, a dermal secretion perhaps, which is toxic to the eggs, rather than the perivisceral fluid which has been often considered toxic (see under *Perivisceral Fluid*, Part IV, especially reference to Pequegnat, 1948). If one has a female half covered with sea water into which the eggs are being shed, one finds that the shed eggs will not form a fertilization membrane or be fertilized on addition of sperm, whereas the eggs from inside the animal give 100% fertilization. The same observations had been made previously by Ohshima (1921). Professor Runnström, during the summer of 1950 at Woods Hole, found that the toxic effect could be overcome by the addition of sodium periodate (5×10^{-5} M) to the sea water (personal communication).

There is of course a great advantage in procuring large quantities of eggs by the KCl method, without destroying the animals. Forced shedding by the electrical method has already been described in *Section c* of this chapter.

h. Removal of Gonads

The old method of obtaining eggs and sperm, practiced for many years, was to remove the ovaries and testes intact from the animal. If animals are not scarce, or if for some reason shedding with KCl or electricity is contra-indicated, the following procedure is advocated (E. B. Harvey, 1939b).

Wash the animal with running cold fresh water for a moment or two to kill any sperm possibly adhering to the shell. Take the animal, oral (teeth) side up, and cut around the shell with scissors, at about its widest circumference; then remove the upper (oral) part of the shell. The five gonads are now in view in the lower part, the ovaries red, the testes white.

To prepare the eggs for experimental work, run a pair of curved forceps gently under an ovary, to snip the duct, and then remove it intact to a finger bowl about a quarter full of fresh sea water. Remove the other four ovaries in the same way. Contrary to the prevalent opinion, the fluid in the body cavity (in a small amount), is not toxic to the eggs. Let the bowl stand for five or ten minutes so that the ripe eggs may flow out of the ovaries. Then put a piece of cheese cloth whose holes are five to ten times the diameter of the egg (i.e. about 0.5 mm.) and which has been wet with sea water, over another finger bowl and pour the egg suspension through. The débris and pieces of tissue will be be held back and you will have eggs free and clean in the dish, ready for use. To keep the eggs for several hours, there should not be too many eggs in the dish, just enough to form a thin layer on the bottom. The bowls of eggs are best kept on the floor of the cement aquarium tables where cool water will flow around them, and they should be kept covered to prevent evaporation. The eggs treated in this way are suitable for use throughout the day though they change slightly on standing. Individual batches of eggs vary greatly in shape, percentage of fertilization, reaction to centrifugal force, etc. Any batch which does not give 98% fertilization membranes or which shows abnormalities in cleavage should be discarded.

The testes should be removed with curved forceps in the same way as the ovaries, but put into a small salt-cellar or a very small Stender dish (3 cm. diameter) without water. This dish should be covered and kept cool. The sperm are inactive when kept concentrated, but become active in sea water, soon wearing themselves out. If the dish of sperm is placed immediately in the refrigerator and kept at about 8° C, the sperm will keep perfectly for 4–5 days. After dilution, the sperm are in optimum condition for only an hour or so.

After opening a male, care should be taken to prevent contamination of females opened subsequently. Scissors and forceps should be put into a bowl of tap water and one's hands should be thoroughly washed, immediately after opening an animal.

CHAPTER 8

Species of Echinoidea near Woods Hole, Mass, and Arbacia lixula (pustulosa)

There are three species of *Echinoidea* which occur in the vicinity of Woods Hole. *Arbacia punctulata* is, or has been, the most common species and the one most used for experimental work as the eggs are ripe most all summer.

The "green" urchin, *Strongylocentrotus dröbachiensis*, has been taken in deep water in Vineyard Sound, off Gay Head, Nantucket, and many places off Cape Cod, Barnstable, Chatham, Provincetown, and off George's Bank 75 miles east of Cape Cod. It is a northern species, being circumpolar in the Arctic, and occurs in the north on both the Atlantic and Pacific coasts of North America, on the east coast as far south as New Jersey. It breeds, however, in the early spring, and is spent and of no use for experimental work at Woods Hole during the summer. It occurs in great abundance at the Mt. Desert Laboratory at Salisbury Cove, Maine, and is ripe there also in the spring, March and April. It is full of (mostly) unripe eggs in January and February, full of ripe eggs in March and April, and is spent by May 15, having no ripe eggs in the summer and fall, though some males have ripe sperm. The animals were shipped to me to Princeton for several years (1936–1942) in the spring, for work on hybridization (E. B. Harvey, 1942). The eggs were fertilized and developed normally to plutei, provided they were kept in the cold, at about 10° C.

The egg of *Strongylocentrotus dröbachiensis* is 160 μ in diameter, is unpigmented and has a jelly coat about 96 μ thick. After fertilization, there is a large perivitelline space of about 30 μ; the hyaline layer is well formed after an hour (at 10° C.) when it is about 3 μ thick. First cleavage takes place in 2–3 hours at 10° C. With centrifugal force, the unfertilized mature egg stratifies with oil at the centripetal pole, then yolk granules, clear layer, and mitochondria at the centrifugal pole; the nucleus (14 μ in diameter) lies under the oil cap. There is also, sometimes, a clear layer under the oil instead of, or in addition to, the clear layer beneath the yolk. The egg breaks with a centrifugal force of 12,000 × g for twelve minutes, just above the clear layer under the

yolk, into a large nucleate half of 152 μ diameter and a very small lower half of 80 μ diameter. When fertilized, the lower non-nucleate half containing a little yolk, clear layer, and mitochondria, develops much better than the upper half containing oil and yolk and the nucleus.

Further information concerning the distribution, morphology, and larval development may be found in Mortensen's *Monograph*, 1943, vol. III, Part. 3, p. 198.

The sand dollar, *Echinarachnius parma*, is abundant at Woods Hole and breeds during the summer, so that it is excellent material for experimental work at the Marine Biological Laboratory. The eggs are best early in the season, June and July. The egg is large, 145 μ in diameter, and is unpigmented. It is surrounded by a jelly coat 95 μ thick or less, in which are imbedded large red pigment granules 6–8 μ in diameter. After fertilization there is a large perivitelline space 30 μ across; the hyaline layer is so thin as to be unmeasureable at any time. Development is a little slower than in Arbacia; first cleavage occurs at 23° C. in 1¼ hours (*Arbacia* 50 min.), and they swim in 16 hours (*Arbacia* 8 hours). *Echinarachnius parma* from Maine is a larger animal, averaging 8 cm. in diameter while the one at Woods Hole averages 5 cm., but the egg measurements are the same. With centrifugal force, the mature unfertilized egg stratifies with yolk granules above, then the clear layer, then granules consisting of two kinds, the upper, finer granules being mitochondria, staining with methyl green and Janus green, and the bottom layer of coarser granules and staining with neutral red and methylene blue. Usually there is no oil cap, but sometimes there are a few oil drops at the centripetal pole; the nucleus lies as usual at the centripetal pole. There is sometimes a clear layer here as well as below, as in photograph 14 of Costello (1939). With sufficient force, 10,000 × g for twelve minutes, the egg breaks across the clear layer into a large upper half (126 μ diameter) and a smaller lower half (100 μ diameter). Both parts develop after fertilization. See Costello, 1932, 1939.

Two other species of *Echinoidea* were dredged by fishermen in August 1954, about 600 ft. down, 90 miles south east of No Man's Land. One was a large dull grey urchin with heavy spines, tentatively identified as *Cidaris abyssicola* A. Agassiz. The other was a rather beautiful smaller urchin, reddish with long spines, tentatively identified as *Coelopleurus floridanus* A. Agassiz. These were quite new to the Woods Hole region. Both are described by Mortensen, the first in his *Monograph* I : 301, and the second in II : 612.

There is another species of Arbacia, *A. lixula (pustulosa)*, accessible

to investigators who work at the Stazione Zoologica in Naples and some other marine stations in Europe. The eggs of the two species are similar, but that of *A. lixula* is a little larger (79 μ diameter) than that of *A. punctulata* (74 μ diameter), is more heavily pigmented, has a smaller perivitelline space (1–2 μ), and a thicker hyaline layer (3 μ on fertilization) E. B. Harvey, 1933a, 1934, 1938a. It stratifies similarly with centrifugal force and is broken into two halves with about the same force (10,000 × g for three minutes), but with this force into almost equal halves, the upper half containing oil, clear layer, mitochondria, a little yolk, and the nucleus, and the lower half containing yolk and pigment (E. B. Harvey, 1933a, 1938a). See also Mortensen's *Monograph* II : 566.

There are four other species of *Arbacia* besides *A. punctulata* and *A. lixula*. These are: *A. stellata*, or *incisa*, *A. spatuligera*, *A. crassispina*, and *A. dufresni*. These are listed in the Classification, with localities where they occur and references to Mortensen's *Monograph*.

CHAPTER 9

Classification

There are about 6,700 species of Echinoidea (Grassé, 1948, p. 162) of which about 600 species are living (H. L. Clark, 1946, p, 277), the rest fossil. In the family *Arbaciidae*, there are 8 recent and 14 fossil genera (H. L. Clark, 1946, p. 306; Mortensen, 1935, M II, p. 547). In the genus *Arbacia* there are 6 species, all living. All Echinoderms live in salt water.

The following classification of the *Echinoidea* is based on H. L. Clark's *Catalogue of the Recent Sea Urchins* (1925), together with some later papers and personal communications with Prof. Clark in 1936 and 1947. He differs in certain cases from Th. Mortensen, whose classification is given in the *Handbook of the Echinoderms of the British Isles* (1927), and in his *Monograph* (1928–1951). For simplification, only species are here listed which occur in regions most visited by investigators from Woods Hole, or which are referred to in this Monograph. All species of the genus *Arbacia* are listed.

Much of the data has been taken from Mortensen's comprehensive *Monograph* of 1928–1951, referred to in the text as M followed by the appropriate volume and page; these references are given after each species, and the reader may here obtain practically all the available information, and references to all the literature relative to that particular species. Many of the references on egg size are from sources not published but obtained through correspondence or from my own notes. When several authorities agree on the size of egg, only one authority is given.

CLASS ECHINOIDEA (OUTLINE)

Order I Cidaroida
  Family 1 Cidaridae
Order II Centrechinoida (Diadematoida [1])
  Suborder A Aulodonta
    Family 1 Centrechinidae (Diadematidae)
  Suborder B Stirodonta
    Family 1 Arbaciidae
      Genus a Arbacia
      Genus b Tetrapygus
      Other genera (6) not described
  Suborder C Camarodonta
    Family 1 Temnopleuridae
    Family 2 Echinidae
    Family 3 Strongylocentrotidae
    Family 4 Echinometridae
Order III Exocycloida
  Suborder A Clypeastrina
    Family 1 Clypeastridae

[1] Diadematoida, Diadema, etc. should be used instead of Centrechinoida, etc. See footnote in chapter 2 d, p. 17.

Family 2 Laganidae
Family 3 Fibulariidae
Family 4 Scutellidae
Suborder B Echinoneina
Family 1 Echinoneidae
Suborder C Cassidulina
Family 1 Neolampadidae
Suborder D Spatangina
Family 1 Hemiasteridae
Family 2 Spatangidae

Class Echinoidea

Order I Cidaroida
Family 1 Cidaridae
Cidaris cidaris (Linné) (Dorocidaris papillata). See M I : 300. Iceland, Norway, Southeast England, Ireland, Spain, Mediterranean, Naples, Malta, Azores, Madeira, Canaries, Cape Verde. Egg 160–180 μ (Prouho, 1887). M I : 289.
Stylocidaris affinis (Philippi) (Dorocidaris papillata). Confused, especially at Naples, with Cidaris cidaris. See Mortensen, M I, p. 293, 341. Florida, Bermuda, West Indies, Gulf of Mexico, Mediterranean, Naples, Malta, Nice, Madeira, Canaries, Cape Verde. M I : 336.
Eucidaris (Cidaris) tribuloides (Lamarck). "Slate pencil urchin." South Carolina to Brazil, Bermuda, Bahamas, West Indies, Jamaica, Mexico, Azores, Africa, Cape Verde. Egg Bermuda 70 μ (E.B.H., 1947, unpub.); Jamaica 70 μ (Tennent, 1922). M I : 400.
Ctenocidaris (Cidaris, Eurocidaris, Stereocidaris) nutrix (Wyv. Thomson). Kerguelen Island. Egg 2 mm. (Hesse and Doflein, 1910–1914, Bd. I : 583; II : 619. M I : 128.
Goniocidaris canaliculata A. Agassiz (Aporocidaris antarctica Mortensen). Falkland and Kerguelen Islands. M I : 116.
Goniocidaris umbraculum Hutton. New Zealand. M I : 164.
Order II Centrechinoida (Diadematoida) see footnote on preceding page.
Suborder A Aulodonta
Family 1 Centrechinidae (Diadematidae, old name, still used by Mortensen)
Centrechinus (Diadema) antillarum (Philippi). Florida, Bermuda, West Indies, Jamaica to Surinam, Mexico, Azores, Madeira, Canaries to Cape Verde. Very long spines, can be 40 cm. Egg Bermuda 71 μ (E.B.H., 1947 unpub.); Tobago 70 μ (Mortensen, 1921). M III, 1 : 269.
Centrechinus (Diadema) setosus (Jackson). Suez, Red Sea, African coast to Durban, Madagascar, Australia, Java, Amboina, South Seas, Japan, Misaki. Egg about 100 μ, Amboina (Mortensen, 1931), 91.6 μ Misaki (Dan, 1952 unpub.). M III, 1 : 256.
Centrostephanus longispinus (Philippi). Mediterranean, Naples, Nice, Morocco to Cape Verde, Azores, Canaries. M III, 1 : 300.
Centrostephanus coronatus (Verrill). Southern California, Corona del Mar, Santa Catalina Island, Gulf of California. M III, 1 : 314.
Suborder B Stirodonta
Family 1 Arbaciidae M II : 529
Genus a Arbacia
Arbacia punctulata (Lamarck). East coast, Cape Cod to Florida, Woods Hole, Beaufort, Tortugas, Yucatan, Cuba, Curaçao, Tobago, Trinidad; *not* Bermuda, Jamaica, Puerto Rico or Lesser Antilles. Egg Woods Hole 74 μ (E.B.H. 1936); Beaufort 80 μ (E.B.H., 1942 unpub.). M II : 573.
Arbacia lixula (Linné) (Echinocidaris des Moulins, A. pustulosa (Leske, A. aequituberculata (Blainville), A. africana (Troschel). Mediterranean, Naples, Monaco, Nice, Marseilles, Spain, Atlantic Coast of North Africa, Guinea, Gold Coast, Azores, Madeira, Canaries, Brazil. Egg 79 μ (E.B.H., 1933a; 1938a). M II : 566.

Arbacia incisa or stellata (Blainville; ? Gmelin); incisa preferred by Clark, stellata by Mortensen; see Clark, 1948, p. 245. Lower California to Peru, Gulf of California, Mexico, Gulf of Panama. M II : 575.

Arbacia spatuligera (Valenciennes). West Coast of South America, Peru. M II : 577

Arbacia crassispina Mortensen. Only from Tristan da Cunha, Nightingale Island. M II : 580.

Arbacia dufresnii (Blainville) (A. alternans (Troschel)). South Coast of South America, Chile, Strait of Magellan. M II : 579.

Genus b Tetrapygus

Tetrapygus (Arbacia, Echinocidaris) niger (Molina). South America, Peru, Chile, M II : 582.

There are 6 other living genera and 14 fossil genera listed by Mortensen, 1935, M II : 547. See also Clark, 1946, p. 306. The living genera are: Arbaciella, Coelopleurus, and the rare genera, Pygmaeocidaris, Dialithocidaris, Habrocidaris, and Podocidaris.

Suborder C Camarodonta

Family 1 Temnopleuridae

Temnopleurus toreumaticus (Leske). Indo-West Pacific, Japan to African East Coast, Amakusa, Misaki, Korea, China, Philippines, Singapore, East Indies, Australia, Ceylon, India, Iran. Egg 80 μ, preserved (Onoda, 1936). M III, 2 : 76.

Temnopleurus hardwickii (Gray). Japan, Hakodate, Asamushi, Misaki, Korea, China. Egg 115 μ. (Motomura, 1950 unpub.). M III, 2 : 84.

Mespilia globulus (Linné). Japan, Seto, Misaki, Korea, Phillipines, Malay region, Samoa. Egg 80 μ (Onoda, 1936); 110.8 μ (Dan, 1952 unpub.). M III, 2 : 177.

Salmacis virgulata L. Agassiz. Java Sea, Singapore; Indian Ocean to Ceylon, Torres Str., East Australia. May be confused with Temnopleurus alexandri. M III, 2 : 134.

Salmacis bicolor L. Agassiz. Indian Ocean, Java Sea. Egg 100 μ (Aiyar, 1935). M III, 2 : 112.

Hypsiechinus coronatus Mortensen. Brood protecting. Southwest of Iceland, Denmark Strait. M III, 2 : 296.

Family 2 Echinidae (Mortensen recognizes a family Toxopneustidae which seems superflous to Clark.)

Echinus esculentus Linné (E. sphaera). England, Plymouth, Ireland, Scotland, Denmark, Sweden, Norway, Iceland, France, Roscoff, to Portugal, southern limit. One of largest urchins may measure 20 cm. Egg 180 μ Plymouth (Shearer, de Morgan, and Fuchs, 1914, p. 267); 145.9 μ Sweden (Borei, 1935 unpub.). M III, 3 : 25.

Echinus melo Lamarck. Mediterranean, Naples, Portugal, West Africa to Cape Verde, Canaries, Azores, West Ireland. M III, 3 : 53.

Echinus acutus Lamarck (E. flemingii). England, Plymouth, Ireland, Denmark, Norway, Iceland, Mediterranean, Naples, Nice. Egg 130–140 μ (Shearer, de Morgan, and Fuchs, 1914; p. 267). M III, 3 : 41.

Psammechinus (Echinus, Parechinus) microtuberculatus (Blainville). Western Mediterranean, Naples, Adriatic, Trieste. Egg 102 μ Naples (E.B.H., 1933a; 1938a). M III, 3 : 139.

Psammechinus (Echinus, Parechinus) miliaris (P. L. S. Müller; Gmelin). Two distinct types, S- and Z- (Lindahl and Runnström, 1929). Iceland, Norway, Sweden, Denmark, Scotland, Ireland, England, Plymouth, N. W. coast of Africa to Cape Verde Isl.; *not* Mediterranean. Egg: S-type 114.8 μ, Z-type 98.3 μ (Lindahl and Runnström, 1929). Eggs variable in size (Borei, 1948). M III, 3 : 127.

Lytechinus (Toxopneustes) variegatus (Lamarck). Typical form is West Indian (H. L. C.). Jamaica, Puerto Rico. M III, 2 : 437.

Subspecies atlanticus (A. Agassiz). Bermuda. Usually brown, some are white. Egg 103 μ (E. B. H., 1947 unpub.). M III, 2 : 444.

Subspecies carolinus (A. Agassiz). Beaufort, Tortugas. Usually white. Egg 112 μ, Beaufort (E. B. H., 1942 unpub.). 100–120 μ, Tortugas (Tennent *et al.*, 1929). M III, 2 : 444.

Clark says Toxopneustes is a good but very different genus in the Pacific.

Lytechinus anamesus H. L. Clark. Southern California, Corona del Mar., Lower California, Guadeloupe Island. Egg 111 μ (Tyler, 1949 unpub.). M III, 2 : 452.

Lytechinus pictus (Verrill). Southern California, Corona del Mar, Lower California, Gulf of California. Egg 111 μ (Tyler, 1949 unpub.). M III, 2 : 450.

Tripneustes (Hipponoë) esculentus (Leske), (T. ventricosus (Lamarck). See M III 2, p. 488, 497. Called "sea eggs" in West Indies. Large form, test may be 15 cm. Bermuda, Bahamas, Florida, Tortugas, West Indies, Jamaica, Trinidad, to Brazil. W. coast of Africa. Egg 84 μ (E. B. H., 1933 a). M III, 2 : 490.

Tripneustes (Hipponoë) gratilla (Linné). Indo-Pacific, South Seas, Australia, Hawaii, Japan, Seto. Egg 90 μ (Onoda, 1936). M III, 2 : 500.

Toxopneustes pileolus (Lamarck). Indo-West Pacific, Madagascar, Fiji, Japan, Misaki, Seto. Egg 80 μ preserved (Onoda, 1936). M III, 2 : 472.

Family 3 Strongylocentrotidae

Paracentrotus (Strongylocentrotus) lividus (Lamarck). Mortensen places this in the Echinidae. Entire Mediterranean, Nice, Naples, Alexandria, Canaries, Madeira, Azores, African coast to Rio de Oro, Roscoff, Ireland and Scotland. Egg at Roscoff 100 μ, at Naples 90 μ (Runnström, 1933); at Roscoff 90 μ (Lindahl and Lundin, 1948). (See also E. B. H., 1933a; 1938a). M III, 3 : 157.

Strongylocentrotus dröbachiensis (O. F. Müller). Named for Dröbak, a seaside town in Norway, near Oslo. Known as the "green urchin." Circumpolar in the Arctic. Atlantic coast from Arctic to New Jersey, abundant in Maine, occurs at Woods Hole. North Pacific coast, Alaska, Aleutian Islands, Puget Sound, N. E. Russia, Norway, Sweden, Denmark, Scotland. Egg Maine 160 μ (E. B. H., 1942). Sweden 136 μ (Vasseur, 1949). M III, 3 : 198.

Strongylocentrotus franciscanus (A. Agassiz). Pacific Coast to Alaska, Puget Sound, Pacific Grove, Corona del Mar, Northern Japan, Hakodate. Egg 120 μ (E. B. H., 1942). M III, 3 : 242.

Strongylocentrotus purpuratus (Stimpson). Pacific Coast to Alaska, Corona del Mar, Pacific Grove. Egg 80 μ (E. B. H., 1942). M III, 3 : 236.

Strongylocentrotus (Allocentrotus) fragilis Jackson. Pacific Coast from Lower California to Vancouver, Corona del Mar. M III, 3 : 255.

Strongylocentrotus (Hemicentrotus) pulcherrimus (A. Agassiz). Japan, Hakodate, Asamushi, Misaki, Seto, North coast of China. Egg 96.55 μ (J. C. Dan, 1952 unpub.). M III, 3 : 248.

Strongylocentrotus nudus (A. Agassiz). Northern Japan, Asamushi, Vladivostok. M III, 3 : 232.

Strongylocentrotus intermedius (A. Agassiz). Northern Japan, Hokaido, Asamushi, Onagawa, Vladivostok. M III, 3 : 225.

Pseudocentrotus depressus (A. Agassiz). Placed by Mortensen in family Toxopneustidae. Southern Japan, Misaki. Egg 105.9 μ (Endo, 1952 unpub.). M III, 2 : 541.

Heliocidaris (Anthocidaris) crassispina (A. Agassiz). Placed by Mortensen

in family Echinometridae. Southern Japan, Misaki. Egg 100 μ (Motomura, 1950 unpub.); 95.5 μ (Dan, 1952 unpub.). M III, 3 : 328.

Sphaerechinus granularis (Lamarck). Mortensen places this with Tripneustes and Lytechinus in family Toxopneustidae. Large form, may be 16 cm. Mediterranean, Naples, Nice, Channel Isls. Guernsey to Cape Verde, Spain, Portugal, Azores, Madeira, Canaries. Egg 98 μ (E. B. H., 1933a; 1938a). M III, 2 : 515.

Family 4 Echinometridae, usually elongate, elliptical

Echinometra lucunter (Linné) (E. subangularis (Leske)). Known as "rock" or "reef" urchin. Bermuda, West Indies, Jamaica, Florida to Brazil, Mexico, West Africa, Dakar to Angola. Egg 80 μ, 95 μ Tortugas (Leitch, 1934a;1936); 85 μ and 97 μ Bermuda (E. B. H., 1947 unpub.); 120 μ Tobago (Mortensen, 1921, p. 71). M III, 3 : 357.

Echinometra viridis A. Agassiz. Southern Florida, Tortugas, Curaçao, Venezuela, Greater Antilles, St. Thomas. M III, 3 : 368.

Echinometra mathaei (Blainville). Clark says this is the most abundant sea urchin in the world. Indo-West Pacific, Suez to Hawaii, Egypt, E. African coast to Durban, Madagascar, Ceylon, Torres Strait, Murray Isl., Australia, Samoa, Japan, Seto, etc. M III, 3 : 381.

Echinometra vanbrunti A. Agassiz. Rock-borer. West coast of North America from central California to Peru, Mazatlan, Mexico; Gulf of California Gulf of Panama. M III, 3 : 373.

Order III Exocycloida

Suborder A Clypeastrina. Usually flattened.

Family 1 Clypeastridae

Clypeaster rosaceus (Linné). N. Carolina, Florida to Brazil, Tortugas, Bahamas, West Indies, Puerto Rico, Jamaica, Barbadoes, Curaçao. Egg 200 μ (in Bouin) (Gardiner, 1927). M IV, 2 : 40.

Clypeaster subdepressus (Gray). Florida, Tortugas, West Indies, Jamaica, Brazil. M IV, 2 : 112.

Clypeaster japonicus Döderlein. Southern Japan, Misaki. Egg 125 μ (Motomura, 1950 unpub.); 115.3 μ (Dan, 1952 unpub.). M IV, 2 : 99.

Family 2 Laganidae

Peronella (Laganum) lesueuri (A. Agassiz) or (Valenciennes). Confusion of species and several varieties. Australia, Torres Strait, Japan, Misaki, China. Egg 300–400 μ (Mortensen, 1921, p. 111; Tennent, 1924); 276 μ (Dan, 1952 unpub.). M IV, 2 : 263.

Family 3 Fibulariidae

Echinocyamus minutus (Pallas) (E. pusillus (O. F. Müller) is Mortensen's preference). Iceland, Norway, Sweden, Scotland, England, Plymouth, Ireland, Mediterranean, Naples, Marseilles, North Africa, Azores. Egg 88 μ (Théel, 1892). M IV, 2 : 178.

Family 4 Scutellidae "Sand dollars"

Echinarachnius parma (Lamarck). East Coast Labrador to Maryland, Maine, Woods Hole, West Coast Alaska to Puget Sound, Aleutian Islands, Japan. Egg 145 μ (E. B. H., 1942). M IV, 2 : 367).

Dendraster (Echinarachnius) excentricus (Eschscholtz). Pacific Coast, Alaska to Lower California, Nanaimo. Egg Pacific Grove 120 μ (Snyder, 1925; Chase, 1935); 115 μ (Needham and Needham, 1930); Corona del Mar 114 μ (Tyler, 1937). M IV, 2 : 382.

Mellita quinquiesperforata (Leske) (M. pentapora (Gmelin), M. testudinata Klein). 5 holes in test. Known as the "keyhole urchin." Vineyard Sound to Brazil, Florida, Bermuda, Jamaica, Puerto Rico, Mexico. Egg Bermuda? 110 μ (Crozier 1918); Beaufort 150 μ (E. B. H., 1942 unpub.). M IV, 2 : 422.

Mellita (Leodia) sexiesperforata (Leske) (M. hexapora (Gmelin), M. sexforis (Lamarck)). 6 holes in test. "Keyhole urchin." South Carolina to Uraguay, Bermuda, West Indies, Jamaica. Egg 260 μ (Crozier, 1918). M IV, 2 : 429.

Encope michelini L. Agassiz. Gulf of Mexico, Florida to Yukatan. M IV, 2 : 441.

Encope emarginata (Leske). East Coast, Florida to Argentina, West Indies, Trinidad. M IV, 2 : 438.

Astriclypeus manni Verrill. Known in Japan as the "perforated pancake urchin." Southern Japan, Misaki. Egg 182.1 μ (Dan, 1952 unpub.). M IV, 2 : 416.

Suborder B Echinoneina

Family 1 Echinoneidae

Echinoneus cyclostomus Leske (E. semilunaris (Gmelin)). Cosmopolitan in tropics, Tortugas, Bermuda, West Indies, Jamaica, Tobago, Indo-Pacific, Madagascar, Torres Strait, Australia, Hawaii. M IV, 1 : 75.

Suborder C Cassidulina

Family 1 Neolampadidae

Anochanus sinensis Grube. Brood protecting. China Sea. M IV, 1 : 344.

Suborder D Spatangina "Heart urchins"

Family 1 Hemiasteridae

Moira atropos (Lamarck). North Carolina, Beaufort, South Carolina, Florida, Texas, West Indies, Jamaica, Puerto Rico. M V, 2 : 329.

Hemiaster (Abatus) cordatus Verrill, or cavernosus. Brood protecting. Kerguelen Island. Egg 1 mm. (Hesse and Doflein, 1910, Bd. 1, p. 583; 1914, Bd. II, p. 619). M V, 2 : 257.

Family 2 Spatangidae

Brissopsis lyrifera (Forbes). Norway, Sweden, Scotland, Ireland, England, Mediterranean, Naples, South Africa, Cape of Good Hope. M V, 2 : 380.

Brissus brissus (Leske) (B. unicolor Klein). Tortugas, Bermuda, West Indies, Jamaica, Mediterranean, Naples, Cape Verde, Madeira, Azores. M V, 2 : 509.

Meoma ventricosa (Lamarck). Southern Florida, Bahamas, West Indies, Jamaica. M V, 2 : 529.

Spatangus purpureus O. F. Müller. Norway, Sweden, Scotland, Ireland, England, Plymouth, Mediterranean, Naples, Malta, Algiers, Azores, M V, 2 : 10.

Spatangus raschi Lovén. Norway, Ireland. M V, 2 : 14.

Lovenia cordiformis A. Agassiz. Southern California, Corona del Mar, Gulf of California, Mexico, Panama, Galapagos Islands. M V, 2 : 104.

Echinocardium (Amphidetus) cordatum (Pennant). Burrows. Norway, Sweden, England, Plymouth, Ireland, Mediterranean, Naples, Nice, Japan, Asamushi, Australia. Egg at Kristineberg, Sweden is pear shape, 125 μ (Gustafson, 1945; Runnström, 1948). "Ellipsoidal" at Millport, Scotland (MacBride, 1914b); at Asamushi 110 μ (Motomura, 1950 unpub.). M V, 2 : 152.

Echinocardium mediterraneum (Forbes). Mediterranean, Naples, Spain, Portugal. Egg 40 μ (Hamman, 1887, p. 140). M V, 2 : 162.

Echinocardium pennatifidum Norman. Norway, Bergen, Faroe Islands to Channel Islands. M V, 2 : 163.

PART II
NORMAL DEVELOPMENT

CHAPTER 10

The immature egg and its maturation

a. Growth and Differentiation of the Oocyte. Plate II

The *Arbacia* egg matures in the ovary, so that when the eggs are shed they have lost their polar bodies and are fully ripe and ready for fertilization. The various stages of growth of the young oocyte and the polar body formation are best studied at Woods Hole in early or middle June. One can obtain immature eggs among the mature ones later in the summer, but they are usually not abundant.

The very young oocytes, of about 14 μ in diameter, have a very large germinal vesicle with a vesicular nucleolus, very little cytoplasm, sometimes granular, sometimes quite clear, and no pigment. (Plate II, Photograph 1). From the data given in Table 1, it will be seen that at this stage the nuclear material (germinal vesicle) occupies about two thirds of the whole cell and that there is more than twice as much nuclear material as cytoplasmic. As the immature egg increases in size, the germinal vesicle also increases, but not so much as the cytoplasm. The largest immature egg is the same size as the mature egg, but the mature nucleus is very much smaller than the germinal vesicle of the immature egg, about 1/40th the volume.

The young oocytes are still without pigment till they reach a diameter of about 33 μ (Photograph 3). They then become slightly pigmented, the pigment granules, gradually increasing in quantity with growth. The oil, recognizable as an oil cap after centrifuging, appears about the same time as the pigment. The mitochondria appear around the germinal vesicle at about the same time, as can be determined by staining with methyl green. A few scattered mitochondrial granules are sometimes present in smaller eggs, of about 23 μ in diameter. Jelly is formed when the young oocyte is about 60 μ in diameter (Photograph 6). The data are given in Table 1.

No study has been made of the oogenesis and spermatogensis of *Arbacia*, but Tennent and Ito (1941) have published a very complete account of the oogenesis of the Japanese form, *Mespilia globulus*, which is probably similar.

TABLE 1

SIZE OF OOCYTE, GERMINAL VESICLE AND NUCLEOLUS DURING GROWTH

STAGE	OOCYTE		GERMINAL VESICLE		NUCLEOLUS		CYTOPLASM SUBTRACT COLUMN 4 FROM COLUMN 2)
	1	2	3	4	5	6	7
	D (μ)	V (μ³)	D (μ)	V (μ³)	D (μ)	V (μ³)	V (μ³)
Very young	14.3	1,531	12.8	1,098	4.8	57.9	433
Pigment present	33	18,820	22	5,575	9.6	463.2	13,245
Jelly present	60	113,100	30	14,140	10	523.6	98,960
Largest	74	212,200	38.4	29,650	11.2	735.6	182,550

SIZE OF MATURE EGG AND NUCLEUS

MATURE EGG	NUCLEUS	CYTOPLASM
D 74 V 212,200	D 11.5 V 796.3	V 211,404

b. Structure of the Oocyte. Plate II

The immature egg when fully developed consists of cytoplasm and germinal vesicle, usually excentric. The cytoplasm consists, like that of the mature egg, of a matrix or ground substance in which are scattered spherical oil droplets, yolk granules, spherical red pigment granules or vacuoles, mitochondria, and microsomes. All the granules are scattered through the cytoplasm except the mitochondria which form a thick layer around the outside wall of the germinal vesicle; this can easily be seen in eggs stained with methyl green. The fully formed germinal vesicle is about 38 μ in diameter, and contains a vesicular nucleolus, usually excentric, about 11 μ in diameter, often with one or many small bodies or nucleolini within it (see also R. D. Allen, 1951 b).

When fixed in Bouin's solution and stained with Heidenhain's haemotoxylin, the nucleolus is quite black, and there is a black staining network through the germinal vesicle (E. B. Harvey and Lavin, 1944); (Photographs 17 and 18). Excellent photographs of sections of the immature *Lytechinus (Toxopneustes)* egg thus prepared are to be found in Wilson's (1895) *Atlas of Fertilization*, Plate I. The appearance of the immature egg with ultraviolet light is similar to that in the stained preparations (E. B. Harvey and Lavin, 1944). The nucleolus and chromatin network are absorbing and appear black in photographs

showing the presence of nucleic acid compounds. Casperson and Schultz (1940) think these are of the ribose type (in *Psammechinus miliaris*). The appearance of the immature *Arbacia* egg with other stains is described and illustrated by E. B. Wilson (1926); Benda-osmic, Benda-Kull, Champy-Kull, Champy-osmic, thionin, toluidin blue have been used. In some of these figures, bodies which appear to be nucleolini inside the nucleolus show up clearly but are not mentioned in the text. Some cytochemical techniques have been described by Krugelis (1947b): Gomori, fast green, toluidin blue, and Feulgen.

With certain intravitam stains, the immature egg stains more intensely than the fertilized egg, and this more intensely than the unfertilized (Lyon and Shackell, 1910b; E. N. Harvey, 1910c).

The immature egg is surrounded, like the mature egg, by a layer of jelly. In the older oocytes it is the same in amount as in the mature egg and may be as much as 32 μ thick. It is thinner in younger oocytes; in an oocyte whose diameter was 60 μ, the jelly was only 3.2 μ. The jelly coat is discussed under the mature egg and further data may be found in Part IV, *Jelly Layer*.

c. Reaction to Sperm

One of the most characteristic features of the immature egg is its reaction to sperm. The formation of papillae or blebs (Photograph 16) wherever sperm hit the surface is a well-known phenomenon in many echinoderms including *Asterias* and holothurians (Hobson, 1927). These papillae have many different forms; they may be blunt or sharply pointed and are sometimes branching; they are always hyaline, without granules. They have been carefully studied by Seifriz (1926) in the sand dollar. The sperm enter the immature *Arbacia* egg as (Photograph 17) they do other sea urchin eggs, e.g., *Lytechinus* as described by Wilson (1895) in his *Atlas*. But no fertilization membrane is raised. The papillae are considered similar to fertilization cones which form on mature eggs after fertilization. They are formed within two minutes after adding sperm, and they last for about 20 minutes and are gradually resorbed. They are formed on eggs with germinal vesicle intact, after the germinal vesicle has broken, during polar body formation and even an hour after the second polar body has been given off. They do not form in Ca-free sea water though the sperm are motile; this has been found also for *Anthocidaris crassispina* by Sugiyama (1938g) and for *Psammechinus miliaris* by Rothschild and Swann (1949). It is of interest that fertilization of mature eggs will not take place in Ca-

free sea water, though the sperm are motile (Loeb, 1915a; E. B. H., unpub.; see also Monroy, 1949 for *Ps. microtuberculatus*). The papillae do not form on immature eggs which have been in hypertonic sea water for 20 minutes and then returned to sea water, a method of producing parthenogenesis of mature eggs (E. B. Harvey, 1938a for *Ps. microtuberculatus*). Sugiyama states that the papillae do not form if the sperm have been treated with ultraviolet light, but this is not true for *Arbacia* (E. B. H., unpub.).

d. Centrifuging and Gravity

On centrifuging, about 10,000 × g for 3 minutes, the mature unfertilized egg is well stratified. The immature egg is only partially stratified, with a small oil cap at the centripetal pole and pigment at the centrifugal pole and above this the yolk but sometimes not well separated from the pigment (Photograph 13). The clear layer is not well defined, and there is no mitochondrial layer. After centrifuging the mitochondria remain as a thick layer around the wall of the germinal vesicle as shown in Photograph 18 (not centrifuged). The egg does not elongate nearly as much as the mature egg, and does not break apart with the forces which are sufficient to break the mature egg into halves. The immature egg is thus shown to be much more viscuous than the mature egg. This has been noted by previous observers (Heilbrunn, 1928, p. 278; 1952, p. 85; Goldforb, 1935b).

The germinal vesicle, in centrifuged eggs, lies at the centripetal pole under the oil, and the nucleolus is heavy and always lies at the bottom of the germinal vesicle (Photograph 13). Gray (1927b) has made some interesting studies on the effect of gravity on the nucleolus of the *Echinus esculentus* egg. He found that the nucleolus descends by gravity at the rate of 0.4 μ per second (1.5 mm. per hour). The nucleolus in the eggs of some species can be driven by strong centrifugal force, with an air turbine, right through the germinal vesicle wall. Such is the case with *Strongylocentrotus dröbachiensis* (E. B. H., unpub.).

e. Respiration

The rate of respiration is less in immature than in mature eggs according to Boell, Chambers, Glancy, and Stern (1940). Borei (1948) found it slightly higher in *Psammechinus miliaris*, and Lindahl and Holter (1941) in *Paracentrotus lividus*.

f. Permeability to Water

According to Churney (1941b, 1942), the permeability to water of the immature egg is the same as the mature egg; the germinal vesicle acts as a perfect osmometer, and the nucleolus swells and shrinks reversibly.

g. Polar Body Formation. Plate II

After the immature egg has reached its full size, it throws off two polar bodies. The first step in this process is the approach of the germinal vesicle to the cell wall (Photograph 7). Then after a period of about 1¼ hours, during which the nucleolus has become smaller and disappeared, the wall of the germinal vesicle breaks down; the red pigment granules become more numerous and very bright in color in this region, just inside the cell wall near the breaking germinal vesicle (Photographs 7 and 8). The mitochondrial granules do not take part, but are heaped up on the opposite side of the breaking germinal vesicle, as can be seen in eggs stained with methyl green. A clear area is seen where the germinal vesicle broke down, and two or two and a half hours (23° C.) after the breakdown, the first polar body is given off. One to one and a half hours later the second polar body is given off. Then about two hours later, the mature nucleus is formed. During the formation of the polar bodies the egg becomes somewhat flattened in the axis of the polar bodies (Photograph 12). This lasts for about 7 minutes; then the egg becomes spherical again.

By watching individual eggs fertilized at intervals (23° C.) after the second polar body has been given off, the following data have been obtained:

½ to 1 hr.	No fertilization membrane, delayed and irregular cleavage, polyspermy, 3 cells at once.
1¼ to 1½ hrs.	Fertilization membrane, polyspermy, irregular cleavage, normal swimming blastulae.
2 to 2½ hrs.	Cleavage delayed and slightly irregular, polyspermy, 4 cells at once.
3 to 5 hrs.	Slight delay in first cleavage; cleavages regular, micromeres normal, normal plutei.

There is apparently a sort of cytoplasmic maturation following nuclear maturation such as has been described for *Paracentrotus lividus* by Paspaleff (1927) and for *Psammechinus miliaris* by Runnström and Monné (1945) and Runnström (1948b). The eggs do not develop

perfectly until several hours after the second polar body has been given off.

If *Arbacia* eggs are centrifuged at the time of polar body formation, it has been found that the polar bodies may come off in any relation to the stratification, in the yolk or pigment zone or even at the oil cap (E. B. H.) (Photographs 14 and 15). It seems that the original polarity of the egg must determine their position and not the polarity imposed by centrifugal force expressed by the stratification.

Usually the polar bodies of *Arbacia* have been thrown off and discarded by the time the eggs are laid or taken from the ovary, but in some batches they are retained in the majority of the eggs. In such a batch, Hoadley (1934) found that they may lie in any position with regard to the (mature) nucleus, and I have confirmed this observation in other batches. The polar bodies in *Paracentrotus lividus* are given off in the micropyle (Boveri, 1901), and also in Lytechinus (Tennent, Taylor, and Whitaker, 1929); this is true also of Arbacia. The first cleavage plane passes through the region of the polar bodies. The micromeres are formed at the vegetal pole, opposite the polar bodies. Previous observers also found that the micromeres formed "approximately opposite to the micropyle" in *Arbacia* (Morgan and Spooner, 1909, p. 116; Spooner, 1911; Hörstadius, 1937a).

Other Species (Additional) and General References

A. Brachet, 1922. *Paracentrotus lividus*, fertilization of immature eggs.
J. Brachet, 1933. *Paracentrotus lividus*, Feulgen stain.
Bryce, 1902. Polar bodies, sections.
Derbès, 1847. "Oursin comestible"; earliest figure of immature egg.
Harris, 1939. *Echinus esculentus*, viscosity and polarity; fall of nucleolus.
Lindahl and Holter, 1941. *Paracentrotus lividus*, respiration.
Lyon and Shackell, 1910b. *Toxopneustes variegatus*, permeability.
Runnström, 1928c. *Paracentrotus lividus*, etc., papillae, surface.
Selenka, 1878. *Toxopneustes variegatus*, early description.
Skowron and Skowron, 1926. *Sphaerechinus granularis*, permeability.
Tennent, Gardiner, and Smith, 1931. *Echinometra lucunter*, stains for micro-chemistry.
von Ubisch, 1950. *Paracentrotus lividus*, general.
Wilson, 1895. *Atlas of Fertilization.*
Wilson, 1899. Protoplasmic structure.
Wilson, 1925. *The Cell*, General.
Wilson and Mathews, 1895. *Toxopneustes variegatus*, polarity.

CHAPTER 11

The mature egg, unfertilized and fertilized

a. Quantity of Eggs in an Arbacia

The number of eggs obtained from one *Arbacia* varies considerably with the season. Even at the height of the season, some females have many more eggs than others. Usually, the eggs obtained from one female in the usual way, by allowing them to ooze out of the excised ovaries and then filtering through cheesecloth, will well cover the bottom of a finger bowl in a thin layer; from exceptionally good females, they cover the bottoms of two finger bowls. When allowed to settle for several hours by gravity, the volume of the eggs from an exceptionally good female amounts to 5 cc. With light centrifuging, this amounts to 3 cc., but many are now without jelly. The 5 cc. of settled eggs with jelly contain approximately 4,000,000 eggs since each egg has a volume of 1,260,300 μ^3 (diameter, egg 74 μ + jelly 60 μ, without interspaces because the jelly of adjacent eggs is contiguous). The volume of the settled eggs is about equal to the volume of eggs left in the ovaries. One *Arbacia*, then, contains about 8,000,000 eggs. The same figure has been arrived at for the number of eggs shed either by KCl or by the electrical method. MacBride (1906, p. 529) states that a well grown *Echinus esculentus* contains 20,000,000 eggs; this compares fairly well with the figures for *Arbacia* since *Echinus esculentus* is a very much larger animal.

Quantity of eggs in 1 cc. settled by gravity (E. B. Harvey)

Eggs with jelly	800,000	(vol. per egg 1,260,000 μ^3; there are no interspaces, eggs are contiguous).
Eggs without jelly	3,500,000	(vol. per egg 212,200 μ^3; allowing 26% for interspaces).
Eggs without jelly	4,700,000	(no allowance for interspaces).

Other figures have been given by Krahl (1950, p. 177).

Eggs per 10 c.mm.	46,500
Eggs per wet gram	4,300,000
Eggs per mg. dry weight	17,600

b. Methods for Estimating Volume and Quantity

Volume. 1. Direct measurement. Measure the diameters of many eggs of a sample, convert to volume[1], and take average.

2. Diffraction method. Described by Lucké, Larrabee and Hartline, (1935). Used by Lucké *et al.* in subsequent papers; Korr (1937); Ballentine (1940b); *et al.*

Number of eggs in a suspension. The main error in determining numbers of eggs is caused by the jelly coat which varies in different batches of eggs, and may be removed from some or all eggs by agitation, centrifuging, etc. The presence of the jelly coat makes a difference of about 60 μ in the diameter of each egg, and it has a volume of about 1,050,000 μ^3.

The following methods have been reviewed and appraised by Shapiro (1935c).

1. Haemocytometer. Used by Tang and Gerard (1932), Gerard and Rubenstein (1934); *et al.*

2. Centrifuge, using haematocrit tubes. Used by Whitaker (1933a IV; 1935); Clowes *et al.* (1936, etc.); Mazia (1937); criticized by Gerard and Rubenstein (1934).

3. Dilution method of Parpart, described by Shapiro (1935c). This seems to be the best method. Immerse quickly a capillary of 1 mm. bore into a uniform suspension of experimental eggs, filling to a length of 8–10 cm. Lay the capillary on its side under a binocular microscope, and count the number of eggs. Do this three times. If the suspension is too thick, dilute to a suitable amount and allow for the dilution factor in calculation of number of eggs in original suspension. Used by Korr (1937); Ballentine (1940b) *et al.*

Number of cleaving eggs. Place the eggs at the desired stage in a weak formol solution, 0.04 to 0.5%, and count at leisure for percentage (Morgan, 1895b and many others).

c. Shape of Eggs

Arbacia eggs are usually spherical when shed or removed from the ovary. Sometimes they are aspherical due to crowding in the ovary, especially late in the season; they become spherical on standing or on fertilization (see also Goldforb, 1935a). They become amoeboid with ethyl urethane, urea, etc. See *Amoeboid Eggs*, Part. IV.

They do not flatten by gravity (McCutcheon, Lucké, and Hartline, 1931; Cole, 1932; E. N. Harvey, 1933; E. B. Harvey, 1934). It was claimed by Chambers (1921a) that *Arbacia* eggs do flatten by gravity, and by Vlès (1926) that *Paracentrotus lividus* eggs flatten. Rothschild and Barnes (1953) state that *P. lividus* eggs do not flatten by gravity.

The egg of *Echinocardium cordatum* (Kristineberg, Sweden) is peculiar in being pear-shaped, but becomes spherical on fertilization (Gustafson, 1945; Runnström, 1948). MacBride (1914b) describes it as "ellipsoidal" at Millport, Scotland. The eggs of the sea urchins at Naples, *Sphaerechinus granularis*, *Psammechinus microtuberculatus*, and *Paracentrotus lividus* are much more aspherical than those of *Arbacia punctulata* at Woods Hole (E. B. Harvey, 1933a).

[1] A good conversion table from diameters to volumes of spheres is given in the Chemical Engineers' Handbook, 3rd edition, 1950, p. 34, 35; 2nd edition, 1941, p. 90, 91. J. H. Perry, editor.

d. Size, Weight and Density of Egg

SIZE	DIAMETER	SURFACE AREA	VOLUME
Egg without jelly	74 μ	17,200 μ^2	212,200 μ^3
Egg with jelly	134 μ (74 + 60)	56,430 μ^2	1,260,000 μ^3
Fertilized egg including fertilization membrane, without jelly	84μ (74 + 10)	22,170 μ^2	310,300 μ^3
Nucleus	11.5 μ	415.6 μ^2	796 μ^3

WEIGHT

1 cc eggs, dry wt. 265 mg. (Ballentine, 1940a)
10^6 eggs, dry wt. 58.0 mg.

10 c. mm. eggs, dry wt. 2.63 mg. (Krahl, 1950)
10 c. mm. eggs, wet wt. 10.9 mg.

Dry weight is approximately 24% of wet weight (Krahl, 1950).

DENSITY

1.081 to 1.087; same to 16 cell stage; blastula lighter than 16 cell stage, but heavier than sea water; plutei 1.055 to 1.066 (Lyon, 1907).
1.0485 to 1.0656, unfertilized (Heilbrunn, 1926a, 1928, p. 71).
1.090, unfertilized, with jelly (E. N. Harvey, 1931, 1932a).
1.084, unfertilized, without jelly.

e. Size of Fertilized Egg

It is generally accepted that the fertilized egg (without fertilization membrane) is the same size as the unfertilized (Whitaker, 1933b), though it is larger by the addition of the hyaline layer, which is extraneous to the egg proper (E. B. Harvey, 1933a for *A. lixula*). But Glaser (1913; 1914a, b, c; 1924) found the egg smaller by 2.5 μ immediately after fertilization, then larger, and also R. S. Lillie (1916a) smaller by 1.0 μ. Chambers (1921a) found it slightly larger, and Shapiro (1948b) found an increase of 2.7% in volume. There is no appreciable increase in diameter until the gut is complete in the gastrula stage, about 17 hours after fertilization (E. B. Harvey, 1949).

f. Variation in Size

Average size of unfertilized egg as recorded by some investigators is shown in the following tabulation. The figures in brackets are computed from the figure given by the author.

DIAMETER, μ	VOLUME, μ^3	AUTHOR
74.1	(213,000)	Glaser, 1914a
74.6	(217,400)	Glaser, 1924
75.0	(220,900)	Heilbrunn, 1915a
74.1	213,000	R. S. Lillie, 1916a
74.1	213,000	McCutcheon, Lucké, and Hartline, 1931
(72.4)	198,900	McCutcheon, Lucké, and Hartline, 1931
(75.4)	224,500	McCutcheon, Lucké, and Hartline, 1931
73	203,700	E. B. Harvey, 1932
74	212,200	E. B. Harvey, 1936, 1941a, 1946
(72.9)	202,640	Goldforb, 1935a

Variation in size by one investigator is shown by:

Goldforb's (1935a) figures for 1,000 eggs from 25 females: 68.3 μ to 77.4 μ diameter.

Shapiro's (1935c) figures for eggs measured through summer of 1934: 64 μ to 81 μ diameter.

Variation in size has also been studied by Glaser (1914a, 1924) and by R. S. Lillie (1916a).

Variation is great for different females but is quite small for the eggs of one female, as noted by many observers and studied especially by Goldforb (1935a).

g. Aberrant Sized Eggs and Nuclei

Aberrant sized eggs and nuclei. Occur in some batches of normal eggs; are uniform in size with no gradations in any one batch (E. B. Harvey).

1. Giant egg with giant nucleus. Egg D. 91 μ, Nucleus 14 μ, (1939); in 1% of eggs; normal development. Egg D. 96 μ, Nucleus 14.4 μ; in a few eggs (1950).

2. Normal egg with giant nucleus, Egg D. 72 μ, Nucleus 17 μ; in 1% of eggs; normal development (1940). Egg D. 71 μ, Nucleus 29 μ; in .01% of eggs; abnormal development (1939).

3. Small egg with giant nucleus. Egg D. 57.6 μ, Nucleus 16.5 μ; normal cleavage, blastulae, no plutei (1940).

4. Small egg with normal nucleus. Egg D. 50 μ, Nucleus 11.5 μ (1940).

5. Normal egg with 2 nuclei, same volume together as a normal nucleus (1940).

6. Immature egg with two germinal vesicles (1943).

h. Effect of Age, Temperature, etc. on Size of Eggs

Effect of:

Age. No effect for 4 hours (Glaser, 1914a). Increase in size for first 23–50 hrs., measurable by 3rd hr., then decrease (Goldforb, 1918a, b; 1935a).

Temperature. No effect 5.4–29.3 °C. (Lucké and McCutcheon, 1926a; 1935).

pH. No effect 4.0–9.8° (Lucké and McCutcheon, 1926a, b).

Amino acids. No swelling (Lucké and McCutcheon, 1926a).

Ether. No effect unless injured (Lucké and McCutcheon, 1926a).

Urethanes. No effect (Lucké, 1931).

Oxygen lack. Become slightly smaller (Hunter, 1936).

i. Structure of the Egg

Like the immature egg, the mature egg contains oil drops, yolk granules, mitochondria, red pigment granules, and microsomes, all scattered in a matrix or ground substance, and a small nucleus. The alveolar structure of the egg protoplasm has been described by E. B. Wilson (1899, 1926). Data on the various granules will be found in Part IV, under the appropriate heading. Photographs of the living unfertilized egg and of a stained section are shown on Plate III, Photograph 1, and Plate V, Photograph 1.

Nucleus. – The nucleus of the mature egg is excentric, has a diameter of approximately 11.5 μ, is lighter than the cytoplasm, i.e., goes to the centripetal pole when the egg is centrifuged. Not much structure is seen in the living nucleus, and it does not take any vital dyes (E. B. H., 1941c). A network of chromatin material is seen in the nucleus of eggs sectioned and stained with Heidenhain's haematoxylin (see Plate V). In photographs taken with ultraviolet light, the network is absorbing and appears black, showing the presence of nucleic acid compounds, generally considered to be of the desoxyribose type (E. B. Harvey and Lavin, 1944).

The nucleus is permeable to water and swells and shrinks in hypo- and hypertonic sea water (E. B. Harvey, 1943) (Table 11; Plate XIV). In 100% sea water the volume is 796 μ^3 (diameter 11.5 μ); in 60% sea water the volume is 2,145 μ^3 (diameter 16.0 μ); in 125% sea water the volume is 382 μ^3 (diameter 9.6 μ). They recover perfectly and return to normal size when replaced in sea water. The swelling and shrinking of the germinal vesicle of the immature egg has been studied by Churney (1941b, 1942).

Jelly. – The mature egg, like the immature, is surrounded by a jelly coat which may be as thick as 32 μ. It has the same refractive index as sea water and cannot be seen with a light microscope, dark field, or phase contrast microscope. It is readily demonstrated with a slight tinge of Janus green or toluidin blue in the sea water. The old method of Boveri (1901) may also be used. He rubbed a stick of India or Chinese ink in a drop of sea water to make a thick solution and added the eggs. The jelly is perfectly clear and the India ink particles remain in the surrounding sea water. Also when many sperm are added to eggs in a dish of sea water, many of them are caught in the jelly and form a halo around the eggs. The even spacing of eggs as they are viewed with the microscope is caused by the invisible jelly. If the eggs are contiguous, the jelly is absent and the eggs are in poor condition. The jelly coat may be made to disappear under many experimental conditions, e.g., acid, ultraviolet, x-rays, etc. (see under *Jelly Layer* in Part IV).

Micropyle. – A funnel shaped micropyle can sometimes be demonstrated in the jelly of both mature and immature eggs by the use of India or Chinese ink or the ink from living squid. I have found the squid ink the most satisfactory, and it is best to use very fresh eggs, not washed. The squid ink makes the jelly swell, often to 60 μ, twice its normal thickness, almost equalling the diameter of the egg. (See under *Jelly Layer*, Part IV). Originally observed by Boveri (1901) in *Paracentrotus lividus*, the micropyle has been shown in colored drawings in *Arbacia* eggs, both normal and centrifuged, by Morgan and Spooner (1909); they state that it is difficult to detect after the early cleavages. It is much better seen in *Psammechinus microtuberculatus* and *Paracentrotus lividus* than in *Arbacia*, according to Plough (1929). *Boveri* (1901 a, b) and others found that the micropyle marks the place where the polar bodies were given off, the animal pole, and thought it marked the point of attachment of the egg to the ovarian wall. But Jenkinson (1911), Lindahl (1932 b), and Hörstadius (1939), for *Paracentrotus*, and Tennent and Ito (1941) for *Mesipilia* have found that the polar bodies come off from the free end, and the egg is attached to the ovarian wall at the opposite or vegetal pole.

The micropyle has been used as a landmark for the localization of materials in the *Arbacia* and other eggs in polarity studies. Morgan (1909) found that both in the normal and centrifuged egg of *Arbacia* the micromeres lie approximately opposite the micropyle. (See also Morgan and Spooner, 1911). Harnley, (1926); Tennent, Taylor, and Whitaker (1929), and Plough (1927, 1929) have also used the micro-

pyle in their studies on the localization of materials in the *Arbacia* egg.

Though the micropyle functions as the place of entry of the sperm in some forms, this is not true for sea urchins, for the sperm can enter at any point. This can readily be determined in centrifuged, stratified eggs.

j. Membranes and Layers. Figures 9 and 10

1. Unfertilized Egg

Egg diameter, without membranes, 74 μ.

Jelly layer. On outside of egg. 28 to 32 μ thick (E. B. H.). Plate XVI, Photograph 3.

Vitelline membrane. Outside of plasma membrane; difficult to distinguish from plasma membrane. Lifts off on fertilization to form fertilization membrane. Not measurable with light microscope; 25 mμ with electron microscope (E. B. Harvey and Anderson, 1943).

Plasma membrane. Lies over cortical layer. 10 mμ (Danielli, 1942, p. 72). Located inside cortical layer according to Parpart and Laris (1954).

Cortical layer. Location of cortical granules which disappear on fertilization. Thickness of layer in *A. punctulata* is 0.8 μ; 1 to 2 μ in other eggs (Monroy and Oddo, 1946; Runnström, 1949b; Mitchison, 1952).

2. Fertilized Egg

Jelly layer. As in unfertilized egg.

Fertilization membrane. 25 mμ when first formed from vitelline membrane; becomes thicker and tougher (E. B. Harvey and Anderson, 1943). Less than .03 μ with electron microscope (Hillier, Lansing, and Rosenthal, 1952).

Perivitelline space. Between fertilization membrane and hyaline layer 3 to 5 μ (E. B. H.).

Hyaline layer (or ectoplasmic layer). An investing layer which binds the blastomeres together, 2 to 3 μ thick when fully formed, 15 to 20 minutes after fertilization (E. B. H.).

Plasma membrane. As in unfertilized egg.

Cortical layer. As in unfertilized egg but no cortical granules. Most of pigment granules located here after fertilization (McClendon, 1909b *et al.*).

Data on the various layers and membranes will be found under the appropriate heading in Part IV.

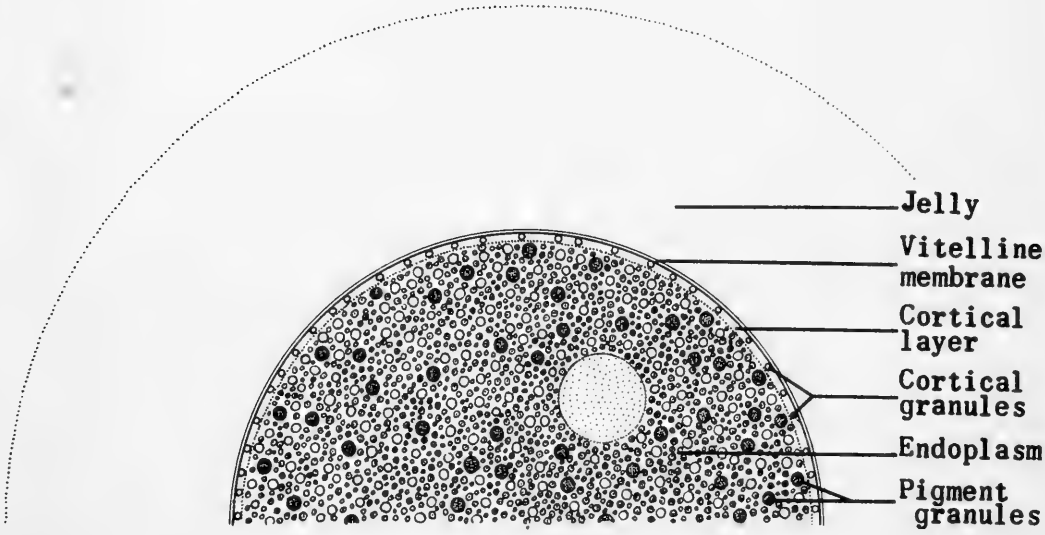


FIG. 9. Diagram of membranes and layers of unfertilized egg of Arbacia punctulata. E. B. Harvey and K. Dan. The plasma membrane is not shown; it lies just outside the cortical layer, and inside the vitelline membrane; or, according to Parpart and Laris (1954), inside the cortical layer.

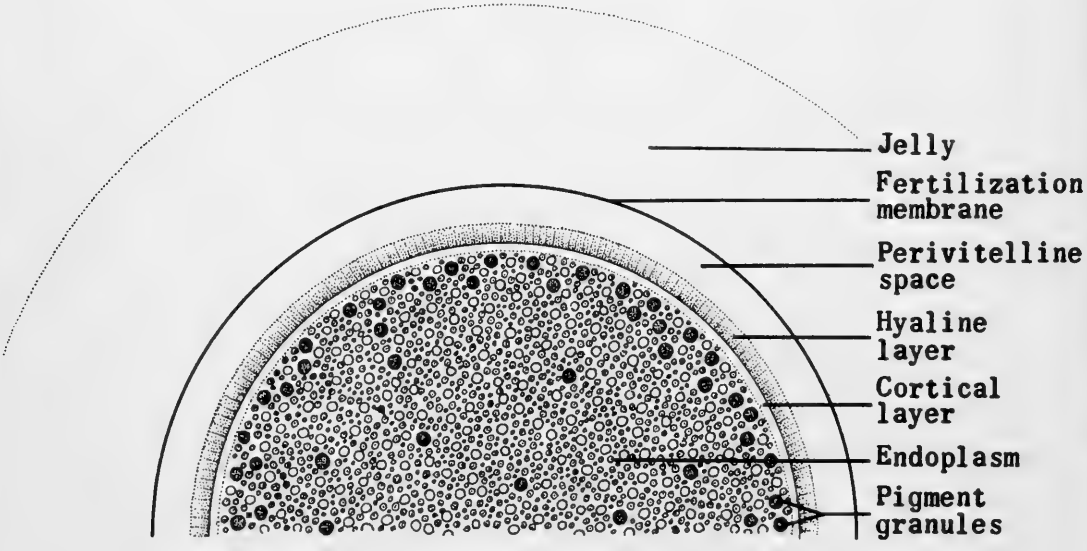


FIG. 10. Diagram of membranes and layers of fertilized egg of Arbacia punctulata. E. B. Harvey and K. Dan.

k. Diagrams of Surface Layers of Arbacia and other Eggs

Chambers, 1938b. *Am. Nat.* 72 : 146. Fertilized sea urchin egg.
Dan and Dan, 1940. *Biol. Bull.* 78 : 488. Fertilized *Strongylocentrotus pulcherrimus* egg.
Danielli, 1942. *Bourne's Cytology* p. 69. Fertilized *Arbacia* egg.
Kopac, 1940a. *Cold Spring Harbor Symp.* 8 : 168. Unfertilized and fertilized *Arbacia* egg.
Moser, 1939a. *J. Exp. Zool.* 80 : 432. Unfertilized *Arbacia* egg.
Motomura, 1941b. *Sci. Rep. Tohoku Imp. Univ.* 16 : 355. Unfertilized eggs of *S. pulcherrimus.*
Öhman, 1945. *Archiv f. Zool.* 36A, no. 7 : 39. Unfertilized sea urchin egg.
Runnström, 1949a. *Adv. Enzymol.* 9 : 245; 1949b. *Pub. Staz. Zool. Nap.* 21 : 13. Unfertilized sea urchin egg.
Runnström and Monné, 1945a. *Arkiv f. Zool.* 36A, no. 18 : 2. Sea urchin oocyte.

l. Granules

Oil globules. 0.6 to 1.0 μ (spherical) (E. B. H.).

Mitochondria. 0.6 to 1.0 μ (spherical) (E. B. H.).

Yolk granules. 0.7 to 1.1 μ (irregular, polyhedral) (E. B. H.).

Pigment vacuoles. All sizes up to 1.7 μ (spherical). Unevenly spaced all through unfertilized egg; most go to periphery on fertilization (McClendon, 1909b; E. B. Wilson, 1926, Fig. 2; Cannan, 1927; E. B. H.; *et al*).

Cortical granules. 0.8 μ (spherical). (Moser, 1939a; E. B. H., 1946a; *et al.* At periphery in unfertilized eggs; disappear on fertilization.

Microsomes. 0.4 μ or less (E. B. H.) Very small granules not visible in normal living egg, but seen in the centrifuged egg throughout the clear layer when stained with Heidenhain's haematoxylin. (Lyon, 1907; E. B. Harvey, 1940c; E. B. Wilson's 1925 *The Cell* p. 32).

(Golgi bodies, described by E. B. Wilson (1926), but by no one else; Just (1927) thinks they are oil drops).

Percentage of formed bodies based on measurements in centrifuged eggs (E. N. Harvey, 1932a):

Nucleus	0.4%
Oil g obules	1.0%
Mitochondria	4.8%
Yolk	27.2%

Pigment vacuoles	5.5%
Fluid	61.1%

Data on the various granules will be found in Part IV.

General References

Wilson, 1925. *The Cell;* older literature.
Runnström, 1952. *Modern Trends in Physiology and Biochemistry;* recent literature on cytoplasm.
Mazia, 1952. *Modern Trends in Physiology and Biochemistry;* recent literature on the nucleus.

CHAPTER 12

Sperm

a. Morphology

The spermatozoon of *Arbacia punctulata* consists of a pointed conical head, a middle piece the shape of a flattened cylinder and a long thin tail (Fig. 11). Measurements of the living sperm are (approximately):

	HEAD	MIDDLE PIECE	TAIL
Height or length	3.25 μ	0.75 μ	45.0 μ
Diameter (maximum)	2.0 μ	2.0 μ	0.2 μ
Volume (calculated)	3.4 μ^3	2.4 μ^3	1.4 μ^3

Total volume 7.2 μ^3
(See E. B. Harvey and Anderson, 1943).

Since the volume of the egg is 212,000 μ^3, the sperm is only about 1/30,000th the volume of the egg. Using another egg and other data, Needham (1931, *Chem. Emb.* p. 1251) arrived at the same figure.

The spermatozoon of *Echinus esculentus*, roughly the same shape, has been studied by Rothschild. He has calculated from photomicrographs that the volume of the whole sperm is about 18 μ^3, of the head and middle piece about 10 μ^3 and of the middle piece not more than 5 μ^3; he found difficulties in measuring the radius of the sperm tail, but says it lies between 0.3 and 0.1 μ (Rothschild, 1950b, d; 1951a; 1952). According to Pictet (1891) the diameter of the tail of all sea urchins is about 0.2 μ. It has been found, in general, that the species of sea urchin which have large eggs also have large sperm (E. B. Harvey, 1942). The egg of *Echinus esculentus* has a diameter of 180 μ and that of *Arbacia punctulata* 74 μ, so that one would expect the spermatozoon of *Echinus* to be larger.

The conical head of the spermatozoon of *Arbacia punctulata* appears to be a mass of chromatin, staining black with Heidenhain's haematoxylin, without any special structure. The tip, the acrosome or perforatorium, however, stains with methyl green and Janus green, mitochondrial stains, and with methylene blue. Popa (1927, p. 251) says

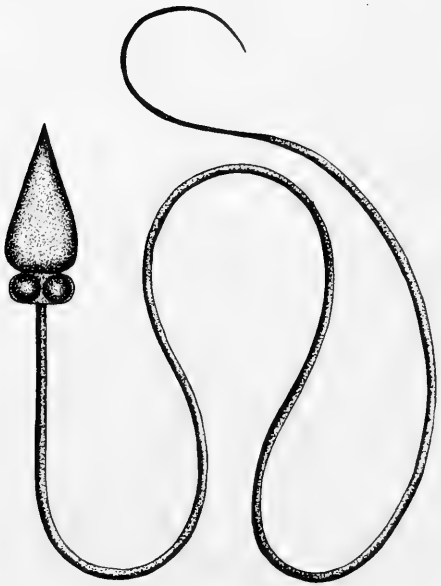


FIG. 11. Drawing of *Arbacia punctulata* spermatozoon.

"there is an exceedingly minute opening in the point of the head", through which "one gets the impression that the spermatozoon eliminates very small amounts of an extremely sticky substance". The head of the sperm contains the haploid group of chromosomes which have a volume, if they are the sole components of the sperm head, of about $3.4\,\mu^3$. A diploid group of 38 chromosomes from a first cleavage cell in a smear preparation stained with aceto-carmine has a volume of about $10.2\ \mu^3$ (average diameter of a spherical chromosome is about $0.8\ \mu$), a little more than double that of the sperm head. The expected double relation is fairly close when one considers the uncertainty as to how much of the material is pure chromatin both in the diploid plate of chromosomes and in the haploid sperm head. The amount of DNA in the *Arbacia* sperm is 0.9×10^{-6} micrograms, calculated as 1.3 to $1.4\ \mu^3$ according to Mazia (personal communication, 1955), or about one third of the volume (or weight) of the head. Marshak and Marshak (1953) give 7.9×10^{-7} micrograms in one sperm.

The middle piece of the spermatozoon of *Arbacia punctulata* contains two large spheres, probably centrosomes, especially well seen with the phase microscope. These bodies in the middle piece stain purple with methyl green and blue with Janus green, indicating the presence of

mitochondrial material. They also stain with methylene blue, not a mitochondrial stain. Rothschild (1952) thinks that the middle piece probably contains enzyme complexes, such as the cytochrome system and that this is suggested by the presence in it of mitochondria-like structures. The middle piece of *Arbacia* can be isolated by a special technique involving the removal of heads and tails (Di Stefano and Mazia, 1952); these authors think that the middle pieces are the center of ribonucleic acid activity. A preliminary paper on the isolated middle pieces has been published recently by Neff (1953). Popa (1927) also isolated certain components of the sperm by destroying others.

The tail of the *Arbacia* spermatozoon consists of about ten separate fibrils of uniform thickness, each fibril having a diameter of about 50 mμ (0.05 μ), as shown in electron microscope pictures (E. B. Harvey and Anderson, 1943). Electron microscope pictures of sperm of other animals show that the fibrillar structure is characteristic of sperm tails, e.g., the squid (Schmitt, Hall, and Jakus, 1943) and the bull (Baylor, Nalbandov, and Clark, 1943). Many years ago, separate fibrils were observed (and figured) in the tails of many different kinds of sperm, of birds, insects, fish, amphibia, and reptiles, by Ballowitz (1888, 1890) in teased material, with an ordinary light microscope. In the chaffinch, he found 7–11 fibrils, somewhat as are found in *Arbacia*.

The structure and reaction to stains of the sperm of *Arbacia punctulata* have been especially studied by Popa (1927). He was particularly interested in the distribution of fats, which he found present in the head, middle piece, and tail. The peculiar forms of the spermatozoa which he observed with different stains can be duplicated by treatment of living sperm with distilled water, drying, etc. (E. B. H.). In distilled water, the heads swell to double their volume (E. B. Harvey and Anderson, 1943).

Figures of the spermatozoon of *Arbacia lixula* given by Pictet (1891) and by Retzius (1910) show it to be similar to that of *A. punctulata*. The acrosome or perferatorium and the two spherical bodies in the middle piece are figured by Retzius on his Plate XV, no. 2. The measurements of *A. lixula* sperm given by Field (1895) are similar to those of *A. punctulata*.

Recent photographs of sperm of other species are those of Vasseur (1947) of *Echinocardium cordatum;* of Tyler (1949c) of *Lytechinus pictus* taken with the electron microscope; of Rothschild (1951a) of *Echinus esculentus;* of J. C. Dan (1952) of two Japanese species (also Dan, 1954, Biol. Bull. 107 : 335–349 and Afzelius, 1954, Zeit. f. Zellforsch. 42 : 134–148).

b. Swimming Rate

The swimming rate of the *Arbacia punctulata* spermatozoon is, according to Grave and Downing (1928) and Grave (1934), about 1 mm. per minute at 22.5° C., or 20 times its length (50 μ) per minute, and slightly faster at a higher temperature. Gemmill (1900) gives a little higher rate for *Echinus esculentus*. For *Psammechinus miliaris*, with a slightly different technique, Rothschild (1951a) estimates 200 μ per second (translatory speed), or about 12 mm. per minute at 18° C.

The human sperm swims, in the uterus and Fallopian tube (a distance of about 190 mm.), at the average rate of about 2.7 mm. per minute (R. L. Brown, 1944) or about 45 times its own length (60 μ) in one minute. A good swimmer swims a mile in 20 minutes, so that a six-foot man swims 44 times his own length in one minute. A human sperm and a man thus swim at about the same rate, length for length (E. B. Harvey, 1946b).

For a further comparison, a gastrula of *Arbacia lixula*, whose diameter is approximately 0.2 mm., swims in a tube of its own diameter according to Needham (1931, Vol. 2, p. 1247) at the rate of 1.17 meters per hour, or 20 mm. per minute, 100 times its diameter. The *Arbacia* lixula gastrula swims about 20 times as fast as the *Arbacia punctulata* sperm, or 5 times as fast, length for diameter. A greater rate would be expected on account of the numerous organs of propulsion, the cilia, though it is not as streamlined. *Paramoecium caudatum* swims about 3 mm. per second or 15 times its length (ca. 200 μ) according to Ludwig (1928); this makes the rate about 180 mm. per minute or 900 times its length. *Paramoecium* therefore swims nine times as fast as the gastrula of *A. lixula*. This greater rate might be expected since *Paramoecium caudatum* is streamlined. Other rates have been given for *Paramoecium* by other investigators. *Tabulae Biologicae* (IV p. 480, Metzner, 1927) give 1.3 mm. per second for *P. caudatum*, length 216 μ. Chase and Glaser (1930) give 833 μ per second, species not mentioned but probably *P. caudatum*. Wichterman (personal communication 1953) has found that for a given species, the rate varies with the phase in its growth and life cycle.

c. Motility

The effect of dilution in activating the sperm and the loss of fertilizing power with time have been studied in *Arbacia punctulata* by F. R. Lillie (1915b), though they were observed earlier in *Echinus esculentus* by

Gemmill (1900). Cohn (1918) found that increased alkalinity increased the activity with a corresponding decrease in length of life; and that increased CO_2 decreased activity and increased length of life. In concentrated sperm, more CO_2 is produced which inactivates the sperm and they live longer. Increasing the temperature increases the activity as one would expect. KCN inactivates them and prolongs their life (Cohn, 1918).

The motility of sperm is increased by egg water or egg extracts (F. R. Lillie, 1913b, 1915a, 1919, p. 111, etc.; Woodward, 1918; Cohn, 1918; Sampson, 1922; *et al*). According to Hartmann *et al.* (1939), it is the echinochrome in the egg water of *A. lixula* that causes the activation. Tyler (1939), however, found this not to be true for *Strongylocentrotus purpuratus*, and Cornman (1941) for *A. punctulata*.

Other sperm-stimulating substances have been found by Clowes and Bachman (1921), including propyl, allyl, and cinnamyl alcohol, propylene, and a "volatile substance derived from marine eggs."

The sperm of *Arbacia* are immobile when in the testes, and become motile only when diluted. The sperm are immotile in absence of oxygen and therefore cannot fertilize the eggs (E. B. Harvey, 1930); they are also immotile in KCN (Cohn, 1918); Loeb (1915a) had found this for *Strongylocentrotus purpuratus*. Motility is also suppressed by halo- and nitrophenols (Clowes, 1951) and by dilute $HgCl_2$ (F. R. Lillie, 1921b), and by the SH reagent PCMB (p-chloro-mercuri-benzoate) (Runnström, 1952).

Sperm are more resistant to anaesthetics than are the eggs, but they can be anaesthetized. In 0.2 M ethyl urethane they lose their fertilizing power in $\frac{1}{2}$ hour and their motility in 2 hours (E. B. H.). Chloretone is efficacious in quieting them.

In Ca-free sea water, the sperm are motile and surround the eggs but will not fertilize them (E. B. H.; *et al.*); this was observed many years ago by Loeb (1915a).

d. Longevity

Although there are many references in the literature to increasing the life span of sperm, it would seem that the length of life depends on their activity, and this is dependent on many factors as described above, and especially on temperature. The less active they are, the longer they live. However, Budington (1935) says that acetylsalicylic acid (aspirin) causes sperm to retain their fertilizing power longer. Tyler (1950, 1955) found that various amino acids and peptides, ver-

sene, and other metal-chelating agents extend the functional life span very greatly. For *Lytechinus pictus* and *S. purpuratus* see Tyler and Atkinson (1950).

e. Injury

Arbacia sperm are injured by hypo- and hypertonic sea water; alcohol 1–12% added to sea water; sea water slightly acidified with HCl; ageing for 6–24 hours (Dungay, 1913). The injured sperm caused irregular cleavage, abnormal blastulae, gastrulae and plutei, the results being non-specific. The sperm are also harmed by $CuCl_2$ and $HgCl_2$ (F. R. Lillie, 1921b).

f. Agglutination

A very noticeable phenomenon in motile *Arbacia* sperm is agglutination, the collection of numbers of sperm into clumps. This was observed by Buller (1900b) in *A. lixula* sperm, and has been studied intensively in *A. punctulata* by F. R. Lillie (1912, 1913a, b, 1914, 1915a, 1919, p. 112, etc.); and by Glaser (1914b); Cohn (1918); Woodward (1918); Sampson (1922); Popa (1927); *et al.* The substance in suspensions of eggs causing this reaction has been called by F. R. Lillie (1913a) *fertilizin* and earlier was called *iso-agglutinin* (F. R. Lillie, 1912). Sperm extracts agglutinate the eggs and inactivate fertilizin (Frank, 1939). The agglutination is usually between the heads of the spermatozoa (F. R. Lillie, 1919, p. 119; Sampson, 1922; Tyler, 1948). See Part IV, *Fertilizin and Agglutinin* for recent reviews, especially Tyler (1948, 1949c).

g. Chemotaxis

The classical studies of Pfeffer (1884) showed without much question that the sperm (antherozoids) of ferns were attracted to the archegonium (and egg), and that this was due to the malic acid and its salts occurring there, a definite chemotaxis. But whether the sperm of animals are attracted to the eggs by chemotaxis has never been proven. Buller (1900a, b, 1902), working in Pfeffer's laboratory, confirmed the chemotaxis in the case of ferns, but when working on sea urchins, including *Arbacia lixula*, at Naples, could find no evidence of chemotaxis, and concluded that in these forms the eggs and sperm meet by chance. Other investigators (e.g. von Dungern, 1902) confirmed Buller's re-

sults. However, F. R. Lillie (1913b, 1919 p. 102) stated that in *Arbacia punctulata*, the sperm are attracted to CO_2 and to egg water which contains "fertilizin". But Loeb (1914c) thought that Lillie's explanation of his experiments was incorrect, and that they did not necessarily show chemotaxis. Later, Loeb took a decided stand against chemotaxis of eggs and sperm and expressed his views in two of his books, *The Organism as a Whole* (1916, p. 92) and *Forced Movements, Tropisms* and *Animal Conduct* (1918, p. 139). The subject has been taken up again recently by Rothschild (1951a, b, 1952), who has come to the conclusion that "in the animal kingdom spermatozoa probably meet or collide with eggs by chance" (1952, p. 1), and that "chemotaxis of spermatozoa toward eggs has never been observed with certainty" (1951b, p. 40). For the older work see Morgan's *Experimental Embryology*, Chapter II, 1927.

h. Polyspermy

See Part IV, *Polyspermy*.

i. Physiology

For references to the physiology of the sperm of *Arbacia punctulata*, such as respiration, enzymes, etc., see under the appropriate topic in Part IV which treats of both eggs and sperm.

j. Seminal fluid

The seminal fluid of *Arbacia punctulata* has been especially studied by Hayashi (1945, 1946) with the following results. Sperm are as motile in seminal fluid as in sea water; they retain their fertilizing power longer. The pH of the seminal fluid is 7.6–7.9; its osmotic pressure is 10% lower than sea water; it contains less than 10 μg reducing sugar in 5 cc.; it contains 2.5 mg. protein per cc.; it does not act as a nutrient for the sperm. It does not contain anti-fertilizin, but increases agglutination. It delays the fall in respiration following the increase after dilution.

The seminal fluid or "seminal plasma" of *Echinus esculentus* has been studied by Rothschild (1948b) with special regard to O_2 tension; he also noted the large amount of potassium in the seminal fluid.

k. Techniques

Method of keeping sperm

Remove the testes from a freshly opened animal as intact as possible, and place them, undiluted, in a small covered stender dish (about 3 cm. diameter). Place

immediately in the refrigerator at about 8 °C. They keep perfectly for several (4–5) days and give nearly 100 % fertilizations.

To Kill Sperm

Formalin, 0.1 to 1 % (Tyler). Distilled water kills *Arbacia* sperm almost immediately (E. B. H.). Keep at 40 °C. for 20 minutes (E. B. H.). Rothschild and Swann (1951) use for *Ps. miliaris*, hypotonic sea water, 45 % sea water with distilled water, to kill spermatozoa in presence of eggs and prevent fertilization without harmful effect on the eggs.

To Prepare Sperm Suspensions for Fertilization

One drop (0.1 cc.) of concentrated ("dry") sperm to 100 cc. sea water, then one drop of this to 10 cc. sea water which contains two drops of concentrated eggs; this is the amount for a Syracuse watch glass (E. B. H.). Just (1939a) recommends one drop of "dry" sperm to 10 cc. sea water, then two drops of this to inseminate eggs in 250 cc. sea water.

The dilute suspensions of sperm last only a short time after preparation, owing to their activity, so that fresh dilutions must be made frequently.

A simple and convenient method of fertilizing a small dish of eggs is to take a small amount of sperm on a toothpick and agitate the eggs with it. With experience, this becomes fairly accurate (E. B. H.).

Determination of Number of Sperm in a Suspension

Barron (personal communication) obtains a constant suspension by measuring the turbidity with a Coleman Junior spectrophotometer, and checks this against the dry weight. Mazia (personal communication) counts the number of sperm in a dilute suspension, fixed in 0.1 % formalin, with a haemocytometer.

There are 5 cc. of concentrated sperm in one *Arbacia*, obtained by allowing the sperm from the five ruptured testes to settle. Tyler found 2×10^{10} spermatozoa in 1 cc. by haemocytometer measurements (personal communication, July 1954). This is the same number as he had obtained from *S. purpuratus* and *Lytechinus* (Tyler and Rothschild, 1951; Tyler, 1953). The complete number of sperm in one *Arbacia* then, is 10^{11}, approximately a million million; the number of eggs in one *Arbacia* is about 8,000,000. One sperm has been calculated to be about 1/30,000 the volume of the egg.

The various methods of counting spermatozoa have been discussed and evaluated by Rothschild (1950a), who advocates a photoelectric absorptionometer.

Other Species (Additional) and General References

Bernstein and Mazia, 1953a. *S. purpuratus*, DNA.

Fuchs, 1914c. *Paracentrotus lividus*, *A. lixula*, etc., fertilizing power increased by egg secretions.

Gray, 1931. *Experimental Cytology* p. 408–421. References to older literature.

Morgan, 1927. *Experimental Embryology* p. 15–93. Chemotaxis, activity etc.

Rothschild, 1951a. Review of recent literature, with good bibliography, with titles.

Runnström, 1949a. Many references to sperm in comprehensive review *The Mechanism of Fertilization*.

Southwick, 1939. *Echinometra subangularis*, activity of sperm.

Tyler, 1948. Review.

CHAPTER 13

Fertilization and Cleavage

a. Fertilization. Plates III and V

The normal development of the *Arbacia punctulata* egg, from fertilization through the cleavages to the early pluteus of four days, is shown in a series of photographs of the living eggs and of stained sections (Plates III, IV, and V). This was done many years ago for *Lytechinus (Toxopneustes) variegatus* in the classic "*Atlas of Fertilization*" by E. B. Wilson (1895), and the two species are quite similar. For *P. lividus* see Boveri (1895) and Hörstadius (1935).

When a spermatozoon touches the surface of a mature Arbacia egg, after penetrating the jelly coat, it is engulfed, and a fertilization cone or entrance cone is formed within 20 seconds. The fertilization membrane rises over this and quickly spreads around the egg; this takes only about five seconds. The fertilization membrane lifts off, leaving a perivitelline space, at first very narrow, but gradually becoming wider so as to be 3–5 μ when fully formed. At first it is usually not equidistant from the surface of the egg, but later becomes so. The whole process from the time that the sperm touches the egg until the fertilization membrane is fully raised takes about two minutes at 23 °C. The fertilization membrane is now generally believed to form from the vitelline membrane with the addition of the cortical granule material. See Part IV under *Fertilization Membrane*, *Vitelline Membrane*, *Cortical Layer*.

The fertilization cone which is raised at the point of entry of the sperm is at first conical or flattened, but later becomes flame-like (Plate III, Photograph 3). It may disappear quickly but often lasts 4 to 5 minutes, especially in eggs early and late in the season, and in eggs kept cold.

In sectioned and stained material, Mathews (Wilson and Mathews, 1895) has described the rotation of the sperm head soon after its entrance into the egg. It rotates through an angle of 180° so that the base containing the sperm aster becomes directed inwards, toward the egg nucleus. (See Wilson's *The Cell*, 3 ed., p. 397).

The sperm aster becomes quite large, easily visible in the living egg, indicating the position of the male pronucleus as it approaches the female pronucleus. The small sperm nucleus flattens over and unites with the large egg nucleus and the astral rays spread through the egg; this is the monaster stage. The rays disappear and the centrosome (probably) divides, forming a curved disk over the nucleus; this is the "streak" stage (Plate III, Photograph 6; Plate V, Photograph 4). The cell wall at this stage is not smooth but somewhat crenate. The hyaline layer which starts to form soon after fertilization has now become 2 to 3 μ thick.

The nucleus enlarges from 11.5 μ to 16 μ in diameter, nearly three times in volume; it becomes elliptical, and then the nuclear membrane breaks (Plate III, Photograph 7). The chromatin material, meanwhile, forms into chromosomes, and a spindle with an aster at each pole is formed on which the chromosomes lie, at first irregularly scattered (prophase), then lined up at the equator (metaphase) (Plate III, Photograph 8; Plate V, Photograph 6). Then the chromosomes divide, half of each chromosome going to each pole (anaphase). After reaching the pole, each chromosome becomes vesicular and the vesicles fuse (telophase). The centrosphere is largest and the astral rays most extended during the late anaphase and telophase (in *Arbacia*).

b. Cleavage. Plates III and V

Now the egg elongates and the cleavage furrow comes in, usually asymmetrically, on one side first, the side nearest the excentrically placed spindle. There is a heaping up of the hyaline layer in the furrow and a corresponding thinning at the poles. The red pigment granules also tend to accumulate in the furrow, as noted by many observers, e.g., McClendon, 1910b. See under *Chromatophores*, Part IV.

The two cells are at first well separated but later become closely pressed together owing probably to the formation of the next mitotic figure (Plate III, Photographs 10, 11). The first cleavage plane passes through the polar axis, i.e., in the region where the polar bodies formed, the animal pole. The second cleavage plane is also meridional and at right angles to the first. The third cleavage plane is equatorial, and we have eight equal blastomeres. The fourth cleavage plane is differential, cutting off four small unpigmented cells, the micromeres, at the vegetal pole (Plate III, Photographs 14, 15). More cleavages follow, and a blastula of many cells is formed. It will be noticed in the photographs that the spindles in the later cleavages have no asters, a characteristic of plant cells (Plate V, Photograph 12).

c. Schedule of Development

A time table of cleavage in a normal batch of *Arbacia* eggs at 23 °C, is given in Table 2, and photographs on Plates III, IV and V.

Other schedules of cleavage times in *Arbacia punctulata* have been given by Fry and Parkes (1934); Fry (1936); Hoadley and Brill (1937), and Blum and Price (1950b). The rates of first cleavage at different temperatures and the Q_{10} for cleavage as determined by Loeb and Wasteneys (1911a) and Loeb and Chamberlain (1915) are given in Table 3, together with a few figures by Fry (1936). The rate of the first three cleavages at different temperatures as obtained by Hoadley and Brill (1937) is given in Table 4.

For a comparison of Arbacia *punctulata* with some other species, a few references for cleavage rates in other species are given in Table 5.

TABLE 2

SCHEDULE OF DEVELOPMENT (E. B. HARVEY)

Time Table of Cleavage and Development of *Arbacia punctulata* Eggs at 23 °C. Times after fertilization for 50 % of the eggs to reach the stage designated.

Completion of fertilization membrane	2 min.
Sperm aster	8 min.
Union of pronuclei (monaster stage)	10 min.
Completion of hyaline layer (begins 2 min.)	20 min.
Streak stage	20–35 min.
Nuclear membrane breaks	35 min.
Prophase	35 min.
Metaphase	40 min.
Anaphase	42 min.
Telophase	45 min.
1st cleavage; 2-cell	50 min.
2nd cleavage; 4-cell — 28 min. after 1st	78 min.
3rd cleavage; 8-cell — 25 min. after 2nd	103 min.
4th cleavage; (12-cell) micromeres; 27 min. after 3rd	130 min.
16-cell — 32 min. after 3rd	135 min.
5th cleavage; (20-cell) — 22 min. after 4th	157 min.
(28-cell) — 30 min. after 4th	165 min.
32-cell — 32 min. after 4th	167 min.
Hatch as blastulae, ca. 1,000 cells	7–8 hrs.
Gastrulae	12–15 hrs.
Skeleton begins	19 hrs.
Plutei	1 day
Plutei, maximum without special feeding	3–4 days

There is a slight variation in cleavage times in different batches of eggs and considerable variation at different times of the year, irrespective of temperature. The above table is for a standard batch in mid-season at 23 °C. It is based on both living eggs and stained sections. There is a greater variation in the later stages of development, e.g., time of hatching.

TABLE 3

TIME IN MINUTES FROM FERTILIZATION TO FIRST CLEAVAGE

TEMPERATURE	LOEB AND WASTENEYS (1911a) LOEB (1913a)	LOEB AND CHAMBERLAIN (1915)	FRY (1936)
7.0°	498.0	—	—
8.0°	410.0	411.0	—
9.0°	308.0	297.5	—
10.0°	217.0	208.5	—
11.0°	—	175.0	—
12.0°	147.0	148.0	—
13.0°	—	129.0	—
14.0°	—	116.0	—
15.0°	100.0	100.0	113.0
16.0°	85.5	—	—
17.5°	70.5	—	—
18.0°	68.0	68.0	—
19.0°	—	65.0	—
20.0°	56.0	56.0	67.0
21.0°	—	53.3	—
22.0°	47.0	46.0	—
23.0°	—	45.5	—
24.0°	—	42.0	—
25.0°	40.0	39.5	42.0
26.0°	33.5	—	—
27.5°	34.0	—	—
30.0°	33.0	—	—
31.0°	37.0	—	—
32.0°	no cl.	—	—

TABLE 4

TIME IN MINUTES FROM FERTILIZATION TO FIRST, SECOND AND THIRD CLEAVAGE (FROM HOADLEY AND BRILL, 1937)

TEMPERATURE	1ST CLEAVAGE	2ND. CLEAVAGE	3RD. CLEAVAGE
9.6°	295	486	528?
12.0°	180	288	399
15.2°	110.5	174.5	239
18.1°	78.5	126	172
21.3°	57.5	94	130
24.1°	46.5	72.5	99
26.9°	41.3	65.5	92
30.0°	40	64	89

TABLE 5

TIME OF FIRST CLEAVAGE OF SOME SPECIES, IN MINUTES AFTER FERTILIZATION

SPECIES	LOCATION	TEMP.	TIME	REFERENCE
Arbacia punctulata	Woods Hole	23°	50	Many
Arbacia lixula	Naples	16°	110	E. B. H., 1933a
	Naples	18°	99	Callan, 1949
Dendraster excentricus	Pacif. Grove	20°	55	Moore, 1933
	Pasadena	22°	47	Tyler, 1936a
Echinometra lucunter	Bermuda	25°	90	E. B. H., 1947 (unp.)
Lytechinus variegatus	Beaufort	22°	55	E. B. H., 1942 (unp.)
	Bermuda	24°	55	E. B. H., 1947 (unp.)
	Tortugas	28°	40	Tennent, 1911a
Paracentrotus lividus	Roscoff	18°	71	Ephrussi, 1933
	Naples	16°	90	E. B. H., 1933a
	Naples	18°	76	Callan, 1949
Ps. microtuberculatus	Naples	16°	70	E. B. H., 1933a
	Naples	18°	61	Callan, 1949
Psammechinus miliaris	Millport	17°	67	Gray, 1927a
	Sweden	18°	56	Borei, 1948
Sphaerechinus granularis	Naples	16°	105	E. B. H., 1933a
	Naples	18°	100	Callan, 1949
S. franciscanus	Pacif. Grove	20°	95	Moore, 1933
	Pacif. Grove	19°	80	E. B. H., 1941 (unp.)
S. purpuratus	Pasadena	20°	77	Tyler, 1936a
	Pacif. Grove	19°	70	E. B. H., 1941 (unp.)
Tripneustes esculentus	Bermuda	22°	90	E. B. H., 1932 (unp.)
	Tortugas	26°	75	Tennent, 1911a

It will be seen that the eggs of some species cleave more rapidly than those of *Arbacia punctulata*, some less rapidly. There seems to be no relation between the size of the egg and the rate of cleavage. One might think that the cleavage rate of a small egg would be faster than that of a large egg. But the small egg of *Tripneustes esculentus*, 84 μ diameter, cleaves more slowly (90 min. at 22 °C.) than the large egg of *Dendraster excentricus*, 114 μ diameter which cleaves in 47 min. at 22 °C. It may be noted that large eggs may come from small species, (e.g.,

Psammechinus microtuberculatus, and small eggs from large species, e.g., *Tripneustes esculentus*. *Arbacia* has a fairly small egg (for sea urchins) which cleaves at an intermediate rate. For sizes of eggs, see Classification, Part I, Chapter 9.

Fox (1938) has found that the cleavage rate of some species *(Paracentrotus lividus)* is different in different localities. Hörstadius (1925) found that in the same locality, Naples, the cleavage rate of this species differs in different seasons of the year; winter eggs and summer eggs have a different rate when kept at the same temperature. This is true also, to a limited extent, of *Arbacia punctulata;* the eggs obtained late in the season (September) are slower to cleave, irrespective of temperature, than those obtained at mid-season (July, August).

d. Cleavage Without Membranes

If the fertilization membranes are removed soon after fertilization, by shaking, the eggs develop quite normally, though somewhat spread out (Plate XVI, Photograph 7). They are held together by the hyaline layer. They cleave at about the same rate but become free-swimming earlier, since the fertilization membrane does not have to be dissolved by the "hatching enzyme". Normal plutei are formed.

If the fertilization membranes are removed and the eggs placed in sea water without calcium, the hyaline layer does not form and the cells are no longer held together. The isolated cells cleave several times and among them, micromeres may be distinguished by their size (Plate XVI, Photograph 9). Soon the isolated masses of cells go to pieces.

e. Sperm Entrance and Cleavage Planes

A sperm may enter at any point on the surface of the egg, as noted by many observers. This can be best demonstrated in centrifuged *Arbacia* eggs, where it may be observed to enter in any zone of the stratified egg, pigment, yolk, clear layer, or even the oil cap.

The tail of the sperm has not been observed to enter the *Arbacia* egg, but is left outside, though the portion adjacent to the head is often seen within the fertilization membrane, in the perivitelline space. This portion as well as the distal end soon disappears as though dissolved. The tail is left outside in the *Lytechinus (Toxopneustes)* egg (Wilson's *Atlas* 1895, p. 14), and probably in other sea urchins, as stated by Wilson, more positively in the earlier editions of *The Cell* (Compare the first edition 1896, p. 136, 149 and second edition 1911, p. 188, 200

with the third edition 1925, p. 395). Recently J. C. Dan (1950) has stated that in six species of Japanese sea urchins and starfish, the tail enters the egg, though none of her figures show it actually inside the egg, only in the perivitelline space, as frequently seen in *Arbacia*. The question does not seem to be of much importance since the tail has completed its function and is probably of no further use.

The first cleavage plane has been observed to cut through without any relation to the entrance point of the sperm, not necessarily through it. This has been found to be the case also in *Paracentrotus lividus* by Hörstadius (1928, 1939) who used the Vogt (1925) vital staining technique to locate the sperm entry. However, Wilson (Wilson and Mathews, 1895, p. 324) says that in *Toxopneustes (Lytechinus)* "the plane of first cleavage is in the great majority of cases at least approximately through the entrance point of the sperm." See also Wilson's *Cell*, 1925, p. 1104.

Though one sperm usually enters the egg, the question has been raised whether fertilization is possible with only one sperm. Kite (1912) injected with a micropipette several sperm into the jelly of an *Arbacia* egg and obtained fertilization from a single sperm. Glaser (1915), however, held that more than one sperm is required to bring about changes in the membrane necessary for one sperm to enter the egg. Tyler (1949c) likewise considers more than one sperm necessary.

f. The Centriole

There is no definite single granule or centriole observable in the center of the aster in *Arbacia punctulata*, either in the sperm aster or the cleavage aster, but a group of small granules. This was stated many years ago by Mathews (Wilson and Mathews, 1895), and was believed to be true also for artificial astrospheres (cytasters) by Morgan (1899). See the discussion by Morgan (1899) and Wilson (1901a). A very skeptical view concerning the centriole in *Echinoderm* eggs, especially in *Echinarachnius parma*, has been held by Fry (1929 and many other papers). See also Wilson's *Cell*, 1925, p. 677.

g. Mid-Bodies

Mid-bodies (Zwischenkörper), thickenings of the disappearing spindle fibers at the equator, are characteristic of plant cells and are very prominent in many animal cells, e.g., the spermatogonial cells of the Orthopteran, *Rhomalium* (see Fig. 60, p. 138 in Wilson's *Cell* 1925). These mid-bodies are not conspicuous in *Arbacia*, though Fry (1937)

describes them as occurring in this form. They are mentioned as occurring in *Lytechinus* by Wilson in his *Atlas* (1895, p. 27 and Fig. XVII), but do not appear conspicuous or typical.

h. Chromosomes

The chromosomes of *Arbacia punctulata* are small and crowded and difficult to count. The diploid number, in cleavage cells, is probably 38. This number, 38, is also given, with drawings, by Matsui (1924); and Morgan (1927, p. 627 footnote) says that 36–38 is recorded by E. B. Wilson and students. E. B. Harvey (1940c) gives 32–38 as the diploid number, and half that number for the parthenogenetic egg. Tennent (1912b, p. 397) says "about 40" (this article was incorrectly attributed to Jordan, 1912, in my tabulation, 1920, p. 12).

In *Arbacia lixula*, the Naples species, Baltzer (1910) gives 40 as the number of chromosomes in cleavage cells. In *Lytechinus (Toxopneustes) variegatus* which closely resembles *Arbacia* in cytological details, the number is 36 or 38 (E. B. Wilson, 1895, *Atlas*, p. 23; 1901a, b; Tennent, 1912b). The chromosome numbers of the other *Echinoidea* are given in the tabulation of E. B. Harvey, 1920, p. 12–14. This list (including a mistake) has been copied in *Tabulae Biologicae*, vol. IV, 108 (1927) by Breslau-Harnish, and in vol. 18, p. 34 (1939) by McClung. Makino, 1951, has added four more references to this list, including *Arbacia punctulata* (38 chromosomes) and *Echinarachnius parma* (52 chromosomes) by Matsui, 1924; *Clypeaster rosaceus* (44 chromosomes) by Gardiner, 1927; and *Strongylocentrotus intermedius* (50 chromosomes) by Niiyama and Makino, 1947. To make the list complete there should be added *Cidaris tribuloides* with 37, 38 diploid, 18, 19 haploid, 19 parthenogenetic eggs (Tennent, 1922); and *Mespilia globulus* with 38 diploid, 19 haploid (Tennent and Ito, 1941). It will be seen that most sea urchins have 36–38 chromosomes, diploid, including *Arbacia punctulata*.

There are apparently no sex chromosomes in *Arbacia;* at least none have been reported. But there are sex chromosomes in *Lytechinus variegatus* and *Tripneustes esculentus* (Tennent, 1911b, 1912a, b, c, 1922); in *Paracentrotus lividus* and *Psammechinus microtuberculatus* (Baltzer, 1913); and in *Cidaris tribuloides* (Tennent, 1922). The digametic sex is the male and not the female as was once thought. (Baltzer, 1909, 1910, 1913).

The chromosomes of *Arbacia* are small and of different sizes and shapes; some are spherical and some rod-like (Tennent, 1912b; Matsui, 1924; E. B. Harvey, 1940c, Photograph 130 on Plate VIII). From

measurements of metaphase plates of first cleavage stained with aceto-carmine, a medium-sized, spherical chromosome has a diameter of ca. 0.8 μ, giving a volume of ca. 0.268 μ^3. All the 38 chromosomes would have a volume of ca. 10.2 μ^3, or 1/20,000 the volume of the egg.

The sperm head, containing the haploid number of chromosomes, has a volume of ca. 3.4 μ^3, as calculated from the data (length 3.25 μ, thickness 2 μ) of E. B. H. (see under *Sperm*, Chapter 12a). The volume of desoxyribose nucleic acid in the *Arbacia* sperm head is 1.3 to 1.4 μ^3 calculated by Mazia (personal communication 1955). For *Drosophila*, Sturtevant (personal communication 1950) calculated that the diploid chromosomes have a volume of ca. 1.0 μ^3, and the sperm head (haploid) about one quarter of this. Other figures for *Drosophila* from which similar volumes can be calculated have been given by Muller (1929) and Gowen and Gay (1933).

i. Visibility of the Cleavage Figure

In the living egg of *Arbacia*, asters are plainly visible, but spindles and chromosomes cannot be observed with the usual light microscope even with the best apochromatic objectives and compensating oculars. When no granules are present, as in the clear quarters of the centrifuged egg, the asters cannot be seen. In photographs taken with ultraviolet light, the chromatin material and chromosomes of the living egg appear as they do in fixed and stained sections (Harvey and Lavin, 1944). With infrared light, photographs of the living egg show the configuration of the mitotic figure in a striking manner as a brilliant white area against a dark granular cytoplasmic background (Harvey and Lavin, 1951b). The spindle of *Arbacia* has not been studied with a polarizing microscope, but the mitotic figures of other sea urchin eggs show a beautiful birefringence (Swann, 1951a, b; Innoué and Dan, 1951; see the fine photographs in these papers and Hughes', 1952, book, Plate XIII).

The structure of the mitotic figure is best studied in sectioned material. The best fixative is probably Bouin, although the egg shrinks from 74 to 50 μ; the most satisfactory stain is Heidenhain's haematoxylin. Very good total mounts can be made with aceto-carmine and acetic orcein. A photograph of a very thin section of a spindle taken with the electron microscope, has been made by Geren and McCulloch (1951).

Fading out of cleavage figure or furrow (reversible) is caused by
Colchicine, Nebel 1937; Nebel and Ruttle, 1938; Beams and Evans, 1940.

Cold, Heilbrunn, 1920b.
Ether, Heilbrunn, 1920b. See E. B. Wilson, 1901b for *Lytechinus*.
Hydrostatic pressure, Marsland, 1938, 1950, 1951 for *A. punctulata;* 1939 for *A. lixula.*
Micromanipulation, Chambers, 1919, 1938c, 1951.
Oxygen-lack, Mathews, 1907; E. B. Harvey, 1927 for other species; 1930 for *A. punctulata.*
Podophyllin, etc., Cornman and Cornman, 1951.
Quinine, Mathews, 1907.
Urethane, Painter, 1918; E. B. H., unpub.

j. Isolation of Mitotic Apparatus

An important method has been recently devised by Mazia for isolating the mitotic apparatus from the rest of cell and has been applied to *Arbacia punctulata* (Mazia and Dan, 1952; Dan, Ito, and Mazia, 1952). This technique, the details of which are given in their papers, allows a much better understanding of the structure, function, and chemistry of different parts of the mitotic figure.

k. Elongation at Cleavage

Elongation of the *Arbacia* egg at the time of cleavage is easily observed in the living egg, as seen in the photographs. Studies have been made on the elongation especially by Churney (1936, 1940). He found that with the fertilization membrane present, the egg elongates from 74 to 82.5 μ, an elongation of 11.5% (at 22.2–26.0 °C.); without the fertilization membrane it elongates from 74 to 103.2 μ, an elongation of 39.4%; see also A. Scott (1946). For the effect of mechanical pressure, see Chambers (1946, 1951). A mathematical treatment of the elongation of the *Arbacia* egg has been made by Buchsbaum and Williamson (1943). It will be observed from the photographs (Plate III) that the elongation takes place at telophase (see also Just, 1928b). A special study of elongation at cleavage in *Echinus esculentus* has been made by Gray (1931, p. 194).

l. Asynchrony in Cleavage

It will be noticed from the schedule of development (Table 2) and from the photographs that there is an asynchrony in cleavage beginning after the 8-cell stage, when one quartet (vegetal) divides horizon-

tally into 4 large cells, macromeres, and 4 small cells, micromeres, some 5 minutes before the other quartet (animal) divides meridionally into 8 equal cells, mesomeres (Plate III, Photographs 14, 15). There is thus a definite 12-cell stage preceding the 16-cell stage by about 5 minutes at 23 °C. The interval is somewhat variable in different batches of eggs and very rarely (in one batch only out of many hundreds) does not occur at all, the 16-cell stage following directly on the 8-cell. In *Lytechinus (Toxopneustes) variegatus* also this fourth cleavage is asynchronous according to Tennent (1911a) and Tennent, Taylor, and Whitaker (1929), so that there is a definite 12-cell stage.

In the next cleavage also, the three types of cells, mesomeres, macromeres and micromeres divide asynchronously, so that there is a 20- and a 28-cell stage preceding the 32-cell stage by 10 and 2 minutes respectively (23 °C.). The 4 macromeres divide first, then the 8 mesomeres and then the 4 micromeres.

It is difficult to follow the division of the different types of cells further, into the 64-cell stage, but sections of later cleavages definitely show that at any particular time the cells are in different stages of mitosis; that asynchrony continues in the later cleavages. There is, however, at least at first, a greater interval between the division of certain sets of cells than between others. There seems to be a major rhythm with considerable spread.

Recently an abstract on this subject, also for *Arbacia*, has been published by Scott and Fox (1952) Their results are similar to mine, and they have carried the asynchrony through the sixth and seventh cleavages.

It is of interest that many years ago Tennent (1911a) published a detailed table of the stages and times in the development of the *Lytechinus* egg, similar to the one for *Arbacia* presented here. He has given the times for asynchronous divisions up to 124 cells. The earlier asynchronous cleavages correspond exactly with those of Arbacia.

The asynchrony in cleavage becomes important in studies of rhythms of oxygen consumption in relation to mitoses, such as those of Zeuthen (1951, etc.). It may be that in some species the rhythms are more marked than in *Arbacia* and *Lytechinus*, and Zeuthen (1951, p. 52) found that in *Psammechinus microtuberculatus* "the micromeres have multiplied slower than the rest of the cells."

m. Micromeres

The micromeres of *Arbacia* are colorless in contrast to the rest of the

cells which are pigmented. Apparently the pigment recedes from the lower parts of the lower four cells (vegetal) at the eight-cell stage (Morgan, 1893). There has never been a very good explanation for this, nor has any experimental work been done on it. McClendon (1910b, p. 243) thinks that "the pigment entirely disappears from the micromere pole, indicating spreading movements due to the surface tension being less here than in the region of the future cleavage furrow. Similar movements of granules have been observed in the cutting off of polar bodies in various eggs, and it may be concluded that for the separation of a very small cell from a large mass of protoplasm a very great difference in surface tension between the pole of the small cell and the cleavage furrow is required."

In some cases, it was found by Morgan (1893) that the retreat of pigment to form the micromeres takes place long before the actual cutting off of the cells—in the four or even two-cell stages. An early appearance of micromeres, in the 8-cell stage, was found by Painter (1915) in eggs treated with phenyl urethane. In eggs treated with mustard gas, the micromeres are formed early and one (colorless) micromere is often present in the two-cell stage (E. B. H., 1943 unpub.).

According to most investigators, the micromeres of *Arbacia*, as in other sea urchins, come off at the vegetal pole, nearly opposite the funnel in the jelly which marks the position of the polar bodies (Morgan and Spooner, 1909; Spooner, 1911; Hörstadius, 1937a). The micromere-forming material was located by Harnley (1926) in the unfertilized *Arbacia* egg, between the nucleus and the center of the egg. But Tennent, Taylor, and Whitaker (1929) could not confirm this, and held that there was no localization of micromere-forming material in the unfertilized eggs, and that in the fertilized egg the micromeres formed at the cut surface of any fragment. Hörstadius (1937a) could not confirm this, nor could he confirm Harnley's work. He believes that in *Arbacia* the micromere-forming material is in the vegetal half of a cut egg, and that the animal half never forms micromeres, the condition he and others have found for other sea urchin eggs. But he admits that "micromere formation is very sensitive to mechanical injury" and "In *Arbacia* fragments the micromere-formation seems to be inhibited very often" (Hörstadius, 1937a, p. 304). Plough (1927) had, some years before, maintained that there was a localization of skeleton-forming material even before first cleavage in *Arbacia* eggs as well as in those of *Echinarachnius*, *Echinus*, and *Paracentrotus* (1927, 1929), just as maintained by Hörstadius (1937a). A study of micromeres in egg fragments has been made by Tennent, Taylor, and Whitaker (1929).

In centrifuged whole eggs, after fertilization, the micromeres may come off at any place, even at the oil cap (E. B. H.). Their position is probably determined by the original polarity of the egg. They may be pigmented but are usually not. See Plate VIII, Photographs 5, 6, 15, 16.

There may be not four micromeres, but three, two, one or none, and yet normal development may follow. This has been found by Tennent, Taylor, and Whitaker (1929) and also by Hörstadius (1937a). Hörstadius also calls attention to the fact that not all small cells are micromeres, but that small cells may be formed as the result of entirely different factors from those leading to micromere formation. These facts must be born in mind in any investigations involving micromeres.

n. Absence of Oxygen

Whether an egg can be fertilized in absence of oxygen has always been an intriguing question. Unfortunately for its answer, oxygen is necessary for motility of the sperm, so that in its absence they cannot swim to the eggs and fertilize them. If there is the slightest trace of oxygen so that a few sperm are very slightly motile, a fertilization membrane is thrown off, but no further development takes place (E. B. Harvey, 1930). That it is the lack of motility of the sperm and not the absence of oxygen is shown by an experiment of Kitching and Moser (1940). *Arbacia* eggs were kept in absence of oxygen beside a drop of a parthenogenetic agent, and then the two drops were mixed in absence of oxygen. A fertilization membrane was formed.

o. Refertilization

It has been the experience of many investigators that an egg, once fertilized, cannot be fertilized again, even if the fertilization membrane has been removed (Loeb, 1916, p. 85; F. R. Lillie, 1919, p. 161; *et al.*). Until recently an only exception for sea urchins is the report of Bury (1913) that if the eggs of *Strongylocentrotus lividus* and *Echinus microtuberculatus*, already fertilized and with fertilization membranes, are kept in the cold, 0° C., they can be refertilized. Recently, Sugiyama (1947, preliminary; 1951) has found that in *Strongylocentrotus pulcherrimus* and other Japanese species, after the fertilization membranes of fertilized eggs have been mechanically removed, and the eggs are washed in Ca-Mg-free sea water, they could be refertilized. The sperm penetrated the eggs and took part in the formation of the mitotic figures producing irregular cleavages characteristic of polyspermy; the

fertilization membranes were not replaced. Refertilization could take place even without removing the original fertilization membrane if treated soon enough; and also in the 2-cell stage.

p. Fertilization after Parthenogenesis

Loeb thought this possible if the fertilization membrane was removed from the parthenogenetic egg *(Strongylocentrotus purpuratus)*; he thought even blastomeres could be fertilized (Loeb, 1913a, p. 234, 237; 1914b; 1915a, b). A reversal of parthenogenetic development and subsequent fertilization could take place also in *Arbacia* eggs (Loeb, 1913c; Wasteneys, 1916). But others found that a subsequent fertilization was not possible unless parthenogenetic treatment was incomplete (C. R. Moore, 1916, 1917; F. R. Lillie, 1919, p. 167, 1921a; Just, 1922a; Lillie and Just, 1924, p. 502). However, more recently Ishida and Nakano (1947, 1950) have found that if the eggs of *S. pulcherrimus* were treated with a parthenogenetic agent (butyric acid) and the fertilization membranes removed mechanically, and were then placed in a Ca-Mg-free medium, they could be fertilized. Sperm entered and cleavage took place similar to that characteristic of polyspermy.

CHAPTER 14

Blastula, Gastrula and Pluteus

a. Blastula and Gastrula. Plates III and IV

By counting the number of cells at the periphery of an optical section of a living blastula, it has been calculated that there are approximately 1,000 cells in an *Arbacia* blastula just before hatching; this would represent approximately 2^{10} or ten cleavages. MacBride (1914) estimated 1,000 for *Psammechinus microtuberculatus* and Morgan (1895c) 500–525 for *Sphaerechinus granularis*, about nine divisions. Soon each cell acquires a cilium and rotates inside the fertilization membrane. About 8 hours after fertilization at 23° C. ($7\frac{1}{2}$ to $9\frac{1}{2}$ hours in different batches) it breaks through the fertilization membrane and becomes free-swimming. This is done by means of a "hatching enzyme" which dissolves the membrane. Such an enzyme was found many years ago in fish eggs, in *Lepidosiren* by Kerr in 1900, and was especially studied in the Ascidians by Berrill (1929). In sea urchins its occurrence was reported by Ishida (1936) in *Strongylocentrotus pulcherrimus*, and later by Kopac (1941) in *Arbacia punctulata*. The Japanese species was studied again more recently by Sugawara (1943a).

The blastocoel of the *Arbacia* egg is usually small on hatching, in contrast to many other sea urchins, e.g., *Strongylocentrotus dröbachiensis*, *Lytechinus variegatus*. In *Arbacia* the blastocoel gradually becomes larger leaving only a thin layer of ciliated cells at the periphery of the blastula. The cilia are longer at the apical pole as early as hatching, forming the apical tuft.

After the blastula has become free-swimming, it remains spherical for about seven hours, swimming very actively. Then it begins to invaginate (about 15 hours after fertilization) at the pole where the micromeres came off, the original vegetal pole, and opposite the pole where the polar bodies were given off, the animal pole (Morgan and Spooner, 1909; Hörstadius, 1937a). This relationship was beautifully shown for the *Paracentrotus lividus* egg in the classic studies of Boveri (1901, etc.) and is the same for *Arbacia*.

The invagination continues until it approaches the anterior end

where the mouth is formed, making the gut complete (about 17 hours after fertilization). The original in-pocketing remains as the anus. Meanwhile (about 16 hours) the skeleton has appeared as a pair of triradiate spicules, one on each side of the (incomplete) gut. During this period there has been no appreciable increase in the size of the organism over that of the egg (without the fertilization membrane), and one would not expect an increase before the alimentary canal is complete and it can take in food from the outside. Then growth occurs and further differentiation. The axis of the larva changes, giving the "prism" stage, and one of the prongs of the triradiate spicules elongates (dark field photograph, Plate IV, Photograph 2). With further elongation, these become rods on each side of the gut, and the early pluteus is formed, at first without arms (Photograph 4, about 20 hours after fertilization). At about this time, the large red pigment spots begin to appear. The arms then grow out, increasing in length with time. The pluteus is quite well formed a day after fertilization, and increases in size during the next two or three days (Plate IV, Photographs 5–9).

b. The Pluteus, Description. Plate IV

The pluteus which we are accustomed to see in our cultures three or four days after fertilization is roughly triangular in shape, swimming with its arms forward, and its pointed end behind, by means of cilia which cover the surface of the body. There are two pairs of arms, a shorter pair on the dorsal side near the mouth, the oral or dorsal arms; and a much longer pair on the ventral side, the anal or ventral arms which may measure 400 μ from base to tip (Plate IV, Photographs 7-9).

The skeleton consists or rods running into the arms, thinner ones into the oral arms, and thicker ones into the anal arms; these long rods meet at the base in a heavy spiny mass. There is also a transverse connecting rod. The rods in the long anal arms of Arbacia are not solid, but fenestrate or ladder-like. Many other sea urchins have this same type of skeleton in their long arms, e.g., *Tripneustes*, *Sphaerechinus*, and *Echinarachnius* (sand dollar). In other sea urchins, the skeleton of the anal arm is a solid rod, e.g., *Lytechinus*, *Psammechinus*, *Paracentrotus*, *Strongylocentrotus*. These two types of arm skeleton have been of great value in hybridizing experiments, in determining maternal and paternal inheritance. In *Arbacia*, another pair of arms which come in later, the postero-dorsal arms, are also fenestrate. According to Fell (1948), the fenestrate rods represent a primary structure since they are found in the larvae of the more primitive forms.

It was found many years ago by Pouchet and Chabry (1889) that calcium is necessary for the formation of the pluteus skeleton. Development would not take place in sea water without any calcium, since the cells break apart (Herbst, 1900). They found that if 1/10th the normal amount of Ca in sea water was replaced by Na, no skeleton was formed. Loeb (1900a) found that Mg and CO_3 ions are also necessary for a normal skeleton. He could obtain plutei of *Arbacia* with normal skeleton in a solution of: 95 cc. 5/8 n NaCl + 1 cc. 10/8 n $MgCl_2$ + 1 cc. 5/8 n KCl + 2 cc. 10/8 n $CaCl_2$ + 1 cc. 1/8 n Na_2CO_3. Herbst, in a series of papers (1892–1904) studied the relation of the composition of the sea water to the development of the pluteus. He showed that SO_4 is necessary for the development of the skeleton (1904). See J. D. Robertson's review (1941).

The development of the triradiate spicules in *Echinus esculentus* has been studied by Woodland (1906, 1907). The spicule arises in an early mesenchyme cell as a granule which becomes three-cornered.

The digestive tract of the pluteus is J-shape consisting of a mouth on the dorsal side between the two shorter arms, an oesophagus, stomach, and intestine and ending in the anus between the two long arms on the ventral side (Plate IV, Photographs 7, 8).

There are large red pigment spots scattered irregularly over the body and often more abundantly along the arms, especially at their tips. They are sometimes irregular in shape, sometimes spherical (E. B. H.). Each spot consists of 20 to 30 individual granules about 2 μ in diameter. The pigment spot itself is variable in size, an average spherical one measuring about 7 μ in diameter. The red pigment is echinochrome, the same that is found in the chromatophores of the egg, having the same composition and absorption spectrum (Ball and Cooper, 1949). The granules swell in distilled water, like the chromatophores of the egg, having similar osmotic properties (E. B. H.). The permeability value has not been studied, and it would be interesting to compare this with the permeability value of the chromatophores as given by D. L. Harris (1943). The gradual decrease in number of the chromatophores and appearance of the pigment spots in the very early pluteus stage following the prism stage might also prove to be an interesting study.

Photographs of a well developed pluteus are shown on Plate IV, Photographs 7–9. These are anal (ventral) and oral (dorsal) views of the same animal, and a side view.

When photographed with ultraviolet light (2537 Å), certain regions appear much darker than others (E. B. Harvey and Lavin, 1951a).

The most absorbing regions are the digestive tract and the two transverse ciliated bands, the oral band around the mouth and the postoral (or ventral) band between the two long(anal) arms above the anal opening. These regions are the most active physiologically, being concerned with procuring and digestion of food, and there may be some correlation between physiological activity and ultraviolet absorption. In fixed preparations stained with haematoxylin, these same regions, the alimentary canal and the two transverse ciliated bands, are deeply stained.

When photographed by infrared light (8,000–10,000 Å), the structures of the pluteus appear as they do with visible light except that the red pigment spots are not distinguishable (E. B. Harvey and Lavin, 1951a).

An interesting though somewhat involved mathematical or geometrical explanation of the form of a pluteus larva may be found in D'Arcy Thompson's *Growth and Form* (1948, p. 625).

The normal pluteus is quite uniform in shape, both in different batches and in individuals of the same batch. There do, however, occur in some cultures, and usually in the entire culture, plutei of a different shape. Sometimes the anal arms are widely divergent, and sometimes they are close together. The cause for these abnormalities has not been determined. Such abnormalities can, however, be produced by experimental conditions, such as KCN, acids, alkalis, salts, alcohols, etc. These have been studied by Child (1916b, 1941, p. 197–211) with relation to axial gradients, and also by Medes (1917). Abnormal plutei have also been produced by acetylsalicylic acid (aspirin), probably an acid effect (Budington, 1935); dinitrophenol, iodoacetic acid, pyocyanine, methylene blue (Waterman, 1938); malonic acid (Rulon, 1948). Tennent (1910a) has made a statistical study of variations in *Lytechinus* plutei.

Most substances have a harmful effect on the plutei, but M. M. Brooks (1943) reports that methylene blue increased the length of the anal arms; those treated with methylene blue averaged 420 μ while the controls averaged 280 μ.

An interesting effect of KCN on the plutei has been reported by Lyon (1902). Ciliary motion is stopped, and when the larvae are returned to sea water, it starts up again, but the cells, either singly or in small masses, break loose and swim for a moment; then the pluteus disintegrates. The same result follows anaerobiosis caused by prolonged exposure to hydrogen gas.

c. Development of Pluteus When Not Fed

At Woods Hole, the pluteus reaches its maximum size in three to four days, and will not continue to grow unless it is specially fed. At its maximum (without feeding), the long anal arms measure about 400 μ; the longest ones in my cultures were 442 μ from base to tip. Cultures of these plutei may be kept in the laboratory, if the sea water is changed every day or so, for three or four weeks. The plutei gradually get smaller by resorption of the arms, and the body takes on a bloated appearance (Plate XVI, Photograph 10). There is apparently sufficient food material in the sea water for them for three of four days after hatching, and there is considerable growth, but after this they degenerate unless supplied with additional food. It seems to make no difference in their growth if supplied with extra food before the fourth day.

d. Food for Pluteus

The best food for sea urchin larvae has been found to be the diatom *Nitzschia closterium*, but they will grow on other diatoms, e.g., *Lichmophora* (E. B. H.). The *Nitzschias* themselves must be raised in pure culture and require a special diet, Miquel's solution[1]. The method has been worked out by Allen and Nelson (1910) in Plymouth, England, and has been used by many investigators at the Plymouth laboratory. Shearer, de Morgan, and Fuchs (1914) have in this way succeeded not only in raising the normal plutei of several species of sea urchin to maturity, but have also raised some hybrid plutei to maturity. Fuchs (1914b) has even obtained the next or F_2 generation of these hybrids.

[1] Miquel's solution as modified by Allen and Nelson (1910), consists of:

Solution A	KNO_3	20.2 gm.
	Distilled water	100 cc.
Solution B	$Na_2HPO_4 \cdot 12\ H_2O$	4 gm.
	$CaCl_2 \cdot 6\ H_2O$	4 gm.
	$FeCl_3$ (melted)	2 cc.
	HCl (concentrated)	2 cc.
	Distilled water	80 cc.

To each liter of sea water add 2 cc. Solution A and 1 cc. Solution B, and sterilize by heating to 70° C. When cool, decant off the clear liquid from the precipitate, which will have formed when Solution B is added to the sea water.

Ketchum and Redfield (1938) have used a slight modification.

A very simple medium for growing *Nitzschia* has been used by John Ryther at the Woods Hole Oceanographic Institute (personal communication, Sept. 1954): Add to sea water at 20° C. or below

$Na_2HPO_4 \cdot 12\ H_2O$	.002%
KNO_3	.01%

Unfortunately the late larval characters of *Echinus esculentus* × *E. acutus*, from which the F_2 generation was obtained, are alike in the two species, so that no information as to the inheritance could be obtained; none of the F_2 hybrids between *E. esculentus* or *E. acutus* × *Ps. miliaris* which would have given the information, reached maturity.. Miss Gordon from MacBride's laboratory raised some *Arbacia* plutei at Woods Hole in 1926, using this method, but she was particularly interested in the later development of the test, and gives no account of the changes in the pluteus in her publication (1929).

There are several varieties of *Nitzschia closterium*. There is a very small form, *Nitzschia closterium minutissima*, the one used in the Plymouth laboratory and now cultured at the Oceanographic Institution at Woods Hole.[1] This is about 24 μ long. A larger form from the New Jersey coast has been cultured at Rutgers University; this measures about 100 μ. A still larger form grows in the Eel Pond at Woods Hole; this measures about 200 μ. In the small variety, there are three types of cells, the normal spindle-shaped cell, a triradiate cell and an oval cell (D. P. Wilson, 1946). The relation of these three types to each other and their division has been studied by Wilson.

The method of feeding *Echinoderm* larvae with diatoms originated with Caswell Grave (1902a, b) at the Beaufort, N. C. laboratory, where he was rearing the sand dollar *Mellita testudinata*. The diatoms are swept into the mouth and oesophagus of the *Arbacia* pluteus by means of very active cilia. The plutei thrive equally well on the small and the large varieties of *Nitzschia closterium* (E. B. H.).

The very young sea urchins just after metamorphosis thrive better on the calcareous protozoon *Trichospherium*, which furnishes the calcareous matter for the shell; a little later they flourish on the red alga *Corallina*, according to Shearer, de Morgan, and Fuchs (1914, p. 276), for *Echinus*. These have not been tried with *Arbacia punctulata*.

In some places the plutei do not require extra food for growth and development, but apparently obtain sufficient food from the sea water. This is the case with *Arbacia punctulata* at Beaufort, N. C. Brooks (1882), and two of his students, Garman and Colton (1883) apparently raised the plutei through metamorphosis without extra food. The sea water there is rich in diatoms.

[1] In a recent paper of N. I. Hendey (1954) entitled *Note on the Plymouth "Nitzschia" Culture* in J. Marine Biol. Assoc'n, U. K. 33 : 335–339, the identification of the original culture of *Nitzschia* of Allen and Nelson (1910) has been questioned, and identified by him as *Phaeodactylum tricornatum* Bohlin, not a diatom but might be related to the *Chrysophyceae*.

CHAPTER 15

Metamorphosis

a. Arbacia Punctulata. Plate VI

After the 3 or 4 day old pluteus, raised in the laboratory, is fed *Nitzschias*, it increases in size, and the anal arms grow to about 600 μ (from 400 μ) when a week old. Then little knobs appear toward the base on the pluteus, which, by the eleventh day have grown out into a new pair of arms extending backward, the postero-lateral (Mortensen) or ventral-lateral (Brooks) (Plate VI, Photograph 3). These arms have very red tips owing to accumulation of red pigment bodies, and they have much longer and stronger cilia than the other arms. These arms grow longer and another pair of knobs appears between the original anal arms and the new red-tipped arms (2 to 3 weeks); these become the postero-dorsal (Mortensen) or dorsal-lateral (Brooks) arms (Photograph 4). These arms have fenestrate rods like the anal arms, unlike the red-tipped arms which have solid rods. All the arms grow much longer, and the animal is easily visible to the naked eye, looking like a small spider (Photograph 5). The animal tumbles about on the tips of its arms and also swims by means of its cilia. The arms are variable in length individually and relatively to each other. They are very fragile and are easily broken off when the animal bumps into something or when transferred to another dish. They have great regenerative capacity, the arms growing out again when broken off. One pluteus from which I had cut off the red-tipped arm about half way down, had completely regenerated it together with the red pigment in five days, so that it looked exactly like its mate. When three or four weeks old, two pairs of tubular processes appear, two dorsal, two ventral, the auricular lobes (Photograph 6). Tho more pairs of arms arise in the head end, the antero-lateral and the antero-dorsal, so that there are now six pairs of arms, three long pairs and three shorter pairs. The body of the adult *Arbacia* is now seen as a yellowish green mass in the pluteus, the dark area in Photograph 6 and thereafter. There are areas of dark red pigment on the surface of the body. The young adult is formed in the body of the pluteus and grows at the expense of the pluteus.

When about two months old, five tube feet appear at one side of the body in a sort of pocket, but soon extend out radially; they have suckers at their extremities and are very active, expanding and contracting (Photograph 7). Within the next two weeks, 15 petal-like structures appear, three adjacent to each tube foot; these are the primitive spines (Photograph 8). The pluteus had already reached its maximal development, and the arms their maximal length, about 1.6 mm.; the whole animal including arms was over 3 mm., maximal diameter. A good diagram of this stage is given by Gordon (1929, p. 29). The length of the anal arm at different ages is given in Table 6. A mathematical treatment of the data has been given by Glaser (1950), showing that they fit closely with a modified version of Huxley's allometric equation.

TABLE 6

APPROXIMATE LENGTH OF LONG (ANAL) ARM FROM BASE TO TIP (IN μ)

ANAL ARM	FED	NOT FED
1 day	180	180
2 days	300	300
3 days	380	380
4 days	410	410
5 days	450	330
6 days	480	250
1 week	600	200
11 days	700	180
2 weeks	750	150
3 weeks	800	
1 month	1000	
1½ months	1300	
2 months	1400	
2½ months	1600	

The head of the pluteus has remained for some time. The arms now or sometimes before this begin to degenerate, the flesh peels off leaving the bare skeleton (Photograph 6), and they are gradually lost. The metamorphosed animal now consists of the greenish spherical body with 5 tube feet and 15 primitive spines (Photograph 9, 10). The tube feet soon increase in number, the newer ones being more slender (Photograph 11). This is the latest stage obtained in the laboratory; the animal measured about 1 mm. including spines, and was about 3½ months old. The times given for different stages are only approximate as they vary greatly in different lots.

A very young adult Arbacia was found in July 1952 in a clam shell

brought in from the sea. This measured 6 mm. including spines. The spines are more numerous and more slender than in the metamorphosed animal, but the structure of these spines and the primitive spines is the same. They are transparent, with veins running through them, something like an insect's wing. The stages between the animal metamorphosed in the laboratory and the youngest animal found outside are much desired. Plutei have not been found in the tow above the beds.

The account given above has been revised from an earlier account (E. B. Harvey, 1949), which did not include the later stages with well formed primitive spines. Earlier studies on the *Arbacia* metamorphosis, as well as that of other forms have been treated in Part I (Chapter 2, Section f) under *Historical*. The best early study of *Arbacia punctulata* is that of Brooks (1882) and his students, Garman and Colton (1883), published by Brooks in his *Handbook of Invertebrate Zoology* (1883), in which excellent drawings of the different stages are given. The works of Agassiz (1872–1874, 1883, 1904) and of Miss Gordon (1929) should also be consulted.

b. Other Species

Many studies on the metamorphosis of other species of sea urchins have been made. Among the most complete studies are those on *Echinus esculentus* by MacBride (1903, 1914a *Text-book of Embryology* vol. 1, p. 504) and by Shearer, De Morgan, and Fuchs (1914); the development of *Psammechinus miliaris* and *Ps. microtuberculatus* is similar (MacBride, 1914a); *Arbacia lixula* and *Paracentrotus lividus* by von Ubisch (1913a, b, c, 1927, 1932, 1950); see also Busch (1849); and Müller (1854); *Echinocyamus pusillus* by Théel (1892); *Echinocardium cordatum* by MacBride (1914b); *Salmacis bicolor* by Aiyar (1935); *Mespilia globulus*, *Strongylocentrotus pulcherrimus*, and other Japanese forms by Onoda (1931, 1936). A very good list of studies on larval forms is given by Mortensen (1921, p. 12), and a more recent list arranged phylogenetically by Fell (1948). Studies made during expeditions to Kei Islands (Amboina), Java, S. Africa (Onrust, Mauritius) and Egypt were published by Mortensen (1931, I and II; 1937, III; 1938 IV); and there are many references in his *Monograph* (1928–1951). See also this Monograph under *Historical*, Part I, Chapter 2, Section f. (p. 18).

A very good general treatise on larval development may be found in Grassé's *Traité de Zoologie*, t. 11, p. 307–312, 1948.

PART III
CENTRIFUGED EGGS

CHAPTER 16

Methods

a. Whole Eggs

When *Arbacia punctulata* eggs are centrifuged in sea water, they are thrown to the bottom of the tube and crushed, because they are heavier than the sea water. They must be centrifuged in a medium of the same density as themselves, in which they will be suspended. The solution must also be of the same osmotic pressure as the eggs, so that they will not swell or shrink. Since individual eggs vary in density, it is best to centrifuge the eggs in a medium of graded density, made by partially mixing sea water and 0.85 M cane sugar. The sugar solution[1] is osmotic with but of slightly greater density than the eggs. Two parts of the cane sugar solution are placed in the bottom of a small slender centrifuge tube and one part of sea water containing the eggs on top. By a slight rotation of the tube the solution can be partly mixed and a density gradient established, so that when centrifuged, the eggs come to lie in a region of their own density. As they break apart during centrifuging, the half-eggs move to a new region equal to their density. They are consequently separated into layers, the heavy (red) halves at the bottom and the light (white) halves at the top (the unbroken eggs are in the middle), as shown in Plate VII, Photograph 10. With a fine pipette the eggs of each layer can be removed without contamination with the eggs of other layers, and large numbers can be collected for experimental work.

A small electric centrifuge has been used with two (or four) glass tubes 6.5 cm. long and 0.4 cm. inside diameter, narrow in order to prevent mixing by convection currents. The centrifugal force, F, in terms of force of gravity (g), is given by the equation $F = 0.04 \times \text{radius (in cm.)} \times (\text{r.p.s.})^1$. A centrifugal force of 10,000 × g for four minutes

[1] The 0.85 M sugar (molecular weight 342.24) solution is prepared by adding 29 gm. sugar (commercial samples are as good as "chemically pure") to sufficient tap water to make 100 cc. Tap water is used rather than distilled water as the slight alkalinity prevents stickiness of the eggs. I have found it best to weigh out several lots of 5.8 gm. sugar and to add tap water to make 20 cc. when needed; the sugar solution will keep for several days in the refrigerator (8 °C.), but becomes acid on standing at room temperature.

is sufficient to break the eggs into halves, and a much smaller force, 3,000 × g for two minutes will stratify them (Plate VII; also Fig. 12).

In working with other eggs at other places, corrections must be made for differences in density of the sea water and the eggs.

The sugar-sea water medium described, in which the eggs are centrifuged, is quite harmless. Eggs may be kept in the solution for five hours, and, when returned to sea water, cleave and develop as well as the controls, and at the same rate. The eggs, however, cannot be fertilized in the sugar solution, though the sperm are active and surround the eggs as normally. This may be due to lack of sufficient calcium; it has been found that eggs cannot be fertilized in Ca-free sea water, though the sperm are active.

In his pioneer experiments, Lyon (1907) used gum arabic for suspending the eggs. He used a centrifugal force of 6,400 × g for one or two minutes, the eggs remaining spherical, though he apparently had a few eggs break into halves which he said could not be fertilized.

b. Crushed Eggs. Homogenates

When *Arbacia* eggs are crushed in a mortar after they have been frozen, and then centrifuged, McClendon (1909a) found that the material separated into two layers, a fluid centripetal layer and a jelly-like centrifugal layer. The chemical composition of the layers is given in Table 7. References to more recent methods of obtaining egg homogenates by freezing and thawing are given by Runnström (1935a). Kopac (1943) has recommended the following treatment:

1. Remove jelly layer by washing in two or more changes of 0.52 M NaCl.
2. Transfer to 1.0 M solution of urea.
3. Within 1 or 2 minutes wash eggs free of urea with 0.53 M KCl.
4. Transfer eggs to a measured volume of citrated KCl solution containing 9 volumes 0.53 M KCl and 1 volume of 0.35 M Na-citrate.
5. Immediately disintegrate eggs by flushing in the above solution through a fine bore pipette.

"The resulting suspension which includes all granules and nongranular residue of the cells is then centrifuged gently to remove unbroken eggs and foreign particulate debris. The supernatent is again centrifuged, at high speed, to separate the granules and other formed elements. The sediment now contains pigment vacuoles, yolk granules, and some mitochondria. The oil globules collect at the meniscus of the centrifuge tube. In the absence of Ca ions, the granules are stable and may be preserved intact for considerable periods. The more or less nongranular supernatent fluid may be separated from the sedimented granules by pipette transfer. This contains most of the residue of the cytoplasmic matrix."

TABLE 7

COMPOSITION OF LAYERS IN CRUSHED ARBACIA EGGS IN PERCENT OF WHOLE EGG
(McClendon 1909 a)

Layer	Centripetal 32.5	Centrifugal 67.5	Whole egg
Water	28.6	53.3	81.9
Solids	3.9	14.2	18.1
Ether ext.	0.308	1.946	2.254
P in ether ext.	0.00154	0.06760	0.06914
Alcohol ext.	1.6	3.48	5.08
P in alcohol ext.	0.0434	0.0814	0.1248
Water ext.	0.78	1.42	2.20
P in water ext.	0.130	0.1822	0.3122
Residue of water ext.	1.309	7.29	8.599
P in residue	0.0392	0.1167	0.1559
N in residue	0.1625	0.775	0.9375
Ash in residue	0.0162	0.0264	0.0426
Total P in layers	0.21414	0.4478	0.66194

Many other methods of preparing a homogenate or a suspension of the contents of *Arbacia* eggs have been used. The important consideration in obtaining a particulate system is to break up the eggs in a medium devoid of calcium, since the yolk and pigment granules disintegrate in its presence. The eggs may be ruptured by forcing through a hypodermic needle from a syringe (Keltch, Strittmatter, Walters, and Clowes, 1950), by grinding with sand in a mortar (Krahl, Keltch, Neubeck, and Clowes, 1941), or by use of a Waring blender. Subsequent centrifuging of the "brei" results in separation of the various constituents, which can then be studied separately. Monroy recommends for A. lixula, for jelly-free eggs: LiCl 1 M + $NaHCO_3$ 0.04 M + Na_2CO_3 0.01 M + Versene 0.01 M, in distilled water. Use a Potter homogenizer (Personal communication July 1955).

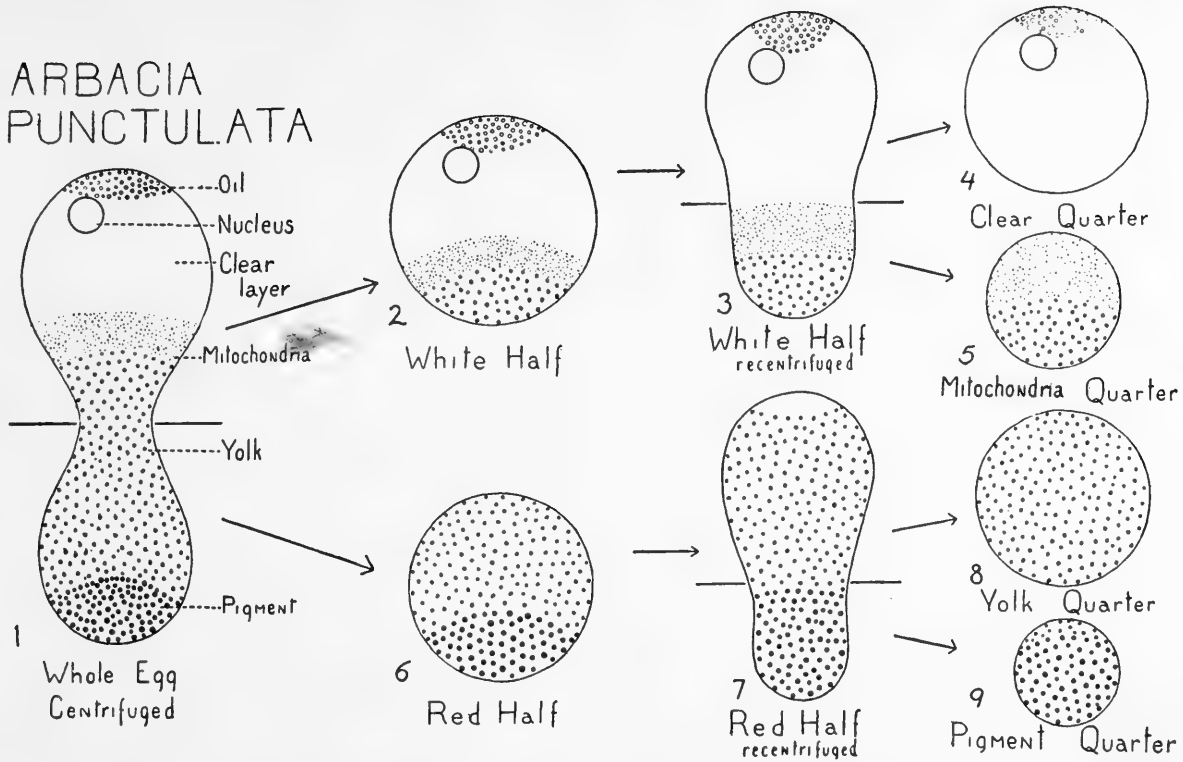


FIG. 12. The unfertilized egg of *Arbacia punctulata*, stratified by centrifugal force (about 3 minutes at 10,000 × g), and the halves and quarters into which it breaks. The drawings are from camera lucida sketches and photographs, made as accurately as possible to scale. Magnified about 450 ×. The clear area in No. 7 at the centripetal pole is due to further packing of the granules with longer centrifuging.

CHAPTER 17

Stratification of the egg granules

a. Unfertilized Eggs. Plate VII

The stratification of the well-centrifuged egg of *Arbacia*, beginning at the light (centripetal) pole, is (1) oil, (2) clear layer without visible granules (see Part IV, *Clear Layer*), (3) a thin finely granular layer of mitochondria, (4) a large yellowish heavy layer of larger yolk granules, (5) a red pigment layer at or near the (centrifugal) pole. The nucleus is always at the centripetal pole just under the oil cap. (E. B. Harvey, 1932, 1936, etc.) (Plate VII, Photograph 1 and Figure 12).

In the older work on *Arbacia* eggs, only four layers were described, the mitochondrial layer being omitted, owing probably to insufficient force (Lyon, 1906a, 1907; Morgan, 1909). In still earlier work on *Arbacia lixula*, Sanzo (1904) obtained only two zones, a protoplasmic and a granular zone, and he figures an oil cap; this led him to believe that he had changed a homolecithal into a telolecithal egg.

b. Vital Staining of Stratified Egg

The different layers of the centrifuged egg stain differently with different vital dyes (E. B. Harvey, 1941c). A table is given (Table 8) of a number of vital dyes and their effect on the different layers. Many other stains were tried, especially acid dyes, without effect. Some dyes, e.g., rose bengal, stained only after the egg was dead. The nucleus does not stain, vitally, in any dye; and the cytoplasm only in basic dyes as noted long ago by Mathews (1907).

Quinine, cinchonine, and cinchonadine stain the pigment almost black like methylene green, with no effect on development (E. B. H., unpub.).

c. Stained Sections of Stratified Eggs

Eggs were fixed in Bouin's fluid, sectioned and stained with iron hematoxylin; some were counterstained with eosin and orange G (E. B. Harvey, 1940c). The clear protoplasmic layer which is optically

empty in the living egg, stains blue and consists of very small granules, the microsomes; this was described in the early paper of Lyon (1907). The yolk stains orange with a rose tinge and the pigment orange. The mitochondria can sometimes be distinguished as a darker bluish band between the protoplasm and the yolk. The oil cap does not show; it is probably dissolved in the fixing fluid. The two half-eggs show the same stratification except that one usually sees a blue cap of protoplasm on the red half-egg, especially when well centrifuged so as to

TABLE 8

VITAL DYES ON CENTRIFUGED UNFERTILIZED EGGS OF ARBACIA PUNCTULATA
(From E. B. Harvey, 1941c, with a few additions)

Dye	*Jelly*	*Oil*	*Clear Layer*	*Mitochondria*	*Yolk*	*Pigment*	*Remarks*
Bismarck brown	o	o	Yellow (upper part more intense)	Yellow	Yellow	Brown	Slightly soluble in sea water
Brilliant cresyl blue	o	o	o	o	Blue	Blue	Very innocuous
Chrysoidin	o	o	Light yellow (upper part more intense)	Light yellow	Yellow	Reddish brown	
Crystal violet	o	o	o	Purple	o	o	
Dahlia	o	o	o	Purple	o	o	
Gentian violet	o	o	o	Purple	o	o	
Janus dark blue B	Purple	o	o	o	o	o	
Janus green or Janus green B	Purple	o	o	Blue	o	o	Rather toxic
Methyl green	o	o	o	Purple	o	o	
Methyl violet	o	o	Upper part violet	Purple	Purple (later)	Purple (later)	
Methylene blue	o	o	o	o	Blue	Blue	Very innocuous
Methylene green	o	o	o	o	o	Almost black	
Neutral red	o	o	Pinkish yellow (lower part more intense)	Pinkish yellow	Brick red	Blood red, almost black	Slightly soluble in sea water
Nile blue sulphate	o	o	Light blue (upper part more intense)	Light blue	Blue	Bluish brown to blue black	Slightly soluble in sea water
Rhodamine	o	o	Pink (upper part more intense)	Pink	Pink	Deep red	Very innocuous
Safranin O	Yellow (few cases)	o	o	Pink (after 1–2 hours)	o	Blood red	Not soluble in sea water
Thionin	Pinkish (few cases)	o	o	Lavender (few cases)	o	o	Not soluble in sea water
Toluidin blue	Pinkish lavender	o	Pinkish lavender	Lavender	Lavender	Purple to blue black	More intense if stained after centrif.

allow further packing of the granules at the centrifugal pole. In the living egg this is seen as a clear layer (Plate XIII, Photograph 5; also Fig. 12, No. 7).

d. Stratification of Other Species

Diameters of the eggs in micra are given. The layers in square brackets may or may not be present. Data from E. B. Harvey (1933a, 1938a, 1947, and unpublished) unless otherwise noted.

Arbacia lixula (79). Stratifies like A. punctulata (E. B. H.; Callan, 1949, with diagram).

Arbacia punctulata (74). Oil, clear, mitochondria, yolk, pigment.

Echinarachnius parma (145). Oil, absent in some, [clear], yolk, clear, mitochondria, neutral red-staining granules (or methylene blue).

Echinocardium cordatum (125). Yolk, clear, mitochondria; apparently no oil (Monné, 1944b).

Echinometra lucunter (85, 97). Oil, clear, mitochondria, yolk.

Lytechinus (Toxopneustes) variegatus (103, 112). Oil, (little), yolk, clear, mitochondria, [clear].

Mellita quinquiesperforata (150). Oil, clear, mitochondria, yolk.

Paracentrotus lividus (90). Oil, [clear], yolk, clear, mitochondria, [clear] (E. B. H.; Callan, 1949, with diagram).

Psammechinus microtuberculatus (102). Oil, [clear], yolk, clear, mitochondria (E. B. H.; Callan 1949, with diagram; Lindahl, 1932c).

Psammechinus miliaris (98, 115). Oil, yolk, clear, mitochondria (Holter and Linderström-Lang, 1940; Runnström and Kriszat, 1950a, with figures).

Sphaerechinus granularis (98). Oil, clear, mitochondria, yolk, [clear]. (E. B. H.; Callan, 1949, with diagram).

Strongylocentrotus dröbachiensis (160). Oil, [clear], yolk, clear, mitochondria.

Strongylocentrotus franciscanus (120). Oil, [clear], yolk, clear, mitochondria, [clear].

Strongylocentrotus purpuratus (80). Oil, [clear], yolk, mitochondria, clear.

Tripneustes (Hipponoë) esculentus (84). Oil, [clear], yolk, [clear], mitochondria, clear.

In all the forms listed above, the nucleus is at the centripetal pole under the oil cap, except that Monné (1944b) shows it near the centrifugal pole in *Echinocardium cordatum.* In *Temnopleurus sp?* Motomura (1935) states that it is at the centrifugal end.

e. Stratification and Polarity

The *Arbacia* eggs fall at random in the centrifuge tubes. There is no orientation as they fall. If there is any polarity in the unfertilized egg, the stratification by centrifugal force bears no relation to it. This was stated by Morgan and Lyon (1907) and proven by Morgan (1909) by comparing the position of the micropyle, which indicates the original axis of the egg, with the stratification; there was no relation.

The position of the polar bodies, if given off during centrifugation bears no relation to the stratification but they may be formed in any region, in the pigment zone or yolk or even at the oil cap (Plate II, Photographs 14, 15).

f. Redistribution of Stratified Material and Return to Spheres

If the eggs, after centrifuging, are returned to sea water, the stratification gradually disappears, and the granules are again scattered throughout the egg. The first layer to be lost is the mitochondrial layer; this becomes indistinct almost immediately and cannot be distinguished after 10–15 minutes. The oil cap remains longest, often for 24 hours.

If the eggs have become dumbbells when the centrifugal force is removed they gradually return to the original spherical shape. Shapiro (1941) has studied the kinetics of the return in sea water, and in sea water without calcium. In sea water they round up, at first rapidly (several minutes), later more slowly. In sea water without calcium they elongate more and contract more quickly.

Normal eggs, uncentrifuged, when deformed by squeezing through a capillary tube (diameter 50 μ; egg 74 μ) round up too quickly to be accurately timed by eye. Moving pictures indicate that the return takes about 0.1 second (E. N. Harvey and Shapiro, 1941).

If fertilized, the elongate eggs do not round up, but remain elongate during cleavages and as blastulae (Plate VIII, Photographs 1–8).

CHAPTER 18

Breaking of the Egg
Egg Fractions and Contents

When centrifuged with low forces, 3000 × g for two minutes, the eggs remain spherical but become stratified. When centrifuged at 10,000 × g for four minutes, most batches of eggs stratify, become dumbbell-shaped and then break across the yolk into two slightly unequal spheres or "half-eggs" (Plate VII and Fig. 12). In the centrifuge tubes (Photograph 10) there are three distinct layers, the white half-eggs toward the top (centripetal end) of the tube, a pinkish layer of elongate unbroken eggs a little below, and a layer of red half-eggs at or near the bottom (centrifugal end) of the tube. In some cases there are five layers with this force due to the breaking of the red half-eggs into quarters. The layers now are: white half-eggs at the centripetal pole, unbroken whole eggs, yolk quarters, unbroken red halves, and pigment quarters at the centrifugal pole (E. B. Harvey, 1936, photo 6).

These quarters can also be obtained by recentrifuging the red halves. The white half-eggs may also be broken into clear and mitochondrial quarters by pipetting them off and recentrifuging them in a slightly less dense sugar solution for about 45 minutes. The nucleus is always in the white half-egg and in the clear quarter-egg. It is lighter than the granules and always lies just under the oil cap at the centripetal pole.

The white halves contain oil cap, nucleus, clear layer, mitochondria, and a little yolk; the slightly smaller red half contains most of the yolk and all the pigment granules; the clear quarter contains oil cap, nucleus, and clear layer; the mitochondrial quarter contains all the mitochondria and some yolk; the yolk quarter contains only yolk and the pigment quarter, all the pigment granules, and a little yolk.

The sizes of the fractions obtained with a force of 10,000 × g are given in Table 9; Photographs 1–9, Plate VII, and a chart drawn from the photographs in Figure 12 (E. B. Harvey, 1932, 1936, 1940c, 1951).

Although the size of the fractions obtained by centrifuging at 10,000 times g for 4 minutes is usually as given, there do occur batches of

TABLE 9

SIZE OF THE ARBACIA EGG AND ITS FRACTIONS IN MICRA WITH 10,000 × G
E. B. Harvey, 1936

Parts	Diameter	Volume	Approximate proportion of whole egg
	μ	μ^3	per cent
Whole egg (nucleate)	74	212,200	
White half (nucleate)	62	124,800	58
Clear quarter (nucleate)	56	91,950	43
Mitochondrial quarter	40	33,510	15
Red half	56	91,950	43
Yolk quarter	52	73,620	35
Pigment quarter	32	17,160	8
Nucleus	11.5	796	0.4

apparently normal eggs which break differently. Sometimes the white half is much larger than normal and the red half correspondingly very small; rarely the white half is smaller than normal and the red half correspondingly large. Usually the entire batch breaks in the same way, but occasionally the eggs in one batch will break in the three different ways, without intermediates (E. B. Harvey, 1936, photos 7–9).

CHAPTER 19

Factors affecting stratification and breaking

a. Centrifugal Force. Plate XIII

With low forces, it takes longer to break the eggs apart, and the eggs have become well stratified before breaking. The white half is much larger than the red half.

With high forces obtained by the air turbine, the egg breaks quickly before it has become well stratified. The white half is much smaller than the red half. The red half can be restratified like the whole egg if recentrifuged (Plate XIII, Photograph 12).

With intermediate forces, the time for breaking and the stratification is intermediate, and the white half and red half are more nearly equal in size. These results are given in Table 10 and in Photographs on Plate XIII (see E. B. Harvey, 1941a).

b. Hypo- and Hypertonic Sea Water. Plate XIV

When eggs are kept and centrifuged in hypotonic sea water (60%, 80%), it takes longer to break them apart. The granules are well packed so that few remain in the white half, which is much larger than the red half. It is also much larger than the control (100% sea water), while the red half remains about the same size.

When eggs are kept and centrifuged in hypertonic sea water (125%), the clear area is small, the mitochondrial layer very thick, being spread over a smaller area and in many cases well marked. The pigment is not well separated from the yolk, there being no clear line of demarcation. It is inaccurate to speak of "well-stratified" eggs, since they may be well stratified with regard to one layer (mitochondria) and poorly stratified with regard to others (yolk and pigment). The white halves are much smaller than the controls, the red halves about the same size. The eggs break more readily in hypertonic than in normal sea water. These results are shown in Table 11 and on Plate XIV (see E. B. Harvey, 1943).

TABLE 10

EFFECT OF VARYING THE CENTRIFUGAL FORCE ON TIME TO BREAK AND SIZE OF HALVES

E. B. Harvey, 1941a

Whole egg		Diameter (μ) 74		Volume (μ³) 212,000		Ratio (approx.)
Minutes to break	Force (× g)	W	R	W	R	W : R
20	4,000	70	41	180,000	36,000	5 : 1
4	10,000	62	56	125,000	92,000	4 : 3
1	60,000	59	59	107,000	107,000	1 : 1
¾	80,000	56	62	92,000	125,000	3 : 4
½	100,000	41	70	36,000	180,000	1 : 5

TABLE 11

SIZE OF HALVES WHEN CENTRIFUGED IN HYPO- AND HYPERTONIC SEA WATER

E. B. Harvey, 1943

Sea water	Whole egg Diam. μ	Whole egg Vol. μ³	White half Diam. μ	White half Vol. μ³	Red half Diam. μ	Red half Vol. μ³	Nucleus Diam. μ	Nucleus Vol. μ³	Per cent broken
Eggs in hypo- and hypertonic sea water, then centrifuged 10,000 × g for 4 minutes									
60 %	82.4	(292,900)	70.4	(182,700)	58.0	(102,200)	16.0	(2,145)	10 %
80 %	74.9	(220,000)	62.1	(125,400)	56.3	(93,400)	12.8	(1,098)	20 %
100 %	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)	11.5	(796)	70 %
125 %	66.6	(154,700)	51.7	(72,360)	53.8	(81,540)	9.6	(382)	98 %
Recovery in 100 per cent sea water									
60 %–100 %	72.0	(195,400)	59.2	(108,600)	55.7	(90,480)	11.2	(736)	
80 %–100 %	72.0	(195,400)	59.2	(108,600)	54.7	(86,170)	11.5	(796)	
100 %	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)	11.5	(796)	
125 %–100 %	72.0	(195,400)	56.3	(93,940)	57.6	(100,060)	11.2	(736)	

The return of whole eggs to normal size is approximately perfect, as found also by McCutcheon, Lucké, and Hartline, 1931, p. 402.

c. Single Salt Solutions. Plate XV

Eggs were kept for 40 minutes in solutions of different salts isosmotic with the sea water, 0.52 M NaCl, 0.53 M KCl, 0.34 M $CaCl_2$, 0.37

M $MgCl_2$, and centrifuged in fresh similar solutions with a measured amount of isosmotic sugar solution at the bottom of each tube to keep the eggs suspended (E. B. Harvey, 1945). It was found that they stratify with decreasing readiness (indicating increasing viscosity) in the following order: $CaCl_2 > MgCl_2 >$ Sea water $>$ NaCl $>$ KCl. They break into "halves" with decreasing readiness in the reverse order, those in $CaCl_2$ which stratify best break least readily. In the bivalent salts they stratify better and break less readily than in sea water, and in the monovalent salts they stratify less and break more readily than in sea water. The ease of breaking must be determined by an effect of the salts on the surface layers rather than by their effect on the interior viscosity. Heilbrunn (1923, 1928) gives a similar series for viscosity, except that Na and K are reversed.

No change in size of halves was noted in the different salt solutions, but the percentage of eggs which broke into two when centrifuged at 10,000 $\times$ g for 4 minutes was as follows: KCl, 99%; NaCl, 90%; sea water, 50%; $MgCl_2$, 20%; $CaCl_2$, none. The rate of stratification was the reverse of the above, i.e., the eggs in $CaCl_2$ were best stratified (E. B. Harvey, 1945 and Plate XV).

d. Temperature, Pressure, Radiation

According to Costello (1934, 1938) the amount of stratification decreases with decrease in temperature, the eggs break less readily, and the red halves are smaller. I have obtained somewhat different results, due probably to difference in procedure. There seems no doubt, however, that eggs kept for three or more hours at 8° C. stratify better and a larger percentage break into two than among those kept at 23°, when centrifuged at the same time. Moreover, eggs are usually considerably stratified by gravity on standing overnight at 8° C., but do not stratify at room temperature.

Under increased hydrostatic pressure unfertilized eggs stratify more rapidly (Brown, 1934) and break into halves more easily (E. B. Harvey and Brown, unpub.).

After treatment with x-rays or ultraviolet light the eggs break more readily (E. B. Harvey, 1940, 1950, unpub.). According to Cheney (1949a) they stratify less easily and break less readily in 0.1% caffeine than in sea water.

e. Fertilization

When the eggs are fertilized first and then centrifuged, they stratify

like the unfertilized ones, but in not so well-defined layers (Plate XII, Photograph 15; cf. with Plate VII, Photograph 1). As is well known, they become more viscous on fertilization (Heilbrunn, 1915, 1920a, 1928, p. 264; E. B. Harvey, 1932, 1933b; Goldforb, 1935b). Except for a short period immediately after fertilization, they break apart into white and red halves like the unfertilized eggs; these are usually of the same relative size as the unfertilized halves, the white half a little larger than the red, except if centrifuged just before cleavage when the white half is quite small. If centrifuged one or two minutes after fertilization, the eggs break into very small pieces (E. B. Harvey, 1933b, 1940b) (Plate XII, Photograph 14).

The fertilization membrane must be removed by vigorous shaking just after it is formed, in order to allow the eggs to elongate and break. If centrifuged during the monaster stage, 6–20 minutes after fertilization, the eggs form long streamers which retract into spheres on removal from the centrifuge. This is particularly noticeable with low forces and readily observed with the centrifuge microscope (E. B. Harvey, 1933b) (Plate XII, Photograph 17). If centrifuged slowly as the fertilization membrane is forming, this may be greatly stretched to a length of 128 μ from a diameter of 80 μ, and the egg may break into two parts inside the stretched membrane. If the force is removed, the two parts may coalesce or may remain and develop separately; in the latter case, the female nucleus may be in the white half and the male nucleus in the red half; the red sphere with the male nucleus usually develops better than the white sphere with the female nucleus.

When eggs are centrifuged after fertilization, the hyaline layer is thrown off as a ring or crescent into the perivitelline space. This is much more striking in eggs with a large perivitelline space, e.g., *Psammechinus microtuberculatus*, and is observed better in *Arbacia punctulata* if the fertilization membrane is broken (E. B. Harvey, 1934) (Plate XVI, Photograph 6). The hyaline layer is later replaced, and if the fertilization membrane remains intact, the egg develops normally.

CHAPTER 20

Properties of egg fractions

Egg fractions have been used to analyze the effect of ultraviolet light and x-rays, etc. on the egg cell (see Part IV); quite a number of studies have been made of the chemical and physical properties of the half-eggs in order to evaluate the part which the various granular layers may play in the life of the cell. Some of the results are given in condensed form below.

Consistency. – When manipulated with a needle the red halves are found to be sticky and glutinous and can be pulled out in strands, while the white halves explode if punctured (E. B. Harvey, 1932; see Chambers, 1938a). The fertilization membrane which forms on the white half is much thinner than that on the red half.

Density. – White half, 1.076; red half $>$ 1.1 when obtained with 10,000 $\times$ g. Whole egg without jelly 1.084. (E. N. Harvey, 1932).

Osmotic Behavior. – Closely parallels the whole egg. The value for osmotically inert material b is twice as great in the red half as in the white half, but computation of b magnifies experimental error and the difference in white and red halves may not be as great (Lucké, 1932). Actual values for the volume change in hypo- and hypertonic sea water are given in Table 12. Water enters white halves several times more rapidly after fertilization than before (Shapiro, 1939a).

Electrical Properties. – The electrical capacity of the white half and red half is the same as that of the whole egg, about 1 microfarad per cm^2. On fertilization, the capacity of whole eggs and white halves increases 3–4 times (Cole and Curtis, 1938; Cole, 1941). The details are given in Part IV.

The internal resistance of whole eggs and white halves, fertilized and unfertilized, is about the same, 180 ohms per cm^3, but the internal resistance of red halves (unfertilized) is 3 to 6 times that of the whole egg (Cole and Curtis, 1938; Cole, 1941). The details are given in Part IV.

The surface charge (zeta potential) of whole eggs is -30.8 ± 0.54 millivolts; white halves, -20.9 ± 0.69 millivolts; red halves, -27.6 ± 0.35 millivolts (Dan, 1936, IV).

TABLE 12

OSMOTIC BEHAVIOR OF FRACTIONS

E. B. Harvey, 1943

Sea Water	Whole Egg		White Half		Red Half	
	diam. μ	vol. μ³	diam. μ	vol. μ³	diam. μ	vol. μ³
Eggs centrifuged in sea water, then placed in hypo-hypertonic sea water						
100 %– 60 %	82.4	(292,900)	67.4	(160,300)	63.4	(133,400)
100 %– 80 %	74.9	(220,000)	61.8	(123,600)	57.2	(97,990)
100 %	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)
100 %–125 %	66.6	(154,700)	54.4	(84,300)	51.2	(70,300)
Lucké's (1932) mean values for above						
100 %– 60 %	(84.6)	317,380	(69.5)	175,560	(63.9)	136,700
100 %– 70 %	(80.6)	274,020	(66.3)	152,320	(61.7)	123,060
100 %	(72.2)	197,440	(59.2)	108,600	(55.7)	90,680

Only the volumes are given by Lucké; the diameters are calculated from the volumes. Lucké found the swelling of whole eggs and fractions completely reversible.

Respiration. – The white half consumes oxygen at about the same rate (0.135 cm^3/hour/cm^3 eggs) as the whole egg, while the red half has about twice that rate. On fertilization the rate for whole eggs and white halves increases about 2.5 times, while the red halves increase 1.2 times (Shapiro, 1935b, 1939b). See Part IV for details. Addition of paraphenylene diamine to white and red halves causes a greater oxygen uptake by the white halves (Boell, Chambers, Glancy, and Stern, 1940). KCN does not affect oxygen uptake of white halves but decreases oxygen consumption of red halves and of both white and red halves after fertilization (Shapiro, 1940).

Enzymes. – Peptidase is found mostly in the white half localized in the matrix; ratio of peptidase activity of white: red halves = 3.4 (Holter, 1936, 1949).

Indophenol (cytochrome) oxidase is more abundant in the red half; ratio of activity white: red halves = 0.5 (Navez and E. B. Harvey, 1935). See Hutchens, Kopac, and Krahl (1942) for association with matrix in disintegrated eggs (see Part IV).

Dehydrogenases (measured by reduction of ferricyanide) are in greater concentration in the red half. Activity ratio of white to red halves 45/55. (Ballentine, 1940c).

Desoxyribonuclease (DNase) activity of white halves is greater than red but not per unit volume. (Mazia, Blumenthal, and Benson, 1948; Mazia, 1949a, b).

Phosphatase activity (acid or alkaline) is not restricted to white halves (Mazia, Blumenthal, and Benson, 1948).

For distribution of peptidase and catalase in fractions of other eggs *(Paracentrotus lividus, Psammechinus miliaris, Echinarachnius, Dendraster)* see Holter and Linderström-Lang (1940) and Holter (1949).

Lipids. – 75% of free fats and sterols, 56% of bound lipids, and 61.6% of total lipids are present in the red halves (Hunter and Parpart, 1946).

CHAPTER 21

Development of centrifuged eggs and fractions

a. Spherical Centrifuged Eggs. Plate VIII

If the eggs of *Arbacia* are centrifuged with a low force (e.g., 3,000 times g for two minutes), they stratify but do not elongate or break apart; they remain spherical. When these spherical eggs are fertilized, the cleavages are quite normal (E. B. Harvey, 1932, 1940c, 1951). The first cleavage usually (but not always) comes through the axis established by centrifuging, at right angles to the stratification; the second cleavage is at right angles to the first and parallel to the stratification, and the third at right angles to the first two and to the stratification. The micromeres may come off anywhere without regard to the stratification, but approximately opposite the micropyle, which marks the original axis of the egg (Plate VIII, Photographs 15, 16). Plutei developed from these eggs, normal in every respect except that the pigment was localized somewhere, in any part of the larva. These facts were established by the early work of Lyon (1906a, 1907) and Morgan and Lyon (1907). Further studies with regard to polarity and development were made by Morgan (1908, 1909), Morgan and Spooner (1909), and Spooner (1911), and I can confirm them. A general summary of cleavage in these eggs and in egg fractions will be found in the paper of E. B. Harvey (1951). A resumé and able discussion of polarity and centrifuged eggs is given in Morgan's *Experimental Embryology*, 1927, p. 496–500.

b. Elongate Centrifuged Eggs. Plate VIII

As mentioned, the elongate eggs when unfertilized resume their spherical shape within a few hours. If, however, they are fertilized immediately in sea water they retain their elongate or dumbbell shape (E. B. Harvey, 1932, 1940c, 1951). The fertilization membrane follows the contour of the egg, but there is usually left a slight bulge of the membrane at the centripetal pole, owing to a slight shrinking of the egg immediately after the fertilization membrane has formed. There must be a gelation or setting of the protoplasm following fertilization, for

if the membranes are removed by drawing up the eggs in a fine capillary, the eggs retain their aspherical shape (and develop).

Cytological details can be observed in the living elongate egg: the sperm aster, union of the pronuclei, enlargement and breaking of the nucleus, cleavage spindle, can all be observed, except that astral radiations cannot be seen unless there are granules. The spindle forms in the long axis of the egg where there is room for it. The first cleavage plane almost always comes across the short axis of the egg, parallel with the stratification but not at the narrowest part, dividing the egg into a smaller clear cell and a larger granular cell. This is in contrast to the first cleavage of the spherical centrifuged egg described, where the first cleavage plane is usually perpendicular to the stratification. The shape of the egg determines the position of the first cleavage plane. The second cleavage plane usually comes in perpendicular to the first in the clear cell, and either perpendicular or parallel with the first in the granular cell. The second and following cleavages are asynchronous, the smaller clearer cells dividing more rapidly than the larger pigmented cells. A slipper-shaped blastula is formed with unequal-sized cells. The pigmented area remains distinct through the cleavages and in the early pluteus as in the spherical egg.

Micromeres may be formed in any region of the elongate egg, even at the oil cap (E. B. H.) (Plate VIII, Photographs 5, 6). They probably form in relation to the original axis of the egg. They may be pigmented but usually are not.

c. White Half-Eggs. Plate IX

The white half-egg which has been allowed to stand in sea water after centrifuging, and become spherical, develops after fertilization, exactly like the whole uncentrifuged egg cleaving at the same rate, sometimes a little faster (E. B. Harvey, 1932, 1940c, 1951). The nuclear phenomena accompanying fertilization can be observed in the living egg with great clearness, and they parallel those described by Wilson (1895, *The Atlas of Fertilization*) in the unpigmented egg of *Lytechinus (Toxopneustes) variegatus*. In the clear, nongranular portion of the egg, however, no astral rays can be seen. The first cleavage plane usually comes in through the oil cap, but may be parallel with the stratification, or at an angle. Following cleavages come in quite normally, but micromeres have not been observed. Blastulae are often formed, normal except for size and color, and these may develop into plutei with well developed arms and normal skeleton but more slowly. In many of the cultures, however, the white halves develop abnormally, and especially

characteristic are the permanent blastulae with imperfect skeleton, the "Dauerblastulae". There seems to be no constant percentage of normal development in any one batch of eggs, but certain whole batches develop better than others. Pigment granules characteristic of normal plutei appear in the white plutei after a few days.

White half-eggs which are fertilized while still elongate after centrifuging develop like the elongate whole egg. The first cleavage plane goes across the short axis and divides the egg unequally. By subsequent cleavages, a white slipper-shaped blastula is formed.

White half-eggs will also develop parthenogenetically if treated with concentrated sea water, and develop into white plutei.

d. Red Half-Eggs, Fertilized Merogones. Plate X

The red half-egg has no nucleus at the time of fertilization, but after fertilization the sperm nucleus with its accompanying aster may often be seen 15–20 minutes after the fertilization membrane has been given off. The fertilization membrane and the hyaline layer are much thicker than in the white half-eggs. The sperm nucleus is at first very small, but enlarges and is quite noticeable about 30 minutes after fertilization. The radiations disappear, the nucleus enlarges and breaks down about an hour after fertilization. A dumbbell-shaped nucleus or a very small amphiaster can sometimes be seen in the living egg, and then two nuclei (about 80 minutes after fertilization). In stained preparations one sees a very narrow spindle in metaphase and anaphase with chromosomes, and coarse astral fibers (Plate XVI, Photographs 12, 15). A two-celled stage may occur, especially in eggs fertilized while elongate, but often cell boundaries fail to come in. The first cleavage plane in the elongate egg comes across the short axis of the egg. Sometimes fairly regular cleavages result in a blastula of many cells with a small blastocoel, but often the blastula is multinucleate without cell boundaries. Hatching has been observed 11 hours after fertilization, and they become active swimmers. Several perfectly normal plutei with lattice-like skeletons have been raised; they were very heavily pigmented, and of course much smaller than those from whole eggs (see E. B. Harvey, 1932, 1940c, 1951). (Plate X, Photograph 16).

e. Red Half-Eggs, Unfertilized. Parthenogenetic Merogones. Plate XI

The red half-eggs, though having no nucleus, can be activated by a parthenogenetic agent, hypertonic sea water or distilled water. They

develop quite like the fertilized red half. They develop best if activated just after centrifuging while still elongate, but spherical ones can also be activated. The fertilization membrane and hyaline layer are thick, as in the fertilized red halves. A clear sphere resembling a nucleus is often present 20 minutes after activation; it is difficult to say whether there is a membrane around it, but there seems to be a phase boundary. The monaster which is often very conspicuous, arises near the "pseudonucleus." The monaster stage is followed by an amphiaster, and a cleavage plane may come in between the two asters in typical fashion. It seems curious that there should be a monaster stage preceeding the amphiaster when there is no nucleus. The first cleavage plane may divide the egg equally, in any relation to the stratification though more often parallel with it. In elongate eggs the first cleavage plane comes across the short axis as in other types of elongate eggs and half-eggs. Successive cleavages may be fairly regular and multicellular organisms of some 500 cells have been obtained. Often cell walls fail to come in, and in later stages the egg is peppered with asters and small spheres which resemble nuclei. Development is very slow and they hatch only after 24 hours, whereas the whole egg hatches in 8 to 9 hours; the fertilized red half hatches in 11 hours. No further development has been obtained; there is apparently no growth or differentiation without nuclear material, though these organisms have lived for a month; unfertilized eggs live at room temperature only a day or two. Many substances have been added to the sea water in an effort to get further development of the parthenogenetic merogones without success: killed *Arbacia* sperm, living frog nuclei, nuclei acid, phage, auxin, adenine, guanine, uracil.

In stained sections there are of course no chromosomes, and there are no spindles, but there are large asters arranged in pairs with very coarse rays (Plate XVI, Photograph 13). The *Feulgen* reaction, specific for chromatin, was negative for the parthenogenetic merogones; there was no red staining material, whereas the fertilized merogones prepared in the same way at the same time showed it very clearly. A comparison of the blastula of a fertilized merogone and a parthenogenetic merogone in aceto-carmine preparations shows very clearly the presence of chromosomes in the former, and their absence in the latter (see E. B. Harvey, 1936, 1938a, 1940b, c, 1951, Figs. 72, 73).

Compare photographs on Plate XI with corresponding ones on Plate X to see how similar is the development of parthenogenetic merogones and fertilized merogones up to a certain stage.

f. Clear Quarter. Plate XII

The clear quarter can be obtained by centrifuging the white half-egg for 20–30 minutes at 10,000 × g; to break the whole egg into halves takes only about 4 minutes. It is best to remove the white halves from the first centrifuging and recentrifuge in a fresh graded sugar-sea water mixture of the same density as the halves. The clear quarters are easy to distinguish from the white halves in the one and two-cell stages, but later it is difficult to distinguish them; they should therefore be segregated from the white halves. The clear quarter has an oil cap but no granules; it contains the bulk of the ground substance or matrix (about two-thirds). The total liquid in the egg has been computed as 61.1% of the total volume (E. N. Harvey, 1932a). The clear quarter is about as near pure protoplasm, without granular inclusions, as it is possible to obtain in the living condition. Although the clear quarter-egg is, in the living condition, free of the usual granules, mitochondria, yolk, and pigment, one can observe a trace of granules appearing as a fine line across the egg, somewhat below the oil cap, if examined with high magnification (700 ×) just after centrifuging. This line separates the clear area into two distinct portions. Sometimes small fibers are observable among the granules (E. B. Harvey, 1946a). These two portions of the clear layer stain with different intensity with many vital dyes (E. B. Harvey, 1941c). They have been studied by McCulloch (1952a), and the fibers studied with the electron microscope.

In order to get any development, it is necessary to allow the clear quarters to stand for an hour or two before they are fertilized. Otherwise the fertilization membrane is not formed or is so thin that it ruptures. The stages following fertilization are exactly like those of a normal egg except that the fertilization membrane is thin, the perivitelline space small, and the astral fibers are not visible because of lack of granules. The whole process is slower. A normal cleavage may take place in 2 hours instead of 50 minutes, which is the time for the whole egg and white half. The cleavage plane may be in any relation to the oil cap, so that one of the blastomeres contains all, none, or any portion of the oil. The second cleavage, resulting in four equal cells, usually takes place in about 3 hours instead of 1½ hours as in the normal egg. Successive cleavages follow, all delayed, till a normal blastula is formed. The blastula hatches from the fertilization membrane and becomes free-swimming in about 20 hours or longer (instead of 8 hours). These blastulae are much less granular than those from white halves. Some of these clear quarter blastulae have formed normal gastrulae

and have developed into small normal plutei with short arms after about three days, with normal *Arbacia* skeleton with a spiny base and lattice-like arm skeleton but with short arms (Plate XII, Photograph 13). After about four days, pigment spots appear. These must be a new formation in both the clear quarter and white-half plutei, since the egg brought in no pigment. Many of the clear quarters develop abnormally as loose clusters of cells caused by the thin hyaline layer, and the nuclei often become very large, three times the normal diameter. Many of the plutei are abnormal with abnormal skeleton. There are no mitochondrial granules in the clear quarter-egg, and none are brought in by the sperm in any appreciable amount, as shown by staining with methyl green. The blastulae also have none, but the plutei have them, localized especially in the cells around the gut, as they are in plutei from whole eggs and white halves. These mitochondria must be a new formation, just as are the pigment granules which appear in the pluteus from the white half-egg.

The slow development of the clear quarter, though having two nuclei, argues against Whitaker's (1929) idea that the ratio of the amount of nuclear and cytoplasmic material is the determining factor in cleavage rate.

When treated with a parthenogenetic agent, hypertonic sea water, a fertilization membrane may be formed, and early nuclear changes may occur, but no pluteus is formed as in the case of the white half.

g. Mitochondrial (Granular) Quarter

The mitochondrial or granular quarter (lower part of the white half) contains all the mitochondria and some yolk and no nucleus. It may cleave after fertilization quite regularly, and typical 2, 4, 16, etc. cell stages have been obtained, but usually cleavage planes fail to come in, so that multi-astral and multinucleate cells are common. The development is a little slower than in normal eggs. Swimming blastulae have been formed in 9 hours, but no skeleton and no plutei.

h. Yolk Quarter

The yolk quarter (upper part of the red half) contains only yolk granules and no nucleus. It develops when fertilized, like the mitochondrial quarter with sometimes regular cleavages and sometimes it is multinucleate, lacking cleavage planes. It develops further, however, forming plutei with irregular skeletons and pigment, but no perfect

plutei have been obtained. There is more of the ground substance in these quarters than in the mitochondrial quarters, as may be ascertained by the clear layer often seen at the centripetal pole when the red half is recentrifuged (Plate XIII, Photograph 5). This quarter is also considerably larger than the mitochondrial quarter.

i. Pigment Quarter

The pigment quarter, containing all the pigment and a little yolk, is very much smaller than any of the other fractions, only one twelfth the volume of the whole egg. It develops after fertilization like the other granular quarters, usually with multinucleate cells, though some regular 2-, 4-, 8-, 16 cell stages have been found. The development is very much delayed. Some swimmers were found after a day, but there was no further development. It is of interest that Morgan (1893) found that fragments of the *Arbacia* egg 1/70 the volume of the egg would cleave. These fragments were obtained by violent shaking of the whole eggs. According to Tennent, Taylor, and Whitaker (1929) for *Lytechinus variegatus*, fragments 1/50 the volume of the whole egg could not be fertilized, fragments 1/35 the volume could be fertilized but segmented irregularly, 1/24 gave regular cleavage, 1/17 formed blastulae with mesenchyme, 1/10 formed gastrulae and fragments about 1/4 the volume of the whole egg formed plutei.

j. Eggs Fertilized, Then Centrifuged

When the whole egg is fertilized and then centrifuged (but not elongated) at any stage after fertilization, it may develop quite normally, and give rise to plutei normal in every respect except for pigmentation. The first cleavage plane usually passes through the oil cap, perpendicular to the stratification but may be parallel with it (E. B. Harvey, 1934). If centrifuged after the spindle has formed, this is thrown to the centripetal pole, and the cleavage plane comes in through the oil. By observing with the centrifuge microscope, one can see the cleavage plane coming in during rotation at 6,000 × g (E. B. Harvey, 1933b). Spooner (1909) obtained the same percent of development, no matter at what stage after fertilization the eggs were centrifuged.

When the fertilized eggs are broken apart after fertilization, at any stage, the white half, containing the combined ♂ and ♀ nuclei, may cleave and develop quite normally through the blastula stage. A few acquire a skeleton and later, pigment, and may become very abnormal

plutei, but no normal plutei have been obtained (Plate XII, Photograph 16). They usually remain "Dauerblastulae." Often the first two white blastomeres develop independently forming twins; this is doubtless due to the lack of the hyaline layer which is thrown off by centrifugal force as a ring or crescent. This can be readily seen in the perivitelline space if the fertilization membrane is ruptured (Plate XVI, Photograph 6). The red half does not develop. This is in marked contrast to the red half obtained from unfertilized eggs which may cleave quite normally after parthenogenetic treatment, though having no nucleus or nuclear material. The red half from the fertilized egg has nevertheless been in contact with nuclear material. Parthenogenetic agents do not have any effect on this red half. It is only if the fertilized egg is broken apart before the ♂ and ♀ nuclei have united, that the red half may divide, owing to the presence of the ♂ nucleus (E. B. Harvey, 1933b, 1940b).

k. Conclusions

An artificial distribution of granules by centrifugal force does not radically change the course of development after the egg is fertilized. No special granules are necessary for cleavage and development (oil, yolk, pigment, and mitochondria), since any fraction may develop without one or more of these, and the clear quarter, which lacks all of them (except oil), may develop into a normal pluteus. As for the nuclei, it is apparent that the male nucleus is not necessary for cleavage and development, since parthenogenetic development takes place in many forms, and, in *Arbacia* results in a perfect pluteus in both the whole eggs and the white halves. The female nucleus is not necessary, since merogonic development of fertilized non-nucleate fragments takes place in many forms and may result in a perfect pluteus in the red halves of *Arbacia*.

It has been shown that cleavage and early development may take place, in the parthenogenetic merogones, without any nucleus at all. The essential material for cleavage and early development, therefore, must be the matrix or clear material which is present in all the fractions. In the living state, this is optically empty, except for the thin line of granules segregated out under high centrifugal forces. When fixed and stained with haematoxylin, the matrix appears filled with very small granules, microsomes (Lyon, 1907, E. B. Harvey, 1940c, Figure 124). It contains nucleoproteins, as shown by ultraviolet of a wave length of 2537 Å (E. B. Harvey and Lavin, 1944). It contains the

greater part of the enzyme peptidase, as determined by Holter (1936), and probably other enzymes. It can be separated by centrifugal force into a lighter portion and a heavier portion, which react differently to various vital dyes (E. B. Harvey, 1941 c). The ordinary granules must have some importance and may be used in early development, but they are not essential and can be replaced. At least one nucleus seems to be necessary for differentiation and complete development, but the early stages of development involving cell multiplication can take place without any nucleus.

PART IV

COMPILATION OF EXPERIMENTAL WORK ARRANGED ALPHABETICALLY*

* Statements always refer to *Arbacia punctulata* unless otherwise noted. Preliminary papers are not listed when followed by a complete paper.

AGEING OF EGGS

Size.—Increase in volume with age (Goldforb, 1918a, b, 1935a; Smith and Clowes, 1924b). Decrease when still older (Goldforb, 1935a).

Shape.—More globular with age (Goldforb, 1935a).

Jelly Layer.—Disappears (Goldforb, 1918a, b, and many others).

Vitelline Membrane.—Stretches more and bursts more rapidly in hypotonic sea water, with age (Goldforb and Schechter, 1932, Goldforb, 1937).

Fertilization Membrane.—Retarded or lacking and closer to the egg, none after 36–52 hours at 20–22° (E. N. Harvey, 1910b, 1914; Goldforb, 1918a, b; Tyler, Ricci, and Horowitz, 1938).

Longevity.—Of unfertilized egg is indicated by ability to form fertilization membrane, i.e. 36 to 52 hours after shedding (See above). Longevity can be increased by (1) sterile conditions (Gorham and Tower, 1902; Tyler *et al.*, 1938, to 10 days); (2) KCN (Loeb and Lewis, 1902, to 7 days; Loeb, 1911, 1913a, p. 26); (3) 2% alcohol (Tyler *et al.*, 1938, to double the time); (4) chloral hydrate (Loeb, 1913a, p. 91); (5) dinitro-o-cresol (Clowes and Krahl, 1936a); (6) acid sea water of pH 5.8 to 6.0 (Smith and Clowes, 1924b); (7) low calcium content of sea water (Schechter, 1937); (8) anaerobiosis (Loeb and Lewis, 1902; Loeb, 1911, 1913a, p. 26, slight increase). See Cyanides.

Polyspermy.—Increases with age (Goldforb, 1918b; Smith and Clowes, 1924b).

Oil Coalescence.—Increases (Chambers and Kopac, 1937, Kopac and Chambers, 1937).

Cytolysis.—Increases with age (Goldforb, 1918a, b; Page, 1929a).

Agglutination.—And fusion of eggs (Goldforb, 1918a, b, 1929b, c).

Separation of Blastomeres.—(Goldforb, 1918b).

Cleavage.—Slower, more irregular and fewer eggs cleave (Goldforb, 1918a, b; Smith and Clowes, 1924b).

Respiration.—Increase (Wasteneys, 1916; Gerard and Rubenstein, 1934; Tyler, Ricci, and Horowitz, 1936, due to bacteria).

Permeability[1].—Increase (Goldforb and Schechter, 1932; Goldforb, 1935c).

Viscosity.—Increase for 35 hours, then decrease (Goldforb, 1935b).

Breaking with Centrifugal Force.—Less readily (Shapiro, 1935b; E. B. H. unpub.).

Ageing of Eggs in Animal.—Due to lateness of season or to keeping animals in aquaria one to three months. Cleavage takes longer, irrespective of temperature; eggs are more viscous, take longer to stratify; break less readily with centrifugal force; some red granules remain in white half after centrifuging (E. B. Harvey, 1939b). Oxygen consumption is reduced (Shapiro, 1935c). Amount of carbohydrate and phosphorus is greater, and of nitrogen is less (Hutchens, Keltch, Krahl, and Clowes, 1942; Crane, 1947). Eggs lose pigment with time and become pale (E. B. H.).

AGGLUTININ

see Fertilizin

AMOEBOCYTES (ELAEOCYTES)

(See Chromatophores, Echinochrome, Perivisceral Fluid)

Definition.—Amoebocytes are small amoeboid cells in the perivisceral fluid, white and red, the red ones being filled with chromatophores containing echinochrome (Plate XVI, Photograph 5).

[1] Permeability means permeability to water throughout this Compilation unless otherwise indicated.

Historical.—Observed by Valentin in 1841 (published in one of the *Monographies d'Échinodermes* of L. Agassiz) in *Echinus lividus (Paracentrotus lividus);* by Williams (1852) in *E. spaera (E. esculentus);* by Geddes (1880) and Gamgee (1880) in *Arbacia lixula;* by MacMunn (1883, 1885) in *P. lividus;* by Cuénot (1891a, b) in many species; *et al.* In *Arbacia punctulata*, McClendon (1912a) and McClendon and Mitchell (1912) *et al.*, refer to them as "elaeocytes." This term really means oil or fat cells, and was originally applied to yellowish lymphocytes, not amoeboid, containing fat, in the coelomic fluid of certain oligochaetes (Rosa, 1896).

Occurrence.—In Perivisceral Fluid, q.v., (Mathews, 1900; McClendon, 1910b, 1912a, McClendon and Mitchell, 1912; Kindred, 1921, 1926; H. V. Wilson, 1924; Donnellon, 1938; *et al.*). Throughout body, especially around testes and ovaries (E. B. H. unpub.).

Color.—Red and white amoebocytes occur in about equal numbers. The red ones owe their color to Echinochrome, present in Chromatophores, q.v.

Amount of Echinochrome.—In amoebocytes: 3.78 gm. per 100 cc. of packed volume of body cells; the eggs contain on the average 0.58 gm. of pigment per 100 cc. of eggs packed by centrifuging (Ball and Cooper, 1949).

Pigment Released.—By water, tissue extracts, salts especially potassium, hypertonic solutions, fat solvents, mechanical and electrical stimulation, ultraviolet light, cold, heat; but it is not released if the body fluid is oxalated or citrated, under most of these conditions (Donnellon, 1938). Release by electric current was earlier observed by McClendon (1910b).

Colorless Amoebocytes.—Of body fluid swell and dissolve if NaCl, KCl or $MgCl_2$ are added to the sea water (Mathews, 1900).

Shape.—Cylindrical or spherical. Decidedly amoeboid. Become spherical on addition of water and with ultraviolet light and x-rays (E. B. H. unpub.), and with hydrostatic pressure (Marsland, 1938).

Size.—Cylinders average 30 μ long by 7.3 μ diameter; volume 1256 μ^3. Spheres average 13.3 μ diameter; volume 1232 μ^3 (E. B. H. unpub.).

Stain.—Colorless amoebocytes are basophilic, red ones acidophilic (Kindred, 1926).

Function.—Not certain. Red ones may be respiratory (Geddes, 1880; Gamgee, 1880; MacMunn, 1885; in other species). See under Echinochrome. Cause clotting of perivisceral fluid (Heilbrunn, 1928, p. 228; Donnellon, 1938).

Fertilization.—Inhibited by amoebocytes (Pequegnat, 1948), probably due to echinochrome (Couillard, 1952).

Other Species (additional)

Davidson, 1953. *Echinarachnius parma.*
Kindred, 1924. *Strongylocentrotus dröbachiensis, S. franciscanus, Echinarachnius excentricus.*

AMOEBOID EGGS

Amoeboid Eggs.—These are caused by
Standing overnight (E. B. H. unpub.).
Urea (R. S. Lillie, 1903; Moser, 1940; Kitching and Moser, 1940; A. R. Moore, 1929, in *S. purpuratus*).
Sucrose (Kitching and Moser, 1940).
Ethyl urethane, 0.4 M for 1 hr. (E. B. H. per E. N. Harvey, 1933).
KCl (Churney, 1940).
$MgCl_2$ (Loeb, 1900a; Churney, 1940).
Electric current, at anode (McClendon, 1910b).
Photodynamic action, light + rose bengal or eosin (Alsup, 1941).

Trypsin, in *Dendraster excentricus* (A. R. Moore, 1951a).

Hypertonic sea water, in *Ps. microtuberculatus* (E. B. Harvey, 1938a).

Clear Quarter-Eggs.—Fertilized, become amoeboid if not cleaved (E. B. Harvey, 1946). Clear quarter-eggs and white half-eggs, made parthenogenetic with ultraviolet light, become amoeboid (E. B. Harvey and Hollaender, 1938).

Arrest of Amoeboid Movement.—By high hydrostatic pressure on urea-treated eggs. By lack of oxygen (Kitching and Moser, 1940).

Other Species (additional)

Dendraster excentricus with trypsin (A. R. Moore, 1951a).

Paracentrotus lividus and *Ps. microtuberculatus*, upper half (with nucleus) of centrifuged egg (E. B. Harvey, 1934, p. 239).

Sphaerechinus granularis, whole centrifuged egg and lower (non-nucleate) half of centrifuged egg (E. B. Harvey, 1938a, p. 184).

ANAEROBIOSIS

See Oxygen-Lack

ANAESTHETICS (NARCOTICS)

Effect on Size of Egg.—Volume increase in 3% ether (Heilbrunn, 1928, p. 203); in chloroform (Heilbrunn, 1915a).

Effect on Shape of Egg.—Eggs become amoeboid if kept in 0.2 M ethyl urethane for one hour (E. B. H. per E. N. Harvey, 1933). Elongate, centrifuged eggs also become amoeboid. These can be fertilized, the fertilization membrane following the irregular contour; they cleave in the irregular shape on return to sea water. If not fertilized the amoeboid eggs become spherical on return to sea water (E. B. H., 1931 unpub.).

Fertilization.—Eggs can be fertilized in alcohols, but do not cleave; cannot be fertilized in ethyl acetate though sperm surround the eggs (Blumenthal, 1928). Can be fertilized in 0.15 M ethyl urethane, sometimes forming a fertilization membrane (E. B. H.).

Surface Precipitation Reaction.—May be prevented by anaesthetics (Heilbrunn, 1934).

Cleavage.—Effective concentrations of many anaesthetics for reversible arrest of cleavage are given in Table 13, from R. S. Lillie (1914b). Effective concentrations of some urethanes for reversible arrest of cleavage are given in Table 14 (E. B. H. per E. N. Harvey, 1932a).

Mitotic Figure.—Anaesthetics (many) may prevent formation of spindle and cause rays to disappear (Heilbrunn, 1920a, b). Urethanes cause astral eays to fade out and cleavage planes to disappear (Painter, 1918; E. B. H., 1930 unpub.). When placed in 0.2 M ethyl urethane at metaphase, cleavage planes fade out and when returned to sea water are replaced irregularly, but normal blastulae may be formed (E. B. H.). These phenomena are similar to those described and figured by me (1927, 1930) for eggs kept without oxygen. A similar disappearance of cleavage planes also is caused by high hydrostatic pressures (Marsland, 1938, 1939, 1950, 1951) and in *Ps. microtuberculatus* by mechanical pressure (Boveri, 1897). Disappearance of astral rays by various agents was first found by O. and R. Hertwig in 1887 in *Paracentrotus lividus*, and very carefully studied by E. B. Wilson (1901b) in etherized eggs of *Toxopneustes (Lytechinus) variegatus;* he also studied obliteration of cleavage furrows.

Monasters formed with chloral hydrate were described in *P. lividus* by O. and R. Hertwig (1887); in *Lytechinus* in etherised eggs (E. B. Wilson, 1901b); in *P. lividus* and *A. punctulata* with phenyl urethane (Painter, 1915, 1916, 1918).

TABLE 13

EFFECTIVE CONCENTRATIONS OF ANAESTHETICS TO ARREST CLEAVAGE OF ARBACIA PUNCTULATA EGGS

From R. S. Lillie 1914b, *J. Biol. Chem.* 17 : 139

Ethyl alcohol	*ca.* 5 v. % (0.87 M)
n-Propyl alcohol	*ca.* 2 v. % (0.27 M)
Iso-propyl alcohol	*ca.* 3 v. % (0.4 M)
n-Butyl alcohol	*ca.* 0.8 v. % (0.086 M)
i-Amyl alcohol	*ca.* 0.4 v. % (0.037 M)
Capryl alcohol	*ca.* 0.015 v. % (0.001 M)
Methyl urethane	2–2.5 % (0.27–0.33 M)
Ethyl urethane	1.5–1.75 % (0.15–0.19 M)
Phenyl urethane	0.08–0.1 % (0.005–0.006 M)
Ethyl ether	0.5–0.6 v. % (0.05–0.06 M)
Chloroform	*ca.* 0.06 % ($^1/_{12}$ sat.) (0.005 M)
Chloral hydrate	0.1–0.12 % (0.006–0.007 M)
Chloretone	0.2–0.25 % (0.008–0.01 M)
Nitromethane	*ca.* 2. v. % (0.42 M): unfavorable
Acetonitrile	*ca.* 2. v. % (0.5 M): unfavorable
Ethyl nitrate	*ca.* 0.25 v. % (0.025 M): unfavorable
Paraldehyde	2–3 v. % (0.15–0.2 M): unfavorable
Chloralose	ineffective in sat. sol. (*ca.* 0.6 %)
Acetanilide	ineffective in sat. sol. (*ca.* 0.5 %)
Phenyl urea	ineffective in sat. sol. (*ca.* 0.5 %)

TABLE 14

CRITICAL ANAESTHETIC CONCENTRATIONS OF URETHANES FOR REVERSIBLY SUPPRESSING CLEAVAGE IN ARBACIA PUNCTULATA EGGS

From E. B. Harvey per E. N. Harvey, 1932a, *Biol. Bull.* 52 : 151

Ethyl urethane	0.15–0.2 M
N-propyl urethane	0.07 M
Isopropyl urethanes	0.1 M
N-butyl carbamate	0.025 M
Isoamyl carbamate	0.01 M
Phenyl urethane	0.00125–0.0025 M

Spiral asters formed in *Paracentrotus lividus* with phenyl urethane (Painter, 1916); with KCN (Runnström, 1930, p. 155). Spiral asters formed with high centrifugal force in *Psammechinus microtuberculatus* (E. B. Harvey, 1935a). See classical study of Mark (1881) on spiral asters normally occuring in *Limax campestris*.

Rhythms.—In mitotic cycle, of sensitivity to anaesthetics. With ether (Spaulding, 1904); higher alcohols (Baldwin, 1920); many anaesthetics (Heilbrunn, 1920a).

Gastrulation.—Effect of alcohols (Waterman, 1936).

Ciliary Activity.—Of blastulae and plutei stopped by chloretone (chlorobutanol); use a small amount of saturated solution in sea water added to swimming larvae (E. B. H.). By magnesium sulphate (E. B. H.); widely used to quiet marine organisms (Mayor, 1909; R. S. Lillie, 1916c; Heilbrunn, 1920b, 1934, 1952, p. 528); magnesium anaesthesia antagonised by calcium (Heilbrunn, 1952, p. 589).

Recovery from Anaesthetics.—Unfertilized eggs may be kept two hours in optimum concentration of any urethane listed in Table 14 and recover in sea water immediately; fertilized eggs undergo slight development while in urethane and time of first cleavage on recovery is independent of duration of exposure, 40 minutes to two hours in ethyl urethane (E. B. H., 1930 unpub.). Similar results for alcohols (Blumenthal, 1928).

Differential susceptibility and recovery (with ethyl alcohol) with relation to axial gradients (Child, 1916a, b).

Acceleration of cleavage on return of anaesthetised eggs to sea water (Blumenthal, 1928).

Prolongation of Life.—Of unfertilized egg. By chloral hydrate (Loeb, 1913a, p. 91). With 2% ethyl alcohol, life is doubled, and this is not due to bactericidal action (Tyler, Ricci, and Horowitz, 1938). See also under Cyanides.

Salts and Anaesthetics.—Antagonism (R. S. Lillie, 1912, 1914a, 1917).

Effect of Temperature.—On carbamate narcosis (Cornman, 1950b).

Respiration and Cleavage.—Anaesthetics prevent cleavage with little effect on respiration. See Respiration A III 1. First shown by Warburg (1910) for *Paracentrotus lividus* with phenyl urethane; 0.0005 M phenyl urethane blocks cleavage and reduces oxygen consumption to 80% of normal. More fully studied in *S. purpuratus* with ethyl urethane and other anaesthetics by Loeb and Wasteneys (1913b). In *Arbacia*, 0.001 M phenyl urethane blocks cleavage and reduces oxygen consumption to 70% of control (Clowes and Krahl, 1940, Krahl and Clowes, 1940). 0.1 M ethyl urethane stops cleavage and reduces oxygen consumption to 75% (Fisher and Henry, 1944, Fisher, Henry, and Low, 1944). 0.0045 M chloral hydrate blocks cleavage, leaving 45% oxygen consumption, (and has practically no effect on unfertilized eggs) (Fisher and Henry, 1944). See Table VIII of Krahl (1950). For barbiturates and local anaesthetics (benzoates, etc.) on cleavage and oxygen consumption see Clowes and Krahl (1940); Krahl and Clowes (1940); Clowes Keltch and Krahl (1940); Krahl, Keltch and Clowes (1940a); see Tables VI and VII of Krahl (1950). For urethane and methylene blue on respiration (Barron, 1929).

Permeability.—Decreased by anaesthetics (R. S. Lillie, 1912, 1914a, b, 1916c, 1918b, etc.; Lucké and McCutcheon, 1932, but see 1926 a and Lucké, 1931). Increase due to fertilization is prevented by anaesthetics (R. S. Lillie, 1918a, b; Lucké and McCutcheon, 1932. For Table of concentrations effective in preventing increase of permeability, which are similar to those arresting cleavage see R. S. Lillie (1918b, p. 426). According to Heilbrunn (1925c, 1943, pp. 144, 531), permeability is not decreased by ether. According to McClendon (1909b) permeability is increased by all agents causing parthenogenesis, including ether.

Viscosity.—Decreased if anaesthetic is dilute, increased if concentrated (Heilbrunn, 1920a, b, 1925c, 1927, 1928, p. 205; by centrifuge tests). See Table 15 from Heilbrunn (1928) giving concentrations and lethal doses of many anaesthetics, with regard to viscosity. Decrease in 2.5% ether according to Heilbrunn (1920a), increase according to Chambers (1924, p. 250); but Heilbrunn (1925c, p. 474 and 1928, p. 207) thinks Chambers' results were due to heating by microscope lamp. Decrease in 0.2 M ethyl urethane by centrifuge tests (E. B. H., 1930 unpub.). Chloretone increases viscosity (Heilbrunn, 1920a, b).

Parthenogenesis.—Caused by chloroform, ether, alcohol (Mathews, 1900; McClendon, 1909b, 1910b; Heilbrunn, 1928, p. 261). By acetone, chloretone, urethane, chloral hydrate, methyl acetate, ethyl acetate, ethyl butyrate, methyl salicylate (Heilbrunn, 1913; Just, 1929a for acetone). Parthenogenesis by hypertonic sea water prevented by anaesthetics (R. S. Lillie, 1914a, b, 1917; Heilbrunn, 1920a). Reversal of parthenogenesis with chloral hydrate (or NaCN); (Loeb, 1913c, 1914b, 1915b); this is denied by F. R. Lillie and Just (1924, p. 505). See Cyanides.

Sperm Respiration.—Ethyl urethane, 0.1 M increases respiration by 23%; 0.01 M

TABLE 15

CONCENTRATIONS OF ANAESTHETICS FOR FERTILIZED ARBACIA PUNCTULATA EGGS VISCOSITY

From Heilbrunn, 1928, *Colloid Chemistry of Protoplasm*, p. 206, amended from Heilbrunn, 1920b, p. 311

Reagent	Concentration of solution found to decrease viscosity Vol. % Anaesthetic conc.	Length of exposure in minutes	Concentration of solution found to cause coagulation Vol. % Lethal conc.	Length of exposure in minutes
Ether	2.5	9	3.5	40½
Chloroform	0.13	6½	1 (emulsion)	8½
Chloral hydrate	0.08	13	1	35
Chloral hydrate	0.25	28	—	—
Nitromethane	2	24½	3	16
Paraldehyde	4	7, 12½	8	6½
Acetone	5	29	10	9
Ethyl nitrate	0.3	6½	—	—
Ethyl nitrate	0.5	29	—	—
Ethyl acetate	3	41½	5	19½
Ethyl acetate	4	6½	—	—
Ethyl butyrate	0.25	33½	0.5	5
Ethyl butyrate	0.33	18	—	—
Acetonitrile	4	6½	5	13½
Propyl alcohol (n)	1	10	—	—
Propyl alcohol (n)	1.33	25	—	—
Amyl alcohol	0.66	28½	1	40
Phenyl urethane	4/5 sat.	6	saturated (= <0.5 %)	15
Ethyl urethane	1.5	8	3	29½
Ethyl urethane	2.5	18	—	—

has no effect. See Respiration B I 8. Phenyl urethane saturated solution inhibits respiration 60 %, one fourth saturated inhibits 12 %, one tenth saturated has no effect (see Respiration B II 6) (Barron, Nelson, and Ardao, 1948). Sperm are more resistant to anaesthetics than eggs, but they can be anaesthetised (Blumenthal, 1928). Sperm lose fertilizing power after one half hour in 0.2 M ethyl urethane; lose motility after two hours (E. B. H., 1930 unpub.).

Other Species (additional) and General References

Cornman, 1950a. *Tripneustes esculentus, Lytechinus variegatus* with carbamates.
Cornman, Skipper, and Mitchell, Jr., 1951. *Tripneustes esculentus*, urethane.
Fisher, 1942. General.
Führner, 1904. *Paracentrotus lividus* and *Psammechinus miliaris* with alcohols etc.
R. S. Lillie, 1916c. *Theory of Anaesthesia.*
Monné, 1947. *Ps. miliaris*, etc.; anaesthetics on structure of protoplasm.
Runnström, 1928b, 1930. *P. lividus, A. lixula* with ethyl and phenyl urethanes.

ARTIFICIAL PARTHENOGENESIS

See Parthenogenesis

CALCIUM

Amount in egg.—1.90 mg Ca per 10^6 eggs (10^6 eggs = 0.124 gm. dry weight) or 0.047 millimols (Page, 1927b).

Total Ca is about 0.3 mg. per cc. eggs; same in unfertilized and fertilized eggs. Exchangeable Ca per cell is 19.2×10^{-9} mg. The concentration of free Ca in the unfertilized egg is of the order of 0.0005 M and increases by 0.001 M on fertilization; bound Ca decreases on fertilization by about 15%. Ca release on fertilization and cytolysis (Mazia, 1933, 1937, 1940; Heilbrunn, Mazia, and Steinbach, 1934).

Sea water.—At Woods Hole contains 0.428 gm. Ca per liter at 20° C. (Page, 1927c, 1928).

Perivisceral Fluid.—Contains 0.395 mg./cc. of Ca as against 0.41 mg./cc. in sea water (Schechter, 1937, from analysis by Mazia).

$CaCl_2$.—Isotonic with sea water at Woods Hole is 0.30 M (M. B. L. Chemical room) usually given as 0.34 M.

Radioactive Ca (Ca^{45}).—Accumulated only by eggs with jelly coats and for only six hours after fertilization (Rudenberg, 1953).

Surface Precipitation Reaction.—With breakdown of pigment granules; calcium necessary (Heilbrunn, 1928, Chapt. 13; 1930, 1943, p. 86; 1952, p. 102; Costello, 1932; *et al.*). Effect of anaesthetics (Mg and ether) on surface precipitation reaction (Heilbrunn, 1934). For other references to calcium and breakdown of pigment granules see Gross (1951); and of other granules (yolk) see Costello (1932) and (cortical) see Moser (1939a, b).

Vitelline membrane.—Made brittle by Ca (Heilbrunn, 1928, p. 149; Chambers, 1944, 1949, 1950; see Kopac, 1940a).

Surface potential of egg.—Effect of Ca (Dan, 1936).

Hyaline layer.—Lack of calcium prevents formation of, or causes dissolution of, hyaline layer of fertilized egg so that blastomeres fall apart. First described by Herbst in 1900 in *Echinus microtuberculatus* and subsequently by many others. Effect of lack of Ca on hyaline layer in Arbacia punctulata and other species described by E. B. Harvey (1933b, 1934). See Hyaline Layer. (Plate XVI, Photograph 9).

Jelly.—Dissolves in Ca-free sea water (E. B. H.).

Ca Necessary for Fertilization.—No fertilization membrane is formed if fertilized in Ca-free sea water though sperm are motile (Loeb, 1915a; Glaser, 1915; E. B. H. unpub.; also Monroy, 1949 for *Psammechinus microtuberculatus*). But see Shapiro (1941).

Cleavage.—Addition of calcium (3 times amount in artificial sea water) delays cleavage, lack of calcium accelerates cleavage (Shapiro, 1941). Delay and arrest with calcium (Schechter, 1937). Effect on furrowing (Scott and Pollen, 1951).

Effect on Cytolysis.—$CaCl_2$ prevents cytolysis (R. S. Lillie, 1911a, b, 1912; Page, 1924; Schechter, 1936). When used for a long time, $CaCl_2$ causes cytolysis (Heilbrunn, 1928, p. 147).

Antagonism.—Ca counteracts NaCl and other Na and K salts (Loeb, 1900a; Mathews, 1905; R. S. Lillie, 1911a, b, 1912, 1914a; McCutcheon and Lucké, 1928; Heilbrunn, 1943, p. 463). Ca antagonises Mg (Heilbrunn, 1934).

Respiration.—For other species (Hultin and Vasseur, see list below).

Permeability to water.—Decreased by isotonic $CaCl_2$ (R. S. Lillie, 1910; McCutcheon and Lucké, 1928; Lucké and McCutcheon, 1929, 1932); no effect on fertilized eggs (R. S. Lillie, 1918b). Permeability to ethylene glycol not significantly changed by Ca (Stewart and Jacobs, 1936).

Viscosity.—Decreased by isotonic $CaCl_2$ (Heilbrunn, 1923, 1928, p. 146, 232;

1943, p. 81; 1952, p. 96; E. B. Harvey, 1945). The cations arranged according to their effect in decreasing viscosity (better stratification) are

Ca > Mg > sea water > Na > K (E. B. Harvey, 1945, p. 74)
Ca > Mg > sea water > K > Na > NH_4 (Heilbrunn, 1928, p. 147).

Added Ca decreases viscosity (Wilbur and Recknagel, 1943).

Breaking of eggs.—Isotonic $CaCl_2$ causes eggs to break with centrifugal force less readily than in sea water; the better stratified, the less easily they break. The cations arranged for ease in breaking are in the reverse order to that given above for decreasing viscosity (E. B. Harvey, 1945).

Calcium-Free Sea Water.—As compared with eggs in sea water, no difference in rate of stratification, indicating change in viscosity, could be detected by observation with a double head centrifuge microscope (E. B. Harvey 1933b) (Table 16). Wilbur and Recknagel (1943) found increased viscosity when calcium was removed from the sea water by potassium citrate. According to Kriszat and Runnström (1951) viscosity is increased in calcium-free sea water in *Ps. miliaris*. Unfertilized *Arbacia* eggs elongated more and broke more readily in calcium-free sea water, and fertilized eggs broke more readily than in sea water (E. B. Harvey, 1933b, 1945). Shapiro (1941) found that unfertilized eggs elongated more and rounded up more rapidly in calcium-free sea water, whereas they elongated less and rounded up more slowly in increased calcium (3 times amount in artifical sea water).

Parthenogenesis.—Caused by adding $CaCl_2$ to the sea water (Loeb, 1900a, 1913a, p. 59, etc.); by 0.3 M $CaCl_2$ (Hollingsworth, 1941). But R. S. Lillie (1910, 1911a, b, 1914a) found that isotonic $CaCl_2$ did not activate, and prevented activation by NaCl. Ca is necessary for activation by ultraviolet light (Heilbrunn and Young, 1930; Heilbrunn and Mazia, 1936) and other parthenogenetic agents (Moser, 1939b). For the necessity of calcium for parthenogenesis in general see Tyler (1941a, p. 322) and Heilbrunn (1943, p. 661).

Longevity of Egg.—Increased by reduction of Ca (Schechter, 1937).

TABLE 16

CALCIUM-FREE AND ARTIFICIAL SEA WATER

M. B. L. at Woods Hole, Mass. as prepared in Chemical Room, for salinity 31 (grams per liter)

	NaCl	KCl	$CaCl_2.2H_2O$	$MgCl_2.6H_2O$	$MgSO_4.7H_2O$	NaHCO-
grams per liter	24.72	0.67	1.36	4.66	6.29	0.18
ml. of 1 M solution*	423.0	9.00	9.27	22.94	25.50	2.15

pH of sea water at Woods Hole 8.14 (Ball and Stock 1937). △ is 1.805.

(Herbst (1900) at Naples, for salinity 38 (grams per liter)

NaCl	KCl	$CaCl_2$	$MgSO_4$	$MgHPO_4$
3% + 0.07% to replace $CaCl_2$	0.08%	0.13%	0.66%	in excess, for alkalinity

Calcium can be removed from sea water with potassium oxalate (or citrate), about 1.5 gm. per liter of sea water.

For other formulae for calcium-free sea water, see McClung's *Microscopical Technique*, 3rd ed., p. 559, 1950; Hörstadius, 1935 and Tyler, 1953.

* Total mixture to be diluted to one liter.

Agglutination.—Of sperm caused by calcium (Loeb, 1915a). See also Vasseur (1949a) for *S. dröbachiensis.*

Formation of Skeleton.—Calcium necessary for pluteus (probably *P. lividus*); if 1/10th of Ca in sea water is precipitated by sodium oxalate a recognizable alteration in development occurs (Pouchet and Chabry, 1889 a, b).

Other Species (additional) and General References

Bialaszewicz, 1927, 1929. *A. lixula, Paracentrotus lividus;* electrolytes.
Costello, 1932. *Echinarachnius parma,* surface precipitation reaction.
Fukuda, 1934. *Pseudocentrotus depressus,* membrane and s.p.r.
Heilbrunn, 1952. *General Physiology,* p. 530.
Herbst, 1904. Salts necessary for development.
Hultin, 1949b. *Psammechinus miliaris,* respiration of egg homogenates.
Hultin, 1950a. *Paracentrotus lividus,* same.
Hultin, 1950b. *Paracentrotus lividus,* viscosity of egg homogenates.
Lepeschkin, 1941. *A. lixula etc.* s.p.r. etc.
Monroy-Oddo, 1946. *A. lixula,* change of Ca content after fertilization.
Örstrom and Örstrom, 1942. *P. lividus,* Ca content of eggs.
Robertson and Webb, 1939; Robertson, 1941. Amount in sea water and body fluids.
Rothschild and Barnes, 1953. *P. lividus,* amount in egg; table of salts and species.
Rulon, 1941a. *Dendraster excentricus,* development in Ca-free sea water.
Runnström, 1925. *P. lividus, Ps. micr., Ps. miliaris,* calcium-lack.
Runnström and Kriszat, 1950a. *Ps. miliaris,* centrifuging.
Sugiyama, 1938d. *Pseudocentrotus depressus, S. pulcherrimus,* fertilization membrane.
Tyler, 1941a, p. 322. Parthenogenesis in general.
Vasseur, 1949b. *S. dröbachiensis, E. esculentus, Ps. miliaris;* oxygen uptake of sperm.

CARBOHYDRATE METABOLISM

Total Carbohydrate.—Determined as glucose. Amount. About 50 mg. glucose per gm. egg protein (Perlzweig and Barron, 1928). About 110 mg. glucose per gm. egg protein or 7% of dry weight of egg, 65% being protein; mostly in egg, little in jelly, (Hutchens, Keltch, Krahl, and Clowes, 1942).

Glycogen.—Identified in eggs by Blanchard in 1927 as noted by Perlzweig and Barron (1928), published by Blanchard in 1935. Amount: 40–57 mg. glycogen per gm. egg protein in unfertilized eggs or 46% of total carbohydrate (Hutchens *et al.* 1942).

Free Reducing Sugar.—Absent (Perlzweig and Barron, 1928; Hutchens, *et al.*, 1942).

Pyruvate.—Amount. 70 μg. pyruvate per gm. dry weight of eggs (Goldinger and Barron, 1946). Eggs utilize 64 μg. pyruvic acid per gm. dry weight per hour, unfertilized; 7 times as much (445 μg.), fertilized (Goldinger and Barron, 1946). 160 μg pyruvate per gm. dry weight per hour, unfertilized; 310 μg. fertilized, in cytolysates (Krahl, Jandorf, and Clowes, 1942).

Lactic Acid.—Amount. 3.14 mg. lactic acid per gm. protein in unfertilized eggs, increase to 5.68 mg. with KCN; slight increase 1–2 hours after fertilization, from 2.71 to 3.23 mg. (Perlzweig and Barron, 1928). Lactic acid negligible during first 24 hours of development (Hutchens, *et al.*, 1942).

Phosphorylation.—Of carbohydrate intermediates (Keltch, *et al.*, 1950, 1951; Strittmatter, *et al.*, 1950; Clowes, *et al.*, 1950, 1951a, b; Clowes, 1951).

Iodoacetate.—Affects carbohydrate metabolism (Runnström, 1935c).

Other Species (additional) and General References

Barron, 1952b. General.
Cleland and Rothschild; 1952a, b. *Echinus esculentus,* glycolysis.

Ephrussi, 1933. *Paracentrotus lividus*, amount of carbohydrate.
Immers, 1952. *Echinus esculentus*, *Echinocardium*, etc., sugars.
Krahl, 1950. Review.
Lindberg and Ernster, 1948. *Strongylocentrotus dröbachiensis*, phosphorylation.
Monné and Slautterback, 1950. Staining of *P. lividus*.
Örström and Lindberg, 1940. *P. lividus*, glycogen, etc., before and after fertilization.
Rothschild and Mann, 1950. *Echinus esculentus*, sperm.
Runnström, 1949b. General.
Spikes, 1949. *S. purpuratus*, *Lytechinus pictus*, sperm.
Stott, 1931. *Echinus esculentus*.
Zielinski, 1939. *P. lividus*.

CHROMATOPHORES (PIGMENT GRANULES)

See also Echinochrome

Chromatophores.—Spherical vacuoles containing echinochrome (Chambers, 1935a, 1938a; D. L. Harris, 1943; *et al.*). See E. B. Wilson (1899, p. 7 and p. 26, Plate I, Fig. 2; 1926, p. 113), who described them as alveoli and showed them as hollow spheres.

Occurrence.—In eggs, unfertilized and fertilized, plutei and in red Amoebocytes, q.v.

Location.—In unfertilized eggs, they are distributed throughout the cytoplasm (Lyon, 1907; E. N. Harvey, 1909, 1910b; McClendon, 1910b, 1912a; R. S. Lillie, 1911b; *et al.*). But they are not evenly distributed (Heilbrunn, 1926a; E. B. H. unpub.).

On fertilization or parthenogenesis, they migrate to periphery of egg (McClendon, 1909b, 1910b, 1912a; E. N. Harvey, 1909, 1910b; Heilbrunn, 1928, p. 264; K. Dan, 1951a; *et al.*). See E. B. Wilson (1926, Fig. 2a, b).

In fertilized eggs, they are located especially at periphery and in cleavage furrow (Loeb, 1895, p. 274, 1905, p. 401; G. F. Andrews, 1897b, p. 85; Mathews, 1906b; McClendon, 1910b, 1912a; Cannan, 1927; *et al.*). Return to uniform distribution after each cleavage (K. Dan, 1951a, b). After 8-cell stage, they move away from the micromere pole, and the micromeres are therefore colorless (Morgan, 1893; Lyon, 1907; E. N. Harvey, 1909; McClendon, 1910b; *et al.*). Location in monaster eggs (Painter, 1918).

In plutei, 20 to 30 chromatophores, each about 2 μ in diameter, are grouped together to form pigment spots, distributed irregularly over the body. These may be spherical or irregular in shape, and vary in size; the average spherical ones are about 7 μ in diameter. They swell and break in distilled water (E. B. H. unpub.).

Size.—Chromatophores are all sizes up to 1.7 μ (E. B. H. unpub.). See also Harris (1943) who says 1 to 2 μ.

Weight.—Heaviest bodies in the egg; go to centrifugal pole on centrifuging (Lyon, 1906a, 1907; McClendon, 1909a; E. B. Harvey, 1932, 1936, etc.; *et al.*).

Specific Gravity.—Approximately 1.1035; of whole egg is approximately 1.0485 (Heilbrunn, 1926a).

Amount in Egg.—5.5% (E. N. Harvey, 1932a); 10% (Costello, 1939).

Origin.—Pigment begins to form in immature egg when about 33 μ in diameter; younger oocytes are colorless (E. B. H. unpub.).

Stain.—With vital dyes. Methylene blue, neutral red (McClendon, 1909b, 1912a; E. N. Harvey, 1910c; Lucké, 1925; E. B. Harvey, 1941c; *et al.*). Bismark brown, brilliant cresyl blue, chrysoidin, methyl violet, Nile blue, rhodamine, saffranin o, toluidin blue (E. B. Harvey, 1941c). They stain black with methylene green and also with quinine, cinchonine and cinchonidine, with no effect on development (E. B. H. unpub.). E. B. Wilson (1926) states that they stain intensely with Janus

Green B, but I have not succeeded in staining them with any of many samples of Janus green and Janus green B. Table 8.

With histological stains. Not stained with most protoplasmic stains (Lyon 1907; E. B. Wilson, 1926); not stained with iron haematoxylin, but are stained with orange G (E. B. Harvey, 1940c); stained with Delafield's haematoxylin (McClendon, 1909b, 1912a). Appearance in electron microscope preparations (McCulloch, 1952a).

Ultraviolet Light.—Somewhat absorbing (E. B. Harvey and Lavin, 1944).

Release of Pigment from Chromatophores in Unfertilized Eggs.—On standing (F. R. Lillie, 1912; Glaser, 1914b, c, 1921a, 1924; Heilbrunn, 1928, p. 246; *et al.*). But Shapiro (1946) thinks not. Rupture or puncture of vitelline membrane (E. N. Harvey, 1910b; Heilbrunn, 1928, p. 70; Chambers, 1938a; Churney, 1941a; D. L. Harris, 1943; *et al.*). Puncture of chromatophore membrane (Chambers, 1935a, 1938a; D. L. Harris, 1943). Electrical, mechanical, or chemical stimulation (McClendon, 1910a, b, 1912a; Heilbrunn, 1928, p. 244; Chambers, 1938a). Heat (E. N. Harvey, 1910b; Chambers, 1938a).

Hypotonic sea water or distilled water (E. N. Harvey, 1910b; and many others). Process described by D. L. Harris (1943). Hypertonic sea water (E. N. Harvey, 1910b; Glaser, 1914b). Parthenogenetic agents (McClendon, 1909b; R. S. Lillie, 1910, 1911a, b; E. N. Harvey, 1910b). Calcium, and to a much less extent magnesium and strontium (Heilbrunn, 1926a, 1928, p. 223, 1930, 1934, etc. in surface precipitation reaction; D. L. Harris, 1943; Gross, 1951, 1953). Acids (E. N. Harvey, 1910b; Barth, 1929). Iodosobenzoic acid (Monroy and Runnström, 1950, 1952). Alkalis (D. L. Harris, 1943). Soaps (Heilbrunn, 1928, p. 242; Page, Shonle, and Clowes, 1933; Gross, 1951). Fat solvents (Heilbrunn, 1828, p. 242). Urea, acetamide, sucrose, ethylene glycol (D. L. Harris, 1943); but see Churney (1938) for urea. Anaerobiosis (Shapiro, 1946).

Pigment Not Released in absence of calcium; chromatophores remain intact (Heilbrunn, 1926a, 1928, p. 224, etc.); D. L. Harris (1943) uses 0.35 M sodium citrate to keep chromatophores intact. In homogenates, chromatophores remain intact in absence of calcium (Gross, 1951).

Release of Pigment on Fertilization or Parthenogenesis.—(McClendon, 1909b, 1910b, 1912a; McClendon and Mitchell, 1912; E. N. Harvey, 1909, 1910b, c; R. S. Lillie, 1910, 1911a, b, 1914b; Lyon and Shackell, 1910b; Glaser, 1914b, c, 1923, 1924; Just, 1922, I; Shapiro, 1946; *et al*).

Release of Pigment from Chromatophores in Fertilized Eggs.—On standing. More released from fertilized than from unfertilized eggs (Loeb, 1910; Lyon and Shackell, 1910b; Glaser, 1914b, c, 1923; Shapiro, 1946; *et al.*). Pressure (Churney 1941a). Mercuric chloride, with clumping of pigment (Hoadley, 1930). Potassium chloride, oxalate and citrate, ammonium chloride, urea; but no effect on unfertilized eggs (Churney, 1938; Churney and Moser, 1940). During cleavage (R. S. Lillie, 1914b; see Churney, 1940).

Effect on Fertilization Membrane.—Coagulating (Monroy and Runnström, 1950, 1952; Runnström, 1950–1951, p. 143; 1952b, p. 67).

Osmotic Properties.—Permeability constant: 0.38 cubic micra of water enter the vacuole per square micron of surface area per minute per atmosphere difference in osmotic pressure; higher than for the cell (D. L. Harris, 1943). Pigment vacuoles therefore do not constitute part of osmotic dead space postulated by Lucké, Hartline, and McCutcheon (1931).

Coalescence of Chromatophores.—With chloroform (E. N. Harvey, 1910c). In presence of calcium, magnesium or strontium ions (D. L. Harris, 1943).

Movement.—Of individual chromatophores in unfertilized eggs. Observed by McClendon (1910b). With T. V. microscopy; translatory motion of 5 μ per second at 24° C. (Parpart, 1953). Under hydrostatic pressure (D. E. S. Brown, 1934; Marsland, 1950). Through cleavage (Dan, 1951a, b).

No Fading with Anaerobiosis (i.e. not reduced).—(Korr, 1939, p. 83 and Ball in same paper, p. 92). See under Echinochrome.

Electric Charge.—Possibility of (E. N. Harvey 1909, 1910b; McClendon, 1910b, 1912a, Heilbrunn, 1926b).

Contain Copper.—(Glaser, 1923).

Other Species

Fischel, 1906b. *A. lixula.*
E. B. Harvey, 1933a, 1938a. *A. lixula.*

CLEAR LAYER OF CENTRIFUGED EGGS

Position in Arbacia.—Layer in centrifuged eggs between oil (centripetal) and granules: mitochondria, yolk and pigment (centrifugal); contains nucleus (early investigators Lyon, 1906a, 1907; Morgan, 1909; McClendon, 1909a *et al.*; later investigators E. N. Harvey, 1932a; E. B. Harvey, 1932, 1936, 1940c, etc.; *et al.*).

Structure.—Optically empty in living egg except after prolonged centrifuging. A narrow band of granules then appears across the clear layer of white halves and clear quarters, forming two zones (E. B. Harvey, 1946a; McCulloch, 1952a). Birefringent fibrils in lower part of clear zone (McCulloch, 1952a). Electron microscope preparations (McCulloch, 1952a; Lansing, Hillier, and Rosenthal, 1952). Some pigment granules remain in clear layer of eggs late in the season (E. B. H.).

Amount.—61.1 % (E. N. Harvey, 1932a); 45 % (Costello, 1939); these figures include fluid between the packed granules; the difference between the two figures is probably due to the amount of centrifuging. About 2/3 of total fluid is in clear layer (E. B. Harvey, 1946a).

Specific Gravity.—About 1.0358; of whole egg is about 1.0485 (Heilbrunn, 1926a).

Viscosity.—Two centipoises, determined by centrifuge tests and return of granules (Heilbrunn, 1952, p. 81; see also Heilbrunn, 1926a, b, 1927, 1928, p. 67).

Stain.—With vital dyes. Faint stain with Bismark brown, chrysoidin, methyl violet, neutral red, Nile blue, rhodamine, toluidin blue (E. B. Harvey, 1941c). Two portions stain differentially (E. B. Harvey, 1946a). Table 8.

Fixed material.—Layer is filled with very small granules which stain blue with iron haematoxylin (Lyon, 1907; Morgan and Lyon, 1907; E. B. Harvey, 1940c; E. B. Harvey and Lavin, 1944; *et al.*); these are probably the "microsomes" of Wilson (1925, p. 32), they measure about 0.2 μ in diameter (E. B. H.). Clear layer stains with protoplasmic stains, alum cochineal (Lyon, 1907). Stains differentially (two kinds of material) with haematoxylin-orange-G-eosin (E. B. Harvey, 1946a, Figs. 32, 33).

Ultraviolet Light.—Absorbed by clear layer in formalin-fixed sections (E. B. Harvey and Lavin, 1944).

Localization of Peptidase.—In clear layer (Holter, 1936).

Other Species

In some other species, e.g., *Sphaerechinus granularis*, the clear layer is at the centripetal pole as it is in *Arbacia punctulata* and *A. lixula*, being lighter than the granules. In other species, e.g., *Psammechinus microtuberculatus*, it is below the yolk but above the mitochondria. In still other species, e.g., *Tripneustes esculentus*, it is beneath the yolk and mitochondria, being the heaviest material in the egg. In some species, e.g., *Paracentrotus lividus*, the clear material may be in more than one zone (E. B. Harvey, 1933a, 1938a).

Other references for clear layer:
Harvey, E. B. per E. N. Harvey, 1939. *Echinarachnius parma, Strongylocentrotus dröbachiensis*, etc.
Harvey, E. B., 1947. *Lytechinus variegatus* and other Bermuda forms.

Lindahl, 1932c. *Psammechinus microtuberculatus*, fine colored picture, p. 330; probably *Ps. miliaris* is the same.
Linderström-Lang, 1938–1939. *Ps. miliaris*, diagram.
Monné, 1944b. *Echinocardium cavdatum*, *Ps. miliaris*.
See under Centrifuged Eggs, Part III, Chapter 17d, p. 127.

CLEAVAGE, ACCELERATION

Many of the substances listed under Cleavage are treated more fully under Respiration

Acetyl Choline.—Acceleration in low concentrations, inhibition in high (Balzer and Villee, 1951; Villee and Villee, 1952.

Alcohols.—Slight acceleration (Blumenthal, 1928); in low concentrations (Waterman, 1936).

Alkali.—Slightly alkaline sea water accelerates cleavage 1½ or 2 cc. of 1/10-normal NaOH to 100 cc. sea water (Loeb, 1898, but see 1913a, p. 35). Also see Glaser (1914b) and Medes (1917). Acceleration at pH 8.2 to 9.2, maximum at 8.8. Delay at pH 9.4 (Smith and Clowes, 1924c). For *Echinus esculentus* see B. Moore, Roaf and Whitley (1905).

Calcium-free Sea Water.—Accelerates cleavage; excess Ca delays cleavage (Shapiro, 1941; Scott and Pollen, 1951).

Carcinogenic Hydrocarbons.—(Choleic acid compounds). Acceleration of cleavage (Keltch, Krahl, and Clowes, 1937). Retarded and atypical cleavage (Lucké, Parpart, and Ricca, 1941).

Copper Chloride.—Acceleration in very dilute $CuCl_2$, 10^{-13} M (Finkel, Allee, and Garner, 1942; Allee, Finkel, *et al.*, 1942). See under Delay.

Crowding.—Early cleavages accelerated if eggs moderately dense, delayed if very dense (Allee and Evans, 1937a, b, c). For *S. dröbachiensis* see Frank and Kurepina (1930); for *P. lividus* see Maxia (1933); for *Anthocidaris crassispina*, *Pseudocentrotus depressus*, *S. pulcherrimus* see Sugiyama (1938e).

Cyanide, Potassium.—Accelerates cleavage in very weak concentrations, 10^{-6} M; arrests cleavage in stronger concentrations, 10^{-5} M to 10^{-4} M (Lyon, 1902). See under Delay and Cyanides.

Cystin.—Acceleration of cleavage and development (Mathews, 1909); not confirmed by King (1912).

Egg Extracts.—"*Homotypic extracts.*" Some accelerate cleavage, some do not (Allee, Finkel, *et al.*, 1942). Acceleration if fats removed (Peebles, 1929).

Heat.—Cleavage accelerated as temperature is raised up to about 31° C. (Loeb and Wasteneys, 1911a; Loeb, 1913a, p. 32; Loeb and Chamberlain, 1915; Hoadley and Brill, 1937). See Tables 3 and 4. Highest temperature permitting cleavage is about 28 °C.; killed at 32° C. (E. B. H.). For *Ps. microtuberculatus* and *Sphaerechinus granularis* see Peter (1905). For *P. lividus* see Ephrussi (1933); for *S. purpuratus*, *Lytechinus anamesus* and *Dendraster excentricus* see Tyler (1936a, b). See under Delay.

Hydrogen Ion Concentration.—See below under pH.

Hypotonic Sea Water.—94–98 %. Accelerates first cleavage 5 % of normal time (Cornman, 1943).

Mechanical Shocks and Vibrations.—Accelerate cleavage (Meltzer, 1903; Mathews and Whitcher, 1903). Whitney (1906) thinks this is due to rise in temperature. Shaking accelerated cleavage in *A. lixula* also (Lyon 1903).

Methylene Blue.—Cleavage accelerated with 5×10^{-5} %, retarded with 5×10^{-4} % (M. M. Brooks, 1933, 1943). Shapiro (1948a) finds delay; also Clowes and Krahl (1936a). See Krahl (1950, Tables VIII and IX). See under Delay.

Oxygen Lack, Partial.—May accelerate cleavage (Loeb, 1895a, 1905 translation).

"I will not commit myself definitely to the statement that in case of a partial lack of oxygen a transitory acceleration of cleavage occurs" (Loeb, 1905, p. 403).

pH.—Acceleration at pH 8.2 to 9.2, maximum at 8.8. Delay above pH 9.4 and below 6.0. (Smith and Clowes, 1924c, d).

Pilocarpine.—In low concentrations accelerates development, in high concentrations delays (Mathews, 1901a; Sollman, 1904a). See Balzer and Villee (1951) and Villee and Villee (1952). For *Dendraster excentricus* see Rulon (1941b).

Radium.—Alpha rays accelerate cleavage, beta rays delay (Packard, 1915, 1916).

CLEAVAGE, DELAY OR ARREST

Acetyl choline.—Delay in high concentrations, acceleration in low concentrations (Balzer and Villee, 1951; Villee and Villee, 1952).

Acid.—Delay (Loeb, 1898, 1913a, p. 35; Glaser, 1914b; Medes, 1917). Retarded below pH 6.0 (Smith and Clowes, 1924c). For *Echinus esculentus* see B. Moore, Roaf, and Whitley (1905).

Ageing.—(Goldforb, 1918a, b; Smith and Clowes, 1924b). No delay if kept, for at least six hours (Fry, 1936).

Alanin.—(King, 1912).

Alcohols.—Delay except in very weak concentrations (Blumenthal, 1928; Waterman, 1936. See below under Anaesthetics.

Alkali.—Acceleration in weak concentrations (Loeb, 1898, 1913a, p. 35; Glaser, 1914b; Medes, 1917). Delay above pH 9.4 (Smith and Clowes, 1924c). For *Echinus esculentus* see B. Moore, Roaf, and Whitley (1905).

Ammonium chloride.—(Kopac, 1948b).

Anaerobiosis.—See below under Oxygen-Lack.

Anaesthetics.—For anaesthetic and lethal doses of common anaesthetics see Table 13 (from R. S. Lillie, 1914b), Table 15 (from Heilbrunn, 1928, p. 206); for urethanes see Table 14 (E. B. Harvey per E. N. Harvey, 1932a). See below Barbiturates, Benzoates, Dithiocarbamates, Urethanes. See also Anaesthetics (Separate topic).

Antibiotics.—Penicillin, aspergillus, etc. (Cornman, 1949); actinomycin, aureothrycin, etc. (Miura, 1953).

Ascorbic Acid.—Or vitamin C (Shapiro, 1948c).

Asparagine.—Development slow, cleavage normal (King, 1912).

Aspartic acid.—King, 1912).

Aspergillus Filtrates.—Antibiotic (Cornman, 1949).

Atropin.—(Mathews, 1901a; Sollman, 1904a; Villee and Villee, 1952).

Azide.—Complete and reversible inhibition of cleavage, and respiration inhibited by 50% at 5×10^{-3} M (Krahl, 1950, p. 195); also Krahl, Keltch Neubeck, and Clowes (1941); Fisher, Henry, and Low (1944). For *Dendraster excentricus* see Rulon (1950a).

Barbiturates.—Or barbituric acid derivatives (Clowes and Krahl, 1940; Clowes, Keltch, and Krahl, 1940; Krahl, 1950, p. 200 and his Table VI).

Benzoates.—And other local anaesthetics (Clowes and Krahl, 1940; Krahl, Keltch, and Clowes, 1940a; Krahl, 1950, p. 200 and his Table VII).

Bile Salts.—(Genther and Schmidt, 1931).

Brilliant Cresyl Blue.—(Shapiro, 1948a).

Caffeine.—(Cheney, 1945, 1948).

Calcium.—Added to sea water delays cleavage, calcium-free sea water accelerates (Shapiro, 1941).

Carbamates.—See Urethanes.

Carbon Dioxide.—Clowes and Smith, 1923; Smith and Clowes, 1924a; Haywood, 1927; Haywood and Root, 1930, 1932; Allee and Evans, 1937b; Allee, Finkel, *et al.*, 1942).

Carbon Monoxide.—M. M. Brooks, 1933; Clowes and Krahl, 1940; Krahl and Clowes, 1940; Krahl, p. 194, 1950). For *P. lividus* see Runnström (1930); for *Dendraster excentricus* see Pease (1942c).

Carcinogenic Hydrocarbons.—Choleic acid compounds. Delayed cleavage (Lucké, Parpart, and Ricca, 1941). Accelerated cleavage (Keltch, Krahl, and Clowes, 1937).

Chloral Hydrate.—Fisher and Henry, 1944). See under Anaesthetics above and Tables 13 and 15.

Chloretone.—Cleavage checked by 0.08% (Heilbrunn, 1920a, p. 233).

Clavacin.—Antibiotic (Cornman, 1949).

Colchicine.—About 10^{-4} M. Mitosis stopped in late prophase or metaphase (Nebel, 1937; Nebel and Ruttle, 1938; Beams and Evans, 1940; Wilbur, 1940; Cornman and Cornman, 1951). For *Ps. microtuberculatus* see Zeuthen (1951, p. 59). For a historical review, see Eigsti, Dustin and Gay-Winn (1949).

Cold.—Progressive delay as temperature is lowered from about 31° C. (Loeb and Wasteneys, 1911a; Loeb, 1913a, p. 32; Loeb and Chamberlain 1915; Hoadley and Brill, 1937; Villee and Villee, 1952; Tables 3, 4, and 19). For *Ps. microtuberculatus, Sphaerechinus granularis* see Peter (1905); for *P. lividus* see Ephrussi (1933); for *S. purpuratus, Lytechinus anamesus* and *Dendraster excentricus* see Tyler (1936a, b).

Copper Chloride.—1/62,500 $CuCl_2$ in sea water prevents cleavage; 1/500,000 prevents fertilization (F. R. Lillie, 1921b). See also Hoadley (1923); Glaser (1923); Waterman (1937). Runnström (1939) removes poisonous effect of copper by shaking the eggs. Acceleration in very dilute solutions, 10^{-13} M (Finkel, Allee, and Garner, 1942; Allee, Finkel, *et al.*, 1942).

Corticotropin.—ACTH. (Menkin, 1952, 1953, Proc. Exp. Biol. Med. 82 : 189–194).

Cortisone.—And desoxycorticosterone (Cornman, 1950c; Menkin, 1952).

Cresols.—See Phenols.

o-Cresol Indophenol.—(Clowes and Krahl, 1936a; Krahl, 1950, Table VIII).

Crowding.—Cleavage delayed if eggs are very dense; accelerated if moderately dense (Allee and Evans, 1937a).

Cyanides.—Cleavage arrested by about 10^{-5} M to 10^{-4} M (Krahl, 1950, p. 192 and his Table V). Data of different observers are given under Cyanides. The required concentration depends on the pH (Krahl). Acceleration of cleavage in very weak concentrations, about 10^{-6} M (Lyon, 1902). Reversal of cleavage inhibition by adenosine triphosphate (ATP). (Barnett, 1953).

Desoxycorticosterone.—(Cornman, 1950c; Menkin, 1952).

Deuterium oxide.—See Heavy Water.

Dimethyl-p-phenylene diamine.—(Clowes and Krahl, 1936a; Krahl, 1950, Table VIII). For *P. lividus* see Runnström (1930, 1932).

Dinitrophenols.—And dinitrocresols. See Phenols.

Dinoflagellate Contaminated Sea Water.—See "Red tide."

Dithiocarbamates.—(Krahl, 1950, Table VIII).

Echinochrome.—(Woodward, 1918). But Allee, Finkel, *et al.* (1942) find no effect.

Egg or Embryo Extracts or "Water".—(Glaser, 1913, 1914b; F. R. Lillie, 1921b; Springer, 1922; Peebles, 1929; Allee and Evans, 1937b, c; R. D. Allen, 1951). Some extracts accelerate (Allee, Finkel, *et al.*, 1942).

Eserine.—Inhibition of cleavage (Villee and Villee, 1952). Stated to accellerate in low concentrations (Balzer and Villee, 1951).

Gliotoxin.—Antibiotic (Cornman, 1949).

Glutamic Acid.—(King, 1912).

Glycerine.—(R. S. Lillie, 1903).

Glycocol.—Cleavage normal, later development slow (King, 1912).

Halophenols and Halocresols.—See Phenols.

Heat.—Highest temperature permitting any cleavage lies between 30.4° C. and 32.7° C.; optimum 24° C. or 25° C. (Hoadley and Brill, 1937). See Acceleration.

Heavy Water.—Deuterium oxide. Delay (Lucké and E. N. Harvey, 1935).

Heparin.—And heparin-like substances (Gagnon, 1950; Heilbrunn and Kelly, 1950; C. V. Harding, 1951).

Hydrogen Ion Concentration.—See acid, alkali, pH.

Hypertonic Sea Water.—Loeb, 1892, 1904; Norman, 1896; Mathews, 1905; Medes, 1917; R. S. Lillie, 1918b; Heilbrunn, 1920a; Churney, 1940).

Hypotonic Sea Water.—(Medes, 1917; Heilbrunn, 1920a, b; Just, 1929b; Churney, 1940). Retardation in 84% sea water or less, acceleration if slightly hypotonic, 94 to 98% (Cornman, 1943).

Iodoacetate.—Iodoacetic acid. Runnström (1935c) used 0.03 M iodoacetate for retarding development. See also Waterman (1938, 1941); Needham and Needham (1940); Clowes and Krahl (1940); Krahl and Clowes (1940); Krahl (1950, p. 199). For *P. lividus, Ps. microtuberculatus, Ps. miliaris* see Tchakotine (1938); for *Dendraster excentricus* see Pease (1941).

Janus Green B.—(R. D. Allen, 1950).

Leucin.—(Mathews, 1909). Delay and pecularities, of later development. (King, 1912).

Leucotaxine.—(Menkin, 1940).

Local Anaesthetics.—See above under Benzoates.

Malonic Acid.—Delay (Rulon, 1948). Krahl and Clowes (1940) found no effect with concentrations used. Malonate inhibition reversed by adenosine triphosphate, ATP (Barnett, 1953). For *Dendraster excentricus* see Pease (1941); Rulon (1951).

Malononitrile.—(Villee, Lowens, *et al.*, 1949).

Mercuric Chloride.—1/625,000 $HgCl_2$ in sea water prevents cleavage, 1/125,000 prevents fertilization (F. R. Lillie, 1921b; also Hoadley, 1923, 1930; Waterman, 1937; Barron and Seki, 1952). Cf. Copper chloride above. For *Paracentrotus lividus* see Rapkine (1931).

Metal Salts (Heavy).—For copper and mercury, see above. For effect of other heavy metal salts on cleavage and development, see Hoadley (1923); Waterman (1937).

Metal Salts (Alkali and Alkaline Earth).—Toxic effects, Li > Na > Mg or Ca > K > Rb > Cs (Page, 1929b). See under Calcium, Lithium, Magnesium, Potassium, Sodium.

Methylene Blue.—Cleavage delayed in 5×10^{-4}%, accelerated in 5×10^{-5}% (M. M. Brooks, 1933, 1943). Delay (Clowes and Krahl, 1936a; Krahl, 1950, Tables VIII and IX; Shapiro, 1948a). Waterman (1938, 1941) finds delay except possibly in very weak concentrations. For effect on development of *Dendraster excentricus* and *S. purpuratus*, see Child (1950b). See under Acceleration.

Morphine.—No effect with 2×10^{-2} M (Krahl, 1950, p. 206).

Napthoquinones.—(Anfinsen, 1947). See Krahl (1950, p. 199).

Necrosin.—(Menkin and Pirovane, 1949).

Neutral Red.—(Clowes and Krahl, 1936a; Krahl, 1950, Table VIII).

Nitrogen Mustards.—Delay at prophase (E. B. Harvey and Cannan, 1943, unpub. except as a confidential report of a war project, see Gilman, and Phillips 1946; E. B. Harvey, 1946a). Delay (Barron, Seegmiller, Mendes, and Narahara, 1948; Hutchens and Podolsky, 1948; Villee, Lowens, *et al.*, 1949; Cornman, 1950d; Krahl, 1950, p. 205). For *A. lixula* and *Sphaerechinus granularis*, see Tau and de Nicola (1949).

Nitrophenols and Nitrocresols.—See Phenols.

Nitrous Oxide.—Delay under pressure of 2.3 atmospheres. No delay with nitrogen or helium even under pressure of 61 atmospheres (Haywood, 1953).

Osmotic Pressure.—Pressure less than 13.2 atmospheres or greater than 28.7 prevents cleavage (Churney, 1940).

Oxygen-lack or Low Oxygen Tension.—See topic Oxygen-Lack and Low Oxygen Tension. Cleavage retarded at 11 mm. Hg; arrested below 4 mm. Hg. (Amberson, 1928). Slight lack of oxygen may possibly accelerate cleavage (Loeb, 1895, 1905

translation, p. 403, see above under Acceleration. Inhibition reversed by adenosine triphosphate, ATP (Barnett, 1953).

Parthenogenetic Treatment.—Delay; first cleavage (at 23° C.) occurs 1½ to 5 hours after activation; fertilized eggs cleave 50 minutes after fertilization (E. B. Harvey, 1936, 1951; E. B. Harvey and Hollaender, 1938). Delay also noted by Loeb (1900a, 1904; Moser (1940) and others. For general information about *Arbacia* and other species see Loeb's *Artificial Parthenogenesis* (1913a).

Penicillin and Penichromin.—(Henry and Henry, 1945; Cornman, 1949). Slight inhibition of cleavage with very weak concentrations of noncrystalline samples of penicillin, but virtually no effect with highly purified samples (Clowes and Keltch, 1946 unpub., per Krahl, 1950, p. 206).

Perivisceral Fluid.—(F. R. Lillie, 1913a, 1914, 1919, p. 173; A. E. Woodward, 1918; Just, 1922a, III; Goldforb, 1935b).

pH.—Delay of cleavage below pH 6.0 and above 9.4; acceleration pH 8.2 to 9.2 (Clowes and Smith, 1923; Smith and Clowes, 1924b, c, d). See under Acid and Alkali above and under Alkali in Cleavage: Acceleration.

Phenols, Cresols and Related Substances.—(Clowes and co-workers, 1934–1951, especially Clowes and Krahl, 1936a; Krahl and Clowes, 1936a, 1938; Clowes 1951). See the comprehensive review of Krahl (1950, p. 196 and his Tables III, IV). Also Waterman (1938, 1941); Villee, Lowens *et al.* (1949); A. Scott (1950). Incomplete reversal of inhibition of dinitrophenol by ATP (Barnett, 1953). For *Dendraster excentricus* see Pease (1941).

Phosphorus, P^{32}.—(Green and Roth, 1950).

Pilocarpine.—In high concentrations, delay; in low concentrations accelerate cleavage (Mathews, 1901a; Sollman, 1904a; Balzer and Villee, 1951, but see Villee and Villee, 1952). For *Dendraster excentricus* see Rulon (1941b); Pease (1942b).

Podophyllin, Podophyllotoxin, Quercitin and Derivatives.—(Cornman, 1947a; Cornman and Cornman, 1951).

Polysaccharides.—Heparin, etc. (C. V. Harding, 1951).

Pressure, Hydrostatic.—(Marsland, 1938, 1950, 1951; Kitching and Moser, 1940; E. B. H., 1933 unpub.). For *Arbacia lixula* see Marsland (1939).

"*Purple X.*"—(Glaser, 1914c; A. E. Woodward, 1915, 1918).

Pyocyanine.—(Runnström, 1935a; Clowes and Krahl, 1936a; Krahl, 1950, Table VIII; Waterman, 1938, 1941). For *Dendraster excentricus* see Pease (1942b); Moore, Bliss, and Anderson (1945) also for *S. purpuratus.*

Quinine.—Very poisonous; one part of quinine sulphate in 17,000 parts sea water stops cleavage (Mathews, 1907). Poisonous action noted by O. and R. Hertwig in 1887.

Radium.—Beta rays delay, alpha rays accelerate cleavage (Packard, 1915, 1916).

"*Red Tide.*"—Dinoflagellate-contaminated sea water. (Cornman, 1947b).

Rhodamine B and Light.—(L. B. Clark, 1940).

Rotenone.—And related compounds (Cornman and Rogers, 1951; Rogers and Cornman, 1951).

Season.—Cleavage delay late in the season (Medes, 1917; Woodward, 1918; Fry, 1936; E. B. Harvey, 1939b).

Sperm Extracts.—(Frank, 1939).

Sugar.—(R. S. Lillie, 1903; Loeb, 1913a, p. 130 for *Strongylocentrotus purpuratus* with good discussion of sugar effects; R. S. Lillie and Cattell, 1923). No fertilization in isotonic sugar solution, but cleavage when returned to sea water even after 5 hours in sugar (E. B. Harvey, 1932).

Sulfide, Sodium.—(Krahl, Keltch, Neubeck, and Clowes, 1941; Krahl, 1950, p. 195).

Sulphanilamide.—And related compounds (Fisher, Henry, and Low, 1944; Krahl, 1950, Table VIII).

Sulphur, S^{35}.—(Green and Roth, 1953, Biol. Bull. 105 : 364)

Temperature.—See under Cold, delay; and Heat, acceleration.

Theobromine and Theophylline (Cheney, 1949b).

Thiurea.—(Bevelander, 1946). For *Dendraster excentricus* see Rulon (1950b).

Toluyene Blue.—(Shapiro, 1948a).

Tyrosin.—(Mathews, 1909; King, 1912). Slight acceleration in some cases with weak solutions (Mathews, 1909).

Ultraviolet Light.—See Ultraviolet Light. Cleavage and development delayed.

Uranyl Nitrate.—(Villee, Lowens, *et al.*, 1949).

Urea.—(R. S. Lillie, 1903).

Urethanes.—Carbamates. Anaesthetic doses given in Table 14. See under Anaesthetics. See Krahl, 1950, p. 199, and his Table VIII.

Usnic Acid.—Antibiotic (Marshak and Harting, 1948; Marshak, 1949a; Marshak and Fager, 1950).

Vitamin C.—Ascorbic acid (Shapiro, 1948c).

Washing Eggs Repeatedly.—Delays cleavage (F. R. Lillie, 1914; Woodward, 1918; *et al.*).

X-Rays.—Cleavage and development delayed. See X-Rays.

COELOMIC FLUID

See Perivisceral Fluid

COMPOSITION OF EGGS

For general analyses of centrifuged layers of crushed eggs, see Table 7 (McClendon, 1909a).

Solids.—26.5 % (Ballentine, 1940a); 23.9 % (Hutchens, Keltch, *et al.*, 1942).

Ash.—8.5–10 % dry weight eggs (Page, 1927b); 7–8 % (Blanchard in E. N. Harvey, 1932a).

Electrolytes.—In millimoles per kilogram water in eggs, calculated by Rothschild and Barnes (1953): Na 321, K 354, Ca 269, Mg 1044, Cl^{-} 30, sulphate 0.0301 (Page, 1927b); K 96, Ca 38, Mg 17 (Blanchard, quoted by E. N. Harvey, 1932a). For *Arbacia lixula;* Na 280, K 162, Ca 16, Mg 41, Cl 384 (Bialaszewicz, 1929); Ca 17, Mg 21 (Monroy-Oddo, 1946). For other species, especially *Paracentrotus lividus*, see Rothschild and Barnes (1953).

Sea Water Electrolytes.—Millimoles per kilogram) for 19 per thousand salinity are: Na 475, K 10, Ca (+Sr) 10, Mg 54 Cl 554, sulphate 29 (Rothschild and Barnes, 1953).

Iron.—0.030 mg. per 10^6 eggs; 0.0005 millimoles (Page, 1927b).

Copper.—17 μg. per cc. of unripe ovarian eggs; 175 μg. per cc. of unfertilized and 21 μg. per cc. of fertilized eggs (Glaser, 1923).

Total Phosphorus.—In millimoles per kilogram water in eggs is 181 (Page, 1927b). For *Arbacia lixula* 132 (Bialaszewicz, 1929). For other species see Rothschild and Barnes (1953). See also Phosphorus Metabolism for phosphorus fractions of egg.

Total Nitrogen.—0.107 mg. per mg. dry weight (Ballentine, 1940a); 0.10 mg. N_2 per mg. dry weight (Hutchens, Keltch, *et al.*, 1942). See Nitrogen.

Total Protein.—65 % dry weight (Hutchens, Keltch, *et al.*, 1942). See Protein and Nucleoprotein.

Total Fat.—See Oil and Lipids.

Carbohydrate.—50 mg. (Perlzweig and Barron, 1928); 110 mg. acid hydrolyzable carbohydrate determined as glucose (7 % of dry egg weight) per gm. egg protein, of which 46 % is glycogen (Hutchens, Keltch, *et al.*, 1942). Practically none from jelly. Glycogen content, 50–80 mg. per gm. egg protein (Blanchard, 1935). See Carbohydrate Metabolism.

Reducing Sugar.—Absent (Perlzweig and Barron, 1928; Hutchens, Keltch, *et al.*, 1942).

Cholesterol.—Present (Mathews, 1913).

Lactic Acid.—3.14 mg. per gm. egg protein. 19 % increase in 4–8 cell fertilized eggs (Perlzweig and Barron, 1928), not confirmed by Hutchens, Keltch, *et al.* (1942).

Echinochrome.—See Echinochrome.

Enzymes.—See Enzymes and Cytochrome.

Other Species and General References

Bialaszewicz, 1927, 1929. *A. lixula and Paracentrotus lividus*, electrolyte content.
Ephrussi and Rapkine, 1928, and Ephrussi, 1933. *Paracentrotus lividus*, protein, fat, carbohydrate, ash.
Harvey, E. N., 1932a. Constants, general.
Krahl, 1950. General.
Leitch, 1934b, 1936. *S. franciscanus, S. purpuratus*, nitrogen, lipoid, ash.
Lindberg, 1943. *Echinocardium cordatum*, phosphate fractions.
Malm and Wachtmeister, 1950. *Psammechinus miliaris, S. dröbachiensis*, potassium content.
Mitchinson and Swann, 1953. *Ps. miliaris*, 25.5% solids.
Needham, 1931 and 1942. General.
Örström and Lindberg, 1940. *Paracentrotus lividus*, carbohydrate content and metabolism.
Pantin, 1931. Body fluids, composition.
Rothschild and Barnes, 1953. Electrolyte content of many species.
Stott, 1931. *Echinus esculentus*, carbohydrates.
Tennent, Gardiner, and Smith, 1931. *Echinometra lucunter*, lipids and glycogen.
Wetzel, 1907. *Paracentrotus lividus*, per cent of solid, fat, N_2, P, ash.
Zielinsky, 1939. *Paracentrotus lividus*, phosphate fractions and carbohydrate metabolism.

CORTICAL LAYER

Position.—A layer just beneath the plasma membrane and above the mass of cytoplasm (Figs 9, 10). According to Parpart and Laris (1954) it is outside the plasma membrane.

Properties.—A relatively rigid gel, comparatively clear; may be liquified reversibly (Danielli, 1942, p. 86). Its thickness is 0.8 μ in *A. punctulata;* 1 μ to 2 μ in some forms (Runnström and Monné, 1945a; Mitchison, 1952). Cortex of centrifuged unfertilized egg is bright in dark field (Moser, 1939a) and is birefringent (McCulloch, 1952a). Cortical layer contains calcium proteinate (Heilbrunn, 1952, p. 465, 538). Bound calcium in cortex of fertilized eggs can be released by potassium (Churney and Moser, 1940).

Liquefaction.—By Micromanipulation (Chambers, 1935b, 1938b). Hydrostatic pressure (Brown, 1934; Marland, 1938, 1950, 1951). Decreased temperature (Marsland, 1950).

Stiffens.—With calcium (Heilbrunn, 1952, p. 97).

Cortical Granules.—In cortical layer, they disappear 20 seconds after fertilization or parthenogenesis and help form the fertilization membrane. The granules are approximately 0.8 μ in diameter and are not displaced by high centrifugal forces, 6,000 × g (Moser, 1939a, b, 1940); 10,000 × g (E. B. Harvey, 1946a). The cortical reaction takes about 10 seconds at 25.7° C. (Moser, 1939a). These granules were first observed and described in *Arbacia* by E. N. Harvey (1911, p. 523) as minute granules unmoved by the centrifuge, which disappear on fertilization and whose substance helps to form the fertilization membrane. These granules were observed and figured in the unfertilized egg of *Echinus esculentus*, and their absence in the fertilized egg was noted by Gray (1924, p. 169; by an error they are called "micromeres" instead

of "microsomes" in the legend. They were described in stained sections of the *Lytechinus* (formerly *Toxopneustes*) egg by Hendee (1931), a student of Tennent's, and are beautifully shown in the color of the stain used. Their disappearance upon fertilization is shown in her pictures of unfertilized and fertilized eggs. Apparently these same granules were described as "Janus green" granules in *S. pulcherrimus* by Motomura (1936).

More recently and apparently without knowledge of previous literature, the cortical granules have been re-discovered and carefully studied in *Arbacia* by Moser (1939a, b, 1940), especially with regard to fertilization and parthenogenesis and to the relation of the granules to the fertilization membrane. Similar studies have been carried on by the Swedish school (See Runnström, Monné, and Wicklund, 1946; Runnström, 1948c; etc.). It seems now to be generally agreed that the fertilization membrane is formed by a combination of the cortical granule material with the vitelline membrane.

Before maturation, the cortical granules are located inside the egg, not at the periphery (McCulloch, 1952b). This has been found to be the case also in *Psammechinus miliaris* and *Brissopsis lyrifera* (Runnström and Monné, 1945a; Monné and Härde, 1951), and in the Japanese species *Strongylocentrotus pulcherrimus* (Motomura, 1936, 1941b).

For the structure of the cortical granules in *Arbacia punctulata* as shown by the electron microscope see McCulloch (1952a, b); Lansing, Hillier, and Rosenthal (1952). The cortical granules of *Arbacia punctulata* stain with Janus green (Motomura, personal communication 1954).

Pigment Granules.—(Chromatophores) which have migrated to the periphery on fertilization are located in the cortex (Brown, 1934 and see under Chromatophores, location).

Cytolysis.—Of cortical layer believed by Loeb to cause activation and membrane formation (Loeb, 1913a, Chap. 17, etc.).

Movement.—Of cortical layer in fertilized eggs (Dan, 1951a, b.).

Effect of Methylated Xanthine.—On cortical granules (Cheney, 1951).

Ribonucleic Acid.—Present in cortex (Lansing and Rosenthal, 1949).

Other Species and General References

Endo, 1952. Japanese sea urchins, cortical granules.

Just, 1939b. Biology of the Cell Surface.

Mitchison and Swann, 1952. *Psammechinus miliaris, Paracentrotus lividus,* birefringence and light scattering.

Monné, 1948. General. Other references to Monné and co-workers given here.

Monroy, 1947. *Ps. microtuberculatus,* birefringence. Other references to Monroy and co-workers given here.

Monroy and Montalenti, 1947. *Ps. miliaris,* birefringence.

Moore, A. R., 1949a, b, 1951b. *Strongylocentrotus purpuratus,* pro-membranes in cortex; and general.

Rothschild and Swann, 1949. *Ps. miliaris,* cortical layer on fertilization.

Runnström, 1948a, c. *Ps. miliaris,* membranes and cortical granules.

Runnström, 1949a, b; 1952a. General. Other references to Runnström and co-workers given here.

Runnström, Monné, and Broman, 1944. *Ps. miliaris* etc. birefringence.

Runnström, Monné, and Wicklund, 1946. *Ps. miliaris, Echinocardium cordatum, S. dröbachiensis,* membranes and cortical granules.

CYANIDES

Fertilization.—Eggs can be fertilized while in KCN (Blumenthal, 1927, 1928; Just, 1928a; A. Scott, 1950). Effect on fertilization membrane (Just, 1928a).

Development.—Of fertilized eggs continues slightly in KCN (Heilbrunn, 1920a; Blumenthal, 1928, 1930; Just, 1928a).

Cleavage.—a. Arrest. Effective concentration to arrest cleavage in *Arbacia punctulata* according to different observers:

M/10,000 KCN (Lyon, 1902).

Few drops of 1/10 % NaCN to 50 cc sea water (Loeb and Wasteneys, 1911a, Loeb, 1913a, p. 26.

M/8,000 KCN (R. S. Lillie, 1914b).

0.000,625 % KCN (Heilbrunn, 1920a).

N/1,000 KCN, least concentration used (Blumenthal, 1928).

5×10^{-5} M KCN (M. M. Brooks, 1933).

1.6×10^{-4} M KCN (Clowes and Krahl, 1940).

1.5×10^{-5} M HCN (Robbie, 1946b).

5×10^{-2} M NaCN; very slow in 10^{-4} M NaCN (A. Scott, 1950).

10^{-5} to 10^{-4} M cyanide agreed upon by all methods; the required concentration depends on the pH (Krahl, 1950).

b. Acceleration in very weak concentrations of KCN, M/1,000,000; delay if stronger than 4 M/1,000,000 (Lyon, 1902).

Cytology.—Of KCN treated eggs (Blumenthal, 1930, with photographs; A. Scott, 1950).

Rhythms.—In mitotic cycle of sensitivity to cyanide (Lyon, 1902; Mathews, 1906a; Heilbrunn, 1920a; Just, 1928a; Clowes and Krahl, 1934b, 1935; Krahl, 1950; Runnström, 1935a). No phase sensitivity (Blumenthal, 1930; A. Scott, 1950).

Later Stages.—Of development to plutei as affected by cyanide (Lyon, 1902; Child, 1916a, b, 1941, p. 199, axial gradients and differential inhibition).

Cilia of Blastulae and Plutei.—Paralyzed by KCN, recovery in sea water (Lyon, 1902).

Recovery.—Delay in cleavage after return to sea water depends on concentration of KCN and is independent of length of exposure (Blumenthal, 1928, 1930; Brinley, 1930). Cleavage inhibition caused by KCN is reversed by addition of ATP (Barnett, 1953).

Prolongation of Life of Unfertilized Eggs.—With N/1000 KCN from one day (at 20 °C) to 7 days (Loeb and Lewis, 1902; Loeb, 1913a, Chapt. X, 1915a; etc.) But Gorham and Tower (1902) think this is due to killing of bacteria among the eggs by KCN; this was answered by Loeb (1911). The same question is discussed by Tyler, Ricci, and Horowitz (1938) for ethyl alcohol.

Salts and Cyanides.—Sodium cyanide prevents toxic action of salts etc. (Loeb, 1910). M/1000 KCN decreases toxicity of Na salt, increases toxicity of K salt (R. S. Lillie, 1912).

Temperature and KCN.—More effective at higher temperatures Korr, 1937).

Oxygen Tension and KCN.—Greater inhibition at low tensions (Robbie, 1946b).

Injection.—M/100 KCN into fertilized eggs does not delay cleavage, but immersion in this does delay it (Brinley, 1930).

Methyl Cyanide.—Acts differently from KCN (Blumenthal 1928, 1930).

Measurements.—Of cyanides (Robbie, 1946a).

Respiration.—Reduced, with cleavage arrest. See Respiration A IV 4. First shown by Warburg (1910) for *P. lividus* with N/10,000 NaCN which arrested cleavage and reduced respiration to 20 % of normal. First shown for *Arbacia punctulata* by Loeb and Wasteneys (1911a) with a few drops of 1/10 % NaCN to 50 cc. sea water which reduced respiration to $\frac{1}{4}$ or $\frac{1}{3}$ normal. Later data of Korr (1937): 2.5×10^{-3} M KCN

arrested cleavage and reduced oxygen consumption to about 50 % at 20 °C.; Clowes and Krahl (1940): 1.6×10^{-4} M KCN arrested cleavage and reduced respiration to 34 % of control. Of Robbie (1946b): 1.5×10^{-5} M HCN arrested cleavage and reduced respiration to 35 % of control. See Krahl (1950, p. 192 and his Table V).

Effect of Cyanides together with other substances affecting respiration: Methylene blue and KCN (Barron, 1929; Runnström, 1935a; M. M. Brooks, 1943). Toluidin blue and KCN (Barron and Hamburger, 1932). Pyocyanine and HCN or KCN Barron and Hamburger, 1932; (Runnström, 1935a; Korr, 1937, with temperature effects). Cytochrome oxidase and NaCN or KCN (Krahl, Keltch, Neubeck, and Clowes, 1941; Ball, 1942). Phenols and KCN (Clowes and Krahl, 1934a, b, 1935, 1936b; Krahl and Clowes, 1940; Clowes, 1951).

Respiration of unfertilized eggs. Not much affected by cyanide (Runnström, 1935a; Korr, 1937; Ball, 1942; M. M. Brooks, 1943, 1946b). Reduced to 50 % of control for unfertilized eggs with 1.3×10^{-3} M KCN, 25 % of control for fertilized eggs (Clowes and Krahl, 1934b). Residual oxygen consumption reduced to 43 % of control for unfertilized eggs with 10^{-4} M HCN, 18 % of control for fertilized eggs (Robbie, 1946b). For pyocyanine and cyanide on unfertilized eggs, see Runnström, 1935a); Korr (1937).

Respiration and Cleavage of half-eggs. Unfertilized white halves little affected; in fertilized white halves, O_2 uptake decreased and cleavage inhibited; in unfertilized and fertilized red halves, O_2 consumption decreased (Shapiro, 1940).

Permeability.—Increase (McClendon, 1909b). KCN decreases, HCN increases permeability (Blumenthal, 1927, 1928). M/800 to M/1000 has no effect on increased permeability following fertilization, though cleavage is inhibited; high concentrations, M/200, prevent the increased permeability following fertilization (R. S. Lillie, 1918a, b; Lucké and McCutcheon, 1932).

Viscosity.—Increase (Heilbrunn, 1920a, b).

Parthenogenesis.—Caused by KCN (McClendon, 1909b, 1910b; M. M. Brooks, 1946a, fertilization membrane, no cleavage). KCN aids parthenogenesis started with isotonic salt solutions (R. S. Lillie, 1911b). KCN does not prevent parthenogenesis caused by salts (R. S. Lillie, 1914a; Heilbrunn, 1915a). NaCN does not prevent parthenogenesis caused by ultraviolet light (Loeb, 1914a).

Reversal of Parthenogenesis.—After parthenogenetic treatment, NaCN causes eggs to return to resting stage according to Loeb (1913a, p. 234, 1913c, 1914b, 1915a, b 1916, p. 190, etc.) with decrease in oxidations (Wasteneys, 1916). These eggs can be fertilized, with increase of oxidations. These results have been questioned by F. R. Lillie and Just (1924, p. 505) and others. See Parthenogenesis, and p. 108.

Effect on Sperm.—KCN inactivates and prolongs life of sperm (Cohn, 1918). 1×10^{-4} M HCN inhibits completely sperm respiration (Barron, Nelson, and Ardao 1948). See Respiration B II 4.

Other Species

Drzewina and Bohn, 1912. *Paracentrotus lividus*, sperm.
Lindahl, 1941. *Paracentrotus lividus.*
Loeb, 1907. *Strongylocentrotus purpuratus.*
Loeb and Wasteneys, 1913b. *S. purpuratus.*
Örström, 1932a, b. *P. lividus.*
Pease, 1941. *Dendraster excentricus.*
Robbie, 1948, 1949. *Echinarachnius parma, Tripneustes esculentus.*
Runnström, 1928b, 1930. *Arbacia lixula, P. lividus.*

CYTOCHROME AND CYTOCHROME OXIDASE

Cytochromes a, b, c.—Occur in sperm, not detectable in eggs, of Arbacia (Ball and Meyerhoff, 1940; Ball, 1942; Clowes and Krahl, 1940; Krahl, Keltch, Neubeck, and Clowes, 1941). Cytochromes a and b_1 found spectroscopically in unfertilized eggs of *Ps. miliaris* (Rothschild, 1949). No cytochrome c yet detected (Borei, 1951, in *Echinus esculentus* egg).

Cytochrome Oxidase.—(Indophenol oxidase, probably "Atmungsferment" of Warburg). Occurs in sperm and eggs (Ball and Meyerhoff, 1940, eggs doubtful; Ball, 1942; Krahl, Keltch, Neubeck, and Clowes, 1941). In unfertilized and fertilized eggs in about same amount (Krahl *et al.*, 1941). Indophenol oxidase activity as determined by Nadi reaction (Navez and E. B. Harvey, 1935):

Whole eggs unfertilized	1.0	Whole eggs fertilized	1.4
White halves unfertilized	2.4	Whole eggs stretched by centrifugal force	3.2
Red halves unfertilized	4.8		

Part of oxidase activity may be due to oil (Navez, 1938). In disintegrated eggs, more cytochrome oxidase associated with matrix than with mitochondria, yolk or pigment granules (Hutchens, Kopac, and Krahl, 1942). For temperature effects see Korr (1937). Inhibited by cyanide, carbon monoxide in the dark (reversal with light), sodium azide, sodium sulfide, 0.6 M NaCl (Krahl, Keltch, Neubeck, and Clowes, 1941; Ball, 1942).

Other Species (additional) and General References

Brachet, 1950. *Chemical Embryology* (scattered references.
Deutsch and Gustafson, 1952. *Ps. miliaris*, in development.
Krahl, 1950. Review, p. 182.
Rothschild, 1948a. *Echinus esculentus*, sperm.
Rothschild, 1949. *Ps. miliaris*, light and carbon monoxide on eggs.
Rothschild, 1951b. General.
Symposium on Respiratory Enzymes, 1942, Univ. Wisconsin.

CYTOLYSIS

Term first used for marine eggs *(Strongylocentrotus purpuratus)* by Loeb (1904), according to Heilbrunn (1928, p. 239).

Caused by—1. Ageing of eggs. (Goldforb, 1918a, b; Goldforb and Schechter, 1932; Schechter, 1936; Page, 1929a).
2. Calcium chloride (isotonic) for a long time. (Heilbrunn, 1928, p. 147).
3. Carcinogenic substances, dibenzanthrene. (Lucké, Parpart, and Ricca, 1941).
4. Electric current. (Lillie and Cattell, 1925; Heilbrunn, 1928, p. 244).
5. Fat solvents. Soaps, saponin, chloroform, alcohol, bile salts etc. (E. N. Harvey, 1910c; Page and Clowes, 1922; Page, 1929a; Page, Shonle, and Clowes, 1933; Heilbrunn, 1928, p. 242; *et al.*).
6. Heat. (Heilbrunn, 1928, p. 249; Dan, 1936). With *S. purpuratus* eggs (von Knaffl-Lenz, 1908; A. R. Moore, 1910, 1917).
7. Hypertonic sea water. (Loeb, 1913a, p. 89 for *S. purpuratus*).
8. Hypotonic sea water, distilled or tap water. 25 cc. sea water + 75 cc. distilled water for 2 to 3 minutes (Glaser, 1913); 40 volumes sea water to 60 volumes tap water for ½ hour (R. S. Lillie, 1916b); distilled water 3–½ minutes (Schechter, 1936); also Page and Clowes (1922); Heilbrunn (1928, p. 242); Just(1928a); *et al.* On fertilized eggs (R. S. Lillie, 1916b; Page, 1929a).
9. Shaking. (Tang and Gerard, 1932).
10. Sodium chloride. (E. N. Harvey, 1910c; Loeb and Wasteneys, 1910; Loeb, 1913, p. 180; Heilbrunn, 1928, p. 251; Mazia, 1933; Dan, 1936; *et al.*).

11. Ultrasonic waves. (E. N. Harvey, E. B. Harvey, and Loomis, 1928; E. N. Harvey, 1930; E. N. Harvey and Loomis, 1931).
12. Ultraviolet light. (Lillie and Baskervill, 1922; Hinrichs, 1927; Heilbrunn and Young, 1930; E. B. Harvey and Hollaender, 1938).
13. Visible light and rhodamine B. (L. B. Clark, 1940).

Prevention.—By $CaCl_2$ and $MgCl_2$ (R. S. Lillie, 1911a, 1912; Page, 1924). Decreased by pretreatment with excess Ca and increased with diminished Ca (Schechter, 1936). Decreased by KCN, chloral hydrate, chloretone, and other anaesthetics (R. S. Lillie, 1912; Loeb, 1913, p. 91 for *S. purpuratus*).

Increase of Free Ca.—In egg on cytolysis (Heilbrunn, Mazia, and Steinbach, 1934).

Acid Formation.—(Runnström, 1935d).

pH.—Of cytolysed eggs (acid of injury), 5.3 ± 0.2; normal eggs, 6.8 ± 0.2 (Pandit and Chambers, 1932).

Methylene blue, etc.—Reduced by cytolysed eggs more rapidly (Ballentine, 1938).

Respiration.—Increased (Loeb, 1913a, p. 14; Tang, 1931a; Whitaker, 1933a, p. 487, footnote; Tyler, Ricci and Horowitz, 1938, but they think it may be due to bacteria present). See Heilbrunn (1915b).

Permeability.—Increased (E. N. Harvey, 1910c; A. R. Moore, 1917 for *S. purpuratus*).

Viscosity.—Increased (Heilbrunn, 1928, p. 245). But said to be decreased in *S. purpuratus* (von Knaffl-Lenz, 1908; Loeb, 1913a, p. 188; A. R. Moore, 1917).

Membrane Formation.—Caused by all cytolytic agents (Loeb, 1913a, p. 8, etc.). Parthenogenetic agents cause incipient cytolysis (Heilbrunn, 1943, p. 661).

Fertilized Eggs.—As compared with unfertilized eggs. Sometimes cytolyse more readily, sometimes less readily according to the agent, time after fertilization (R. S. Lillie, 1916b; Page, 1929a) and the species (compare last references with Loeb, 1913a, p. 92 and Page and Clowes, 1922).

Dark (or Black) and Pale (or White) Cytolysis.—(Loeb, 1913a, p. 89–91 and Chapt. 17 for *S. purpuratus;* Goldforb, 1918b). Dark cytolysis in *Arbacia* with certain soaps (Page, Shonle, and Clowes, 1933); pale cytolysis (Heilbrunn, 1928, p. 232, 239). White and black cytolysis in *Echinus esculentus* (Rothschild, 1938).

Nature of Cytolysis.—(Loeb, 1913a, Chapt. 17; A. R. Moore, 1917; Heilbrunn, 1928, p. 238–254 and 1943, p. 661).

Autolysis.—(Lyon and Shackell, 1910a).

Other Species

There are many other references to cytolysis in *Arbacia* and other species scattered through the literature. Mention is here made only of Hobson (1932) for *Psammechinus miliaris* and Lord Rothschild (1938) concerning the changes in the egg surface in *Echinus esculentus* during cytolysis.

DENSITY — SPECIFIC GRAVITY

Unfertilized Egg.—Density 1.081–1.087 (Lyon, 1907). 1.0485 to 1.0656 (Heilbrunn, 1926a, 1928, p. 71). With jelly 1.090 (E. N. Harvey, 1931c, 1932a). Without jelly 1.084 (E. N. Harvey, as above). Density of unfertilized egg is approximately same as 3 parts isosmotic (0.85 M) cane sugar solution plus one part sea water (E. N. Harvey, 1931c).

Granules.—In unfertilized egg 1.14 (Heilbrunn, 1926a, 1928, p. 72).

Clear Protoplasm.—(Matrix) of unfertilized egg 1.04 (Heilbrunn, as above).

White Half-Egg.—Separated by centrifugal force 1.076 (E. N. Harvey, 1931c, 1932a).

Red Half-Egg.—Separated by centrifugal force > 1.100 (E. N. Harvey, as above).

Nuclear Fluid.—Density about 1.04 (Heilbrunn, 1928, p. 85). Nucleus less dense than cytoplasm, goes to centripetal pole on centrifuging.

Nucleolus.—Density about 1.14 (Heilbrunn, 1928, p. 85).

Fertilized Egg.—2-cell and 16-cell. Same density as unfertilized egg (Lyon, 1907).

Blastula.—Density about 1.060; heavier than sea water, lighter than egg (Lyon, 1906b, 1907).

Pluteus.—Density between 1.055 and 1.066 (Lyon, 1907).

Sea Water. Density in different localities

Woods Hole	1.024 at 19.5° C. (Lyon, 1907)
	1.02426 at 21.5° C. (Garrey, 1915)
	1.0238 at 20° C. (E. N. Harvey, 1931a)
Tortugas	1.0246 (McClendon, 1910b)
	1.0244–1.0253 (Leitch, 1934a)
Pacific Grove	1.0241 at 12.9° C. (Bolin, from records, 1952)
Plymouth, Eng.	1.0266 at 11° C. (Lowndes, 1944a)
Naples, Italy	1.0278 at 16° C. (E. B. Harvey, 1933a)

DENSITY OF EGGS OF OTHER SPECIES

Arbacia lixula Unfertilized egg with jelly	1.101	(E. B. Harvey, 1933a)
without jelly	1.096	
Echinometra lucunter Unfertilized egg	1.04	(Leitch, 1934a)
Echinus esculentus Similar to *Ps. miliaris*		(Lowndes, 1944)
Paracentrotus lividus Unfertilized egg with jelly	1.083	(E. B. Harvey, 1933a)
without jelly	1.079	
Psammechinus microtuberculatus With jelly	1.074	(E. B. Harvey, 1933a)
without jelly	1.072	
Psammechinus miliaris Unfertilized egg	1.0725	(Lowndes, 1944a)
Fertilized, 1 hr.	1.0736	
Blastula, 14 hr.	1.0758	
Blastula, 20 hr.	1.0793	
Gastrula, late, 44 hr.	1.0839	
Echinopluteus, early, 68 hr.	1.09626	
Sphaerechinus granularis Unfertilized egg with jelly	1.083	(E. B. Harvey, 1933a)
without jelly	1.081	
Str. pulcherrimus Without fert. mem.	1.0772	(Hiramoto, 1954)
With fert. mem.	1.056	

DYES

See Vital Dyes

ECHINOCHROME

See Amoebocytes, Chromatophores

Name.—Given by MacMunn (1883, 1885, 1889) for the coloring matter in perivisceral fluid, ovaries and shell of *Echinus esculentus* (?), *E. sphaera*, *Strongylocentrotus (Paracentrotus) lividus* and *Amphidotus (Echinocardium) cordatus*. Called "arbacin" by Vlès and Vellinger (1928) in *Arbacia aequituberculata (A. lixula)*.

Occurrence.—In test, eggs, and red amoebocytes of *Arbacia punctulata* (McClendon, 1912a; Cannan, 1927; *et al.*). In test is probably present as Ca salt or adsorbed on $CaCO_3$; not readily extracted by organic solvents in which echinochrome is soluble. Can be obtained in solution by digesting shells in acid (Ball, personal communica-

tion, Aug. 1954). In amoebocytes, eggs and plutei, is in chromatophores, though R. S. Lillie (1911b) thought some is diffused through the cell. Occurs only in *Echinoidea* among the Echinoderms (Fox and Scheer, 1941).

Amount.—In eggs, 0.58 gm. pigment per 100 cc. eggs packed by centrifuging. In amoebocytes, 3.78 gm. per 100 cc. of packed body cells; same from ♂ and ♀. In test 0.19 grams echinochrome per 100 gm. Average ♀ contains 38 gm. echinochrome, ♂ half this (Ball and Cooper, 1949).

Chemical Structure.—$C_{12}H_{10}O_7$ (Ball, 1936, Ball and Cooper, 1949). Same for *A. lixula* (Lederer and Glaser, 1938; Hartman, Schartau, Kuhn, and Wallenfels, 1939). It is a polyhydroxynaphthoquinone, which in the eggs is bound to proteins (Kuhn and Wallenfels 1939; Wallenfels and Gauhe, 1943 in *A. lixula*).

According to some investigators echinochrome contains copper (Glaser, 1923); fats (McClendon, 1909a, 1912a); fatty acids (Navez, 1939).

Soluble.—In acetone, absolute alcohol, ether (McClendon, 1912a); in chloroform, ether, benzene; insoluble in petroleum ether (Ball, 1934, 1936). Same for *A. lixula* (Lederer and Glaser, 1938); same for *S. purpuratus* (Tyler, 1939).

Adsorbed.—On charcoal (Hinrichs, 1927); on norite (Navez and DuBois, 1940).

Extraction and Crystallization.—Of echinochrome in *A. punctulata* (McClendon, 1912a; Cannan, 1927; Ball, 1934, 1936; Ball and Cooper, 1949). Forms reddish or orange needle-like crystals (McClendon, 1912a; Ball, 1934). Same for *A. lixula* (Lederer and Glaser, 1938; Glaser and Lederer, 1939; Kuhn and Wallenfels, 1939); and for *S. purpuratus* (Tyler, 1939).

Color Change.—Red or orange in acid, violet or green in alkali (McClendon, 1910a, b). Red to yellow; pK_1' value at 26 °C. calculated to be 6.38; unstable in alkaline ranges from pH 6.8 (Ball, 1936; Ball and Cooper, 1949). In sodium citrate at pH 7.4 turns dirty brown then clear green (D. L. Harris, 1943). Faded by ultraviolet (Hinrichs, 1927; E. B. H., 1950 unpub.). Change of color used as a natural indicator of pH in *A. lixula* (Vlès and Vellinger, 1938).

Absorption Spectra.—Studied by MacMunn (1883, 1885) in other species. In *A. punctulata* by McClendon (1912a); Ball (1936); Ball and Cooper (1949). Absorption spectra same for eggs, amoebocytes, and tests (McClendon, 1912a); also for spines and plutei (Ball and Cooper, 1949). Red acid form has peaks at λ 255, 335, and 475 mμ; yellow form at λ 275, 400 and 475 mμ (Ball, 1936; Ball and Cooper, 1949). For absorption spectra of *A. lixula* see Runnström (1928b); Lederer and Glaser (1938); Kuhn and Wallenfels (1939).

Oxidation-Reduction.—Echinochrome does not form a dissociable compound with oxygen, but can be reduced (with sodium hydrosulphite) and re-oxidized. $E_0 = +0.1995$ volts; and E_0' at pH 7 and 30 °C. = —0.221 volts (Cannan, 1927). For *A. lixula* see Lederer and Glaser (1938). In eggs, it is not reduced by anaerobiosis (McClendon, 1910b, 1912a; Cannan, 1927; Korr, 1939, p. 83; and Ball, same paper, p. 92). With regard to a species having very little pigment, Cannan (1927, p. 187) says "it would appear that *E. esculentus* holds its pigment in the partially reduced state, since the perivisceral fluid is almost colorless but rapidly turns red when removed from the animal. In *Arbacia*, the echinochrome is in the oxidized state and I know of no observation of the spontaneous decolorization of the cells in vivo."

Function.—Not known. Was thought to be an oxygen carrier in other species by Geddes (1880); MacMunn (1885); Griffiths (1892a); but this was denied by Cuénot (1891a). Has been questioned for *A. punctulata* by McClendon (1912a); Cannan (1927).

Stated to increase respiration, 16 times, in *Paracentrotus lividus* and *Sphaerechinus granularis* (Friedheim, 1932), but this was denied by Tyler (1939) for *S. purpuratus*.

Stated to be a sperm activating agent in *A. lixula* (Hartman, Schartau, Kuhn, and Wallenfels, 1939), but this was denied by Tyler (1939) for *Strongylocentrotus purpuratus*

and by Cornman (1941) for *A. punctulata.* See Hartmann, Schartau, and Wallenfels (1940).

Fertilization.—Inhibited by echinochrome from red amoebocytes; has no effect on cleavage if eggs exposed after fertilization; binds calcium; causes agglutination of sperm (Couillard, 1952). Allee *et al.* (1942) also found no effect on cleavage.

For release.—Of echinochrome from chromatophores, see under Chromatophores.

Other Species (additional) and General References

Baldwin, 1952, p. 184 General.
Fox, 1953. Biochromes.
Fox and Scheer, 1941. Pacific coast forms.
Goodwin, Lederer, and Musago, 1951. Spinochromes, nomenclature.
Goodwin and Srisukh, 1950. *E. esculentus, P. lividus,* 5 pigments.
Goodwin and Srisukh, 1951. *Echinocardium cordatum,* echinochrome almost absent.
Krahl, 1950. Review.
Lederer, 1940. Review.

ECTOPLASMIC LAYER

See Hyaline Layer

ELECTRICAL PROPERTIES AND EFFECTS

I. Electrical properties of eggs.

A. Membrane capacity and internal resistance (From Cole's 1941 Table in *Tabulae Biologicae,* vol. 19, Part II, p. 25).

Membrane Capacity $\mu f/cm^2$		Internal Resistance ohm/cm^3	
	Suspensions		
Unfertilized	ca. 1.0	90	(Cole, 1928)
Fertilized	ca. 1.0	90	
Unfertilized	0.73	186	(Cole and Cole, 1936;
Fertilized	3.1	186	Cole, 1937, 1938)
Unfertilized	0.86	147	(Cole and Spencer, 1938)
Fertilized	3.3	180	
	Single eggs		
Unfertilized	1.1	180	(Cole and Curtis, 1938)
Fertilized	2.8	210	
	White halves		
Unfertilized	0.63	125	(Cole and Curtis, 1938)
Fertilized	2.25	125	
	Red halves		
Unfertilized	0.62–2.3	360–775	(Cole and Curtis, 1938)

B. Surface charge; Zeta potential (Dan, 1933; see also 1931).
Unfertilized eggs with jelly —34.1 ± 0.47 millivolts
Unfertilized eggs without jelly —30.3 ± 0.47 millivolts
Fertilized eggs without jelly —28.7 ± 0.42 millivolts
Unfertilized eggs without jelly, in dead sperm suspension —26.7 ± 0.56 millivolts
Cleaving eggs without jelly —27.2 millivolts

Centrifuged eggs and fractions without jelly (Dan, 1936, IV)
Whole eggs —30.8 ± 0.54 millivolts
Light halves —20.9 ± 0.69 millivolts
Heavy halves —27.6 ± 0.35 millivolts
Effect of dilution and medium on Zeta potential (Dan, 1936, III).

C. Miscellaneous.
Membrane resistance, unfertilized and fertilized eggs (calculated) > 25 ohm/cm² (Cole and Curtis, 1938); "1,000 a reasonable value" (Cole, 1940); > 100 ohm/cm² (Cole 1941).
Electrical properties of cell membrane (McClendon, 1910b; R. S. Lillie, 1911b, 1916b).
Granules have +charge, surface layer — charge (Heilbrunn, 1923, 1926b, 1928, p. 183).
Electrical changes on stimulation and cleavage (R. S. Lillie, 1903, 1909, 1916b).
Increase (about one fourth) of electric conductivity of eggs on fertilization or parthenogenesis (McClendon, 1910a, c, 1912b).

II. Electrical properties of sperm. Negatively charged. Cataphoretic velocity 1.75 μ/sec. per volt/cm. Surface p.d. 22.0 millivolts (Mudd, Mudd, and Keltch, 1929).

III. Electrical effects on
A. Unfertilized eggs.
Move to anode (Moser 1939b). See above under B. Surface charge (Dan).
Disintegrate, at anode first; unfertilized before fertilized (McClendon, 1910a, b, c; Moser, 1939b).
Parthenogenesis caused by induction shocks (McClendon, 1909b, 1910b).
Parthenogenesis and development not caused by direct current (R. S. Lillie and Cattell, 1925).
Cortical response and membrane elevation caused by direct current, at side toward anode first (Moser, 1937, 1939b).
Permeability increased (McClendon, 1910a, b, c, 1914b).
Viscosity; transitory decrease, then increase (Angerer, 1939).

B. Fertilized eggs.
No relation between electrical conductivity of medium and cleavage until concentration reduced to 20 %, then cleavage slower (R. S. Lillie and Cattell, 1923).
Eccentricity of egg in fertilization membrane, nearer the membrane at anode (McClendon, 1910b, 1914a; Dan, 1933).

C. Nucleus.
Goes to anode (McClendon, 1910b).

D. Chromatophores.
Lose pigment (McClendon, 1910a, b.)

Other Species

Cole, 1935. *Tripneustes esculentus.*
Cole, 1941. Tabulation in *Tabulae Biologicae.*
Dan, 1934, *Echinarachnius*, surface charge. Biol. Bull. 66 : 247–256.
Gray, 1916. *Arbacia lixula, Psammechinus miliaris* etc.
Iida, 1943a, b, c. *Pseudocentrotus depressus, Strongylocentrotus pulcherrimus*, capacitance.
McClendon, 1910b. *Lytechinus variegatus, Tripneustes esculentus.*
Rothschild, 1938. *Echinus esculentus*, biophysics of cell surface.
Rothschild and Swann, 1949. *Psammechinus miliaris*, action potential.
Vlès, 1931. *Paracentrotus lividus*, lysis.

ENZYMES

The classification is based on Sumner and Somer's book on Enzymes (1953)

1. Proteolytic

Proteolytic enzyme in granules of unfertilized egg (A. A. Woodward, Jr., 1949).

Peptidase in matrix not in granules (Holter, 1936, 1949).

Trypsin and chymotrypsin. In *Psammechinus miliaris* and *Echinocardium cordatum*, dissolve the vitelline membrane of the unfertilized egg, so that when fertilized, no fertilization membrane is formed, but the egg develops (Monné and Broman, 1944; Runnström, 1948a). Trypsin was used for *A. punctulata* by Runnström in 1950 and by Dan and Mazia in 1951 as the best method of obtaining fertilized eggs without fertilization membranes. Dan used 4 mg. crystalline trypsin in 100 cc. sea water for 10 minutes. Effect on membrane was first observed by Kunitz in 1932 (See A. R. Moore, 1949a, footnote p. 243; 1949b, footnote p. 207).

Papain dissolves hyaline layer of developing eggs; no effect of ficin or trypsin (Northrop, 1947; E. B. H.). See under Hyaline Layer.

"Hatching enzyme", a protease (Sugawara, 1943a) dissolves fertilization membrane of normal blastulae, in *S. pulcherrimus* (Ishida, 1936; Sugawara, 1943a, b). In *A. punctulata* (Kopac, 1941). For earlier work on other forms, especially Ascidians, see Berrill (1929).

Autolysis. See Lyon and Shackell (1910a).

In sperm and egg extracts. Negative results (Gies, 1901). See Loeb (1901, 1913a, chapter 19; older references given here). Also see Rothschild, 1952.

2. Nucleases

Polynucleotidase; localized in nucleus of egg, also in sperm; present in white half-egg, not in red half (Mazia, 1941). Withdrawn by Mazia in 1950.

Ribonuclease, RNase. Drop in activity after fertilization which is maintained 20 hours (Bernstein, 1949; Krahl, 1950).

Desoxyribonuclease, DNase. In eggs, half-eggs and sperm; not restricted to nucleus but distributed in cytoplasm in unfertilized egg; about equal in the two half-eggs; no change in development to pluteus, but sedimentable fraction increases; activity in egg is 10 times that of mammalian tissue. Activity in sperm is 10 times that of egg per unit volume, 10^{-4} times per cell (Mazia and Neff, 1947; Mazia, Blumenthal, and Benson, 1948; Mazia, 1949a, b.).

Inhibition of DNase by usnic acid (Marshak, 1949a; Marshak and Fager, 1950).

3. Esterases

Cholinesterase, in *Paracentrotus lividus* (Augustinsson and Gustafson, 1949).

Lipase, lipolysin (A. E. Woodward, 1918, 1921; Glaser, 1921b, 1922a, 1923). Just (1929a, 1930a) thinks this does not exist. Runnström (1949a) could not find it in *A. lixula*.

Phosphatase. In unfertilized, fertilized and developing eggs (Mazia, 1941; Mazia, Blumenthal, and Benson, 1948; Krugelis, 1947b. In oocytes (Krugelis, 1947a, b). Not localized in nucleus as indicated by half-eggs (Mazia, 1941; Mazia, Blumenthal, and Benson 1948).

Adenosintriphosphatase, ATPase. In *S. purpuratus* (Conners and Scheer, 1947); in *P. lividus* (Runnström, 1949a, b).

4. Carbohydrases

Hyaluronidase, in *A. lixula* sperm (Monroy and Ruffo, 1947).

Hyaluronidase? in *A. punctulata* eggs (Chambers, 1949).

These have been questioned by Krauss (1950). See also A. Monroy, L. Tosi, G. Giardina and R. Maggio, 1954, Biol. Bull, 106 : 169–177; and Rothschild, 1952.

5. Oxidizing enzymes and coenzymes

Cytochrome oxidase. See Cytochrome and Cytochrome Oxidase.

Catalase. Greater amount following fertilization; more in sperm than in eggs, observation of A. P. Mathews (Lyon, 1909). Not more after fertilization, but respiration 4–6 times greater (Amberg and Winternitz, 1911). Present in matrix not in granules in *Ps. miliaris* (Holter, 1949). Present in sperm (Evans, 1947; Barron, Gasvoda and Flood, 1949).

Dehydrogenases (Korr, 1937; Ballentine, 1940b, in half-eggs, 1940c). Succinic dehydrogenase present in sperm not in eggs (Ball and Meyerhoff, 1940). Does not appear in eggs even after 24 hours (Goldinger and Barron, 1946).

Flavin-adenine-dinucleotide, FAD, coenzyme (Krahl, Keltch and Clowes, 1940b; Krahl, 1950, p. 183).

Diphosphopyridine nucleotide, DPN, coenzyme (Jahndorf and Krahl, 1942; Krahl, 1950, p. 183).

Diphosphothiamine (cocarboxylase), coenzyme (Krahl, Jahndorf, and Clowes, 1942; Goldinger and Barron, 1946; Krahl, 1950, p. 184).

Enzymes for oxidative phosphorylation (Clowes, 1951; Clowes, Keltch, Strittmatter, and Walters, 1950; Clowes, Keltch, and Walters, 1951a, b; Keltch, Smythe, and Clowes 1951; Keltch, Strittmatter, Walters, and Clowes; 1950, Strittmatter, Keltch, Walters, and Clowes, 1950); Krahl's Review, 1950, pp. 184, 198).

6. Transferases

Hexokinase, in homogenates of eggs and embryos (Krahl, Keltch, Walters, and Clowes, 1953).

Other Species (additional) and General References

Barron, 1952a. General.

Bohus-Jensen, 1950. *P. lividus*, *Sphaerechinus granularis*, ficin, trypsin.

Bohus-Jensen, 1953. *Lytechinus variegatus*, *Mellita sexiesperforata*, *Echinometra lucunter*, trypsin on cross fertilization.

Cleland and Rothschild, 1952a, b. *E. esculentus*, glycolytic.

Deutsch and Gustafson, 1952. *Ps. miliaris*, decrease of catalase during development.

Doyle, 1938. *Ps. miliaris*, peptidase and catalase.

Gustafson and Hasselberg, 1950, 1951. *Ps. miliaris*, *P. lividus* etc., enzymes on developing eggs.

Holter, 1949. General.

Holter and Lindahl, 1941. *P. lividus*, peptidase.

Holter and Linderström-Lang, 1940. General.

Krahl, 1950. Review.

Linderström-Lang, 1939. General.

Lundblad, 1950. *A. lixula*, *P. lividus*, proteolytic enzyme.

Mazia, 1952. General on nucleus.

A. R. Moore, 1951a. *Dendraster excentricus*, trypsin.

Rothschild, 1950b, c. *Echinus esculentus*, catalase in sperm and eggs.

Rothschild, 1951a, 1952. Review of sperm.

Runnström, 1949a, b, c; 1950–1951. General.

FERTILIZATION MEMBRANE

Definition.—The fertilization membrane is the membrane normally formed after fertilization or activation by a parthenogenetic agent. Its precursor is the vitelline membrane.

Historical.—The fertilization membrane was first described by Derbès in 1847 in *Echinus esculentus*, and later by Fol (1877) in *Asterias glacialis*.

Origin.—Many of the older investigators believed it arose from the preexisting

vitelline membrane (R. S. Lillie, 1911a, 1916b; Kite, 1912; Heilbrunn, 1913, 1915a, 1928, p. 259; Glaser, 1913, 1915, 1924; F. R. Lillie, 1914; Chambers, 1921a, 1924; *et al.*). But some thought it arose *de novo*, by secretion of a membrane substance (E. N. Harvey, 1909, 1910b, 1914) or by precipitation of oppositely charged colloids (McClendon, 1909b, 1911, 1914a; see Garrey, 1919).

More recently it has been definitely shown that the fertilization membrane comes from the vitelline membrane, since it does *not* form when the vitelline membrane has been removed by KCl, urea, or trypsin. (See below under "Removable"); development takes place without a fertilization membrane. (See under Vitelline Membrane). It has also been shown that the cortical granules which disappear on fertilization help in the formation of the fertilization membrane (Moser, 1939a; etc.). See under Cortical Layer. It is now generally accepted that the fertilization membrane is the pre-existing vitelline membrane whose properties have changed, together with cortical granule material, in *Arbacia* and in other species (See Runnström, 1952a, Chapt. VII "The Origin of the Fertilization Membrane").

Formation.—The fertilization membrane starts to form at the sperm entry in about 20 seconds after it touches the surface (E. B. H.; see also Just, 1928a, 1939b, p. 105; Moser, 1939a; *et al.*). See Part II, *Fertilization.* This was first observed in *Asterias glacialis* by Fol (1877). It is fully elevated about two minutes after fertilization at 23 °C. (E. B. H.).

According to Heilbrunn (1913, 1915a, 1924a), membrane elevation is due to a lowering of the surface tension, since all parthenogenetic agents do lower the surface tension. According to Loeb (1913a, p. 212; 1912a, p. 136, 150), it is due to swelling of a colloid and liquefaction of the surface. According to E. N. Harvey (1910b) and R. S. Lillie (1911a) it results from increase in permeability. Jelly is not necessary for its formation (E. N. Harvey, 1914; F. R. Lillie, 1914; F. R. Lillie and Just, 1924, footnote p. 453; *et al.*). Oxygen necessary for formation of fertilization membrane in fertilized eggs, because it is necessary for motility of sperm (E. B. Harvey, 1930; Barron, 1932). Not necessary for membrane formation in parthenogenetic eggs (Loeb, 1913a, p. 215; Kitching and Moser, 1940).

Structure.—No regular structure or pattern is shown by the electron microscope (E. B. Harvey and Anderson, 1943). This is also true of *Ps. miliaris*, according to Mitchison (1953). Hillier, Lansing, and Rosenthal (1952) say that the membrane, in *Arbacia*, is composed of a single layer of loosely packed particles.

Thickness.—Though readily visible, its thickness is not measureable with a light microscope. Measured with an electron microscope, it is 250 Å when first elevated and dried (E. B. Harvey and Anderson, 1943). According to Hillier, Lansing, and Rosenthal (1952), it is less than 300 Å thick. A recent measurement with the electron microscope, of the fertilization membrane of a different species, *Ps. miliaris*, gives its thickness as 100 Å (Mitchison, 1953). The dry thickness as measured with an interference microscope is given as about 160 Å (Mitchison and Swann, 1953). The early membrane is easily ruptured (R. S. Lillie, 1916b; E. B. Harvey, 1933b, *et al.*). It becomes thicker and tougher after about five minutes (Heilbrunn, 1915a; Chambers, 1921a; E. B. Harvey, 1933b; E. B. Harvey and Anderson, 1943; *et al.*). In *Ps. miliaris* its thickness is given as about 1μ (Runnström, Monné, and Wicklund, 1946).

Specific Gravity.—Lighter than the eggs. If placed in distilled water immediately after they are formed, the membranes can be freed of the egg material and form a layer above the eggs (E. B. Harvey and Anderson, 1943). Whitaker (1933a) found that sometimes they were thrown off in the centrifuge and then formed a layer above the eggs.

Elasticity.—Can stretch with centrifugal force from 82 μ diameter (normal) to 140 μ when first formed; they resist stretching after five minutes (E. B. Harvey, 1933b; E. B. Harvey and Anderson, 1943). Expansibility (Chambers, 1942).

Chemical Properties.—A "haptogen" membrane consisting mainly of protein (R. S. Lillie, 1909). Probably an "albuminoid"; insoluble in concentrated H_2SO_4, HCl, KOH, NaOH, etc. (E. N. Harvey, 1910b). Not a lipoid because not soluble in benzol, ether, alcohol, saponin, etc. (Loeb, 1913a, p. 214). A protein gel with little or no lipid (Heilbrunn, 1915a). Insoluble in KCl, urea or trypsin after hardening, soluble while elevating (Kopac, 1940a, 1941a; Chambers, 1942, 1944; A. R. Moore, 1949a, p. 243 footnote, *re* Kunitz, 1932 unpub.). For *Psammechinus miliaris* see Monroy and Runnström (1948). Contains ribonucleic acid (Lansing and Rosenthal, 1949).

Oil Coalescence.—Prevented by fertilization membrane (Kopac, 1940a, 1941a; Chambers, 1944).

Permeability.—Freely permeable to salts of sea water, relatively impermeable to sugar and proteins (E. N. Harvey, 1910b). Freely permeable to salts, impermeable to colloids like egg albumen and difficultly permeable to sugar (R. S. Lillie, 1911a). Permeable to water, salts and sugar, impermeable to colloids (Loeb 1913a, p. 208; 1916, p. 108). Permeable to electrolytes when fully formed (Heilbrunn 1915a).

Distance from Egg Surface.—Normally 3 to 5 μ (E. B. Harvey per E. N. Harvey, 1932a; E. B. Harvey and Anderson, 1943; *et al.*). May be 6.5 μ (E. B. H.). It may be closely adherent under various conditions so as to be difficult to detect (by cold, 32 °C., E. N. Harvey, 1910b; Just, 1928a; *et al.*), or it may be widely separated (by urea, Moser, 1940). Collapses in 1 or 2 % egg albumen (Loeb, 1913a, p. 208; Heilbrunn, 1915a, 1924a; R. S. Lillie, 1918b); in 2 % Witte's peptone (Garrey, 1919); with blood albumen (Chambers, 1942).

Longevity.—Membranes obtained from hatching blastulae dissolve almost at once (due to hatching enzyme present). Membranes obtained in distilled water may remain intact for 12 hours (E. B. Harvey and Anderson, 1943).

Function.—Not to prevent other sperm from entering as was originally suggested by Fol (1877), and maintained by many others (e.g., Kite, 1912). It is not necessary for development (McClendon, 1912b; Glaser, 1913; E. N. Harvey, 1914; Chambers, 1930; Loeb, 1915c, though earlier, 1913a, p. 233, he thought it was necessary). It is probably protective. See Plate XVI, Photograph 7.

A Second Fertilization Membrane.— Many investigators have found that a fertilized egg cannot be fertilized again, even if the fertilization membrane has been removed (Loeb, 1916, p. 85; F. R. Lillie, 1919, p. 25, 161). Loeb (1913a, p. 234) however, thought that fertilization could be superimposed on artificial parthenogenesis, and a second membrane would form after the one due to parthenogenesis had been shaken off (Loeb, 1913a, p. 234, 1914b, 1915a, b). This was shown not to be the case, but to be due to insufficient treatment with the parthenogenetic agent, by C. R. Moore (1916, 1917); F. R. Lillie (1914, 1919, p. 167, 1921a); Just (1922a); F. R. Lillie and Just (1924, p. 502).

But more recently, Sugiyama (1947, 1951) in the case of fertilized eggs *(Strongylocentrotus pulcherrimus)*, and Ishida and Nakano (1947, 1950) in the case of parthenogenetic eggs, state that refertilization can take place if the eggs are washed in Ca-Mg-free sea water after (usually) shaking off the first membrane. See Part II, *Fertilization*, Chapter 13, sections o and p. (p. 107, 108).

Removable by:—(1) Shaking (McClendon, 1910b; E. N. Harvey, 1911; F. R. Lillie, 1914; Plough, 1927; Kopac, 1940a; *et al.*). Shake the eggs immediately after the fertilization membranes have formed, in a test tube about one quarter full of eggs and sea water, violently, with thumb over open end, for about 30 seconds (E. B. H.). (2) Straining through bolting cloth (Just, 1939a, b, p. 199 footnote). (3) Sucking through a fine pipette (Plough, 1927; E. B. Harvey, 1932). (4) Micromanipulation (Chambers, 1942). (5) Distilled water, one minute after formation of membranes; empty membranes are recoverable (E. B. Harvey and Anderson, 1943). (6) Sea water from around hatching blastulae which contains "hatching

enzyme" (Kopac, 1941a); see Part II; Chapter 14a, BLASTULA. (7) Isosmotic KCl while membrane is elevating, 1.5 minutes after insemination (Kopac, 1940a; Chambers, 1942, 1944). (8) 1.0 M urea while membrane is elevating (Chambers, 1940, 1942; Kopac, 1940a, 1943; Moser, 1940; A. R. Moore, 1930a for *Strongylocentrotus purpuratus*). (9) 4 mg. crystalline trypsin in 100 cc. sea water for 10 minutes, or 0.1 % noncrystalline trypsin of Merck, as membrane is elevating (Dan, 1951 unpub.; see Runnström and co-workers for other species, especially Runnström, 1948a). See under Vitelline Membrane.

A method for removing fertilization membranes from large quantities of eggs by bolting silk has been described by Lindahl and Lundin (1948) for *Paracentrotus lividus*.

Effect of Ageing or Repeated washings.—Membrane retarded or prevented (E. N. Harvey, 1914, after 52 hours; Goldforb, 1918a, b, after 42 hours; F. R. Lillie, 1914, after 11 to 33 washings).

Effect of Calcium-free Sea Water.—As medium. No membrane is formed and there is no cleavage though sperm are active (Loeb, 1915a; E. B. H. unpub.). Also for *Ps. microtuberculatus* (Monroy, 1949).

Calcium.—In medium not necessary for hardening of membrane (Chambers, 1942).

Effect of Heparin.—No membrane formed (C. V. Harding, 1951).

Effect of Iodosobenzoic Acid.—And cytoplasmic fraction. Membrane thicker and change of birefringence; hatching inhibited, membranes do not dissolve (Monroy and Runnström, 1952; Runnström and Kriszat, 1952a, c). Effect of other SH-reagents (Runnström and Kriszat, 1952a).

Effect of Electric Current.—On fertilized egg. Fertilization membrane nearer the egg at anode (McClendon, 1910b, 1914a; Dan, 1933).

Effect of Ultraviolet "Blitz".—On unfertilized egg. Fertilization membrane forms on one side only (E. N. Harvey, 1942). See Spikes (1944) for *Lytechinus pictus*.

In centrifuged eggs and fractions.—Membrane thinner at centripetal pole of whole egg and white halves; very thin on clear quarters and breaks at oil cap if fertilized immediately after centrifuging. Thicker and closely investing on red half, mitochondrial quarter and pigment quarter (E. B. Harvey, 1933b, 1940c, 1946).

Other Species and General References

Carter, 1924. *Sphaerechinus granularis*.

Chase, 1935. *Strongylocentrotus purpuratus, Dendraster excentricus*.

Endo, 1952. Japanese species.

Gray, 1922. *Ps. miliaris*.

Hobson, 1932a. *Ps. miliaris*.

Hyman, 1923. *S. purpuratus, S. franciscanus*.

Just, 1919. *Echinarachnius parma*.

Just, 1939b. *The Biology of the Cell Surface*. General.

F. R. Lillie, 1919. *Problems of Fertilization*. General.

F. R. Lillie and Just, 1924. Fertilization in Cowdry's *General Cytology*.

Loeb, 1912a. *The Mechanistic Conception of Life*.

Loeb, 1913a. *Artificial Parthenogenesis and Fertilization*.

Loeb, 1916. *The Organism as a Whole*.

A. R. Moore, 1930a, b; 1949a, b; 1951a. *S. purpuratus, Dendraster excentricus*.

Monroy, 1949. *Psammechinus microtuberculatus*. Other references may be found in review by Runnström 1949a.

Motomura, 1934a, 1941b, 1950c. *S. pulcherrimus*, etc.

Runnström, 1949a, b, c; 1952a, b. General. Other references to Runnström, Monroy, and co-workers may be found especially in 1949a.

FERTILIZIN AND AGGLUTININ

This subject with its vast literature will not be covered in this Monograph. Excellent reviews have recently been published to which the reader is referred.

Bielig, H. J. and F. Medem, 1949. *Experientia* 5 : 11–30.
Rothschild, Lord, 1951a. *Biol. Rev.* 26 : 1–27.
Runnström, J. 1949a. *Adv. in Enzymol.* 9 : 241–327.
Tyler, A. 1948. *Physiol. Rev.* 28 : 180–219; 1949c. *Am. Nat.* 83 : 195–219.

Reviews of the earlier work which the reader may consult are:

Just, E. E. 1930. *Protoplasma* 10 : 300–342.
Lillie, F. R. 1919. *Problems of Fertilization.*
Lillie, F. R. and E. E. Just, 1924. Fertilization, in Cowdry's *General Cytology*, section VIII.

HEAT PRODUCTION

Eggs.—Unfertilized, 0.08 calories per hour per million eggs; fertilized, (2–8 cell), 0.52 calories. At instant of fertilization, rate of heat production is 10–12 times that of unfertilized eggs, then decreases for 20 minutes, then constant until first cleavage, then drops and remains constant to 8-cell stage (Rogers and Cole, 1925).

Sperm.—"The heat production of Arbacia sperm is similar to that of an exothermic chemical reaction of the first order" (Rogers and Cole, 1925, p. 352).

Other Species

Meyerhoff, 1911. *Paracentrotus lividus*, eggs and sperm.
Shapiro, 1948d. Compilation in *Tabulae Biologicae.*
Shearer, 1922b. *Psammechinus miliaris*, eggs.
Trurnit, 1939. *Ps. miliaris*, change during cleavage.

HYALINE LAYER

Hyaloplasmic Layer, Ectoplasmic Layer

Definition.—Hyaline layer is an investing layer of the egg formed after fertilization or parthenogenetic treatment; binds blastomeres together.

Historical.—Though previously observed by O. Hertwig (1876), Fol (1877, 1879) and Selenka (1878); it was first described by Hammar in 1896 as an "ectoplasmatische Schicht" in *Echinus miliaris;* "a clear, colorless homogeneous layer." This was confirmed by E. A. Andrews in 1897. It was described for *Arbacia* by G. F. Andrews earlier in the same year. Its importance as a "Verbindungsmembran" was shown by Herbst in 1900 in *Echinus microtuberculatus.*

Thickness.—Very thin, less than 0.5 μ when first formed, becomes gradually thicker until after 20 minutes it is two to three μ thick (E. B. Harvey, 1934). Its thickness varies in different species. It is thicker in *Arbacia lixula* than in *A. punctulata*, and is here measureable immediately after fertilization; it can be shown by measuring that it is added to the surface (E. B. Harvey, 1933a, 1934). It is very thin in the starfish egg, there being no appreciable hyaline layer (Chambers 1921a, 1930, 1940, etc.). It is intermediate in strength between *A. punctulata* and *Asterias* in the sand dollar egg *(Echinarachnius parma)* (Chambers, 1940). It is very thin in *Echinarachnius parma* (E. B. H. unpub.) and in *Dendraster excentricus* (Moore, 1928a).

It is thin on the centripetal pole of the centrifuged egg, on the white halves and clear quarters; thick over the centrifugal pole and in the red halves, yolk and pigment quarters (E. B. Harvey, 1932, 1940c, 1946a). It is thickened and wrinkled

in the cleavage furrow (McClendon, 1910b; Painter, 1918; Just, 1928b; E. B. Harvey, 1934; Chambers, 1938c). It is increased in thickness by hypertonic sea water (E. B. Harvey, 1940a, Photograph 1, p. 205; Gray, 1924, 1931, p. 198, in *E. esculentus*).

Structure.—Gelatinous film (Loeb, 1913a, p. 19); tough, sticky, fibrous, elastic; can be torn with micro-needles (Chambers, 1921a, 1930, 1940). Is probably a calcium proteinate (A. R. Moore, 1928a, 1949b in *S. purpuratus*); see Chapter 6 of Gray's *Experimental Cytology* (1931).

Stains.—With isamine blue, toluidin blue (Kite, 1912). It does not stain with methylene blue, brilliant cresyl blue or neutral red (E. B. Harvey, 1934).

Function.—Binds blastomeres together (Herbst, 1900 in *Ps. microtuberculatus*). For *Arbacia punctulata* see McClendon, 1910b; Chambers, 1921a, 1930, 1938c, 1940; E. B. Harvey, 1946a; *et al.* Important in cell division (Just, 1928a; Gray, 1924, 1931, p. 196 in *E. esculentus*). See Plate XVI, Photograph 9.

Ca-free sea water.—Dissolves. See Calcium for preparation. For *Arbacia* see (E. B. Harvey, 1934; Chambers, 1940; *et al.*).

Isosmotic KCl.—Dispersed by (Chambers, 1940, 1944; Kopac, 1940a, 1941a). By lithium (Chambers, 1940).

NaCl + KCl.—0.52 M in proportions 19 : 1 at pH 7; is non-toxic (Chambers, 1938c, 1940; Kopac, 1940a).

Papain, Trypsin and Ficin.—Dissolved by enzyme papain, not by trypsin or ficin (Northrop, 1947; E. B. H.). Bohus-Jensen (1950) also found it not dissolved by trypsin or ficin (in *Ps. miliaris*, etc.). There is disagreement about trypsin; Runnström, Monné, and Broman (1944) found that trypsin did dissolve the hyaline layer in *Ps. miliaris;* and A. R. Moore (1951a) in *Dendraster excentricus*, but not (1949a, b) in *S. purpuratus*. There may be differences in the trypsin used or in the reaction of different species, or differences due to the time when the trypsin is used.

Aceto-carmine.—Dispersed by aceto-carmine, forming spheres, which unite and form striations in perivitelline space (E. B. Harvey, 1950, unpub.).

Urea.—Prevents formation (A. R. Moore, 1930a, 1949a, in *S. purpuratus*).

Twins.—When dissolved, in 2-cell stage twins are formed (E. B. Harvey, 1940a; 1935b in *Ps. microtuberculatus;* Loeb, 1909b in *S. purpuratus*). Plate XVI, Photo. 8.

Hatching Enzyme.—Not dissolved by (Kopac, 1941a).

X-ray Effect.—(Kopac, 1941b).

Replaced.—After removal (E. B. Harvey, 1934, 1935b; Kopac, 1940a; Gray, 1931, p. 212, in *E. esculentus*).

Centrifugal force.—Hyaline layer is thrown off as a crescent in *Arbacia*, as a ring in *Ps. microtuberculatus*, lying in the perivitelline space at the centrifugal pole. (Plate XVI, Photograph 6). This can be dissolved in calcium-free sea water, and re-precipitated in sea water (E. B. Harvey, 1934).

Coalescence.—With oil drops, none (Kopac, 1940a, 1941a; Chambers, 1944).

Permeability.—Freely permeable to electrolytes (Chambers, 1940; Gray, 1931, p. 198 in *Echinus esculentus*).

Other Species (additional) and General References

Dan and Ono, 1952. *Mespilia globulus.*
Dan, Yanagita, and Sugiyama, 1937. *Mespilia globulus.*
Goldschmidt and Popoff, 1908. *P. lividus, Ps. microtuberculatus.*
Gray, 1924. *Echinus esculentus.*
Gray, 1931. *Experimental Cytology*, general; chapt. 6 and 9.
Just, 1939b. *The Biology of the Cell Surface.* General.
Moore, A. R., 1949a, b; 1951b. Review.
Morgan, 1927. *Experimental Embryology*, p. 138. General.

HYBRIDS

Hybrids of Arbacia Punctulata.—

Arbacia punctulata ♂ × *Asterias forbesii* ♀. Few gastrulae, not maternal (Morgan, 1893). Mathews, 1901c questions this hybridization. Tyler and Metz (1954) found that *Arbacia* eggs would not cross fertilize with *Asterias* sperm with or without trypsin treatment.

Arbacia punctulata ♀ × *Echinarachnius parma* ♂. Plutei, maternal (E. B. Harvey, 1942). Difficult cross (Just, 1919, Matsui, 1924).

Arbacia punctulata ♂ × *Echinarachnius parma* ♀. Blastulae, maternal (E. B. Harvey, 1942). Plutei, not described (Just, 1919). Plutei, maternal; cytology (Matsui, 1924).

Arbacia punctulata ♀ × *Lytechinus (Toxopneustes) variegatus* ♂. Few plutei, intermediate; cytology (Tennent, 1912b, c). Abnormal plutei (E. B. Harvey, 1942 unpub.).

Arbacia punctulata ♂ × *Lytechinus (Toxopneustes) variegatus* ♀. Few plutei, intermediate; cytology (Tennent, 1912b, c).

Arbacia punctulata ♀ × *Mellita pentapora* ♂. Plutei, more maternal (Tennent, 1910b).

Arbacia punctulata ♀ × *Moira atropos* ♂. Plutei, more maternal; cytology (Tennent, 1908, 1910b).

Arbacia punctulata ♂ × *Moira atropos* ♀. No data (Tennent, 1910b).

Arbacia punctulata ♀ × *Strongylocentrotus dröbachiensis* ♂. Plutei, maternal (E. B. Harvey, 1942).

Hybrids of Other Species.—

Listed to 1910 (Tennent, 1910b, also in Morgan's *Experimental Embryology*, p. 613). See *Tabulae Biologicae* 1930, VI, p. 514 and "Other species", below.

Hybrid (Heterospermic) Merogones.—These are important in affording a means of distinguishing nuclear and cytoplasmic inheritance. There are very few references to hybrid merogones of *Arbacia* in comparison with those of other species. Chambers and Ohshima (1922) were unsuccessful in fertilizing the non-nucleate fraction of *Arbacia* eggs with *Echinarachnius* sperm, though with their own sperm (homospermic merogones) they produced dwarf plutei with small nuclei. Fry (1927) obtained non-nucleate fractions of *Echinarachnius* eggs fertilized with *Arbacia* sperm, but they reached only the blastula stage. Attempts have been made to raise plutei by fertilizing the non-nucleate halves of *Arbacia*, obtained by centrifuging, with the sperm of *Echinarachnius*, but these have been abnormal and underdeveloped (E. B. H., 1941 unpub.). However, the non-nucleate halves of *Sphaerechinus granularis* obtained by centrifuging, have been fertilized with *Psammechinus microtuberculatus* sperm in some preliminary experiments done in collaboration with von Ubisch in 1934 and these gave hybrid (intermediate) skeletons similar to the whole hybrids, thus showing both nuclear and cytoplasmic influence. Further study of the skeleton of these heterospermic merogones seemed desirable before publication. Von Ubisch has recently (1954) published his results on these centrifuged merogones, also finding the skeleton intermediate; he therefore infers that they are complete hybrids and not merogones. In another series of experiments done in 1954 in which he obtained non-nucleate merogones by cutting the *Sphaerechinus* egg, the skeleton was paternal.

The development of *homospermic merogones* of *Arbacia* obtained by cutting has been studied by Tennent, Taylor, and Whitaker (1929) and by centrifuging by the author (1932, 1940c, 1946c, and this Monograph, Part III). Some perfect but small plutei have been raised from the fertilized red half-egg (See Plate X, Photograph 16).

Hybrids Occurring in Nature.—

Echinus esculentus × *E. acutus* (Mortensen, Monograph, III, 3 : 36, 50, 136).

Echinus esculentus × *E. elegans* (Mortensen, Monograph, III 3 : 64).

Echinus esculentus × *Psammechinus miliaris* (Ibid., III 3 : 36).

Strongylocentrotus dröbachiensis × *S. pallidus* (Vasseur, 1952b).
Strongylocentrotus purpuratus × *S. dröbachiensis* (Swan, 1953 Evolution 7 : 269).
Strongylocentrotus purpuratus × *S. franciscanus* (Swan, 1953).

Methods of Increasing Hybridization.—
Heavy insemination (F. R. Lillie, 1921c).
Standing in sea water (Tennent, 1910b, 1923).
Egg water (Hultin, 1948b; Harding and Harding, 1952a).
Hyperalkalinity (Tennent, 1910b, 1923; Loeb, 1913a, p. 225, 1915a).
Heat (Herbst, 1906).
Remove jelly (Harding and Harding, 1952b).
Remove vitelline membrane with trypsin or urea, adding glycine, bovine albumen, etc. (Hultin, 1948a, b; Bohus-Jensen, 1953); does not work with *A. punctulata* (Tyler and Metz, 1954).
Crowd the eggs (E. B. Harvey, 1941d).

Cleavage Rate of Hybrid.—Is that of the egg (Tennent, 1910b, 1922; A. R. Moore, 1933 in *Dendraster* × *S. franciscanus;* De Francesco, 1934, in Naples species).

Variability of Hybrids.—(Shearer, De Morgan, and Fuchs, 1914; Hörstadius, 1940). Tennent (1913, p. 536) writes "It has been established again and again that under some conditions we may obtain larvae of a maternal type with respect to certain characters, under other conditions larvae of a paternal type and under still other conditions larvae of a blended type. This is established. We should accept the fact."

Other Species and General References (Incomplete)

Bohus-Jensen, 1953. Bermuda species.
Bronn's *Tierreich*, 1904, Bd. 2, Abt. 3, Buch IV : 1238–1251.
De Francesco, 1933. Naples species.
Fuchs, 1914b, F_2 *Echinus* hybrids.
Harding and Harding, 1952a, b. *A. lixula* × *P. lividus* and *Echinocardium cordatum* × *Psammechinus miliaris.*
Harvey, E. B., 1933a. Naples species.
Harvey, E. B., 1942. *Echinarachnius parma* × *S. dröbachiensis*, maternal; *S. franciscanus* × *S. purpuratus*, maternal, but see A. R. Moore, 1943.
Harvey, E. B., 1947. *Lytechinus variegatus* × *Tripneustes esculentus*, maternal.
Herbst, 1906–1914.
Hörstadius, 1936c. *Psammechinus microtuberculatus* × *Paracentrotus lividus*
Hultin, 1948a, b. Naples species.
Matsui, 1924. List of hybrids.
Moore, A. R., 1949c. Portugaliae Acta Biologica, Ser. A.
Morgan, 1927. *Experimental Embryology*. General.
Nümann, 1933. Naples species.
Runnström, 1949a, 1952a. General.
Shearer, De Morgan, and Fuchs, 1914. *Psammechinus miliaris, Echinus esculentus* and *E. acutus.*
Tabulae Biologicae, 1930, VI : 514. Grimpe.
Tennent, 1910b. List of hybrids.
Tennent, 1912a. *Lytechinus variegatus* × *Tripneustes esculentus.*
Tennent, 1922. *Cidaris tribuloides.*
Tennent, 1923. Japanese species.
von Ubisch, 1937. *Echinocyamus pusillus* × *Ps. miliaris.*
von Ubisch, 1939. *Echinocardium cordatum* × *Ps. miliaris.*
Wilson, 1925. *The Cell.*

HYDROGEN ION CONCENTRATION, PH

Sea Water at Woods Hole.—pH usually taken as approximately 8.0 (Heilbrunn, 1943, p. 473). Other values are 8.1 to 8.3 (Redfield, 1948, etc.). See Ball and Stock (1937). pH in relation to CO_2 content (Henderson and Cohn, 1916; McClendon, 1916).

Cytoplasm of Arbacia punctulata.—By injection of indicators pH of unfertilized and fertilized egg 6.8 ± 0.2 (Pandit and Chambers, 1932). Wiercinski (1944) gives: protoplasm pH 6.2 ± 0.2, hyaline protoplasm (white half-eggs) 5.8 to 6.8, granular material 5.4, nucleus above 7.0.

Change of Intracellular pH.—In salts of weak acids and weak bases (Jacobs, 1940). See Smith and Clowes, 1924; Haywood and Root, 1930, 1932 for CO_2. For *Echinarachnius parma* eggs with CO_2 and NH_3, see Chambers, 1928.

Other eggs.—Paracentrotus lividus and *Echinocardium cordatum*, pH of interior, unfertilized and fertilized eggs to 16 cell stage is 6.6 (Needham and Needham, 1926a). But according to Vlès (1924), Vlès and Vellinger (1928) *et al.*, the pH of the eggs of *Paracentrotus lividus* and *Arbacia aequituberculata (lixula)* is about 5.5. Rapkine and Wurmser (1926) found the pH of cytoplasm and nucleus of *P. lividus* oocytes the same, around 7.0. For a discussion of the controversy see Reiss' *Monograph* (1926) and Needham's *Chemical Embryology*, Vol. II, p. 839–855 (1931).

Blastocoel.—pH of blastocoel of *A. punctulata*, in blastula, gastrula and pluteus is same as sea water (Chambers and Pollack, 1927). But according to Rapkine and Prenant (1925) the pH of blastocoel of *P. lividus* and *Echinocardium cordatum* is 7.0 to 7.3 in blastula and pluteus; 8.5 in early gastrula when spicules form. See Needham (1931, p. 846–849).

Cytolysed eggs.—pH in *A. punctulata* is 5.3 ± 0.2 (Pandit and Chambers, 1932).

Effect of pH of Surrounding Medium.—On hyaloplasm of egg, none unless injured (Wiercinski, 1944). On fertilization, block to fertilization in medium of pH 6.8 and below (Clowes and Smith, 1923, Smith and Clowes, 1924d). On cleavage and development, normal cleavage rate (if fertilized in sea water) at pH 8.2 to 5.8. Optimum for fertilization, cleavage and viability pH 6.0 (Clowes and Smith, 1923, Smith and Clowes, 1924b, c). Acceleration of cleavage rate or development if medium is slightly alkaline (Loeb, 1898, but see 1913a, p. 35; also Glaser, 1914b; pH 8.2 to 9.2 (Smith and Clowes, 1924c). Retardation of cleavage below pH 6.0 and above 9.4 (Smith and Clowes, 1924c, d). Effect of pH on pluteus (Child, 1916b, Medes, 1917).

On polyspermy, maximum near pH 7.2, none at 7.4 to 9.8 (Clowes and Smith, 1923, Smith and Clowes, 1924d). On respiration, diminished by low pH (Root, 1920; Anfinson, 1947). Increased by alkalinity (Wasteneys, 1916). On permeability, no swelling till injury at pH 4.0; none at 9.8 (Lucké and McCutcheon, 1926b). See Permeability. On viscosity (Barth, 1929; Howard, 1931). On parthenogenesis, slight if pH more than 9.0 (Smith and Clowes, 1924b). On cytolysis, greatest in medium of pH 9.3 (Smith and Clowes, 1924b). On sperm, effects on (Cohn, 1918).

Effects of Substituted Phenols.—In relation to pH (Clowes and Krahl, 1936a; Krahl and Clowes, 1938; Hutchens, Krahl, and Clowes, 1939).

Effects of Barbituric Acid Derivatives.—In relation to pH (Clowes, Keltch, and Krahl, 1940; Krahl, 1950).

Effect of Local Anaesthetic Bases.—In relation to pH (Krahl, Keltch, and Clowes, 1940).

Other Species (additional) and General References

Ashbel, 1931. *A. lixula* and *P. lividus*.

Gray, 1931. *Experimental Cytology*, p. 85–87, general.

Heilbrunn, 1943. *An Outline of General Physiology*, p. 47–55; 473–476; enzymes, p. 199.

Hirabayashi, 1937. *Toxopneustes pileolus*, blastocoel.

Lison, 1935. General; 1941, Tabulation.

Moore, B., Roaf and Whitley, 1905. *Echinus esculentus*, rate of cleavage increases with slight alkalinity.

Needham, 1931. *Chemical Embryology*, vol. II, general.

Needham and Needham, 1926b. Review.

Reiss, 1926. Review Monograph.

INFRARED LIGHT

No experimental data for *Arbacia punctulata*, but in *Strongylocentrotus franciscanus* and *purpuratus*, fertilizability decreased with wave lengths 0.8 to 1.2 μ (Nelson and S. C. Brooks, 1933).

Photographs of eggs, half-eggs, and nuclei of *A. punctulata* with infrared light (E. B. Harvey and Lavin, 1951a, b).

Reference

Giese, 1947. General on radiations.

INTERFACIAL TENSION

Oil-Protoplasm; Oil-Water. See under Oil (Coalescence)

INTRAVITAM DYES

See Vital Dyes

JELLY LAYER

Definition. A gelatinous layer on the outside of the egg, often called the *zona pellucida* or chorion (Plate XVI, Photograph 3 and Figs 9, 10). Observed in *Echinus esculentus* by Derbès in 1847, and called by him *la couche mucilagineuse*. Funnel-shaped micropyle present in jelly.

Visibility.—Not visible in sea water because of its refractive index, but its presence is indicated by the spacing between individual eggs. If eggs are contiguous, there is no jelly. Jelly and micropyle can be demonstrated by India or Chinese ink (old method of Boveri, 1901), or squid ink. The jelly is readily shown by a slight tinge to the sea water of Janus green or toluidin blue (cause shrinkage after some minutes); also by the halo of sperm caught in the jelly after a heavy insemination. It has been described as radially striated as it comes from the ovary, then becoming invisible (McClendon 1914a); as a fibrillar network (Chambers, 1933).

Thickness.—Is 28 to 32 μ; the average volume ca. 1,050,000 μ^3. It swells to 60 μ with squid ink (E. B. H.). The jelly of *Ps. miliaris* has been found to swell to double its width with rabbit serum and certain amino acids (Runnström, Monné, and Wicklund, 1946), and also with versene (Borei and Björklund, 1953); this also caused swelling of the *Lytechinus* egg jelly (Tyler, 1953). For the chemistry of squid ink, see Ball and Ramsdell (1940).

Specific Gravity.—Lighter than the egg, goes to centripetal pole on centrifuging (Shapiro, 1935c; E. B. H. unpub.) (Plate XVI, Photograph 4).

Presence.—In oocytes as well as mature eggs (F. R. Lillie, 1914, 1919, p. 119). Not present in oocytes under 60 μ diameter and may remain till hatching (E. B. H.).

Not necessary for fertilization membrane (F. R. Lillie, 1914, 1915a; E. N. Harvey, 1914, *et al.*). Some early observers thought it necessary (McClendon, 1911, 1914a). See F. R. Lillie and Just, 1924, p. 453, footnote.

Electric Charge.—Negative, is acidic (McClendon, 1911, 1912b). For more recent work see Runnström, Tiselius, and Vasseur (1942).

Permeability changes.—No effect (R. S. Lillie, 1917).

Loss of Jelly.—On standing in sea water (many observers). Loss of jelly with age (R. S. Lillie, 1917; Goldforb, 1918a, b; *et al.*).

Not Replaced.—When removed (R. S. Lillie, 1921; *et al.*).

Contains Agglutinins.—(Fertilizin), but not in immature eggs (F. R. Lillie, 1914, 1915a, 1919, p. 117, etc.; Glaser, 1914b; Loeb, 1915a; Frank, 1939; *et al.*). For

presence of fertilizin in jelly of immature eggs of other species see Vasseur (1952, p. 26). Tyler (1941b) considers fertilizin identical with jelly in other species (*S. purpuratus)*; also Runnström (1952a, p. 45).

Fertilization.—Aided by jelly (F. R. Lillie, 1914; Tyler, 1941b for *S. purpuratus*).

Takes up Chlorine.—From sea water (Glaser, 1922b); takes up copper (Glaser, 1923). Takes up calcium (radioactive) (Rudenberg, 1952).

$CaCl_2$.—Makes it sticky (Page, 1929b).

Is Precipitated.—By cytoplasmic fraction from frozen eggs (antifertilizin) (Monroy and Runnström, 1952), previously shown for *S. purpuratus* by Tyler (1940a).

Chemistry of Jelly.—Jelly is a hyaline proteid dissolving in sea water; mucin (McClendon, 1909a, 1912b). For other species, Vasseur (1948a) gives about 20% protein and 80% polysaccharide esterified with sulphuric acid; the composition varies in different species. Tyler (1949c) gives for the composition of fertilizin (jelly) of *S. purpuratus* approximately equivalent amounts of amino acids (> 20%), reducing sugar (> 25%) and sulphate (23%).

Coalescence with Oil Drops.—Inhibited (Kopac, 1940a).

Stains.—With Janus green, Janus dark blue B, saffranin O, thionin, toluidin blue (E. B. Harvey, 1941c). According to McClendon (1914a) it stains with methylene blue and neutral red. Stains with acridine orange, but this is very toxic. Table 8.

Can Be Removed.—By washing and agitation (McClendon, 1914a; F. R. Lillie, 1914; E. N. Harvey, 1914; R. S. Lillie, 1917; *et al.*). Straining through bolting silk (Just, 1928a, 1939a; Shapiro, 1935c; *et al.*). Fine pipette. Acid; 1.4 cc. N/10 HCl + 50 cc. sea water (F. R. Lillie, 1915a; *et al.*). I use one drop N/10 HCl, from a medium pipette, to 50 cc. sea water, then wash well (E. B. Harvey, 1941c). Alkali (McClendon, 1909b; Barth, 1929). Calcium-free sea water (E. B. H.). NaCl, isosmotic (0.54 M) (R. S. Lillie, 1921; Kopac, 1940a). KCl, isosmotic (0.53 M) (E. B. H. unpub.; Page, 1929b). NaCl + $CaCl_2$ 17 : 1 (R. S. Lillie, 1921). NH_4Cl (Kopac 1948a).

It is also removed by trypsin, chymotrypsin, and papain, in other species (Tyler, 1940b in *S. purpuratus;* Runnström, Tiselius, and Vasseur, 1942, and Minganti, 1953, in *Ps. miliaris*). Centrifuging (McClendon, 1914a; Shapiro, 1935c; E. B. Harvey, 1941c); this is variable and not reliable. X-rays (E. B. Harvey, 1941c; Evans, Beams, and Smith, 1941). Ultraviolet light (E. B. H., 1950 unpub.); in *Paracentrotus lividus* (Tchakotine, 1921a). For centrifuging see Plate XVI, Photograph 4.

Other Species (additional) and General References

Hobson, 1927. *E. esculentus, Ps. miliaris.*
Monné, 1944a. *Ps. miliaris,* birefringence.
Motomura, 1950b. *S. pulcherrimus.*
Runnström, 1949*a*. Review.
Runnström, 1952a. Review in Symp. Soc. Exp. Biol. VI.
Tyler, 1948. Review.
Vasseur *(et al.)*. Many papers summarized in independent publication printed by Kihlströms Tryckeri, 1952; most of these are listed by Runnström 1952a.

LEUCOCYTES

See Amoebocytes, Perivisceral Fluid

Occurrence.—In perivisceral fluid, together with amoebocytes (Geddes, 1880; Cuénot, 1891a in other species; Kindred, 1921, 1926; H. V. Wilson, 1924; Donnellon, 1938 in *A. punctulata*). Two kinds distinguished by Liebman (1950), phagocytes and trephocytes.

Shape.—Irregular with long filamentous processes or pseudopods (Kindred, 1921, 1926; H. V. Wilson, 1924; Liebman, 1950).

Phagocytosis and Clotting.—(Kindred, 1921, 1926; H. V. Wilson, 1924; Donnellon, 1938; Liebman, 1950). Trephocyte material taken up by oocytes (Liebman, 1950).

Other Species

Kindred, 1924. S. dröbachiensis.

LIPIDS

See also Oil

In eggs.—Cholesterol present in *Arbacia* egg, not in *Asterias* (Mathews, 1913; Page, 1923, 1927a).

Cephalin, more; lecithin, less in *Arbacia* than in *Asterias* (Page, 1927a). No change in lecithin (?) content between 2-cell stage and blastula (Shackell, 1911); this was criticised by Robertson and Wasteneys (1913) who found decrease in lecithin in *Strongylocentrotus purpuratus.*

Amount. *A.* In alcohol-ether extract, 8.3 gm. of oil from 183 million eggs; 1.539 gm. of acetone-insoluble material with high percentage of cephalin. Iodine number of oil 146–148, saponification value approximately 606 (Page, 1927a).

B. Total fat (all material soluble in petroleum ether) in a million unfertilized eggs is 5.65 mg; sterol 0.43 mg. or 7.5 % of total fat; phospholipid 2.17 mg. or 38 % of total fat. Total fat decreases up to time of hatching (8 hrs.), increases for 10 hrs., then decreases; sterol unchanged and phospholipid uncertain. Loss of total fat in 43 hrs. is 3.54 mg. per million eggs (Hayes, 1938).

C. Total lipid ("lyophylled" eggs), 5.4 % of whole egg, 26.9 % of solids in egg; 77 % of total lipid is probably bound to protein. No change in total or bound lipid in 5 hrs. development (Parpart, 1941).

Crude oil obtained with fat solvents is reactive with Nadi reagent. I_2 index 180–190, saponification index around 200. Sterols and phospholipids present, also fatty acids and glycerids (Navez, 1938, 1939; Navez and DuBois, 1940).

In Centrifuged Eggs.—(Ether extract). Amount. Whole eggs 2.254 % total fat; centripetal layer of centrifuged crushed eggs 0.3 %; centrifugal layer 1.946 % (McClendon, 1909a).

In Half-Eggs.—(Obtained by centrifuging). White half-eggs: free fats and sterols (ether extract) 2.2 mg. per million halves; bound lipids (alcohol-ether extract) 9.6 mg. per million halves.

Red half-eggs: free fats and sterols 6.6 mg. per million halves; bound lipids 12.2 mg. per million halves. 75 % of free fats and sterols, 56 % of bound lipids are in red half, or 61.6 % of total lipids (Hunter and Parpart, 1946).

In Nucleoli.—Lipids and phospholipids not present in nucleoli of *Arbacia*, as determined by staining (Gates, 1941).

In Sperm.—Lipoids and lipoproteins present in acrosome, middle piece and tail, on the surface (Popa, 1927). Lipids in alcohol-ether extracts do not contain agglutinating substance; this is in protein residue (Frank, 1939).

Effect on The Eggs:—Of fatty acids. Cause parthenogenesis, especially butyric (Loeb, 1913a, p. 71, etc., *et al.*, see under Parthenogenesis. Decrease viscosity (Howard, 1931). Of soaps. Different effects by different soaps on cytolysis, pigment discharge, stratification and breaking with centrifugal force and on response to microdissection (Page, Shonle, and Clowes, 1923).

Other Species (additional) and General References

Cleland and Rothschild, 1952a. *Echinus esculentus*, eggs, analysis.
Ephrussi, 1933. *Paracentrotus lividus*, analysis.

Leitch, 1934b. *Strongylocentrotus purpuratus*, S. *franciscanus*, analysis.
Needham, 1931. *Chemical Embryology*. General, vol. I : 310, 347; vol. II : 1244.
Öhman, 1945. *Ps. miliaris*, *Echinocardium cordatum*, lipid layer, and analysis.
Rothschild and Cleland, 1952. *Echinus esculentus*, sperm, analysis.
Tennent, Gardiner, and Smith, 1931. *Echinometra lucunter*, microchemistry, and analysis.
Wetzel, 1907. *Paracentrotus lividus*, analysis.

LITHIUM

Occurrence.—Lithium is present in tissues of *Paracentrotus lividus* and *Echinus esculentus* (Fox and Ramage, 1931). Li enters eggs and embryos of *Sphaerechinus granularis* (Ranzi and Falkenheim, 1937).

LiCl Isotonic.—With sea water at Woods Hole is approximately 0.56 M (Page, 1929). 0.60 M (M. B. L. Chemical Room).

Effect of Li on Eggs.—General effect is exogastrulation and increase of entoderm (vegetalization) as first shown by Herbst (1892) for *Echinus (Psammechinus) microtuberculatus*, *Strongylocentrotus (Paracentrotus) lividus* and *Sphaerechinus granularis*. Exogastrulation in *Arbacia punctulata* obtained with N/80–N/100 LiCl (MacArthur, 1924); with 20 parts 0.54 M LiCl and 80 parts sea water for 4 hours (Costello, 1948).

Toxicity.—Eggs will develop in 5 cc. of 2.6% LiCl + 100 cc. sea water for 20 hours; more toxic for *Echinarachnius parma;* effect can be counteracted by potassium or pyocyanine (Runnström, 1935b). But Moore, Bliss and Anderson (1945) found this not true for *S. purpuratus* and *Dendraster excentricus*, that pyocyanine effect is additive to Li effect. Toxic effect of Li is mitigated by K, Rb and Cs (Loeb, 1920).

Hyaline Layer.—Is softened and dispersed (Runnström, 1935b; Chambers, 1940).

Pigment Granules.—LiCl prevents breakdown of pigment granules by Ca (surface precipitation reaction) in order of $NH_4 > Na > K > Li$ (Heilbrunn, 1928, p. 230).

Respiration.—No references found for *Arbacia*. Li reduces respiration in *Paracentrotus lividus* (Lindahl, 1936; Lindahl and Öhman, 1938); in *S. purpuratus* and *Dendraster excentricus* (Moore, Bliss, and Anderson, 1945).

Permeability.—Increase (Lucké and McCutcheon, 1929).

Parthenogenesis.—Slight stimulation with LiCl (Loeb, 1900a; Hollingsworth. 1941).

Replacement.—Lithium can be replaced by many substances in causing exogastrulation, e.g., NaCl, $CuCl_2$, $NiCl_2$, methylene blue and other dyes and substances. For specific references see Child's *Patterns and Problems of Development* (1941, p. 222 footnote); and Child (1948).

Other Species (additional) and General References

Child, 1936a, 1940, 1941. *S. franciscanus*, *S. purpuratus*, *Dendraster excentricus;* axial gradient.
Herbst, 1892, 1893, 1896. *Ps. microtuberculatus*, *P. lividus*, *Sphaerechinus granularis*.
Hörstadius, 1936a, b. *P. lividus*.
Lindahl, 1942. Review.
MacArthur, 1924. *S. franciscanus*, *Echinarachnius parma*.
Moore, A. R., 1930c; Moore, Bliss, and Anderson, 1945. *S. purpuratus*, *Dendraster excentricus;* lithium and pyocyanine on development and respiration.
Needham, 1942. *Biochemistry and Morphogenesis*, p. 485; general.
Rulon, 1946. *Dendraster excentricus;* lithium and sodium thyocyanate.
Runnström, 1928a. *P. lividus*.
von Ubisch, 1929. *Echinocyamus pusillus*.
Waterman, 1932. *P. lividus*.

MAGNESIUM

Amount in Egg.—4.48 mg. magnesium per 10^6 eggs (10^6 eggs = 0.124 gm. dry weight), or 0.182 millimoles (Page, 1927b).

Amount in Sea Water.—At Woods Hole 1.3004 gm. per liter at 20° C. (Page, 1927c, 1928).

$MgCl_2$ Isotonic.—With sea water at Woods Hole is 0.30 M (M. B. L. Chemical Room). Also given as 0.37 M. $MgSO_4$ isotonic with sea water at Woods Hole may be 0.81 M (M. B. L. Chemical Room). Also given as 0.52 M.

Surface Precipitation Reaction.—Mg acts like Ca but is less potent (Heilbrunn, 1930, 1934, 1943, p. 470, 538).

Cytolysis.—Prevented by $MgCl_2$ (R. S. Lillie, 1911a, b; Page, 1924).

Mg Counteracts NaCl.—And other Na and K salts (Loeb, 1900a; Mathews, 1905; R. S. Lillie, 1911a, b, 1914a; McCutcheon and Lucké, 1928). Mg is antagonized by Ca (Heilbrunn, 1934; Hollingsworth, 1941).

Colorless Amoebocytes of Body Fluid.—Dissolved by $MgCl_2$ (Mathews, 1900).

Amoeboid Eggs.—Caused by $MgCl_2$ (Loeb, 1900a; Churney, 1940).

Effect of Mg on Fertilized Eggs.—Nuclear division without cell division (Loeb, 1895b, 1900a; Norman, 1896). Astropheres present also (Morgan, 1899).

Mg as an Anaesthetic.—$MgSO_4$ widely used to quiet marine organisms (Mayor, 1909; R. S. Lillie, 1910, 1916c; Heilbrunn, 1934, 1943, p. 460; 1952, p. 528). First used by Tullberg in 1892 (see last reference of Heilbrunn). Mg anaesthesia counteracted by Ca (Heilbrunn, 1943, p. 531). Magnesium sulfate can be used on blastulae and plutei of Arbacia. "Methocoel", methyl cellulose, also quiets cilia (Marsland, 1943); also Chloretone. See McClung's *Microscopical Technique*, 1950, p. 436.

Respiration.—No references found.

Permeability.—Decrease (R. S. Lillie, 1910; McCutcheon and Lucké, 1928; Heilbrunn, 1943, p. 531).

Viscosity.—(Better stratification with decreased viscosity). $MgCl_2$ decreases viscosity (Heilbrunn, 1923, 1928, p. 147, 1943, p. 81; E. B. Harvey, 1945). Isosmotic solutions arranged in order of their effect in decreasing viscosity (better stratification): $CaCl_2$ > $MgCl_2$ > sea water > NaCl > KCl (E. B. Harvey, 1945). Heilbrunn 1928, p. 147) gives them in the same order except that K and Na are reversed.

Breaking with Centrifugal Force.—Break less readily in $MgCl_2$ than in sea water. Isosmotic solutions arranged in order of their effectiveness in causing breaking into halves, proceed in the reverse order from that given above for decrease in viscosity; the greater the viscosity (less stratification), the more readily they break (E. B. Harvey, 1945).

Parthenogenesis.—$MgCl_2$ added to sea water, used first by Morgan (June, 1899, 1900a, b) for parthenogenesis, then by Loeb (October, 1899, 1900a, b); plutei first obtained by Loeb (1899; see Loeb 1913a, p. 53, 57, etc.). R. S. Lillie (1910, 1911a, 1914a) found isotonic $MgCl_2$ did not activate. Hollingsworth (1941) obtained slight parthenogenesis with isotonic $MgCl_2$, but inhibition of its activation by $CaCl_2$.

Other Species (additional) and General References

Bialazewicz, 1927, 1929. *A. pustulosa (lixula)* and *Paracentrotus lividus*, electrolytes.
Heilbrunn, 1952, p. 528. *General Physiology*, general.
Herbst, 1904. Salts necessary for development.
Loeb, 1913a. *Artificial Parthenogenesis*, especially for *S. purpuratus* and *S. franciscanus*.
Robertson and Webb, 1939. Amount of Mg in sea water and body fluids.
Rothschild and Barnes, 1953. *P. lividus*, amount in egg; table of salts and species.
Wilson, 1901a. *Toxopneustes* (*Lytechinus*) *variegatus*, cytology.

METABOLISM

See Respiration

MITOCHONDRIA

Size.—Smallest granules that can be moved by centrifugal force, 0.6 to 1.0 μ (E. B. H. per E. N. Harvey, 1932a; E. B. Harvey, 1936). With electron microscope 0.3 to 0.5 μ, probably (McCulloch, 1952a).

Density.—Lighter than yolk and pigment granules; form a narrow band above the yolk in centrifuged eggs (first described by E. N. Harvey, 1932a as the "fifth layer"; E. B. Harvey, 1932, 1936, etc.; *et al.*). It is the last layer to form with centrifuging, and the first layer to redistribute, disappearing five to ten minutes after centrifuging (E. B. H.).

Amount in Egg.—4.8 % (E. N. Harvey, 1932a); 5 % (Costello, 1939).

Origin.—Normally mitochondria appear in the immature egg when it is about 33 μ in diameter, at the same time as the pigment and oil; a few scattered granules sometimes appear when a little smaller, 23 μ. In the immature egg of all sizes, the mitochondrial granules are concentrated around the germinal vesicle, and are not displaced by strong centrifugal force (10,000 × g) (E. B. H.).

They appear *de novo* in the clear quarter egg in the pluteus. This quarter contains no mitochondria after being separated off from the unfertilized egg by centrifugal force, and there are none throughout cleavage nor in the blastulae, but they do occur in the pluteus. This is true also of the pigment granules in the clear quarter. There are no pigment granules until the pluteus is about 4 days old. (E. B. Harvey, 1946a).

Stain.—Best vital stain is methyl green, stains violet. Janus green stains them green but is rather toxic (E. B. Harvey, 1932, 1941c). Other vital stains are: Bismark brown, chrysoidin, gentian violet, methyl violet, neutral red, Nile blue, rhodamine, saffranin O (later), thionin (few cases), toluidin blue (E. B. Harvey, 1941c). Also crystal violet, dahlia B (E. B. H. unpub.). Fixed material. Mitochondria stain red with Benda-Kuhl (E. B. Wilson, 1926). Slightly stained with iron haematoxylin (E. B. Harvey, 1940c). Table 8.

Other Species (additional) and General References

In some other species, e.g., *Sphaerechinus granularis*, the mitochondria are the lightest granules as in *Arbacia punctulata* and *A. lixula* (E. B. Harvey, 1933 a, 1938 a). In some species they are the heaviest material in the egg, e.g., *Psammechinus microtuberculatus* (E. B. Harvey, 1933a, 1938a, and see colored picture of Lindahl, 1932, p. 330) and *Ps. miliaris* (Linderström-Lang, 1938–1939). In still other species, they are the heaviest granules, but lighter than the clear material, e.g., *Tripneustes esculentus* (E. B. Harvey, 1933a, 1947).

Gustafson, 1952. General.

Gustafson and Lenicque, 1952. *Ps. miliaris*, distribution of mitochondria at different stages of development.

Harvey, E. B., 1933a, 1938a. Naples species.

Harvey, E. B., 1947. Bermuda species.

Runnström, 1952b. General, in Barron's *Modern Trends*.

NARCOTICS

See Anaesthetics

NITROGEN

Nitrogen by weight, times 6.25 is usually assumed to give protein present.

Amount.—Of nitrogen in eggs (Ballentine, 1940a):

0.107 mg. nitrogen per mg. dry weight
265 mg. dry weight per cm.^{3} cells
58.0 mg. dry weight per 10^6 cells
26.8 mg. nitrogen per cm.^{3} cells
5.86 mg. nitrogen per 10^6 cells.

Similar figures have been given by Hutchens, Keltch, Krahl and Clowes (1942); they report also 10% of nitrogen is in the jelly.

Increase.—In soluble nitrogen on autolysis of unfertilized and fertilized eggs, in acid solution; increase in soluble nitrogen on autolysis of sperm in neutral or alkaline media; about one sixth of soluble nitrogen from autolysed and control eggs is of protein origin (Lyon and Shackell, 1910a).

Other Species

Ephrussi, 1933. *Paracentrotus lividus*, 10.7% nitrogen dry weight.
Gustafson and Hjelte, 1950. *P. lividus*, amino acid–N.
Hultin, 1950c. *P. lividus*, uptake of ^{15}N-labeled ammonia.
Wetzel, 1907. *P. lividus*, 1.6% nitrogen wet weight, 7.2% dry weight.

NUCLEAR DIVISION WITHOUT CELL DIVISION

Historical.—First obtained by O. and R. Hertwig in 1887 in *Paracentrotus lividus* with nicotine, chloral hydrate, etc. In *Arbacia punctulata*, with salts on fertilized eggs, with NaCl by Loeb (1892). Questioned by Morgan (1893, 1896). Loeb's results were confirmed by Norman (1896) with $MgCl_2$, and restated by Loeb (1895b, 1900a). Morgan later (1899) agreed that nuclear division could take place without cell division, but was irregular and accompanied by artificial astropheres.

Urea.—(R. S. Lillie, 1903).

Cold.—O° to 2 °C., then room temperature (Lyon, 1904b).

Acid.—And alkali (Smith and Clowes, 1924c).

Carbamates.—(Cornman, 1950b).

Nitrous Oxide.—(Haywood, 1953).

Normally in many fertilized red half-eggs, granular, yolk and pigment quarter-eggs obtained by centrifugal force (E. B. Harvey, 1932, 1940c, 1951, and this Monograph).

For obliteration of cleavage plane, leaving nuclear without cell division see Anaesthetics, Hydrostatic Pressure, Oxygen-Lack.

Other Species (additional) and General References

Boveri, 1897. *Echinus (Psammechinus) microtuberculatus*, pressure and cold.
Driesch, 1892. Same species, pressure and heat.
Godlewski, 1908. Same species, CO_2.
Korschelt and Heider, 1902, p. 215. General.
Moore, Roaf, and Whitley, 1905. *Echinus esculentus*, alkali.
Polowzow, 1924. *P. lividus*, alcohol.
Sugawara, 1943a, b. *S. pulcherrimus*, "hatching enzyme", trypsin, pepsin, papayotin, $CeCl_3$, ageing.
Sugiyama, 1938a, b, c. *S. pulcherrimus*, *Pseudocentrotus depressus*, *Mespilia globulus* etc., egg albumen, heparin, hirudin; oxygen consumption.
E. B. Wilson, 1901a. *Toxopneustes (Lytechinus) variegatus*, parthenogenetic eggs with $MgCl_2$.
E. B. Wilson, 1901b. Fertilized eggs with shaking and ether.
E. B. Wilson, 1925. *The Cell*, p. 174. Analysis, separability of factors.
Ziegler, 1894. *Echinus (Ps.) microtuberculatus*, pressure.

NUCLEOPROTEINS

See also Proteins and Phosphorus Metabolism

Historical.—Mathews (1897) discovered a substance in *Arbacia lixula* sperm, not a protamine, but similar to a histone, in combination with nucleic acid, which he called "arbacin". Mathews (1915, p. 174) extracted a very small amount of nucleic acid (?) from unfertilized *Arbacia punctulata* eggs.

Amount in Eggs and Embryos.—Blanchard (1935) obtained 12.75 gm. of crude nucleic acid from 4,820 gm. of unfertilized eggs; 1.08 gr. of desoxyribose nucleic acid (DNA) and about same amount of ribose nucleic acid, its pentose derivative (RNA).

For more recent figures on amounts of DNA and RNA in eggs and embryos see Table 17 under Phosphorus Metabolism.

Amount of RNA phosphorus in unfertilized egg is 20×10^{-5} micrograms; DNA phosphorus 0.7 to 1×10^{-5} micrograms. DNA/RNA = 0.05 in unfertilized egg, 0.17 in 8-hour embryo (blastula), 0.46 in 24-hour embryo (pluteus). RNA in unfertilized egg does not change in early development, but DNA increases during cleavage until pluteus stage; hence DNA of fertilized egg probably does not come from RNA of unfertilized egg as earlier postulated by Brachet in other species (Schmidt, Hecht, and Thannhauser, 1948; see also Villee, Lowens, Gordon, Leonard and Rich, 1949; Villee, Villee and LaPlace, 1953). DNA of blastula is 10 × RNA (Abrams, 1951; see Schmidt *et al.*, 1948). Marshak and Marshak (1953) give amounts in an unfertilized egg as RNA 2.4×10^{-3} micrograms, DNA 8.1×10^{-6} micrograms, and state that there is no detectible Feulgen-positive material in the nucleus of the mature ovum.

Location in Egg.—In other species *(Paracentrotus lividus, Psammechinus miliaris,* etc.) it has been shown that DNA is located in the nucleus, but there is also some RNA; RNA is mostly in cytoplasm near nuclear membrane and in germinal vesicle of immature egg (work of Brachet and Casperson). See Runnström in *Modern Trends* (1952, p. 65); Brachet's *Chemical Embryology* (1950, p. 212); Brachet (1947, 1952); Casperson and Schultz (1940).

In *A. punctulata.*—Location of RNA-proteins in eggs (Tsuboi, 1953). Location of nucleic acid compounds in immature, mature and centrifuged eggs and half-eggs as photographed by ultraviolet light (E. B. Harvey and Lavin, 1944, 1951a). Feulgen reaction negative in parthenogenetic merogones, positive in fertilized merogones (E. B. Harvey, 1940c, p. 186). RNA present in cell cortex, vitelline and fertilization membranes (Lansing and Rosenthal, 1949, 1952).

Feulgen Reaction.—For determination of DNA. See Gray's *Experimental Cytology* (1931, p. 84); Brachet (1933, 1937, 1950); Pasteels and Lison (1950, p. 448); McClung's *Microscopical Technique* (1950, p. 135); Gomori (1952). For recent appraisals of the Feulgen reaction see Brachet (1952, p. 176); Lesler (1953); Lumb (1950).

Amount and Location in Sperm.—Nucleic acid 29.66% (in *A. lixula,* Mathews, 1897). RNA 1% of amount in egg; DNA 3% of amount in egg (Schmidt, *et al.*, 1948). Sperm contributes 1/30th as much DNA to fertilized egg as does the egg (Mazia, 1949b). DNA, about 0.9×10^{-6} micrograms in one *Arbacia* sperm (Mazia, personal communication, July 1951). Marshak and Marshak (1953) give 7.9×10^{-7} micrograms DNA in one sperm. RNA activity localized in middle piece of sperm (Di Stefano and Mazia, 1952). Two DNA fractions in sperm (Barton, 1951, 1952).

Analysis and Synthesis.—Methods of extraction and analysis (Schmidt and Thannhauser, 1945; Schmidt, *et al.*, 1948; Villee, *et al.*, 1949; Mazia, 1949b; Abrams, 1951, with isotope tracers; Marshak and Vogel, 1951). Analysis of phosphorus fractions (Crane, 1947; Schmidt, *et al.*, 1948; Villee, *et al.*, 1949, 1950, see Table 17 under Phosphorus Metabolism). Purines, adenine and guanine extracted from unfertilized eggs (Blanchard, 1935). Purines and pyrimidines, adenine, cytosine, thymine ex-

tracted from sperm nucleic acid (Daly, Allfrey and Mirsky, 1950). From sperm and eggs, but no uracil in sperm, no thymine in eggs (Marshak and Vogel, 1951). Synthesis of nucleic acid purines, adenine and guanine (Abrams, 1951). See Marshak and Marshak, 1953.

Effect of Various Drugs.—On nucleic acid metabolism (Villee, *et al.*, 1949; Villee and Villee, 1952). Inhibition of DNA and DNase by usnic acid (Marshak and Fager, 1950).

Other Species (additional) and General References

Bernstein and Mazia, 1953a, b. *S. purpuratus.*
Brachet, 1947, 1952. General.
Brachet, 1950. *Chemical Embryology*, Chapt. 6, general.
Callan, 1949. Naples species, RNA in eggs.
Caspersson and Schultz, 1940. *Psammechinus miliaris*, ultraviolet.
Elson and Chargaff, 1952a, b. *Paracentrotus lividus*, DNA in eggs and sperm; PNA in embryos.
Hultin, 1949a. *A. lixula*, *Ps. miliaris* etc., agglutination by nucleoproteins.
Krahl, 1950. Review, p. 187.
Lison and Pasteels, 1951. *P. lividus*, amount DNA at different stages.
Loeb, 1907. Nuclein synthesis, general.
Masing, 1910. *A. lixula*, no nuclein synthesis in development.
Mirsky and Ris, 1951. *Echinometra* and general.
Needham and Needham, 1930. *Dendraster excentricus*, nuclein synthesis.
Rothschild, 1951a. Purines and pyrimidines in sperm of *Echinus esculentus.*
Runnström, 1952. General, in Barron's *Modern Trends.*
Symposium on nucleic acids, 1947. Cold Spring Harbor Symposia, vol. 12.
Symposium on nucleic acid, 1947. Symposia of Society for Experimental Biology, no. 1.
Symposium (Discussion) on nucleic acids, 1951. *J. Cell. and Comp. Physiol.* 38, supplement no. 1.
Vendreley, C. and R., 1949. *A. lixula*, *P. lividus.*

OIL

See also Lipids

Size.—Of oil globules in egg. Diameter 0.6–1.0 μ (E. B. H. per E. N. Harvey, 1932a; E. B. Harvey, 1936). Smaller than 1 μ (Chambers, 1938a); *et al.*

Density.—Lightest material in egg; goes to centripetal pole in centrifuged eggs, forming an oil cap (Lyon, 1906a, 1907); McClendon, 1909a; E. B. Harvey, 1932, 1936, etc.; *et al.*).

Amount.—Of oil in oil cap. 1 % of egg (E. N. Harvey, 1932a); 2 % (Costello, 1939). In *Lytechinus variegatus* there is usually no oil cap but sometimes a few oil drops at centripetal pole; good oil cap in dilute (80 %) sea water (E. B. H.).

Decrease.—In number of oil drops (in sections) from unfertilized egg to 4-cell stage, slight increase in blastula (Pelluet, 1938).

Stain.—Oil cap does not stain with any vital dyes tried (E. B. Harvey, 1941c). In fixed preparations; oil cap does not blacken much with osmic acid and does not stain (Lyon, 1907). Black with osmic acid, subsequently bleaches with turpentine; black with Benda-Kuhl (E. B. Wilson, 1926); cf. Pelluet (1938). Oil cap does not show in preparations fixed in Bouin but does after formalin and Flemming (E. B. Harvey, 1940c; E. B. Harvey and Lavin, 1944).

Ultraviolet Light.—Oil cap is slightly absorbing (E. B. Harvey and Lavin, 1944).

Effect of Ammonium Salts.—"Lipophanerosis" (see Needham, 1942, p. 206), increase in size of oil cap, probably due to release of bound oil (Heilbrunn, 1936; Wiercinski, 1944; cf. Kopac (1948a); see also Navez 1938; Navez and Du Bois, 1940;

Parpart (1941) found no dec rease in bound lipid).

Effect of $CaCl_2$.—On oil cap, agglutination; of NaCl and KCl, dispersion (Chambers, 1938a).

Coalescence.—Of oil globules of centrifuged egg into a single mass by heat or formalin then compression (Chambers, 1938a). Coalescence of egg with oil drops on the surface and interfacial tension of oil-protoplasm and oil-water (Chambers, 1935b, 1936, 1937a, 1938b; Chambers and Kopac, 1937a; Kopac, 1938, 1939a, b; 1940a, b, 1943, 1944, 1948a, 1950; Kopac and Chambers, 1937, 1938).

Other Species

Chambers and Kopac, 1937c, *Lytechinus variegatus*, *Echinometra lucunter*, coalescence of oil drops.

Tennent, Gardiner, and Smith, 1931. *Echinometra lucunter*, staining of oil.

OSMOTIC PRESSURE

Freezing Point Depression. Δ, of a gram molecular solution of a nonelectrolyte = 1.86°C. and corresponds to an osmotic pressure of 22.4 atmospheres at 0 °C. Since osmotic pressure varies directly with the freezing point depression, 22.4/1.86 (or 12) × Δ gives the osmotic pressure. Osmotic pressure of sea water at Woods Hole is 21.7 atmospheres at 0 °C. (Chemical room, M. B. L.). According to Garrey (1915) it is 21.9 atmospheres. Osmotic pressure of eggs is same as sea water.

Freezing point depression, Δ, of sea water in different localities in —°C.:

Beaufort, N. C.	—2.04 (Garrey, 1915)
Naples, Italy	—2.29 (Botazzi, 1897)
	—2.2–2.3 (E. B. Harvey, 1933a)
	—2.2–2.43 (D'Amora, 1937)
New York (old aquarium)	—1.85 (W. H. Cole, 1940, per H. W. Smith)
Pacific Grove, Cal.	—1.905 (Garrey, 1905, 1915)
Salisbury Cove, Me.	—1.759 (W. H. Cole, 1940)
Tortugas, Florida	—2.03 (McClendon, 1910b)
Woods Hole, Mass.	—1.805 (Garrey, 1905; Chemical room, M. B. L.)

Osmotic pressure calculated for a molecular solution does not always equal osmotic pressure as ascertained by measuring volume changes of the eggs, e.g., sugar, $CaCl_2$ (Loeb, 1913a, p. 130; Heilbrunn, 1952, p. 127).

Salt Solutions.—Isomotic with the sea water and eggs at Woods Hole (salinity 31, Δ 1.805 °C.). Solutions used in Chemical room of M. B. L.: (Kindness of G. M. Cavanaugh).

NaCl	0.52 M	LiCl	0.60 M
KCl	0.53 M	CsCl	0.53 M
$CaCl_2$	0.30 M	RbCl	0.58 M
$MgCl_2$	0.30 M	NaBr	0.53 M
$MgSO_4$	0.81 M?	Na_2HPO_4	0.40 M
NH_4Cl	0.53 M (Heilbrunn)	Na_2SO_4	0.53 M

Cane Sugar Solution.—Isosmotic with the eggs at Woods Hole, as ascertained by swelling and shrinking: 0.85 M (E. B. H.). Loeb (1913a, p. 130) gives 0.75 M and Garrey (1915) gives 0.73 M. For centrifuging use $\frac{2}{3}$ isosmotic sugar solution to $\frac{1}{3}$ eggs in sea water. See Part III, Centrifuging. Cane sugar solution isosmotic with eggs in sea water at Naples is 1.0 molar (E. B. Harvey, 1933a).

Dextrose Solution.—Isosmotic with Woods Hole sea water is 0.95 molal (Lucké, 1931).

Cleavage.—Is arrested if osmotic pressure is less than 13.2 atmospheres or more than 28.7 (Churney, 1940).

OXYGEN CONSUMPTION

See Respiration

OXYGEN-LACK (ANAEROBIOSIS) AND LOW OXYGEN TENSION

Size of Egg.—Slightly smaller in absence of oxygen (Hunter, 1936), but not statistically significant (Keckwick and E. N. Harvey, 1934).

Life-Span.—Of unfertilized egg increased, slightly, much less than with KCN (Loeb and Lewis, 1902; Loeb, 1911, 1913a, p. 26).

Unfertilized Egg.—Remains normal for 8 hours in absence of oxygen, with slight delay in formation of fertilization membrane and cleavage when fertilized (in air) after 3 or more hours without oxygen (E. B. Harvey, 1930); normal for 5 hours (Barron, 1932).

Fertilization Membrane.—No fertilization membrane formed if fertilized in absence of oxygen, but sperm are not motile (E. B. Harvey, 1930; see Barron, 1932). Fertilization membrane is formed on parthenogenetic eggs in absence of oxygen (Kitching and Moser, 1940).

Cleavage Arrested.—Reversibly (Loeb, 1895, translated 1905; 1913a, p. 25; Lyon, 1902, susceptible period 10–12 minutes after fertilization; E. B. Harvey, 1927, for *P. lividus* and *Ps. microtuberculatus*, susceptible period just before cleavage; E. B. Harvey, 1930, for *A. punctulata;* Amberson, 1928; Tang and Gerard, 1932; Runnström, 1935a, even with pyocyanine; Clowes and Krahl, 1940).

Low Oxygen Tension.—On respiration and cleavage. Oxygen consumption practically constant between 228 and 20 mm. Hg, reduced below 20 mm. Hg. Cleavage not retarded until below 11 mm. Hg, and arrested below 4 mm. Hg (Amberson, 1928). Similar results by Tang and Gerard (1932); Kitching and Moser 1940; Clowes and Krahl (1940).

Reversed by ATP (Barnett, 1953).

On respiration of unfertilized eggs. Oxygen consumption begins to fall at 20 mm. Hg (Tang 1931a).

Asters.—Not formed, or disappear in absence of oxygen; reversible (Mathews, 1907; E. B. Harvey, 1921, 1930).

Cleavage Planes.—Obliterated by oxygen-lack, but on admitting air, irregular cleavage planes come in and go (Loeb, 1905, p. 401; E. B. Harvey, 1927, 1930) and result in normal blastulae and plutei. Similar results with urethane and ether (see Anaesthetics), and high hydrostatic Pressures, q.v.).

Embryos.—Disintegrate in lack of oxygen (Lyon, 1902).

X-Rayed Eggs.—Have less cleavage delay in absence of oxygen (Anderson, 1939)

Toxicity.—Of salts, etc. reduced (Loeb, 1910).

Amoeboid Motion Produced by Urea.—Is arrested; also arrested by high hydrostatic pressures (Kitching and Moser, 1940).

Echinochrome.—Is released from unfertilized eggs in O_2 lack (Shapiro, 1946). Echinochrome not changed to the reduced form by O_2 lack. See under Echinochrome.

Permeability.—To water and ethylene glycol not affected by oxygen-lack (Hunter and E. N. Harvey, 1936; Hunter, 1936, correcting Keckwick and E. N. Harvey, 1934, Hunter and E. N. Harvey, 1935).

Parthenogenesis.—Caused by oxygen-lack (Mathews, 1900; McClendon, 1909b, 1910b).

Sperm.—Immobilized by absence of oxygen, reversible till after 3 to 4 hours exposure. Fertilizing power lost and no recovery after 4 hours (E. B. Harvey, 1930; see also Barron, 1932)..Motility and fertilizing capacity maintained by glycine and other amino acids (Tyler and Lord Rothschild, 1951; Tyler, 1953).

Other Species and Reviews

Loeb, 1913a, and many other references. *Strongylocentrotus purpuratus.*
Tang, 1933, 1941. Reviews.

PARTHENOGENESIS (ARTIFICIAL PARTHENOGENESIS)

Historical.—A. Natural parthenogenesis of Echinoderms (to gastrula stage) was first described by Greeff in 1876 in *Asteracanthium rubens (Asterias glacialis);* though doubted by some, this was generally conceded correct (O. Hertwig, 1890, p. 304). It was described in several species of sea urchins, including *Arbacia lixula* by Viguier (1900a, b), working in Algiers. He claimed that the eggs even developed to plutei in normal sea water. This was questioned by Loeb (1901) and others. Lyon (1903) found that *A. lixula* had a natural tendency to parthenogenesis, but only after 20 to 24 hours in sea water. Loeb (1900a, p. 469) states that *A. punctulata* eggs reach a 2- or 4-cell stage after standing about 24 hours in sea water. Mathews (1907, p. 107) says that *Arbacia* has a typical non-parthenogenetic ovum, and *Asterias* is almost parthenogenetic.

B. Artificial parthenogenesis in sea urchins was first studied by O. and R. Hertwig in 1887, who found that eggs of *P. lividus* formed a membrane when shaken with chloroform. R. Hertwig (1895, 1896) obtained 2-cell stages with strychnine. In *Arbacia punctulata*, cleavages to about 64 cells, were obtained first by Morgan who read a paper before the Morphological Society Dec. 26, 1897, and published his results in Feb. 1898 in a preliminary paper, and in June 1899 in a complete paper; he used NaCl, KCl or $MgCl_2$ in sea water. Loeb's first paper was published in Oct. 1899; he was the first to obtain parthenogenetic *plutei (Arbacia punctulata)*, using $MgCl_2$ in sea water. He extended the work to other species, especially the Californian *Strongylocentrotus purpuratus*, and devised many other methods, seeking a physico-chemical explanation of development. He published about 75 papers on this subject, and his book on *Artificial Parthenogenesis* (1909, translated in 1913) is a classic.

Experimental.—A. Best method for *Arbacia punctulata.* This is hypertonic sea water for about 20 minutes. The sea water is made hypertonic either by boiling to half its volume, or by adding 30 gm. NaCl per liter. Different batches respond differently, as also do the different egg fractions. A slight variation in time of exposure may give better results in some batches (E. B. Harvey, 1936, 1940c). Some of the methods listed give only fertilization membranes, others give a few cleavages and others give normal plutei.

B. Methods which have proved effective for *Arbacia punctulata*

a. Physical.

1. Mechanical agitation (McClendon, 1909b, 1910b). Effective for *Asterias* but not for *Arbacia* (Mathews, 1901b, 1907).

2. Puncture (Moser, 1939b; Kitching and Moser, 1940).

3. Electricity. Induction shocks (McClendon, 1909b, 1910b). Direct current (Moser, 1939b). No effect of electric current (R. S. Lillie and Cattell, 1925).

4. Heat, around 32 °C. (Mathews, 1900; McClendon, 1909b, 1910b; Loeb, 1913a, p. 185; Heilbrunn, 1925a, 1928, p. 262).

5. Cold, 0°–10 °C. (Morgan, 1900b; Greeley, 1902; McClendon, 1909b, 1910b; E. B. Harvey, 1936, 24 hrs. at 8°).

6. Photodynamic action (visible light) (R. S. Lillie and Hinrichs, 1923; Hinrichs, 1926a; + rose bengal or eosin, Alsup, 1941).

7. Ultraviolet light (Loeb, 1914a; R. S. Lillie and Baskervill, 1922; Heilbrunn and Young, 1930; E. B. Harvey and Hollaender, 1937, 1938; Nebel, E. B.

Harvey, and Hollaender, 1937; Hollaender, 1938; Moser, 1939b; E. N. Harvey, 1942).

8. X-rays (E. B. Harvey unpub.; negative, Richards, 1915).

b. Chemical.

1. Oxygen lack or diminished oxygen (Mathews, 1900; McClendon, 1909b, 1910b).

2. Hypertonic sea water, by evaporation (S. J. Hunter, 1901, 1903; Greely, 1903; McClendon, 1909b, 1910b; Glaser, 1913; Just, 1928c; E. B. Harvey, 1936, 1940c).

3. Hypotonic sea water or distilled water (McClendon, 1909b, 1910b; Glaser, 1913; Just, 1928a, 1939a, p. 45, distilled water 15 seconds; 1939b, p. 233; Heilbrunn, 1928, p. 261; E. B. Harvey, 1940c).

4. Salts.

NaCl (Morgan, 1898, 1899, 1900a, b; Loeb, 1900b, 1901, 1913a, p. 60, etc.; Greeley, 1903; McClendon, 1909b, 1910b; Heilbrunn, 1915a, 1928, p. 261; C. R. Moore, 1917; Just, 1922a I, 1939a, b, p. 224; E. B. Harvey, 1936, 1940c; *et al.*).

NaCl, NaBr, $NaNO_3$, NaI, NaCNS (R. S. Lillie; 1910, 1911b).

Na_2SO_4 (Just, 1929a).

KCl (Morgan, 1899, 1900b; Loeb, 1900a, b, 1901, 1913a, p. 60, etc.; Just, 1922a I, 1939a, b, p. 224; E. B. Harvey, 1936).

KI, KCNS (R. S. Lillie, 1910, 1911b).

$CaCl_2$ (Loeb, 1900a, 1913a, p. 59, *etc.*; Hollingsworth, 1941).

$MgCl_2$ (Morgan, 1899, 1900a, b; Loeb, 1899, 1900a, b, 1901, 1913a, p. 57, etc.; Greeley, 1903; Hollingsworth, 1941; *et al.*).

$BaCl_2$ (Just, 1929a; Hollingsworth, 1941).

$SrCl_2$ (Hollingsworth, 1941; see Heilbrunn, 1915a).

$HgCl_2$ (F. R. Lillie, 1921b; Hoadley, 1923, 1930; Heilbrunn, 1925d).

5. Acids.

HCl, HNO_3, feeble; H_2SO_4, negative (Loeb, 1900a, 1901, 1913a, p. 57, 138).

CO_2 (McClendon, 1909b, 1910b; McClendon and Mitchel, 1912; Jacobs, 1922).

Acetic acid (McClendon, 1909b, 1910b; D. Harding, 1951).

Lactic, phosphoric, butyric acids and injury substances (D. Harding, 1951).

Butyric acid, alone. Fertilization membrane only (Loeb, 1913a, p. 71, 1915a; F. R. Lillie, 1914; Heilbrunn, 1915a; C. R. Moore, 1916; Just, 1939b, p. 222). 2-cell (A. R. Moore, 1915). *Et al.*

Butyric acid + hypertonic sea water, Loeb's double method (Loeb, 1913a, p. 71, 1916, p. 99: 50 cc. sea water + 2 cc. N/10 butyric acid for 2–4 min.; sea water 10–15 min.; 50 cc. sea water + 8 cc. $2\frac{1}{2}$ m NaCl for $17\frac{1}{2}$–$22\frac{1}{2}$ min.; sea water; at 23 °C.) Heilbrunn (1915a, p. 170) advises only $\frac{1}{2}$ min. in 50 cc. sea water + 2.8 cc. N/10 butyric, then hypertonic sea water. This method has been used by Just (1939b, p. 222) and many others, but hypertonic sea water alone is much simpler and gives good results.

Many other fatty acids have been used by Loeb (1913a, pp. 67, 134, 185) for *Strongylocentrotus purpuratus*, and would probably work for *Arbacia*.

6. Alkalis and amines.

NaOH, KOH, NH_4OH (Loeb, 1900a, 1913a, p. 57; McClendon, 1909b, 1910b); membranes form in solutions more alkaline than pH 9.0 (Smith and Clowes, 1924b). NH_4OH (weak base) better than NaOH, KOH or tetraethylammonium hydroxide (strong bases) (Loeb, 1912b, 1913a, p. 147).

Amines (butylamine, benzylamine, protamine (Loeb, 1913a, p. 149).

7. Fat solvents, esters and narcotics.

Toluol (Heilbrunn, 1915a, 1928, p. 261; Heilbrunn and Young, 1930;

Moser, 1939b).
Chloroform (Mathews, 1900; Heilbrunn, 1915a, 1928, p. 261).
Ether (Mathews, 1900; McClendon, 1909b, 1910b).
Alcohol (Mathews, 1900).
Benzol, toluol, amylene, chloroform, aldehyde, salicylaldehyde, ether, alcohol, propyl alcohol, given by Loeb, 1913a, pp. 181–183, probably refer to *Strongylocentrotus purpuratus*, but some may have been tried also on Arbacia.
Acetone (Heilbrunn, 1913; Just, 1929a).
Chloretone, urethane, chloral hydrate, esters: —methyl acetate, ethyl acetate, ethyl butyrate, methyl salicylate (Heilbrunn, 1913).

8. Detergents.

Bile salts and soap (Loeb, 1913a, p. 177, probably *S. purpuratus*).
"Dreft" (E. B. Harvey unpub.).

9. Glucosides

Saponin (Heilbrunn, 1915a, 1928, p. 261; Moser, 1939b; Kitching and Moser, 1940).
Saponin, solanin, digitalin (Loeb, 1913a, p. 174, probably refers to *Strongylocentrotus purpuratus*).

10. Alkaloids.

Strychnine (Morgan, 1900a, b; Mathews, 1900).
Quinine, pilocarpine (Mathews, 1900).

11. Nonelectrolytes.

Sucrose (Loeb, 1900b, 1913a, p. 60; E. B. Harvey, 1936; Moser, 1940).
Urea (Loeb, 1900b, 1913a, p. 60; Moser, 1940); Kitching and Moser, 1940).
Thiurea, glycerine (Moser, 1940).
Acetamide (Heilbrunn, 1913).

12. Proteins, enzymes, organ extracts.

Egg albumen (Heilbrunn, 1915a, 1921, 1928, p. 261).
Blood serum (Loeb, 1912b, 1913a, p. 194 footnote; see Heilbrunn, 1915a).
Thrombin from ox blood (Just, 1929a).
Ovarian extract (Glaser, 1913, 1914c).
Sperm extract (Sampson, 1926, but see Loeb, 1901, 1913a, p. 201; Gies, 1901; Frank, 1939).
Extract of injured tissues (D. Harding, 1951).
Lipolysin (A. E. Woodward, 1918).
Hirudin (Just, 1929a).
Papain? (Loeb, 1901).

13. Miscellaneous.

KCN (McClendon, 1909b, 1910b; see Heilbrunn, 1915a; M. M. Brooks, 1946a, b, Lyon, 1903 for *P. lividus*.
Picric acid (trinitrophenol) (Heilbrunn, 1913).
Chlorine (Heilbrunn, 1925d).
Iodine (Woodward and Hague, 1917).
Tannine and ammonia (R. S. Lillie, 1910; McClendon, 1910b).
"Hexaresorcinol" (E. B. Harvey unpub.).
Vitamin K (2-methyl-1,4-naphthoquinon) (Halaban, 1949).

C. Fertilization after parthenogenesis. See Part II, Chapter 13, paragraph *p*, p. 108.

D. Development of parthenogenetic eggs. Some batches develop much better than others. Development same as in fertilized eggs, but slower (many observers); first cleavage 1½ to 5 hrs. for parthenogenetic, 50 min. for fertilized (E. B. Harvey, 1936, and unpub., E. B. Harvey and Hollaender, 1938). Parthenogenetic plutei of *A. punctulata* have been obtained in early stages but not carried through metamorphosis, though this could undoubtedly be done. Shearer and Lloyd (1913) raised 15 parthenogenetic *Echinus esculentus* through metamorphosis; this took 8 weeks, whereas

normal eggs take 5–6 weeks; they died soon after. Delage (1909) raised two parthenogenetic *Paracentrotus (Strongylocentrotus) lividus* through metamorphosis (60 days) to the young adult of 18 months; they then measured 3.1 and 3.6 cm. overall, the tests 1.5 and 2.2 cm. One was a ♂ with ripe sperm, the other immature, but probably ♂. The ♂ is digametic in sea urchins (Tennent, 1911b, 1912a, b, c in *Toxopneustes* and *Hipponoë*, and Baltzer, 1913, in *P. lividus* and *Ps. microtuberculatus*).

E. Physiology of parthenogenetic eggs. Same as fertilized eggs for increase of oxidation rate (McClendon and Mitchell, 1912; Keltch and Clowes, 1947; Warburg, 1910 for *S. lividus*); for increase of viscosity (Heilbrunn, 1915a, 1928, p. 261), for increase of permeability (R. S. Lillie, 1916a) etc.

F. Cytology of parthenogenetic eggs. Different from fertilized eggs on account of absence of ♂ nucleus (Morgan, 1899, 1900b; E. B. Harvey and Hollaender, 1937, 1938; Nebel, E. B. Harvey, and Hollaender, 1937). See careful study of *Toxopneustes* (E. B. Wilson, 1901a); also Hindle (1910 for *S. purpuratus*). Half number of somatic chromosomes (16–18) in early cleavage (E. B. Harvey, 1940c); 18 in *Toxopneustes* (E. B. Wilson, 1901a) and in *S. purpuratus* (Hindle, 1910).

G. Parthenogenetic merogones. Development (parthenogenetic) of non-nucleate half and quarter eggs, broken by centrifugal force; also of parthenogenetic nucleate fractions (E. B. Harvey, 1936, 1940b, c, 1946a, 1951; E. B. Harvey and Hollaender, 1937, 1938). See Part III, Chapt. 21 *e* and Plate XI.

Other Species and General Rerefences

Bronn's *Thier-Reich*, 1904, pp. 1213–1223. Review and methods.
Dalcq, 1928. *Les Bases Physiologiques de la Fécondation et de la Parthénogénèse.*
Delage, 1901. 1908. *Paracentrotus lividus;* methods and cytology.
E. B. Harvey, 1938a. Naples species, parthenogenetic merogones.
E. N. Harvey, 1910a, b. Methods; also *Lytechinus variegatus.*
Heilbrunn, 1913, p. 349. Methods; 1915a. General.
F. R. Lillie, 1919. *Problems of Fertilization.*
R. S. Lillie, 1941. Review.
Loeb, 1913a. *Artificial Parthenogenesis and Fertilization.*
Loeb, 1916. *The Organism as a Whole*, pp. 95–127.
Lyon, 1903. Naples forms, including *A. lixula.*
McClendon, 1910b, p. 245. Methods.
Morgan, 1927. *Experimental Embryology*, pp. 537–593.
Tabulae Biologicae, 1927, Bd. IV, p. 216.
Tyler, 1941a. Review.
Vandel, 1931. *La Parthénogénèse.*
E. B. Wilson, 1925. *The Cell*, pp. 467–487.

PERIVISCERAL FLUID (COELOMIC FLUID, BLOOD)

See Amoebocytes, Leucocytes

Historical.—Studied in other species by Quatrefages (1850); Williams (1852) who called it "cyclaqueous fluid"; Geddes (1880); Gamgee (1880); MacMunn (1883, 1885); Cuénot (1891a, b). Referred to in *Arbacia punctulata* by Mathews (1900); McClendon (1910b), etc.

Contains.—Amoebocytes, Leucocytes (McClendon, 1912a; McClendon and Mitchell, 1912; Kindred, 1921, 1926; Donnellon, 1938; *et al.*). Also other kinds of cells (H. V. Wilson, 1924).

Inorganic Composition.—Identical with sea water in *Echinus esculentus* (J. D. Robertson, 1939; he thinks the small differences found by Bethe and Berger, 1931, due to inaccurate methods). Identical with sea water in *Strongylocentrotus dröbachiensis* and

Echinarachnius parma (W. H. Cole, 1940). For calcium in *A. punctulata* Schechter (1937) gives, from analysis by Mazia, 0.395 mg./cc. of calcium in coelomic fluid against 0.41 mg./cc. in sea water.

Chemistry.—For *A. punctulata*, see Van der Hyde (1922). For *Paracentrotus lividus* see Mourson and Schlagdenhauffen (1882).

Toxicity to Eggs.—Perivisceral fluid harmful to eggs (Fol, 1879, p. 86). Plasma (filtered) inhibits fertilization (F. R. Lillie, 1914, 1919, p. 173; Lillie and Just, 1924; Just, 1922a, III; A. E. Woodward, 1918). But others found it is not the serum in the perivisceral fluid but material from the pigmented amoebocytes (echinochrome?) that prevents fertilization (Pequegnat, 1948; Couillard, 1952). Still others found that the toxic substance is not the perivisceral fluid but a dermal secretion or material from the outside of the shell (Ohshima, 1921; E. B. Harvey, 1939b). The toxic substance was found to have no effect on cleavage if eggs were exposed after fertilization (Ohshima, 1921; Pequegnat, 1948; Couillard, 1952). Runnström (1950–1951) says toxic effect can be removed by sodium periodate.

Toxicity to Sperm.—Serum not harmful (F. R. Lillie, 1912, 1919, p. 174; Just, 1922a, III; Lillie and Just, 1924, p. 495; Ohshima, 1921).

Contains Agglutinin.—(F. R. Lillie, 1912, 1914; Couillard, 1952).

Increases Viscosity of Eggs.—(Goldforb, 1935b).

Clotting.—Caused by cells or cell extracts, not plasma (Heilbrunn, 1928, p. 228; Donnellon, 1938). Caused by leucocytes (Kindred, 1921; H. V. Wilson, 1934). For effect of various substances on clot formation (Donnellon, 1938).

Other Species (additional) and General References

Barnes and Rothschild, 1950. *Echinus esculentus*, copper content.
Bialaszewicz, K., 1933. *P. lividus*, *Sphaerechinus granularis*, mineral content.
Bogucki, 1930. *Paracentrotus lividus*, re harmful action.
Davidson, 1952. *Echinarachnius parma*, clotting.
Ephrussi, 1925. *P. lividus*, fertilization membrane.
Grassé, 1948. *Traité de Zoologie*, general.
Kindred, 1924. *S. dröbachiensis*, *S. franciscanus*, *Echinarachnius excentricus;* cellular elements.
Pantin, 1931. General.
Robertson and Webb, 1939. Estimation of inorganic content.
Tyler, 1946. *S. franciscanus*, *S. purpuratus*, *Lytechinus pictus*, *Dendraster excentricus;* heteroagglutinins.
Webb, 1937. *E. esculentus*, *P. lividus;* inorganic content.

PERIVITELLINE SPACE AND CONTENTS

Definition.—The perivitelline space is the space between the egg surface or vitelline membrane and the fertilization membrane.

Width.—3 to 5 μ (E. B. Harvey per E. N. Harvey, 1932a). May be 6.5 μ (E. B. H.). Decreased under various conditions so as to be practically obliterated (E. N. Harvey, 1910b; Just, 1928a; *et al.*). With 1 to 2% egg albumin (Loeb, 1913a, p. 208; Heilbrunn, 1915a; R. S. Lillie, 1918b). With 2% Witte's peptone (Garrey, 1919). Increased, 2 or 3 times, with urea treatment (Moser, 1940). Differs in width in different species (E. B. Harvey, 1933a, 1934; *et al.*).

Contents.—Chiefly sea water (E. N. Harvey, 1910b). Sea water and a colloid (Loeb, 1913a, p. 207; 1916, p. 108). A liquid probably containing colloids (R. S. Lillie, 1911a; Garrey, 1919; Glaser, 1924: *et al.*). A jelly-like liquid, as concluded by Fol in 1879 (E. B. Wilson, 1925, p. 413). A gel containing colloids; appears striated when under tension or when extended by electric current (McClendon, 1910b, 1914a). Striated with aceto-carmine; striations are formed by coalescence of spheres from hyaline layer (E. B. Harvey unpub.).

Liquid removed with pipette from perivitelline space by Chambers causing collapse of membrane (Garrey, 1919). Fine suspension of carbon in sea water injected in perivitelline space (Chambers, 1942).

Electrical Properties.—Bears positive charge (McClendon, 1910b, 1912b, 1914a).

Other Species (additional)

Gray, 1927b. *E. esculentus, Psammechinus miliaris.*
Hiramoto, 1954. *Hemicentrotus (Strongylocentrotus) pulcherrimus.*
Hobson, 1927. *E. esculentus, Ps. miliaris.*
Mitchison and Swann, 1953. *Ps. miliaris,* colloid.

PERMEABILITY

A. Unfertilized Egg.—

1. Penetration of water; osmotic properties. Change in size in hypo- and hypertonic sea water (Sollman, 1904b; R. S. Lillie, 1910–1918; Loeb, 1913a, p. 61; Lucké, *et al.* including McCutcheon, Hartline, Larrabee, Ricca, Parpart, 1926–1951; Northrop, 1927; Jacobs, 1933a, b, c; Stewart and Jacobs, 1936; E. B. Harvey, 1943. See Table 12; Shapiro, 1948b, *et al.* Reviews by Lucké and McCutcheon, 1932; Lucké, 1940; Wilbrandt, 1941 in *Tabulae Biologicae,* Vol. 19 (Pt. 2), pp. 371–389 for tables. See Plate XIV.

Equilibrium size given by $(V_o - b)P_o = (V_{ex} - b)P_{ex}$, where V_o = volume in sea water, P_o = osmotic pressure sea water, V_{ex} = volume in concentrated or diluted sea water, P_{ex} = osmotic pressure in concentrated or diluted sea water, b = osmotically inactive material (Lucké and McCutcheon, 1932; Lucké, 1940). Complete recovery on return to sea water. Temperature has no effect on equilibrium (Lucké, 1935).

Osmotically inactive material b, 6–20 %, average 12 % (McCutcheon, Lucké, and Hartline, 1931; Lucké, Larrabee, and Hartline, 1935). Increase on fertilization (Shapiro, 1948b).

Rate of water penetration. Permeability to water is defined as:

$$\frac{dV}{dt} = kA(P - P_{ex}),$$

where dV/dt = rate of change in volume, A = surface area, P = osmotic pressure at time t, P_{ex} = osmotic pressure within egg or of solution with which cell is in equilibrium, and k the permeability constant. For integrated equations and discussion of derivation see Lucké and McCutcheon, 1932, Lucké, 1940.

Endosmosis and exosmosis. For water entering eggs at 20 °C., k = 0.087 μ^3 per μ^2 surface per atmosphere difference of pressure per minute; for water leaving eggs, k = 0.141 μ^3. At 15 °C., k = 0.05 — 0.06 for endosmosis and 0.07–0.08 for exosmosis. Values are independent of osmotic pressure but depend on kind and proportions of salt in medium, injury, narcotics, temperature, etc. (Lucké, Hartline, and McCutcheon, 1931). At 22 °C. by diffraction method of study, k = 0.106 for endosmosis and 0.127 for exosmosis (Lucké, Larrabee, and Hartline, 1935); also Stewart and Jacobs, 1936.

2. Penetration of heavy water same as water (Lucké and E. N. Harvey, 1935).

3. Penetration of non-electrolytes. Permeability to a solute (S) may be defined as

$$\frac{dS}{dt} = kA\left(C_S - \frac{S}{V}\right)$$

where dS/dt = rate of change of amount of solute; A = surface area; C_S = external concentration; V = volume of the egg., k = number of moles that will penetrate

1 μ^2 of surface in 1 minute with a concentration difference between exterior and interior of 1 mole per liter (Jacobs and Stewart, 1932). For additional methods of study see Jacobs, 1933a, b, c. Values of k: ethylene glycol, 3.6×10^{-15}; acetamid, 5.8×10^{-15}; propionamid, 14.2×10^{-15}; butyramid, 36.6×10^{-15}; glycerol, 05×10^{-15} (Stewart and Jacobs, 1932a). For large effect of temperature, see Stewart and Jacobs, 1932b. At 21.5–23 °C. k for diethylene glycol, 2.6×10^{-15}; ethylene glycol 4.4×10^{-15}; propylene glycol 7.7×10^{-15} (Stewart and Jacobs, 1936). Ethylene glycol k at 24° by diffraction method, 4.0×10^{-15} (Lucké, Larrabee, and Hartline, 1935); See also Stewart, 1931a and Lucké, Hartline, and Ricca, 1939. No effect of lack of oxygen on penetration of ethylene glycol (Hunter, 1936).

4. Penetration of ammonium salts. Salts of strong acids do not penetrate while salts of weak acids penetrate due to entrance of undissociated acid and ammonia (Stewart, 1931b, Jacobs and Stewart, 1936).

5. Penetration of fatty acids and their salts. Deduced from effect on viscosity (Howard, 1931). See also Hydrogen Ion.

6. Penetration of various agents active in suppressing cleavage and other egg activities, see Krahl review, 1950, pp. 189–192.

7. Penetration of ions. Potassium (Shapiro and Davson, 1941). Radioactive phosphate (Na_2HPO_4); P^{32} absorption is connected with cell activity, 40 times greater in fertilized egg (Abelson, 1947, 1948).

8. Penetration of dyes. See Vital Dyes.

9. Factors affecting permeability.

Age. Increase (Goldforb, 1935c).

Anaesthetics and narcotics. For general statements see R. S. Lillie, 1912, 1914a, b, 1916c, 1917, 1918a, b, and Heilbrunn, 1925c. Urethanes, decrease in isotonic glucose, no change in sea water (Lucké and McCutcheon, 1926a, 1932, Lucké, 1931). See Anaesthetics.

Caffeine. No effect (Cheney, 1948).

Carcinogens. Choleic acids of 10-methyl benzanthrene, 20-methylcholanthrene, 1, 2, 5, 6 dibenzanthrene do not affect k for water although they retard or prevent cleavage (Lucké, Parpart, and Ricca, 1941).

Cyanide. McClendon, 1909b; R. S. Lillie, 1918a, b; HCN increases, KCN decreases (Blumenthal, 1927, 1928).

Electric current. 60 cycle A. C. has no effect in sea water but causes a slight decrease in isotonic glucose containing small amounts of NaCl, KCl and $CaCl_2$ (Fowler, 1934).

Electrolytes. For general statement see Mathews, 1905; R. S. Lillie, 1910, 1911a, b, 1912, 1914a; Lillie and Baskervill, 1921, 1922; McClendon, 1910a; Lucké and McCutcheon, 1926a, b, 1929, 1932.

Absence of ions (glucose solution) increases k for water from 0.05 to 0.1 at 12 °C, and 0.0001 M $CaCl_2$ or $MgCl_2$ added to glucose solution maintains k same as in sea water (McCutcheon and Lucké, 1928).

Cations decrease permeability to water, the effectiveness increasing with the valence of the cation. In 0.38 M dextrose solution containing 0.005 M K_3 citrate (in which solution cells have high water permeability), the following concentrations of cobaltamine chlorides were required to reduce permeability to the value obtained in sea water: —0.00005 M of the 6 valent salt, more than twice as much of the 4 valent salt, more than eight times as much of the 3 valent, and 64 times as much of the 2 valent salt, while this amount of the 1 valent salt was incompletely effective. Temperature 12° ± 0.5 °C. (Lucké and McCutcheon, 1929).

Anions increase permeability to water, the effectiveness increasing rapidly with the valence of the anions. In 0.38 M dextrose solution containing 0.0005 M $CaCl_2$, 0.001 M of potassium ferrocyanide was required to definitely increase permeability, twice as much ferricyanide, four times as much potassium sulphate, and eight times

as much chloride. Temperature 12° ± 0.5 °C. (Lucké and McCutcheon, 1929).

H-ion concentration. No effect on penetration of water (Lucké and McCutcheon, 1926b). See also Hydrogen Ion.

For the effect of pH on penetration of many active compounds which are salts of weak acids or bases, see Smith and Clowes, 1924, and Haywood and Root, 1930, 1932, for bicarbonates; see Krahl and Clowes, 1938 and Hutchens, Krahl, and Clowes, 1939, for substituted phenols; see Krahl, 1940, Clowes, Keltch, and Krahl, 1940 for barbiturates and see Krahl, Keltch, and Clowes, 1940a for local anaesthetics.

Injury. Increase (Lucké and McCutcheon, 1926a, b, 1930, 1932; decrease, Goldforb, 1935c).

Jelly coat. No effect (R. S. Lillie, 1917).

Leucotaxine. Increases k for water from 0.12 to 0.19 (Menkin, 1940).

Non-electrolytes. Increase k for water from 0.05 for sea water to 0.097 for glucose, 0.103 for saccharose, and 0.142 for glycocoll (McCutcheon and Lucké, 1928). See also McClendon, 1910a.

Organic extracts. *Arbacia* egg extracts increase (Glaser, 1914c); manmalian testis and spleen increase (Favilli, 1932).

Oxygen lack. Slight increase in k to water, no effect on osmotic equilibrium (Keckwick and E. N. Harvey, 1934); no effect on k for water or ethylene glycol but slight decrease in volume of egg (Hunter and E. N. Harvey, 1936; Hunter, 1936).

Sea water concentration. No effect on k for water, as was once supposed (McCutcheon and Lucké, 1926; Lucké and McCutcheon, 1927, 1932; Lucké, Hartline, and McCutcheon, 1931; Lucké, Larrabee, and Hartline, 1935.

Temperature. Higher temperatures greatly increase k to water. Q_{10}, 2 to 3 and μ values, 13000–17000 (Lucké and McCutcheon, 1926a, 1932; McCutcheon and Lucké, 1926, 1927, 1932; Lucké, Hartline, and McCutcheon, 1931). No effect on equilibrium (Lucké, 1935). k for ethylene glycol, propionamid and butyramid also greatly increased by rise of temperature (Stewart and Jacobs, 1932b).

Ultraviolet light. Increase (Heilbrunn and Mazia, 1936, p. 650). No effect with eggs of *Strongylocentrotus purpuratus* (Reed, 1948). See Heilbrunn, 1952, p. 164 and Part IV.

X-rays. No effect (Lucké, Ricca, and Parpart, 1951).

B. Egg Fractions.—White and red halves. See Chapter 20. (Lucké, 1932; Shapiro, 1939a; E. B. Harvey, 1943, and Table 12 and Plate XIV).

C. Fertilized Eggs and Parthenogenetic Eggs.—Increase in permeability as compared with unfertilized eggs (McClendon, 1909b, 1910a, b; E. N. Harvey, 1909, 1910c; R. S. Lillie, 1910, 1911a, b; Lyon and Shackell, 1910b; Glaser, 1913; Loeb, 1913a, p. 92, 1916, p. 119; Heilbrunn, 1915). k for penetration of water increases about 4 times (R. S. Lillie, 1916a, 1918a; McCutcheon and Lucké, 1932; Lucké and McCutcheon, 1932. k for penetration of ethylene glycol increases three times after fertilization and somewhat less after distilled water activation (Stewart and Jacobs, 1932a); k for diethylene glycol and propylene glycol doubled in fertilized eggs (Stewart and Jacobs, 1936).

Change in osmotically inert fraction on activation from 7.3 to 27.4% (Shapiro, 1948b).

For permeability rhythms see R. S. Lillie, 1910, 1911a, b, 1914b, 1916b, 1917.

D. Nucleus of Unfertilized Egg.—See Table 11 and Plate XIV for changes in size in hypo- and hypertonic sea water (E. B. Harvey, 1943).

E. Cytoplasmic Granules.—Yolk granule behavoir complicated. Pigment granules act like leaky osmometers with k for water penetration somewhat higher than for cell (Harris, 1943). For release of pigment on fertilization, see Chromatophores. The large pigment spots of plutei swell from 5 to 10 μ diam. and break in distilled water (E. B. H.).

F. Immature Egg.—More permeable than mature (Lyon and Shackell, 1910b).

G. Germinal Vesicle.—Approximates a perfect osmometer in behavoir (Churney, 1942).

H. Sperm.—The volume doubles in distilled water before bursting (E. B. Harvey and Anderson, 1943). See Shapiro, 1948e. Proc. Soc. Exp. Biol. Med. 67 : 180–182.

I. Amoebocytes.—Become spherical in distilled water before bursting (E. B. H.). See Amoebocytes.

Other Species (additional) and General References

Brooks and Brooks, 1941. The *Permeability of Living Cells.* General.
Brooks and Chambers, 1948. *S. franciscanus, S. purpuratus,* P^{32} uptake.
Chambers and White, 1949. *S. purpuratus,* P^{32} uptake.
Chambers, Whiteley, Chambers, and Brooks, 1948. *Lytechinus pictus,* P^{32} uptake.
Davson and Danielli, 1952. *The Permeability of Natural Membranes.* General.
Dorfman, 1932, 1933. *Strongylocentrotus dröbachiensis,* rhythmic changes in osmotic properties.
Fukuda, 1935. *Anthocidcris crassispina* and *Pseudocentrotus depressus,* water penetration and ionic composition of medium.
Herlandt, 1914, 1918b, 1920. *Paracentrotus lividus, Sphaerechinus granularis,* rhythmic changes in permeability.
Hobson, 1932a. *Psammechinus miliaris,* permeability to water after fertilization.
Jacobs, 1924. Cowdry's *General Cytology.* General; 1952a in *Modern Trends.* General.
Krahl, 1950. General.
Leitch, 1931, 1934a, b, 1936. *S. franciscanus, S. purpuratus, Echinometra lucunter, Dendraster excentricus,* permeability to water and non-solvent volume.
Lindberg, 1949. *Psammechinus miliaris,* P^{32} uptake.
Lucké and McCutcheon, 1932. General.
Lyon and Shackell, 1910b. *Lytechinus,* dyes.
Skowron and Skowron, 1926. *Sphaerechinus granularis,* immature egg.
Thörnblom, 1932. *Paracentrotus lividus,* change in permeability on fertilization.
Whitaker, 1936. *S. franciscanus,* water and dyes after fertilization.
Wilbrandt, 1941. General in *Tabulae Biologicae* 19, pt. 2.

PHOSPHORUS METABOLISM

See also Nucleoproteins

Amount.—Of phosphorus in unfertilized *Arbacia* egg.s 0.9064 mg. total phosphate per 10^6 eggs; 0.0291 millimoles; 10^6 eggs = 0.124 gm. dry weight (Page, 1927b). In centrifuged eggs (McClendon, 1909a). Table 17. 130 mg. phosphorus per gram nitrogen or 0.31 mg. per cent of wet weight (Crane, 1947). 96×10^{-5} micrograms phosphorus per egg (Schmidt, Hecht, and Thannhauser, 1948).

Of phosphorus in fertilized *Arbacia* eggs. Same in 2–4 cell and blastula (Shackell, 1911). This was criticised by Robertson and Wasteneys (1913) for *S, purpuratus,* and answered by Masing (1914). Uptake of P^{32} by fertilized egg is 40 times that by the unfertilized; 1.0×10^{-3} mg. per million eggs one hour after fertilization. At 10 °C. 1/7th amount phosphorus taken up as at 23° (Abelson, 1947, 1948). Uptake of P^{32} increases rapidly on fertilization and rate of uptake is not affected by x-rays (Evans, 1950).

Analysis.—Of phosphorus fractions. Table 7 in centrifuged eggs (McClendon, 1902a) and Table 17 (Villee *et al.*, 1949).

Amount of Phosphorus in Sperm.— 2.86 % (in *A. lixula,* Mathews, 1897).

Phospholipids.—(Lecithin) in unfertilized eggs. 2.17 mg. per 10^6 eggs or 38 % of total fat which is 5.65 mg. per 10^6 eggs (Hayes, 1938); he calculates 3.84 mg. phospholipid per 10^6 eggs from McClendon's (1909a) data and 8.4 mg. from Page's

(1927a, b) data. For later references, see Table 17 of Analyses of phosphorus fractions.

ATP and ADP.—In eggs (Abelson, 1948; see Whiteley, 1949). ATP restores division rate reduced to 50 % by lowered oxygen tension (Barnett, 1953).

Radioactive Phosphate P^{32}.—Used for metabolism studies by Brooks (1940, 1943; Abelson (1947, 1948); Villee, *et al.* (1949, 1950, 1952, 1953).

Effect of P^{32}.—On division rate; delay (Green and Roth, 1950).

Phosphorus Metabolism.—Inhibited by (1) usnic acid (Marshak and Harting, 1948); (2) dinitrophenol, malononitrile, uranyl nitrate, nitrogen mustard, cold; high concentrations of pilocarpine, atropine, eserine, acetylcholine, with an acceleration of phosphorus metabolism using low concentrations (Villee, *et al.*, 1949, 1952). See also Abelson (1947).

Phosphorylation.—(Clowes, 1951; Clowes, Keltch, Strittmatter, and Walters, 1950; Clowes, Keltch, and Walters, 1951a, b; Keltch, Smythe, and Clowes, 1951; Keltch, Strittmatter, Walters, and Clowes, 1950; Strittmatter, Keltch, Walters, and Clowes, 1950; Krahl's *Review*, 1950, pp. 184, 198).

TABLE 17

ANALYSES OF THE PHOSPHORUS FRACTIONS OF ARBACIA EGGS

In milligrams P per gram wet weight of egg or embryo (from Villee, Lowens, Gordon, Leonard, and Rich, 1949, p. 98

Investigator	Stage	Acid sol. P	Phospho-lipid P	Acid in-sol. P	DNA P	RNA P	Phospho-protein P
Crane (1947)	Unfertilized eggs	1.26	1.26	0.84	0.034	0.63	0.155
Schmidt, Hecht and Thann-hauser (1948)	Unfertilized eggs	—	1.52	1.06	0.039	0.924	0.108
Villee et al. (1949)	3 hr. embryos	1.42	—	0.86	0.05	0.74	0.06

See also Abelson (1947, 1948). The above table is given in a slightly modified form by Krahl (1950, p. 188). Another table including *Arbacia* and other sea urchins is given by Whiteley (1949).

Other Species (additional) and General References

Brooks and Chambers, 1948. *S. purpuratus, S. franciscanus.*
Chambers and Mende, 1953. *S. dröbachiensis.*
Krahl, 1950. Review, p. 187.
Lindberg, 1949, 1950. ATP in *Ps. miliaris* and *P. lividus.*
Masing, 1910, 1914. *A. lixula.*
Needham and Needham, 1930. *Dendraster excentricus.*
Rothschild and Barnes, 1953. Phosphorus fractions of *Paracentrotus lividus.*
Rothschild and Mann, 1950. ATP in sperm of *Echinus esculentus.*
Runnström, 1952b. General.
Wetzel, 1907. *P. lividus.*
Whiteley, 1949. *Lytechinus pictus* and General.
Zielinski, 1939. *P. lividus.*

PHOTODYNAMIC ACTION

See Visible Light

PIGMENT GRANULES

See Chromatophores, Echinochrome

PLASMA MEMBRANE

Definition.—Plasma membrane is the membrane at the surface of the protoplasm,* over the cortical layer, supposed to be responsible for the permeability of the cell. The older investigators made no distinction between plasma membrane and vitelline membrane, calling the two together the "pellicle." It is difficult to distinguish the two optically in an unfertilized *Arbacia* egg. It has been called the "luminous" layer by Runnström and Monné (1945a) in *Psammechinus miliaris*, since it is luminous in dark field. See Figs 9 and 10.

Thickness.—Less than 10 mμ (Danielli, 1942, 1951b, p. 154). The dried plasma membrane of the red blood cell is 50–60 Å (Parpart and Ballentine, 1952; Hillier and Hoffman, 1953).

Structure.—A liquid film (Chambers, 1935b, 1938b, 1944, 1949). Fatty (Chambers, 1935b). Lipoid with adsorbed protein (E. N. Harvey and Danielli, 1938; Danielli 1951b, p. 151; Davson and Danielli, 1952, p. 57).

Oil Coalescence.—Coalescence of egg with oil drops on the surface (Chambers, 1935b, 1944; Chambers and Kopac, 1937a; Kopac and Chambers, 1937; Kopac, 1940a, 1943; etc.).

Other Properties.—Very delicate, easily ruptured, becomes more fluid on churning with needle and with calcium; cannot be removed; is necessary for life of cell; can repair itself (Chambers, 1938b, 1940, 1944, 1949, 1950; Kopac, 1940a).

Other References (General)

Just, 1939b. *The Biology of the Cell Surface.*
Runnström, 1949a.

POLYSPERMY

Definition.—Polyspermy is the fertilization of an egg by two or more sperm, the egg dividing into three or more cells at first cleavage.

Historical.—Observed by Fol (1877) and studied by O. and R. Hertwig (1887) in *Paracentrotus lividus*. A good account of the early work of the Hertwigs, Boveri (1902, 1907), etc. is given in Morgan's (1927) *Experimental Embryology*, Chapt. VII. It was concluded that "in the sea urchin the division of the protoplasm is strictly regular, but the chromosomal distribution is disturbed" (p. 87) and "swimming blastulae develop from these eggs, but only very rarely a normal embryo" (p. 86). See also Wilson's (1925). *The Cell*, p. 416 and 917.

Occurs.—In *Arbacia* in unripe eggs fertilized soon after the extrusion of the second polar body (E. B. H.). See Part II, Chap. 10, sect. g.

Caused.—In Arbacia by Stale eggs, i.e., eggs left standing in sea water (Hoadley 1923; J. M. Clark, 1936, after 16 hours). Shaking (Morgan, 1893, 1895b). Cold

* Recent investigations of A. K. Parpart and P. C. Laris with a television microscope, indicate that the plasma membrane of the unfertilized *Arbacia* egg lies *inside* the cortical, layer which is between the vitelline membrane and the plasma membrane (*Biol. Bull.* 107 : 301, 1954).

5 °C. (Just, 1939b, p. 202). Excess sperm (F. R. Lillie, 1919, p. 260; J. M. Clark, 1936). But Just (1928a) thinks not. At pH 7.4–6.8, none at 7.4–9.8 (Clowes and Smith, 1923, Smith and Clowes, 1924d; J. M. Clark, 1936); A. Scott, 1946). Organic acids; aspartic (King, 1912); butyric (Just, 1939b, p. 202). SH-reagent, p-chloromercuribenzoate (Runnström and Kriszat, 1952a). Substances used by Hertwigs (1887): chloral hydrate 0.2 %; cocaine hydrochloride 0.025 %; nicotine 1 drop to 200 cc.; morphine sulphate 0.6 %; strychnine sulphate 0.1 %; quinine sulphate 0.05 %. (J. M. Clark, 1936). Salts of Na, K, Ca, Mg (J. M. Clark, 1936). Fat solvents, alcohol, ether, chloroform, ethyl urethane (J. M. Clark, 1936). Cleavage of polyspermic eggs (Scott, 1946).

Other Species (additional)

Baltzer, 1908. *Paracentrotus lividus, Psammechinus microtuberculatus.*
Brachet, 1922. *P. lividus*, during, maturation.
Bury, 1913. *P. lividus, Ps. microtuberculatus*, cold.
Ishida and Nakano, 1950. *Strongylocentrotus pulcherrimus*, fertilization after parthenogenesis.
Rothschild, 1953a. *P. lividus*, nicotine; 1954, Quart. Rev. Biol. 29: 332–342.
Rothschild and Swann, 1950, 1951. *Ps. miliaris*, nicotine.
Runnström and Monné, 1945a. *Ps. miliaris, Brissopsis lyrifera*, during maturation.
Sugiyama, 1947, 1951. *S. pulcherrimus* etc., after refertilization.
Wilson and Mathews, 1895. *Lytechinus variegatus.*

POTASSIUM

Amount in Egg.—2.445 mg. potassium per 10^6 eggs (10^6 eggs = 0.124 gm. dry weight) or 0.063 millimoles (Page, 1927b).

Amount in Sea Water.—At Woods Hole. 0.412 gm. per liter at 20 °C. (Page, 1927c).

K : Na in Eggs.—As 1.90 : 1; in sea water K : Na as 0.0213 : 1 (Blanchard per Howard, 1931). Twenty times as much K in eggs as in sea water; about same amount in unfertilized and fertilized eggs (Shapiro and Davson, 1941).

KCl Isotonic.—With sea water at Woods Hole is 0.53 M (M. B. L. Chemical Room).

Loss of K.—By eggs in sea water and accumulation of K in sea water with excess K. (Shapiro and Davson, 1941).

Uptake.—And loss of K^{42} by unfertilized and fertilized eggs (E. L. Chambers, White, Jeung, and S. C. Brooks, 1948; E. L. Chambers, 1949; Chambers and Chambers, 1949); also for *S. purpuratus.*

Replacement.—Of K ion by rubidium and cesium, but not by thorium and uranium (R. F. Loeb, 1920).

Chemical Character.—And physiological action of potassium ion (J. Loeb, 1920).

Toxicity.—KCl less than NaCl (Loeb, 1900a; R. S. Lillie, 1910, 1911a, b, 1912; Page, 1924, 1929b; Chambers and Chambers, 1938; *et al.*). For toxicity on *Arbacia* eggs, Page (1929b) arranges cations thus: Li > Na > Mg or Ca > K > Rb > Cs, used as chlorides. Toxic effect can be counteracted by $CaCl_2$ and $MgCl_2$ and certain anaesthetics (R. S. Lillie, 1911b, 1912).

Isotonic K Cl has little effect on unfertilized eggs, and only in susceptible periods on fertilized eggs; also for other K salts and for *S. purpuratus* (Chambers and Chambers, 1938, 1949).

Pigment Granules.—In unfertilized egg are not affected by isotonic KCl; in fertilized egg they break down after monaster stage (Churney and Moser, 1940). Isotonic KCl prevents action of Ca on pigment granules which causes them to break down in the surface precipitation reaction (Heilbrunn, 1928, p. 230).

Hyaline Layer.—Absent in isotonic KCl (Chambers and Chambers, 1949).

Sperm.—Immobilised in KCl at pH 6.0 (Chambers and Chambers, 1949).

Clotting of Body Fluid and Liberation of Pigment.—Hastened in the order of $SO_4 <$ $Cl < NO_3 <$ SCN (Donnellon, 1938).

Colorless Amoebocytes of Body Fluid.—Dissolved by KCl. (Mathews, 1900).

Amoeboid Eggs.—Caused by KCl (Churney, 1940).

Respiration.—No references found.

Permeability.—K causes increase of permeability in the order of KCl < KBr < KNO_3 < KCNS < KI (R. S. Lillie, 1910, 1911a, b). Increase of permeability with increase in valence of anions (McCutcheon and Lucké, 1928; Lucké and McCutcheon, 1929). Increased permeability can be counteracted by $CaCl_2$, $MgCl_2$ and certain anaesthetics (R. S. Lillie, 1911b, 1912; McCutcheon and Lucké, 1928, by $CaCl_2$ and $MgCl_2$.)

Viscosity.—KCl increases viscosity in the order: KCl > NaCl > sea water > $MgCl_2$ > $CaCl_2$ (E. B. Harvey, 1945). Heilbrunn gives the same order except that KCl and NaCl are reversed (Heilbrunn, 1923, 1928, p. 146, 1943, p. 81).

Breaking with Centrifugal Force.—Break more readily than in sea water in the order of increasing viscosity as above (reverse order of stratification), that is, the most viscous (least stratified) break most readily (E. B. Harvey, 1945).

Parthenogenesis.—Caused by KCl and other K salts. KCl added to sea water was first used by Morgan (1899) as a parthenogenetic agent and subsequently by many others. K salts listed above (under permeability) by R. S. Lillie, cause parthenogenesis, effective in the order given, but are more effective if followed by hypertonic sea water (R. S. Lillie, 1910, 1911a, b.).

Other Species (additional) and General References

Bialaszewicz, 1927, 1929. *A. lixula, P. lividus;* electrolytes in eggs.

Heilbrunn, 1952. *General Physiology*, p. 523.

Herbst, 1904. Salts necessary for development.

Loeb, 1913a. *Artificial Parthenogenesis. S. purpuratus, S. franciscanus,* especially.

Malm and Wachtmeister, 1950. *Ps. miliaris, S. dröbachiensis;* amount K in unfertilized and fertilized eggs.

Oddo and Esposito, 1951. *A. lixula, P. lividus;* changes in K content after fertilization.

Robertson, 1939; Robertson and Webb, 1939; Webb, 1939. Inorganic composition of sea water and body fluids.

Rothschild, 1948b. *E. esculentus;* K in seminal, perivisceral fluids and sea water.

Rothschild and Barnes, 1953. *P. lividus;* inorganic constituents of eggs; table of salts and species.

PRESSURE (HYDROSTATIC, INTERNAL AND MECHANICAL)

A. Hydrostatic (External) Pressure.

Unfertilized eggs.—Decrease in viscosity as shown by stratification of centrifuged eggs, 408 atmospheres (about 6,000 lbs/in^2) with force of 7,200 × g (Brown, 1934). Eggs break into halves more readily, with centrifugal force (E. B. H., 1933 unpub.). No ill effects if compressed to 680 atmospheres (10,000 lbs/in^2) for several minutes (Kitching and Moser, 1940). Eggs which had been made amoeboid (by urea) stop movement at 340 atmospheres; also stop in O_2 lack (Kitching and Moser, 1940).

Fertilized eggs.—Decrease in viscosity especially of the cortical zone as shown by displacement of peripheral pigment granules by centrifuging at 408 atmospheres with force of 7,200 × g (Brown, 1934). Pressure arrests and obliterates cleavage furrow reversibly, at 450 atmospheres (Marsland, 1938, 1942, 1950, 1951). Effect similar to Oxygen-Lack, q.v., and urethanes and ether (see Anaesthetics).

Causes unequal first cleavage of centrifuged spherical eggs along the stratification (E. B. H., 1933 unpub.); without pressure first cleavage of spherical eggs is equal

and usually perpendicular to stratification. Delays cleavage (E. B. H., 1933 unpub.; Marsland, 1938; Kitching and Moser, 1940).

Temperature and pressure.—Lowered temperature acts like increased pressure (Marsland, 1950); see Brown (1934).

Adenosine triphosphate.—Counteracts inhibiting effects of pressure (Marsland, Landau, and Zimmerman, 1953).

Insemination.—Prevented at 6,000 lbs/in^2, but sperm are active (Marsland, 1948).

Amoebocytes.—Become spherical at 400 atmospheres (Marsland, 1938).

Moderately High Pressures.—(61 atmospheres) of nitrogen and helium have no effect on cleavage; nitrous oxide delays cleavage at 2.3 atmospheres (Haywood, 1953).

B. Internal Pressure.

Internal Pressure.—Of unfertilized egg is 40 dynes per cm.^{2} (Cole, 1932). Internal pressure of unfertilized egg necessary to rupture the membrane, is of order of 1/100 atmosphere. Effect of hypotonic sea water, salts, pH, anaesthetics, ultraviolet light, trypsin on resistance to internal pressure (Rieser, 1950).

Internal pressure.—Of fertilized egg, calculated from Chambers' experiment of rupturing one of two blastomeres, 62 dynes per cm.^{2} (Sichel and Burton, 1936; see Chambers, 1938c).

C. Mechanical Pressure.

Method.—Cover slip or compressorium.

Position of Cleavage Planes and Micromeres.—Changed, cells forming flat plates (Morgan, 1893).

Obliteration of Cleavage Plane.—In *Psammechinus microtuberculatus* (?) (Boveri, 1897).

Other Species (additional) and General References

Driesch, 1892. *Echinus (Psammechinus) microtuberculatus*, mechanical pressure.
Lepeschkin, 1941a. *A. lixula* etc., mechanical pressure.
Marsland, 1939, 1951. *A. lixula*, hydrostatic pressure.
Marsland, 1942. General.
Marsland and Landau, 1950. *Echinarachnius parma*, hydrostatic pressure.
Morgan, 1927, p. 468. *Experimental Embryology*. Mechanical pressure, general.
Pease, 1942a. *Strongylocentrotus purpuratus*, hydrostatic pressure.
Ziegler, 1894. *Echinus (Psammechinus) microtuberculatus*, mechanical pressure.

PROTEINS

See also Nucleoproteins

Protein is assumed to be 6.25 times the nitrogen present, by weight.

Amount in Egg.—About 65 % of egg dry weight is protein (Hutchens, Keltch, Krahl, and Clowes, 1942).

Change on Fertilization.—12 % of total protein becomes insoluble 3 to 10 minutes after fertilization (Mirsky, 1936; known as "Mirsky protein"). See Monroy and Oddo (1951) for *A. punctulata* and *A. lixula*.

Amount in Seminal fluid.—About 2.5 mg. per cc. undiluted seminal fluid (Hayashi, 1945, 1946).

On Sperm Surface.—(Hayashi, 1945, 1946; Popa, 1927, lipoprotein).

Basic Proteins.—(Protamines and histones) extracted from sperm, cause agglutination (Metz, 1949); see Frank (1939).

Other Species and General References

Conners and Scheer, 1947. *S. purpuratus*, analysis of protein.
Ephrussi, 1933. *P. lividus*, protein is 66.88% of dry weight.
Hultin, 1949a. *A. lixula*, *P. lividus*, *Ps. miliaris*, *Echinocardium cordatum*, basic proteins.

Kavanau, 1953. *S. purpuratus*, amino acids in development.
Leitch 1934b. *S. franciscanus*, *S. purpuratus*, amount of protein.
Lindvall and Carsjö, 1951. *E. esculentus*, protein fractions.
Monroy, 1950. *P. lividus*, protein fractions by electrophoresis.
Runnström, 1949a. General.
Tyler, 1948. Agglutinins.

RADIUM

Effect on Cleavage.—Beta rays retard, gamma rays accelerate cleavage; most susceptible in metaphase (Packard, 1915, 1916).

Rays cause cytolysis.—(Packard, 1916).

Other Species

Bohn, 1903. *Strongylocentrotus lividus.*
Hertwig, G., 1912. *Parechinus miliaris.*
Miwa, Yamashita, and Mori, 1939, 1940, 1941. *Pseudocentrotus depressus* and *Strongylocentrotus pulcherrimus.*
Reiss, 1925. Probably *Paracentrotus lividus.*

RESPIRATION, OXYGEN CONSUMPTION, METABOLISM

Index

A. Eggs

I. Increase of O_2 consumption (Table 18).
II. Increase of O_2 consumption with reversible blocking or delay of cell division.
III. Practically no effect on O_2 consumption with blocking (sometimes reversible) of cell division.
IV. Decrease or inhibition of O_2 consumption and of cell division.
V. Half-eggs (Table 18).
VI. Homogenates (cell-free system).
VII. Carbon dioxide production.
VIII. Respiratory quotient, RQ.
IX. Temperature coefficient, Q_{10}, Table 19.

B. Sperm

I. Increase of O_2 consumption.
II. Decrease or inhibition of O_2 consumption.
III. Carbon dioxide production.
IV. Respiratory quotient.

Location in Text. A. Eggs

Anaesthetics, A III 1; see also topic Anaesthetics.
Azide, A III 6.
Barbiturates A III 1 (Barbituric acid).
Benzoic acid A III 1 (Local anaesthetic bases).
Caffeine A IV 8.
Carbamates (Urethane).
Carbon dioxide A IV 2.
Carbon monoxide A IV 3.
Centrifuged eggs A I 7 (Stretched).
Chloral hydrate A III 1.
Cleavage A I 3.

Location in Text. B. Sperm

Nitrogen mustards B I 7.
Seminal fluid B I 2.
Sulfhydryl groups B I 4, II 7.
Uranyl nitrate B II 5.
Urethane B I 8, II 6.
Usnic acid B I 6, II 3.
X-rays B I 5, II 2.

TABLE 18

OXYGEN CONSUMPTION (CM^3/HOUR/CM^3 EGGS)

From Ballentine 1940b

Species	Unfert.	Fert.	F/Unf.	Temp.	Reference
Arbacia punctulata	0.158	0.790	5.0	25°	Tang, 1931
		0.550		25°	Tang and Gerard, 1932
	0.040	0.200	5.0	21°	Whitaker, 1933a, b
	0.038	0.148	3.9	24°	Rubenstein and Gerard, 1934
	0.023	0.101	4.4	21°	Rubenstein and Gerard, 1934
	0.149	0.385	2.6	26°	Shapiro, 1935b
	0.060	0.320	5.3	21°	Korr, 1937
	0.090	0.450	5.0	25°	Korr, 1937
	0.100	0.450	4.5	25°	Ballentine, 1940b
Arbacia lixula	0.028	0.168	6.0	20°	Warburg, 1908
Psammechinus miliaris	0.021	0.79	3.8	15°	Shearer, 1922b
Paracentrotus lividus	0.036	0.216	6.0	23°	Warburg, 1915
		0.216		21°	Runnström, 1930
Asterias	0.080	0.080	1.0	23°	Tang, 1931b
Arbacia punctulata, half-eggs					Shapiro, 1935b, 1939b
Whole egg	0.149	0.385	2.6	26°	
Light half	0.123	0.349	2.37		
Heavy half	0.281	0.266	1.2		

To these references should be added: Shapiro (1935c) for eggs kept four weeks in the laboratory; Clowes and Krahl (1936a), Keltch and Clowes (1947) who give for both fertilized and parthenogenetic eggs of *A. punctulata* 2.7 times the unfertilized; Robbie (1946b) who gives F/Unf. = 4.5 at 23°; Shearer (1922a) for *Echinus microtuberculatus*. A list similar to Ballentine's is given by Krahl (1950, Table I). For *Psammechinus miliaris*, immature, unfertilized and fertilized see Borei (1948).

RESPIRATION

A. Eggs

I. Increase of O_2 Consumption (Table 18)

1. After Fertilization and Parthenogenesis.—First described by Warburg (1908) for *Arbacia lixula* as 6 to 7 times more for the fertilized than for the unfertilized egg. For *A. punctulata*, 3 to 4 times (Loeb, 1910; Loeb and Wasteneys, 1911a; Loeb, 1913a, p. 27; etc.). For the parthenogenetic egg of *Strongylocentrotus lividus* as about

the same as for the fertilized egg (Warburg, 1910); of *A. punctulata* as double (McClendon and Mitchell, 1912). Later, more accurate data were tabulated by Ballentine (1940b), Table 18. He has converted some of the original data, given in millions of eggs into cm^3 of eggs for uniformity in the equation: $QO_2 = cm^3\ O_2$ consumed per hour per cm^3 cells.

2. After Maturation.—Mature egg consumes more oxygen than immature (Boell, *et al.*, 1940). But Lindahl and Holter (1941) found that the oocytes of *P. lividus* consumed more oxygen than the unfertilized or fertilized eggs; Borei (1948) found that the oocytes of *Ps. miliaris* consumed more than the unfertilized eggs.

3. During Development.—Increase hourly to fifth hour after fertilization (Loeb, 1913a, p. 29). Increase 1.6 to 4.5 hours (Chesley, 1934). Gradual increase to 95 minutes (Whitaker, 1933a). Increase to 22nd hour (Tyler, Ricci, and Horowitz, 1938). Gradual increase till hatching, then more marked increase (Hutchens, Keltch, *et al.*, 1942; Krahl, 1950). Tang (1931) found no change through third cleavage, but he (1948) found mitotic rhythms. Rhythms have also been found in *Ps. miliaris*, etc. by Zeuthen (1947, 1949, 1950, 1951). For increased respiration in later stages see M. M. Brooks (1943).

4. On Standing.—(Wasteneys, 1916; Gerard and Rubinstein, 1934, p. 376 footnote; *et al.*). Tyler, Ricci and Horowitz (1938) find increase is due to bacteria; see also Gorham and Tower (1902).

5. On Shaking.—(Whitaker, 1933a, p. 488 footnote; Velick, 1941, also for centrifuged eggs).

6. On Cytolysis.—(Loeb, 1913a, p. 14; Tang, 1931; Whitaker, 1933a, p. 487 footnote; Tyler, Ricci, and Horowitz, 1938; *et al.*). Decrease found by Heilbrunn (1915b). See Rubenstein and Gerard (1934) and Ballentine (1940c).

7. In Stretched Eggs.—By centrifuging, 60 to 100% greater oxygen consumption (Velick, 1941). Cytochrome oxidase activity 3.2 times greater (Navez and E. B. Harvey, 1935, see under Cytochrome Oxidase).

8. By Redox Indicators.—Methylene blue or toluidin blue (Barron, 1929; Barron and Hamburger, 1932; Chesley, 1934; Runnström, 1935a; Clowes and Krahl, 1936a; Ballentine, 1938, 1940b). Increased oxidation with block or delay of cell division (Clowes and Krahl, 1936a, Shapiro, 1948a). But M. M. Brooks (1943) found increased oxidation with acceleration of development and larger plutei. Neutral red (Clowes and Krahl, 1936a). O-cresol-indophenol (Clowes and Krahl, 1936a). See Krahl, (1950, Table VIII).

9. By OH Ions.—(Loeb and Wasteneys, 1911b, 1915; Wasteneys, 1916, see Loeb, 1913a, p. 37; McClendon and Mitchell, 1912).

10. By Hexose Phosphate.—(Runnström, 1935a).

11. By Sodium Chloride.—And other parthenogenetic agents (Mitchell and McClendon, 1911; McClendon and Mitchell, 1912; Keltch and Clowes, 1947).

12. By Naphthoquinones.—In low concentrations; decrease of respiration in higher concentrations (Anfinsen, 1947).

II. Increase of O_2 Consumption with Reversible Blocking or Delay of Cell Division by

1. Pyocyanine.—Increased O_2 consumption, cell division reversibly blocked in high concentrations; iron-containing enzyme not involved (Barron and Hamburger, 1932; Runnström, 1935a; Clowes and Krahl, 1936a; Korr, 1937). Also in thawed frozen eggs (Runnström, 1935a).

2. Dimethylparaphenylenediamine.—Cell division reversibly blocked in high concentrations (Runnström, 1935a; Clowes and Krahl, 1936a). Effect on O_2uptake of half-eggs (Boell, Chambers, Glancy, and Stern, 1940).

3. Nitro- and Halophenols.—And related compounds; cell division reversibly blocked at optimum respiratory concentration (Clowes and Krahl, 1934a, b, 1935,

1936a, b, c; Krahl and Clowes, 1935, 1936a, b, 1938, 1940; Hutchens, Krahl, and Clowes, 1939; Krahl, Keltch, and Clowes, 1937; Clowes, 1951). On parthenogenetic eggs (Keltch and Clowes, 1947; Keltch, Walters, and Clowes, 1947). On cell-free system, see under VI. See Krahl (1950, p. 196 and his Table IV.)

4. Mercuric Chloride, Hydrogen Peroxide and X-rays.—In small doses increase respiration with delay of cell division; large doses decrease respiration with delay of cell division (Barron, Flood, and Gasvoda, 1949; Barron and Seki, 1952; Barron, Seki, and Johnson, 1952).

5. Methylene blue.—See above A I 8.

III. Practically No Effect on O_2 Consumption, or Slight Decrease, with Blocking (Sometimes Reversible) of Cell Division by

1. Anaesthetics.—(Narcotics). See topic Anaesthetics.

2. Nitrogen Mustards.—(Barron, Seegmiller, Mendes, and Narahara, 1948; Hutchens and Podolsky, 1948).

3. Penicillin.—(Henry and Henry, 1945). See Krahl (1950, p. 206).

4. Usnic Acid.—Antibiotic (Marshak and Harting, 1948).

5. Sulphanilamide.—(0.04 M) blocks cleavage and reduces oxygen consumption to 55 %. No effect on unfertilized eggs (Fisher, Henry, and Low, 1944). (See Krahl, 1950, Table VIII).

6. Sodium Azide.—(5×10^{-3} M) cell division blocked while respiration is inhibited 50 % (Krahl, Keltch, Neubeck, and Clowes, 1941; Fisher, Henry, and Low, 1944).

7. Sodium Sulfide.—(2×10^{-4} M) cell division blocked while respiration is inhibited 50 % (Krahl, Keltch, Neubeck, and Clowes, 1941).

IV. Decrease or Inhibition of O_2 Consumption and of Cell Division

1. Low oxygen Tension.—O_2 consumption practically constant from 228 to 20 mm. Hg., then decreases; same for cell division; no cleavage below 4 mm. Hg. (Amberson, 1928). See also Tang (1931, 1933, 1941; Gerard (1931); Tang and Gerard (1932); Clowes and Krahl (1940); Krahl (1950). For unfertilized eggs, see Tang (1931a).

2. Carbon Dioxide.—(Root, 1930; Haywood and Root, 1930, 1932).

3. Carbon Monoxide.—Action reversed by light (Clowes and Krahl, 1940; Krahl, 1950). On cytochrome oxidase (Krahl, Keltch, Neubeck, and Clowes, 1941). See M. M. Brooks (1943).

4. Cyanides.—See under topic Cyanides.

5. Iodoacetate.—(Iodoacetic acid). (Runnström, 1935c; Clowes and Krahl, 1940; Krahl and Clowes, 1940).

6. Naphthoquinones.—Inhibit respiration and cleavage in high concentrations, stimulate respiration in low concentrations (Anfinsen, 1947).

7. Malononitrile.—Depresses respiration, inhibits cleavage (Villee, *et al.*, 1949).

8. Caffeine.—(Cheney, 1945, 1946).

9. Mercuric Chloride, Hydrogen Peroxide and X-rays.—In large doses decrease respiration with delay of cell division; in small doses increase respiration with delay of cell division (Barron, Flood, and Gasvoda, 1949; Barron and Seki, 1952; Barron, Seki, and Johnson, 1952). For x-rays see also Chesley (1934) and Evans (1950).

V. Oxygen Consumption of Half-Eggs

Unfertilized white half, same as whole egg; unfertilized red half 88 % in excess of whole egg; fertilized white half 2.7 fold increase at 25.9 °C, like whole egg; fertilized red half same as unfertilized (Shapiro, 1935b, 1939b). See under A I, 1 Table 18. (Also Navez and E. B. Harvey, 1935; Ballentine, 1940c).

VI. Oxygen Consumption in Cell-Free System

In homogenates or in thawed frozen eggs (Runnström, 1935a, also with pyocyanine or methylene blue). Oxidase activity but no respiration (Boell, Chambers, Glancy, and Stern, 1940). Cytochrome oxidase activity and content of fractions (Krahl,

Keltch, Neubeck, and Clowes, 1941; Hutchens, Kopac, and Krahl, 1942). Oxygen consumption of cell-free system of unfertilized eggs about three times that of same weight of intact unfertilized eggs and about same as that of equal weight of fertilized eggs (Keltch, Strittmatter, Walters, and Clowes, 1950). Slightly different figures given by Crane and Keltch (1949). Stimulation by dinitrocresol and phosphate; also methods (Crane and Keltch, 1949). Oxidative phosphorylation (Keltch, *et al.*, 1950; Clowes, Keltch, *et al.*, 1950; Strittmatter, *et al.*, 1950; Clowes, *et al.*, 1951a, b; Keltch, *et al.*, 1951).

VII. Carbon Dioxide Production

Nearly equivalent increase on increase of O_2 consumption (Clowes and Krahl, 1936a). See Ballentine (1940b, c). Carbon dioxide production occurs in rhythms in segmenting eggs, greatest at time of active division (Lyon, 1904a, b).

VIII. Respiratory Quotient

RQ, of fertilized eggs 0.78 (Amberson, 1928); 0.71 (Root, 1930). Of eggs one to 25 hours after fertilization 0.86 (Hutchens, Keltch, Krahl, and Clowes, 1942; Krahl, 1950).

IX. Temperature Coefficient, Q_{10} (Table 19)

Fertilized eggs, about 2 (3°–27 °C.) (Loeb and Wasteneys, 1911a; Loeb, 1913a, p. 33).

Unfertilized eggs, 4.1; fertilized eggs 1.8; cytolysed eggs 1.9 (13°–30 °C.). Increase of O_2 consumption on fertilization depends on temperature, 8 times as great at 11 °C., and twice as great at 29.9 °C.; there should be no increase at 32 °C. (Rubenstein and Gerard, 1934).

TABLE 19

TEMPERATURE COEFFICIENTS, Q_{10} FOR VELOCITY OF OXIDATIONS AND OF FIRST CLEAVAGE OF ARBACIA PUNCTULATA

Loeb and Wasteneys, 1911a; Loeb, 1913a, p. 33; Loeb and Chamberlain, 1915

Interval of temperature	Q_{10} for respiration of fertilized egg	Q_{10} for cleavage	Q_{10} for cleavage (Loeb and Chamberlain 1915)
3–13°	2.18	—	—
5–15°	2.16	—	—
7–17°	2.0	7.3	—
8–18°	—	6.0	6.0
9–19°	—	>4.0	4.5
10–20°	2.17	3.9	3.7
11–21°	—	—	3.3
12–22°	—	3.3	3.2
13–23°	2.45	—	2.8
14–24°	—	—	2.8
15–25°	2.24	2.6	2.5
16–26°	—	2.6	—
17–27°	2.0	—	—
17.5–27.5°	—	2.2	—
20–30°	1.96	1.7	—
22–32°	1.40	—	—

Unfertilized eggs 3.7 (13°–23 °C.), 4.5 (18°–28 °C.); fertilized eggs 2.2 (13°–23 °C) 2.5 (18°–28 °C.) (Korr, 1937).

For μ values see Rubenstein and Gerard (1934) and Korr (1937). For figures on other eggs see Borei and Lybing (1949, p. 113).

B. Sperm

I. Increase of O_2 Consumption by

1. Dilution of Sperm.—(Gray, 1928a for *Echinus esculentus* and *E. miliaris;* Barron and Goldinger; 1941; Barron, Gasvoda, and Flood, 1949; Hayashi, 1946; *et al.*).

2. Seminal Fluid.—(Hayashi, 1946; Nelson, 1949).

3. Iodoacetate.—And malonate, in concentrations not affecting respiration of fertilized eggs (Barron and Goldinger, 1941).

4. –SH Reagents.—In small concentrations; inhibit in large concentrations (Barron, Nelson, and Ardao, 1948; Nelson, 1949).

5. X-rays and Hydrogen Peroxide.—Decrease in large doses, increase in small doses (Barron, Flood, and Gasvoda, 1949; Barron, Gasvoda, and Flood, 1949; Barron and Seki, 1952; Barron, Seki, and Johnson, 1952).

6. Usnic Acid.—(Antibiotic) in high concentrations of sperm, decrease in low concentrations (Nelson, 1948). Little effect on respiration of fertilized eggs, but inhibits cleavage (Marshak and Harting, 1948).

7. Nitrogen Mustards.—Increase respiration of sperm, that of eggs not affected (Barron, Seegmiller, Mendes, and Narahara, 1948).

8. Ethyl Urethane.—0.1 M (Barron, Nelson, and Ardao, 1948). See topic Anaesthetics.

II. Decrease or Inhibition of O_2 Consumption by

1. Egg Water.—(Gray, 1928c found increase in *Echinus esculentus*, no effect in *E. miliaris;* Hayashi, 1946).

2. X-rays and Hydrogen Peroxide.—Decrease in large doses; increase in small doses (Barron, Flood, and Gasvoda, 1949; Barron, Gasvoda, and Flood, 1949; Barron and Seki, 1952; Barron, Seki, and Johnson, 1952).

3. Usnic Acid.—(Antibiotic) in low concentrations of sperm, increase in high concentrations (Nelson, 1948). Little effect on respiration of fertilized eggs, but inhibits cleavage (Marshak and Harting, 1948).

4. HCN.—(1×10^{-4}) inhibits (Barron, Nelson, and Ardao, 1948).

5. Uranyl Nitrate.—Uranium (Benedict and Barron, 1946; Barron, Muntz, and Gasvoda, 1948).

6. Phenyl Urethane.—Saturated solution inhibits, 1/10th saturation no effect (Barron, Nelson, and Ardao, 1948). See Anaesthetics.

7. –SH Reagents.—Inhibit in large concentrations, increase in small (Barron, Nelson and Ardao, 1948; Nelson, 1949).

III. Carbon Dioxide Production (Cohn, 1918).

IV. Respiratory Quotient, RQ. 1.08 (Barron and Goldinger, 1941); 1.01 (Barron, Seegmiller, Mendes, and Narahara, 1948).

General References and Reviews

Ball, 1942. Oxidative mechanisms.
Barron, 1952a. Mechanism of enzymatic oxidation-reductions, in *Modern Trends.*
Brachet, 1950. *Chemical Embryology.*
Krahl, 1950. Metabolic activities and cleavage in *Arbacia;* a review.
Loeb, 1913. *Artificial Parthenogenesis and Fertilization.*

Needham, 1931. *Chemical Embryology.*
Needham, 1942. *Biochemistry and Morphogenesis.*
Runnström, 1949a. Metabolic changes following fertilization; a review with good bibliography, no titles.
Smith and Kleiber, 1950. Size of egg and oxygen consumption.
Whitaker, 1933b. Rate of oxygen consumption; comparisons and interpretation.

Other Species (additional)

Ashbel, . 130 *A. pustulosa*, RQ.
Ashbel, [illegible] *A. pustulosa*, *P. lividus*, hydrogen ion concentration.
Borei, 1948, 1949. *Ps. miliaris*, fall of respiration after removal from ovary.
Callan, 1949. Naples species, respiration and cleavage rates.
Carter, 1931. *E. esculentus*, *E. miliaris*, sperm.
Ephrussi, 1926, 1933. *Paracentrotus lividus*, temperature on respiration, different stages and RQ.
Gray, 1925, 1927a. *E. esculentus*, *E. miliaris*, cleavage and growth.
Hultin, 1949b, 1950a. *Ps. miliaris*, *P. lividus*, homogenates and calcium.
Laser and Rothschild, 1939. *Ps. miliaris*, respiration during fertilization.
Lindahl, 1939. *P. lividus*, respiration at different stages.
Lindahl, 1941. *P. lividus*, cyanide. Many other papers.
Lindahl and Holter, 1940. *P. lividus*, respiration of animal and vegetative halves.
Lindberg and Ernster, 1948. *S. dröbachiensis*, homogenates.
Öhman, 1940. *P. lividus*, RQ, at different stages.
Örström, 1932a. *P. lividus*, dimethylparaphenylenediamine.
Robbie, 1948, 1949. *Echinarachnius parma*, *Tripneustes esculentus*, cyanide.
Rothschild, 1948a, 1950d, 1951a. *E. esculentus*, sperm.
Rothschild, 1949. *Ps. miliaris*, carbon monoxide on eggs.
Runnström, 1930, 1932. *P. lividus*, *Arbacia pustulosa*, respiration of egg.
Tyler and Humason, 1937. *S. purpuratus*, *Dendraster excentricus*, temperature coefficients.
Vasseur, 1949b. *S. dröbachiensis*, *E. esculentus*, *Ps. miliaris*, calcium and jelly on respiration of sperm.

RHYTHMS OR PERIODS OF SUSCEPTIBILITY DURING MITOTIC CYCLE

Acids.—HCl, most susceptible at cleavage, first and second (Spaulding, 1904); HCl and oxalic acid, during resting period; acetic, propionic butyric and valeric acids at anaphase (Keltch, Wade, and Clowes, 1934); see also Clowes, Keltch, and Wade (1933).

Alcohols, Higher.—Most susceptible 10 to 15 minutes after fertilization and immediately before cleavage, 45 to 48 minutes after fertilization (Baldwin, 1920); see Blumenthal (1930).

Ammonia.—And organic amines, at beginning of prophase (Keltch, Wade, and Clowes, 1934); see also Clowes, Keltch, and Wade (1933).

Carbon Dioxide Production.—Maximum at cleavage, first and second (Lyon, 1904, a, b).

Catalase.—No rhythmical change in content or action during cleavage and development, but increase just after fertilization (Lyon, 1909).

Colchicine.—About 10^{-4} M. Mitosis stopped at late prophase or metaphase (Nebel, 1937, Nebel and Ruttle, 1938; Beams and Evans, 1940; Wilbur, 1940). See Cornman and Cornman (1951).

Cold.—0° to 2 °C. Most susceptible 10 to 15 minutes after fertilization; different from heat, similar to KCN (Lyon, 1904b).

Cyanides.—Most susceptible (M/50 to M/200 KCN) 10 to 15 minutes after fertilization, and just after first and second cleavage; same as for oxygen-lack; probably each cleavage increases susceptibility (Lyon, 1902, 1904a). But Mathews (1906a)

finds susceptible period (M/25 to M/100 NaCN) at time of astral development, sperm and cleavage asters; and Heilbrunn (1920a) with 0.005% KCN at spindle stage. Cleavage blocked (with M/200 KCN) in monaster stage (Just, 1928a); (with 1.6×10^{-4} M KCN) in early prophase (Clowes and Krahl, 1934b, 1935; Krahl, 1950, Clowes, 1951); in early prophase or "streak" stage, with or without pyocyanine (Runnström, 1935a). No definite rhythm (Blumenthal, 1930; A. Scott, 1950).

Ether.—Most susceptible during cleavage, first and second (Spaulding, 1904).

Heat.—32° to 36 °C. Most susceptible just before first and second cleavage; different from cold (Lyon, 1904b).

Hypertonic Sea Water.—Most susceptible 5 to 15 minutes after fertilization and immediately before and during each cleavage (A. R. Moore, 1915).

Hypotonic Sea Water.—Most susceptible and most cytolysis during formation of the cleavage furrow, due to increased permeability of plasma membrane, decreased electrical polarization and increased surface tension; same for first, second and third cleavages (R. S. Lillie, 1916b). During "pause" and especially during late anaphase just before egg elongates (Just, 1928b). Increasingly susceptible, with cytolysis from 2 to 6 minutes after fertilization till cleavage; and same for saponin (Page, 1929a). Most susceptible in anaphase and early telophase, least in prophase; reverse for saponin (Keltch, Wade, and Clowes, 1934).

Mechanical Shocks.—Most susceptible immediately after fertilization (Mathews and Whitcher, 1903).

Nitrogen Mustard.—Mitosis stopped in early prophase, "streak" stage (E. B. H. and Cannan, 1943 unpub., but see Gilman and Phillips, 1946, literature list # 52, p. 436, incorrectly quoted on p. 413; correctly stated by E. B. Harvey, 1946a, p. 261). Cornman (1950d), however, found no phase block but overall slowing.

Oxygen-Lack.—Most susceptible (hydrogen atmosphere) 10 to 15 minutes after fertilization; same as for KCN (Lyon, 1902). Cleavage blocked (nitrogen atmosphere) before fusion of pronuclei, with or without pyocyanine (Runnström, 1935a); at prophase (Clowes and Krahl, 1940).

Phenols And Derivatives.—Block to cleavage in early prophase (Clowes and Krahl, 1934a, b, 1935, 1936a; Krahl, 1950; Clowes, 1951). A. Scott (1950) finds no phase inhibition.

Radium.—Most susceptible in metaphase (Packard, 1916).

Salts.—NaCl, KCl, $CaCl_2$, $MgCl_2$. Most susceptible during cleavage, first and second (Spaulding, 1904). Effect of salts on resistant period (Page, 1929a). See Chambers and Chambers (1949).

Saponin.—Same as for hypotonic sea water (Page, 1929a); most susceptible in prophase, least in telophase, reverse of hypotonic sea water (Keltch, Wade, and Clowes, 1934).

Ultraviolet Light.—(Blum and Price, 1950a).

X-Rays.—Most susceptible at prophase (Henshaw, 1938c; E. B. Harvey, 1946a). Refractory period (Blum, *et al.*, 1951).

Other Species and General References

Brachet, 1950. *Chemical Embryology*, p. 157, general.
Herlant, 1914, 1918b, 1920. *Paracentrotus lividus.*
Just, 1922b, c. *Echinarachnius parma*, hypotonic sea water.
Runnström, 1933. *P. lividus*, respiration rhythms.
Zeuthen, 1949, 1950. *S. franciscanus, Dendraster, Ps. miliaris*, respiration rhythms.
Zeuthen, 1951. *Ps. microtuberculatus*, colchicine.

ROENTGEN RAYS

See X-Rays

SHELL

Chemical composition of shell of *Arbacia lixula* (Terentieva, 1932; tabulation by Vinogradov, 1953, p. 252).

$CaCO_3$	90.08 % of ash residue
$MgCO_3$	7.72
$CaSO_4$	2.20

Chemical composition of plates of *Echinus esculentus* (Bütschli, 1908, p. 81; tabulated by Vinogradov, 1953, p. 252; also in Grassé's *Traité de Zoologie*, 1948, vol. 11, p. 8).

$CaCO_3$ (calcite)	86.40	89.64 % of ash residue
$MgCO_3$	8.53	8.84
Phosphate	0.08	0.08
$CaSO_4 + 2H_2O$	1.70	1.40
SiO_2	0.04	0.04
Organic material	0.03	

Chemical composition of other Echinoid skeletons and skeletal parts (Tabulation by Vinegradov, 1953, p. 252, 255 etc.).

Red pigment (echinochrome) in shell of *Arbacia* is present as a Ca salt or adsorbed on $CaCO_3$ (Ball, see under Echinochrome); as a Ca salt in *S. purpuratus* (Tyler, 1939).

SODIUM

Amount in Egg.—1.301 mg. Na per 10^6 eggs (10^6 eggs = 0.124 gm. dry weight), or 0.056 millimoles (Page, 1927b).

Amount in Sea Water.—At Woods Hole. 8.80 gm. per liter at 20 °C. (Page, 1927c, 1928).

Na : K.—In eggs as 1 : 1.90; in sea water Na : K as 1 : 0.0213 (Blanchard per Howard, 1931).

NaCl Isotonic.—With sea water at Woods Hole is 0.53 M (M. B. L. Chemical Room).

Toxicity.—NaCl more toxic than KCl. Delays or prevents cleavage (Loeb, 1900a, R. S. Lillie, 1910, 1911a, b, 1912; Page, 1924, 1929b; Chambers and Chambers, 1938; *et al.*). Toxic effects counteracted by $CaCl_2$ and $MgCl_2$ (Mathews, 1905; R. S. Lillie, 1911a, b, 1912); also by some anaesthetics (R. S. Lillie, 1912).

Surface Precipitation Reaction.—Prevented by NaCl; prevents breakdown of pigment granules by Ca (Heilbrunn, 1928, p. 229).

Surface Potential of Egg.—(Dan, 1936).

Cytolysis.—Caused by NaCl (E. N. Harvey, 1910c; Loeb and Wasteneys, 1910; Loeb, 1913a, p. 180; R. S. Lillie, 1912; Heilbrunn, 1928, p. 251; Mazia, 1933; Dan, 1936; *et al.*).

Jelly.—NaCl (0.54 M) dissolves jelly and causes agglutination of eggs; prevented by $CaCl_2$. (R. S. Lillie, 1921).

Colorless Amoebocytes of Body Fluid. Dissolved by NaCl (Mathews, 1900).

Fertilized Egg.—When NaCl added to sea water, nuclear division without cell division (Loeb, 1892, 1895b, 1900a, etc.). Also astrospheres (Morgan, 1899).

Respiration.—Increase (Mitchell and McClendon, 1911; McClendon and Mitchell, 1912; Heilbrunn, 1943, p. 455; but see Loeb and Wasteneys, 1910).

Permeability.—Increase, as indicated by dissolving of pigment granules in the order of: NaCl < NaBr < $NaNO_3$ < NaCNS < NaI; also toxic and effective as parthenogenetic agents in this order. Na salts more effective (and more toxic) than K salts. Increased permeability can be antagonized by $CaCl_2$ and $MgCl_2$ and some anaesthetics (R. S. Lillie, 1910, 1911a, b. 1912; McCutcheon and Lucké, 1928; also Heilbrunn, 1943, p. 142).

Viscosity.—NaCl increases viscosity in the order: KCl > NaCl > sea water > $MgCl_2$ > $CaCl_2$ (E. B. Harvey, 1945). Heilbrunn gives the same order except that KCl and NaCl are reversed (Heilbrunn, 1923, 1928, p. 146, 1943, p. 81).

Breaking with Centrifugal Force.—Break more readily than in sea water in the order of increasing viscosity (reverse order of stratification), that is, the most viscous (least stratified) break most readily (E. B. Harvey, 1945).

Parthenogenetic Agent.—NaCl added to sea water; first used by Morgan (1898, 1899, 1900a, b, etc.); subsequently by Loeb, R. S. Lillie, and many others. NaOH has also been used (Loeb, 1913a, p. 148).

The best parthenogenetic agent for *Arbacia punctulata* is: 30 gm. NaCl to one liter of sea water for 20 minutes, then sea water. Or increase the salt content of sea water by boiling to half its volume, leave eggs in this solution for 20 minutes, then sea water (E. B. Harvey, 1936). Results vary with different batches of eggs, and one can vary the solution and time slightly.

Other Species and General References

Bialaszewicz, 1927, 1929. *A. lixula, P. lividus;* electrolytes in eggs.
Harvey, E. B., 1938a. Parthenogenetic agent in Naples sea urchins.
Heilbrunn, 1952. *General Physiology*, p. 519.
Herbst, 1904. Salts necessary for development.
Loeb, 1913a. *Artificial Parthenogenesis. S. purpuratus, S. franciscanus,* especially.
Malm and Wachtmeister, 1950. *Ps. miliaris, S. dröbachiensis,* amount Na in unfertilized and fertilized eggs.
Morgan, 1896. *Sphaerechinus granularis,* astrospheres.
Robertson, 1939; Robertson and Webb, 1939; Webb, 1939. Inorganic composition of sea water and body fluids.
Rothschild and Barnes, 1953. *P. lividus,* inorganic constituents of egg; table of salts and species.

SPECIFIC GRAVITY

See Density

SURFACE FORCES

See Tension at the Surface

SURFACE TENSION

See Tension at the Surface

TENSION AT THE SURFACE

Surface Tension, Surface Forces

Unfertilized Egg.—0.2 dyne/cm., by centrifuge method (E. N. Harvey, 1931c, 1932a, b, 1937, 1938). 0.08 dyne/cm., by compression method; internal pressure 40 dynes/cm.^{2} (Cole; 1932). Agreement with Harvey and Cole, by stretching (Norris, 1939).

Fertilized Egg.—(Without fertilization membrane). Same as unfertilized till just before cleavage, then increase. 0.03–0.05 dyne/cm. (for eggs late in season) (Cole and Michaelis, 1932).

Cleaving Egg.—0.09 dyne/cm.; 62 dynes/cm.^{2} excess internal pressure when one of two blastomeres is punctured (Sichel and Burton, 1936).

Fertilization Membrane.—Formation due to lowered surface tension (Heilbrunn, 1913, 1915a, 1924a, 1925d).

Cytolysis.—Due to lowering of surface tension of plasma membrane (Heilbrunn, 1915a).

Cell Division.—Explained by change in surface tension (R. S. Lillie, 1903, 1909; McClendon, 1910b; see Wilson's *The Cell*, 1925, p. 158, 192–197 for general references and discussion; also Gray, 1931, p. 214).

For interfacial tension, oil-protoplasm, oil-water, see under Oil, references to Chambers and Kopac.

Other Species and Reviews

Danielli, 1942, Bourne's *Cytology and Cell Physiology*. Review.
E. N. Harvey, 1954. *Protoplasmatologia*. Review.
E. N. Harvey and Danielli, 1938. Review.
Vlès, 1926. *Paracentrotus lividus*.

TWINS, TRIPLETS, QUADRUPLETS

Twins, triplets, quadruplets may be produced through destruction of hyaline layer which binds blastomeres together by:

Shaking.—In *Echinus* (Driesch, 1891, and later).

Hypotonic Sea Water.—(Loeb, 1905b, p. 303, translation of 1894 paper). See Just, 1939a, p. 13.

Hypertonic Sea Water.—Just after cleavage is best method. (E. B. Harvey, 1940a). (Plate XVI, Photograph 8).

$MgCl_2$.—Added to sea water (Loeb 1900a).

Lack of K.—Na or Ca in sea water, in *S. purpuratus*, (Loeb, 1909b, 1912, p. 204).

Ethyl Urethane.—(0.2 M) 3 to 60 min. then sea water and fertilize; fertilization membrane pinches the egg into two parts (E. B. H. unpub.).

Centrifuging.—In 2-cell stage, in *Ps. microtuberculatus* (E. B. Harvey, 1935b); in *Arbacia* (1940a). Hyaline layer is removed by centrifugal force.

Each twin has two micromeres (E. B. H. unpub.).

Natural twins formed in *Prionocidaris baculosa* by separation of first two blastomeres which develop separately into half sized larvae. (Mortensen, 1938, p. 14).

Twins, triplets and quadruplets from a single egg may all develop into perfect dwarf plutei (E. B. Harvey, 1940a). Plate XVI, Photograph 8.

ULTRASONIC WAVES

High frequency sound waves on *Arbacia punctulata* eggs (E. N. Harvey, E. B. Harvey, and A. L. Loomis, 1928; E. N. Harvey, 1930). High speed photographs show eggs can be cytolysed in less than 1/1200 second (E. N. Harvey and Loomis, 1931).

Other Forms

Schmidt, F. O., 1929, California starfish.

ULTRAVIOLET LIGHT

Cleavage and Development (Eggs or Sperm Radiated).—Delayed cleavage and abnormal development (Child, 1924, p. 109 footnote; Hinrichs, 1926b, c, 1927; Nebel, E. B. Harvey, and Hollaender, 1937; E. N. Harvey, 1942; Giese, 1946). Delayed cleavage and enhanced recovery with visible light (Blum, *et al.*, 1949a, b, 1950a, c, d, 1951; Marshak, 1949b, c). Of centrifuged eggs. Delay greater if irradiated through oil cap, less if through pigment (C. V. Harding and Thomas, 1950). Of half-eggs. Delay in cleavage of white halves; and of red (non-nucleate) halves except when exposed before fertilization, to wave lengths of 2700–3130 Å (Blum, *et al.*, 1949a, 1950c, d, 1951). Photorecovery of white halves (Blum, *et al.*, 1949a, 1950c, d, 1951; Marshak, 1949b, c).

Parthenogenetic Agent.—(Loeb, 1914a; R. S. Lillie and Baskervill, 1922; Heilbrunn and Young, 1930; E. B Harvey and Hollaender, 1937, 1938; Moser, 1939b). For cytology, multipolar and anastral mitoses see Nebel, E. B. Harvey, and Hollaender (1937); E. B. Harvey and Hollaender (1938). For method, Hollaender (1938). Fertilization membrane on one side (Moser, 1939b; E. N. Harvey, 1942); also Spikes (1944) for *Lytechinus pictus* and Reed (1943, 1948) for *S. purpuratus*. Both white and red (non-nucleate) half-eggs are activated with wave lengths of 2260–2480 Å, and the red halves with 2650–3300 Å also (E. B. Harvey and Hollaender, 1937, 1938).

Cytolytic Agent.—R. S. Lillie and Baskervill, 1922; Heilbrunn and Young, 1930).

Permeability.—Uncertain (See Heilbrunn's 1952 *General Physiology*, p. 164). Increase in *P. lividus* (Tchakhotine, 1921b) and in *Lytechinus pictus* (Spikes, 1944), but Reed (1948) found no change in permeability in *S. purpuratus*.

Viscosity.—Decrease for 15 minutes then increase (Heilbrunn and Young, 1930).

Breaking with Centrifugal Force.—Break more readily after radiation (E. B. H., 1950 unpub.).

Loss of Fertilizin.—And agglutinating power (Hinrichs, 1926c, 1927).

Echinochrome.—Fades (Hinrichs, 1927). Pigment granules in egg become clumped (E. B. H., 1950 unpub.).

Loss of Jelly.—(E. B. H., 1950 unpub.).

Sperm.—Reduced motility and fertilizing power and agglutination (Hinrichs, 1926b, c, 1927). Sperm more sensitive than eggs (Giese, 1946). Sperm treated with ultraviolet before fertilization delay cleavage; no photorecovery if outside the egg (Marshak, 1949b, c; Blum, *et al.*, 1950c, 1951).

Amoebocytes.—They round up and red ones become pale (E. B. H., 1950, unpub.).

Chromatin.—As photographed by ultraviolet light (E. B. Harvey and Lavin, 1944).

Eggs.—Half-eggs and plutei as photographed by ultraviolet light (E. B. Harvey and Lavin, 1951a).

Ultraviolet and Heat.—(Hinrichs, 1927; Hutchings, 1948).

Other Species (additional) and General References

Casperson and Schultz, 1940, *Psammechinus miliaris*, absorption spectra.

Chase, 1938. *Dendraster excentricus.*

Giese, 1939. *Strongylocentrotus purpuratus*, sperm.

Giese, 1945, 1946, 1947, 1949, 1950. General and reviews; see especially for references to Giese on Pacific Coast forms.

Giese and Wells, 1952. *S. purpuratus.*

Heilbrunn and Mazia, 1936. Review in Duggar's *Biological Effects of Radiation*, vol. I, p. 625–676.

Hollaender, 1954. *Radiation Biology.* Review.

Tchakhotine, 1921b. *Paracentrotus lividus*, puncture method.

Tchakhotine, 1937. *P. lividus*, parthenogenesis, change in permeability.

Vlès and Gex, 1928, 1934. *P. lividus.*

Wells and Giese, 1950. *S. purpuratus*, photoreactivity.

VISCOSITY

Water.—At 20 °C. = 1 centipoise.

Clear Protoplasm.—Of *Arbacia* egg = 3 centipoises; determined by centrifuging granules and by Brownian movement of granules in centrifuged egg. (Heilbrunn, 1926a, b, 1927, 1928, p. 67, 1943, p. 69).

Entire Protoplasm.—Equals 6–7 centipoises (Heilbrunn, *ibid.*).

Nuclear Fluid.—About 10 centipoises (Heilbrunn, 1943, p. 73).

Immature Egg.—Is more viscous than mature. (Heilbrunn, 1921, 1928, p. 278, 1943, p. 70; Goldforb, 1935b; E. B. H.).

Maturation.—During, decrease in viscosity (Goldforb, 1935b; E. B. H.).

Fertilized Egg.—More viscous than unfertilized. (Heilbrunn, 1915, 1920a, 1928, p. 264; E. B. Harvey 1932, 1933b).

Prophase.—After breaking of nuclear membrane, increase (E. B. H.).

Parthenogenetic Egg.—More viscous than unfertilized. (Heilbrunn, 1915, 1928, p. 261).

Stages in Mitosis.—Changes in viscosity (Heilbrunn, 1920a, 1921, 1927, 1928, p. 265, 1943, p. 653; Chambers, 1919, 1924; Fry and Parks, 1934; Fry, 1936; Page, 1929a).

Red Halves.—More viscous than white halves (E. B. Harvey, 1932; Chambers, 1938a).

Decrease in Viscosity by

Ageing. Decrease after 35 hours (Goldforb, 1935b).

Alkalies (Barth, 1929).

Anaesthetics. Ether (Heilbrunn, 1920a and b, 1925c, 1927, 1928, p. 205, 288; see Chambers, below under Increase). Ether and other anaesthetics (Heilbrunn, 1920a and b, 1928, p. 206). See Table 15.

Carbon dioxide (Howard, 1931, 1932; Jacobs, 1922, after short exposure).

Colchicine (Beams and Evans, 1940, prevents gelation and aster disappears after fertilization; Wilbur, 1940).

Electric current; direct and alternating; transitory decrease, then increase (Angerer, 1939).

Fatty acids and salts of fatty acids (Howard, 1931).

Heparin and heparin-like substances (Heilbrunn and Kelly, 1950).

Hypotonic sea water (Heilbrunn, 1920a and b, 1928, p. 211; Chambers, 1924; E. B. Harvey, 1943).

Mechanical agitation (Chambers, 1924).

Nitrogen mustards (E. B. H. and Cannan, unpub.).

Pressure, hydrostatic; on unfertilized eggs and on cortex of fertilized eggs (Brown, 1934; Marsland, 1938, 1939, 1942, 1950, 1951.)

Salts. $CaCl_2$ (Heilbrunn, 1923, 1927, 1928, p. 146, 1943, p. 81; E. B. Harvey, 1945; see Chambers, 1949). $MgCl_2$ *(Ibid)*. Aluminum chloride (Heilbrunn, 1925b, 1928, p. 151). Copper chloride; latent period 21 min, then decrease, then increase (Angerer, 1937). (See also Heilbrunn, 1928, p. 143).

Temperature. Heat, decreasing viscosity with increasing temperature —1.8 °C. to +28 °C. (Costello, 1934). Cold, about —3 °C. (Heilbrunn, 1920a and b, 1928, p. 106). Eggs stratify by gravity if kept overnight at 8° but not at 21.5°. They break more readily on centrifuging at low temperatures (E. B. H.); Costello (1938) found the reverse.

Ultraviolet rays, decrease at first, then increase. (Heilbrunn and Young, 1930).

Increase in Viscosity by

Acids (Barth, 1929; list of acids and effective pH on p. 510).

Ageing. Viscosity increases for first 35 hrs., then decreases (Goldforb, 1935b).

Anaesthetics. Chloretone (Heilbrunn, 1920a, b). Ether (Chambers, 1924; see Heilbrunn and above under Decrease).

Body fluid (Goldforb, 1935b).

Carbon dioxide (Jacobs, 1922 after long exposure).

Cytolysis (Heilbrunn, 1928, p. 245).

Electric current; direct and alternating; transitory decrease, then increase (Angerer, 1939).

Formalin (Heilbrunn, 1927).

Hypertonic sea water (Heilbrunn, 1915, 1920a, 1943, p. 81; Chambers, 1924; E. B. Harvey, 1943).

Injury substances (D. Harding, 1951).

Light with dyes, eosin and rose bengal (Alsup, 1941).

Potassium cyanide (Heilbrunn, 1920a, b).

Salts. NaCl (Heilbrunn, 1923, 1927, 1928, p. 146, 1943, p. 81; E. B. Harvey 1945; see Chambers, 1949). KCl *(Ibid.)*. Potassium citrate (Wilbur and Recknagel, 1943). NH_4Cl (Heilbrunn, 1923, 1928, p. 146). $CuCl_2$; increase after 20 min. (Heilbrunn, 1928, p. 143; see also Angerer, 1937). $CuSO_4$ (Heilbrunn, 1927, 1928, p. 143). $HgCl_2$ *(Ibid.)*.

Saponin (Heilbrunn, 1915).

Temperature. Cold, 0 °C. to 5 °C. (Payne, 1928, 1930). Cold, increasing viscosity with increasing cold 28 °C. to —1.8 °C. (Costello, 1934). Cold, extreme, below —3 °C. (Heilbrunn, 1920b). Heat, 31.5 °C. to 32.9 °C. (Heilbrunn, 1925a, 1928, p. 116).

Ultraviolet rays, decrease of interior at first, then increase. (Heilbrunn and Young 1930).

X-rays, increased viscosity following fertilization is prolonged; no effect on unfertilized eggs (W. L. Wilson, 1950).

No Effect on Viscosity of

External pH (Heilbrunn, 1923, pH 7–9; Goldforb, 1935b).

Shearing (centrifugal) forces (Heilbrunn, 1928, p. 47, 1943, p. 70; Howard, 1932).

X-rays on unfertilized eggs (W. L. Wilson, 1950).

Other Species and General References

J. E. Harris, 1939. *Echinus esculentus*, nucleus.
Heilbrunn, 1928, 1943, 1952. General.
Hyman, 1923. *Strongylocentrotus franciscanus* and *S. purpuratus*.
Marsland and Landau, 1950. *Echinarachnius parma*.
Mitchell, 1941. General.
Runnström, 1928c, d. *Echinocardium, Paracentrotus, Psammechinus microtuberculatus, Ps. miliaris, Arbacia lixula*. See his literature list for other references.
Runnström and Kriszat, 1950a. *Ps. miliaris*.
Seifriz, 1920, 1924. *Tripneustes, Echinarachnius;* 1929. General.

VISIBLE LIGHT AND PHOTODYNAMIC ACTION

Visible Light.—On cleavage, little effect (Giese, 1947; Marshak, 1949c). On parthenogenesis. Induces membrane formation (R. S. Lillie and Hinrichs, 1923; Hinrichs, 1926a).

Photosensitization.—Visible light and dyes. On cleavage and development (eggs or sperm treated), abnormal development or delayed cleavage with

Benzoflavin (Hinrichs, 1926a, b).
Eosin (Hinrichs, 1923; 1926a, b.; Child, 1924, p. 109 footnote; Pereira 1925).
Methylene blue (Hinrichs, 1926a, b).
Neutral red (Hinrichs, 1926a, b).
Rhodamine B (L. B. Clark, 1940).
Riboflavin, on sperm (Marshak, 1949c).
Rose bengal (Alsup, 1941).

Causes parthenogenesis with Eosin (R. S. Lillie and Hinrichs, 1923; Alsup, 1941) and Rose bengal (Alsup, 1941). Causes cytolysis with rhodamine (L. B. Clark, 1940),

Photoreactivity.—Enhanced recovery with visible light after ultraviolet light (Blum *et al.*, 1949a, b, 1950c, d, 1951; Marshak, 1949b, c).

Reversal.—Inhibition by carbon monoxide of cytochrome oxidase is reversed by light (Krahl, Keltch, Neubeck, and Clowes, 1941).

Viscosity.—Increase with eosin or rose bengal (Alsup, 1941).

Other Species

Bohn and Drzewina, 1923. *Strongylocentrotus lividus,* neutral red and light.
A. R. Moore, 1928b. *S. purpuratus,* eosin and light.
Rothschild, 1949. *Psammechinus miliaris,* light and CO,
Tennent, 1942. *Lytechinus variegatus,* many dyes and light. Also, many short articles in this literature list.
Wells and Giese, 1950. *S. purpuratus,* on sperm.

VITAL DYES

On Stratified, Centrifuged Eggs.—See Part III on Centrifuging, Chapter 17 and Table 8. (Also E. B. Harvey, 1941c).

On granules of egg.—See under Chromatophores, Clear Layer, Mitochondria, Yolk Granules. (This section).

Immature eggs.—*(Toxopneustes)* stain with certain intravitam dyes more than fertilized and fertilized more than unfertilized (Lyon and Shackell, 1910b; E. N. Harvey, 1910c).

Increased Oxygen Consumption.—With methylene blue, toluylene blue and the effect of KCN and urethanes (Barron 1929, Barron, and Hamburger, 1932). Methylene blue, neutral red, o-cresol-indophenol, dimethyl paraphenylenediamine, with block of cell division (Clowes and Krahl, 1936a: Krahl, 1950, Tables VIII, IX). Methylene blue, effect on cleavage and development (Waterman, 1938, 1941). Methylene blue with acceleration of development; effect of KCN and CO (M. M. Brooks, 1943). Methylene blue, toluylene blue, brilliant cresyl blue with delay of first cleavage (Shapiro, 1948a). Methylene blue; x-rays have no effect (Chesley, 1934).

With pyocyanine not affected by KCN (Barron and Hamburger, 1932). More increase in respiration in unfertilized than fertilized eggs; more with pyocyanine than with methylene blue; effect of HCN and of lithium with pyocyanine (Runnström, 1935a, b). Increase of respiration with block to cell division (Clowes and Krahl, 1936a; Krahl, 1950, his Table VIII). Effect of KCN and temperature (Korr, 1937). Effect on cleavage and development (Waterman, 1938, 1941).

Janus Green B.—Delays or inhibits cleavage and development. (R. D. Allen, 1950).

Photosensitizing Action.—Neutral red and methylene blue (Hinrichs, 1926a, b); rhodamine B (L. B. Clark, 1940). See extensive study on *Lytechinus variegatus* by Tennent (1942; and many short papers).

Exogastrulae.—Produced by two indigo sulphonate dyes, not by methylene blue (M. M. Brooks, 1951).

Vital Staining.—Of parts of eggs or embryos to trace development (Vogt method); not used for *Arbacia* but for other sea urchin eggs by von Ubisch (1925), Lindahl (1932c) and Hörstadius (1935), *et al.*; see article by Hörstadius (1950) in McClung's *Microscopical Technique,* 3rd ed., p. 561–563.

Injection.—Of vital dyes for pH determination, see under Hydrogen Ion Concentration.

General References

Standard books on cytological technique:
Conn's *Biological Stains,* 1946, 5th ed.
Lee's *Vade Mecum,* 1950, 11th ed.
McClung's *Microscopical Technique,* 1950, 3rd ed

Technical books on dyes:
Rowe, 1924, Editor of *Color Index*.
Schultz, 1928–1934, *Farbstofftabellen*.
Vital staining, general:
von Möllendorff, 1920, 1928.

Other Species

Bank, 1933. *Arbacia lixula;* nucleus.
Becker, 1936. Review.
Child, 1936b. *S. purpuratus, S. franciscanus, Dendraster excentricus;* dyes and axial gradients.
Gellhorn, 1931. *S. purpuratus;* dyes and permeability.
Gersch and Ries, 1937. *A. lixula, Sphaerechinus granularis, Paracentrotus lividus, Psammechinus miliaris;* determination.
Lepeschkin, 1941*b*. *P. lividus;* neutral red.
Monné, 1945. *Ps. miliaris, Echinocardium cordatum* etc.; eggs centrifuged and stained. Also other papers.
Moore, Bliss, and Anderson, 1945. *S. purpuratus, Dendraster excentricus;* pyocyanine.
Örström, 1932a. *P. lividus;* dimethylparaphenylenediamine.
Ranzi and Falkenheim, 1937. *Sph. granularis;* determination. Good literature list with titles.
Runnström, 1930, 1932. *P. lividus;* methylene blue and dimethylparaphenylenediamine; 1935b. *Echinarachnius parma;* pyocyanine.
Runnström and Thörnblom, 1938. *P. lividus;* pyocyanine.

VITELLINE MEMBRANE

Definition.—It is the membrane on the exterior of the egg proper, outside the plasma membrane. The older investigators made no distinction between plasma membrane and vitelline membrane referring to them together as a "pellicle". It is difficult to distinguish the two optically in an unfertilized *Arbacia* egg. The vitelline membrane is now generally believed to elevate on fertilization or parthenogenesis, and become, somewhat modified, the fertilization membrane (Chambers, 1942, 1944; Kopac, 1940a). See under Fertilization Membrane and Fig, 9 and 10.

Thickness.—Readily visible but not measurable with a light microscope. As determined by the electron microscope, it is (dried) about 250 Å thick just after being elevated as the fertilization membrane (E. B. Harvey and Anderson, 1943). This is considerably thicker than the dried membrane of the red blood cell which according to the latest data is 50–60 Å (Parpart and Ballentine, 1952; Hillier and Hoffman, 1953). However, Mitchison (1953) has recently given a lower figure for the (fertilization) membrane of *Psammechinus miliaris*, 100 Å, with electron microscope. See Fertilization Membrane.

Structure.—According to Heilbrunn (1915a, 1926b, it is a protein gel with little or no lipid, slightly rigid. According to Kopac (1940a), it is soft, plastic and gelatinous, a delicate film-like membrane. In electron microscope photographs, no structure is evident (E. B. Harvey and Anderson, 1943).

Centrifuging.—Causes it to flow toward centrifugal pole, becoming thinner at centripetal pole (E. B. Harvey, 1932).

Microdissection.—Can be torn or pulled out with a needle, or removed (Kite, 1912; Chambers, 1921a, 1930, 1942, 1949; Kopac, 1940a).

Re-Formed.—When slightly broken (Heilbrunn, 1915a; Chambers, 1917, 1921a).

Elasticity.—(Heilbrunn, 1928, p. 99, 250; 1943, p. 70, 92; Cole, 1932; E. N. Harvey, 1936, 1937; Chambers, 1942). On the unfertilized egg it stretches from the diameter of a sphere, 74 μ, to the length of a spheroid, 140 μ, on centrifuging (E. B. Harvey and Anderson, 1943). About 25 % increase in surface on centrifuging (E. N. Harvey, 1931c). Can be stretched between two needles (Norris, 1939).

Tension at Surface.—0.2 dyne/cm. (E. N. Harvey, 1931c, 1937; Norris, 1939), when stretched; 0.08 dyne/cm. (Cole, 1932); 0.09 dyne/cm. (Sichel and Burton, 1936). Tension of unfertilized and fertilized eggs, without fertilization membranes, (until just before cleavage) is the same (Cole and Michaelis (1932). See Tension at the Surface, and E. N. Harvey (1954).

Electrical Properties.—Of two membranes together (McClendon, 1910b; R. S. Lillie, 1911b, 1916b; Heilbrunn, 1923, 1926b, 1928, p. 183). Membrane resistance is > 100 ohms/cm² (Cole, 1941; see also 1940 and Cole and Curtis, 1938). Capacity about one microfarad/cm² (Cole, 1941). Zeta potential without jelly, about 30.3 millivolts (Dan, 1933). See Electrical Properties.

Oil Coalescence.—(Kopac and Chambers, 1938; Kopac, 1940a; Chambers, 1944).

Removal.—By (1) micromanipulation (Kite, 1912; Chambers, 1921a, 1930, 1938b, 1942; Chambers and Kopac, 1937a; Kopac and Chambers, 1938). (2) Washing in isosmotic NaCl or KCl when membrane is being lifted off to form the fertilization membrane (transitional membrane), 1.5 minutes after insemination (Chambers, 1942, 1944; Kopac, 1940a). (3) Urea (1 M) (Chambers, 1940, 1942; Kopac, 1940a, 1943; Moser, 1940). Pioneer work on development without fertilization membrane by action of urea on sirface of egg *(Strongylocentrotus purpuratus)* was done by A. R. Moore (1929, 1930a). He used a molar solution of urea at pH 7 for two minutes, then fertilized the eggs in sea water. Moser (1940) and others (Kopac, 1940a) have used a similar technique for *Arbacia.* (4) Trypsin, used for *A. punctulata* by Runnström in 1950 and by Dan and Mazia in 1951. Dan used (1951 unpub.) 0.1% non-crystalline trypsin of Merck in sea water for a few minutes or 4 mg. crystalline trypsin in 100 cc. sea water for 10 minutes; this dissolves the vitelline membrane, and is probably the best way of obtaining fertilized eggs without fertilization membranes. Trypsin had been used previously for *Psammechinus miliaris* and *Echinocardium cordatum* (Runnström, Monné, and Broman, 1944). In 1932 (unpub.) Kunitz had found that trypsin had an effect on the precursor of the fertilization membrane in *Arbacia*, preventing its appearance (See A. R. Moore, 1949a, footnote, p. 243; 1949b, footnote, p. 207). Best medium for keeping denuded eggs is mixture of NaCl and KCl in proportion of 19 to 1 in concentrations isotonic with sea water and pH 7.0 (Chambers, 1940, 1944).

Effect of Calcium.—Brittleness increased (Heilbrunn, 1928, p. 149; Chambers, 1944, 1949, 1950; see Kopac, 1940a).

X-Rays.—(Kopac, 1940c, 1941b).

Anaesthetics.—(R. S. Lillie, 1914b).

Ageing.—(Goldforb, 1937).

Centrifugal Force on Breaking.—Break more readily when vitelline membrane is removed (Solano and Mazia, 1953).

Copper.—Present and absorbed (Glaser, 1923).

Ribonucleic Acid.—Present (Lansing and Rosenthal, 1949).

Strength.—Vitelline membrane of different species differs in strength; that of *Arbacia* is weaker than that of *Echinarachnius* and *Asterias* (Kopac, 1940a).

Other Species (additional) and General References

Carter, 1924. *Sphaerechinus granularis.*

Chase, 1935. *Strongylocentrotus purpuratus, Dendraster excentricus.*

Hobson, 1932b. *Psammechinus miliaris.*

Hultin, 1948a, b. *Ps. miliaris, Sphaerechinus granularis, Paracentrotus lividus, A. lixula;* trypsin and urea, cross fertilization.

Hyman, 1923. *S. franciscanus, S. purpuratus,* and review of earlier work.

Just, 1939b. *The Biology of the Cell Surface,* general.

Moore, A. R., 1930b. *Dendraster.*

Moore, A. R., and M. M. Moore, 1931. *Paracentrotus lividus.*
Motomura 1941b. *S. pulcherrimus.*
Runnström, 1948a. *Ps. miliaris, Echinocardium cordatum,* trypsin.
Runnström, 1949a, 1952a. General.
Runnström and Monné, 1945a. *Ps. miliaris, Echinocardium cordatum;* trypsin.
Runnström, Monné, and Broman, 1944. *Ibid.*
Runnström, Monné, and Wicklund, 1946. *Ibid.*

X-RAYS

Cleavage and Development (Eggs or Sperm Radiated).—Delayed cleavage and development (Mavor and De Forrest, 1924; Henshaw, *et al.*, Francis, C. T. Henshaw, Cohen, 1932–1941; Chesley, 1934; Heilbrunn and Young, 1935; Evans and Beams, 1939; Little and Evans, 1940; Evans, 1940, 1942, 1947, 1950; Rugh, 1949; Lucké, Ricca, and Parpart , 1951; Barron and Seki, 1952). Cleavage delay occurs mostly in prophase (Henshaw, 1938c, 1940 II; Henshaw and Cohen, 1940, see Henshaw, 1940 IV). Multipolar cleavage (Henshaw, 1938b, 1940 VI, 1941). Enlarged nuclei with nucleoli in prophase with delay in cleavage (E. B. Harvey, 1946a). Effect on gastrulation, form exogastrulae (Waterman, 1934).

Recovery (Henshaw, 1932, 1938b, etc.; White, 1938; Evans, 1950). Recovery not affected by visible light, thus differing from ultraviolet (Blum, *et al.*, 1949a, 1950c, 1951).

Delay greater with addition of ovarian tissue (Heilbrunn and Young, 1935); with lowered temperature (Henshaw, 1940 V). Delay inhibited by potassium citrate (Wilbur and Recknagel, 1943); delay lessened by packing of eggs (Cohen, 1940); by oxygen-lack (Anderson, 1939). Delay in cleavage of white halves but not of (non-nucleate) red halves if radiated before fertilization (Henshaw, 1938a; Blum, *et al.* 1950c, 1951). Enlarged nuclei with nucleoli in early prophase of white halves (E. B. Harvey, 1946a).

Parthenogenetic Agent.—In whole eggs, white halves and (non-nucleate) red halves (E. B. Harvey, 1940 unpub., but see reference in Giese, 1947, p. 269, line 33 of first column).

Cause Cytolysis.—(Lucké, Ricca, and Parpart, 1951).

Permeability.—No effect (Richards, 1915; Lucké, Ricca and Parpart, 1951).

Respiration.—Of eggs. No effect (Chesley, 1934). Slight effect (Evans, 1940). Of sperm. Inhibition (Barron, Gasvoda, and Flood, 1949; Barron, Flood, and Gasvoda, 1949). Of eggs and sperm, inhibition in large doses, increase in small (Barron and Seki, 1952).

Viscosity.—No effect on unfertilized eggs (Wilbur and Recknagel, 1943; W. L. Wilson, 1950). Period of increased viscosity after fertilization continues longer (W. L. Wilson, 1950).

Breaking with Centrifugal Force.—Break more readily after radiation (E. B. H., 1940 unpub.).

Loss of Fertilizin.—And agglutinating power (Richards and Woodward; 1915; Evans, Beams, and Smith, 1941; Metz, 1942).

Loss of Jelly.—(Evans and Beams, 1939; Evans, 1940b; M. E. Smith and Evans, 1940; Evans, Beams, and Smith, 1941; E. B. H., 1940 unpub.).

Sperm.—Loss of motility and fertilizing power (Evans and Beams, 1939; Evans, 1942, 1947); effect lessened by proteins, egg albumen, etc., in the sea water (Evans and Slaughter, 1941; Evans, Slaughter, Little, and Failla, 1941). No recovery (Henshaw, 1936, 1938b). Sperm is more sensitive than the egg to x-rays (Mavor and De Forrest, 1924; Henshaw, 1936).

Vitelline Membrane.—(Kopac, 1940c, 1941b).

Amoebocytes.—Become spherical (E. B. H., 1940 unpub.).

Other Species and Reviews

Giese, 1947. Review.
Hollaender, 1954. *Radiation Biology*. Review.
Langendorff, 1931. *Psammechinus miliaris*.
Lee, 1947. Review.
Miwa, Yamashita, and Mori, 1939, 1940, 1941. *Pseudocentrotus depressus, Strongylocentrotus pulcherrimus*.
Reiss, 1925. Probably *Paracentrotus lividus*.

YOLK GRANULES

Size.—0.7 to 1.1 μ diameter (E. B. H. per E. N. Harvey, 1932a, E. B. Harvey, 1936); 0.3 μ (Heilbrunn, 1926a); 1.0 to 1.5 μ with electron microscope (McCulloch, 1952a).

Shape.—Irregular or polyhedral (E. B. H. per E. N. Harvey, 1932a, E. B. Harvey, 1936). Elliptical (McCulloch, 1952a).

Density.—Lighter than chromatophores; form a layer above pigment layer and below mitochondrial layer in centrifuged eggs (E. N. Harvey, 1932a; E. B. Harvey, 1932, 1936, etc.).

Approximately 1.1035; the whole egg is approximately 1.0485 (Heilbrunn, 1926a).

Amount in Egg.—27.2 % (E. N. Harvey, 1932a). 38 % (Costello, 1939).

Stain.—With vital stains: Bismark brown, brilliant cresyl blue (faint), chrysoidin, methyl violet (later), methylene blue (faint), neutral red, Nile blue, rhodamine, toluidin blue (E. B. Harvey, 1941c). Fixed material. Yolk granules darken with osmic acid (Lyon, 1907; E. B. Wilson, 1926); not stained with most protoplasmic stains (Lyon, 1907; E. B. Wilson, 1926); pale blue with Benda-Kuhl (E. B. Wilson, 1926); stain with orange G but not iron haematoxylin (E. B. Harvey, 1940c). Table 8.

Break Down.—In hypotonic sea water (D. L. Harris, 1943). On rupture of vitelline membrane, and with cytolytic agents, e.g. saponin; no break down in absence of calcium (Costello, 1932).

BIBLIOGRAPHY

BIBLIOGRAPHY

The following list includes all the important papers and books written on *Arbacia punctulata* and also many on other sea urchins during the last century, from about 1850 until 1954. Every paper and book listed has been looked over by the author except that of W. Busch (1849) which was inaccessible

Abelson, P. H. 1947. Permeability of eggs of Arbacia punctulata to radioactive phosphorus. *Biol. Bull.* 93 : 203 (Abstract).

———. 1948. Studies of the chemical form of P^{32} after entry into the Arbacia egg. *Biol. Bull.* 95 : 262 (Abstract).

Abrams, R. 1951. Synthesis of nucleic acid purines in the sea urchin embryo. *Exp. Cell Res.* 2 : 235–242.

Agassiz, A. 1867 (1864). On the embryology of the Echinoderms. *Mem. Amer. Acad. Arts and Sci.* 9 : 1–30.

———. 1869. On the young stages of Echini. *Bull. Mus. Comp. Zool. Harvard* 1 : 279–296.

———. 1872–74. Revision of the Echini. *Mem. Mus. Comp. Zool. Harvard* 3, no. 7 : 11–762.

———. 1876. On viviparous Echini from the Kerguelen Islands. *Proc. Amer. Acad. Arts and Sci.* 11 : 231–236.

———. 1881. Report on the Echinoidea, dredged by H. M. S. Challenger 1873–1876. Report on the scientific results of H. M. S. Challenger, *Zoology* III, Part IX : 1–321.

———. 1883. a. Selections from embryological monographs. II. Echinodermata. *Mem. Mus. Comp. Zool. Harvard* 9, no. 2 : 1–45.

———. 1883. b. Reports on the results of dredging by the U. S. Coast Survey Steamer Blake. XXIV Part I. Report on the Echini. *Mem. Mus. Comp. Zool. Harvard* 10, no. 1 : 1–94.

———. 1904. The Panamic deep sea Echini. *Mem. Mus. Comp. Zool. Harvard* 31 : 53–55 and Plates 53, 54.

——— and Clark, H. L. 1908. Hawaiian and other Pacific Echini. *Mem. Mus. Comp. Zool. Harvard* 34 : 43–133.

Agassiz, L. 1836. Prodrome d'une monographie des radiares. *Mém. Soc. Sci. Nat. Neuchâtel* 1 : 168–199.

———. 1838. *Monographies d'Échinodermes. Des Salénies.* Neuchâtel.

———. 1842–1846. *Nomenclator Zoologicus.* Soloduri; Jent et Gassman..

———. 1857. Essay on classification. *Contributions to the Natural History of the U.S.A.* Vol. 1. Boston. Little, Brown & Co.

Aiyar, R. G. 1935. Early development and metamorphosis of the tropical Echinoid Salmacis bicolor Agassiz. *Proc. Indian Acad. Sci.* Sect. B, 1 : 714–728.

———. 1938. Salmacis. *Indian Zoological Mem.*, ed. by K. N. Bahl, VII. Lucknow Pub. House.

Aldrovandi, U. 1606. *De Reliquis Animalibus.* Lib. III. De testaceis, sive conchyliis. Cap. XL : 400–416. De Echinis. Bononiae.

Allee, W. C. 1919. Note on animal distribution following a hard winter. *Biol. Bull.* 36 : 96–104.

———. 1923. a. Studies in marine ecology. I. The distribution of common littoral invertebrates of the Woods Hole region. *Biol. Bull.* 44 : 167–191.

———. 1923. b. Studies in marine ecology. III. Some physical factors related to the distribution of littoral invertebrates. *Biol. Bull.* 44 : 205–253.

———. 1923. c. Studies in marine ecology: IV. The effect of temperature in limiting the geographical range of invertebrates of the Woods Hole littoral. *Ecology* 4 : 341–354.

——— and Evans, G. 1937. a. Some effects of numbers present on the rate of cleavage and early development in Arbacia. *Biol. Bull.* 72 : 217–232.

——— and Evans, G. 1937. b. Further studies on the effect of numbers on the rate of cleavage in eggs of Arbacia. *J. Cell. and Comp. Physiol.* 10 : 15–28.

——— and Evans, G. 1937. c. Certain effects of numbers present on the early development of the purple sea urchin, Arbacia punctulata: a study in experimental ecology. *Ecology* 18 : 337–345.

———, Finkel, A. J., Garner, H. R., Evans, G. and Merwin, R. M. 1942. Some effects of homotypic extracts on the rate of cleavage of Arbacia eggs. *Biol. Bull.* 83 : 245–259.

Allen, E. J. and Nelson, E. W. 1910. On the artificial culture of marine plankton organisms. *Q. J. M. S.* 55 : 361–431. Also *J. Marine Biol. Assoc.* 8 : 421–474 (1910).

Allen, R. D. 1950. The effect of Janus green B on cleavage and protoplasmic viscosity. *Biol. Bull.* 99 : 353 (Abstract).

———. 1951. a. Antimitotic substances seccreted from eggs. *Biol. Bull.* 101 : 214 (Abstract).

———. 1951. b. The role of the nucleolus in spindle formation. *Biol. Bull.* 101 : 214 (Abstract).

Alsup, F. W. 1941. Photodynamic studies on Arbacia eggs. *Biol. Bull.* 81 : 297 (Abstract).

Amberg, S. and Winternitz, M. C. 1911. The catalase of sea urchin eggs before and after fertilization with especial reference to the relation of catalase to oxidation in general. *J. Biol. Chem.* 10 : 295–302.

Amberson, W. R. 1928. The influence of oxygen tension upon the respiration of unicellular organisms. *Biol. Bull.* 55 : 79–91.

Anderson, R. S. 1939. The X-ray effect on the cleavage time of Arbacia eggs in the

absence of oxygen. *Biol. Bull.* 77 : 325 (Abstract).

ANDREWS, E. A. 1897. Hammar's Ectoplasmic layer. *Amer. Natur.* 31 : 1027–1032.

ANDREWS, G. F. 1897. a. Some spinning activities of protoplasm in starfish and sea-urchin eggs. *J. Morph.* 12 : 367–389.

———. 1897. b. The living substance as such: and as organism. *J. Morph.* 12, suppl. to Part 2 : 1–176.

ANFINSEN, C. B. 1947. The inhibitory action of naphthoquinones on respiratory process: the inhibition of cleavage and respiration in the eggs of Arbacia punctulata. *J. Cell. and Comp. Physiol.* 29 : 323–332.

ANGERER, C. A. 1937. The effect of salts of heavy metals on protoplasm. I. The action of cupric chloride on the viscosity of sea urchin eggs. *J. Cell. and Comp. Physiol.* 10 : 183–197.

———. 1939. The effect of electric current on the relative viscosity of sea urchin egg protoplasm. *Biol. Bull.* 77 : 399–406.

APICIUS, M. G(ABIUS). 80 B.C.–A.D. 40? *De re coquinaria.* Trans. as *Cookery and Dining in Imperial Rome* by J. D. Vehling. 1936. W. M. Hill Pub., Chicago.

ARCHIPPUS, written ca. 415 B.C. *The Fishes.*

ARISTOTLE. 384–322 B.C. *Historia Animalium.* The works of Aristotle trans. into English under the editorship of J. A. Smith and W. D. Ross, Vol. IV. Trans. by D'Arcy Thompson. 1910. Oxford, Clarendon Press.

———. *De Partibus Animalium.* The works of Aristotle trans. into English under the editorship of J. A. Smith and W. D. Ross, Vol. V. Trans. by Wm. Ogle, 1911. Oxford, Clarendon Press.

———. *De Generatione Animalium.* The works of Aristotle trans. into English under the editorship of J. A. Smith and W. D. Ross, Vol. V. Trans. by A. Platt. 1910. Oxford, Clarendon Press.

ASHBEL, R. 1930. Sul quoziente respiratorio della uova fecondate e non fecondate dei ricci di mare (Arbacia pustulosa Gray). *Boll. Soc. Biol. Sperim.* 5 (2) : 72–74.

———. 1931. L'azione della concentrazione degli idrogenioni sulla respirazione della uova fecondate e non fecondate dei ricci di mare. *Pub. Staz. Zool. Napoli* 11 : 194–203.

ATHENAEUS. After A.D. 228. *Deipnosophistae,* or *Banquet of the Learned.* Trans. by C. D. Yonge. London. H. G. Bohn. 1854.

AUGUSTINE, ST. A.D. 354–431. *De Civitate Dei.*

AUGUSTINSSON, K. B. and GUSTAFSON, T. 1949. Cholinesterase in developing sea urchin eggs. *J. Cell. and Comp. Physiol.* 34 : 311–321.

BAGLIONI, S. 1905. Über das Sauerstoff Bedürfnis des Zentralnervensystems bei Seetieren. *Zeits. Allgem. Physiol.* 5 : 415–434.

BALDWIN, E. 1952. *Dynamic Aspects of Biochemistry.* 2nd ed. Cambridge Univ. Press.

BALDWIN, F. M. 1920. Susceptible and resistant phases of the dividing sea urchin egg when subjected to various concentrations of lipoid-soluble substances, especially the higher alcohols. *Biol. Bull.* 38 : 123–140.

BALL, E. G. 1934. Isolation of crystalline echinochrome. *Biol. Bull.* 67 : 327–328. (Abstract).

———. 1936. Echinochrome, its isolation and composition. *J. Biol. Chem.* 114 : VI, after p. 355.

———. 1942. Oxidative mechanisms in animal tissues. *Symposium on Respiratory Enzymes.* Univ. Wisconsin Press pp. 16–32.

——— and COOPER, O. 1949. Echinochrome: its absorption spectra; pK_1' value; and concentration in the eggs, amoebocytes and test of Arbacia punctulata. *Biol. Bull.* 97 : 231–232 (Abstract).

——— and MEYERHOF, B. 1940. On the occurrence of iron-porphyrin compounds and succinic dehydrogenase in marine organisms possessing the copper blood pigment hemocyanin. *J. Biol. Chem.* 134 : 483–493.

——— and RAMSDELL, P. A. 1940. Squid ink, a study of its composition and enzymatic production. *Biol. Bull.* 79 : 353 (Abstract).

——— and STOCK, C. C. 1937. The pH of sea water as measured with the glass electrode. *Biol. Bull.* 73 : 221–226.

BALLENTINE, R. 1938. Reducing activity of fertilized and unfertilized Arbacia eggs. *Biol. Bull.* 75 : 368 (Abstract).

———. 1940. a. Total nitrogen content of the Arbacia egg. *J. Cell. and Comp. Physiol.* 15 : 121–122.

———. 1940. b. Analysis of the changes in respiratory activity accompanying the fertilization of marine eggs. *J. Cell and Comp. Physiol.* 15 : 217–232.

———. 1940. c. Quantitative cytochemistry. The distribution of the reducing systems in the egg of Arbacia punctulata. *J. Cell and Comp. Physiol.* 16 : 39–47.

BALLOWITZ, E. 1888, 1890. Untersuchungen über die Struktur der Spermatozoen, zugleich ein Beitrag zur Lehre vom feineren Bau der contraktilen Elemente. I. Birds, *Arch. f. Mikr. Anat.* 32 : 401–473 (1888). II. Insects, *Zeit. f. Wissen. Zool.* 50 : 317–

407 (1890). III. Fish, Amphibia, Reptiles, *Arch. f. Mikr. Anat.* 36 : 225–290 (1890).

Baltzer, F. 1908. Über mehrpolige Mitosen bei Seeigeleiern. *Verh. Phys.-Med. Gesells. Würzburg* 39 : 291–330.

———. 1910. Über die Beziehung zwischen dem Chromatin und der Entwicklung und Vererbungsrichtung bei Echinodermenbastarden. *Arch. f. zell for.* 5 : 497–621.

———. 1913. Über die Herkunft der idiochromosomen bei Seeigeln. *Sitz.-Ber. Physik.-med. Gesell. Würzburg.* 1913 : 90–94.

Balzer, D. T. and Villee, C. A. 1951. Drugs inhibiting growth and nucleic acid synthesis in Arbacia embryos. *Biol. Bull.* 101 : 204 (Abstract).

Bank, O. 1933. Die umkehrbare Entmischung des Kernes bei Allium cepa und im Arbacia-ei. *Protoplasma* 19 : 125–131.

Barabasch-Nikiforov, I. L. 1933. Der Kalan oder Seeotter. (Russian). See *Mertens*, R. 1935.

Barnes, H. and Lord Rothschild. 1950. A note on the copper content of sea urchin semen and sea water. *J. Exp. Biol.* 27 : 123–125.

Barnett, R. C. 1953. Cell division inhibition of Arbacia and Chaetopterus eggs and its reversal by Krebs cycle intermediates and certain phosphate compounds. *Biol. Bull.* 104 : 263–274.

Barron, E. S. G. 1929. Studies on blood cell metabolism. III. The effect of methylene blue on the eggs of the sea urchin and starfish. The mechanism of the action of methylene blue on living cells. *J. Biol. Chem.* 81 : 445–457.

———. 1932. The effect of anaerobiosis on the eggs and sperm of sea urchin, starfish and Nereis and fertilization under anaerobic conditions. *Biol. Bull.* 62 : 46–53.

———. 1943. The application of biological oxidation-reduction systems to the study of cellular respiration. *Biol. Symposia* 10 : 27–69.

———. 1952. (Editor) *Modern Trends in Physiology and Biochemistry.* Academic Press Pub.

———. 1952. a. The mechanism of enzymatic oxidation-reductions. *Modern Trends*, ed. by Barron : 1–24.

———. 1952. b. The oxidative pathways of carbohydrate metabolism. *Modern Trends*, ed. by Barron: 471–507.

———, Flood, V. and Gasvoda, B. 1949. Studies on the mechanism of ionizing radiations. V. The effect of hydrogen peroxide and of X-radiated sea water on the respiration of sea urchin sperm and eggs. *Biol. Bull.* 97 : 51–56.

———, Gasvoda, B. and Flood, V. 1949. Studies on the mechanism of action of ionizing radiations. IV. Effect of X-ray irradiation on the respiration of sea urchin sperm. *Biol. Bull.* 97 : 44–50.

——— and Goldinger, J. M. 1941. Effect of iodoacetate and malonate on respiration of sea urchin sperm. *Proc. Soc. Exp. Biol. and Med.* 48 : 570–574.

——— and Hamburger, M., Jr. 1932.The effect of cyanide upon the catalytic action of dyes on cellular oxygen consumption. *J. Biol. Chem.* 96 : 299–305.

———, Muntz, J. A. and Gasvoda, B. 1948. Regulatory mechanisms of cellular respiration. I. The role of cell membranes: Uranium inhibition of cellular respiration. *J. Gen. Physiol.* 32 : 163–178.

———, Nelson, L. and Ardao, M. I. 1948. Regulatory mechanisms of cellular respiration. II. The role of soluble sulphydryl groups as shown by the effect of sulphydryl reagents on the respiration of sea urchin sperm. *J. Gen. Physiol.* 32 : 179–190.

———, Seegmiller, J. E., Mendes, E. G. and Narahara, H. T. 1948. The effect of nitrogen mustards on the respiration and fertilization of sea urchin sperm and eggs. *Biol. Bull.* 94 : 267–274.

——— and Seki, S. L. 1952. Studies on the mechanism of action of ionizing radiations. VII. Cellular respiration, cell division and ionizing radiations. *J. Gen. Physiol.* 35 : 865–871.

———, Seki, L. and Johnson, P. 1952. Ibid. VIII Effect of hydrogen peroxide on cell metabolism, enzymes and proteins. *Arch. Biochem. and Biophys.* 41 : 188–202.

Barth, L. G. 1929. The effects of acids and alkalis on the viscosity of protoplasm. *Protoplasma* 7 : 505–534.

Bartholomaeus, A. or Bartholomew (ca. 1190–1260). *De Proprietatibus Rerum*, written ca. 1240, trans. 1397 or 1398 by J. Trevisa as *Properties of all Thyngs.*

Barton, J., II. 1951. A new DNA fraction of the cell nucleus. *Biol. Bull.* 101 : 236 (Abstract).

———. 1952. The action of DN-ase on cell nuclei. *Biol. Bull.* 103 : 319–327.

Baylor, M. R. B., Nalbandov, A. and Clark. G. L. 1943. Electron microscope study of sperm. *Proc. Soc. Exp. Biol. and Med.* 54 : 229–232.

Beams, H. W. and Evans, T. C. 1940. Some effects of colchicine upon the first cleavage

in Arbacia punctulata. *Biol. Bull.* 79 : 188–198.

Becker, W. A. 1936. Vitale Cytoplasma- und Kernfärbungen. *Protoplasma* 26 : 439–487.

Bedford, F. P. 1900. On Echinoderms from Singapore and Malacca. *Proc. Zool. Soc. London* 1900: 271–299.

Bell, F. J. 1889. Note on the relative claims to recognition of the generic names Arbacia, Gray and Echinocidaris, Des Moulins. *Ann. Mag. Nat. Hist.* 6th Ser. 3 : 290.

Belon, P. 1553. *De Aquatilibus.* Paris.

Benedict, D. and Barron, E. S. G. 1946. The effect of uranyl nitrate on the respiration of Arbacia sperm. *Biol. Bull.* 91 : 220 (Abstract).

Bernasconi, I. 1947. Distribucion geografica de los Equinoideos Argintinos. *Gaea*, Buenos Aires 8 : 97–114.

Bernstein, M. H. 1949. Ribonucleinase activity in the development of the sea urchin, Arbacia punctulata. *Biol. Bull.* 97 : 255 (Abstract).

——— and Mazia, D. 1953. a. The desoxyribonucleoprotein of sea urchin sperm. I. Isolation and analysis. *Biochim. et Biophys. Acta* 10 : 600–606.

——— and Mazia, D. 1953. b. Ibid. II. Properties. *Biochim. et Biophys. Acta* 11 : 59–68.

Berrill, N. J. 1929. Studies in tunicate development. Part I. General physiology of development of simple Ascidians. *Phil. Trans. Roy. Soc. London* B 218 : 37–78.

Bethe, A. and Berger, E. 1931. Variationen im Mineralbestand verschiedener Blutarten. *Pflüger's Arch. f. ges. Physiol.* 227 : 571–584.

Bevelander, G. 1946. Effect of thiurea on development of the sea urchin, Arbacia punctulata. *Proc. Soc. Exp. Biol. and Med.* 61 : 268–270.

Bialaszewicz, K. 1927. Contributions à l'étude de la composition minérale des cellules œufs. *Pub. Staz. Zool. Nap.* 8 : 355–369.

——. 1929. Recherches sur la répartition des électrolytes dans le protoplasme des cellules ovulaires. *Protoplasma* 6: 1–50.

———. 1933. Contribution à l'étude de la composition minéral des liquides nourriciers chez les animaux marins. *Arch. Internat. Physiol.* 36 : 41–53.

Bielig, H. J. and Medem, F. 1949. Wirkstoffe der tierischen Befruchtung. *Experientia* 5 : 11–30.

Blanchard, K. C. 1935. The nucleic acid of the eggs of Arbacia punctulata. *J. Biol. Chem.* 108 : 251–256.

Blum, H. F., Price, J. P., Robinson, J. C. and Loos, G. M. 1949. a. Effect of ultraviolet radiation on the rate of cell division of Arbacia eggs. *Biol. Bull.* 97 : 232 (Abstract).

———, Loos, G. M., Price, J. P. and Robinson, J. C. 1949. b. Enhancement by "visible" light of recovery from ultraviolet irradiation in animal cells. *Nature* 164 : 1011.

——— and Price, J. P. 1950. a. Delay in cleavage of the Arbacia egg by ultraviolet radiation. *J. Gen. Physiol.* 33 : 285–304.

——— and ———. 1950. b. Time relationships in the cleavage of the normal fertilized egg of Arbacia punctulata. *J. Gen Physiol.* 33 : 305–310.

———, Robinson, J. C. and Loos, G. M. 1950. c. The loci of action of ultraviolet and X-radiation and of photorecovery, in the egg and sperm of the sea urchin. *Proc. Nat. Acad. Sci.* 36 : 623–627.

———, Loos, G. M. and Robinson, J. C. 1950. d. The accelerating action of illumination in recovery of Arbacia eggs from exposure to ultraviolet radiation. *J. Gen. Physiol.* 34 : 167–181.

———, Robinson, J. C. and Loos, G. M. 1951. The loci of action of ultraviolet and X-radiation and of photorecovery in the egg and sperm of the sea urchin Arbacia punctulata. *J. Gen. Physiol.* 35 : 323–342.

Blumenthal, R. 1927. The effects of cyanides on the swelling of protoplasm. *Biol. Bull.* 52 : 313–324.

———. 1928. The effects of certain anaesthetics on the cleavage reaction in sea urchin eggs. *Physiol. Zool.* 1 : 269–285.

———. 1930. Mitotic development of Arbacia eggs in cyanides. *Physiol. Zool.* 3 : 539–563.

Boell, E. J., Chambers, R., Glancy, E. A. and Stern, K. G. 1940. Oxidase activity and respiration of cells and cell-fragments. *Biol. Bull.* 79 : 352 (Abstract).

Bogucki, M. 1930. À propos de la prétendue action inhibitrice du liquide cœlomique sur la membranogénèse et sur la segmentation des œufs d'oursin. *Protoplasma* 11 : 432–439.

Bohn, G. 1903. Influence des rayons du radium sur les œufs vierges et fécondés, et sur les premiers stades du développement. *C. R. Acid. Sci. Paris* 136 : 1085–1086.

——— and Drzewina, A. 1923. Action toxique du rouge neutre en presence de la lumière. *C. R. Soc. Biol. Paris* 89 : 386–388.

Bohus-Jensen, A. A. 1950. Action of ficin on sea urchin eggs. *Exp. Cell Res.* 1 : 351–352.

——— 1953. The effect of trypsin on the

cross fertilizability of sea urchin eggs. *Exp. Cell. Res.* 5 : 325–328.

Bölin, L. 1926. Der Geotropismus von Psammechinus miliaris O.F.M. *Internat. Rev. Gesam. Hydrobiol. u. Hydrograph.* 16 : 125–129.

Boone, L. 1928. Echinodermata from tropical East American Seas. *Bull. Bingham Oceanog. Collect.* 1, Art. 4 : 1–22.

Borei, H. 1948. Respiration of oocytes, unfertilized eggs and fertilized eggs from Psammechinus and Asterias. *Biol. Bull.* 95 : 124–150.

———. 1949. Independence of post-fertilization respiration in the sea urchin egg from the level of respiration before fertilization. *Biol. Bull.* 96 : 117–122.

———. 1951. Cytochrome c in sea urchin eggs. *Acta Chem. Scand.* 4 : 1607–1608.

——— and Lybing, S. 1949. Temperature coefficients of respiration in Psammechinus eggs. *Biol. Bull.* 96 : 107–116.

Borei, H. G. and Björklund, U. 1953. Effect of versene on sea urchin eggs. *Exp. Cell Res.* 5 : 216–219.

Bottazzi, F. 1897. La pression osmotique du sang des animaux marins. *Arch. Ital. de Biol.* 28 : 61–72.

Bourne, G. H. 1942. (Editor). *Cytology and Cell Physiology.* Oxford Press.

———. 1951. (Editor). *Cytology and Cell Physiology.* 2nd ed. Oxford Press.

Boveri, Th. 1889. Ein geschlechtlich erzeugter Organismus ohne mütterliche Eigenschaften. *Sitz. Gesel. Morph. u. Physiol. München* 5 : 73–83. Trans. by T. H. Morgan, 1893, as "An organism produced sexually without characteristics of the mother." *Amer. Naturalist* 27 : 222–232.

———. 1897. Zur Physiologie der Kern- und Zellteilung. *Sitz. Ber. Physik,-Med. Gesell. Würzburg* 1896 : 133–151.

———. 1901. Die Polarität von Ovocyte, Ei und Larve des Strongylocentrotus lividus. *Zool. Jahrb.*, Abt. f. Anat. 14 : 630–653.

———. 1902. Über mehrpolige Mitosen. *Verhand. Phys.-Med. Gesell. Würzburg* 35 : 67–90.

———. 1907. Zellenstudien VI. Die Entwickelung dispermer Seeeigeleier. *Jenais. Zeits.* 36 (N. F.): 1–292.

———. 1918. Zwei Fehlerquellen bei Merogonieversuchen und die Entwicklungsfähigkeit merogonischer und partiell-merogonischer Seeigelbastarde. *Arch. f. Entwick.* 44 : 417–471.

Brachet, A. 1922. Recherches sur la fécondation prématurée de l'œuf d'oursin (Paracentrotus lividus). *Arch. de Biol.* 32 : 205–244.

———. 1933. Recherches sur la synthèse de l'acide thymonucléique pendant le développement de l'œuf d'oursin. *Arch. de Biol.* 44 : 519–576.

———. 1937. Remarques sur la formation de l'acide thymonucléique pendant le développement des œufs à synthèse partielle. *Arch. de Biol.* 48 : 529–548.

———. 1947. The metabolism of nucleic acids during embryonic development. *Cold Spring Harbor Symposium* 12 : 18–27.

———. 1950. *Chemical Embryology.* Trans. by L. G. Barth. Interscience Pub., N.Y. Chapt. VI. Synthesis, localization and physiological role of the nucleic acids.

———. 1952. The role of the nucleus and the cytoplasm in synthesis and morphogenesis. *Symp. Soc. Exp. Biol.* 6 : 173–200.

Brehm, A. E. *Brehm's Tierleben.* 1925. Leipzig. Bd. 1 : 359–373. 1878, Abt. 4, Bd. 2, p. 431.

Breynius, J. P. 1732. *Schediasma de Echinis et Echinitis.* Gedani.

Brinley, F. J. 1930. The effect of intracellular cyanide on cleavage of Arbacia eggs. *Physiol. Zool.* 3 : 366–372.

Bronn, H. G. 1904. *Klassen und Ordnungen des Thier-Reichs.* Bd. 2, Abt. 3, Buch IV. Die Seeigel. Leipzig.

Brooks, M. M. 1933. The effect of respiratory poisons and methylene blue on cleavage of certain eggs. *Biol. Bull.* 65 : 356. (Abstract).

———. 1943. Methylene blue, potassium cyanide and carbon monoxide as indicators for studying the oxidation-reduction potentials of developing marine eggs. *Biol. Bull.* 84 : 164–177.

———. 1946. a. Oxidation-reduction studies as a clue to the mechanism of fertilization of marine eggs. *Biol. Bull.* 91 : 212 (Abstract).

———. 1946. b. The mechanism of fertilization of eggs. *Growth* 10 : 391–410.

———. 1951. Oxidation-reduction dyes and "lithium larvae". *Biol. Bull.* 101 : 217. (Abstract).

Brooks, S. C. 1940. The intake of radioactive isotopes by living cells. *Cold Spring Harbor Symp.* 8 : 171–180.

———. 1943. Intake and loss of ions by living cells. I. Eggs and larvae of Arbacia punctulata and Asterias forbesi exposed to phosphate and sodium ions. *Biol. Bull.* 84 : 213–225.

——— and Brooks, M. M. 1941. *The Permeability of Living Cells.* Borntraeger, Berlin. Reprinted 1944 by Edwards, Ann Arbor, Mich.

——— and Chambers, E. L. 1948. Penetration of radioactive phosphate into the

eggs of Strongylocentrotus purpuratus, S. franciscanus and Urechis caupo. *Biol. Bull.* 95 : 262–263 (Abstract).

Brooks, W. K. 1882. *Handbook of Invertebrate Zoology*. Cassino, Boston.

Brown, D. E. S. 1934. The pressure coefficient of "viscosity" in the eggs of Arbacia punctulata. *J. Cell. and Comp. Physiol.* 5 : 335–346.

Brown, F. A. Jr. 1950. *Selected Invertebrate Types*. Wiley & Sons, N.Y. Echinodermata by W. M. Reid; Arbacia punctulata pp. 528–540.

Brown, R. L. 1944. Rate of transport of spermia in human uterus and tubes. *Am. J. Obst. and Gyn.* 47 : 407–411.

Browne, Sir T. 1658. *The Garden of Cyrus*.

Bruguières, J. G. 1789. Histoire naturelle des Vers Échinodermes. *Encyclopédie Méthodique*.

Bryce, T. H. 1902. Maturation of the ovum in Echinus esculentus. *Q. J. M. S.* 46 : 177–224.

Buchsbaum, R. and Williamson, R. R. 1943. The rate of elongation and constriction of dividing sea urchin eggs as a test of a mathematical theory of cell division. *Physiol. Zool.* 16 : 162–171.

Budington, R. A. 1935. The tolerance of acetyl-salicylic acid by sperm and eggs of Arbacia. *Biol. Bull.* 69 : 335 (Abstract).

Bull, H. O. 1939. The growth of Psammechinus miliaris (Gmelin) under aquarium conditions. *Rep. Dove Marine Lab.*, Ser. 3, no. 6 : 39–42.

Buller, A. H. R. 1900. a. Contributions to our knowledge of the physiology of the spermatozoa of ferns. *Ann. of Botony* 14: 543–582.

———. 1900. b. The fertilization process in Echinoidea. *Rep. British Assoc. 70th Meeting*: 387–388.

———. 1902. Is chemotaxis a factor in the fertilization of the eggs of animals? *Q. J. M. S.* 46 : 145–176.

Bury, H. 1889. Studies in the embryology of the Echinoderms. *Q. J. M. S.* 29 : 409–449.

———. 1895. The metamorphosis of Echinoderms. *Q. J. M. S.* 38 : 45–135.

Bury, J. 1913. Experimentelle Untersuchungen über die Einwirkung der Temperatur o °C. auf die Entwicklung der Echinideneier. *Arch. f. Entwick.* 36 : 537–594.

Busch, W. 1849. *Beobachtungen über Anatomie und Entwickelung einiger wirbellosen Seethiere*. Berlin.

Bütschli, O. 1908. Untersuchungen über organische Kalkgebilde, nebst Bemerkungen über organische Kieselgebilde. *Abhandl. König. Gesel. d. Wissen. zu Göttingen.* Math. Physik. Kl. N.F. 6 : no. 3 : 1–177.

Byron, Lord. 1821. *Sardanapalus: A Tragedy*.

Cailliaud, F. 1856. Observations sur les oursins perforants de Bretagne. *Rev. et Mag. de Zool.*, Ser. II, 8 : 158–179.

Callan, H. G. 1949. Cleavage rate, oxygen consumption and ribose nucleic acid content of sea urchin eggs. *Biochim. et Biophys. Acta* 3 : 92–102.

Camerano, L. 1890. Osservazioni intorno al dimorfismo sessuale degli Echinodermi. *Boll. Mus. Zool. Anat. Comp. Torino* 5, no. 91 : 1–3.

Camerarius, J. 1654. *Symbolorum et Emblematum ex Re Herbaria Desumtorum Centuria IV, Una Collecta*. Frankfurt.

Cannan, R. K. 1927. Echinochrome. *Biochem. J.* 21 : 184–189.

Carter, G. S. 1924. On the early development of the Echinoderm egg. *Proc. Cambridge Phil. Soc.* (= *Biol. Rev.*) 1 : 79–107.

———. 1931. Iodine compounds and fertilization. II. The oxygen consumption of suspensions of sperm of Echinus esculentus and Echinus miliaris. *J. Exp. Biol.* 8 : 176–193.

Caso, M. E. 1953. Estado actual de los conocimientos acerca de la fauna de los Equinodermis de Mexico. Mexico, D. F. Earlier papers, 1946, 1948, 1949. *Anal. Inst. Biol. Mexico*, vol. 17, 19, 20.

Caspersson, T. and Schultz, J. 1940. Ribonucleic acids in both nucleus and cytoplasm, and the function of the nucleolus. *Proc. Nat. Acad. Sci.* 26 : 507–514.

Cerami, C. 1924. Dimorfismo sessuale nei ricci di mare. *Ist. Zool. Anat. Comp. Palermo, Diss.* See La Cascia 1930.

Chadwick, H. C. 1929. Regeneration of spines in Echinus esculentus. *Nature* 124 : 760–761.

Chambers, E. L. 1949. The uptake and loss of K^{42} in the unfertilized and fertilized eggs of Strongylocentrotus purpuratus and Arbacia punctulata. *Biol. Bull.* 97 : 251–252 (Abstract).

——— and Chambers, R. 1938. The resistance of fertilized Arbacia eggs to immersion in KCl and NaCl solutions. *Biol. Bull.* 75 : 356–357 (Abstract).

——— and Chambers, R. 1949. Ion exchanges and fertilization in Echinoderm eggs. *Am. Nat.* 83 : 269–284.

———, White, W., Jeung, N. and Brooks, S. C. 1948. Penetration and the effects of low temperature and cyanide on penetration of radioactive potassium into the eggs of Strongylocentrotus purpuratus

and Arbacia punctulata. *Biol. Bull.* 95 : 252–253.

CHAMBERS, E. L. and MENDE, J. T. 1953. a. The adenosine triphosphate content of the unfertilized and fertilized eggs of Asterias forbesii and Strongylocentrotus dröbachiensis. *Arch. Biochem. and Biophys.* 44 : 46–56.

——— and WHITE, W. E. 1949. The accumulation of phosphate and evidence for synthesis of adenosine triphosphate in the fertilized sea urchin egg. *Biol. Bull.* 97 : 225–226.

———, WHITELEY, A., CHAMBERS, R. and BROOKS, S. C. 1948. Distribution of radio active phosphate in the eggs of the sea urchin, Lytechinus pictus. *Biol. Bull.* 95 : 263 (Abstract).

CHAMBERS, R. 1917. Microdissection Studies. I. The visible structure of cell protoplasm and death changes. *Am. J. Physiol.* 43 : 1–12.

———. 1919. Changes in protoplasmic consistency and their relation to cell division. *J. Gen. Physiol.* 2 : 49–68.

———. 1921. a. Microdissection Studies. III. Some problems in the maturation and fertilization of the Echinoderm egg. *Biol. Bull.* 41 : 318–350.

———. 1924. The physical structure of protoplasm as determined by micro-dissection and injection. *Cowdry's General Cytology*: 237–309.

———. 1928. Intracellular hydrion concentration studies. I. The relation of the environment to the pH of protoplasm and of its inclusion bodies. *Biol. Bull.* 55 : 369–385.

———. 1930. Enveloping membranes of echinoderm ova. *Science* 71 : 340.

———. 1933. The manner of sperm entry in various marine ova. *J. Exp. Biol.* 10 : 130–141.

———. 1935. a. The living cell. *Harrow & Sherwin's Textbook of Biochemistry*, Chapt. I: 17–39.

———. 1935. b. Studies on the physical properties of the plasma membrane. *Biol. Bull.* 69 : 331 (Abstract).

———. 1936. Experimental studies on the oil wetting property of the plasma membrane. *Biol. Bull.* 71 : 398 (Abstract).

———. 1937. a. Cortical cytolysis of the Echinoderm egg. *Biol. Bull.* 73 : 353–354 (Abstract).

———. 1937. b. The physical state of the wall of the furrow in a dividing cell. *Biol. Bull.* 73 : 367–368 (Abstract).

———. 1938. a. Cytoplasmic inclusions and matrix of the Arbacia egg. *Biol. Bull.* 75 : 350–351 (Abstract).

CHAMBERS, R. 1938. b. The physical state of protoplasm with special reference to its surface. *Am. Nat.* 72 : 141–159.

———. 1938. c. Structural and kinetic aspects of cell division. *J. Cell. and Comp. Physiol.* 12 : 149–165.

———. 1940. The relation of extraneous coats to the organization and permeability of cellular membranes. *Cold Spring Harbor Symp.* 8 : 144–153.

———. 1942. The intrinsic expansibility of the fertilization membrane of Echinoderm ova. *J. Cell. and Comp. Physiol.* 19 : 145–150.

———. 1944. Some physical properties of protoplasm. *Colloid Chemistry* ed. by J. Alexander 5 : 864–875.

———. 1946. Karyokinetic lengthening and cleavage. *Anat. Rec.* 94 : 373 (Abstract).

———. 1949. Micrurgical studies of protoplasm. *Biol. Rev.* 24 : 246–265.

———. 1950. The cell as an integrated functional body. *Ann. N.Y. Acad. Sci.* 50 : 817–823.

———. 1951. Micrurgical studies on the kinetic aspects of cell division. *Ann. N.Y. Acad. Sci.* 51 : 1311–1326.

——— and KOPAC, M. J. 1937. a. The coalescence of living cells with oil drops. I. Arbacia eggs immersed in sea water. *J. Cell. and Comp. Physiol.* 9 : 331–343.

——— and KOPAC, M. J. 1937. c. The coalescence of sea urchin eggs with oil drops. *Carnegie Inst. Washington Year Book.* 36 : 88–89.

——— and OHSHIMA, H. 1922. Merogony experiments on sea urchin eggs. *Proc. Soc. Exp. Biol. & Med.* 19 : 320–321.

——— and POLLACK, H. 1927. The pH of the blastocoel of Echinoderm embryos. *Biol. Bull.* 53 : 233–238.

CHASE, A. M. and GLASER, O. 1930. Forward movement of Paramoecium as a function of the hydrogen ion concentration. *J. Gen. Physiol.* 13 : 627–636.

CHASE, H. Y. 1935. The origin and nature of the fertilization membrane in various marine ova. *Biol. Bull.* 69 : 159–184.

———. 1938. The effect of ultraviolet light upon cleavage in certain marine eggs. *Biol. Bull.* 75 : 134–144.

CHAUCER, G. (1340?–1400) *Boethius* (Skeat ed.).

CHENEY, R. H. 1945. The effects of caffeine on oxygen consumption and cell division in the fertilized egg of the sea urchin, Arbacia punctulata. *J. Gen. Physiol.* 29 : 63–72.

———. 1948. Caffeine effects on fertilization and development in Arbacia punctulata. *Biol. Bull.* 94 : 16–24.

CHENEY, R. H. 1949. a. Stratification and deformation of Arbacia punctulata eggs centrifuged in caffeine solutions. *Biol. Bull.* 96 : 70–73.

———. 1949. b. Theobromine and theophylline effects upon the rate and form of Arbacia development. *Biol. Bull.* 97 : 234 (Abstract).

———. 1950 Viability of Arbacia gametes in relation to time. *Biol. Bull.* 99 : 354–355 (Abstract).

———. 1951. Behavior of cortical granules in methylated xanthine effects. *Biol. Bull.* 101 : 207 (Abstract).

CHESLEY, L. C. 1934. The effect of radiation upon cell respiration. *Biol. Bull.* 67 : 259–272.

CHILD, C. M. 1916. a. Axial susceptibility gradients in the early development of the sea urchin. *Biol. Bull.* 30 : 391–405.

———. 1916. b. Experimental control and modification of larval development in the sea urchin in relation to the axial gradients. *J. Morph.* 28 : 65–133.

———. 1924. *Physiological Foundations of Behavior.* Holt and Co.

———. 1936. a. A contribution to the physiology of exogastrulation in Echinoderms. *Arch. f. Entwick.* 135 : 457–493.

———. 1936. b. Differential reduction of vital dyes in the early development of Echinoids. *Arch. f. Entwick.* 135 : 426–456.

———. 1940. Lithium and Echinoderm exogastrulation: with a review of the physiological-gradient concept. *Physiol. Zool.* 13 : 4–12.

———. 1941. *Patterns and Problems of Development.* Univ. Chicago Press, Chicago, Ill.

———. 1948. Exogastrulation by sodium azide and other inhibiting conditions in Strongylocentrotus purpuratus. *J. Exper. Zool.* 107 : 1–38.

———. 1950. a. Differential modification of sand dollar development in relation to temperature. *Physiol. Zool.* 23 : 140–168.

———. 1950. b. Differential inhibition and modification of Echinoderm development by methylene blue. *Physiol. Zool.* 23 : 269–287.

CHURNEY, L. 1936. The quantitative determination of mitotic elongation. *Biol. Bull.* 70 : 400–407.

———. 1938. The effect of potassium and of urea on the breakdown of the pigment granules in the fertilized egg of Arbacia. *Anat. Rec.* 72, Suppl. : 65–66.

———. 1940. Mitotic elongation. II. Osmotic and salt effects. *Physiol. Zool.* 13 : 95–101.

———. 1941. a. Effect of shearing forces on the cortex of the fertilized egg of Arbacia punctulata. *Proc. Soc. Exp. Biol. and Med.* 46 : 393–395.

CHURNEY, L. 1941. b. The physico-chemical properties of the nucleus. *Univ. Penn. Bicent. Conf.*, Cytology, Genetics and Evolution: 113–128. Univ. Penn. Press, Philadelphia.

———. 1942. The osmotic properties of the nucleus. *Biol. Bull.* 82 : 52–67.

——— and MOSER, F. 1940. The effect of excess potassium on the cortex of the fertilized egg of Arbacia punctulata. *Physiol. Zool.* 13 : 212–217.

CLARK, H. L. 1899. Further notes on the Echinoderms of Bermuda. *Ann. N.Y. Acad. Sci.* 12 : 117–138.

———. 1902. The Echinoderms of the Woods Hole Region. *Bull. U.S. Fish Comm.* 22 : 545–576. Arbacia punctulata, p. 563.

———. 1913. Echinoderms from Lower California, with descriptions of new species. *Bull. Am. Mus. Nat. Hist.* 32 : 185–236.

———. 1921. Report on the Echinoidea of the Barbados-Antigua Expedition from Univ. Iowa in 1918. *Univ. Iowa Studies in Natural Hist.* 9, no. 5 : 103–121.

———. 1923. The distribution and derivation of some New England Echinoderms. *Am. Nat.* 57 : 229–237.

———. 1925. *A catalogue of The Recent Sea-urchins (Echinoidea).* The British Museum, London.

———. 1932. The ancestry of Echini. *Science* 76 : 591–593.

———. 1933. A handbook of the littoral Echinoderms of Porto Rico and the other West Indian Islands. *N.Y. Acad. Sci.* 16, Part 1 : 1–147.

———. 1942. The Echinoderm fauna of Bermuda. *Bull. Mus. Comp. Zool. Harvard* 89 : 367–391.

———. 1946. The Echinoderm fauna of Australia. *Carneg. Inst. Washington, Pub.* no. 566 : 1–567.

———. 1948. A report of the Echini of the warmer Eastern Pacific, based on the collections of the Valero III. *Alan Hancock Exp.* 8 : 225–352. Univ. S. Cal.

CLARK, J. M. 1936. An experimental study of polyspermy. *Biol. Bull.* 70 : 361–384.

CLARK, L. B. 1940. Effects of visible radiation on Arbacia eggs sensitized with rhodamine B. *Biol. Bull.* 79 : 368–369.

CLELAND, K. W. and LORD ROTHSCHILD. 1952. a. The metabolism of the sea urchin egg. Anaerobic breakdown of carbohydrate. *J. Exp. Biol.* 29 : 285–294.

——— and ———. 1952. b. The metabolism of the sea urchin egg. Oxidation of carbohydrate. *J. Exper. Biol.* 29 : 416–428.

CLOWES, G. H. A. 1951. The inhibition of cell division by substituted phenols with special reference to the metabolism of dividing cells. *Ann. N.Y. Acad. Sci.* 51 : 1409–1431.

——— and BACHMAN, E. 1921. A volatile sperm stimulating substance derived from marine eggs. *J. Biol. Chem.* 46 : Proceedings XXXI–XXXII (Abstract).

———, KELTCH, A. K. and KRAHL, M. E. 1940. Extracellular and intracellular hydrogen ion concentration in relation to anesthetic effects of barbituric arid derivatives. *J. Pharm. and Exp. Therap.* 68 : 312–329.

———, KELTCH, A. K., STRITTMATIER, C. F. and WALTERS, C. P. 1950. Action of nitro- and halophenols upon oxygen consumption and phosphorylation by a cell-free particulate system from Arbacia eggs. *J. Gen. Physiol.* 33 : 555–561.

———, KELTCH, A. K. and WADE, L. 1933. Variation in the sensitivity of eggs following fertilization. *Biol. Bull.* 65 : 367. (Abstract).

———, KELTCH, A. K. and WALTERS, C. P. 1951. a. On a dinitrocresol-resistant, phosphorylating mechanism present in cell-free homogenates of unfertilized Arbacia eggs. *Biol. Bull.* 101 : 219 (Abstract).

———, ——— and ———. 1951. b. On the effects exerted by calcium chloride and by freezing and thawing on the anaerobic, dinitrocresol-resistant, phosphorylating mechanism of homogenates of Arbacia eggs. *Biol. Bull.* 101 : 220 (Abstract).

——— and KRAHL, M. E. 1934. a. Action of dinitro compounds on sea urchin eggs. *Science* 80 : 384–385.

——— and ———. 1934. b. Action of certain oxidative stimulants and depressants on respiration and cell division. *The Lilly Research Laboratories Dedication*: 73–79.

——— and ———. 1935. Action of certain oxidative stimulants and depressants on respiration and cell division. *J. Biol. Chem.* 109 : XXI.

——— and ———. 1936. a. Studies on cell metabolism and cell division. I. On the relation between molecular structures, chemical properties, and biological activities of the nitrophenols. *J. Gen. Physiol.* 20 : 145–171.

——— and ———. 1936. b. On the action of dinitro compounds on cell division. *Am. J. Cancer* 26 : 192–193 (Abstract).

——— and ———. 1936. c. Antagonistic action of dinitrothymol and dinitrocresol on cellular respiration. *Proc. Soc. Exp. Biol. and Med.* 34 : 565–566.

CLOWES G. H. A. and KRAHL M. E. 1940, Studies on cell metabolism and cell division. III. Oxygen consumption and cell division of fertilized sea urchin eggs in the presence of respiratory inhibitors. *J. Gen. Physiol.* 23 : 401–411.

——— and SMITH, H. W. 1923. The influence of hydrogen ion concentration on the fertilization and growth of certain marine eggs. *Am. J. Physiol.* 64 : 144–159.

COE, W. R. 1912. Echinoderms of Connecticut. *Conn. Geol. and Nat. Hist. Survey*, Bull. no. 19 : 1–152.

COHEN, I. 1940. Cleavage delay in Arbacia punctulata eggs irradiated while closely packed in capillary tubes. *Radiology* 35 : 561–562.

COHN, E. J. 1918. Studies in the physiology of spermatozoa. *Biol. Bull.* 34 : 167–218.

COLE, K. S. 1928. Electric impedance of suspensions of Arbacia eggs. *J. Gen. Physiol.* 12 : 37–54.

———. 1932. Surface forces of the Arbacia egg. *J. Cell. and Comp. Physiol.* 1 : 1–9.

———. 1935. Electric impedance of Hipponoë eggs. *J. Gen. Physiol.* 18 : 877–887.

———. 1937. Electric impedance of marine egg membranes. *Trans. Faraday Soc.* Part 8: 33 : 966–972.

———. 1938. Electric impedance of marine egg membranes. *Nature* 141 : 79.

———. 1940. Permeability and impermeability of cell membranes for ions. *Cold Spring Harbor Symp.* 8 : 110–122.

———. 1941. Impedance of single cells. *Tabulae Biologicae* 19 : Part II : 24–27.

——— and COLE, R. H. 1936. Electric impedance of Arbacia eggs. *J. Gen. Physiol.* 19 : 625–632.

——— and CURTIS, H. J. 1938. Electric impedance of single marine eggs. *J. Gen. Physiol.* 21 : 591–599.

——— and MICHAELIS, E. M. 1932. Surface forces of fertilized Arbacia eggs. *J. Cell. and Comp. Physiol.* 2 : 121–126.

——— and SPENCER, J. M. 1938. Electric impedance of fertilized Arbacia egg suspensions. *J. Gen. Physiol.* 21 : 583–590.

COLE, W. H. 1940. The composition of fluids and sera of some marine animals and of the sea water in which they live. *J. Gen. Physiol.* 23 : 575–584.

CONN, H. J. 1946. *Biological Stains*; fifth ed. Biotech. Pub., Geneva, N.Y.

CONNORS, W. M. and SCHEER, B. T. 1947. Adenosinetriphosphatase in the sea urchin egg. *J. Cell. and Comp. Physiol.* 30 : 271–283.

COOPER, K. W. 1941. Visibility of the primary spindle fibers and the course of mitosis in the living blastomeres of the mite,

Pediculopsis graminum (Reut.). *Proc. Nat. Acad. Sci.* 27 : 480–484.

Cornman, I. 1941. Sperm activation by Arbacia egg extracts, with special reference to echinochrome. *Biol. Bull.* 80 : 202–207.

———. 1943. Acceleration of cleavage of Arbacia eggs by hypotonic sea water. *Biol. Bull.* 84 : 244–261.

———. 1947. a. Inhibition of Arbacia egg cleavage by podophyllotoxin and related substances. *Biol. Bull.* 93 : 192 (Abstract).

———. 1947. b. Retardation of Arbacia egg cleavage by dinoflagellate-contaminated sea water (red tide). *Biol. Bull.* 93 : 205 (Abstract).

———. 1949. Alteration and inhibition of sea urchin egg division by antibiotics. *J. Clin. Invest.* 28 : 1045.

———. 1950. a. Inhibition of sea urchin egg cleavage by a series of substituted carbamates. *J. Nat. Cancer Inst.* 10 : 1123–1138.

———. 1950. b. Temperature alteration of carbamate narcosis of dividing Arbacia eggs. *Biol. Bull.* 99 : 338 (Abstract).

———. 1950. c. Dividing Arbacia eggs as test objects for the effects of cortisone and desoxycorticosterone on cell division, potassium permeability, and glucose utilization. *Biol. Bull.* 99 : 357 (Abstract).

———. 1950. d. Retardation throughout the mitotic cycle of dividing sea urchin eggs by the "prophase poisons", in urethanes and nitrogen mustards. *Anat. Rec.* 108 : 535 (Abstract).

——— and Cornman, M. E. 1951. The action of podophyllin and its fractions on marine eggs. *Ann. N.Y. Acad. Sci.* 51 : 1443–1487.

——— and Rogers, E. F. 1951. Alleviation of anti-mitotic effect of rotenone by alpha tocopherol. *Biol. Bull.* 101 : 208 (Abstract).

——— and ———. 1952. Chemical characteristics of rotenone antagonists as determined by effects on dividing sea urchin eggs. *Biol. Bull.* 103 : 298 (Abstract).

———, Skipper, H. S. and Mitchell, J. H., Jr. 1951. The fixation of urethan carbon atoms in sperm and in resting and rapidly dividing cells. *Cancer Research* 11 : 195–199.

Costello, D. P. 1932. The surface precipitation reaction in marine eggs. *Protoplasma* 17 : 239–257.

———. 1934. The effects of temperature on the viscosity of Arbacia egg protoplasm. *J. Cell. and Comp. Physiol.* 4 : 421–433.

———. 1938. The effect of temperature on the rate of fragmentation of Arbacia eggs subjected to centrifugal force. *J. Cell. and Comp. Physiol.* 11 : 301–307.

Costello, D. P. 1939. The volumes occupied by the formed cytoplasmic components in marine eggs. *Physiol. Zool.* 12 : 13–20.

———. 1948. Oöplasmic segregation in relation to differentiation. *Ann. N.Y. Acad. Sci.* 49 : 663–683.

Cotte, J. 1944. *Poissons et Animaux Aquatiques au Temps de Pline.* Paris.

Couillard, P. 1952. The fertilization inhibitor in the blood cells of Arbacia. *Biol. Bull.* 103 : 299 (Abstract).

Cowdry, E. V. 1924. *General Cytology.* Univ. Chicago Press, Chicago, Ill.

Cowles, R. P. 1911. Reactions of the sea urchin and the starfish to changes in light intensity. *Johns Hopkins Univ. Circ. 30,* no. 232 : 3–9.

Crane, R. K. 1947. The distribution of phosphorus in the unfertilized egg of Arbacia punctulata. *Biol. Bull.* 93 : 192–193 (Abstract).

——— and Keltch, A. K. 1949. Dinitrocresol and phosphate stimulation of the oxygen consumption of a cell-free oxidative system obtained from sea urchin eggs. *J. Gen. Physiol.* 32 : 503–509.

Crozier, W. J. 1918. On the pigmentation of a clypeastroid, Mellita sesquiperforatus Leske. *Am. Nat.* 52 : 552–556.

Ctesias. 5th cent. B.C.

Cuénot, L. 1891. a. Études sur le sang et les glandes lymphatiques dans la série animale. Part. 2: invertébrés. *Arch. Zool. expér. et gén.* Sér. II, t. 9 : 593–670.

———. 1891. b. Études morphologiques sur les Échinodermes. *Arch. de Biol.* 11 : 313–680.

———. 1912. Contributions à la faune du bassin d'Arcachon. V. Échinodermes. *Bull. Sta. Biol. d'Arcachon.* 14 : 17–116.

Cuvier, G. 1805. *Leçons d'Anatomie Comparée,* t. 3 : 329–335. Paris.

———. 1817. *Le Règne Animal.* Paris.

———. 1834. *The Animal Kingdom.* Additions by E. Griffith. Vol. 12. London.

Dahremberg, C., Saglio, E. et Pottier, E. 1904. *Dictionaire des Antiquités Grecques et Romaines.* T. III. pt. 2. Paris.

Dalcq, A. 1928. *Les Bases Physiologiques de la Fécondation et de la Parthénogénèse.* Les Presses Universitaires de France. Paris.

Daly, M. M., Allfrey, V. G. and Mirsky, A. E. 1950. Purine and pyrimidine contents of some desoxypentose nucleic acids. *J. Gen. Physiol.* 33 : 497–510.

D'Amora, T. 1937. La pressione osmotica degli embrioni di Sepia officinalis. *Arch. di Scienze Biol.* 23 : 513–516.

Dan, J. C. 1950. Sperm entrance in Echino-

derms observed with the phase microscope. *Biol. Bull.* 99 : 399–411.

DAN, J. C. 1952. Studies on the acrosome. I. Reaction to egg-water and other stimuli. *Biol. Bull.* 103 : 54–56.

DAN, K. 1931. The electric properties of sea urchin eggs and amoebae. *Anat. Rec. Suppl.* 51 : 28 (Abstract).

———. 1933. Electrokinetic studies of marine ova. I. Arbacia punctulata. *J. Cell. and Comp. Physiol.* 3 : 477–492.

———. 1934. Electrokinetic studies of marine ova. II. Cumingea tellinoides, Asterias forbesii, Echinarachnius parma, Nereis limbatus and Cerebratulus lacteus. *Biol. Bull.* 66 : 247–256.

———. 1936. Electrokinetic studies of marine ova. III and IV. *Physiol. Zool.* 9 : 43–65.

III. The effect of dilution of sea water, and of sodium and calcium upon the surface potentials of Arbacia eggs. pp. 43–57

IV. The surface potentials of centrifuged fragments of Arbacia eggs. pp. 58 – 65.

———. 1951. a. The movement of the cortical layer of Arbacia eggs in Ca-free medium through cleavage and the resting stage, as revealed by the shift of the pigment granules. *Biol. Bull.* 101 : 208 (Abstract).

———. 1951. b. Cyto-embryology in sea urchins. *Biol. Bull.* 101 : 237–238 (Abstract).

——— and DAN, J. C. 1940. Behavior of the cell surface during cleavage. III. On the formation of the cell surface during cleavage. *Biol. Bull.* 78 : 486–501.

———, ITO, S. and MAZIA, D. 1952. Study of course of formation of the mitotic apparatus in Arbacia and Mactra by isolation techniques. *Biol. Bull.* 103 : 292 (Abstract).

——— and ONO, T. 1952. Cyto-embryological studies of sea urchins. I, II. *Biol. Bull.* 102 : 58–89.

———, YANAGITA, T. and SUGIYAMA, M. 1937. Behavior of the cell surface during cleavage. I. *Protoplasma* 28 : 66–81.

DANIELLI, J. F. 1942. The cell surface and cell physiology. Chapt. III : 68–98 of *Bourne's Cytology and Cell Physiology*. Oxford Press.

———. 1951. a. Physical and physicochemical studies of cells. Chapt. II of *Bourne's Cytology and Cell Physiology*, 2nd ed. Oxford Press.

———. 1951. b. The cell surface and cell physiology. Chapt. IV of *Bourne's Cytology and Cell Physiology*, 2nd ed. Oxford Press.

DAVIDSON, E. 1953. Clotting of the perivisceral fluid of the sand dollar, Echinarachnius parma. *Biol. Bull.* 105 : 372 (Abstract).

DAVSON, H. and DANIELLI, J. F. 1943. *The Permeability of Natural Membranes*. Macmillan Co.

——— and ———. 1952. *The Permeability of Natural Membranes*. 2nd ed. Cambridge Univ. Press.

DE FRANCESCO, C. 1933. Sulla velocità di sviluppo negli incroci degli Echinodermi. *Pubb. Sta. Zool. Napoli* 13 : 279–288.

DELAGE, Y. 1901. Études expérimentales sur la maturation cytoplasmique et sur la parthénogénèse artificielle chez les Échinodermes. *Arch. Zool. expér. et gén. Sér. III*, t. 9 : 285–326.

———. 1908. Les vrais facteurs de la parthénogénèse expérimentale. Élevage des larves parthénogénétiques jusqu'à la forme parfaite. *Arch. Zool. expér. et gén. Sér. IV*. t. 7 : 445–506.

———. 1909. Le sexe chez les oursins issus de parthénegénèse expérimentale. *C. R. Acad. Sci. Paris* 148 : 453–455.

——— et HÉROUARD, E. 1903. *Traité de Zoologie Concrète*. Paris. T. 3.

DERBÈS, M. 1847. Observations sur le méchanisme et les phénomènes qui accompagnent la formation de l'embryon chez l'oursin comestible. *Ann. de Sci. Nat. III Sér. Zool.* 8 : 80–98.

DES MOULINS, C. 1835–1837. *Études sur les Échinides*. Bordeaux.

DEUTLER, F. 1926. Über das Wachstum des Seeigelskeletts. *Zool. Jahrb. Abt. f. Anat. u. Ontog. der Tiere* 48 : 119–200.

DEUTSCH, H. F. and GUSTAFSON, T. 1952. The changes in catalase and cytochrome oxidase activity in developing sea urchin eggs. *Arkiv f. Kemi* 4 : 221–231.

DIOSCORIDES, PEDACIUS, first cent. A.D. Translated 1655 by John Goodyear. Edited and first printed 1933 by Univ. Press Oxford.

DI STEFANO, H. and MAZIA, D. 1952. The role of the midpiece in the incorporation of P^{32} into the ribonucleic acid of Arbacia spermatozoa. *Biol. Bull.* 103 : 299–300 (Abstract).

DOHRN, A. 1875. Mittheilung aus und über die Zoologische Station von Neapel. *Zeits. wiss. Zool.* 25 : 457–480.

DONNELLON, J. A. 1938. An experimental study of clot formation in the perivisceral fluid of Arbacia. *Physiol. Zool.* 11 : 389–398.

DORFMAN, W. A. 1932. Permeability and cytolysis of the sea urchin egg with regard to some fertilization problems. *Protoplasma* 16 : 56–78.

———. 1933. Osmotic behavior of the sea

urchin egg as a criterion of its permeability. *Protoplasma* 19 : 578–586.

Dorfman, W. A. 1934. Rhythmical changes of the cortical layer of the developing sea urchin egg. *Protoplasma* 21 : 235–240.

Doyle, Wm. L. 1938. Studies on enzymatic histochemistry XXVII. Peptidase and catalase activity of marine ova. *J. Cell. and Comp. Physiol.* 11 : 291–300.

Driesch, H. 1891. Entwicklungs-mechanische Studien. I. Der Werth der beiden ersten Furchungszellen in der Echinodermentwicklung. Experimentelle Erzeugung von Theil- und Doppelbildungen. *Zeits. wiss. Zool.* 53 : 160–178.

———. 1892. Entwicklungsmechanische Studien IVb. Von der Veränderung der Furchung durch Druckwirkungen. *Zeit. wiss. Zool.* 55 : 10–29.

———. 1893. Entwicklungsmechanische Studien. VII. Exogastrula und Anenteria. *Mitth. Zool. Sta. Neapel* 11 : 221–226.

Drzewina, A. and Bohn, G. 1912. Effets de l'inhibition des oxydations sur les spermatozoïdes d'oursin et, par leur intermédiare, sur le développement. *C. R. Acad. Sci. Paris* 154 : 1639–1641.

——— and ———. 1924. Un nouveau cas d'hermaphrodisme chez l'oursin, Strongylocentrotus lividus. *C. R. Acad. Sci. Paris.* 178 : 662–663.

Dubois, R. 1900. Sur la spermase et l'ovulase. *C. R. Soc. Biol. Paris* 52 : 197–199.

Dufossé. 1847. Observations sur le developpement des oursins. *Ann. de Sci. Nat. Sér. III Zool.* 7 : 44–52.

Duggar, B. M. 1936. *Biological Effects of Radiation*, 2 vols. McGraw Hill Book Co. N.Y. Chapt. 18. p. 625–676 of Vol. 1.The action of radiations on living protoplasm by Heilbrunn, L. V. and Mazia, D.

Dujardin, M. F. and Hupé, M. H. 1862. *Histoire Naturelle des Zoophytes Échinodermes.* Paris.

Dungay, N. S. 1913. A study of the effects of injury upon the fertilizing power of sperm. *Biol. Bull.* 25 : 213–260.

Eigsti, O. J., Dustin, P., Jr. and Gay-Winn, N. 1949. On the discovery of the action of colchicine on mitosis in 1889. *Science* 110 : 692.

Elmhirst, R. 1922. Habits of Echinus esculentus. *Nature* 110 : 67.

Elson, D. and Chargaff, E. 1952. a. On the desoxyribonucleic acid content of sea urchin gametes. *Experientia* 8 : 143–145.

——— and ———. 1952. b. Observations on pentose nucleic acid composition in sea urchin embryos and in mamalian cell fractions. *Symposium on Phosphorus Metabolism.* McElroy and Glass. vol. II : 329–338.

Endo, Y. 1952. The role of cortical granules in the formation of the fertilization membrane in eggs from Japanese sea urchins. I. *Exp. Cell Res.* 3 : 406–418.

Ennius. 239–169 B.C. *Hedyphagetica* (Varia).

Ephrussi, B. 1925. Sur la membrane de fécondation de l'œuf d'oursin (Paracentrotus lividus Lk.). Action du liquide cœlomique. *C. R. Acad. Sci. Paris.* 180 : 775–778.

———. 1926. Sur les coefficients de température des différent phases de la mitose des œufs d'oursin (Paracentrotus lividus Lk.) et de l'Ascaris megalocephala. *Protoplasma* 1 : 105–123.

———. 1933. Contribution à l'analyse des premiers stades du développement de l'œuf. Action de la température. *Arch. de Biol.* 44; 1–147.

——— and Rapkine, L. 1928. Composition chimique de l'œuf d'oursin (Paracentrotus lividus Lk.) et ses variations au cours du développement. *Ann. de Physiol. et de Physicochim. Biol.* No. 3 : 386–399.

Epicharmus. Born ca. 540 B.C. *The Marriage of Hebe.*

Evans, T. C. 1940. a. Oxygen consumption of Arbacia eggs following exposure to Roentgen radiation. *Biol. Bull.* 79 : 361 (Abstract).

———. 1940. b. Effects of roentgen radiation on the jelly of the Arbacia egg. I. Disintegration of the jelly. *Biol. Bull.* 79 : 362 (Abstract).

———. 1942. Effects of irradiated water on Arbacia sperm. *Biol. Bull.* 83 : 298 (Abstract).

———. 1947. Effects of hydrogen peroxide produced in the medium by radiation on spermatozoa of Arbacia punctulata. *Biol. Bull.* 92 : 99–109.

———. 1950. Some effects of X-rays on the mechanisms of cell division. *Biol. Bull.* 99 : 317 (Abstract).

——— and Beams, H. W. 1939. Effects of roentgen radiation on certain phenomena related to cleavage in Arbacia eggs (Arbacia punctulata). *Biol. Bull.* 77 : 331 (Abstract).

———, ——— and Smith, W. E. 1941. Effects of roentgen radiation on the jelly of the Arbacia egg. *Biol. Bull.* 80 : 363–370.

—— and Slaughter, J. C. 1941. Effect of sea water on the radiosensitivity of Arbacia sperm. *Biol. Bull.* 81 : 282 (Abstract); *The Collecting Net* 16 : 101, 113–114.

———, Slaughter, J. C., Little, E. P. and Failla, G. 1941. The influence of the medium on the radiosensitivity of sperm. *Biol. Bull.* 81 : 291–292 (Abstract).

FAVILLI, G. 1932. The influence of some organ extracts on the permeability to water of sea urchin eggs (Arbacia punctulata). *J. Cell. and Comp. Physiol.* 2 : 1–10.

FABER, G. L. 1883. *The Fisheries of the Adriatic and the Fish Thereof.* London.

FELL, H. BARRACLOUGH. 1945. A revision of the current theory of Echinoderm embryology. *Trans. Roy. Soc. New Zealand* 75, Part 2 : 73–101.

———. 1948. Echinoderm embryology and the origin of the chordates. *Biol. Rev.* 23 : 81–107.

FEWKES, J. W. 1881. On the development of the pluteus of Arbacia. *Mem. Peabody Acad. Sci.* Vol. 1. No. 6 : 1–10.

———. 1890. a. On excavations made in rocks by sea urchins. *Am. Nat.* 24 : 1–21.

———. 1890. b. Sea urchin excavations at Guaymas, Mexico. *Am. Nat.* 24 : 478–480.

FIELD, G. W. 1895. On the morphology and physiology of the Echinoderm spermatozoon. *J. Morph.* 11 : 235–270.

FINKEL, A. J., ALLEE, W. C. and GARNER, H. R. 1942. Copper and the acceleration of early cleavage of Arbacia eggs. *J. Cell. and Comp. Physiol.* 20 : 179–187.

FISCHEL, A. 1906. a. Über Bastardierungversuche bei Echinodermen. *Arch. f. Entwick.* 22 : 498–525.

———. 1906. b. Zur Entwicklungsgeschichte der Echinodermen. I. Zur Mechanik der Zellteilung. II. Versuche mit vitaler Färbung. *Arch. f. Entwick.* 22 : 526–541.

FISCHER, P. 1864. Note sur les perforations de l'Echinus lividus (Lamk.). *Ann. d. Sci. Nat. Ser. V. Zool.* 1 : 321–332.

FISHER, K. C. 1942. Narcosis. *Canad. Med. Ass. J.* 47 : 414–421.

——— and HENRY, R. J. 1944. The effects of urethane and chloral hydrate on oxygen consumption and cell division in the egg of the sea urchin, Arbacia punctulata. *J. Gen. Physiol.* 27 : 469–481.

———, HENRY, R. J. and LOW, E. 1944. The effects of sulfanilamide and azide on O_2 consumption and cell division in the egg of the sea urchin, Arbacia punctulata. *J. Pharm. and Exper. Therap.* 81 : 58–66.

FOL, H. 1877. Sur le commencement de l'hénogénie chez divers animaux. *Arch. de Zool. expér. et gén. Sér. I*, t. 6 : 145–169; 180–192.

———. 1879. Recherches sur la fécondation et le commencement de l'hénogénie chez divers animaux. *Mém. Soc. de physique et d'hist. nat. de Genève* 26 : 89–397.

FORBES, E. 1841. *A History of British Starfishes, and other Animals of the Class Echinodermata.* London, Van Voorst.

FOWLER, C. 1934. Effect of the alternating current on the permeability of sea urchin eggs to water. *Biol. Bull.* 67 : 330–331 (Abstract).

FOX. D. L. 1953. *Animal Biochromes and Structural Color.* Cambridge Univ. Press, London and New York.

——— and SCHEER, B. T. 1941. Comparative studies of the pigments of some Pacific Coast Echinoderms. *Biol. Bull.* 80 : 441–455.

FOX, H. M. 1924. a. Lunar periodicity in reproduction. *Proc. Roy. Soc. London B* 95 : 523–550. See also *Nature* 109 : 237–238 (1922).

———. 1924. b. The spawning of echinoids. *Proc. Camb. Phil. Soc. Biol. Sci.* 1 : 71–74 (= *Biol. Rev.*).

———. 1938. The activity and metabolism of poikilothermal animals in different latitudes. III. *Proc. Zool. Soc. London, Ser. A* 108 : 501–505.

——— and RAMAGE, H. 1931. A spectrographic analysis of animal tissues. *Proc. Roy. Soc. London B* 108 : 157–173.

FRANK, G. and KUREPINA, M. 1930. Die gegenseitige Beeinflussung der Seeigeleier als mitogenetischer Effekt betrachtet. *Arch. f. Entwick.* 121 : 634–638.

FRANK, J. A. 1939. Some properties of sperm extracts and their relationship to the fertilization reaction in Arbacia punctulata. *Biol. Bull.* 76 : 190–216.

FRIEDHEIM, E. A. H. 1932. Sur deux ferments respiratoires accessoires d'origine animale. *C. R. Soc. de Biol. Paris* 111 : 505–507.

FRY, H. J. 1927. The cross fertilization of enucleated Echinarachnius eggs by Arbacia sperm. *Biol. Bull.* 53 : 173–178.

———. 1929. The so-called central bodies in fertilized Echinarachnius eggs I, II, III. *Biol. Bull.* 56 : 101–128; 57 : 131–150; 57 : 151–158 with collaboration of M. Jacobs and H. M. Lieb.

———. 1936. Studies of the mitotic figure. V. The time schedule of mitotic changes in developing Arbacia eggs. *Biol. Bull.* 70 : 89–99.

———. 1937. Studies of the mitotic figure. VI. Mid-bodies and their significance for the central body problem. *Biol. Bull.* 73 : 565–590.

——— and PARKS, M. E. 1934. Studies of the mitotic figure. IV. Mitotic changes and viscosity changes in eggs of Arbacia, Cumingia and Nereis. *Protoplasma* 21 : 473–499.

FUCHS, H. M. 1914. a. On the conditions of self-fertilization in Ciona. *Arch. f. Entwick.* 40 : 157–204.

FUCHS, H. M. 1914. b. On F_2 Echinus hybrids. *J. Marine Biol. Assoc.* 10 : 464–465.

———. 1914. c. The action of egg secretions on the fertilizing power of sperm. *Arch. f. Entwick.* 40 : 205–252.

FÜHNER, H. 1904. Pharmakologische Studien an Seeigeleiern. Der Wirkungsgrad der Alkohole. *Arch. f. Exp. Path. u. Pharm.* 52 : 69–82.

FUKUDA, T. R. 1934. The membrane stretching in the eggs of sea urchin and the surface precipitation reaction. *J. Fac. Sci. Imp. Univ. Tokyo* Sect. IV, 3 : 335–341.

———. 1935. Ionic antagonism in the water permeability of sea urchin eggs. *J. Cell. and Comp. Physiol.* 7 : 301–312.

GADD, G. 1907. Ein Fall von Hermaphroditismus bei dem Strongylocentrotus droebachiensis O.F. Müll. *Zool. Anz.* 31 : 635.

GAGNON, A. 1950. The action of heparin on fertilization and cleavage in some marine eggs. *Biol. Bull.* 99 : 341 (Abstract).

GALEN. A. D. 130–200.

GAMGEE, A. 1880. *Textbook of Physiological Chemistry of the Animal Body.* Vol. I. Macmillan Co.

GARDINER, M. S. 1927. The chromosomes of Clypeaster rosaceus. *J. Morph.* 43 : 547–555.

GARMAN, H. & COLTON, B. P. 1883. Some notes on the development of Arbacia punctulata, Lam. *Studies Biol. Lab. Johns Hopkins Univ.* 2 : 247–255.

GARREY, W. E. 1905. The osmotic pressure of sea water and of the blood of marine animals. *Biol. Bull.* 8 : 257–270.

———. 1915. Some cryoscopic and osmotic data. *Biol. Bull.* 28 : 77–86.

———. 1919. The nature of the fertilization membrane of Asterias and Arbacia eggs. *Biol. Bull.* 37 : 287–293.

GATES, R. R. 1941. Tests of nucleoli and cytoplasmic granules in marine eggs. *Biol. Bull.* 81 : 298 (Abstract).

GEDDES, P. 1880. Observations sur le fluide périviscérale des oursins. *Arch. Zool. expér. et gén. Sér. I.* t. 8 : 483–496.

GELLHORN, E. 1931. Vital staining and permeability. II. *Protoplasma* 12 : 66–78; III. *Protoplasma* 14 : 28–35.

GEMMILL, J. F. 1900. On the vitality of the ova and spermatozoa of certain animals. *J. Anat. and Physiol.* 34 : 163–181.

———. 1912. The locomotor function of the lantern in Echinus with observations on other allied lantern activities. *Proc. Roy. Soc. London* 85B: 84–108.

GENTHER, I. T. and SCHMIDT, L. H. 1931. The effect of bile salts on development of Arbacia. *Anat. Record* 51 Suppl.: 29 (Abstract).

GERARD, R. W. 1931. Oxygen diffusion into cells. *Biol. Bull.* 60 : 245–268.

——— and RUBINSTEIN, B. B. 1934. A note on the respiration of Arbacia eggs. *J. Gen. Physiol.* 17 : 375–381.

GEREN, B. B. and MCCULLOCH, D. 1951. Development and use of the Minot rotary microtome for thin sectioning. *Exp. Cell Res.* 2 : 97–102.

GERSCH, M. and RIES, E. 1937. Vergleichende Vitalfärbungsstudien. *Arch. f. Entwick.* 136 : 169–209.

GESNER, C. 1558. *Historia Animalium.* IV. Piscium et aquatilium Animantium Natura. Tiguri.

———. 1563. Fischbuch. In *Tierbuch.* Tiguri.

GIARD, A. 1900. À propos de la parthénogénèse artificielle des œufs d'Échinodermes. *C. R. Soc. Biol. Paris.* 52 : 761–764.

GIES, W. J. 1901. Do spermatozoa contain enzyme having the power of causing development of mature ova? *Am. J. Physiol.* 6 : 53–76.

GIESE, A. C. 1939. Ultraviolet radiation and cell division. Nuclear sensitivity: effect of irradiation on sea urchin sperm. *J. Cell. and Comp. Physiol.* 14 : 371–382.

———. 1945. Ultraviolet radiations and life. *Physiol. Zool.* 18 : 223–250.

———. 1947. Radiations and cell division. *Quart. Rev. Biol.* 22 : 253–282.

———. 1946. Comparative sensitivity of sperm and eggs to ultraviolet radiations. *Biol. Bull.* 91 : 81–87.

———. 1949. Activation of eggs, fertilization and early development as affected by ultraviolet rays. *Am. Nat.* 83 : 165–183.

———. 1950. Action of ultraviolet radiation on protoplasm. *Physiol. Rev.* 30 : 431–458.

——— and WELLS, P. H. 1952. Protective effect of glycine on sperm exposed to light. *Science* 115 : 239–240.

GILMAN, A. and PHILLIPS, F. S. 1946. The biological actions and therapeutic applications of the B-chloroethyl amines and sulfides. *Science* 103 : 409–415 and 436.

GLASER, O. 1913. On inducing development in the sea urchin (Arbacia punctulata), together with considerations on the initiatory effect by fertilization. *Science* 38 : 446–450.

———. 1914. a. The change in volume of Arbacia and Asterias eggs at fertilization. *Biol. Bull.* 26 : 84–91.

———. 1914. b. A qualitative analysis of the egg-secretions and extracts of Arbacia

and Asterias. *Biol. Bull.* 26 : 367–386.

GLASER, O. 1914. c. On auto-parthenogenesis in Arbacia and Asterias. *Biol. Bull.* 26 : 387–409.

———. 1915. Can a single spermatozoon initiate development in Arbacia? *Biol. Bull.* 28 : 149–153.

———. 1921. a. Note on the pigment of Arbacia egg-secretion. *Biol. Bull.* 41 : 256–258.

———. 1921. b. The duality of egg-secretion. *Am. Naturalist* 55 : 368–373.

———. 1922. a. The hydrolysis of higher fats in egg-secretion. *Biol. Bull.* 43 : 68–74.

———. 1922. b. The temporary concentration of sea-salts about Arbacia eggs. *Biol. Bull.* 43 : 175–183.

———. 1923. Copper, enzymes and fertilization. *Biol. Bull.* 44 : 79–104.

———. 1924. Fertilization, cortex, and volume. *Biol. Bull.* 47 : 274–283.

———. 1950. Note on the growth of arms in the pluteus of Arbacia punctulata. *Biol. Bull.* 98 : 34–35.

GLASER, R. et LEDERER, E. 1939. Échinochrome et spinochrome; dérivés méthylés; distribution; pigments associés. *C. R. Acad. Sci. Paris* 208 : 1939–1942.

GLYCAS, M(ICHAEL). 12th cent. *Annales a mundi exordio usque ad obitum Alexii Comneni.* Paris.

GODLEWSKI, E. 1908. Plasma und Kernsubstanz in der normalen und der durch äussere Factoren veränderten Entwicklung der Echiniden. *Arch. f. Entwick.* 26 : 278–328.

GOLDFORB, A. J. 1918. a. Effects of ageing upon germ cells and upon early development. Part II. Changes in moderately aged eggs and sperm. *Biol. Bull.* 34 : 372–409.

———. 1918. b. Effects of ageing upon germ cells and upon early development. Part III. Changes in very aged eggs. *Biol. Bull.* 35 : 1–32.

———. 1929. b. Changes in agglutination of ageing germ cells. *Biol. Bull.* 57 : 350–387.

———. 1929. c. Factors that change agglutinability of ageing sperm. *Biol. Bull.* 57 : 389–411.

———. 1935. a. Change in size and shape of ageing eggs (Arbacia punctulata). *Biol. Bull.* 68 : 180–190.

———. 1935. b. Viscosity changes in ageing unfertilized eggs of Arbacia punctulata. *Biol. Bull.* 68 : 191–206.

———. 1935. c. Increased permeability to water of ageing unfertilized eggs (Arbacia punctulata). *J. Gen. Physiol.* 19 : 149–165.

GOLDFORB, A. J. 1937. Effects of age on Arbacia eggs. VI. Increased stretching and bursting of egg membrane in hypotonic sea water. *Physiol. Zool.* 10 : 59–70.

——— and SCHECHTER, V. 1932. Studies in ageing eggs. Change in resistance of egg membrane to bursting in hypotonic solutions. *Proc. Soc. Exp. Biol. and Med.* 29 : 1271–1272.

GOLDINGER, J. M. and BARRON, E. S. G. 1946. The pyruvate metabolism of sea urchin eggs during the process of cell division. *J. Gen. Physiol.* 30 : 73–82.

GOLDSCHMIDT, R. and POPOFF, M. 1908. Über die sogenannte hyaline Plasmaschicht der Seeigeleier. *Biol. Centralb.* 28 : 210–223.

GOMORI, G. 1952. *Microscopic Histochemistry.* Univ. Chicago Press, Chicago, Ill.

GOODWIN, T. W., LEDERER, E. and MUSAJO, L. 1951. The nomenclature of the spinochromes of sea urchins. *Experientia* 7 : 375–376.

——— and SRISUKH, S. 1950. A study of the pigments of the sea-urchins, Echinus esculentus L. and Paracentrotus lividus Lamarck. *Biochem. J.* 47 : 69–76.

——— and ———. 1951. Caroteniods in the heart urchin Echinocardium cordatum. *Nature* 167: 358–359.

GORDON, I. 1929. Skeletal development in Arbacia, Echinarachnius and Leptasterias. *Phil. Trans. Roy. Soc. London,* B 217 : 289–334.

GORHAM, F. P. and TOWER, R. W. 1902. Does potassium cyanide prolong the life of the unfertilized egg of the sea urchin? *Am. J. Physiol.* 8 : 175–182.

GOWEN, J. W. and GAY, E. H. 1933. Gene number, kind and size in Drosophila. *Genetics* 18 : 1–31.

GRANT, U. S. IV. and HERTLEIN, L. G. 1938. The West American Cenozoic Echinoidea. *Pub. Univ. Calif., Los Angelos, Math. and Phys. Sci.* 2 : 1–209.

GRASSÉ, P. P. 1948. *Traité de Zoologie,* Tome XI, Échinodermes: 1–363. Masson et Cie, Paris.

GRAVE, B. H. and DOWNING, R. C. 1928. The longevity and swimming ability of spermatozoa. *J. Exp. Zool.* 51 : 383–388.

GRAVE, B. H. 1934. Further studies on the longevity and swimming ability of spermatozoa. *Biol. Bull.* 67 : 513–518.

GRAVE, C. 1902. a. A method of rearing marine larvae. *Science* 15 : 579–580.

———. 1902. b. Some points in the structure and development of Mellita testudinata. *Johns Hopkins Univ. Circulars,* no. 157: 1–8.

GRAY, J. 1916. Electrical conductivity of

Echinoderm eggs and its bearing on the problems of fertilization and artificial parthenogenesis. *Phil. Trans. Roy. Soc. London* B 207 : 481–529.

GRAY, J. 1921. Note on true and apparent hermaphroditism in sea urchins. *Proc. Camb. Phil. Soc.*, 20 : 481.

———. 1922. A critical study of the facts of artificial fertilization and normal fertilization. *Q. J. M. S.* 66 : 419–437.

———. 1924. The mechanism of cell division. I. The forces which control the form and cleavage of the eggs of Echinus esculentus. *Proc. Camb. Phil. Soc.* (Biol. Rev.) 1 : 164–188.

———. 1925. The mechanism of cell division. II. Oxygen consumption during cleavage. *Proc. Camb. Phil. Soc.* (Biol. Rev.) 1 : 225–236.

———. 1927. a. The mechanism of cell-division. III. The relationship between cell-division and growth in segmenting eggs. *J. Exp. Biol.* 4 : 313–321.

———. 1927. b. Ibid. IV. The effect of gravity on the eggs of Echinus. *J. Exp. Biol.* 5 : 102–111.

———. 1928. a. The effect of dilution on the activity of spermatozoa. *J. Exp. Biol.* 5 : 337–344. 1928. b. The senescence of spermatozoa. *Ibid.* p. 345–361. 1928. c. The effect of egg secretions on the activity of spermatozoa. *Ibid* p. 362–365.

———. 1931. *Textbook of Experimental Cytology.* Macmillan Co., and Cambridge Univ. Press.

GRAY, J. E. 1835. On the genera distinguishable in Echinus, Lam. *Proc. Zool. Soc. London* Part 3 : 57–58.

GREEFF, R. 1876. Über den Bau und die Entwicklung der Echinodermen. 5. Mittheilung, Parthenogenesis bei den Seesternen. *Sitz. Gesells. z. Beförd. Gesammt. Naturwiss. zu Marburg.*: 83–85.

GREELEY, A. W. 1902. Artificial parthenogenesis in starfish produced by a lowering of temperature. *Am. J. Physiol.* 6 : 296–304.

———. 1903. On the effect of variations in the temperature upon the process of artificial parthenogenesis. *Biol. Bull.* 4 : 129–136.

GREEN, J. W. and ROTH, J. S. 1950. The effect of P^{32} on the division time of Arbacia eggs. *Biol. Bull.* 99 : 358 (Abstract).

GRIFFITHS, A. B. 1892. a. Sur l'échinochrome: un pigment respiratoire. *C. R. Soc. Biol. Paris* 115 : 419–420.

GROSS, P. R. 1951. The lytic action of calcium ions on the pigment granules of the Arbacia egg. *Biol. Bull.* 101 : 209 (Abstract).

GROSS, P. R. 1953. The effect of calcium on the proteins of sea urchin egg homogenates. *Biol. Bull.* 105 : 365 (Abstract).

GRUBE, E. 1868. Über einen lebendig gebärenden Seeigel. *Monatsber. Akad. Wiss. Berlin* 1868: 178–180.

GUDGER, E. W. 1933. Echinoderm enemies of fishes. *Bull. N. Y. Zool. Soc.* 36 : 72–77.

GUSTAFSON, T. 1945. Contribution to the knowledge of the determination of the dorso ventral axis of the sea urchin egg. *Arkiv f. Zool.* 37 B, no. 1 : 1–2.

———. 1952. Nitrogen metabolism, enzymatic activity and mitochondrial distribution in relation to differentiation in the sea urchin egg. From *Wenner-Gren Inst. Exp. Biol.*, Uppsala.

——— and HASSELBERG, I. 1950. Alkaline phosphatase activity in developing sea urchin eggs. *Exp. Cell Res.* 1 : 371–375.

——— and ———. 1951. Studies on enzymes in the developing sea urchin egg. *Exper. Cell Res.* 2 : 642–672.

——— and Hjelte, M.-B. 1950. The amino acid metabolism of the developing sea urchin egg. *Arkiv f. Kemi* 2 : 363–366.

——— and LENICQUE, P. 1952. Studies on mitochondria in the developing sea urchin egg. *Exp. Cell Res.* 3 : 251–274.

HAGEDOORN, A. L. 1909. On the purely motherly character of the hybrids produced from the eggs of Strongylocentrotus. *Arch. f. Entwick.* 27 : 1–20.

HALABAN, A. 1949. Vitamin K as a protoplasmic coagulant and parthenogenetic agent. *Biol. Bull.* 97 : 240–241 (Abstract).

HAMANN, O. 1887. Beiträge zur Histologie der Echinodermen. *Jen. Zeits. N. F.* 14 (= 21) : 87–266.

HAMMAR, J. A. 1896. Über einen primären Zusammenhang zwischen den Furchungszellen des Seeigeleies. *Arch. mikr. Anat.* 47 : 14–23.

HARDING, C. V. 1951. The action of certain polysaccharides on fertilization in the sea urchin egg. *Exp. Cell Res.* 2 : 403–415.

——— and HARDING, D. 1952. a. Cross fertilization with the sperm of Arbacia lixula. *Exp. Cell Res.* 3 : 475–484.

——— and ———. 1952. b. The hybridization of Echinocardium cordatum and Psammechinus miliaris. *Arkiv f. Zool.* 4 : 91–93.

——— and THOMAS, L. J., Jr. 1950. Ultraviolet light induced delay in cleavage of centrifuged Arbacia eggs. *J. Cell. and Comp. Physiol.* 35 : 403–411.

HARDING, D. 1951. Initiation of cell division in the Arbacia egg by injury substances. *Physiol. Zool.* 24 : 54–69.

HARNLEY, M. H. 1926. Localization of the micromere material in the cytoplasm of the egg of Arbacia. *J. Exp. Zool.* 45 : 319–333.

HARRIS, D. L. 1943. The osmotic properties of cytoplasmic granules of the sea urchin egg. *Biol. Bull.* 83 : 179–192.

HARRIS, J. E. 1939. Studies on living protoplasm. II. The physical structure of the nucleus of the Echinoderm oocyte. *J. Exp. Biol.* 16 : 258–277.

HARTMANN, M., SCHARTAU, O., KUHN, R. und WALLENFELS, K. 1939. Über die Sexualstoffe der Seeigel. *Naturwissen.* 27 : 433.

———, ——— and WALLENFELS, K. 1940. Untersuchungen über die Befruchtungsstoffe der Seeigel II. *Biol. Zentralb.* 60 : 398–423.

HARVEY, E. B. 1920. A review of the chromosome numbers in the Metazoa, Part II. *J. Morph.* 34 : 1–67; for Echinoidea p. 12–14.

———. 1927. The effect of lack of oxygen on sea urchin eggs. *Biol. Bull.* 52 : 147–161.

———. 1930. The effect of lack of oxygen on the sperm and unfertilized eggs of Arbacia punctulata and on fertilization. *Biol. Bull.* 58 : 288–292.

———. 1932. The development of half and quarter eggs of Arbacia punctulata and of strongly centrifuged whole eggs. *Biol. Bull.* 62 : 155–167.

———. 1933. a. Development of the parts of sea urchin eggs separated by centrifugal force. *Biol. Bull.* 64 : 125–148.

———. 1933. b. Effects of centrifugal force on fertilized eggs of Arbacia punctulata as observed with the centrifuge-microscope. *Biol. Bull.* 65 : 389–396.

———. 1934. a. Effects of centrifugal force on the ectoplasmic layer and nuclei of fertilized sea urchin eggs. *Biol. Bull.* 66 : 228–245.

———. 1934. b. Una centrifuga-microscopio. *Boll. Soc. Ital. Biol. Speriment.* 9 : 484–487.

———. 1935. a. The mitotic figure and cleavage plane in the egg of Parechinus microtuberculatus, as influenced by centrifugal force. *Biol. Bull.* 69 : 287–297.

———. 1935. b. Some surface phenomena in the fertilized sea urchin egg as influenced by centrifugal force. *Biol. Bull.* 69 : 298–304.

———. 1935. c. Cleavage without nuclei. *Science* 82 : 277 (Note).

———. 1936. Parthenogenetic merogony or cleavage without nuclei in Arbacia punctulata. *Biol. Bull.* 71 : 101–121.

HARVEY, E. B. 1938. a. Parthenogenetic merogony or development without nuclei of the eggs of sea urchins from Naples. *Biol. Bull.* 75 : 170–188.

———. 1938. b. Development of the sea urchin, Arbacia. (With photographs). *Turtox News* 16 : 149–152.

———. 1939. a. An hermaphrodite Arbacia. *Biol. Bull.* 77 : 74–78.

———. 1939. b. Arbacia. *The Collecting Net* 14 : 180–181.

———. 1939. c. A method of determining the sex of Arbacia, and a new method of producing twins, triplets and quadruplets. *The Collecting Net* 14 : 211.

———. 1940. a. A new method of producing twins, triplets and quadruplets in Arbacia punctulata, and their development. *Biol. Bull.* 78 : 202–216.

———. 1940. b. Development of half eggs of Arbacia punctulata obtained by centrifuging after fertilization, with special reference to parthenogenetic merogony. *Biol. Bull.* 78 : 412–427.

———. 1940. c. A comparison of the development of nucleate and non-nucleate eggs of Arbacia punctulata. *Biol. Bull.* 79 : 166–187.

———. 1940. d. A note on determining the sex of Arbacia punctulata. *Biol. Bull.* 79 : 363 (Abstract).

———. 1941. a. Relation of the size of "halves" of the Arbacia punctulata egg to centrifugal force. *Biol. Bull.* 80 : 354–362.

———. 1941. b. The cytology of fertilization and cleavage of Arbacia punctulata. (With photographs). *Turtox News* 19 : 97–99.

———. 1941. c. Vital staining of the centrifuged Arbacia punctulata egg. *Biol. Bull.* 81 : 114–118.

———. 1941. d. Cross-fertilization of Echinoderms. *Science* 94 : 90–91.

———. 1942. Maternal inheritance in Echinoderm hybrids. *J. Exp. Zool.* 91 : 213–235.

———. 1943. Rate of breaking and size of "halves" of the Arbacia punctulata egg when centrifuged in hypo- and hypertonic sea water. *Biol. Bull.* 85 : 141–150.

———. 1945. Stratification and breaking of the Arbacia punctulata egg when centrifuged in single salt solutions. *Biol. Bull.* 89 : 72–75.

———. 1946. a. Structure and development of the clear quarter of the Arbacia punctulata egg. *J. Exp. Zool.* 102 : 253–276.

———. 1946. b. Fertilization. *Encylop. Brit.* vol. 9, 1946 Copyright. 188–191.

———. 1947. Bermuda sea urchins and

their eggs. *Biol. Bull.* 93 : 217–218 (Abstract).

HARVEY, E. B. 1949. The growth and metamorphosis of the Arbacia punctulata pluteus, and late development of the white halves of centrifuged eggs. *Biol. Bull.* 97 : 287–299.

———. 1951. Cleavage in centrifuged eggs and in parthenogenetic merogones. *Ann. N.Y. Acad. Sci.* 51 : 1336–1348.

———. 1952. Electrical method of "sexing" Arbacia and obtaining small quantities of eggs. *Biol. Bull.* 103 : 284 (Abstract).

———. 1953. A simplified electrical method of determining the sex of sea urchins and other marine animals. *Biol. Bull.* 105 : 365 (Abstract).

———. 1954. Electrical method of determining the sex of sea urchins. *Nature* 173 : 86.

——— and ANDERSON, T. F. 1943. The spermatozoon and fertilization membrane of Arbacia punctulata as shown by the electron microscope. *Biol. Bull.* 85 : 151–156.

——— and HOLLAENDER, A. 1937. Activation of centrifuged whole eggs and their fractions by monochromatic ultraviolet radiation. *Biol. Bull.* 73 : 365 (Abstract).

——— and ———. 1938. Parthenogenetic development of the eggs and egg fractions of Arbacia punctulata caused by monochromatic ultraviolet radiation. *Biol. Bull.* 75 : 258–265.

——— and LAVIN, G. I. 1944. The chromatin in the living Arbacia punctulata egg and the cytoplasm of the centrifuged egg as photographed by ultraviolet light. *Biol. Bull.* 86 : 163–168.

——— and ———. 1951. a. The eggs and half-eggs of Arbacia punctulata and the plutei as photographed by ultraviolet, visible and infrared light. *Exp. Cell Res.* 2 : 393–397.

——— and ———. 1951. b. Nuclei of Arbacia and Chaetopterus eggs as photographed by infrared light. *Exp. Cell Res.* 2 : 398–402.

HARVEY, E. N. 1909. Membrane formation and pigment migration in sea urchin eggs as bearing on the problem of artificial parthenogenesis. *Science* 30 : 694–696.

———. 1910. a. Methods of artificial parthenogenesis. *Biol. Bull.* 18 : 269–280.

———. 1910. b. The mechanism of membrane formation and other early changes in developing sea urchins' eggs as bearing upon the problem of artificial parthenogenesis. *J. Exp. Zool.* 8 : 355–376.

———. 1910. c. The permeability and cytolysis of eggs. *Science* 32 : 565–568.

HARVEY, E. N. 1911. Studies on the permeability of cells. *J. Exp. Zool.* 10 : 507–556.

———. 1914. Is the fertilization membrane of Arbacia eggs a precipitation membrane? *Biol. Bull.* 27 : 237–239.

———. 1930. Biological aspects of ultrasonic waves, a general survey. *Biol. Bull.* 59 : 306–325.

———. 1931. a. A determination of the tension at the surface of eggs of the Annelid Chaetopterus. *Biol. Bull.* 60 : 67–71.

———. 1931. b. Observations on living cells, made with the microscope-centrifuge. *J. Exp. Biol.* 8 : 267–274.

———. 1931. c. The tension at the surface of marine eggs, especially those of the sea urchin, Arbacia. *Biol. Bull.* 61 : 273–279.

———. 1932. a. Physical and chemical constants of the egg of the sea urchin, Arbacia punctulata. *Biol. Bull.* 62 : 141–154.

———. 1932. b. The microscope-centrifuge and some of its applications. *J. Franklin Inst.* 214 : 1–23.

———. 1933. The flattening of marine eggs under the influence of gravity. *J. Cell. and Comp. Physiol.* 4 : 35–47.

———. 1934. The air turbine for high speed centrifuging of biological material, together with some observations on centrifuged eggs. *Biol. Bull.* 66 : 48–54.

———. 1936. The properties of elastic membranes with special reference to the cell surface. *J. Cell. and Comp. Physiol.* 8 : 251–260.

———. 1937. Methods of measuring surface forces of living cells. *Trans. Faraday Soc.* 33, Part 8 : 943–946.

———. 1938. Some physical properties of protoplasm. *J. Applied Physics* 9 : 68–80.

———. 1939. Structure of cells as revealed by centrifugal force. *Arch. Exp. Zellf.* 22 : 463–476.

———. 1942. Stimulation of cells by intense flashes of ultraviolet light. *J. Gen. Physiol.* 25 : 431–444.

———. 1954. Tension at the cell surface. *Protoplasmatologia*, ed. by F. Weber and L. V. Heilbrunn. Springer Verlag, Wien.

——— and DANIELLI, J. F. 1938. Properties of the cell surface. *Biol. Rev.* 13 : 319–341.

———, HARVEY, E. B. and LOOMIS, A. L. 1928. Further observations on the effect of high frequency sound waves on living matter. *Biol. Bull.* 55 : 459–469.

—— and LOOMIS, A. L. 1931. High speed photomicrography of living cells subjected to supersonic vibrations. *J. Gen. Physiol.* 15 : 147–153.

——— and SHAPIRO, H. 1941. The recovery period (relaxation) of marine eggs after

deformation. *J. Cell. and Comp. Physiol.* 17 : 135–144.

HAWKINS, H. L. 1929. In defense of Bothriocidaris. *Geol. Mag.* 66 : 71–79.

———. 1931. The first Echinoid. *Biol. Rev.* 6 : 443–458.

HAYASHI, T. 1945. Dilution medium and survival of the spermatozoa of Arbacia punctulata. I. Effect of the medium on fertilizing power. *Biol. Bull.* 89 : 162–179.

———. 1946. Dilution medium and survival of the spermatozoa of Arbacia punctulata. II. Effect of the medium on respiration. *Biol. Bull.* 90 : 177–187.

HAYES, F. R. 1938. The relation of fat changes to the general chemical embryology of the sea urchin. *Biol. Bull.* 74 : 267–277.

HAYWOOD, C. 1927. Carbon dioxide as a narcotic agent. I. The effect of carbon dioxide upon the fertilized egg of Arbacia. *Biol. Bull.* 53 : 450–464.

———. 1953. The cleavage times of fertilized eggs of the sea urchin, Arbacia punctulata, at high pressures of nitrogen, helium and nitrous oxide. *J. Cell. and Comp. Physiol.* 41 : 335–343.

——— and ROOT, W. S. 1930. A quantitative study of the effect of carbon dioxide upon the cleavage rate of the Arbacia egg. *Biol. Bull.* 59 : 63–70.

——— and ———. 1932. The effect of carbon dioxide in the presence of varying amounts of bicarbonate upon the cleavage rate of the Arbacia egg. *J. Cell. and Comp. Physiol.* 2 : 177–191.

HEILBRUNN, L. V. 1913. Studies in artificial parthenogenesis. I. Membrane elevation in the sea urchin egg. *Biol. Bull.* 24 : 343–361.

———. 1915. a. Studies in artificial parthenogenesis. II. Physical changes in the egg of Arbacia. *Biol. Bull.* 29 : 149–203.

———. 1915. b. The measurement of oxidation in the sea urchin egg. *Science* 42 : 615–616.

———. 1920. a. An experimental study of cell division. I. The physical conditions which determine the appearance of the spindle in sea urchin eggs. *J. Exp. Zool.* 30 : 211–237.

———. 1920. b. The physical effect of anesthetics upon living protoplasm. *Biol. Bull.* 39 : 307–315.

———. 1921. Protoplasmic viscosity changes during mitosis. *J. Exp. Zool.* 34 : 417–447.

———. 1923. The colloid chemistry of protoplasm. II. The electrical charges of protoplasm. *Am. J. Physiol.* 64 : 481–498.

HEILBRUNN, L. V. 1924. a. The surface tension theory of membrane elevation. *Biol. Bull.* 46 : 277–280.

———. 1924. b. The colloid chemistry of protoplasm. III. The viscosity of protoplasm at various temperatures. *Am. J. Physiol.* 68 : 645–648.

———. 1925. a. Studies in artificial parthenogenesis. IV. Heat parthenogenesis. *J. Exp. Zool.* 41 : 243–260.

———. 1925. b. The electrical charges of living cells. *Science* 61 : 236–237.

———. 1925. c. The action of ether on protoplasm. *Biol. Bull.* 49 : 461–476.

———. 1925. d. Studies in artificial parthenogenesis. V. Anomalous action of mercuric chloride. *Biol. Bull.* 49 : 241–249.

———. 1926. a. The absolute viscosity of protoplasm. *J. Exp. Zool.* 44 : 255–278.

———. 1926. b. The physical structure of the protoplasm of sea urchin eggs. *Am. Nat.* 60 : 143–156.

———. 1927. The viscosity of protoplasm. *Quart. Rev. Biol.* 2 : 230–248.

———. 1928. *The Colloid Chemistry of Protoplasm.* Berlin.

———. 1929. Hermaphroditism in Arbacia. *Science* 69 : 427.

———. 1930. The action of various salts on the first stage of the surface precipitation reaction in Arbacia egg protoplasm. *Protoplasma* 11 : 558–573.

———. 1934. The effect of anesthetics on the surface precipitation reaction. *Biol. Bull.* 66 : 264–275.

———. 1936. Protein lipid binding in protoplasm. *Biol. Bull.* 71 : 299–305.

———. 1943. *An Outline of General Physiology.* 2nd ed. Saunders Co., Phila. & London. (1st ed. 1937).

———. 1952. *An Outline of General Physiology,* 3rd ed. Saunders Co., Phila.

———, HARRIS, D. L., LE FEVRE, P. G., WILSON, W. L. and WOODWARD, A. A. 1946. Heat death, heat injury, and toxic factor. *Physiol. Zool.* 19 : 404–429.

——— and KELLY, J. W. 1950. Anti-mitotic substances in living tissues. *Anat. Rec.* 108 : 535 (Abstract).

——— and MAZIA, D. 1936. The action of radiations on living protoplasm. *Biological Effects of Radiation,* vol. 1 : 625–676. Ed. by B. M. Duggar.

———, ——— and STEINBACH, H. B. 1934. Free and bound calcium in stimulation and injury. *Anat. Rec.* 60 : 32 Suppl. (Abstract).

——— and YOUNG, R. A. 1930. The action of ultraviolet rays on Arbacia egg protoplasm. *Physiol. Zool.* 3 : 330–341.

HEILBRUNN, L. V. and YOUNG, R. A. 1935. Indirect effects of radiation on sea urchin eggs. *Biol. Bull.* 69 : 274–278.

HENDEE, E. C. 1931. Formed components and fertilization in the egg of the sea urchin Lytechinus variegatus. *Tortugas Laboratory* Vol. 27 and *Carnegie Inst. Washington Pub.* no. 413 : 99–105.

HENDERSON, L. J. and COHN, E. J. 1916. The equilibrium between acids and bases in sea water. *Proc. Nat. Acad. Sci.* 2 : 618–620.

HENRY, R. J. and HENRY, M. D. 1945. The effect of penicillin on eggs of the sea urchin, Arbacia punctulata. *J. Gen. Physiol.* 28 : 405–413.

HENSHAW, P. S. 1932. Studies of the effect of roentgen rays on the time of the first cleavage in some marine invertebrate eggs. I. Recovery from roentgen-ray effects in Arbacia eggs. *Am. J. Roentgen. and Radium Ther.* 27 : 890–898.

———. 1936. The question of recovery from x-ray effects in Arbacia sperm. *Biol. Bull.* 71 : 399 (Abstract).

———. 1938. a. The action of x-rays on nucleated and non-nucleated egg fragments. *Am. J. Cancer* 33 : 258–264.

———. 1938. b. The effect of x-rays on Arbacia punctulata sperm. *Biol. Bull.* 75 : 347 (Abstract).

———. 1938. c. The question of whether the delay in cleavage of Arbacia eggs produced with x-rays is caused by a general slowing of the cleavage process or by a block at some particular stage. *Biol. Bull.* 75 : 356 (Abstract).

———. 1939. Fixation of x-ray effect by fertilization in Arbacia eggs. *Biol. Bull.* 77 : 325–326 (Abstract).

———. 1940. Further studies on the action of roentgen rays on the gametes of Arbacia puntulata.

I. Delay in cell division caused by exposure of sperm to roentgen rays, p. 899–906.

II. Modification of the mitotic time schedule in the eggs by exposure of the gametes to roentgen rays, p. 907–912.

III. Fixation of irradiation effect by fertilization in the eggs, p. 913–916.

IV. Co-author with I. Cohen. Changes in the radiosensitivity during the first cleavage cycle, p. 917–920.

V. The influence of low temperature on recovery from roentgen ray effects in the eggs. p. 921–922.

VI. Production of multipolar cleavage in the eggs by exposure of the gametes to roentgen rays, p. 923–933.

Am. J. Roentgen. and Radium Ther. 43 : 899–933.

HENSHAW, P. S. 1941. The induction of multipolar cell division with x-rays and its possible significance. *Radiology* 36 : 717–724.

——— and FRANCIS, D. S. 1933. A response of Arbacia eggs to x-rays. *Biol. Bull.* 65 : 361 (Abstract)

——— and ——— 1936. The effect of x-rays on cleavage in Arbacia eggs: evidence of nuclear control of division rate. *Biol. Bull.* 70 : 28–35.

———, HENSHAW, C. T. and FRANCIS, D. S. 1933. The effect of roentgen rays on the time of first cleavage in marine invertibrate eggs. II. Differential recovery and its influence when different methods of exposure are used. *Radiology* 21 : 533–541.

HERBST, C. 1892. Experimentelle Untersuchungen über den Einfluss der veränderten chemischen Zusammensetzung des umgebenden Mediums auf die Entwickelung der Thiere. I. Versuche an Seeigeleiern. *Zeits. f. wiss. Zool.* 55 : 446–518.

———. 1893. *Ibid.* II. Weiteres über die morphologische Wirkung der Lithiumsalze und ihre theoretische Bedeutung. *Mitth. Zool. Sta. Neapel* 11 : 136–220.

———. 1896. *Ibid.* III. Über das Ineinandergreifen von normaler Gastrulation und Lithiumentwickelung. *Arch. f. Entwick.* 2 : 455–516.

———. 1897–1904. Über die zur Entwickelung der Seeigel Larven notwendigen anorganischen Stoffe, ihre Rolle und ihre Vertretbarkeit. I. 1897. *Arch. f. Entwick.* 5 : 649–793, II. 1901. *Ibid.* 11 : 617–689. III. 1904. *Ibid.* 17 : 306–520.

———. 1900. Über das Auseinandergehen von Furchungs- und Gewebezellen in kalkfreiem Medium. *Arch. f. Entwick.* 9 : 424 463.

———. 1904. Über die zur Entwickelung der Seeigel larven notwendigen anorganischen Stoffe, ihre Rolle und ihre Vertretbarkeit. III. *Arch. f. Entwick.* 17 : 306–520.

———. 1906. Vererbungsstudien. II. Über den Einfluss der Temperatur auf die Ausbildung der Seeigelbastarde. *Arch. f. Entwick.* 21 : 177–293.

———. 1906–1914. Vererbungsstudien I–III. *Arch. f. Entwick.* 21 : 173–305; IV. 22 : 473–497; V. 24 : 185–238; VI. 27 : 266–308; VII. 34 : 1–89; X. 39 : 617–650.

———. 1925. Laut mündlichen Mittheilungenvon C. Herbst; Neapel 1925. See *Tabulae Eiol.* VI : 501.

HERLANT, M. 1914. Sur l'existence d'un rythme périodique dans le déterminisme

des premiers phénomènes du développement parthénogénétique expérimental chez l'oursin. *C. R. Acad. Sci. Paris* 158 : 1531–1533.

Herlant, M. 1918. a. Un cas d'hermaphrodisme complet et fonctionnel chez Paracentrotus lividus. *Arch. Zool. expér. et gén.* 57: notes et revue: 28–31.

———. 1918. b. Variations cycliques de la perméabilité chez l'œuf activé. *C. R. Soc. Biol. Paris.* 81 : 151–155.

———. 1920. Le cycle de la vie cellulaire chez l'œuf activé. *Arch. de Biol.* 30 : 517–600.

Herodotus. 484–425 B.C.

Hertwig, G. 1912. Das Schicksal des mit Radium bestrahlten Spermachromatins im Seeigelei. *Arch. mikr. Anat.* 79 II : 201–241.

Hertwig, O. 1876. Beiträge zur Kentniss der Bildung, Befruchtung und Theilung des thierischen Eies. *Morph. Jahrb.* 1 : 347–434.

———. 1890. Experimentelle Studien am tierischen Ei vor, während und nach der Befruchtung. *Jena. Zeitsch. N.F.* 17 (= 24): 268–313.

Hertwig, O. and R. 1886. Experimentelle Untersuchungen über die Bedingungen der Bastardbefruchtung. *Jena. Zeitsch. N.F.* 12 (= 19) : 121–165.

———. 1887. Über den Befruchtungs- und Teilungsvorgang des tierischen Eies unter dem Einfluss äusserer Agentien. *Jena. Zeitsch. N.F.* 13 (= 20) : 120–241 and 477–510.

Hertwig, R. 1895. Über Centrosoma und Centralspindel. *Sitzungs. Ges. Morph. und Physiol. München* 11 : 41–59.

———. 1896. Über die Entwicklung des unbefruchteten Seeigeleies. *Festschr. f. Gegenbaur* Bd II : 21–86. Leipzig.

Hesse, R., Allee, W. C. and Schmidt, K. P. 1937. *Ecological Animal Geography.* John Wiley & Sons.

——— und Doflein, F. 1910–1914. *Tierbau und Tierleben,* Bd. I, II. Leipzig.

Hill, John. 1752. An History of Animals. 3rd part of *A General Natural History.*

Hillier, J. and Hoffman, J. F. 1953. On the ultrastructure of the plasma membrane as determined by the electron microscope. *J. Cell. and Comp. Physiol.* 42 : 203–247.

———, Lansing, A. I. and Rosenthal, T. B. 1952. Electron microscopy of some marine egg surfaces. *Biol. Bull.* 103 : 293 (Abstract).

Hindle, E. 1910. A cytological study of artificial parthenogenesis in Strongylocentrotus purpuratus. *Arch. f. Entwick.* 31 : 145–163.

Hinrichs, M. A. 1923. Modification of larval development in Arbacia on the basis of differential susceptibility to light. *Anat. Rec.* 26 : 372–373 (Abstract).

———. 1926. a. Modification of development on the basis of differential susceptibility to radiation. II. Arbacia and visible light following sensitization. *Biol. Bull.* 50 : 1–16.

———. 1926. b. *Ibid.* III. Arbacia germ cells, and (a) ultraviolet radiation, (b) visible radiation following sensitization. *Biol. Bull.* 50 : 455–472.

———. 1926. c. The effect of radiation on the fertilizing power of Arbacia sperm. *Biol. Bull.* 50 : 473–489.

———. 1927. Ultraviolet radiation and the fertilization reaction in Arbacia punctulata. *Biol. Bull.* 53 : 416–437.

Hippocrates, ca. 460–377 B.C.

Hirabayashi, K. 1937. Change of blastocoelic pH during gastrulation of the Echinoid embroyos. *Annot. Zool. Jap.* 16 : 205–209.

Hiramoto, Y. 1954. Nature of the perivitelline space in sea urchin eggs. *I. Jap. J. Zool.* 11 : 227–243.

Hoadley, L. 1923. Certain effects of the salts of the heavy metals on the fertilization reaction in Arbacia punctulata. *Biol. Bull.* 44 : 255–279.

———. 1930. Some effets of $HgCl_2$ on fertilized and unfertilized eggs of Arbacia punctulata. *Biol. Bull.* 58 : 123–144.

———. 1934. The relation between the position of the female pronucleus and the polar bodies in the unfertilized egg of Arbacia punctulata. *Biol. Bull.* 67 : 220–222.

——— and Brill, E. R. 1937. Temperature and the cleavage rate of Arbacia and Chaetopterus. *Growth* 1 : 234–244.

Hobson, A. D. 1927. A study of the fertilization membrane in the Echinoderms. *Proc. Roy. Soc. Edinburgh* 47 : 94–117.

———. 1932. a. The effect of fertilization on the permeability to water and on certain other properties of the surface of the egg of Psammechinus miliaris. *J. Exp. Biol.* 9 : 69–92.

———. 1932. b. On the vitelline membrane of the egg of Psammechinus miliaris and of Teredo norvegica. *J. Exp. Biol.* 9 : 93–106.

Hollaender, A. 1938. Monochromatic ultraviolet radiation as an activating agent for the eggs of Arbacia punctulata. *Biol. Bull.* 75 : 248–257.

———. 1954. *Radiation Biology,* editor. National Research Council. Vol. I. McGraw Hill Co. N.Y.

HOLLINGSWORTH, J. 1941. Activation of Cumingia and Arbacia eggs by bivalent cations. *Biol. Bull.* 81 : 261–276.

HOLMES, F. S. 1860. *Post-Pleiocene Fossils of South Carolina.* Charleston, S.C.

HOLMES, S. J. 1912. Phototaxis in the sea urchin Arbacia punctulata. *J. Animal Behavior* 2 : 126–136.

HOLTER, H. 1936. Studies on enzymatic histochemistry. XVIII. Localization of peptidase in marine ova. *J. Cell. and Comp. Physiol.* 8 : 179–200.

———. 1949. Problems on enzyme location and development. *Pub. Staz. Zool. Napoli* 21 Suppl. 60–76.

——— and LINDAHL, P. E. 1941. The distribution of peptidase in Paracentrotus embryos. *J. Cell. and Comp. Physiol.* 17 : 235–241.

——— and LINDERSTRØM-LANG, K. 1940. Enzymatische Histochemie. *Handbuch der Enzymologie,* p. 65–114.

HORACE. 65–8 B.C. *Epodes V.*

HÖRSTADIUS, S. 1925. Temperaturanpassung bei den Eiern von Paracentrotus lividus Lk. *Biologia Generalis* 1 : 522–536.

———. 1928. Über die Determination des Keimes bei Echinodermen. *Acta Zoologica* 9 : 1–191.

———. 1935. Über die Determination im Verlaufe der Eiachse bei Seeigeln. *Pubb. Staz. Zool. Napoli* 14 : 251–479.

———. 1936. a. Über die zeitliche Determination im Keim von Paracentrotus lividus Lk. *Arch. f. Entwick.* 135 : 1–39.

———. 1936. b. Weitere Studien über die Determination im Verlaufe der Eiachse bei Seeigeln. *Arch. f. Entwick.* 135 : 40–68.

———. 1936. c. Studien über heterosperme Seeigelmerogone nebst Bemerkungen über einige Keimblattchimären. Mem. Musée royal d'hist. natur. de Belgique, Ser. 2, Fasc. 3 : 801–880.

———. 1937. a. Investigations as to the localisation of the micromere-, the skeleton-, and the entoderm-forming material in the unfertilized egg of Arbacia punctulata. *Biol. Bull.* 73 : 295–316.

———. 1939. The mechanics of sea urchin development, studied by operative methods. *Biol. Rev.* 14 : 132–179.

———. 1940. Change in inheritance in Echinoid hybrids. *J. Marine Biol. Assoc.* 24 : 99–113.

———. 1950. Free-hand manipulations. *McClung's Handbook of Microscopical Technique* 3rd ed. : 555–563.

HOWARD, E. 1931. The effect of fatty acid buffer systems on the apparent viscosity of the Arbacia egg, with especial reference to the question of cell permeability to ions. *Biol. Bull.* 60 : 132–151.

HOWARD, E. 1932. The structure of protoplasm as indicated by a study of the apparent viscosity of sea urchin eggs at various shearing forces. *J. Cell. and Comp. Physiol.* 1 : 355–369.

HSIA, W. P. 1948. On the relations between the number of coronal plates and the diameter of the test in three species of sea urchins. *Contrib. Inst. Zool. National Acad. Peiping* 4 : 25–31.

HUGHES, A. 1952. *The Mitotic Cycle.* Academic Press, N.Y. and Butterworths, London.

HULTIN, T. 1948. a. Species specificity in fertilization reaction. The role of the vitelline membrane of sea urchin eggs in species specificity. *Ark. f. Zool.* 40A, no. 12 : 1–9.

———. 1948. b. Species specificity in fertilization reaction. II. Influence of certain factors on the cross-fertilization capacity of Arbacia lixula (L.). *Ark. f. Zool.* 40A, no. 20 : 1–8.

———. 1949. a. Agglutination of sea urchin eggs by nucleoproteins. *Arkiv f. Kemi* 1 : 419–423.

———. 1949. b. Effect of calcium on respiration and acid formation in homogenates of sea urchin eggs. *Arkiv. f. Kemi, Mineral. o. Geol.* 26A no. 27 : 1–10.

———. 1950. a. On the oxygen uptake of Paracentrotus lividus egg homogenates after the addition of calcium. *Exp. Cell Res.* 1 : 159–168.

———. 1950. b. On the acid formation, breakdown of cytoplasmic inclusions and increased viscosity in Paracentrotus egg homogenates after the addition of calcium. *Exp. Cell Res.* 1 : 272–283.

———. 1950. c. The protein metabolism of sea urchin eggs during early development ment studied by means of ^{15}N-labelled ammonia. *Exp. Cell Res.* 1 : 599–602.

———. 1952. Incorporation of N^{15}-labelled glycine and alanine into the proteins of developing sea urchin eggs. *Exp. Cell Res.* 3 : 494–496.

HUNTER, F. R. 1936. The effect of lack of oxygen on cell permeability. *J. Cell. and Comp. Physiol.* 9 : 15–27.

———. 1938. The measurement of the permeability constants for water and for a non-electrolyte using an isosmotic solution. *J. Cell. amd Comp. Physiol.* 12 : 313–318.

——— and HARVEY, E. N. 1935. The effect of lack of oxygen on the permeability of the egg of Arbacia punctulata to ethylene glycocol. *Biol. Bull.* 69 : 344 (Abstract).

——— and ———. 1936. The effect of lack

of oxygen on the permeability of the egg of Arbacia punctulata. *Biol. Bull.* 71 : 396–397 (Abstract).

HUNTER, F. R. and PARPART, A. K. 1946. The distribution of lipid between the light and heavy halves of the Arbacia egg. *Biol. Bull.* 91 : 222 (Abstract).

HUNTER, S. J. 1901. On the production of artificial parthenogenesis in Arbacia by the use of sea water concentrated by evaporation. *Am. J. Physiol.* 6 : 177–180.

———. 1903. On the conditions governing the production of artificial parthenogenesis in Arbacia. *Biol. Bull.* 5 : 143–151.

HUTCHENS, J. O., KELTCH, A. K., KRAHL, M. E. and CLOWES, G. H. A. 1942. Studies on cell metabolism and cell division. VI. Observations on the glycogen content, carbohydrate consumption, lactic acid production, and ammonia production of eggs of Arbacia punctulata. *J. Gen. Physiol.* 25 : 717–731.

———, KOPAC, M. J. and KRAHL, M. E. 1942. The cytochrome oxidase content of centrifugally separated fractions of unfertilized Arbacia eggs. *J. Cell. and Comp. Physiol.* 20 : 113–116.

———, KRAHL, M. E. and CLOWES, G. H. A. 1939. Physiological effects of nitro- and halo-substituted phenols on Arbacia eggs in the presence of ammonia. *J. Cell. and Comp. Physiol.* 14 : 313–325.

——— and PODOLSKY, B. 1948. The effect of nitrogen mustards on cleavage and development of Arbacia eggs. *Biol. Bull.* 95 : 251 (Abstract).

HUTCHINGS, L. M. 1948. Combined effect of ultraviolet light and heat upon first cleavage of Arbacia eggs. *Biol. Bull.* 95 : 259–260 (Abstract).

HYMAN, L. H. 1923. Some notes on the fertilization reaction in Echinoderm eggs. *Biol. Bull.* 45 : 254–278.

IIDA, T. T. 1943. a. Changes of electric capacitance following fertilization in sea urchin eggs. *J. Fac. Sci. Imp. Univ. Tokyo.* Sec. IV. 6 : 141–151.

———. 1943. b. Correlative changes of capacitance and permeability following fertilization in sea urchin eggs. *J. Fac. Sci. Imp. Univ. Tokyo.* Sec. IV., 6 : 153–163.

———. 1943. c. Effects of diluted sea water on membrane capacitance of sea urchin eggs. *J. Fac. Sci. Imp. Univ. Tokyo.* Sec. IV, 6 : 165–173.

IKEDA, H. 1931. a. A biometric study of the sexual dimorphism and sex ratio in Temnopleurus toreumaticus (Klein). *Annot. Zool. Jap.* 13 : 233–242.

IMHOOF-BLUMER, F. und KELLER, O. 1889. *Tier- und Pflanzenbilder auf Münzen und Gemmen des Klassischen Altertums.* Leipzig, Teubner.

IMMERS, J. 1952. Carbohydrate components in unfertilized sea urchin eggs. *Arkiv f. Zool.* Ser. 2, vol. 3 : 367–371.

INOUÉ, S. and DAN, K. 1951. Birefringence of the dividing cell. *J. Morph.* 89 : 423–455.

IRWIN, M. 1953. Steel-boring sea urchins. *Pacific Discovery* 6 : 26–27.

ISHIDA, J. 1936. An enzyme dissolving the fertilization membrane of sea urchin eggs. *Annot. Zool. Jap.* 15 : 453–459.

——— and NAKANO, E. 1947. The fertilization of the activated sea urchin eggs deprived of the fertilization membrane. *Zool. Mag.* (Dobutsugaku Zasshi) 57 : 117–120. In Japanese with English summary. Preliminary report.

——— and ———. 1950. Fertilization of activated sea urchin eggs deprived of fertilization membrane by washing with Ca-Mg-free media. *Annot. Zool. Jap.* 23 : 43–48.

IWATA, K. S. 1950. A method of determining the sex of sea urchins and of obtaining eggs by electric stimulation. *Annot. Zool. Jap.* 23 : 39–42.

JACKSON, H. W. 1939. Notes on marine aquarium animals. *Am. Midland Nat.* 22 : 654–659.

JACKSON, R. T. 1912. Phylogeny of the Echini, with a revision of palaeozoic species. *Mem. Boston Soc. Nat. Hist.* 7 : 1–491.

———. 1927. Studies of Arbacia punctulata and allies, and of nonpentamerous Echini. *Mem. Boston Soc. Nat. Hist.* 8, no. 4 : 437–565.

———. 1929. The status of Bothriocidaris. *Bull. Mus. Comp. Zool. Harvard* 69 : 481–512.

JACOBS, M. H. 1922. The effects of carbon dioxide on the consistency of protoplasm. *Biol. Bull.* 42 : 14–30.

———. 1924. Permeability of the cell to diffusing substances. *Cowdry's General Cytology* pp. 99–164.

———. 1933. a. The simultaneous measurement of cell permeability to water and to dissolved substances. *J. Cell. and Comp. Physiol.* 2 : 427–444.

———. 1952. The measurement of cell permeability with particular reference to the erythrocyte. *Modern Trends*, ed. by Barron: 149–171.

——— and STEWART, D. R. 1932. A simple method for the quantitative measurement

of cell permeability. *J. Cell. and Comp. Physiol.* 1 : 71–82.

Jacobs, M. H. and Stewart, D. R. 1936. The distribution of penetrating ammonium salts between cells and their surroundings. *J. Cell. and Comp. Physiol.* 7 : 351–365.

Jacobs, M. H. 1933. b. The relation between cell volume and penetration of a solute from an isosmotic solution. *J. Cell. and Comp. Physiol.* 3 : 29–43.

———. 1933. c. Volume changes in solutions containing both penetrating and nonpenetrating solutes, and their relation to the "permeability ratio." *J. Cell. and Comp. Physiol.* 3 : 121–129.

———. 1940. Some aspects of cell permeability to weak electrolytes. *Cold Spring Harbor Symp.* 8 : 30–39.

Jandorf, B. J. and Krahl, M. E. 1942. Studies on cell metabolism and cell division. VIII. The diphosphopyridine nucleotide (cozymase) content of eggs of Arbacia punctulata. *J. Gen. Physiol.* 25 : 749–754.

Jenkinson, J. W. 1911. On the origin of the polar and bilateral structure of the egg of the sea urchin. *Arch. f. Entwick.* 32 : 699–716.

John, G. 1889. Über bohrende Seeigel. *Arch. f. Naturges.* 55, Bd. 1 : 268–302.

Johnson, M. E. and Snook, H. J. 1927. *Seashore Animals of the Pacific Coast.* Macmillan Co., N.Y.

Just, E. E. 1919. The fertilization reaction in Echinarachnius parma. I, II, III. *Biol. Bull.* 36 : 1–53.

———. 1922. a. Initiation of development in the egg of Arbacia. I–III. *Biol. Bull.* 43 : 384–422.

———. 1922. b. Studies of cell division. I. The effect of dilute sea water on the fertilized egg of Echinarachnius parma during the cleavage cycle. *Am. J. Physiol,* 61 : 505–515.

———. 1922. c. The fertilization reaction in Echinarachnius parma. V. The existence in the inseminated egg of a period of special susceptibility to hypotonic sea water. *Am. J. Physiol.* 61 : 516–527.

———. 1927. Bodies in the egg of Arbacia described by E. B. Wilson as Golgi. *Anat. Rec.* 37 : 158 (Abstract).

———. 1928. a. Initiation of development in Arbacia. IV. Some cortical reactions as criteria for optimum fertilization capacity and their significance for the physiology of development. *Protoplasma* 5 : 97–126.

———. 1928. b. Studies of cell division. II. The period of maximum susceptibility to dilute sea water in the cleavage cycle of the fertilized egg of Arbacia. *Physiol. Zool.* 1 : 26–36.

Just, E. E. 1928. c. Initiation of development in Arbacia. V. The effect of slowly evaporating sea water and its significance for the theory of auto-parthenogenesis. *Biol. Bull.* 55 : 358–368.

———. 1929. a. Initiation of development in Arbacia. VI. The effect of sea water precipitates with special reference to the nature of lipolysin. *Biol. Bull.* 57 : 422–438.

———. 1929. b. Hydration and dehydration in the living cell. II. Fertilization of eggs of Arbacia in dilute sea water. *Biol. Bull.* 57 : 443–448.

———. 1930. The present status of the fertilizin theory of fertilization. *Protoplasma* 10 : 300–342.

———. 1939. a. *Basic Methods for Experiments on Eggs of Marine Animals.* Blakiston's Son & Co., Philadelphia.

———. 1939. b. *The Biology of the Cell Surface.* Blakiston's Son & Co., Philadelphia.

Kavanau, J. L. 1953. Metabolism of free amino acids, peptides and proteins in early sea urchin development. *J. Exper. Zool.* 122 : 285–337.

Keckwick, R. A. and Harvey, E. N. 1934. The effect of anaerobic conditions on the permeability of the egg of Arbacia to water. *J. Cell. and Comp. Physiol.* 5 : 43–51.

Keller, O. 1889. See Imhoof-Blumer, F., und Keller, O.

———. 1913. *Die Antike Tierwelt.* Bd. II. Leipzig, Englemann. Echinoderma pp. 571–574.

Kellogg, V. L. 1899. The Hopkins Seaside Laboratory. *Am. Nat.* 33 : 629–634.

Keltch, A. K. and Clowes, G. H. A. 1947. On the relation between oxygen consumption, fertilization membrane formation, and cell division in artificially fertilized Arbacia eggs. *Biol. Bull.* 93 : 195–196 (Abstract).

———, Krahl, M. E. and Clowes, G. H. A. 1937. Stimulation of the rate of cell division of Arbacia eggs by carcinogenic hydrocarbons. *Biol. Bull.* 73 : 377 (Abstract).

———, Smythe, M. P. and Clowes, G. H. A. 1951. Phosphorus uptake of cell-free homogenates of unfertilized Arbacia eggs under aerobic and anaerobic conditions. *Biol. Bull.* 101 : 220 (Abstract).

———, Strittmatter, C. F., Walters, C. P. and Clowes, G. H. A. 1950. Oxidative phosphorylation by a cell-free particulate system from unfertilized Arbacia eggs. *J. Gen. Physiol.* 33 : 547–553.

———, Wade, L. W. and Clowes, G. H.

A. 1933. On the relative sensitivity of Arbacia eggs and sperm to various acids and bases. *Biol. Bull.* 65 : 366–367 (Abstract).

KELTCH, A. K., WADE, L. W. and CLOWES G. H. A. 1934. Contrasting curves of resistance exhibited by Arbacia eggs exposed to various chemical agents during the cleavage cycle. *Biol. Bull.* 67 : 331–332 (Abstract).

———, WALTERS, C. P. and CLOWES, G. H. A. 1947. Effect of dinitrocresol on oxygen consumption and cell division in artificially fertilized as compared with sperm fertilized Arbacia eggs. *Biol. Bull.* 93 : 196–197 (Abstract).

KERR, J. G. 1900. External features in the development of Lepidosiren paradoxa, Fitz. *Phil. Trans. Roy. Soc. London B* 192 : 299–330.

KETCHUM, B. H. and REDFIELD, A. C. 1938. A method for maintaining a continuous supply of marine diatoms by culture. *Biol. Bull.* 75 : 165–169.

KIDDER, J. H. 1875. Contributions to the Natural History of Kerguelen Island made in connection with the American Transit of Venus Expedition 1874–75. *Bull. U.S. National Museum.* Wash. Gov. Printing Office. no. 3 : 69–71.

KINDRED, J. E. 1921. Phagocytosis and clotting in the perivisceral fluid of Arbacia. *Biol. Bull.* 41 : 144–152.

———. 1924. The cellular elements in the perivisceral fluid of Echinoderms. *Biol. Bull.* 46 : 228–251.

———. 1926. A study of the genetic relationships of the "amoebocytes with spherules" in Arbacia. *Biol. Bull.* 50 : 147–154.

KING, H. D. 1912. The effects of some amido-acids on the development of the eggs of Arbacia and of Chaetopterus. *Biol. Bull.* 22 : 273–290.

KITCHING, J. A. and MOSER, F. 1940. Studies on a cortical layer response to stimulating agents in the Arbacia egg. IV. Response to chemical and physical agents in the absence of oxygen, and observations of the effects of low oxygen tensions and high hydrostatic pressures upon amoeboid eggs. *Biol. Bull.* 78 : 80–91.

KITE, G. L. 1912. The nature of the fertilization membrane of the egg of the sea urchin (Arbacia punctulata). *Science* 36 : 562–564.

KLEIN, J. T. 1734. *Naturalis dispositio Echinodermatum.* Gedani.

KLEINHOLZ, L. H. 1938. Color changes in Echinoderms. *Pub. Staz. Zool. Napoli* 17 : 53–57.

KNAFFL-LENZ, E. See under *von* Knaffl-Lenz.

KOEHLER, O. 1915–1916. Über die Ursachen der Variabilität bei Gattungsbastarden von Echiniden. *Zeits. f. Induk. Abstam. u. Vererbungs.* 15 : 1–163; 177–295.

KOEHLER, R. 1883. Recherches sur les Échinides des côtes de Provence. *Ann. Mus. d'hist. nat. Marseille,* Zool. 1, mem. no. 3 : 1–167.

———. 1927. *Les Échinodermes des Mers d'Europe.* T. 2. Doin et Cie. Paris.

KOPAC, M. J. 1938. Effect of the hyaline plasma layer on coalescence of Arbacia eggs with oil drops. *Anat. Rec. Suppl.* 72 : 113 (Abstract).

———. 1939. a. Coalescence with oil drops of Arbacia eggs immersed in KCl or $CaCl_2$ solutions. *Anat. Rec. Suppl.* 75 : 142–143 (Abstract).

———. 1939. b. Interfacial reactions between oils and disintegrated Arbacia eggs. *Anat. Rec. Suppl.* 75 : 143 (Abstract).

———. 1940. a. The physical properties of the extraneous coats of living cells. *Cold Spring Harbor Symp.* 8 : 154–170.

———. 1940. b. Some properties of the residue from rapidly disintegrated Arbacia egg cytoplasm. *Biol. Bull.* 79 : 341 (Abstract).

———. 1940. c. The effect of x-radiation on the vitelline membrane of Arbacia eggs. *Anat. Rec. Suppl.* 78 : 169.

———. 1941. a. Disintegration of the fertilization membrane of Arbacia by the action of an "enzyme." *J. Cell. and Comp. Physiol.* 18 : 215–220.

———. 1941. b. The effect of 200-kv x-radiation on the extraneous coats of Arbacia eggs. *J. Appl. Physics* 12 : 348.

———. 1943. Micrurgical application of surface chemistry to the study of living cells. *Micrurgical and Germ-free Techniques,* Chapt. II : 26–71. J. A. Reyniers, ed., Univ. Notre Dame.

———. 1944. Some surface chemical properties of protoplasmic proteins. *Colloid Chemistry,* ed. by J. Alexander. Vol. 5 : 875–883.

———. 1948. a. The action of NH_4Cl on the surface membranes of Arbacia eggs. *Biol. Bull.* 95 : 267 (Abstract).

———. 1948. b. The inhibition of development of Arbacia eggs by NH_4Cl. *Biol. Bull.* 95 : 267–268 (Abstract).

———. 1950. The surface chemical properties of cytoplasmic proteins. *Ann. N.Y. Acad. Sci.* 50 : 870–909.

——— and CHAMBERS, R. 1937. The coalescence of living cells with oil drops. II. Arbacia eggs immersed in acid or alkaline calcium solutions. *J. Comp. and Cell. Physiol* 9 : 345–361.

KOPAC, M. J. and CHAMBERS, R. 1938. Effect of the vitelline membrane on coalescence of Arbacia eggs with oil drops. *Biol. Bull.* 75 : 372–373 (Abstract).

KORR, I. M. 1937. Respiratory mechanisms in the unfertilized and fertilized sea urchin egg. A temperature analysis. *J. Cell. and Comp. Physiol.* 10 : 461–485.

———. 1939. Oxidation-reductions in heterogeneous systems. *Cold Spring Harbor Symp.* 7 : 74–93.

KORSCHELT, E. and HEIDER, K. 1902. *Lehrbuch der Vergleichenden Entwicklungsgeschichte der Wirbellosen Thiere.* Allgemeiner Theil, erste Lieferung. Jena.

——— and ———. 1936. *Vergleichende Entwicklungsgeschichte der Tiere.* Bd. 1: Echinodermen 482–536. Jena, Gustav Fischer. Older edition (1895) in English, *Textbook of the Embryology of Invertebrates*, Part 1.

KRAHL, M. E. 1950. Metabolic activities and cleavage of eggs of the sea urchin, Arbacia punctulata. A review, 1932–1949. *Biol. Bull.* 98 : 175–217.

——— and CLOWES, G. H. A. 1935. Stimulation of oxygen consumption and suppression of cell division by dihalo and trihalophenols. *Proc. Soc. Exp. Biol. and Med.* 33 : 477–478.

——— and ———. 1936. a. Studies on cell metabolism and cell division. II. Stimulation of cellular oxidation and reversible inhibition of cell division by dihalo- and trihalophenols. *J. Gen. Physiol.* 20 : 173–184.

——— and ———. 1936. b. Influence of temperature on stimulation of oxidation by dinitro-, dihalo- and trihalophenols. *Proc. Soc. Exp. Biol. and Med.* 34 : 567–569.

——— and ———. 1938. Physiological effects of nitro- and halo-substituted phenols in relation to extracellular and intracellular hydrogen ion concentration. I. Dissociation constants and theory. II. Experiments with Arbacia eggs. *J. Cell. and Comp. Physiol.* 11 : 1–39.

——— and ———. 1940. Studies on cell metabolism and cell division. IV. Combined action of substituted phenols, cyanide, carbon monoxide, and other respiratory inhibitors on respiration and cell division. *J. Gen. Physiol.* 23 : 413–427.

———, JANDORF, B. J. and CLOWES, G. H. A. 1942. Studies on cell metabolism and cell division. VII. Observations on the amount and possible function of diphosphothiamine (cocarboxylase) in eggs of Arbacia punctulata. *J. Gen. Physiol.* 25 : 733–747.

———, KELTCH, A. K. and CLOWES, G. H. A. 1937. Respiratory effects of substituted phenols at varying carbon dioxide tensions. *Proc. Soc. Exp. Biol. and Med.* 36 : 700–702.

KRAHL, M. E., KELTCH, A. K. and CLOWES, G. H. A. 1940. a. The role of changes in extracellular and intracellular hydrogen ion concentration in the action of local anesthetic bases. *J. Pharm. and Exp. Therap.* 68 : 330–350.

———, ——— and ———. 1940. b. Flavindinucleotide in eggs of the sea urchin, Arbacia punctulata. *Proc. Soc. Exp. Biol. and Med.* 45 : 719–721.

———, ———, NEUBECK, C. E. and CLOWES, G. H. A. 1941. Studies on cell metabolism and cell division. V. Cytochrome oxidase activity in the eggs of Arbacia punctulata. *J. Gen. Physiol.* 24 : 597–617.

———, ———, WALTERS, C. P. and CLOWES, G. H. A. 1953. Hexokinase and isomerase activity in eggs of the sea urchin, Arbacia punctulata and other marine forms. *Biol. Bull.* 105 : 377 (Abstract).

KRAUSS, M. 1950. On the question of hyaluronidase in sea urchin spermatozoa. *Science* 112 : 759.

KRISZAT, G. and RUNNSTRÖM, J. 1951. Some effects of adenosine triphosphate on the cytoplasmic state, division and development of the sea urchin egg. *Trans. N.Y. Acad. Sci.* Ser. II, vol. 13 : 162–164.

KROHN, A. 1849. *Beitrag zur Entwicklungsgeschichte der Seeigellarven.* Heidelberg.

KRUGELIS, E. J. 1947. a. Alkaline phosphatase activity in oocytes of various marine invertebrates. *Biol. Bull.* 93 : 209 (Abstract).

———. 1947. b. Alkaline phosphatase activity in developmental stages of Arbacia. *Biol. Bull.* 93 : 209 (Abstract).

KUHN, R. and WALLENFELS, K. 1939. Über die chemische Natur des Stoffes, den die Eier des Seeigels (Arbacia pustulosa) absondern, um die Spermatozoen anzulocken. *Ber. der deutsch. chem. Ges.* 72 : 1407–1413.

LA CASCIA, P. 1930. Sul problema del dimorfismo sessuale negli Echinoidi regolari (Centrechinidae). *Bull. Ist. Zool. Palermo* 2, no. 2 : 17–40.

LAMARCK, J. B. P. A. DE. 1816. *Histoire Naturelle des Animaux sans Vertèbres.* T. III : 1–59. Paris.

LAMBERT, J. M. and THIÉRY, P. 1909–1925. *Essai de Nomenclature Raisonnée des Échinides.* Chaumont.

LANGENDORFF, H. and M. 1931. Untersuchungen an befruchteten Seeigeleiern. *Strahlentherapie* 42 (4) : 793–799.

LANKESTER, E. RAY. 1900. *A Treatise on Zoology*. Part 3, The Echinoderma. London.

LANSING, A. I., HILLIER, J. and ROSENTHAL, T. B. 1952. Electron microscopy of some marine egg inclusions. *Biol. Bull.* 103 : 294 (Abstract).

——— and ROSENTHAL, T. B. 1949. Ribonucleic acid at cell surfaces and its possible significance. *Biol. Bull.* 97 : 263 (Abstract).

——— and ———. 1952. The relation between ribonucleic acid and ionic transport across the cell surface. *J. Cell. and Comp. Physiol.* 40 : 337–345.

LASER, H. and LORD ROTHSCHILD. 1939. The metabolism of the eggs of Psammechinus miliaris during the fertilization reaction. *Proc. Roy. Soc. London* B 126 : 539–557.

LAVIN, G. I. 1943. Simplified ultraviolet microscopy. *Rev. Sci. Instr.* 14 : 375–376.

LAVIN, G. I. 1950. Microscopy in the ultraviolet visible and infrared. *Biol. Bull.* 99: 342 (Abstract).

LEDERER, E. 1940. Les pigments des invertébrés (À l'exception des pigments respiratoires). *Biol. Rev.* 15 : 273–306.

——— et GLASER, R. 1938. Sur l'échinochrome et le spinochrome. *C. R. Acad. Sci Paris*, 207 : 454–456.

LEA, D. E. 1947. *Actions of Radiations on Living Cells*. Univ. Cambridge Press and Macmillan Co.

LEE, A. BOLLES. 1950. *The Microtomists Vade-Mecum*. 11th ed.

LEITCH, J. L. 1931. The water exchange of living cells. I. The normal permeability of the eggs of some marine invertebrates. *Univ. California Pub. Zool.* 36 : 127–140.

———. 1934. a. A physico-chemical study of marine eggs. *Tortugas Laboratory, Annual Report, Carnegie Inst., Washington, Year Book* no. 33 : 255–257.

———. 1934. b. The water exchanges of living cells. II. Non-solvent volume determinations from swelling and analytical data. *J. Cell. and Comp. Physiol.* 4 : 457–473.

———. 1936. The water exchanges of living cells. III. The application of a photographic method to the determination of the non-solvent volume of the eggs of Echinometra lucunter. *Tortugas Lab. Vol. 29. Carnegie Inst., Washington, Year Book* no. 452 : 349–358.

LEPESCHKIN, W. W. 1941. a. Über die Resistenz des Protoplasmas der Echinodermen-eier gegen mechanische Eingriffe, seine Fluoideigenschaften und die sogeannte surface precipitation reaction. *Protoplasma* 35 : 364–389.

———. 1941. b. Vitalfärbung der Echinodermen-Eier und selektive Permeabilität der Protoplasmaoberfläche. *Protoplasma* 35 : 588–594.

LESKE, N. G. 1778. *Additamenta ad Klein Naturalem Dispositionem Echinodermatum*. Lipsiae.

LESSLER, M. A. 1953. The nature and specificity of the Feulgen nucleal reaction. *Internat. Rev. Cytology* 2 : 231–247.

LEUCKART, C. G. F. R. 1848. *Über die Morphologie und die Verwandtschaftsverhältnisse der wirbellosen Thiere*. Braunschweig.

LIEBMAN, E. 1950. The leucocytes of Arbacia punctulata. *Biol. Bull.* 98 : 46–59.

LILLIE, F. R. 1912. The production of sperm isoagglutinins by ova. *Science* 36 : 527–530.

———. 1913. a. The mechanism of fertilization. *Science* 38 : 524–528.

———. 1913. b. Studies of fertilization. V. The behavior of the spermatozoa of Nereis and Arbacia with special reference to egg-extractives. *J. Exp. Zool.* 14 : 515–574.

———. 1914. Studies of fertilization. VI. The mechanism of fertilization in Arbacia. *J. Exp. Zool.* 16 : 523–590.

———. 1915. a. Sperm agglutination and fertilization. *Biol. Bull.* 28 : 18–33.

———. 1915. b. Studies of fertilization. VII. Analysis of variations in the fertilizing power of sperm suspensions of Arbacia. *Biol. Bull.* 28 : 229–251.

———. 1919. *Problems of Fertilization*. Univ. Chicago Press, Chicago, Ill.

———. 1921. a. Studies of fertilization. IX. On the question of superposition of fertilization on parthenogenesis in Strongylocentrotus purpuratus. *Biol. Bull.* 40 : 23–31.

———. 1921. b. Studies of fertilization. X. The effects of copper salts on the fertilization reaction in Arbacia and a comparison of mercury effects. *Biol. Bull.* 41 : 125–143.

———. 1921. c. Studies of fertilization. VIII. On the measure of specificity in fertilization between two associated species of the sea urchin genus Strongylocentrotus. *Biol. Bull.* 40 : 1–22.

——— and JUST, E. E. 1913. Breeding habits of the heteronereis form of Nereis limbata at Woods Hole, Mass. *Biol. Bull.* 24 : 147–169.

——— and ———. 1924. Fertilization. *Cowdry's General Cytology* pp. 451–536.

LILLIE, R. S. 1903. Fusion of blastomeres and nuclear division without cell division in solutions of non-electrolytes. *Biol. Bull.* 4 : 164–178.

———. 1909. The general biological signi-

ficance of changes in the permeability of the surface layer or plasma membrane of living cells. *Biol. Bull.* 17 : 188–208.

Lillie, R. S. 1910. The physiology of cell division. II. The action of isotonic solutions of neutral salts on unfertilized eggs of Asterias and Arbacia. *Am. J. Physiol.* 26: 106–133.

———. 1911. a. The physiology of cell division. III. The action of calcium salts in preventing the initiation of cell division in unfertilized eggs through isotonic solutions of sodium salts. *Am. J. Physiol.* 27 : 289–307.

———. 1911. b. The physiology of cell division. IV. The action of salt solutions followed by hypertonic sea-water on unfertilized sea urchin eggs and the role of membranes in mitosis. *J. Morph.* 22 : 695–730.

———. 1912. Antagonism between salts and anaesthetics. II. Decrease by anaesthetics in the rate of toxic action of pure isotonic salt solutions on unfertilized starfish and sea urchin eggs. *Am. J. Physiol.* 30 : 1–17.

———. 1914. a. Antagonism between salts and anesthetics. IV. Inactivation of hypertonic sea water by anesthetics. *J. Exp. Zool.* 16 : 591–616.

———. 1914. b. The action of various anaesthetics in suppressing cell-division in sea urchin eggs. *J. Biol. Chem.* 17 : 121–140.

———. 1916. a. Increase of permeability to water following normal and artificial activation in sea urchin eggs. *Am. J. Physiol.* 40 : 249–266.

———. 1916. b. The physiology of cell-division. VI. Rhythmical changes in the resistance of the dividing sea urchin egg to hypotonic sea water and their physiological significance. *J. Exp. Zool.* 21 : 369–402.

———. 1916. c. The theory of anaesthesia. *Biol. Bull.* 30 : 311–366.

———. 1917. The conditions determining the rate of entrance of water into fertilized and unfertilized Arbacia eggs, and the general relation of changes of permeability to activation. *Am. J. Physiol.* 43 : 43–57.

———. 1918. a. Comparative permeability of fertilized and unfertilized eggs to water. *Science* 47 : 147–149.

———. 1918. b. The increase of permeability to water in fertilized sea urchin eggs and the influence of cyanide and anaesthetics upon this change. *Am. J. Physiol.* 45 : 406–430.

———. 1921. A simple case of salt antagonism in starfish eggs. *J. Gen. Physiol.* 3 : 783–794.

———. 1941. Further experiments on artificial parthenogenesis in starfish eggs, with a review. *Physiol. Zool.* 14 : 239–267.

Lillie, R. S. and Baskervill, M. L. 1921. The action of neutral isotonic salt solutions in sensitizing Arbacia eggs to the activating influence of hypertonic sea water. *Am. J. Physiol.* 57 : 110–124.

——— and ———. 1922. The action of ultraviolet rays on Arbacia eggs, especially as affecting the response to hypertonic sea water. *Am. J. Physiol.* 61 : 272–288.

——— and Cattell, W. 1923. The relation between the electrical conductivity of the external medium and the rate of cell division in sea urchin eggs. *J. Gen. Physiol.* 5 : 807–814.

——— and ———. 1925. The conditions of activation of unfertilized starfish eggs by the electric current. *Biol. Bull.* 49 : 100–110.

——— and Hinrichs, M. A. 1923. Activation of starfish and sea urchin eggs by photodynamic action. *Anat. Rec.* 26 : 370 (Abstract).

Lindahl, P. E. 1932. a. Über den feineren Bau der Rinde des Seeigeleies. *Protoplasma* 16 : 378–386.

———. 1932. b. Zur Kenntnis des ovarialeies bei dem Seeigel. *Arch. f. Entwick.* 126: 373–390.

———. 1932. c. Zur experimentellen Analyse der Determination der Dorsoventralachse beim Seeigelkeim. I. Versuche mit gestreckten Eiern. II. Versuche mit zentrifugierten Eiern. *Arch. f. Entwick.* 127 : 300–338.

———. 1936. Zur Kenntniss der physiologischen Grundlagen der Determination im Seeigelkeim. *Acta Zool.* 17 : 179–365.

———. 1939. Zur Kenntnis der Entwicklungsphysiologie des Seeigeleies. *Zeits. f. vergl. Physiol.* 27 : 233–250.

———. 1941. Über die CN-resistente Atmung des Seeigeleies. *Arkiv f. Kemi, Miner. och Geolog.* 14A: No. 12 : 1–31.

———. 1942. Contribution to the physiology of form generation in the development of the sea urchin. *Quart. Rev. Biol.* 17 : 213–227.

——— and Holter, H. 1940. Beiträge zur enzymatischen Histochemie XXXIII. Die Atmung animaler und vegetativer Keimhälften von Paracentrotus vividus. *C. R. Travaux Lab. Carlsberg*, Ser. Chim, 23 : 257–288.

——— and ———. 1941. Über die Atmung der Ovozyten erster Ordnung von Paracentrotus lividus und ihre Veränderung während der Reifung. *C. R. Travaux*

Lab. Carlsberg, Ser. Chim. 24, no. 2 : 49–57.

LINDAHL, P. E. and LUNDIN, J. 1948. Removal of the fertilization membranes from large quantities of sea urchin eggs. *Science* 108: 481–482.

——— and OHMAN, L. O. 1938. Weitere Studien über Stoffwechsel und Determination im Seeigelkeim. *Biol. Zentralb.* 58 : 179–218.

——— and RUNNSTRÖM, J. 1929. Variation and ecology of Psammechinus miliaris (Gmelin). *Acta Zool.* 10 : 401–484.

LINDBERG, O. 1943. Studien über das Problem des Kohlehydratabbaus und der Säurebildung bei der Befruchtung des Seeigels. *Ark. f. Kemi, Min. och Geol.* 16A, no. 15.

———. 1949. On the turnover of adenosine triphosphate in the sea urchin egg. *Arkiv f. Kemi, Min. och Geol.* 26B, no. 13 : 1–4.

———. 1950. On surface reaction in the sea urchin egg. *Exp. Cell Res.* 1 : 105–114.

——— and ERNSTER, L. 1948. On carbohydrate metabolism in homogenised sea urchin eggs. *Biochim. et Biophysica Acta* 2 : 471–477.

LINDERSTRØM-LANG, K. 1939. Distribution of enzymes in tissues and cells. *Harvey Lectures* 1938–1939 : 214–245.

LINDVALL, S. 1948. Changes in activity of tributyrin splitting enzyme during early sea urchin egg development. *Arkiv f. Kemi, Min. och Geol.* 26B, no. 9 : 1–3.

——— and CARSJÖ, A. 1951. On protein fractions and inorganic ions in sea urchin eggs, unfertilized and fertilized. *Exp. Cell Res.* 2 : 491–498.

LINNÉ, C. 1758. *Systema Naturae.* 10th ed.

LISON, L. 1935. Sur la determination du pH intracellulaire par les colorants vitaux indicateurs. L'erreur métachromatique. *Protoplasma* 24 : 453–465.

———. 1941. pH and rH intracellulaires. *Tab. Biol.* 19; Part 2 : 1–23.

——— and PASTEELS, J. 1951. Études histophotométriques sur le teneur en acide désoxyribonucléique des noyaux au cours du développement embryonnaire chez l'oursin Paracentrotus lividus. *Arch. de Biol.* 62 : 1–43.

LITTLE, E. P. and EVANS, T. C. 1940. Delay in first cleavage of Arbacia eggs following roentgen irradiation of zygotes. *Biol. Bull.* 79 : 362 (Abstract).

LO BIANCO, S. 1909. Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del golfo di Napoli. *Mitt. Zool. Sta. Neapel* 19 : 513–761.

LOEB, J. 1892. Investigations in physiological morphology. III. Experiments on cleavage *J. Morph.* 7 : 253–262.

LOEB, J. 1894. Über eine einfache Methode, zwei oder mehr zusammengewachsene Embryonen aus einem Ei hervorzubringen. *Arch. ges. Physiol.* 55 : 525–530.

———. 1895. a. Untersuchen über die physiologischen Wirkungen des Sauerstoffmangels. *Pflüger's Archiv f. ges. Physiol.* 62: 249–294. Trans. 1905, Studies in Gen. Physiol. Part I: 370 – 423. Univ. Chicago Press, Chicago, Ill.

———. 1895. b. Über Kernteilung ohne Zellteilung. Briefliche Mitteilung an den Herausgeber. *Arch. f. Entwick.* 2 : 298–300.

———. 1898. Über den Einfluss von Alkalien und Säuren auf die embryonale Entwickelung und das Wachsthum. *Arch. f. Entwick.* 7 : 631–641.

———. 1899 (October). On the nature of the process of fertilization and the artificial production of normal larvae (Plutei) from the unfertilized eggs of the sea urchin. *Amer. J. Physiol.* 3 : 135–138.

———. 1900. a. On the artificial production of normal larvae from the unfertilized eggs of the sea urchin (Arbacia). *Am. J. Physiol.* 3 : 434–471.

———. 1900. b. Further experiments on artificial parthenogenesis and the nature of the process of fertilization. *Am. J. Physiol.* 4 : 178–184.

———. 1901. Experiments on artificial parthenogenesis in Annelids (Chaetopterus) and the nature of the process of fertilization. *Am. J. Physiol.* 4 : 423–459.

———. 1904. Über Befruchtung, künstliche Parthenogenese und Cytolyse des Seeigeleies. *Pflüger's Arch. f. ges. Physiol.* 103 : 257–265. Trans. in Univ. Cal. Pub. 2 : 73–81, 1905.

———. 1905. a. The physiological effects of lack of oxygen. *Studies in General Physiology*, Decennial Pub., Univ. Chicago, 2nd Ser., vol. 15, Part 1 : 370–423. Translation of 1895 paper.

———. 1905. b. On a simple method of producing from one egg two or more embryos which are grown together. *Studies in General Physiology*, Decennial Pub., Univ. Chicago, 2nd Ser., vol. 15, Part 1 : 303–308. Translation of 1894 paper.

———. 1907. The chemical character of the process of fertilization and its bearing upon the theory of life phenomena. *Univ. Cal. Pub. Physiol.* 3 : 61–80.

———. 1909. a. *Die Chemische Entwicklungserregung des Thierischen Eies.* Springer, Berlin.

———. 1909. b. Über die chemischen Be-

dingungen für die Entstehung einelliger Zwillinge beim Seeigel. *Arch. f. Entwick.* 27 : 119–140.

Loeb, J. 1910. The prevention of the toxic action of various agencies upon the fertilized egg through the suppression of oxidation in the cell. *Science* 32 : 411–412.

———. 1911. Auf welche Weise rettet die Befruchtung das Leben des Eies? *Arch. f. Entwick.* 31 : 658–668.

———. 1912. a. *The Mechanistic Conception of Life.* Univ. Chicago Press, Chicago, Ill.

———. 1912. b. The comparative efficiency of weak and strong bases in artificial parthenogenesis. *J. Ex. Zool.* 13 : 577–590.

———. 1913. a. *Artificial Parthenogenesis and Fertilization.* Univ. Chicago Press. A translation from the German edition of 1909.

———. 1913. b. Further experiments on natural death and prolongation of life in the egg. *J. Exp. Zool.* 15 : 201–208.

———. 1913. c. Reversibility in artificial parthenogenesis. *Science* 38 : 749–751.

———. 1914. a. Activation of the unfertilized egg by ultraviolet rays. *Science* 40 : 680–681.

———. 1914. b. Umkehrbarkeit in der Entwicklungserregung des Seeigeleis. *Arch. f. Entwick.* 38 : 277–287.

———. 1914. c. Cluster formation of spermatozoa caused by specific substances from eggs. *J. Exp. Zool.* 17 : 123–140.

———. 1915. a. On the nature of the conditions which determine or prevent the entrance of the spermatozoon into the egg. *Am. Nat.* 49 : 257–285.

———. 1915. b. Reversible activation and incomplete membrane formation of the unfertilized eggs of the sea urchin. *Biol. Bull.* 29 : 103–110.

———. 1915. c. Concerning Brachet's ideas of the role of membrane formation in fertilization. *Biol. Bull.* 28 : 87–92.

———. 1916. *The Organism as A Whole.* Putnam's Sons, N.Y.

———. 1918. *Forced Movements, Tropisms and Animal Conduct.* Monographs on Experimental Biology I. Lippincott Co. Philadelphia.

———. 1920. Chemical character and physiological action of the potassium ion. *J. Gen. Physiol.* 3 : 237–245.

——— and Chamberlain, M. M. 1915. An attempt at a physico-chemical explanation of certain groups of fluctuating variation. *J. Exp. Zool.* 19 : 559–568.

——— and Lewis, W. H. 1902. On the prolongation of the life of the unfertilized eggs of sea urchins by potassium cyanide. *Am. J. Physiol.* 6 : 305–317.

Loeb, J. and Wasteneys, H. 1910. Warum hemmt Natriumcyanid die Giftwirkung einer Chlornatriumlösung für das Seeigelei. *Biochem. Zeits.* 28 : 340–349.

——— and ———. 1911 a. Sind die Oxydationsvorgänge die unabhängige Variable in den Lebenserscheinungen? *Biochem. Zeits.* 36 : 345–356.

——— and ———. 1911. b. Die Beeinflussung der Entwicklung und der Oxidationsvorgänge im Seeigelei (Arbacia) durch Basen. *Biochem. Zeits.* 37 : 410–423.

——— and ———. 1913. a. The influence of hypertonic solution upon the rate of oxidations in fertilized and unfertilized eggs. *J. Biol. Chem.* 14 : 469–480.

——— and ———. 1913. b. Is narcosis due to asphyxiation? *J. Biol. Chem.* 14 : 517–523.

——— and ———. 1915. Further experiments on the relative effect of weak and strong bases on the rate of oxidations in the egg of the sea urchin. *J. Biol. Chem.* 21 : 153–158.

Loeb, R. F. 1920. Radioactivity and physiological action of potassium. *J. Gen. Physiol.* 3 : 229–236.

Lowndes, A. G. 1944. a. Densities of the embryonic stages of sea urchins. *Nature.* 154 : 55–56.

———. 1944. b. Shape of sea urchins. *Nature* 154 : 675–676.

Lucilius. ca. 148–103 B.C. *Satirae.*

Lucké, B. 1925. Observations on intravitam staining of centrifuged marine eggs. *Proc. Soc. Exp. Biol. and Med.* 22 : 305–306.

———. 1931. The effect of certain narcotics (urethanes) on permeability of living cells to water. *Biol. Bull.* 60 : 72–79.

———. 1932. On osmotic behavior of living cell fragments. *J. Cell. and Comp. Physiol.* 2 : 193–199.

———. 1935. The effect of temperature on osmotic equilibrium of living cells (unfertilized eggs of Arbacia punctulata). *J. Cell. and Comp. Physiol.* 5 : 465–471.

———. 1940. The living cell as an osmotic system and its permeability to water. *Cold Spring Harbor Symp.* 8 : 123–132.

———, Hartline, H. K. and McCutcheon, M. 1931. Further studies on the kinetics of osmosis in living cells. *J. Gen. Physiol.* 14 : 405–419.

———, ——— and Ricca, R. A. 1939. Comparative permeability to water and to certain solutes of the eggs of three marine invertebrates, Arbacia, Cumingea and Chaetopterus. *J. Cell. and Comp. Physiol.* 14 : 237–252.

——— and Harvey, E. N. 1935. The permeability of living cells to heavy water

(deuterium oxide). *J. Cell. and Comp. Physiol.* 5 : 473–482.

Lucké, B., Larrabee, M. G. and Hartline, H. K. 1935. Studies on osmotic equilibrium and on the kinetics of osmosis in living cells by a diffraction method. *J. Gen. Physiol.* 19 : 1–17.

——— and McCutcheon, M. 1926. a. Reversible and irreversible swelling of living and dead cells. *Arch. Path.* 2 : 846–854.

——— and ———. 1926. b. The effect of hydrogen ion concentration on swelling of cells. *J. Gen. Physiol.* 9 : 709–714.

——— and ———. 1927. The effect of salt concentration of the medium on the rate of osmosis of water through the membrane of living cells. *J. Gen. Physiol.* 10 : 665–670.

——— and ———. 1929. The effect of valence of ions on cellular permeability to water. *J. Gen. Physiol.* 12 : 571–580.

——— and ———. 1930. The effect of injury on cellular permeability to water. *Arch. Path.* 10 : 662–670.

——— and ———. 1932. The living cell as an osmotic system and its permeability to water. *Physiol. Rev.* 12 : 68–139.

———, Parpart, A. H. and Ricca, R. A. 1941. Failure of choleic acids of carcinogenic hydrocarbons to alter permeability of marine eggs and of mammalian erythrocytes. *Cancer Research* 1 : 709–713.

———, Ricca, R. A. and Parpart, A. 1951. Differential effects of roentgen rays on cell permeability and on cell cleavage. Experiments with egg cells of Arbacia punctulata. *J. Nat. Cancer Inst.* 11 : 997–1024.

Ludwig, W. 1928. Die Betriebsstoffwechsel von Paramoecium caudatum Ehrbg. *Arch. f. Protistenk.* 62 : 12–40.

Lumb, E. S. 1950. Cytochemical reactions of nucleic acids. *Quart. Rev. Biol.* 25 : 278–291.

Lundblad, G. 1950. Proteolytic activity in sea urchin gametes. *Exp. Cell Res.* 1 : 264–271.

Lyon, E. P. 1902. Effects of potassium cyanide and of lack of oxygen upon the fertilized eggs and the embryos of the sea urchin (Arbacia punctulata). *Am. J. Physiol.* 7 : 56–75.

———. 1903. Experiments in artificial parthenogenesis. *Am. J. Physiol.* 9 : 308–318.

———. 1904. a. Rhythms of CO_2 production during cleavage. *Science* 19 : 350–353.

———. 1904. b. Rhythms of susceptibility and of carbon dioxide production in cleavage. *Am. J. Physiol.* 11 : 52–58.

———. 1906. a. Some results of centrifugalizing the eggs of Arbacia. *Am. J. Physiol.* 15 : XXI-XXII, follows p. 301 (Proc. Am. Physiol. Soc.).

Lyon, E. P. 1906. b. Note on the geotropism of Arbacia larvae. *Biol. Bull.* 12 : 21–22.

———. 1907. Results of centrifugalizing eggs. *Arch. f. Entwick.* 23 : 151–173.

———. 1909. The catalase of Echinoderm eggs before and after fertilization. *Am. J. Physiol.* 25 : 199–213.

——— and Shackell, L. F. 1910. a. Autolysis of fertilized and unfertilized Echinoderm eggs. *J. Biol. Chem.* 7 : 371–377.

———. and ———. 1910. b. On the increased permeability of sea urchin eggs following fertilization. *Science* 32 : 249–251.

Lytton, E. Bulwer, 1834. *The Last Days of Pompeii.*

MacArthur, J. W. 1924. An experimental study and a physiological interpretation of exogastrulation and related modifications in Echinoderm embryos. *Biol. Bull.* 46 : 60–87.

MacBride, E. W. 1903. The development of Echinus esculentus, together with some points in the development of E. miliaris and E. acutus. *Phil. Trans. Roy. Soc. London*, B. 195 : 285–327.

———. 1906. Echinodermata in *Cambridge Natural History*. vol. I., Macmillan Co.

———. 1914. a. *Text-Book of Embryology.* Vol. I. Echinodermata 456–567. Macmillan Co.

———. 1914. b. The development of Echinocardium cordatum. *Q. J. M. S.* 59 : 471–486.

MacGinitie, G. E. and MacGinitie, N. 1949. *Natural History of Marine Animals.* McGraw-Hill Book Co. N.Y.

MacMunn, C. A. 1883. Studies in animal chromatology. *Proc. Birmingham Phil. Soc.* 3 : 351–407.

———. 1885. On the chromatology of the blood of some invertebrates. *Q. J. M. S.* 25 : 469–490.

———. 1889. Contributions to animal chromatology. *Q. J. M. S.* 30 : 51–96.

Makino, S. 1951. *An Atlas of the Chromosome Numbers in Animals.* Echinoidea p. 43–46. Iowa State College Press.

——— and Niiyama, H. 1947. A study of chromosomes in Echinoderms. *J. Fac. Sci. Hokkaido Univ.*, Ser. VI, Zool. 9 : 225–232.

Malm, M. and Wachtmeister, L. 1950. A comparison between the potassium content of unfertilized and fertilized sea urchin eggs. *Arkiv f. Kemi* 2 : 443–449.

Mangold, E. 1909. Sinnesphysiologische Studien an Echinodermen. Ihre Reak-

tionen auf Licht und Schatten. *Zeits. f. Allgem. Physiol.* 9 : 112–146.

MARINE BIOLOGICAL ASSOCIATION. 1931. *Plymouth Marine Fauna.* 2nd ed. Plymouth.

MARION, A. F. 1873. Reproductions hybrides d'Échinodermes. *C. R. Acad. Sci. Paris* 76 : 963–966.

MARK, E. L. 1881. Maturation, fecundation and segmentation of Limax campestris, Binney. *Bull. Mus. Comp. Zool. Harvard* 6 : 175–625, Part II, no. 12.

MARSHAK, A. 1949. a. Evidence for activity of desoxyribonuclease in nuclear fusion and mitosis by the use of d-usnic acid. *Biol. Bull.* 97 : 223 (Abstract).

———. 1949. b. Recovery from ultraviolet light-induced delay in cleavage of Arbacia eggs by irradiation with visible light. *Biol. Bull.* 97 : 244 (Abstract).

———. 1949. c. Recovery from ultraviolet light-induced delay in cleavage of Arbacia eggs by irradiation with visible light. *Biol. Bull.* 97 : 315–322.

——— and FAGER, J. 1950. Prevention of nuclear fusion and mitosis and inhibition of desoxyribonuclease by d-usnic acid. *J. Cell. and Comp. Physiol.* 35 : 317–329.

——— and HARTING, J. 1948. Inhibition of cleavage and P^{32} uptake in Arbacia by d-usnic acid. *J. Cell. and Comp. Physiol.* 31 : 321–325.

——— and MARSHAK, C. 1953. Desoxyribonucleic acid in Arbacia eggs. *Exp. Cell Res.* 5 : 288–300.

——— and VOGEL, H. J. 1951. Microdetermination of purines and pyrimidines in biological material. *J. Biol. Chem.* 189 : 597–605.

MARSLAND, D. A. 1938. The effect of high hydrostatic pressure upon cell division in Arbacia eggs. *J. Cell. and Comp. Physiol.* 12 : 57–70.

———. 1939. The mechanism of cell division. Hydrostatic pressure effects upon dividing egg cells. *J. Cell. and Comp. Physiol.* 13 : 15–22.

———. 1942. Protoplasmic streaming in relation to gel structure in the cytoplasm. *Structure of Protoplasm;* ed. by Wm. Seifriz, Iowa State College, Ames, Iowa: 127–161.

———. 1943. Quieting Paramoecium for the elementary student. *Science* 98: 414.

———. 1948. The effects of pressure on the insemination reaction of Arbacia eggs. *Biol. Bull.* 95 : 251–252 (Abstract).

———. 1950. The mechanisms of cell division; temperature-pressure experiments on the cleaving eggs of Arbacia punctulata. *J. Cell. and Comp. Physiol.* 36 : 205–227.

———. 1951. The action of hydrostatic pressure on cell division. *Ann. N.Y. Acad. Sci.* 51 : 1327–1335.

MARSLAND, D. A. and LANDAU, J. 1950. Cell division: a temperature-pressure study of the cleavage potential in the eggs of the sand dollar (Echinarachnius parma). *Anat. Rec.* 108 : 574–575 (Abstract).

———, ——— and ZIMMERMAN, A. 1953. Adenosine triphosphate as an energy source in cell division; pressuretemperature experiments on the cleaving eggs of Arbacia and Chaetopterus. *Biol. Bull.* 105 : 366–367 (Abstract).

MARX, W. 1929. Über sekundäre Geschlechtsmerkmale bei Psammechinus miliaris und Echinocyamus pusillus. *Zool. Anz.* 80 : 331–335.

MASING, E. 1910. Über das Verhalten des Nucleinsäure bei der Furchung des Seeigeleies. *Hoppe-Seyler's Zeits. Physiol. Chem.* 67 : 161–173.

———. 1914. Bemerkungen zu der Arbeit von T. B. Robertson and H. Wasteneys "On the changes in lecithin content which accompany the development of sea urchin eggs." *Arch. f. Entwick.* 40 : 666–667.

MATHEWS, A. P. 1897. Zur Chemie der Spermatozoen. *Hoppe-Seyler's Zeit. f. Physiol. Chem.* 23 : 399–411.

———. 1900. Some ways of causing mitotic division in unfertilized Arbacia eggs. *Am. J. Physiol.* 4 : 343–347.

———. 1901. a. The action of pilocarpine and atropine on the embryos of the starfish and the sea urchin. *Am. J. Physiol.* 6 : 207–215.

———. 1901. b. Artificial parthenogenesis produced by mechanical agitation. *Am. J. Physiol.* 6 : 142–154.

———. 1901. c. The so-called cross fertilization of Asterias by Arbacia. *Am. J. Physiol.* 6 : 216–218.

———. 1905. The toxic and anti-toxic action of salts. *Am. J. Physiol.* 12 : 419–443.

———. 1906. a. A note on the susceptibility of segmenting Arbacia and Asterias eggs to cyanides. *Biol. Bull.* 11 : 137–140.

———. 1906. b. A note on the structure of the living protoplasm of Echinoderm eggs. *Biol. Bull.* 11 : 141–145.

———. 1907. A contribution to the chemistry of cell division, maturation, and fertilization. *Am. J. Physiol.* 18 : 89–111.

———. 1909. The influence of some amino-acids on the development of Echinoderms. *Biol. Bull.* 16 : 44–46.

———. 1913. An important chemical difference between the eggs of the sea urchin and those of the starfish. *J. Biol. Chem.* 14 : 465–467.

MATHEWS, A. P. 1915. *Physiological Chemistry.* 1st ed. Wm. Wood Co., N.Y.

——— and WHITCHER, B. R. 1903. The importance of mechanical shock in protoplasmic activity. *Am. J. Physiol.* 8 : 300–306.

MATSUI, K. 1924. Studies on the hybridization among the Echinoderms with special reference to the behavior of the chromosomes. *J. Coll. Agr. Imp. Univ. Tokyo* 7 : 211–236.

MAYOR, J. W. and DEFORREST, D. M. 1924. The relative susceptibility to x-rays of the eggs and sperm of Arbacia. *Proc. Soc. Exp. Biol. and Med.* 22 : 19–21.

MAXIA, C. 1933. Ulteriori richerche sulle radiazione "mitogenetiche." *Scritti Biol.* 8 : 141–147.

MAYOR, A. G. 1909. On the use of magnesium in stupefying marine animals. *Biol. Bull.* 17 : 341–342.

MAZIA, D. 1933. Sodium chloride cytolysis in the sea urchin egg. *Anat. Rec.* 57 : 39 Suppl. (Abstract).

———. 1937. The release of calcium in Arbacia eggs on fertilization. *J. Cell. and Comp. Physiol.* 10 : 291–304.

———. 1940. The binding of ions by the cell surface. *Cold Spring Harbor Symp.* 8 : 195–203.

———. 1941. Enzyme studies on chromosomes. *Cold Spring Harbor Symp.* 9 : 40–46.

———. 1949. a. The distribution of desoxyribonuclease in the developing embryo (Arbacia punctulata). *J. Cell. and Comp. Physiol.* 34 : 17–32.

———. 1949. b. Desoxyribonuclease and desoxyribonucleic acid in development. *Growth* 13, Supp. 5–31.

———. 1952. Physiology of the cell nucleus. *Modern Trends in Physiology and Biochemistry*; pp. 77–122.

MAZIA, D. 1953. Chemical and electron microscopic observations on the isolated mitotic apparatus. *Biol. Bull.* 105 : 355 (Abstract).

———, BLUMENTHAL, G and BENSON, E. 1948. The activity and distribution of desoxyribonuclease and phosphatases in the early development of Arbacia punctulata. *Biol. Bull.* 95 : 250–251 (Abstract).

——— and DAN, K. 1952. The isolation and biochemical characterization of the mitotic apparatus of dividing cells. *Proc. Nat. Acad. Sci.* 38 : 826–838.

——— and NEFF, R. J. 1947. Desoxyribonuclease in the gametes and developing eggs of Arbacia punctulata. *Anat. Rec.* 99 : 637 (Abstract).

MCCLENDON, J. F. 1908. The segmentation of eggs of Asterias forbesii deprived of chromatin. *Arch. f. Entwick.* 26 : 662–668.

MCCLENDON, J. F. 1909. a. Chemical studies on the effects of centrifugal force on the eggs of the sea urchin (Arbacia punctulata). *Am. J. Physiol.* 23 : 460–466.

———. 1909. b. On artificial parthenogenesis of the sea urchin egg. *Science* 30 : 454–455.

———. 1910. a. Further proofs of the increase in permeability of the sea urchin's egg to electrolytes at the beginning of development. *Science* 32 : 317–318.

———. 1910. b. On the dynamics of cell division. II. Changes in permeability of developing eggs to electrolytes. *Am. J. Physiol.* 27 : 240–275.

———. 1910. c. How could increase in permeability to electrolytes allow the development of the egg? *Proc. Soc. Exp. Biol. and Med.* 8 : 1–3.

———. 1911. The relation between the formation of the fertilization membrane and the initiation of development. *Science* 33: 387–388.

———. 1912. a. Echinochrome, a red substance in sea urchins. *J. Biol. Chem.* 11: 435–441.

———. 1912. b. The osmotic and surface tension phenomena of living elements and their physiological significance. *Biol. Bull.* 22 : 113–162.

———. 1914. a. On the nature and formation of the fertilization membrane of the Echinoderm egg. *Intern. Zeit. f. Physik.-Chem.* 1 : 163–168.

———. 1914. b. Experiments on the permeability of cells. VII. *Carnegie Inst., Washington,* Pub. no. 183 : 125–130.

———. 1916. The composition, especially the hydrogen ion concentration of sea water in relation to marine organisms. *J. Biol. Chem.* 28 : 135–152. See Carnegie Inst. Pub. No. 251, 1917.

——— and MITCHELL, P. H. 1912. How do isotonic sodium chloride solution and other parthenogenetic agents increase oxidation in the sea urchin's egg? *J. Biol. Chem.* 10 : 459–472.

MCCLUNG, C. E. 1939. Chromosome numbers in animals. *Tab. Biologicae* vol. 18 : 34.

———. 1950. *Handbook of Microscopical Technique*; 3rd ed., edited by Ruth McClung Jones. Harper & Bros., N.Y.

MCCULLOCH, D. 1952. a. Fibrous structures in the ground cytoplasm of the Arbacia egg. *J. Exp. Zool.* 119 : 47–63.

———. 1952. b. Note on the origin of the cortical granules in Arbacia punctulata eggs. *Exp. Cell Res.* 3 : 605–607.

MCCUTCHEON, M. and LUCKÉ, B. 1924. The

mechanism of vital staining with basic dyes. *J. Gen. Physiol.* 6 : 501–507.

McCutcheon, M. and Lucké, B. 1926. The kinetics of osmotic swelling in living cells. *J. Gen. Physiol.* 9 : 697–707.

——— and ———. 1927. The kinetics of exomosis of water from living cells. *J. Gen. Physiol.* 10 : 659–664.

——— and ———. 1928. The effect of certain electrolytes and non-electrolytes on permeability of living cells to water. *J. Gen. Physiol.* 12 : 129–138.

——— and ———. 1932. The effect of temperature on permeability to water of resting and of activated cells (unfertilized and fertilized eggs of Arbacia punctulata). *J. Cell and Comp. Physiol.* 2 : 11–26.

———, ——— and Hartline, H. K. 1931. The osmotic properties of living cells (eggs of Arbacia punctulata). *J. Gen. Physiol.* 14 : 393–403.

McDougall, K. D. 1943. Sessile marine invertebrates of Beaufort, N. C. *Ecol. Monogr.* 13 (3) : 321–374.

Medes, G. 1917. A study of the causes and the extent of variations in the larvae of Arbacia punctulata. *J. Morph.* 30 : 317–432.

Meltzer, S. J. 1903. Some observations on the effects of agitation upon Arbacia eggs. *Am. J. Physiol.* 9 : 245–251.

Menkin, V. 1940. Effect of leukotaxine on cellular permeability and on cleavage development. *Proc. Soc. Exp. Biol. and Med.* 44 : 588–593.

———. 1952. Effect and significance of various steroids and of ACTH (corticotropin) on the cleavage pattern of Arbacia ova. *Biol. Bull.* 103 : 305 (Abstract).

——— and Pirovane, L. A. 1949. The effect of necrosin on the cleavage for fertilized sea urchin ova. *Biol. Bull.* 97 : 244–245 (Abstract).

Mertens, R. 1935. Aus dem Leben des Seeotters. *Natur und Volk* 65 : 401–407. From Barabasch-Nikiforov, 1933 (Russian).

Metz, C. B. 1942. The inactivation of fertilizin and its conversion to the "univalent" form by x-rays and ultraviolet light. *Biol., Bull.* 82 : 446–454.

———. 1949. Agglutination of sea urchin eggs and sperm by basic proteins. *Proc. Soc. Exp. Biol. and Med.* 70 : 422–424.

——— and Donovan, J. 1951. Specific fertilizin agglutination of dead sperm. *Biol. Bull.* 101 : 202 (Abstract).

Meyerhof, O. 1911. Untersuchungen über die Wärmetönung der vitalen Oxydationsvorgänge in Eiern. *Biochem. Zeits.* 35: 246–328.

Miller, R. A. and Smith, H. B. 1931. Observations on the formation of the egg of Echinometra lucunter. *Tortugas Laboratory Vol. 27, Carnegie Inst., Washington* Pub. no. 413 : 47–52.

Millot, N. 1950. The sensitivity to light, reactions to shading, pigmentation, and color change of the sea urchin, Diadema antillarum Phillipi. *Biol. Bull.* 99 : 329–330 (Abstract).

———. 1952. Color change in the Echinoid, Diadema antillarum Phillipi. *Nature* 170: 325–326.

Minganti, A. 1953. The action of proteolytic enzymes on the vitelline membrane of Psammechinus miliaris eggs. *Exp. Cell Res.* 5 : 492–499.

Mirsky, A. E. 1936. Protein coagulation as a result of fertilization. *Science* 84 : 333–334.

——— and Ris. H. 1951. Desoxyribonucleic acid and acid content of animal cells and its evolutionary significance. *J. Gen. Physiol.* 34 : 451–462.

Mitchell, J. S. 1941. The colloidal properties of the cell; viscosity. *Tab. Biologicae* 19, part 1: 339–345.

Mitchell, P. H. and McClendon, J. F. 1911. On the increase in oxidation in the egg at the beginning of development. *Science* 34 : 281–282.

Mitchison, J. M. 1952. Cell membranes and cell division. The structure of the cell membrane. *Symp. Soc. Exp. Biol.* 6 : 105–127.

———. 1953. The thickness of the sea urchin fertilization membrane. *Exp. Cell Res.* 5 : 536–538.

——— and Swann, M. M. 1952. Optical changes in the membranes of the sea urchin egg at fertilization, mitosis and cleavage. *J. Exp. Biol.* 29 : 357–362.

——— and ———. 1953. Measurements on sea urchin eggs with an interference microscope. *Q. J. M. S.* 94 : 381–389.

Miura, Y. 1953. Effect of some antibiotics on the cleavage of Arbacia eggs. *Biol. Bull.* 105 : 378–379 (Abstract).

Miwa, M., Yamashita, and Mori, K. 1939. The action of ionizing rays on sea urchin. I–IV. *Gann* 33 : 1–12; 117–121; 316–322; 323–331.

———, ——— and ———. 1940. *Ibid.* V. The mitotic observations on the effects of roentgen rays upon unfertilized eggs and sperm. *Gann* 34 : 239–245.

———, ——— and ———. 1941. The sperm of sea urchin as a biological test object in roentgen dosimetry. *Gann* 35 : 127–132.

Molina, J. I. 1787. *Natural History of Chile.* Trans. 1809. Printed for Longman, Hurst, Rees and Orme.

MONNÉ, L. 1944. a. The induced birefringence of the jelly coat of the sea urchin eggs. *Arkiv f. Zool.* 35B, no. 3 : 1–6.

———. 1944. b. Cytoplasmic structure and cleavage pattern of the sea urchin egg. *Arkiv f. Zool.* 35A, no. 13 : 1–27.

———. 1945. Investigations into the structure of the cytoplasm. *Arkiv f. Zool.* 36A, no. 23: 1–28.

———. 1947. The action of narcotics and of hydrating and dehydrating agents on the structure of the cytoplasm. *Arkiv f. Zool.* 39A, no. 7 : 1-32.

———. 1948. Functioning of the cytoplasm. *Adv. in Enzymol.* 8 : 1–69.

——— and HÄRDE, S. 1951. On the cortical granules of the sea urchin egg. *Arkiv f. Zool.* Ser. 2, Bd. 1. no. 31 : 487–498.

——— and SLAUTTERBACK, D. B. 1950. Differential staining of various polysaccharides in sea urchin eggs. *Exp. Cell Res.* 1: 477–491.

MONROY, A. 1947. Further observations on the fine structure of the cortical layer of unfertilized and fertilized sea urchin eggs. *J. Cell. and Comp. Physiol.* 30 : 105–109.

———. 1949. On the formation of the fertilization membrane in the sea urchin Psammechinus micr. *Exp. Cell Res.* Suppl. 1 : 525–528.

———. 1950. A preliminary electrophoretic analysis of proteins and protein fractions in sea urchin eggs and their changes on fertilization. *Exp. Cell Res.* 1 : 92–104

———, MONROY-ODDO, A. and DE NICOLA, M. 1951. The carotenoid pigments during early development of the egg of the sea urchin Paracentrotus lividus. *Exper. Cell Res.* 2 : 700–702.

——— and MONTALENTI, G. 1947. Variations of the submicroscopic structure of the cortical layer of fertilized and parthenogenetic sea urchin eggs. *Biol. Bull.* 92: 151–161.

——— and ODDO, A. M. 1946. Richerche sulla fisiologia della fecondazione. I. Natura dello strato corticale dell'uovo vergine di riccio di mare. *Pub. Staz. Zool. Napoli* 20 : 46–60.

——— and ———. 1951. Solubility changes of proteins in sea urchin eggs upon fertilization. *J. Gen. Physiol.* 35 : 245–253.

——— and RUFFO, A. 1947. Hyaluronidase in sea urchin sperm. *Nature* 159 : 603–604.

——— and RUNNSTRÖM, J. 1948. Some experiments pertaining to the chemical changes occurring at the formation of the fertilization membrane of sea urchin egg. *Arkiv f. Zool.* 40A, no. 18 : 1–6.

——— and ———. 1950. Further studies on the action of a cytoplasmic component on jelly coat and membrane formation in Arbacia punctulata. *Biol. Bull.* 99 : 339 (Abstract).

MONROY, A. and RUNNSTRÖM, J. 1952. A cytoplasmic fraction acting on the surface layers of the Arbacia punctulata eggs. *Exp. Cell Res.* 3 : 10–18.

MONROY-ODDO, A. Variations in Ca and Mg contents in Arbacia eggs as a result of fertilization. *Experientia* 2 : 371–372.

MOORE, A. R. 1910. The temperature coefficient of cytolysis in the case of the unfertilized egg of the sea urchin, Strongylocentrotus purpuratus. *Quart. J. Exp. Physiol.* 3 : 257–260.

———. 1915. On the rhythmical susceptibility of developing sea urchin eggs to hypertonic sea water. *Biol. Bull.* 28 : 253–259.

———. 1917. The mechanism of cytolysis in sea urchin eggs. *J. Biol. Chem.* 28 : 475–482.

———. 1928. a. On the hyaline membrane and hyaline droplets of the fertilized egg of the sea urchin, Strongylocentrotus purpuratus. *Protoplasma* 3 : 524–530.

———. 1928. b. Photodynamic effects of eosin on the eggs of the sea urchin, Strongylocentrotus purpuratus. *Archiv. Sci. Biol.* 12 : 231–234.

———. 1929. The function of the fertilization membrane in the development of the larva of the sea urchin. *Science* 70 : 360.

———. 1930. a. Fertilization and development without membrane formation in the egg of the sea urchin Strongylocentrotus purpuratus. *Protoplasma* 9 : 9–17.

———. 1930. b. Fertilization and development without membrane formation in the egg of Dendraster excentricus. *Protoplasma* 9 : 18–24.

———. 1930. c. On the invagination of the gastrula. *Protoplasma* 9 : 25–33.

———. 1933. Is cleavage rate a function of the cytoplasm or of the nucleus? *J. Exp. Biol.* 10 : 230–236.

———. 1943. Maternal and paternal inheritance in the plutei of hybrids of the sea urchins Strongylocentrotus purpuratus and Strongylocentrotus franciscanus. *J. Exp. Zool.* 94 : 211–228.

———. 1949. a. The relation of ions to the appearance and persistence of the fertilization and hyaline membranes in the eggs of the sea urchin. *Am. Nat.* 83 : 233–247.

———. 1949. b. On the precursors of the fertilization and the hyaline membranes in the egg of the sea urchin, Strongylocentrotus purpuratus. *Biodynamica* 6 : 197–212.

Moore, A. R. 1951. a. Action of trypsin on the eggs of Dendraster excentricus. *Exp. Cell Res.* 2 : 284–287.

———. 1951. b. On the origin and nature of the membranes which result from fertilization in the eggs of Echinoderms. *Scientia* 6th Ser., 45 : 195–199.

———, Bliss, H. S. and Anderson, E. H. 1945. The effects of pyocyanine and of lithium on the development and respiration of the eggs of two Echinoderms. *J. Cell. and Comp. Physiol.* 25 : 27–35.

——— and Miller W. A. 1937. Occurrence of birefringence in fertilized egg of the sea urchin. *Proc. Soc. Exp. Biol. and Med.* 36: 835–836.

——— and Moore, M. M. 1931. Fertilization and development without membrane formation in the egg of the sea urchin (Paracentrotus lividus). *Arch. de Biol.* 42: 375–388.

Moore, B., Roaf, E. H. and Whitley, E. 1905. On the effects of alkalis and acids and of alkaline and acid salts upon growth and cell division in the fertilized eggs of Echinus esculentus. A study in the relationship to the causation of malignant disease. *Proc. Roy. Soc. London* B 77 : 102–136.

Moore, C. R. 1916. On the superposition of fertilization on parthenogenesis. *Biol. Bull.* 31 : 137–179.

———. 1917. On the capacity for fertilization after initiation of development. *Biol. Bull.* 33 : 258–295.

Moore, H. B. 1932. A hermaphrodite sea urchin. *Nature* 130 : 58.

———. 1934. A comparison of the biology of Echinus esculentus in different habitats. Part I. *J. Marine Biol. Assoc.* 19: 869–881.

———. 1935. a. *Ibid.* Part II. *Ibid.* 20: 109–128.

———. 1935. b. A case of hermaphroditism and viviparity in Echinocardium cordatum. *J. Marine Biol. Assoc.* 20 : 103–107.

———. 1937. A comparison of the biology of Echinus esculentus in different habitats. Part III. *J. Marine Biol. Assoc.* 21 : 711–719.

——— and Moore, D. M. 1946. Preglacial history of Bermuda. *Bull. Geol. Soc. Amer.* 57 : 207–222.

Morgan, T. H. 1893. Experimental studies on Echinoderm eggs. *Anat. Anz.* 9 : 141–152.

———. 1895. a. The formation of one embryo from two blastulae. *Arch. f. Entwick.* 2 : 65–71.

———. 1895. b. A study of a variation in cleavage. *Arch. f. Entwick.* 2 : 72–80.

Morgan, T. H. 1895. c. Studies of the "partial" larvae of Sphaerechinus. *Arch. f. Entwick.* 2 : 81–126.

———. 1895. d. Experimental studies of the blastula and gastrula stages of Echinus. *Arch. f. Entwick.* 2 : 257–267.

———. 1895. e. The fertilization of nonnucleate fragments of Echinoderm eggs. *Arch. f. Entwick.* 2 : 268–280.

———. 1896. The production of artificial astropheres. *Arch. f. Entwick.* 3 : 339–361.

———. 1898. The effect of salt solutions on unfertilized eggs of Arbacia. *Science* 7: 222–223.

———. 1899 (June). The action of salt solutions on the unfertilized and fertilized eggs of Arbacia, and of other animals. *Arch. f. Entwick.* 8 : 448–539.

———. 1900. a. The effect of strychnine on the unfertilized eggs of the sea urchin. *Science* 11 : 178–190.

———. 1900. b. Further studies on the action of salt solutions and of other agents on the eggs of Arbacia. *Arch. f. Entwick.* 10 : 489–524.

———. 1908. The location of embryo forming regions in the egg. *Science* 28 : 287–288.

———. 1909. The effects produced by centrifuging eggs before and during development. *Anat. Record* 3 : 155–161.

———. 1927. *Experimental Embryology.* Columbia Univ. Press.

——— and Lyon, E. P., 1907. The relation of the substances of the egg, separated by a strong centrifugal force, to the location of the embryo. *Arch. f. Entwick.* 24 : 147–159.

——— and Spooner, G. B. 1909. The polarity of the centrifuged egg. *Arch. f. Entwick.* 28 : 104–117.

Morse, M. 1912. Artificial parthenogenesis and hybridization in the eggs of certain invertebrates. *J. Exp. Zool.* 13 : 471–496.

Mortensen, Th. 1898. Die Echinodermen Larven der Plankton-Expedition. *Ergeb. Plankton Exp. d. Humboli-Stiftung* Bd. 2 J : 1–118.

———. 1910. On some West Indian Echinoids. *Smithsonian Inst. U.S. National Mus. Bull.* 74 : 1–28.

———. 1913. a. Echinological notes. V. The phylogeny of the Echini. A critical essay. *Vid. Medd. Dansk. Naturh. Foren.* 65 : 211–240.

———. 1913. b. Die Echiniden des Mittelmeeres. *Mitt. Zool. Sta. Neapel.* 21 : 1–40.

———. 1921. *Studies of the Development and Larval Forms of Echinoderms.* Copenhagen.

———. 1927. a. *Handbook of the Echinoderms of the British Isles.* Oxford Univ. Press.

MORTENSEN, TH. 1927. b. On the postlarval development of some Cidarids. *Kgl. Dansk. Vidensk. Selsk. Skr. Naturv. og Math.*, Ser. 8, vol. 11, no. 5 : 367–387.

———. 1928. Bothriocidaris and the origin of the Echinoids. *Vid. Medd. Dansk. Naturk. Foren.* 86 : 93–122.

———, 1928–1951. *A Monograph of the Echinoidea* I–V; text and plates, 16 vols. Copenhagen. (Arbacia punctulata in vol. II p. 573–575; 1935).

———. 1930. Bothriocidaris and the ancestry of Echinoids. A reply. *Vid. Medd. Dansk. Naturh. Foren.* 90 : 313–352.

———. 1931. Contributions to the study of the development and larval forms of Echinoderms. I–II. *Kgl. Dansk. Vidensk. Selsk. Skr. Naturv. og Math.*, Ser. 9, Vol. 4; no. 1.

———. 1935. *A Monograph of the Echinoidea. II.* Copenhagen and London. Oxford Univ. Press. Arbacia punctulata p. 573–575.

———. 1937. Contributions to the study of the development and larval forms of Echinoderms. III. *Kgl. Dansk. Vidensk. Selsk. Skr. Naturv. og Math.* Ser. 9, vol. 7, no. 1.

———. 1938. Contributions to the study of the development and larval forms of Echinoderms. IV. *Ibid.* vol. 7, no. 3.

———. 1940. *A Monograph of the Echinoidea.* M III, 1. Copenhagen.

———. 1943. *A Monograph of the Echinoidea.* M III, 2, 3. Copenhagen.

MORTINUS, JOHN. 1912. *The Natural History of Northhamptonshire.* London.

MOSER, F. 1937. The cortical response of Arbacia punctulata eggs to direct current. *Biol. Bull.* 73 : 389 (Abstract).

———. 1939. a. Studies on a cortical layer response to stimulating agents in the Arbacia egg. I. Response to insemination. *J. Exp. Zool.* 80 : 423–445.

———. 1939. b. *Ibid.* II. Response to chemical and physical agents. *J. Exp. Zool.* 80 : 447–471.

———. 1940. *Ibid.* III. Response to nonelectrolytes. *Biol. Bull.* 78 : 68–79.

MOTOMURA, I. 1934. a. On the mechanism of fertilization and development without membrane formation in the sea urchin egg, with notes on a new method of artificial parthenogenesis. *Sci. Rep. Tohoku Imp. Univ.* 4th ser. 9 : 33–45.

———. 1934. b. Über die Exogastrulabildung beim Seeigelkeim durch auxin, glykogen und $KClO_3$. *Sci. Rep. Tohuku Imp. Univ.* 4th Ser. 9 : 123–130.

———. 1935. Determination of the embryonic axis in the eggs of Amphibia and Echinoderms. *Sci. Rep. Tohoku Imp. Univ.* 4th Ser. 10 : 211–245.

MOTOMURA, I. 1936. Notes on the cytoplasmic structure of the egg of a sea urchin Strongylocentrotus pulcherrimus. *Zool. Mag.* 48 : 753–758.

———. 1941. a. The sexual character of the sea urchin, Strongylocentrotus pulcherrimus A. Agassiz. *Sci. Rep. Tohoku Imp. Univ.* 4th Ser. 16 ; 431.

———. 1941. b. Materials of the fertilization membrane in the eggs of Echinoderms. *Sci. Rep. Tohoku Imp. Univ.* 4th Ser. 16 : 345–363.

———. 1950. b. On the secretion of fertilizin in the eggs of a sea urchin Strongylocentrotus pulcherrimus (A. Agassiz). *Sci. Rep. Tohoku Univ.* 4th Ser. 18 : 554–560.

———. 1950. c. On a new factor for the toughening of the fertilization membrane of sea urchins. *Sci. Rep. Tohoku Univ.* 4th Ser. 18 : 561–570.

MOURSON, J. and SCHLAGDENHAUFFEN, F. 1882. Nouvelles recherches chimiques et physiologiques sur quelques liquides organiques (eau d'oursin, etc.). *C. R. Acad. Sci. Paris.* 95 : 791–794.

MUDD, E. B. H., MUDD, S. and KELTCH, A. K. 1929. Effect of Echinid egg-waters on the surface potential difference of the sperm. *Proc. Soc. Exp. Biol. and Med.* 26: 392–394.

MULLER, H. J. 1929. The gene as the basis of life. *Proc. Intern. Cong. Plant Sci.* 1: 897–921.

MÜLLER, J. 1846–1855. Nine articles on Echinoderms, mostly metamorphosis. *Abh. König. Akad. Wiss. Berlin.*

———. 1846. a. Bericht über einige neue Thierformen der Nordsee. *Arch. f. Anat. und Physiol.* 1846. : 101–110.

——— 1846 b Über die Larven und die Metamorphose der Ophiuren und Seeigel. *Abh. König. Akad. Wiss. Berlin* 1846: 273–312.

———. 1853. Über den allgemeinen Plan in der Entwicklung der Echinodermen. *Abh. König. Akad. Wiss. Berlin* 1853. 123–219.

———. 1854. Über die Gattungen der Seeigellarven. (Siebente Abhandlung über die Metamorphose der Echinodermen). *Abh. König. Akad. Wiss. Berlin* 1854 : 1–55.

NAVEZ, A. E. 1938. Indophenoloxidase in Arbacia eggs and the Nadi reaction. *Biol. Bull.* 75 : 357 (Abstract).

———. 1939. Fatty acid compounds in the unfertilized egg of Arbacia punctulata. *Biol. Bull.* 77 : 323 (Abstract).

——— and DUBOIS, A. B. 1940. Fatty acid

compounds in the unfertilized egg of Arbacia punctulata. *Biol. Bull.* 79 : 354–355.

Navez, A. E. and Harvey, E. B. 1935. Indophenol oxidase activity in intact and fragmented Arbacia eggs. *Biol. Bull.* 69 : 342 (Abstract).

Nebel, B. R. 1937. Cytological observations on colchicine. *Biol. Bull.* 73 : 351–352 (Abstract).

———, Harvey, E. B. and Hollaender, A. 1937. The cytology of Arbacia punctulata activated by monochromatic ultraviolet radiation. *Biol. Bull.* 73 : 365–366 (Abstract).

——— and Ruttle, M. L. 1938. The cytological and genetical significance of colchicine. *J. Heredity* 29 : 3–9.

Needham, J. 1931. *Chemical Embryology.* Cambridge Univ. Press.

———. 1942. *Biochemistry and Morphogenesis.* Cambridge Univ. Press.

——— and Moore, A. R. 1929. Hermaphroditism in Dendraster. *Science* 70 : 357.

——— and Needham, D. M. 1926. a. The hydrogenion concentration and oxidation reduction potential of the cell interior before and after cleavage. A micro-injection study on marine eggs. *Proc. Roy. Soc. London* B 99 : 173–199.

——— and ———. 1926. b. The oxidation-reduction potential of protoplasm: A review. *Protoplasma* 1 : 255–294.

——— and ———. 1930. On phosphorus metabolism in embryonic life. I. Invertebrate eggs. *J. Exp. Biol.* 7 : 317–348.

——— and ———. 1940. A note on the biochemistry of embryonic determination in Echinoderms. *J. Exp. Biol.* 17 : 147–152.

Neefs, Y. 1937. Sur divers cas d'hermaphrodisme fonctionnel chez l'oursin Strongylocentrotus lividus. *C. R. Acad. Sci. Paris* 204 : 900–902.

———. 1952. Sur le cycle sexuel de Sphaerechinus granularis L. *C. R. Acad. Sci. Paris* 234 : 2233–2235.

———. 1953. Divers cas d'hermaphrodisme chez l'oursin Arbacia lixula L. *Bull. Biol. de la France et de la Belgique* 87 : 461–468.

Neff, R. J. 1953. Isolation of midpieces from the sperm of Arbacia punctulata and observations on the role of the midpiece in sperm motility. *Biol. Bull.* 105 : 367–368 (Abstract).

Nelson, C. and Brooks, S. C. 1933. Effect of infrared on subsequent fertilization of the eggs of certain marine invertebrates. *Proc. Soc. Exper. Biol. and Med.* 30 : 1007–1009.

Nelson, L. 1948. Usnic acid, an antibiotic, and sperm metabolism. *Biol. Bull.* 95: 286–287 (Abstract).

Nelson, L. 1949 Sulfhydryl inhibitors and a seminal fluid factor in sperm respiration. *Biol. Bull.* 97 : 259–260 (Abstract).

Noll, F. C. 1881. Mein Seewasser-Zimmeraquarium. *Der Zoologische Garten* 221 : 138–177.

Norman, W. W. 1896. Segmentation of the nucleus without segmentation of the protoplasm. *Arch. f. Entwick.* 3 : 106–126.

Norris, C. H. 1939. The tension at the surface, and other physical properties of the nucleated erythrocyte. *J. Cell. and Comp. Physiol.* 14 : 117–133.

Northrop, J. H. 1927. Kinetics of the swelling of cells and tissues. *J. Gen. Physiol.* 11 : 43–56.

———. 1947. Action of papain and ficin on tadpoles and Arbacia eggs. *J. Gen. Physiol.* 30 : 375–376.

Nümann, W. 1933. Untersuchungen der Skelette an Varianten, Bastarden und Chimären von regulären und irregulären Seeigeln. *Zeit. f. induk. Abstamm. u. Vererbungsl.* 65 : 447–522.

Oddo, A. M. and Esposito, M. 1951. Changes in the potassium content of sea urchin eggs on fertilization. *J. Gen. Physiol.* 34 : 285–293.

Öhman, L. O. 1940. Über die Veränderung des respiratorischen Quotienten während der Frühentwicklung des Seeigeleies. *Arkiv f. Zool.* 32A, No. 15 : 1–9.

———. 1945. On the lipids of the sea urchin egg. *Arkiv f. Zool.* 36A, no. 7 : 1–95.

Ohshima, H. 1921. Inhibitory effect of dermal secretion of the sea urchin upon the fertilizability of the egg. *Science* 54: 578–580.

Onoda, K. 1931. Notes on the development of Heliocidaris crassispina with special reference to the structure of the larval body. *Mem. Coll. Sci. Kyoto Imp. Univ.* Ser. B. 7; 103–134.

———. 1936. Notes on the development of some Japanese Echinoids with special reference to the structure of the larval body. *Jap. J. Zool.* 6 : 637–654.

Örström, A. 1932. a. Zur Analyse der Atmungssteigerung bei der Befruchtung des Seeigeleies auf der Grundlage von Versuchen über Oxidation und Reduktion von Dimethylparaphenylendiamin in der Eizelle. *Protoplasma* 15 : 566–589.

———. 1932. b. Über die Wirkung KCN-haltiger hypertonischer Lösungen auf den Stoffwechsel und die Entwicklungserregung des Seeigeleies. *Arkiv f. Zool.* 24 B, no. 7 : 1–5.

Örström, A. and Lindberg, O. 1940. Über den Kohlen-hydratstoffwechsel bei der Befruchtung des Seeigeleies. *Enzymologia* 8: 367–84.

——— und Örström, M. 1942. Über die Bindung von Kalzium in Ei und Larve von Paracentrotus lividus. *Protoplasma* 36: 475–490.

Orton, J. H. 1920. Sea temperature and breeding in marine animals. *J. Marine Biol. Assoc.* 12 : 339–366.

———. 1929. On the occurrence of Echinus esculentus on the foreshore in the British Isles. *J. Marine Biol. Assoc.* 16: 289–296.

Osborn, H. L. 1898. A case of variation in the number of ambulacral systems of Arbacia punctulata. *Am. Nat.* 32 : 259–261.

———. 1901. Variations in the apical plates of Arbacia punctulata from Woods Hole, Mass. *Science* 13 : 938–940.

Otter, G. W. 1932. Rock-burrowing Echinoids. *Biol. Rev.* 7 : 89–107.

Packard, C. 1915. The effects of the beta and gamma rays of radium on protoplasm. *J. Exp. Zool.* 19 : 323–353.

———. 1916. The effect of radium radiations on the rate of cell division. *J. Exp. Zool.* 21 : 199–212.

Page, I. H. 1923. Asteriasterol—A new sterol from the starfish and the sterols of certain other marine echinoderms. *J. Biol. Chem.* 57 : 471–476.

———. 1924. Observations on the theory of tetany. *Proc. Soc. Exp. Biol. and Med.* 22 : 117–118.

———. 1927. a. The oils of the sea urchin and starfish egg. *Biol. Bull.* 52 : 164–167.

———. 1927. b. The electrolytic content of the sea urchin and starfish egg. *Biol. Bull.* 52 : 168–172.

———. 1927. c. The composition of Woods Hole sea water. *Biol. Bull.* 52 : 161–163.

———. 1928. Further observations on the chemical composition of Woods Hole sea water. The chlorine content and salt analysis. *Biol. Bull.* 55 : 449–452.

———. 1929. a. Rhythmic susceptibility changes in the cleaving Arbacia egg. *J. Exp. Biol.* 6 : 219–228.

———. 1929. b. The toxicity of monovalent and divalent cations for sea urchin eggs. *Biol. Bull.* 57 : 449–457.

——— and Clowes, G. H. A. 1922. Cytolysis and protoplasmic structure. I. Resistance reversal phenomena in saponin and hypotonic cytolysis. *Am. J. Physiol.* 63 : 117–126.

———, Shonle, H. A. and Clowes, G. H. A. 1933. The relation of interfacial tension to cytolysis of sea urchin eggs by soaps. *Protoplasma* 19 : 213–227.

Painter, T. S. 1915. An experimental study in cleavage. *J. Exp. Zool.* 18 : 299–323.

———. 1916. Contributions to the study of cell mechanics. I. Spiral asters. *J. Exp. Zool.* 20 : 509–527.

———. 1918. Contributions to the study of cell mechanics. II. Monaster eggs and narcotized eggs. *J. Exp. Zool.* 24 : 445–497.

Palmer, L. 1935. The shedding reaction of Arbacia punctulata. *Biol. Bull.* 69 : 336 (Abstract).

———. 1937. The shedding reaction in Arbacia punctulata. *Physiol. Zool.* 10 : 352–367.

Pandit, C. G. and Chambers, R. 1932. Intracellular hydrion-concentration studies. IX. The pH of the egg of the sea urchin, Arbacia punctulata. *J. Cell. and Comp. Physiol.* 2 : 243–249.

Pantin, C. F. A. 1931. The origin of the composition of the body fluids in animals. *Biol. Rev.* 6 : 459–482.

Parker, G. H. 1922. The geotropism of the sea urchin Centrechinus. *Biol. Bull.* 43: 374–383.

———. 1927. Locomotion and righting movements in Echinoderms, especially in Echinarachnius. *Am. J. Psychol.* 39 : 167–180.

———. 1931. The color changes in the sea urchin Arbacia. *Proc. Nat. Acad. Sci.* 17: 594–596.

———. 1932. On certain feeding habits of the sea urchin Arbacia. *Am. Nat.* 66: 95–96.

———. 1936. Direction and means of locomotion in the regular sea urchin Lytechinus. *Mém. Musée Roy. d'Hist. Nat. Belgique.* Ser. 2, Fasc. 3 : 197–208.

——— and Van Alstyne, M. A. 1932. Locomotor organs of Echinarachnius parma. *Biol. Bull.* 62 : 195–200.

Parpart, A. K. 1941. Lipo-protein complexes in the egg of Arbacia. *Biol. Bull.* 81 : 296 (Abstract).

———. 1949. (Editor) *The Chemistry and Physiology of Growth.* Princeton Univ. Press.

———. 1953. The motion of echinochrome granules in the unfertilized egg of Arbacia punctulata. *Biol. Bull.* 105 : 368 (Abstract).

——— and Ballentine, R. 1952. Molecular anatomy of the red cell plasma membrane. *Barron's Modern Trends*: 135–148.

——— and Laris, P. C. 1954. Where is the plasma membrane of the unfertilized egg

of Arbacia punctulata? *Biol. Bull.* 107: 301–302 (Abstract).

Paspaleff, G. 1927. Über Protoplasmareifung bei Seeigeleiern. *Pubb. Staz. Zool. Napoli* 8 : 1–70.

Pasquini, P. 1927. Richerche di embriologia sperimentale sugli Echinodermi. I. Segmentazione atipica e successivo sviluppo delle ova di Arbacia punctulata Grey centrifugate, dopo la fecondazione. *Rend. R. Accad. Naz. Lincei*, Cl. fis mat. e nat., Ser 6 vol 5 : 353–358.

———. 1928. a. *Ibid.* II. Sul differenziamento polare delle ova di Arbacia punctulata Grey, centrifugate subito dopo la fecondazione. *Ibid.* 7 : 590–595.

———. 1928. b. Anisotropia dell'ovo fecondato di Arbacia e analisi degli effetti provocati dalle forza centrifuga sulla sua organizzazione. *Boll. Istit. Zool. R. Univ. Roma* 6 : 3–25.

Pasteels, J. and Lison, L. 1950. Recherches histophotométriques sur la teneur en acide désoxyribosenucléique au cours de mitoses somatiques. *Arch. de Biol.* 61: 445–474.

Payne, F. 1909. The separated blastomeres of centrifuged eggs of Arbacia. *Science* 30: 934–935.

Payne, N. M. 1930. Some effects of low temperature on internal structure and function in animals. *Ecology* 11 : 500–504.

Pease, D. C. 1941. Echinoderm bilateral determination in chemical concentration gradients. I. The effects of cyanide, ferricyanide, iodoacetate, picrate, dinitrophenol, urethane, iodine, malonate, etc. *J. Exp. Zool.* 86 : 381–404.

———. 1942. a. The inhibition of fertilization and membrane elevation by high hydroststic pressures. *J. Cell. and Comp. Physiol.* 19 : 131–133.

———. 1942. b. Echinoderm bilateral determination in chemical concentration gradients. II. The effects of azide, pilocarpine, pyocyanine, diamine, cysteine, glutathione and lithium. *J. Exp. Zool.* 89: 329–345.

———. 1942. c. *Ibid.* III. The effects of carbon monoxide and other gasses. *J. Exp. Zool.* 89 : 347–356.

Peebles, F. 1929, Growth regulating substances in Echinoderm larvae. *Biol. Bull.* 57 : 176–187.

Pelluet, D. 1938. A quantitative study of the changes in the cytosome of eggs of the Echinodermata during early cleavage. *Q. J. M. S.* 80 : 285–291.

Pennant, T. 1777. *British Zoology*. vol. 4. London.

Pequegnat, W. E. 1948. Inhibition of fertilization by blood extracts. *Biol. Bull.* 95: 69–82.

Pereira, J. R. 1925. On the combined toxic action of light and eosin. *J. Exp. Zool.* 42 : 257–262.

Perlzweig, W. A. and Barron, E. S. G. 1928. Lactic acid and carhohydrate in sea urchin eggs under aerobic and anaerobic conditions. *J. Biol. Chem.* 79 : 19–26.

Perry, J. H. 1941. *Chemical Engineer's Handbook*, 2nd ed. McGraw-Hill Book Co. N.Y. & London, 1950, 3rd ed.

Peter, K. 1905. Der Grad der Beschleunigung tierischer Entwicklung durch erhöhte Temperatur. *Arch. f. Entwick.* 20: 130–154.

Petrunkevitch, A. *Morphology of Invertebrate Types*. Macmillan Co.

Pfeffer, W. 1884. Locomotorische Richtungsbewegungen durch chemische Reize. *Untersuchungen aus d. Bot. Inst. Tübingen* 1 : 363–482. Trans., Pfeffers' Physiology of Plants, by A. J. Ewart, vol. 3: 343–355 (1906).

Phile, M(anuel). ca. 1275–1340. *De Animalium Proprietate; de Echino aquatili.*

Pictet, C. 1891. Recherches sur la spermatogénèse chez quelques invertébrés de la Méditerranée. *Mitth. Zool. Sta. Neapel* 10 : 75–152.

Pieri, J. B. 1899. Un nouveau ferment soluble: l'ovulase. *Arch. Zool. exp. et Gen.* 27, Notes et Rev. p. XXIX (or 3rd Ser. t. 7).

Pliny (Caius Plinius Secundus). A. D. 23–79. *Natural History*, trans. by J. Bostock and H. T. Riley 1890–1900, in Bohn's Classical Library, 6 vols. London, and N.Y.

Plough, H. H. 1927. Defective pluteus larvae from isolated blastomeres of Arbacia and Echinarachnius. *Biol. Bull.* 52 : 373–393.

———. 1929. Determination of skeleton forming material at the time of the first cleavage in the eggs of Echinus and Paracentrotus. *Arch. f. Entwick.* 115 : 380–395.

Polowzow, W. 1924. Über die Wirkung der Alkoholnarkose auf die Entwickung der Seeigeleier. *Arch. f. Mikros. Anat. u. Entwick.* 103 : 1–67.

Popa, G. T. 1927. The distribution of substances in the spermatozoon (Arbacia and Nereis). *Biol. Bull.* 52 : 238–257.

Pouchet, G. and Chabry, L. 1889. a. Sur le développement des larves d'oursin dans l'eau de mer privée de chaux. *C. R. Soc. Biol. Paris* 41 : 17–20.

——— and ———. 1889. b. De la production des larves monstrueuses d'oursin, par privation de chaux. *C. R. Acad. Sci. Paris* 108 : 196–198.

PRENANT, M. 1927. Les formes minéralogiques du calcaire chez les êtres vivants, et la problème de leur déterminisme. *Biol. Rev.* 2 : 365–393.

PRINCE, E. E. 1913. Some rare cases of albinism in animals. *Ottawa Naturalist* 27 : 122–127.

PROUHO, H. 1887. Recherches sur le Dorocidaris papillata et quelques autres échinides de la Méditerranée. *Arch. Zool. exper. et gén.* Sér. II t. 5 : 213–380.

QUATREFAGES, A. DE. 1850. Mémoire sur la cavité générale du corps des invertebrés. *Annal. des Sci. Nat.* Ser. III 14 : 302–320.

RANZI, S. and FALKENHEIM, M. 1937. Ricerche sulle basi fisiologische della determinazione nell'embrione degli Echinodermi. *Pubb. Staz. Zool. Napoli* 16 : 436–458.

RAPKINE, L. 1931. Sur les processus chimiques au cours de la division cellulaire. *Ann. Physiol. et Physicochim. Biol.* 7 : 382–418.

——— and PRENANT, M. 1925. Reaction du liquide blastocœlien chez le pluteus d'oursin dans la première phase du développement. *C. R. Acad. Sci. Paris* 181 : 1099–1011.

——— and WURMSER, R. 1926. Sur le potentiel de réduction du noyau et les oxidations cellulaires. *C. R. Soc. Biol. Paris* 94: 989–990.

RÉAUMUR, R. A. F. de. 1712. Observations sur le mouvement progressif de quelques coquillages de mer, sur celui des hérissons de mer et sur celui d'une espèce d'étoile. *Mem. de l'Acad. roy. des Sci. de Paris* (Math. et Phys.): 115–145.

REDFIELD, A. C. 1948. Characteristics of sea water. *Corrosion Handbook*, H. H.Uhlig, ed.

REED, E. A. 1943. Unilateral membrane formation in the sea urchin egg treated with ultraviolet light. *Anat. Rec.* 87 : 467 (Abstract).

———. 1948. Ultraviclet light and permeability of sea urchin eggs. *J. Cell. and Comp. Physiol.* 31 : 261–280.

REID, W. M. 1950. Echinodermata in *Brown's Selected Imvertebrate Types*, pub. by Wiley and Sons, N.Y. Arbacia punctulata pp. 528–540.

REISS, P. 1925. Sur l'action comparée des rayons X et du radium sur le noyau et le cytoplasme. *C. R. Soc. Biol. Paris.* 92: 1403–1406.

———. 1926. *Le pH intérieur cellulaire.* Presses Univ. de France, Paris.

RETZIUS, G. 1910. Zur Kenntnis der Spermien der Echinodermen. *Biol. Untersuch.* 15 : 55–62.

REVERBERI, G. 1940. Su due casi di ermafroditismo in Arbacia pustulosa. *Boll. di Zool.* 11 : 11–15.

———. 1947. Ancora sull' ermafroditismo nei ricci di mare. *Boll. di Zool.* 14 : 15–23.

RICHARDS, A. 1915. Experiments on x-radiation as the cause of permeability changes. *Am. J. Physiol.* 36 : 400–417.

——— and WOODWARD, A. E. 1915. Note on the effect of x-radiation on fertilizin. *Biol. Bull.* 28 : 140–147.

RICKETTS, E. F. and CALVIN, J. 1948. *Between Pacific Tides.* Stanford Univ. Press.

RIESER, P. 1950. The resistance of cell membranes to internal pressure. *Physiol. Zool.* 23 : 199–208.

ROBBIE, W. A. 1946. a. The quantitative control of cyanide in manumetric experimentation. *J. Cell. and Comp. Physiol.* 27: 181–209.

———. 1946. b. The effect of cyanide on the oxygen consumption and cleavage of the sea urchin egg. *J. Cell. and Comp. Physiol.* 28 : 305–324.

———. 1948. Some correlations between development and respiration in the sand dollar egg, as shown by cyanide inhibition studies. *J. Gen. Physiol.* 31 : 217–232.

———. 1949. Respiration of the tissues of some invertebrates and its inhibition by cyanide. *J. Gen. Physiol.* 32 : 655–670.

ROBERTSON, J. D. 1939. The inorganic composition of the body fluids of three marine invertebrates. *J. Exp. Biol.* 16 : 387–397.

———. 1941. The function and metabolism of calcium in the Invertebrata. *Biol. Rev.* 16 : 106–133.

——— and WEBB, D. A. 1939. The microestimation of sodium, potassium, calcium, magnesium, chloride, and sulphate in sea water and the body fluids of marine animals. *J. Exp. Biol.* 16 : 155–177.

ROBERTSON, T. B. and WASTENEYS, H. 1913. On the changes in lecithin-content which accompany the development of sea urchin eggs. *Arch. f. Entwick.* 37 : 485–496.

ROBINS, F. W. 1939. *The Story of the Lamp (and the Candle).* Oxford Univ. Press, London and N.Y.

ROGERS, C. G. and COLE, K. S. 1925. Heat production by the eggs of Arbacia punctulata during fertilization and early cleavage. *Biol. Bull.* 49 : 338–353.

ROGERS, E. F. and CORNMAN, I. 1951. The potency of rotenone and related compounds in inhibiting division of sea urchin eggs. *Biol. Bull.* 101 : 227–228 (Abstract).

ROMANES, G. J. 1885. *Jelly fish, Starfish and Sea Urchins.* Appleton Co., N.Y.

ROMANES, G. J. and EWART, J. C. 1881. Observations on the locomotor system of Echinodermata. *Phil. Trans. Roy. Soc. London* 172 : 829–885.

RONDELET, G. 1554. *Libri de piscibus marinis, in quibus verae piscium effigies expressae sunt.* Lugduni.

ROOT, W. S. 1930. The influence of carbon dioxide upon the oxygen consumption of Paramoecium and the egg of Arbacia. *Biol. Bull.* 59 : 48–62.

ROSA, D. 1896. I linfociti degli Oligocheti. Richerche istologiche. *Mem. R. Accad. Sci. Torino*, Ser. 2, t. 46 : 149–177.

ROTHSCHILD, Lord. 1938. The biophysics of the egg surface of Echinus esculentus during fertilization and cytolysis. *J. Exp. Biol.* 15 : 209–216.

———. 1948. a. The physiology of Echinus esculentus spermatozoa. *J. Exp. Biol.* 25: 15–21.

———. 1948. b. The physiology of sea urchin spermatozoa. Lack of movement in semen. *J. Exp. Biol.* 25 : 344–352.

———. 1948. c. The physiology of sea urchin spermatozoa. Senescence and dilution effect. *J. Exp. Biol.* 25 : 353–368.

———. 1949. The metabolism of fertilized and unfertilized sea urchin eggs. The action of light and carbon monoxide. *J. Exp. Biol.* 26 : 100–111.

———. 1950. a. Counting spermatozoa. *J. Exp. Biol.* 26 : 388–395.

———. 1950. b. The physiology of sea urchin spermatozoa. Catalase. *J. Exp. Biol.* 26 : 396–409.

———. 1950. c. A note on the catalase content of sea urchin eggs. *J. Exp. Biol.* 26: 410–411.

———. 1950. d. The respiration of sea urchin spermatozoa. *J. Exp. Biol.* 27 : 420–436.

———. 1951. a. Sea urchin spermatozoa. *Biol. Rev.* 26 : 1–27.

———. 1951. b. Sperm-egg interacting substances and metabolic changes associated with fertilization. *Biochem. Soc. Symp.* no. 7 : 40–51.

———. 1952. Spermatozoa. *Sci. Progress* 40: 1–10.

———. 1953. The fertilization reaction in the sea urchin. The induction of polyspermy by nicotine. *J. Exp. Biol.* 30: 57–67.

——— and BARNES, H. 1953. The inorganic constituents of the sea urchin egg. *J. Exp. Biol.* 30 : 534–544.

——— and CLELAND, K. W. 1952. The physiology of sea urchin spermatozoa. The nature and location of the endogenous substrate. *J. Exp. Biol.* 29 : 66–71.

ROTHSCHILD, Lord and MANN, T. 1950. Carbohydrate and adenosinetriphosphate in sea urchin semen. *Nature* 166 : 781–782.

——— and SWANN, M. M. 1949. The fertilization reaction in the sea urchin egg. A propagated response to sperm attachment. *J. Exp. Biol.* 26 : 164–176.

——— and ———. 1950. The fertilization reaction in the sea urchin egg. The effect of nicotine. *J. Exp. Biol.* 27 : 400–406.

——— and ———. 1951. The fertilization reaction in the sea urchin. The probability of a successful sperm-egg collision. *J. Exp. Biol.* 28 : 403–416.

ROWE, F. M. 1924. *Colour Index.* Soc. of Dyers and Colourists, Bradford, Yorshire.

RUBENSTEIN, B. B. and GERARD, R. W. 1934. Fertilization and the temperature coefficients of oxygen consumption in eggs of Arbacia punctulata. *J. Gen. Physiol.* 17 : 677–685.

RUDENBERG, F. H. 1953. The role of the jelly coat in the uptake of calcium by eggs of Arbacia punctulata before and after fertilization. *Exp. Cell Res.* 4 : 116–126.

RUGH, R. 1949. Effect of ionizing radiations of gametes, embryos, and the newborn. *Trans. N.Y. Acad. Sci.* Ser. II, vol.12: 55–57.

RULON, O. 1941. a. Modification of development in the sand dollar by NaCNS and Ca-free sea water. *Physiol. Zool.* 14 : 305–315.

———. 1941. b. The alteration of developmental pattern in the sand dollar by pilocarpine. *Physiol. Zool.* 14 : 461–469.

———. 1946. Modifications of sand dollar development by exposure to lithium chloride and sodium thyocyanate before and after fertilization. *Physiol. Zool.* 19 : 58–66.

———. 1948. The experimental modification of developmental patterns in Arbacia with malonic acid. *Physiol. Zool.* 21 : 100–105.

———. 1950. a. The modification of developmental patterns in the sand dollar with sodium azide. *Physiol. Zool.* 23 : 236–247.

———. 1950. b. The modification of developmental patterns in the sand dollar with thiourea. *Physiol. Zool.* 23 : 248–257.

———. 1951. The modification of developmental pattern in the sand dollar by malonic acid. *Physiol. Zool.* 24 : 85–92.

RUMPHIUS, G. E. 1705. *D'Amboinsche Rariteitkamer.* Amsterdam.

RUNNSTRÖM, J. 1925. Über den Einfluss des Kaliummangels auf das Seeigelei. *Pubb. Staz. Zool. Nap.* 6 : 1–200.

———. 1928. a. Zur experimentellen Ana-

lyse der Wirkung des Lithiums auf den Seeigelkeim. *Acta Zool.* 9 : 365–424.

RUNNSTRÖM, J. 1928. b. Struktur und Atmung bei der Entwicklungserregung des Seeigeleies. *Acta Zool.* 9 : 445–499.

———. 1928. c. Die Veränderungen der Plasmakolloide bei der Entwicklungserregung des Seeigeleies. *Protoplasma* 4: 388–514.

———. 1928. d. *Ibid.* II. *Protoplasma* 5: 201–310.

———. 1930. Atmungsmechanismus und Entwicklungserregung bei dem Seeigelei. *Protoplasma* 10 : 106–173.

———. 1932. Die Beeinflussung der Atmung und Spaltung im Seeigelei durch Dimethylparaphenylendiamin und Hydrochinon. *Protoplasma* 15 : 532–565.

———. 1933. a. Stoffwechselvorgänge während der ersten Mitose des Seeigeleies. *Protoplasma* 20 : 1–10.

———. 1935. a. On the influence of pyocyanine on the respiration of the sea urchin egg. *Biol. Bull.* 68 : 327–334.

———. 1935. b. An analysis of the action of lithium on sea urchin development. *Biol. Bull.* 68 : 378–384.

———. 1935. c. Influence of iodoacetate on activation and development of the eggs of Arbacia punctulata. *Biol. Bull.* 69: 351–355.

———. 1935. d. Acid formation in frozen and thawed Arbacia punctulata eggs and its possible bearing on the problem of activation. *Biol. Bull.* 69 : 345–350.

———. 1939. Über den Einfluss von Kupfer auf die Entwicklungserregung des Eies von Arbacia, nebst Bemerkungen über das Problem der Entwicklungserregung. *Protoplasma* 32 : 366–372.

———. 1948. a. On the action of trypsin and chymotrypsin on the unfertilized sea urchin egg. A study concerning the mechanism of formation of the fertilization membrane. *Arkiv f. Zool.* 40 A, no. 17: 1–16.

———. 1948. b. Membrane formation in different stages of cytoplasmic maturation of the sea urchin egg. *Arkiv f. Zool.* 40 A, no. 19 : 1–6.

———. 1948. c. Further studies on the formation of the fertilization membrane in the sea urchin egg. *Arkiv f. Zool.* 40 A, no. 1 : 1–19.

———. 1949. a. The mechanism of fertilization in metazoa. *Adv. in Enzymol.* 9: 241–327.

———. 1949. b. Some results and views concerning the mechanism of initiation of development in the sea urchin egg. *Pubb. Staz. Zool. Napoli* 21 : 9-21, Suppl.

RUNNSTRÖM, J. 1949. c. A survey of studies pertaining to the mechanism of activation in the sea urchin egg. *Exp. Cell Res.* Suppl. 1 : 469–474.

———. 1950–1951. The problems of fertilization as elucidated by work on sea urchins. *Harvey Lectures* 46 : 116–152.

———. 1952. a. The cell surface in relation to fertilization. *Symp. Soc. Exp. Biol.* VI: 39–88.

———. 1952. b. The cytoplasm, its structure and role in metabolism, growth and differentiation. *Barron's Modern Trends in Physiology and Biochemistry*, p. 47–76. Academic Press, N.Y.

——— and KRISZAT, G. 1950. a. On the effect of adenosine triphosphoric acid and of Ca on the cytoplasm of the egg of the sea urchin Psammechinus miliaris. *Exp. Cell Res.* 1 : 284–303.

——— and ———. 1952. a. The action of SH-reagents on the activation process in the sea urchin egg. *Arkiv f. Zool.*, Ser. 2, Bd. 4 : 165–185.

——— and ———, 1952. b. The cortical propagation of the activation impulse in the sea urchin egg. *Exp. Cell Res.* 3 : 419–426.

——— and ———. 1952. c. Animalizing action of iodosobenzoic acid in the sea urchin development. *Exp. Cell Res.* 3 : 497–499.

——— and MONNÉ, L. 1945. On some properties of the surface layers of immature and mature sea urchin eggs, especially the changes accompanying nuclear and cytoplasmic maturation. *Ark. f. Zool.* 36A, no. 18 : 1–27.

———, ——— and BROMAN, L. 1944. On some properties of the surface layers in the sea urchin egg and their changes upon activation. *Ark. f. Zool.* 35A, no. 3 : 1–32.

———, ——— and WICKLUND, E. 1946. Studies on the surface layers and the formation of the fertilization membrane in urchin eggs. *J. Colloid Sci.* 1 : 421–452.

———, TISELIUS, A. and VASSEUR, E. 1942. Zur Kenntnis der Gamonwirkungen bei Psammechinus miliaris und Echinocardium cordatum. *Arkiv f. Kemi, Mineral. och Geol.* 15A, no. 16 : 1–18.

——— and THÖRNBLOM, D. 1938. The influence of pycyanine on the animalization of the sea urchin egg induced by calcium-free sea water and sulphocyanate. *Acta Biol. Latvica* 8 : 97–115. Abstract in Protoplasma 33 : 628–629 (1939).

SAMPSON, M. M. 1922. Iso-agglutination and hetero-agglutination of spermatozoa. *Biol. Bull.* 43 : 267–284.

SAMPSON, M. M. 1926. Sperm filtrates and dialyzates. Their action on ova of the same species. *Biol. Bull.* 50 : 301–338.

SANZO, L. 1904. Trasformazione sperimentale delle uova lecitiche diffuse in uova telolecitiche. *Richer. Lab. Anat. Norm. R. Univ. Roma* 10 : 263–272.

SCHECHTER, V. 1936. Comparative hypotonic cytolysis of several types of invertebrate egg cells and the influence of age. *Biol. Bull.* 71 : 410 (Abstract).

———. 1937. Calcium reduction and the prolongation of life in the egg cells of Arbacia punctulata. *Biol. Bull.* 72 : 366–376.

SCHMIDT, G., HECHT, L. and THANNHAUSER, S. J. 1948. The behavior of the nucleic acids during the early development of the sea urchin egg (Arbacia). *J. Gen. Physiol.* 31 : 203–207.

——— and THANNHAUSER, S. J. 1945. A method for the determination of desoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. *J. Biol. Chem.* 161 : 83–89.

SCHMIDT, O. 1878. *Brehm's Tierleben.* Abt. 4, Bd. 2, Wirbellose Tiere.

SCHMITT, F. O., HALL, C. E. and JAKUS, M. A. 1943. The ultrastructure of protoplasmic fibrils. *Biol. Symp.* 10 : 261–276.

SCHÜCKING, A. 1903. Zur Physiologie der Befruchtung, Parthenogenese und Entwicklung. *Pflüger's Arch. f. ges. Physiol.* 97: 58–97.

SCHULTZ, G. 1931. *Farbstofftabellen.* 7te Auflage. Leipzig.

SCOTT, A. 1946. The effect of low temperature and of hypotonicity on the morphology of the cleavage furrow in Arbacia eggs. *Biol. Bull.* 91 : 272–287.

———. 1950. A cytological analysis of the effects of cyanide and 4,6-dinitro-orthocresol on the mitotic phases in Arbacia punctulata. *Biol. Bull.* 99 : 362–363 (Abstract).

——— and FOX, H. 1952. Cleavage synchrony in individual embryos and in populations of embryos of Arbacia punctulata. *Biol. Bull.* 103 : 288 (Abstract).

——— and POLLEN, R. 1951. The effect of calcium and temperature on furrowing in Arbacia. *Biol. Bull.* 101 : 229 (Abstract).

SEAGER, H. W. 1896. *Natural History in Shakespeare's Time.* 62 Paternoster Row, London.

SEIFRIZ, Wm. 1920. Viscosity values of protoplasm as determined by microdissection. *Bot. Gaz.* 70 : 360–386.

———. 1926. Protoplasmic papillae of Echinarachnius oocytes. *Protoplasma* 1: 1–14.

SEIFRIZ, Wm. 1929. The viscosity of protoplasm. *Bull. Nat. Res. Counc.* 69 : 229–261. Molecular Physics in relation to Biology; Report of sub-committee.

SELENKA, E. 1878. *Zoologische Studien.* I. Befruchtung des Eies von Toxopneustes variegatus. Leipzig.

SEMPER, K. 1881. *Animal Life as Affected by The Natural Conditions of Existence.* Intern. Sci. Series. Appleton, N.Y.

SHACKELL, L. F. 1911. Phosphorus metabolism during early cleavage of the Echinoderm egg. *Science* 34 : 573–576.

SHAKESPEARE, Wm. 1623. *Merry Wives of Windsor.*

———, 1623. *The Tempest.*

SHAPIRO, H. 1935. a. A case of functional hermaphroditism in the sea urchin, Arbacia punctulata, and an estimate of the sex ratio. *Am. Nat.* 69 : 286—288.

———. 1935. b. The respiration of fragments obtained by centrifuging the egg of the sea urchin, Arbacia punctulata. *J. Cell. and Comp. Physiol.* 6 : 101–116.

———. 1935. c. The validity of the centrifuge method for estimating aggregate cell volume in suspensions of the egg of the sea urchin, Arbacia punctulata. *Biol. Bull.* 68 : 363–377.

———. 1939. a. The diffusibility of water into half eggs of the sea urchin, Arbacia punctulata. *Anat. Rec.* Suppl. 75 : 142 (Abstract).

———, 1939. b. Some functional correlatives of cellular metabolism. *Cold Spring Harbor Symp.* 7 : 406–419.

———. 1940. Further studies on the metabolism of cell fragments. *Biol. Bull.* 79: 377 (Abstract).

———. 1941. Centrifugal elongation of cells, and some conditions governing the return to sphericity, and cleavage time. *J. Cell. and Comp. Physiol.* 18 : 61–78.

———. 1946. The extracellular release of echinochrome. *J. Gen. Physiol.* 29 : 267–275.

———. 1948. a. Diminution of rate of cell division by reversible dyes coincident with enhancement of oxidations. *Physiol. Zool.* 21 : 218–224.

———. 1948. b. The change in osmotically inactive fraction produced by cell activation. *J. Gen. Physiol.* 32 : 43–51.

———. 1948. c. Retardation of cell division by Vitamin C in physiological concentrations. *Biol. Bull.* 94 : 79–85.

———. 1948. d. Heat production of different cells. *Tab. Biol.* 19, part 3 : 1–29.

———. 1948. e. Do chromosomes manifest osmotic volume change? *Proc. Soc. Exp. Biol. and Med.* 67 : 180–182.

SHAPIRO, H. and DAVSON, H. 1941. Permeability of the Arbacia egg to potassium. *Biol. Bull.* 81 : 295–296 (Abstract).

SHEARER, C. 1922. a. On the oxidation processes of the Echinoderm egg during fertilization. *Proc. Roy. Soc. London* B 93: 213–229.

———. 1922. b. On the heat production and oxidation processes of the Echinoderm egg during fertilization and early development. *Proc. Roy. Soc. London* B 93 : 410–425.

——— DE MORGAN, W. and FUCHS, H. M. 1914. On the experimental hybridization of Echinoids. *Phil. Trans. Roy. Soc. London* B 204 : 255–362.

——— and LLOYD, D. J. 1913. On methods of producing artificial parthenogenesis in Echinus esculentus and the rearing of the parthenogenetic plutei through metamorphosis. *Q. J. M. S.* 58 : 523–551.

SICHEL, F. J. M. and BURTON, A. C. 1936. A kinetic method of studying surface forces in the egg of Arbacia. *Biol. Bull.* 71 : 397–398 (Abstract).

SINGER, C. 1931. *A Short History of Biology.* Oxford, Clarendon Press. Also entitled The Story of Living Things, Harper & Bros. N.Y.

SKOWRON, S. and SKOWRON, H. 1926. Les changements du rapport plasmo-nucléaire dans des œufs pas mûrs d'oursins sous l'influence de différence de la pression osmotique du milieu. *Bull. Acad. Polonaise Sci. et Lettr.*, Cl. Sci., Math. et Nat. Ser. B: 859–879.

SLOANE, HANS. 1707, vol. 1; 1725, vol. 2. *A Voyage to Jamaica etc.* London.

SMITH, A. H. and KLEIBER, M. 1950. Size and oxygen consumption in fertilized eggs. *J. Cell. and Comp. Physiol.* 35 : 131–140.

SMITH, H. W. and CLOWES, G. H. A. 1924. a. The influence of carbon dioxide on the velocity of division of marine eggs. *Am. J. Physiol.* 68 : 183–202.

——— and ———. 1924. b. The influence of hydrogen ion concentration on unfertilized Arbacia, Asterias and Chaetopterus eggs. *Biol. Bull.* 47 : 304–322.

——— and ———. 1924. c. The influence of hydrogen ion concentration on the development of normally fertilized Arbacia and Asterias eggs. *Biol. Bull.* 47 : 323–332.

——— and ———. 1924. d. The influence of hydrogen ion concentration on the fertilization process in Arbacia, Asterias and Chaetopterus eggs. *Biol. Bull.* 47 : 333–344.

Smith, M. E. and EVANS, T. C. 1940. Effects of roentgen radiation on the jelly of the Arbacia egg. II. Changes in pH of egg media. *Biol. Bull.* 79 : 362 (Abstract).

SNYDER, C. D. 1925. Egg-volume and fertilization membrane. *Biol. Bull.* 49 : 54–60.

SOCIETY OF DYERS AND COLORISTS. 1924. *Color Index.* Ed. by F. M. Rowe. Bradford, Yorkshire.

SOLANO, SISTER F. and MAZIA, D. 1953. The role of the vitelline membrane in the fragmentation of the Arbacia egg by centrifugal force. *Biol. Bull.* 105 : 384 (Abstract).

SOLLMANN, T. 1904. a. The simultaneous action of pilocarpine and atropin on the developing embryos of the sea urchin and star fish. A contribution to the study of the antagonistic action of poisons. *Am. J. Physiol.* 10 : 352–361.

———. 1904. b. Structural changes of ova in anisotonic solutions and saponin. *Am. J. Physiol.* 12 : 99–115.

SOUTHERN, R. 1915. Marine ecology. In Clare Island Survey. *Proc. R. Irish Acad.* 31, Part 67 : 1–110.

SPAULDING, E. G. 1904. The rhythm of immunity and susceptibility of fertilized sea urchin eggs to ether, to HCl, and to some salts. *Biol. Bull.* 6 : 224–240.

SPEK, J. 1918. Differenzen im Quellungszustand der Plasmakolloide als eine Ursache der Gastrulainvagination etc. *Kolloidchem. Beihefte* 9 : 259–399. (esp. 347–366).

SPIKES, J. D. 1944. Membrane formation and cleavage in unilaterally irradiated sea urchin eggs. *J. Exp. Zool.* 95 : 89–103.

———. 1949. Metabolism of sea urchin sperm. *Am. Nat.* 83 : 285–301.

SPOONER, G. B. 1911. Embryological studies with the centrifuge. *J. Exp. Zool.* 10 : 23–49.

SPRINGER, M. G. 1922. The effect upon developing eggs of extracts of embryos of the same species. *Biol. Bull.* 43 : 75–96.

STEWART, D. R. 1931. a. The permeability of the Arbacia egg to non-electrolytes. *Biol. Bull.* 60 : 152–170.

———. 1931. b. The permeability of the Arbacia egg to ammonium salts. *Biol. Bull.* 60 : 171–178.

——— and JACOBS, M. H. 1932. a. The effect of fertilization on the permeability of the eggs of Arbacia and Asterias to ethylene glycol. *J. Cell. and Comp. Physiol.* 1 : 83–92.

——— and ———. 1932. b. The permeability of the egg of Arbacia to ethylene glycol at different temperatures. *J. Cell. and Comp. Physiol.* 2 : 275–283.

——— and ———. 1936. Further studies

on the permeability of the egg of Arbacia punctulata to certain solutes and to water. *J. Cell. and Comp. Physiol.* 7 : 333–350.

Stott, F. C. 1931. The spawning of Echinus esculentus and some changes in gonad composition. *J. Exp. Biol.* 8 : 133–150.

Strittmatter, P., Keltch, A. K., Walters, C. P. and Clowes, G. H. A. 1950. Oxidative phosphorylation by a cell-free, particulate, enzyme system derived from fertilized Arbacia eggs. *Biol. Bull.* 99 : 333–334 (Abstract).

Studer, Th. 1880. Über Geschlechtsdimorphismus bei Echinodermen. *Zool. Anz.* 3 : 523–527 (a) and 543–546 (b).

Sugawara, H. 1943. a. Hatching enzyme of the sea urchin, Strongylocentrotus pulcherrimus. *J. Fac. Sci. Imp. Univ. Tokyo*, Sect. IV, 6 : 109–127.

———. 1943. b. The formation of multinucleate eggs of the sea urchin by treatment with proteolytic enzymes. *J. Fac. Sci. Imp. Univ. Tokyo*, Sect. IV, 6 : 129–139.

Sugiyama, M. 1938. a. Experimental studies on nuclear division without cytoplasmic segmentation in the sea urchin eggs. *J. Fac. Sci. Imp. Univ. Tokyo*, Sect. IV, 4: 471–487.

———. 1938. b. Further studies on the experimental formation of multinucleated eggs of sea urchin. *J. Fac. Sci. Imp. Univ. Tokyo*, Sect. IV, 4 : 489–494.

———. 1938. c. Oxygen consumption during the nuclear division without cytoplasmic segmentation in the sea urchin eggs. *J. Fac. Sci. Imp. Univ. Tokyo*, Sect. IV, 4 : 495–499.

———, 1938. d. Effect of some divalent ions upon the membrane development of sea urchin eggs. *J. Fac. Sci. Imp. Univ. Tokyo*, Sect. IV, 4 : 501–508.

———. 1938. e. Effect of crowding upon the rate of cleavage in the sea urchin egg. *Zool. Mag.* (Dobutsugaku Zasshi) 50 : 48–50.

———. 1938. f. Further studies on the development of the fertilization membrane in sea urchin eggs. *Ann. Zool. Jap.* 17 : 360–364.

———. 1938. g. Experiments on the formation of protoplasmic protrusions from sea urchin oocytes. *J. Fac. Sci. Imp. Univ. Tokyo* Sect. IV, 5 : 127–131.

———. 1947. Some experiments to refertilize the sea urchin eggs which were once fertilized. *Zool. Mag.* (Dobutsugaku Zasshi) 57 : 121–123. In Japanese with English summary. Prelim. report.

———. 1951. Re-fertilization of the fertilized eggs of the sea urchin. *Biol. Bull.* 101 : 335–344.

Sumner, J. B. and Somers, G. F. 1953. *Chemistry and Methods of Enzymes.* 3rd ed. Academic Press, N.Y.

Sumner, F. B., Osburn, R. C. and Cole, L. J. 1911, 1913. A biological survey of the waters of Woods Hole and vicinity. *Bull. Bureau of Fisheries.* Vol. 31 Part I, sect. I, Physical and zoological. Part II, sect. III, A catalogue of the marine fauna.

Swan, E. F. 1952. Regeneration of spines by sea urchins of the genus Strongylocentrotus. *Growth* 16 : 27–35.

Swann, M. M. 1951. a. Protoplasmic structure and mitosis. I. The birefringence of the metaphase spindle and asters of the living sea urchin egg. b. II. The nature and cause of birefringence changes in the sea urchin egg at anaphase. *J. Exp. Biol.* 28: 417–444.

———. 1954. Secondary sex differences in five European species of sea urchin. *Pubb. Staz. Zool. Napoli* 25 : 198–199.

Symposium on nucleic acids and nucleoproteins 1947. *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 12.

Symposium (Discussion) on the Biochemistry of nucleic acids, 1951. *J. Cell. and Comp. Physiol.* 38 : suppl. I.

Symposium on Respiratory Enzymes. 1942. Univ. Wisconsin Press.

Synposium on nucleic acid. *Symposia of the Society for Experimental Biology* I, 1947.

Tabulae Biologicae on Echinodermata
1927 IV: 108 *Chromosome numbers* (Breslau-Harnisch);
1930 VI : 490–535 *Echinodermata*, general (Grimpe), VI : 503 *Egg Size*, VI : 513 *Parthenogenesis*, VI : 514 *Hybrids*, VI : 527 *Chromosome numbers*;
1939 18 : 34 *Chromosome numbers* (Mc Clung);
1941 19: Part II : 25–27 *Impedance* (Cole), 19 : Part II: 371–389 *Permeability* (Wilbrandt);
1948 19 : Part III : 18–20 *Heat production* (Shapiro).

Tamini, E. Richerche sulla vegetativizzazione nello sviluppo dei ricci di mare. *R. Ist. Lombardo di Sci. e Let.* 76 : 363–392.

Tang, P. S. 1931. a. The oxygen tension-oxygen consumption curve of unfertilized Arbacia eggs. *Biol. Bull.* 60 : 242–244.

———. 1931. b. The rate of oxygen consumption of Asterias eggs before and after fertilization. *Biol. Bull.* 61 : 468–471.

———. 1933. On the rate of oxygen consumption by tissues and lower organisms as a function of oxygen tension. *Quart. Rev. Biol.* 8 : 260–274.

TANG, P. S. 1941. Respiration in the living cell. *Quart. Rev. Biol.* 16 : 173–189.

———. 1948. Rhythmic respiration in the sea urchin. *Nature* 162 : 189.

——— and GERARD, R. W. 1932. The oxygen tension-oxygen consumption curve of fertilized Arbacia eggs. *J. Cell. and Comp. Physiol.* 1 : 503–513.

TAU, C. C. and DE NICOLA, M. 1949. The effect of nitrogen mustard on sea urchin eggs. *Experientia* 5 : 164–166.

TCHAKHOTINE, S. 1921. a. Le rôle physiologique de l'enveloppe gelatineuse de l'œuf d'oursin. *C. R. Soc. Biol. Paris* 84 : 330–332.

———. 1921. b. Les changements de la perméabilité de l'œuf d'oursin localisés expérimentalement. *C. R. Soc. Biol. Paris* 84 : 464–466.

———. 1937. Radiations, cell permeability and colloidal changes. *Trans. Faraday Soc.* 33 : 1068–1072.

———. 1938. Cancérisation expérimentale des éléments embryonnaires, obtenue sur des larves d'oursins. *C. R. Soc. Biol. Paris* 127 : 1195–1197.

TENNENT, D. H. 1908. The chromosomes in cross-fertilized Echinoid eggs. *Biol. Bull.* 15 : 127–134.

———. 1910. a. Variation in Echinoid plutei. *J. Exp. Zool.* 9 : 657–714.

———. 1910. b. 1911. a. Echinoderm hybridization. Dept. Marine Biol. *Carnegie Inst. Washington* 3 : 117–152, Pub. no. 132.

———. 1911. b. A heterochromosome of male origin in Echinoids. *Biol. Bull.* 21: 152–154.

———. 1912. a. The behavior of the chromosomes in cross-fertilized Echinoid eggs. *J. Morph.* 23 : 17–29.

———. 1912. b. Studies in cytology. I. A further study of the chromosomes of Toxopneustes variegatus. II. The behavior of the chromosomes in Arbacia-Toxopneustes crosses. *J. Exp. Zool.* 12: 391–411

———. 1912. c. The correlation between chromosomes and particular characters in hybrid Echinoid larvae. *Am. Nat.* 46 : 68–75.

———. 1913. Echinoderm hybridization. *Science* 37 : 535–537.

———. 1922. Studies of the hybridization of Echinoids, Cidaris tribuloides. Dep. Marine Biol. Vol. 18, *Carnegie Inst. Washington*, Pub. no. 312 : 1–42.

———. 1923. Investigations on the hybridization of Echinoids conducted at the Misaki Biological Station of Tokyo Imperial University, from Apr. 24 to Aug. 16, 1923. *Carnegie Inst. Washington*, Year Book no. 22 : 169–171.

TENNENT, D. H. 1924. Specificity in fertilization. *Science* 60 : 162–164.

———. 1942. The photodynamic action of dyes on the eggs of the sea urchin, Lytechinus variegatus. *Tortugas Lab.*, vol. 35, *Carnegie Inst. Washington*, Pub. no. 539: 1–153.

———, GARDINER, M. S. and SMITH, D. E. 1931. A cytological and biochemical study of the ovaries of the sea urchin Echinometra lucunter. *Tortugas Lab.*, vol. 27 and *Carnegie Inst. Washington*, Pub. no. 413 : 1–46.

——— and ITO, T., 1941. A study of the oogenesis of Mespilia globulus (Linné). *J. Morph.* 69 : 347–404.

——, TAYLOR, C. V. and WHITAKER, D. M., 1929. An investigation on organization in a sea urchin egg. *Tortugas Lab.*, vol. 26 and *Carnegie Inst. Washington*, Pub. no. 391: 1–104.

TERENTIEVA, K. F. 1932. La composition minérale des squelettes de certaines espèces d'échinodermes contemporaines. *Trav. Lab. Biogéochim. U. R. S. S.* 2 : 45.

THÉEL, H. 1892. On the development of Echinocyamus pusillus. *Nov. Acta R. Soc. Sci. Upsala*, Ser. III, Vol. 15, VI : 1–57.

THOMPSON, D'ARCY. 1942, 1948. *On Growth and Form.* Cambridge Univ. Press. and Macmillan Co., N.Y.

———. 1947. *A Glossary of Greek Fishes.* Oxford Univ. Press.

THOMSON, C. WYVILLE. 1876–78. Notice of some peculiarities in the mode of propagation of certain Echinoderms of the southern sea. *J. Linn. Soc. Zool.* 13 : 55–79.

———. 1877. *The Voyage of the "Challenger." The Atlantic.* Macmillan Co.

THÖRNBLOM, D. 1932. Über die Veränderung der Permeabilität des Seeigeleies für Anelektrolyte bei der Befruchtung. *Arkiv. f. Zool.* 24 B, no. 4 : 1–5.

TIEDEMANN, F. F. 1816. *Anatomie der Roehren-Holothurie, des pommeranzenfarbigen Seesterns und des Stein-Seeigels.* Landshut.

TORTONESI, E. 1939. Gli Echinodermi nei loro rapporti con l'uomo; specie utili e specie dannose. *Boll. di Pesca, di Piscicol. e di Idrobiol. Rome* 15 : 302–311.

TREWAVAS, E. 1922. Note on the occurrence of Echinus esculentus above low tide mark on the Cornish coast. *J. Marine Biol. Assoc.* 12 : 833–834.

TRURNIT, H. J. 1939. Schwankungen des Energiewechsels während der Zellteilung. *Naturwiss.* 27 : 805–806.

TSUBOI, K. K. 1953. Nucleic acid and pro-

tein distribution in unfertilized and fertilized eggs of Arbacia. *Biol. Bull.* 105 : 392 (Abstract).

TUOMEY, M. and HOLMES, F. S. 1857. *Pleiocene Fossils of South Carolina.* Charleston, S.C.

TYLER, A. 1936. a. On the energetics of differentiation. III. Comparison of the temperature coefficients for cleavage and later stages in the development of the eggs of some marine animals. *Biol. Bull.* 71 : 59–81.

———. 1936. b. On the energetics of differentiation. IV. Comparison of the rates of oxygen consumption and of development at different temperatures of eggs of some marine animals. *Biol. Bull.* 71 : 82–100.

———. 1937. On the energetics of differentiation. V. Comparison of the rates of development and of oxygen consumption of tight membrane and normal echinoderm eggs. *J. Exp. Zool.* 76 : 395–406.

———. 1939. Crystalline echinochrome and and spinochrome: their failure to stimulate the respiration of eggs and sperm of Strongylocentrotus. *Proc. Nat. Acad. Sci.* 25 : 523–528.

———. 1940. a. Agglutination of sea urchin eggs by means of a substance extracted from the eggs. *Proc. Nat. Acad. Sci.* 26: 249–256.

———. 1940. b. Sperm agglutination in the keyhole limpet Megathura crenulata. *Biol. Bull.* 78 : 159–178.

———. 1941. a. Artificial parthenogenesis. *Biol. Rev.* 16 : 291–336.

———. 1941. b. The role of fertilizin in the fertilization of eggs of the sea urchin and other animals. *Biol. Bull.* 81 : 190–204.

———. 1944. Sexual dimorphism in sea urchins. *Anat. Rec.* 89 : 573 (Abstract).

———. 1946. Natural heteroagglutinins in the body fluids and seminal fluids of various invertebrates. *Biol. Bull.* 90 : 213–219.

———. 1948. Fertilization and immunity. *Physiol. Rev.* 28 : 180–219.

———. 1949. a. A simple non-injurious method for incuding repeated spawning of sea urchins and sand dollars. *The Collecting Net* 19 : 19–20.

———. 1949. b. Serological aspects of fertilization. *The Collecting Net* 19 : 6–8.

———. 1949. c. Properties of fertilizin and related substances of eggs and sperm of marine animals. *Am. Nat.* 83 : 195–219.

———. 1950. Extension of the functional life span of spermatozoa by amino acids and peptides. *Biol. Bull.* 99 : 324 (Abstract).

———. 1953. Prolongation of life-span of sea urchin spermatozoa, and improvement of the fertilization reaction, by treatment of spermatozoa and eggs with metal-chelating agents (amino acids, Versene, DEDTC, oxine, cupron). *Biol. Bull.* 104 : 224–239.

TYLER, A. and FOX, S. W. 1939. Sperm agglutination in the keyhole limpet and the sea urchin. *Science* 90 : 516–517.

——— and HUMASON, W. D. 1937. On the energetics of differentiation. VI. Comparison of the temperature coefficients of the respiratory rates of unfertilized and fertilized eggs. *Biol. Bull.* 73 : 261–279.

——— and METZ, C. B. 1954. The action of trypsin on Arbacia and Echinarachnius eggs in homologous and cross fertilization. *Biol. Bull.* 107 : 321 (Abstract).

———, RICCI, N. and HOROWITZ, N. H. 1938. The respiration and fertilizable life of Arbacia eggs under sterile and non-sterile conditions. *J. Exp. Zool.* 79 : 129–143.

——— and LORD ROTHSCHILD. 1951. Metabolism of sea urchin spermatozoa and induced anaerobic motility in solutions of amino acids. *Proc. Soc. Exp. Biol. and Med.* 76 : 52–58.

VALENTIN, G. 1841. Anatomie des Échinodermes. Anatomie du genre Echinus. *Monographies d'Échinodermes* by L. Agassiz. Neuchâtel.

VANDEL, A. 1931. *La Parthénogénèse.* Doin et Cie., Paris.

VAN DER HEYDE, H. C. 1922. *On the Physiology of Digestion, Respiration and Excretion in Echinoderms.* C. de Boer, Jr., Den Helder, Holland.

VASSEUR, E. 1948. a. Chemical studies on the jelly coat of the sea urchin egg. *Acta Chem. Scand.* 2 : 900–913.

———. 1947. The spermatozoon of the sea urchin. Echinocardium cordatum (Pennant). *Arkiv f. Zool.* 40 B no. 3 : 1–3.

———. 1949. a. Effect of calcium ions on the agglutination in Strongylocentrotus dröbachiensis Müll. *Arkiv f. Kemi* 1 : 105–116.

———. 1949. b. Effect of sea urchin jelly coat solution and calcium on the oxygen uptake of sea urchin sperm. *Arkiv f. Kemi* 1 : 393–399.

———. 1952. a. The chemistry and physiology of the jelly coat of the sea urchin egg. Independent publication, Kihlströms Tryck. A. B. Stockholm.

———. 1952. b. Geographic variation in the Norwegian sea urchins, Strongylocentrotus droebachiensis and S. pallidus. *Evolution* 6 : 87–100.

VASSEUR, E. and IMMERS, J. 1949. b. Hexosamine in the sea urchin jelly coat: a misinterpretation of the method of Elson and Morgan. *Arkiv f. Kemi* 1 : 253–256.

——— and WICKLUND, E. 1953. Correlation of some physiological properties of the jelly-coat solution of the sea urchin egg. *Arkiv f. Zool.* Ser 2, Bd. 4 : 363–366.

VELICK, S. F. 1941. The effect of centrifugation upon the oxygen consumption of Arbacia eggs. *Biol. Bull.* 81 : 303–304 (Abstract).

VENDRELEY, C. and R. 1949. Sur la teneur individuelle en acide désoxyribonucléique des gamètes d'oursins Arbacia et Paracentrotus. *C. R. Soc. Biol. Paris* 143 : 1386–1387.

VERRILL, A. E. 1907. The Bermuda Islands. Part IV Geology; Part V Coral Reefs. *Trans. Conn. Acad. Arts and Sci.* 12 : 245–348.

——— and SMITH, S. I. 1874. *Report upon the Invertebrate Animals of Vineyard Sound and Adjacent Waters.* Washington: Government Printing Office.

VIGUIER, C. 1900. a. L'hermaphroditisme et la parthénogénèse chez les Échinodermes. *C. R. Acad. Sci. Paris* 131 : 63–66.

———. 1900. b. La théorie de la fertilisation chimique des œufs, de M. Loeb. Note presented by E. Perrier. *C. R. Acad. Sci. Paris* 131 : 118–121.

VILLEE, C. A., LICHTENSTEIN, R., NATHANSON, N. and ROLANDER, B. 1950. Phosphorus metabolism in embryonic and adult invertebrate tissue. *Biol. Bull.* 99: 366 (Abstract).

———, LOWENS, M., GORDON, M., LEONARD, E. and RICH, A. 1949. The incorporation of P^{32} into the nucleoproteins and phosphoproteins of the developing sea urchin embryo. *J. Cell. and Comp. Physiol.* 33 : 93–112.

——— and VILLEE, D. T. 1952. Studies on phosphorus metabolism in sea urchin embryos. *J. Cell. and Comp. Physiol.* 40 : 57–71.

———, VILLEE, D. and LAPLACE, 1953. Nucleic acid turnover rates in Arbacia eggs and sperm. *Biol. Bull.* 105 : 386 (Abstract).

VINOGRADOV, A. P. 1953. The Elementary Chemical Composition of Marine Organisms. Trans. from Russian. *Sears Foundation for Marine Research. Memoir II,* Yale Univ. New Haven, Conn.

VLÈS, F. 1924. Recherches sur le pH intérieur cellulaire. *Arch. de Physique Biol.* 4 : 1–20.

———. 1926. Les tensions de surfaces et les déformations de l'œuf d'oursin. *Arch. de Physique Biol.* 4 : 263–284.

VLÈS, F. 1931. Notes sur la lyse électrique de l'œuf d'oursin. *Arch. de Physique Biol.* 9: 201–208.

——— et GEX, M. 1928. Recherches sur le spectre ultraviolet de l'œuf d'oursin (Paracentrotus lividus Lk.) et de ses constituants. *Arch. de Physique Biol.* 6 : 255–286.

——— et ———. 1934. Sur la structure des spectres ultraviolets de l'œuf d'oursin. Introduction à une technique de microspectrométrie ultraviolette. *Arch. de Physique Biol.* 11 : 157–190.

——— et VELLINGER, E. 1928. Recherches sur le pigment de l'œuf d'Arbacia envisagé comme indicateur de pH intracellulaire. *Bull. de l'Institut océanog. Monaco,* No. 513 : 1–16. Also Arch. de Physique Biol. 6 : 239–254.

VOGT, W. 1925. Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfarbung. *Arch. f. Entwick.* 106 : 542–610.

VON BAER, K. E. 1847. Auszug aus einem Berichte des Akademikers v. Baer, aus Triest vom 1. (13) November, 1845. *Bull. Physico-Math. Acad. Imp. Sci. St. Pétersbourg* 5 : 231–239.

VON DUNGERN, E. 1902. Neue Versuche zur Physiologie der Befruchtung. I. Die Ursachen der Specificität bei der Befruchtung. *Zeits. f. allgem. Physiol.* 1 : 34–55.

VON KNAFFL-LENZ, E. 1908. Über die Beziehungen zwischen Lipoidverflüssigung und Cytolyse. *Pfluger's Arch. f. gesam. Physiol.* 123 : 279–292.

VON MÖLLENDORFF, W. 1920. Vitale Färbungen an tierischen Zellen. *Ergeb. d. Physiol.* 18 : 141–306.

———. 1928. Methoden zu Studien über vitale Färbungen an Tierzellen. *Abderhalden Handbuch der Arbeitsmethoden,* Abt. V, Teil 2, Hälfte 1 : 97–152.

VON UBISCH, L. 1913. a. Die Anlage und Ausbildung des Skeletsystems einiger Echiniden und die Symmetrieverhältnisse von Larve und Imago. *Zeits. f. wiss. Zool.* 104 : 119–156.

———. 1913. b. Die Entwicklung von Strongylocentrotus lividus. (Echinus microtuberculatus, Arbacia pustulosa.) *Zeits. f. wiss. Zool.* 106 : 409–448.

———. 1913. c. Über das larvale Muskelsystem von Arbacia pustulosa. *Verh. Phys. Med. Ges. Würzburg.* N.F. 42 : 127–133.

———. 1925. Entwicklungsphysiologische Studien an Seeigelkeimen. *Zeit. f. wiss. Zool.* 124 : 361–381.

———. 1927. Über die Symmetrieverhältnisse von Larven und Imago bei regu-

lären und irregulären Seeigeln. *Zeits. f. wiss. Zool.* 129 : 541–566.

Von Ubisch, L. 1929. Über die Determination der larvalen Organe und der Imaginalanlage bei Seeigeln. *Arch. f. Entwick.* 117 : 80–122.

———. 1932. Untersuchungen über Formbildung. III. Ein vorwiegend spekulativer Beitrag zur Frage der Entstehung und systematischen Bedeutung der Seeigelplutei. *Arch. f. Entwick.* 127 : 216–250.

———. 1937. Die normale Skelettbildung bei Echinocyamus pusillus und Psammechinus miliaris und die Bedeutung dieser Vorgänge für die Analyse der Skelette von Keimblattchimären. *Zeits. f. wiss. Zool.* 149 : 402–476.

———. 1939. Keimblattchimärenforschung an Seeigellarven. *Biol. Rev.* 14 : 88–103.

———. 1950. Die Entwicklung der Echiniden. *Verh. Kon. Nederl. Ak. Wet. Afd. Nat.* 2nd Ser., Vol. 47, no 2 : 1–50.

———. 1954. Über Seeigelmerogone. *Pubbl. Staz. Zool. Napoli* 25 : 246–340.

Von Uexküll, J. 1896. Vergleichend sinnesphysiologische Untersuchungen. II. Der Schatten als Reiz für Centrostephanus longispinus. *Zeits. f. Biol.* 34 : 319–339.

———. 1899. Die Physiologie der Pedicellarien. *Zeits. f. Biol.* 37 : 334–403.

———. 1900. Die Physiologie des Seeigelstachels. *Zeits. f. Biol.* 39 : 73–112.

———. 1909. *Umwelt und Innenwelt der Tiere.* Berlin.

Wali enfels, K. 1943. Der Farbstoff der roten Blutzellen des Seeigels Arbacia pustulosa. *Ber. d. deutsch. chem. Gesell.* 76: 323–325.

——— and Gauhe, A. 1943. Synthese von Echinochrom A. *Ber. d. deutsch. chem. Gesell.* 76 : 325–327.

Warburg, O. 1908. Beobachtungen über die Oxydationsprozesse im Seeigelei. *Hoppe-Seyler's Zeits. f. physiol. Chem.* 57: 1–16.

———. 1910. Über die Oxidationen in lebenden Zellen nach Versuchen am Seeigelei. *Hoppe-Seyler's Zeits. f. physiol. Chem.* 66 : 305–340.

———. 1915. Notizen zur Entwicklungspshysiologie des Seeigeleies. *Pflüger's Arch. f. gesam. Physiol.* 160 : 324–332.

Wasteneys, H. 1916. The rate of oxidations in reversed artificial parthenogenesis. *J. Biol. Chem.* 24 : 281–298.

Waterman, A. J. 1932. Effects of isotonic salt solutions upon the development of the blastula of the sea urchin Paracentrotus lividus. *Arch. de Biol.* 43 : 471–519.

Waterman, A. J. 1934. Further studies of exogastrulation in the sea urchin. *Biol. Bull.* 67 : 172–181.

———. 1936. The effect of alcohol on gastrulation in Arbacia. *Biol. Bull.* 71 : 413–420.

———. 1937. Effect of salts of heavy metals on development of the sea urchin, Arbacia punctulata. *Biol. Bull.* 73 : 401–420.

———. 1938. Respiratory stimulants and gastrulation in Arbacia. *Biol. Bull.* 75: 376 (Abstract).

———. 1941. Ectodermization of the larva of Arbacia. *Biol. Bull.* 81 : 304 (Abstract).

Webb, D. A. 1937. Studies on the ultimate composition of biological material. II. Spectrographic analyses of marine invertebrates with special reference to the chemical composition of their environment. *Sci. Proc. R. Dublin Soc.* 21 : 505–539.

———. 1939. The sodium and potassium content of sea water. *J. Exp. Biol.* 16: 178–183.

Wells, P. H. and Giese, A. C. 1950. Photoreactivation of ultraviolet light injury in gametes of the sea urchin Strongylocentrotus purpuratus. *Biol. Bull.* 99 : 163–172.

Wetzel, G. 1907. Die Entwicklung des Ovarialeies und des Embryos, chemisch untersucht mit Berücksichtigung der gleichzeitigen morphologischen Veränderungen. II. *Arch. f. Anat. u. Physiol.*, Physiol. Abt. Suppl. 1907 : 507–542.

Whitaker, D. M. 1929. Cleavage rates in fragments of centrifuged Arbacia eggs. *Biol. Bull.* 57 : 159–171.

———. 1933. a. On the rate of oxygen consumption by fertilized and unfertilized eggs. IV. Chaetopterus and Arbacia punctulata. *J. Gen. Physiol.* 16 : 475–495.

———. 1933. b. *Ibid.* V. Comparisons and interpretation. *J. Gen. Physiol.* 16 : 497–528.

———. 1935. Egg quantity and the respiratory rates of several marine eggs. *Science* 82 : 68.

———. 1936. Permeability to water and staining role of developing and resting sea urchin eggs. *Proc. Soc. Exp. Biol. and Med.* 34: 708–712.

White, T. N. Recovery of Arbacia eggs from high intensity x-ray effects. *Biol. Bull.* 75 : 347 (Abstract).

Whiteley, A. H. 1949. The phosphorus compounds of sea urchin eggs and uptake of radio-phosphate upon fertilization. *Am. Nat.* 83 : 249–267.

Whitney, D. D. 1906. An examination of the effects of mechanical shocks and vi-

brations upon the rate of development of fertilized eggs. *J. Exp. Zool.* 3 : 41–47.
WICHTERMAN, R. 1953. *The Biology of Paramoecium.* Blakiston Co.
WIERCINSKI, F. J. 1944. An experimental study of protoplasmic pH determination. *Biol. Bull.* 86 : 98–112.
WILBRANDT, W. 1941. Die Permeabilität der tierischen Zelle. *Tabulae Biologicae* vol. 19 pt. 2 : 334–389, esp. XII Eizellen 371–389.
WILBUR, K. M. 1940. Effects of colchicine upon viscosity of the Arbacia egg. *Proc. Soc. Exp. Biol. and Med.* 45 : 696–700.
———. and RECKNAGEL, R. O. 1943. The radiosensitivity of eggs of Arbacia punctulata in various salt solutions. *Biol. Bull.* 85 : 193–200.
WILLIAMS, T. 1852. On the blood proper and chylaqueous fluid of invertebrate animals. *Phil. Trans. Roy. Soc. London* 142, (Part II : 595–653.
WILSON, D. P. 1946. The triradiate and other forms of Nitzschia closterium (Ehrenberg) Wm. Smith, forma minutissima of Allen and Nelson. *J. Marine Biol. Ass.* 26 : 235–270.
WILSON, E. B. (and LEAMING, E.) 1895. *An Atlas on the Fertilization and Karyokinesis of the Ovum.* Columbia Univ. Press, Macmillan and Co.
———. 1896. a. Centrosome and middle piece in the fertilization of the egg. *Science* 5 : 390–391.
———. 1896. b. *The Cell in Development and Inheritance.* 1st ed. Macmillan Co., N.Y.
———. 1899. On protoplasmic structure in the eggs of Echinoderms and some other animals. *J. Morph.* 15 Supp. 1–28.
———. 1901. a. Experimental studies in cytology. I. A cytological study of artificial parthenogenesis in sea urchin eggs. *Arch. f. Entwick.* 12 : 529–596.
———. 1901. b. Experimental studies in cytology. II. Some phenomena of fertilization and cell-division in etherized eggs. III. The effect on cleavage of artificial obliteration of the first cleavage-furrow. *Arch. f. Entwick.* 13 : 353–395.
———. 1905. Cytasters and centrosomes in artificial parthenogenesis. *Zool. Anz.* 28: 8–12.
———. 1911. The Cell in Development and Inheritance. 2nd ed. Macmillan Co., N.Y.
———. 1925. *The Cell in Development and Heredity.* 3rd ed. Macmillan Co. N.Y.
———. 1926. Newer aspects of the alveolar structure of protoplasm. *Am. Nat.* 60 : 105–120.
——— and MATHEWS, A. P. 1895. Maturation, fertilization, and polarity in the Echinoderm egg. New light on the "Quadrille of the centers." *J. Morph.* 10 : 319–342.
WILSON, H. V. 1924. Amoeboid behavior of the lymph cells in sea urchins. *J. Elisha Mitchell Sci. Soc.* 40 : 169–170.
WILSON, L. P. (Louise Palmer). 1940. Histology of the gonad wall of Arbacia punctulata. *J. Morph.* 66 : 463–479.
WILSON. W. L. 1950. The effect of roentgen rays on protoplasmic viscosity changes during mitosis. *Protoplasma* 39 : 305–317.
WINTHROP, J. 1670. (Gov. of Conn. in New England.) An extract of a letter to the publisher concerning some natural curiosities, especially a very strange and very curiously contrived fish, sent for the repository of the R. Society. *Phil. Trans. Roy. Soc. London* 5 : 1151–1153.
WOODLAND, W. 1906. 1907. Studies in spicule formation. III. On the mode of formation of the spicular skeleton in the pluteus of Echinus esculentus. V. The schleroblastic development of spicules in Ophiuroidea and Echinoidea. *Q. J. M. S.* 49 : 305–325; 51 : 31–43.
WOODWARD, A. A. Jr. 1949. Proteolytic enzymes in cytoplasmic granule preparations of Arbacia eggs. *Biol. Bull.* 97 : 264 (Abstract).
WOODWARD, A. E. 1915. Note on the nature and source of "purple X". *Biol. Bull.* 29 : 135–137.
———. 1918. Studies on the physiological significance of certain precipitates from the egg secretions of Arbacia and Asterias. *J. Exp. Zool.* 26 : 459–501.
———. 1921. The parthenogenetic effect of Echinoderm egg-secretions on the eggs of Nereis limbata. *Biol. Bull.* 41 : 276–279.
——— and HAGUE, F. S. 1917. Iodine as a parthenogenetic agent. *Biol. Bull.* 33 : 355–360.

YONGE, C. M. 1949. *The Sea Shore.* Pub. by Collins, St. James Place, London.

ZEUTHEN, E. 1947. Respiration and cell division in eggs of the sea urchin, Psammechinus miliaris. *Nature* 160 : 577–578.
———. 1949. Oxygen consumption during mitosis; experiments on fertilized eggs of marine animals. *Am. Nat.* 83 : 303–322.
———. 1950. Respiration during cell division in the egg of the sea urchin Psammechinus miliaris. *Biol. Bull.* 98 : 144–151.
———. 1951. Segmentation, nuclear growth and cytoplasmic storage in eggs of Echinoderms and Amphibia. *Pubbl. Staz. Zool. Napoli* 23 Suppl. 47–69.
ZIEGLER, H. E. 1894. Über Furchung unter

Pressung. *Verhand. d. anat. Gesell.* 8 : 132–146.

ZIELINSKI, M. A. 1939. Carbohydrate metabolism and phosphorus compounds in the fertilized eggs of the sea urchin (Paracentrotus lividus Lm.) *Acta Biol. Exper. Warsaw* 13 : 35–48.

ZIRPOLO, G. 1929. Maturità sessuale e fasi lunari. *Riv. Fisica, Math. e Sci. Nat.*, Napoli Ser. 2, vol. 3 : 393–402.

SUBJECT INDEX

SUBJECT INDEX

The Compilation of Experimental Work in Part IV contains practically everything known about the subjects and is arranged alphabetically, with subheads in logical sequence, in index form. The main topics are given with page numbers in the Index but the subheads (italicized in text) are indexed only when not included in a main topic. Subheads under "Cleavage" and "Respiration" are arranged alphabetically in the text and "Respiration" has a special index to various factors influencing metabolism (p. 212). Many substances not listed in the Index may be found under these main topics, and also under "Parthenogenesis."

References to "Other Species" occur at the end of each topic. These species are not indexed individually.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