# THE GROWTH AND METAMORPHOSIS OF THE ARBACIA PUNCTULATA PLUTEUS, AND LATE DEVELOPMENT OF THE WHITE HALVES OF CENTRIFUGED EGGS

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The pluteus of *Arbacia punctulata* which is well known to many investigators is the early pluteus with four arms, a pair of long anal (post-oral) arms on the ventral side, and a pair of short oral (pre-oral) arms on the dorsal side. In order to obtain further development of the pluteus, it is necessary, at Woods Hole, to feed the animals a rather special diet. In 1882 W. K. Brooks and two of his students, Garman and Colton, raised the plutei of *Arbacia punctulata* at Beaufort, N. Carolina, apparently without any special feeding. The sea water there is rich in diatoms, and the plutei can probably obtain what they require for growth from the sea water. This work was published by Brooks (1882) in his Handbook of Invertebrate Zoology, and by Garman and Colton (1883). The drawings are excellent for detail, but there is no indication of size and not adequate indication of age. A few stages had previously been described and figured by Fewkes (1881) working in A. Agassiz's laboratory at Newport, R. I. The present account with photographs gives the development of the pluteus with regard to size, sequence of events and rate of development at Woods Hole.

The best food for sea urchin larvae has been found to be the diatom, *Nitzschia closterium;* I found that they would also grow on the diatom, Licmophora. The Nitzschias themselves require a special diet, and must be raised in pure culture; they are raised on 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, deMorgan 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 (1914) has even obtained the next or $F_2$ generation of these hybrids. Unfortunately, the late

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

| | | |
|---|---|---|
| Solution A | $KNO_3$ | 20.2 grams |
| | Distilled water | 100 cc. |
| Solution B | $Na_2HPO_4 \cdot 12\ H_2O$ | 4 grams |
| | $CaCl_2 \cdot 6\ H_2O$ | 4 grams |
| | $FeCl_3$ (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.

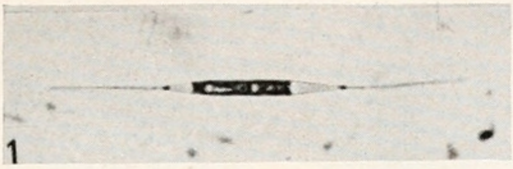
larval characters of *Echinus esculentus* × *E. acutus* from which the $F_2$ generation was obtained are alike in the two species, so that no information as to inheritance could be obtained, and none of the $F_2$ hybrids between *E. esculentus* or *E. acutus* × *E. miliaris* which would have given the 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 two forms of *Nitzschia closterium* both of which are devoured by the plutei. One, the large form (Plate I, Photograph 1) is about 100 μ long; the other, forma *minutissima* (Photograph 2), is about 24 μ long and is the variety used in the Plymouth laboratory. The Nitzschias are swept into the oesophagus and stomach (Photographs 3, 4) by cilia.

Several stages in the early development from the fertilized egg are shown on Plate II (Photographs 1–6). The micromere stage (Photograph 2) is the first sign of differentiation of cells, the micromeres being small and colorless. It also marks the beginning of asynchronous cleavage; in Arbacia there is a definite 12-cell stage preceding the 16-cell stage. With further cleavages a blastula is formed and emerges from the fertilization membrane (Photograph 3) in about 8 hours, the time varying by one or two hours in different batches and with different temperatures. At this stage I have estimated that there are 1,000 to 2,000 cells representing 10 to 11 cleavages ($2^{10}$ to $2^{11}$). The blastocoel becomes larger, leaving a single layer of peripheral, ciliated cells (Photograph 4). Then invagination takes place (Photograph 5), and a gastrula is formed (Photograph 6). At this time the skeleton appears in the form of triradiate spicules, one on each side of the gut. During this period there is no appreciable increase in 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. Now growth occurs and differentiation into the pluteus form with skeletal rods on each side (Photograph 7). At this stage the large pigment spots characteristic of the later plutei begin to form. The young pluteus increases in size and the arms begin to grow out (Photograph 8). The pluteus is well formed in 24 hours (Photograph 9). It is larger on the second day (Photograph 10), usually reaching a maximum in three or four days (Photograph 11). The long anal arms may measure 410 μ from base to tip. Without special feeding, the pluteus may live three or four weeks, gradually getting smaller by resorption of its arms (Photographs 12–14). It has apparently obtained sufficient food for growth from the sea water for the first four days, but then requires additional food. The structure of a two day pluteus is shown in serial photographs (Plate I, Photographs 5, 6, 7). These are taken at different levels through the animal, corresponding to serial sections of imbedded material.

Further development of the three or four day pluteus may take place if *Nitzschia closterium* is added to the cultures of plutei. Only a few plutei in any culture continue to develop. The British investigators have found it expedient to have only a few individuals in a large amount of water, 20 to 30 in a half-gallon jar. (See MacBride 1914, p. 506.) I raised them in Syracuse watch glasses holding about 15 cc. of sea water with about a dozen plutei in each dish. The developing larvae

PLATE I



Nitzschia closterium

Nitzschia closterium forma minutissima

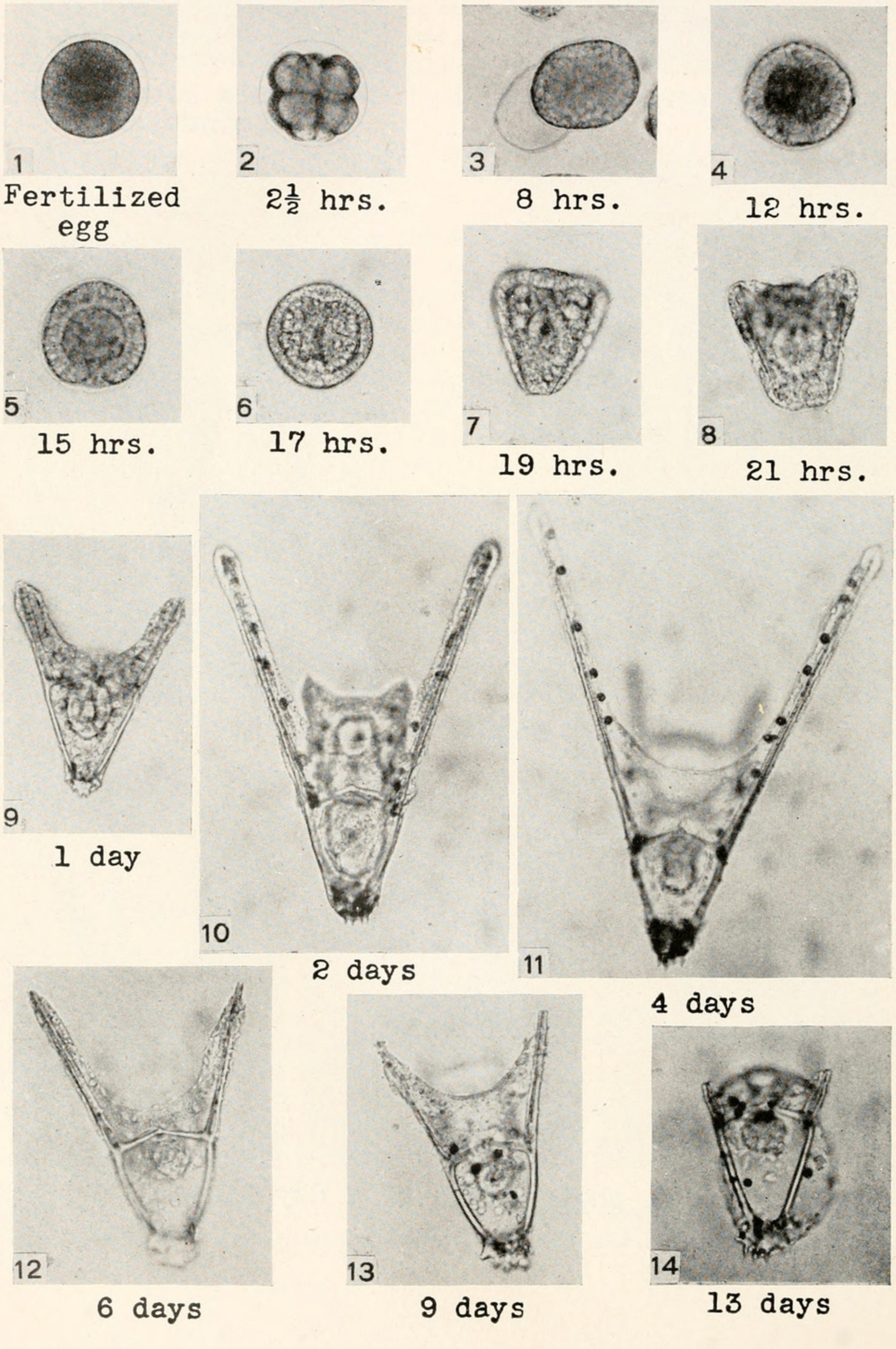
Plutei engulfing Nitzschias

Serial photographs of 2 day pluteus

Dorsal Median Ventral

PLATE II

DEVELOPMENT WHEN NOT FED



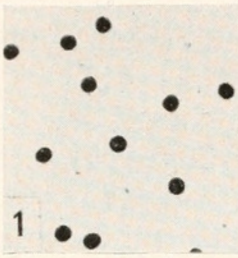
were transferred to fresh sea water with a pipetteful of the Nitzschia culture every few days. The eggs from which the final small adults were obtained were fertilized on July 12th, 1948, at Woods Hole, and taken to Princeton, N. J., on October 3rd. The last four died after completing metamorphosis on November 17th, a little over four months old.

The photographs on Plates III and IV, 1–18 are all to the same scale. The development varies in time in different batches, and in individuals of the same batch, so that the times given are only approximate. The eggs at this magnification (approximately 24 times) are of the size shown in Photograph 1. The one and the three day plutei to this scale are shown in Photographs 2, 3, shown with a larger magnification in Photographs 9, 10, 11 on Plate II (approximately 180 times). For the first four days, the development is the same whether fed Nitzschia or not, the food in the sea water being adequate. When fed, in about a week (Photograph 4, Plate III), the anal arms have become considerably longer. Then little knobs appear toward the base of the animal, which, by the eleventh day have grown out into a definite pair of new arms, the ventral lateral or postero-lateral (Photograph 5). These are always heavily pigmented at the tips which appear very red. These grow longer and a pair of knobs appear between the original anal arms and the new red-tipped arms. These are slightly noticeable in Photograph 6. In a month's time, these have grown into a second new pair of arms (Photograph 7) ; these are the dorsal lateral or postero-dorsal arms. These arms usually do not have red tips, although sometimes all the arms are pigmented at the tips. All the arms become continually longer (Photograph 8). The animals are now easily visible to the naked eye and look like small spiders. The arms are variable in length individually and relatively to each other. They are all ciliated, the cilia on the red-tipped ones being much longer and stronger than those on the other arms. The animal now swims actively by means of its cilia, and also walks or tumbles about on the tips of its arms, which can be readily moved. The arms are quite fragile and are easily broken off when the animal bumps into something or when it is 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 then looked exactly like its mate. The broken piece may seal itself off and swim about actively by means of its cilia like a complete organism, looking something like a paramoecium (Plate VI, Photograph 6). One of these pieces was alive and active for four days when it was inadvertently lost. The body of the adult Arbacia is seen as a yellowish green mass in the pluteus, the dark area in Photographs 7, 8 (Plate III) and thereafter. There are areas of dark red pigment on the surface. The young adult is formed in the body of the pluteus and grows at the expense of the pluteus.

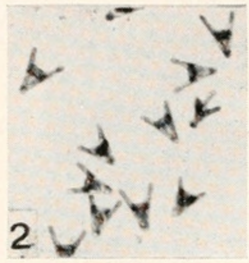
By six weeks the pluteus has become quite complicated, with two pairs of secondary oral arms, and four tubular processes (auricular lobes), two dorsal and two ventral. In Photograph 9, one sees several of the oral arms (at the anterior end) and a pair of the tubular processes (at the posterior end). A diagram of this stage is given by Miss Gordon (1929, p. 291). Soon after this, the five primitive ambulacral feet appear at one side of the body; these have suckers at their extremities and are continually expanded and contracted (Photograph 10).

PLATE III

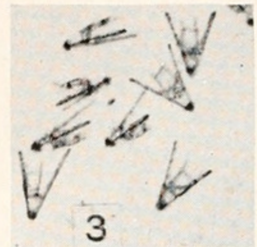
## DEVELOPMENT WHEN FED




Eggs to scale

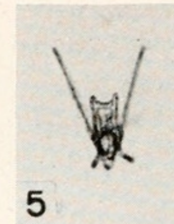



1 day

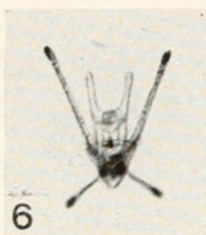



3 days

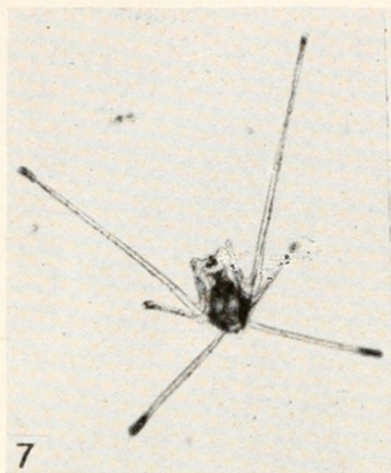

1 week



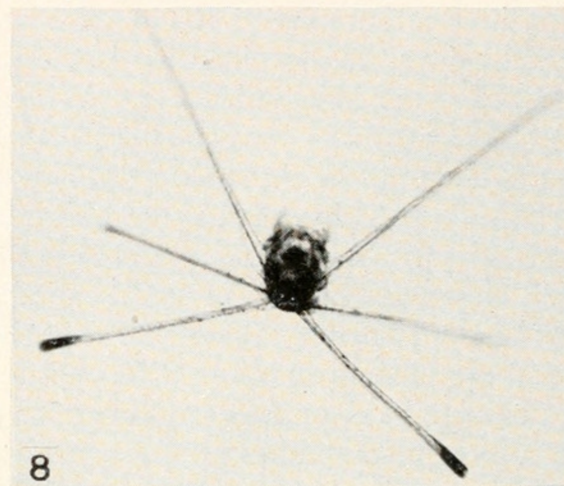

11 days



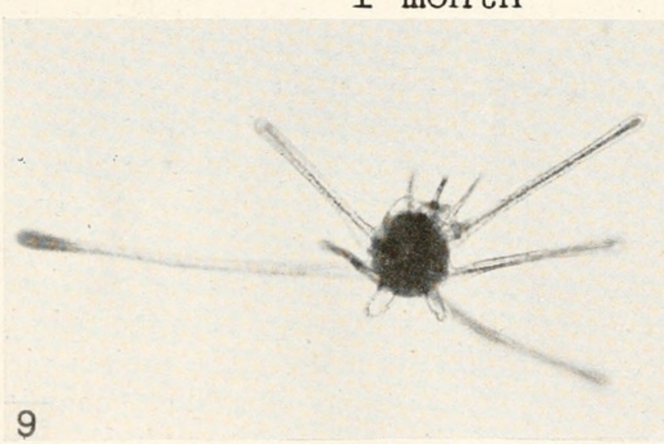

3 weeks



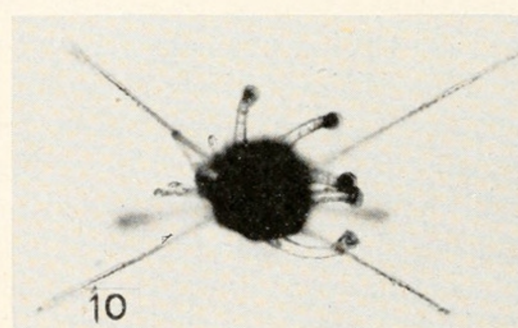

1 month



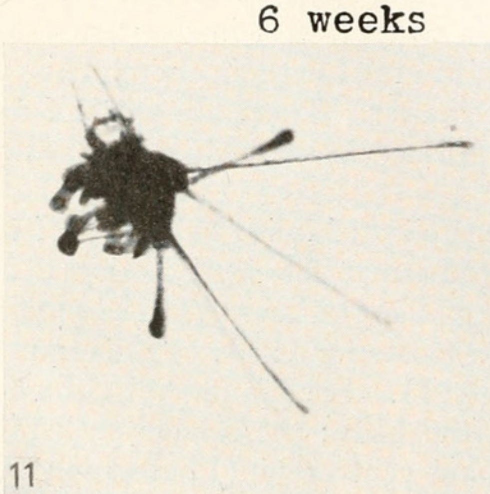

5 weeks



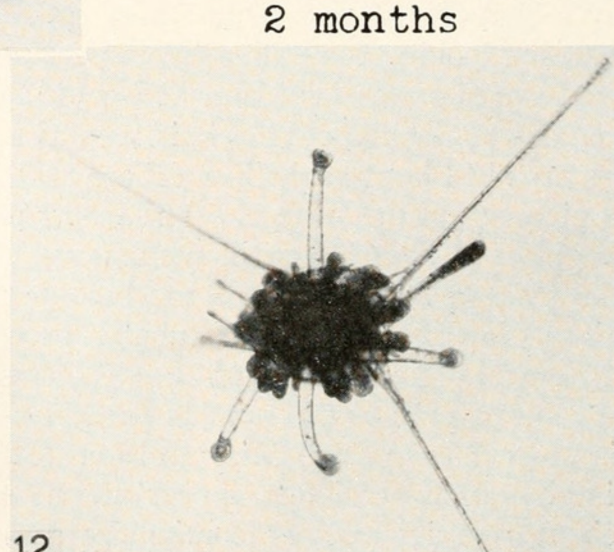

6 weeks




2 months



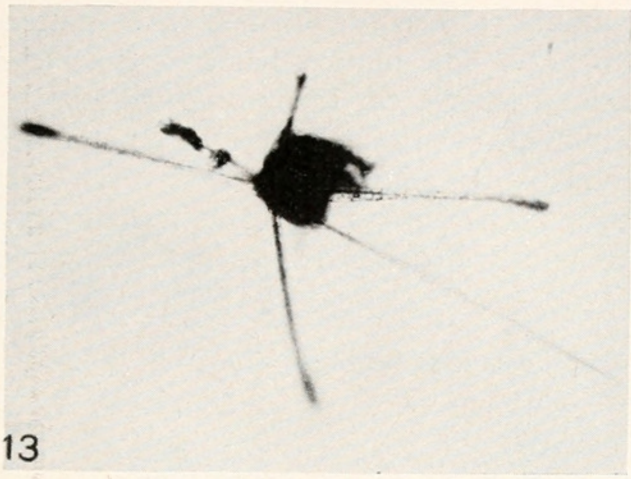

2 months

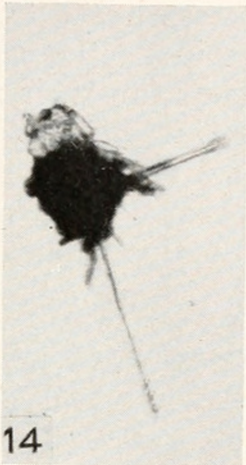


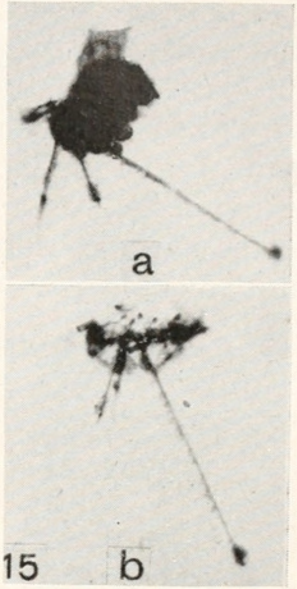

2½ months

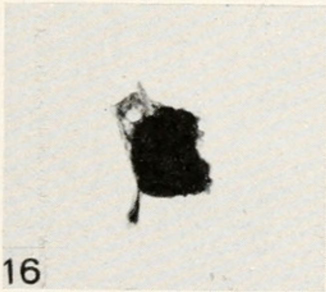
PLATE IV

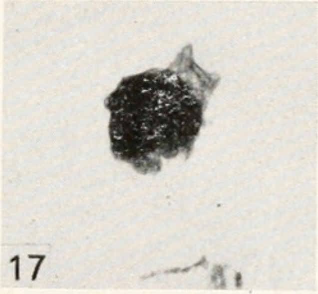
## DEVELOPMENT WHEN FED II


13
3 months


14
4 months

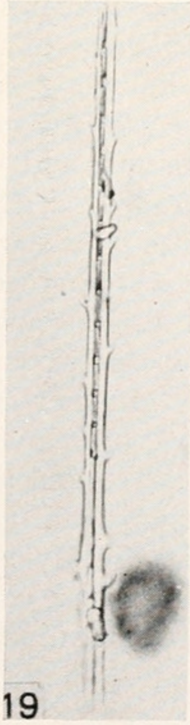

a
15 b
4 months

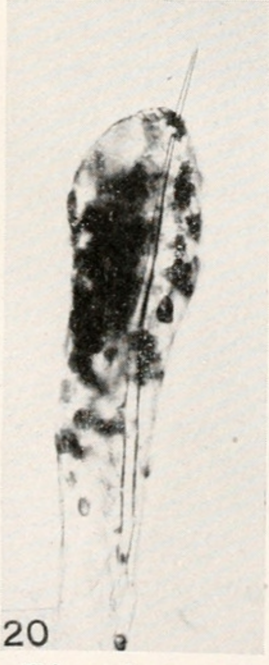

16
4 months

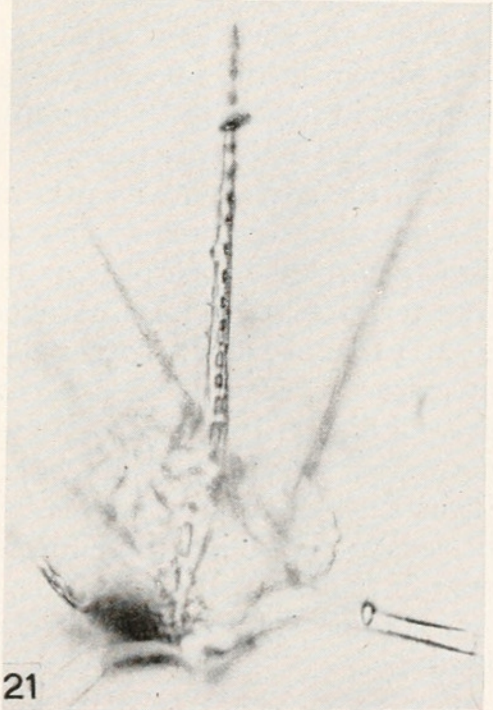

17
4 months


18
4 months

## ARM SKELETON


19
Original


20
First new


21
Second new (middle)

In Photograph 11, also a two months pluteus, one sees the three pairs of long arms, two pairs of the short arms near the head of the pluteus which is still prominent, the five ambulacral feet, with suckers, and the body of the developing adult. After two or more weeks, between each two ambulacral feet are formed three flattened plates, the first set of spines (Photograph 12). The pluteus has now reached its full development and the arms their maximal length, ca. 1.6 mm. The length of the arms is quite variable in different individuals even of the same batch. To give some idea of the increase in length of the arms, the following table is given of the length of the long anal arm at different ages. The figures represent measurements of the average better developed ones, the poorly developed ones not being taken into account.

*Approximate length of long (anal) arm from base to tip* ($\mu$)

| | Fed | Not fed |
|---|---|---|
| 1 day | 180 | 180 |
| 2 day | 300 | 300 |
| 3 day | 380 | 380 |
| 4 day | 410 | 410 |
| 5 day | 450 | 330 |
| 6 day | 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 | |

After reaching their maximal size, and often earlier, the arms begin to go to pieces; the flesh peels off, leaving the bare skeleton (Plate IV, Photograph 13). By four months several of the arms have gone to pieces; the body of the adult is conspicuous, but the head of the pluteus remains (Photograph 14). Sometimes the arms are shed as a whole piece like a shell. In Photograph 15 three of the arms shown in the upper Photograph (a) were shed as a unit the following day, as shown in the lower Photograph (b). The animal was left with one arm (Photograph 16). This was thrown off, but the head of the pluteus remained, and the body of the adult with its primitive spines was developing (Photographs 17, 18). The animal at this time was about a half millimeter in diameter. These later stages in metamorphosis took place in my cultures within a few days. The whole process from fertilization to metamorphosis took over four months (July 12 to November 17). I do not know whether this is the normal period under natural conditions as my cultures were subjected to changes in temperature and food. My last four animals all died at this time. They are known to require a different food after metamorphosis, which I did not have at hand. According to Shearer, deMorgan and Fuchs (1914), the best food is the calcareous protozoan Trichosphaerium; and later the red alga Corallina, these furnishing the calcareous matter needed for the development of the test and spines (l.c., p. 276). Miss Gordon (1929) has given a complete account of the further development of the young adult with especial attention to the test.

PLATE V

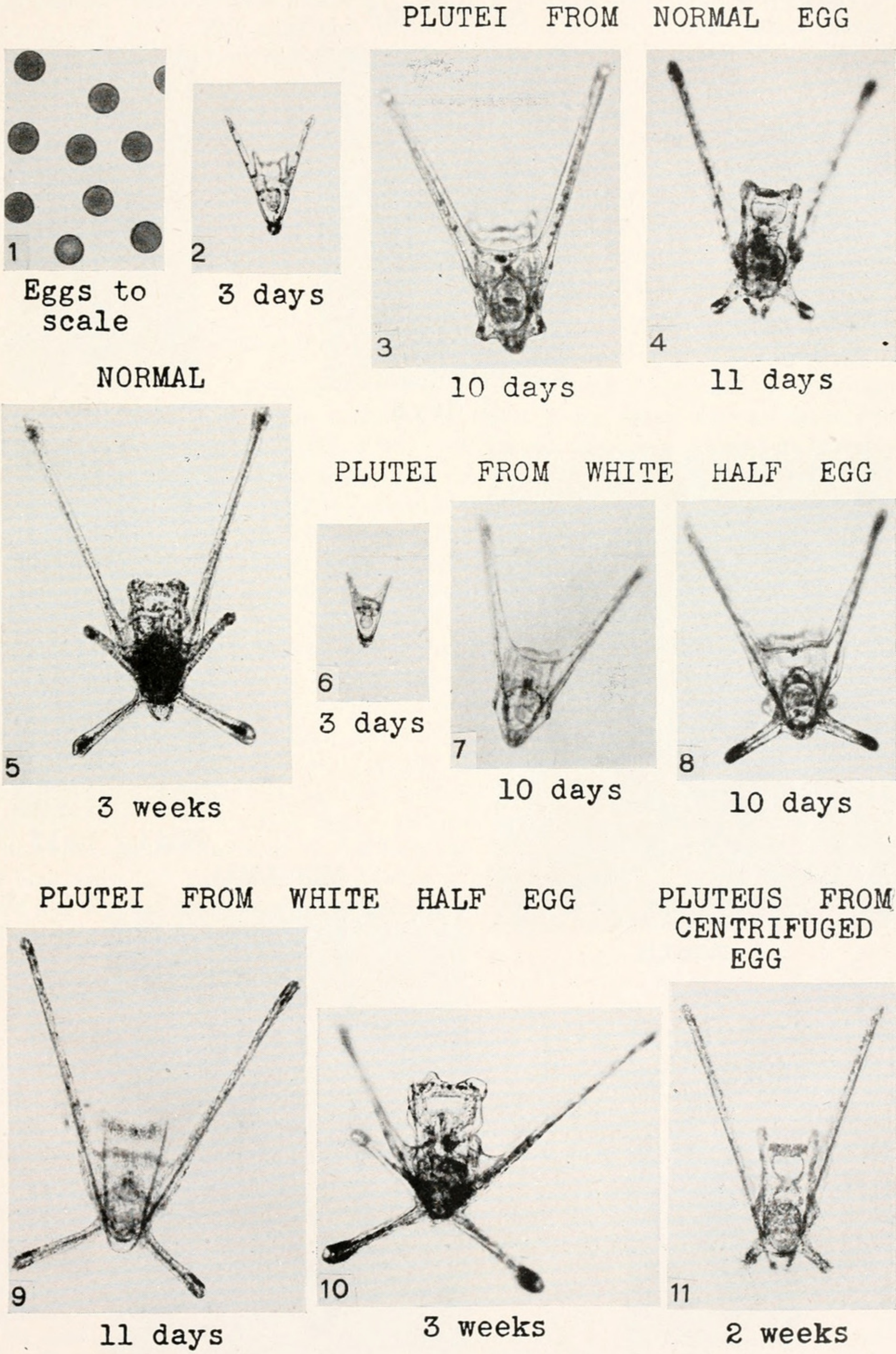
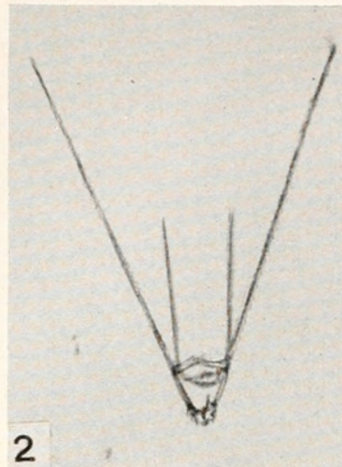

PLUTEI FROM NORMAL EGG
1
Eggs to scale
2
3 days
3
10 days
4
11 days
NORMAL
5
3 weeks
PLUTEI FROM WHITE HALF EGG
6
3 days
7
10 days
8
10 days
PLUTEI FROM WHITE HALF EGG
PLUTEUS FROM CENTRIFUGED EGG
9
11 days
10
3 weeks
11
2 weeks

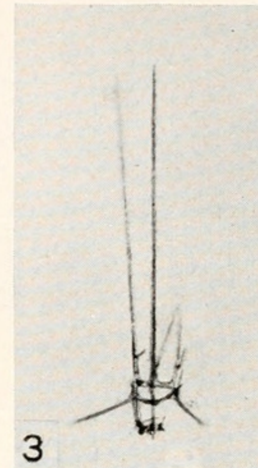
PLATE VI

SKELETONS

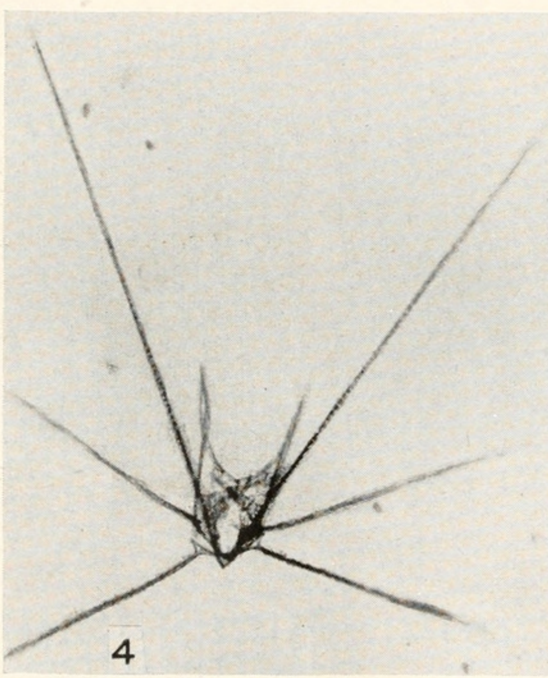
2 days



8 days



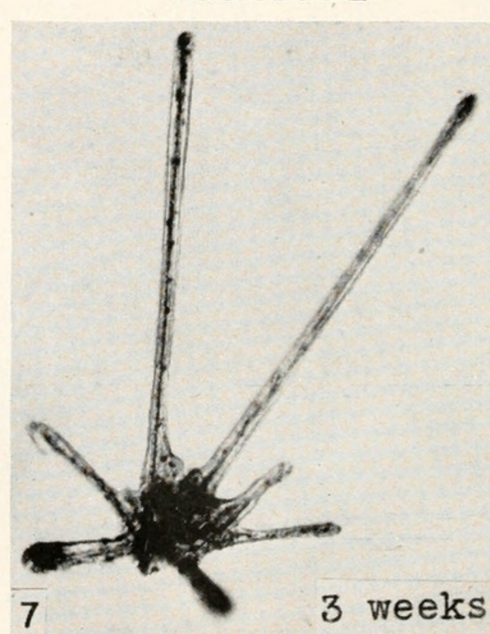
11 days



1 month

White half

ABNORMAL



3 weeks

BROKEN OFF ARM

The arm skeletons of the pluteus are interesting. The skeleton of the original long pair of (anal) arms is fenestrate, that is, it is a rod with holes in it (Plate IV, Photograph 19). The new pair of red-tipped (ventral lateral) arms have a solid skeletal rod with no holes (Photograph 20). This photograph shows also the concentration of pigment at the tip. The rod in the second new pair of long (dorsal lateral) arms, between the other two long pairs, is again fenestrate (Photograph 21). As is well known, the skeletons of the original long (anal) arms are solid in the plutei of many sea urchins. We have, in general, two types of plutei, those with fenestrate arm skeleton such as Arbacia, Tripneustes, Sphaerechinus and the sand-dollar, Echinarachnius; and those with solid arm skeleton, such as Strongylocentrotus, Lytechinus and Psammechinus. This has been of great value in hybridizing experiments, in determining maternal and paternal inheritance.

*Development of the white half egg*

The Arbacia egg can be separated into a white and a red half by centrifugal force. The white half when fertilized develops in the same way as the normal whole egg (E. B. Harvey, 1932, 1940). A normal pluteus is formed in two days similar to that from the whole egg except that it is smaller and lacks pigment. The pigment spots, however, begin to come in on the third day after fertilization, and continually increase. The white plutei, like those from the whole egg, do not develop beyond the four armed stage without special feeding. The photographs on Plate V were taken at a greater magnification than the preceding series on Plates III and IV (i.e. approximately 60 times). The eggs to scale are shown in Photograph 1; the normal three day pluteus in Photograph 2. Several later stages of normal plutei are shown in Photographs 3, 4, 5, for comparison with the same stages of the white plutei (Photographs 6–10). The ten day white plutei (Photographs 7, 8) are in all respects like those from whole eggs (Photographs 3, 4). There is the same massing of red pigment in the tips of the first new pair of ventral lateral arms. There is now no difference in size between the plutei from the half egg and those from the whole egg, in fact the former may be larger (Photograph 9). The three weeks pluteus from the half egg (Photograph 10) has the three pairs of long arms like that from the whole egg (Photograph 5). These were not carried any further, but it seems certain that the later development would be like that of the pluteus from the whole egg.

*Development of the centrifuged egg*

The very young pluteus from the centrifuged egg has the pigment granules concentrated in certain areas, most frequently above the mouth, though they may be in other positions. The pigment spots are also unevenly distributed at first, but after three or four days they are fairly uniformly distributed so that one cannot distinguish between the plutei from centrifuged eggs and those from normal eggs.[2] The later

[2] The pigment spots of the plutei are bright red, in contrast to the brownish red color of the unfertilized eggs. However, in centrifuged eggs, the concentrated mass of pigment granules is bright red. This difference in color is well shown in kodochrome slides when the contrasting objects are taken on the same slide. E. G. Ball (*Biol. Bull.*, **97**: 231) has compared the absorption spectra of acid alcohol extracts of plutei and eggs, and has found the two pigments identical.

development, when fed, is like that of the normal pluteus. In Photograph 11 is shown a two weeks pluteus from a centrifuged egg, which is similar to the pluteus from a normal egg of about the same age shown in Photograph 4.

*Skeletons*

The skeletons from plutei of various ages are shown in the photographs on Plate VI; the fully formed skeleton is shown in Photograph 4. A skeleton from a white half egg is shown in Photograph 5, quite like that from a whole egg of the same age (Photograph 3).

*Abnormalities*

The only abnormal later pluteus occurring in my cultures is shown in Photograph 7, on Plate VI. It had one extra arm on one side.

## Magnification of Photographs

The photographs were taken of the living animal with different objectives, as indicated below in brackets, and a 10X ocular.

Plate I. Photographs 1, 2 about 420X (70X). Photographs 3, 4 about 240X (40X). Photographs 5, 6, 7 about 180X (30X).

Plate II. All photographs about 180X (30X).

Plate III. All about 24X (4X).

Plate IV. Photographs 13–18 about 24X (4X). Photographs 19–21 about 240X (40X).

Plate V. All about 60X (10X).

Plate VI. Photographs 1–5 and 7 about 60X (10X). Photograph 6 about 240X (40X).

Explanations of the plates are given on the plates, and in the text.

## Summary

1. The growth and metamorphosis of the *Arbacia punctulata* pluteus after the four day stage has been traced by means of photographs. The plutei were raised on the diatom, *Nitzschia closterium*.

2. The pluteus from the white half egg develops pigment spots after the third day, and develops in exactly the same way as that from the whole egg, when fed Nitzschia.

3. The pluteus from the centrifuged, stratified, egg becomes like that from the normal egg after the third day and develops similarly when fed.

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