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Studies on the Normal Course of Development on Pelecypoda, Using Mainly the Eggs of *C. gigas* and *C. chinensis*

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ABSTRACT—The echinoderm eggs have been a well known object for the analysis of development (Yamamoto, 1988). This may be based mainly on their moderate size and transparency of the ovum. Moreover, the most important fact is that the eggs can be artificially inseminated.

Hitherto, Yamamoto carried out experiments to learn the mechanism of syngamy (Yamamoto, 1973). As a matter of fact, the course of the syngamy of both pronuclei is known to be different in the kind of species. In the case of the sea urchin, both pronuclei directly fuse together before the initiation of nuclear division. However, in the eggs of certain molluscs, it is known that the direct fusion of both pronuclei does not occur prior to the formation of the mitotic spindle. In fact, the paternal and maternal chromosomes become enclosed by common nuclear membranes in the nuclei of the two daughter cells (Balinsky, 1965).

To learn the mechanism of syngamy in the latter cases, first of all, the writers took up experiment to ascertain the normal development using some species of molluscs. In spite of their expectation, as it was difficult to pursue a course of cell linage because of the opacity of the eggs used, they intended to review the early development and larval differentiation of the Pelecypoda. In the present report, the writers described the normal course of development using mainly the eggs of the oyster, *Crassostrea gigas* and the bivalve, *Caecera chinensis*.

Immediately after the shedding of the ovum, the unfertilized egg showed itself to be polyhedral-shaped. Within 10 to 20 minutes after insemination, the breakdown of the germinal vesicle took place followed by the maturation division. In the case of *C. gigas*, just prior to the first cleavage, conspicuous bulging of the cytoplasm appeared at the vegetal side of the egg. When the cleavage occurred, this protuberance was absorbed into the one side of the daughter blastomere, and the egg was separated into the daughter cells of an unequal size. Continually, the writer first concentrated to pursue the cell linage, then to analyze the course of morphogenesis in the larval stages of development.

INTRODUCTION

Mollusca is a large phylum of invertebrates, consisting of Amphineura, Gastropoda, Scaphopoda and Cephalopoda, characterized by a soft unsegmented body enclosed partly or wholly in a calcareous shell, and having gills, a foot and a mantle. These animals have been more or less bound with human existence. Nowadays, by the remains of shell heaps along the ancient sea coast, it is shown that the antiquities inhabiting near the sea coast, lived on the molluscs consisting of oysters, clams, mussels, roll shells, squids and so forth. Accordingly, Gastropoda and Pelecypoda have been two of the most important groups for our dietry food.

The body structures of the molluscs are quite different to those of the annelids, however, on the phylogenic point of view, the both phylums have a intimate connection following up identical line of evolutionary progress. Consequently, the course of thier early development bears a remarkable resemblance with each other. Generally, the cleavage pattern of both phylums is the spiral with the exception of the cephalopods. And passing through the conspicuous forms of the larval stages, that is to say, the trochophore and the veliger, they metamorphose and gain finally the adult form.

In the phylum Mollusca, without regard to compose enormous kind of animals, there exists a few species capable for artificial insemination. Therefore in the present paper, the writers first intended to analyze the development of the Japanese oyster, *C. gigas* and the others, then made an attempt to review the development and the course of metamorphosis on the Pelecypoda, comparing with the literatures having done by many investigators.

MATERIALS AND METHOD

The Japanese oyster, *Crassostrea gigas* was collected at Matsushima Bay. And the other species, *Caecera chinensis* was gathered at the vicinity of the Marine Biological Station of Asamushi, Tôhoku University.

The spawning season of the oyster, *Crassotrea gigas,* begins on the end of July and reaches its maximum on early in August. The initiation of the spawning is said to introduce with the sea water temparature beyond 15℃, and reaches its maximum about 25℃. In the case of *Caecera chinensis,* the spawning begins in mid-July, and continues to the end of this month. Generally, the spawning of the molluscs has been introduced by several methods of stimulations such as an application of electric, high temperature and chemical treatments. In the present experiment, the writers first removed the shells, then dissected matured ovary into small pieces in the sea water. Afterward, they were filtrated through three sheets of gauze, heaped one over the other. The unfertilized eggs thus obtained were repeatedly washed by an ordinary sea water, and they were stocked in a refrigerator until the time of the experiment. The spermatozoa were picked off with pointed tips of the forceps directly from the dissected body, in which the testis was present as a sheet of whitish tissues on the hepatic lobes. The small amount of spermatozoa was mixed to the egg suspention, then the behavior of the unfertilized eggs was observed at every intervals.

The sermatozoon of *C. gigas* is flagellar shaped consisting of head, middle piese and tail

regions. It must be recognized that the middle pieces of this spermatozoon consist of about four small spheres, hunging directly from its head region. In *Caecera*'s case, the spermatozoon is the typical flagellar shaped similar to that of the Echinodermata.

RESULT AND DISCUSSION

The unfertilized egg immediately after dissection, displays unsettled and changable in shape. Meanwhile, the unfertilized egg transforms gradually into a flask shape. Its pointed tip shows a area where the egg, during oogenesis, hung onto the wall of the ovary. Accordingly, the pointed tip corresponds to the vegetal pole side of the unfertilized egg. During ten to twenty minutes leaving the eggs as they stand, most of the unfertilized eggs transform into spherical, when they are in the full maturity. At this time, the germinal vesicle about 30 μm, is clearly seen in the egg, containing comparably large nucleolus in its nucleoplasm. In adition, the unfertilized egg of *Caecera* seems to somewhat clear than that of *C. gigas* for the sake of appearance.

Generally, as stated previously, it has been known that the artificial insemination of the Pelecypoda is not easily done. In these animals, *Crassostrea, Caecera, Mactra, Mitilus* and others are a few species possible to fertilize by the artificial insemination. The common natures of these species fertilizable, are said to be those that the period of fertilization takes place when their germinal vesicles are vividly present in the ovium. The elevation of the fertilization membrane of the above described species is not conspicuous however, the perivitelline space gradually becomes to increase slightly in thickness. Subsequently, in the advance of development, the breakdown of the germinal vesicle can clearly be observed.

Eliminations of two polar bodies

Within 30 to 40 minutes after insemination, the egg surface in the animal pole region flattens, becomming transparent in the limited area of the elimination of the polar body. Meanwhile, meiosis I spindle is formed just beneath the animal pole. After the completion of the first polar body, the meiosis II spindle is formed at almost the same position, where the meiosis I spindle was formerly present. A small transparent dome like protuberance appears in the central region of the animal pole, then the second polar body is formed. The first and second polar bodies are usually settled sitting close together in the perivitelline space just over the animal pole. The course of the polar body formation is illustrated in the figures (Fig. 1, a–i). After the completion of the second polar body, the female pronucleus becomes to be seen just beneath the animal pole.

As to the course of the maturation division and the mechanism of the elimination of the polar bodies, the writers ask to the readers to refer Yamamoto's previous papers (Yamamoto, 1971, 1972, 1973, 1974 and 1981), and the investigations having done by many Japanese investigators (Kanatani et al, 1980; Sawada, 1960 etc.).

After a while, a sperm aster appears at any places in the egg interior. In most cases, it appears in the cytoplasm in the location close to the animal half. In the case of *C. gigas*, the

course of syngamy is not able to be recognized, because of the opacity based upon the presence of many yolk granules in the egg cytoplasm.

Polar lobe formation to first cleavage

After syngamy, the nuclear membrane of the zygote seems to disintegrate, then the mitotic apparatus begins to be formed. The vegetal portion of the fertilized egg of *C. gigas* gradually becomes transparent as a result of the initiation of the vortex movement occurring in the egg interior. The fertilized egg is deformed like a pear shaped appearance as a result of the formation of the cytoplasmic protuberance from the vegetal side of the egg. This protuberance is denominated to 'polar lobe', very characteristic in the molluscan development. In the progress of development, the initial wide communication between the egg proper and the polar lobe, becomes constricted until only a narrow connection is left. When views from outside, the external appearance of the egg exibits a clover leaf pattern. The egg in this stage is called 'trefoil stage'. In the *Caecera* eggs however, the formation of the polar lobe is not conspicuous, accordingly the course of the cleavage is, in certain aspect, similar to that of the echinoderm eggs. After a while, the furrow appears in the animal pole side, in the plane parallel with the egg axis. The furrow initially deepens slowly, but in the case of *C. gigas*, the egg appears as if it were in three cell-stage. One blastomere, communicating directry with the polar lobe, then absorbs all of the lobular materiales forming finally one large daughter blastomere. In this way, the egg is divided into blastomeres of an unequal size.

On the other hand, the mechanism of the unequal division in the case of *C. chinensis* is different. According to the writer's previous report (1965 and 1971), the mitotic apparatus formed in the central part of the egg, gradually moves toward the egg surface, taking eccentric in position. This will be caused by the unequal development of the two astrospheres. Just prior to the cleavage, unequal astrospheres were clearly observable, and sometimes later the egg was separated into the daughter cells of an unequal size. To know the mechanism, particulary the determination of the position of their mitotic apparatus on one side of the egg, Yamamoto (1965) carried out the experiment to conferm, whether the plane of division was altered when the formation of the astral rays was blocked, or the rays once formed was disintegrated by the action of some mitotic inhibitors. He ascertained, when the mitotic apparatus was settled in a center of the egg in the case of the exposure to the drugs, the egg cleft into the daughter blastomeres of an equal size. On the other hand, when the astral rays once formed and reaching in full growth, were disintegrated just prior to the furrowing stages, the egg could proceed its way regularly into the daughter blastomeres of an unequal size. From these results, he came to the conclusion that the mechanism of unequal cleavage has a close relationship to the position of the mitotic apparatus accompanying with the growth of the elongating astral rays.

Miyazaki (1935 and 1936) stated, that the most of the species having prominent polar lobe, including *Crassostrea, Mithylus* and *Scallops*, later adhere to the sea bed and grow up to the sessil type. In addition to the above description, the role of the polar lobe is thought as follows. The mechanism and the significance of the polar lobe formation have not been elucidated as

yet, however a few experiments have done concerning its role to the normal course of development. Willson (1904), using the eggs of *Dentalium*, carried out the experiment on the removal of the polar lobe until four cell-stage, ascertained that the treated eggs later developed into swimming larva devoid of apical tuft. From the above fact, it became clear that the substances functioning the formation of the apical tuft, reside within the limitted portion of the polar lobe, then they moved to the D-blastomere by way of CD-blastomere. Davidson (1966) also carried out the experiment of the removal of the polar lobe, using the eggs of the Pelecypoda, *Ilyanassa absolea*. He ascertained finally that the RNA synthesis of the operated eggs became lower when compaired with the eggs of the unoperated eggs. From the fact, it became obvious that the substance or substances residing in the polar lobe, have an important role for the later development in the larval differentiation.

Cleavage

The plane of the first cleavage is meridional. In this manner, the egg is separated into two unequal daughter blastomeres. The larger blastomere is termed as CD-blastomere and the other smaller one is as AB-blastomere. The plane of the second cleavage is formed meridional, passing through the egg axis perpendicular to the plane of the first cleavage. At this time, AB-blastomere divides into the two equal blastomere named A and B. On the other hand, CD-blastomere, as in the first cleavage, gives rise to two daughter blastomeres of an unequal size. The smaller blastomere is called C, and the larger one, D. These blastomeres (A-D) arrange in a clockwise order when viewed from the upper side. Galtsoff (1964), by the observation of the normal course of development of the American oyster, *C. virginica*, found that in the four cell stage, A and B represent the anterior, and C and D, the posterior halves of the larva. While, A and D form the left side and B and C, right half of the larval body. The D is the biggest of all the four blastomeres. They are called macromere. In a little while, the third cleavage takes place in the horizontal plane. As the results, the four smaller blastomeres (1a-1d) are formed on the top of the mother cells (1A-1D). The four smaller blastomeres are located slightly aslant to each of the mother cell. The newly formed blastomeres laid upward the mother cells, are designated as the first quartet or micromeres. In the 8 cell-stage, the 1D -blastomere is the largest of all the blastomeres present. As mentioned above, this type of cleavage whose new blastomere is formed in the position slightly oblique to the mother cell, is generally known as the 'spiral cleavage'.

The reasons how to accomplish the spiral cleavage may be based upon the formation of the mitotic apparatus in the mother cell, that it is formed oblique in position to the egg axis. However, its motive power has not yet been established. The first to the third quartets are thought to develope into the ectodermal cells and they give rise to those organs and structures, that maintain contact with the outside world. After the completion of the third cleavage, the division of the four macromeres (1A-1D) takes place sending the second quartet (2a-2d) upward, slightly oblique to each of the mother cell. Therefore, the zygote enters 12 cell-stage. Hitherto, in every cleavage, the dixtral and sinistral spiral cleavages are seen one after the other. At this period of time, the newly formed blastomere termed 2d is

bigger than the mother cell 2D. Accordingly, 2d is the biggest of all the blastomeres in this stage. The 2d cell has been given a name, the first somatoblast or X cell, which was termed by Wilson (1904) in the studies of Nereis development. He described that the first somatoblast gives rise to many organs and structures of the ectodermal origin in the future, such as the visceral plate, the shell gland and the related tissues of the foot. Unfortunately, in the present experiment, the writer could not ascertain any structures derived from the 2d-blastomese, because of the difficulty of pursuing the cell linage in the materials used.

Subsequently, the division of the first quartet takes place making the blastomeres of $1a^1$-$1d^1$ and $1a^2$-$1d^2$. The $1a^1$-$1d^1$ occupy the upermost layer of the developing embryo, and $1a^2$-$1d^2$ are located slightly oblique in the position overlying the second quartet. The blastomeres of $1a^2$-$1d^2$ are designated as the primary trochoblast which later forms the velum of the veliger larva. The velum is the typical motor organ in the swimming larva of the molluscs and the annelids. It is composed of the flat disc projecting fine hairs from the body of the larva itself. The surface of the disc is covered with dense cilia. As the result of the cleavage of the 1q cells, the zygote enters 16 cell-stage.

When the first somatoblast 2d enters the mitotic phase, the $2d^1$ and $2d^2$ are formed lying both sides of the larval body in ventro-lateral position. At this period, the larva becomes to assume the symmetrical coutour.

Before long, the division of the blastomere 2D takes place resulting the formation of 3d and 3D. Consequently, the zygote reaches 18 cell-stage. Meanwhile, the primary trophoblast $1a^2$-$1d^2$ divide and arrange in a form of circle, therefore they heap up in two layers of the quartets, $1a^{21}$-$1d^{21}$ and $1a^{22}$-$1d^{22}$. In this way, the zygote proceeds into 22 cell-stage. Up to this stage, it takes about three hours in the case of *C. gigas*. Continually to the 22 cell-stage, the division of the macromeres (2A-2C) takes place making upward the 3q consisting of 3a-3c. The cells of the 3q overlie in the position slightly oblique to their mother cells (3A-3C). The newly formed micromeres are denominated as the third quartet. The cells of the 3q are somewhat larger comparing to the cells of the other quartets. The formation of the third quartet described above, is the last division of the provision of the new micromeres from the macromeres. The newly formed quartet settles downward in the proximity to the botom of the embryo. The macromeres (3A-3D) later become to from and differentiate into the endoderm and a part of the mesodermal cells. Among them, 3D is termed to the cell of the mesoendoderm, which later differentiate into the part of the endodermal and the mesodermal cells. On the contrary, the ectodermal cells are said to originate from those of the first quartet. With the progress of development, the embryo acquires gradually a characteristic appearance similar to the shape of the mulberry. This is the 'morula'. Korshelt et al (1895) described this type of the embyo as the 'sterreoblastula', because of the irregularity of the embryonic contour when observed from the outworld.

The morula is made up of the irregularly arranged cell cluster. It is constituted with the groups of the segmented cells of the micromeres and the larger macromeres, arranged above and below. Here, it must be noticed that the formation of the morula in the molluscs reaches in the way of the several steps such as 16, 18 and 22 cell-stage, because the every cleavages take place asynchronously. The observations pursuing the cell linage of development of

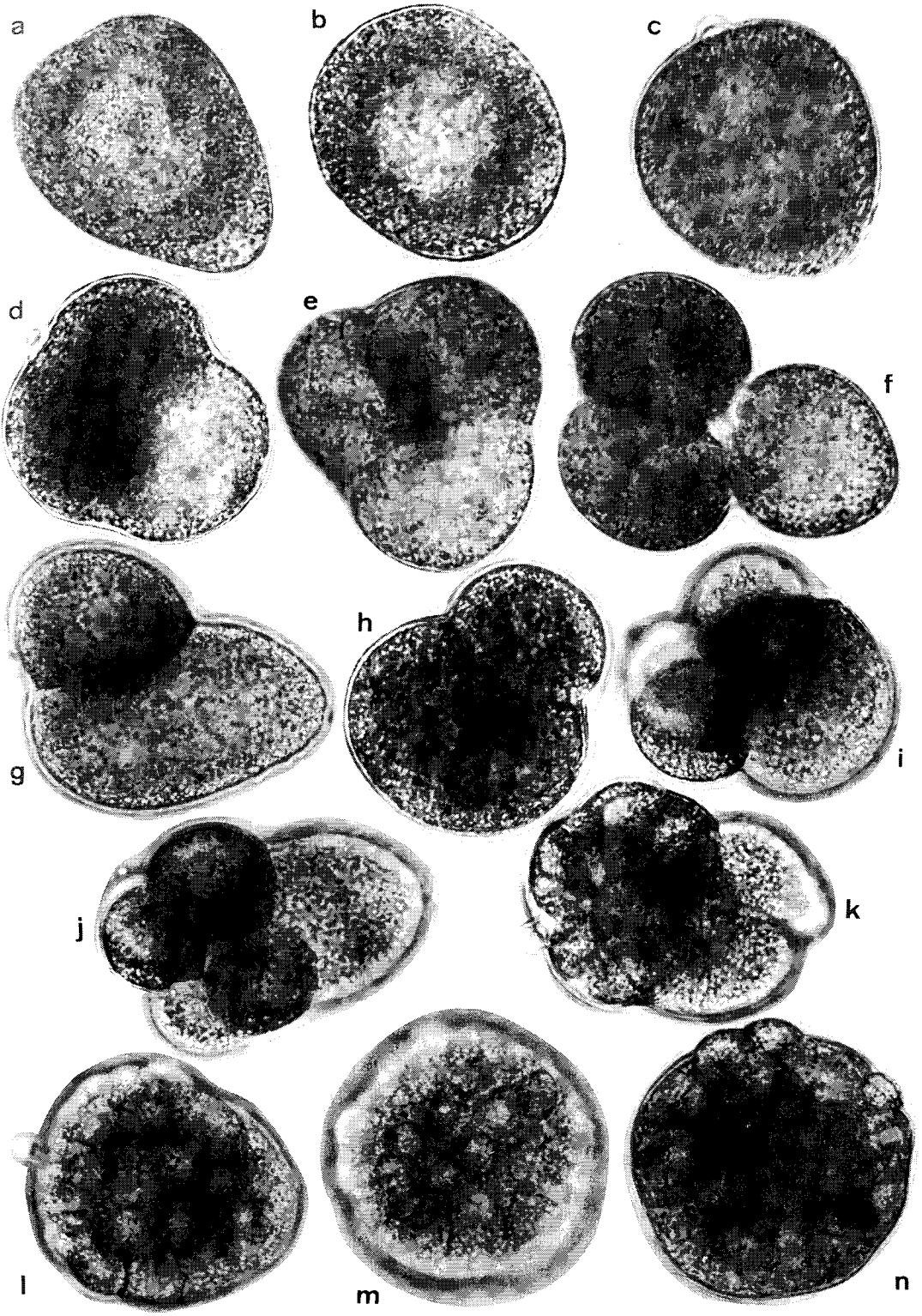

FIGURE 1 : Normal course of development in *Crassostrea gigas*.

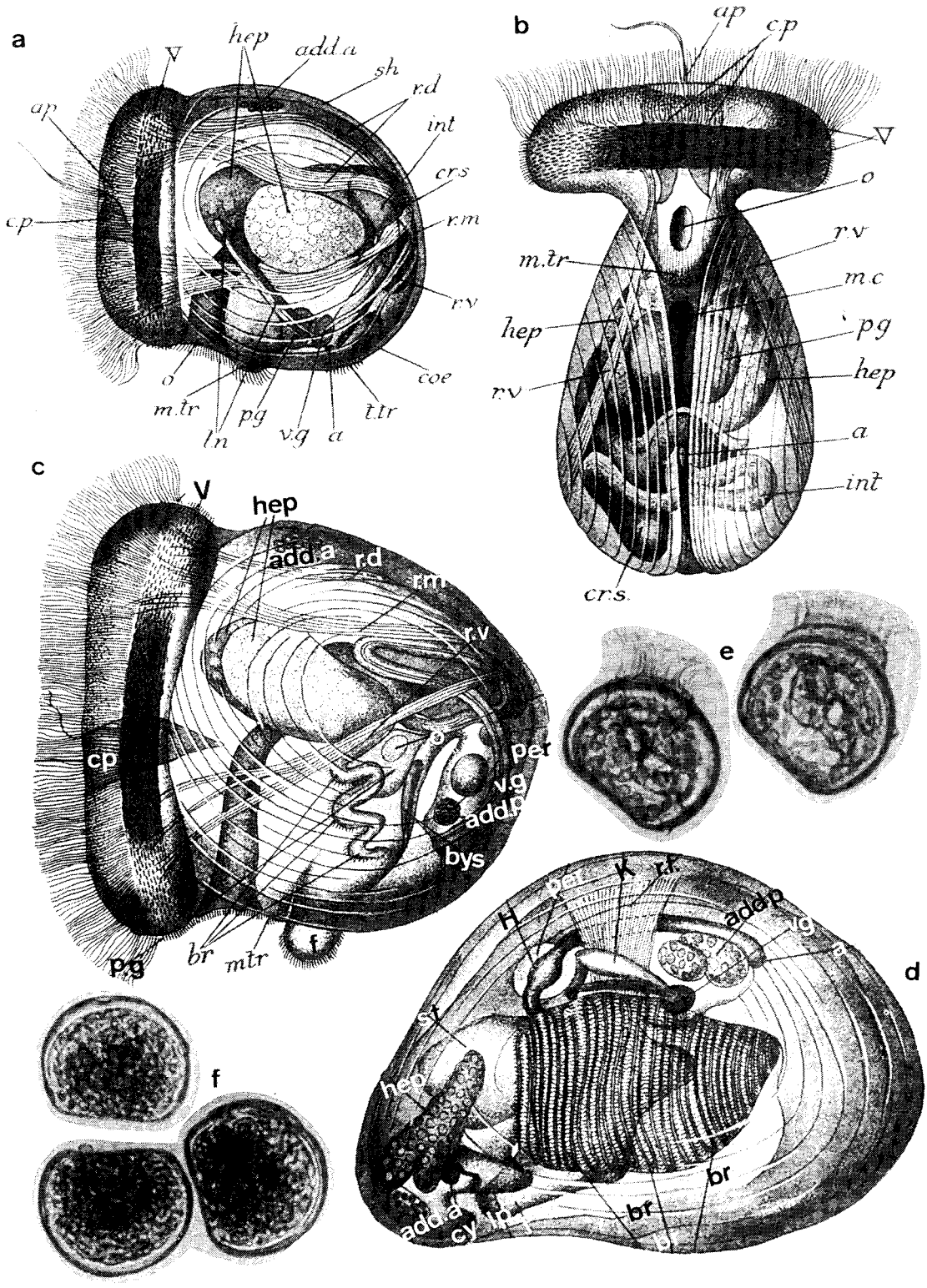

FIGURE 2 : Larval development of *Dreissencia polymorpha* (*after MacBride*).

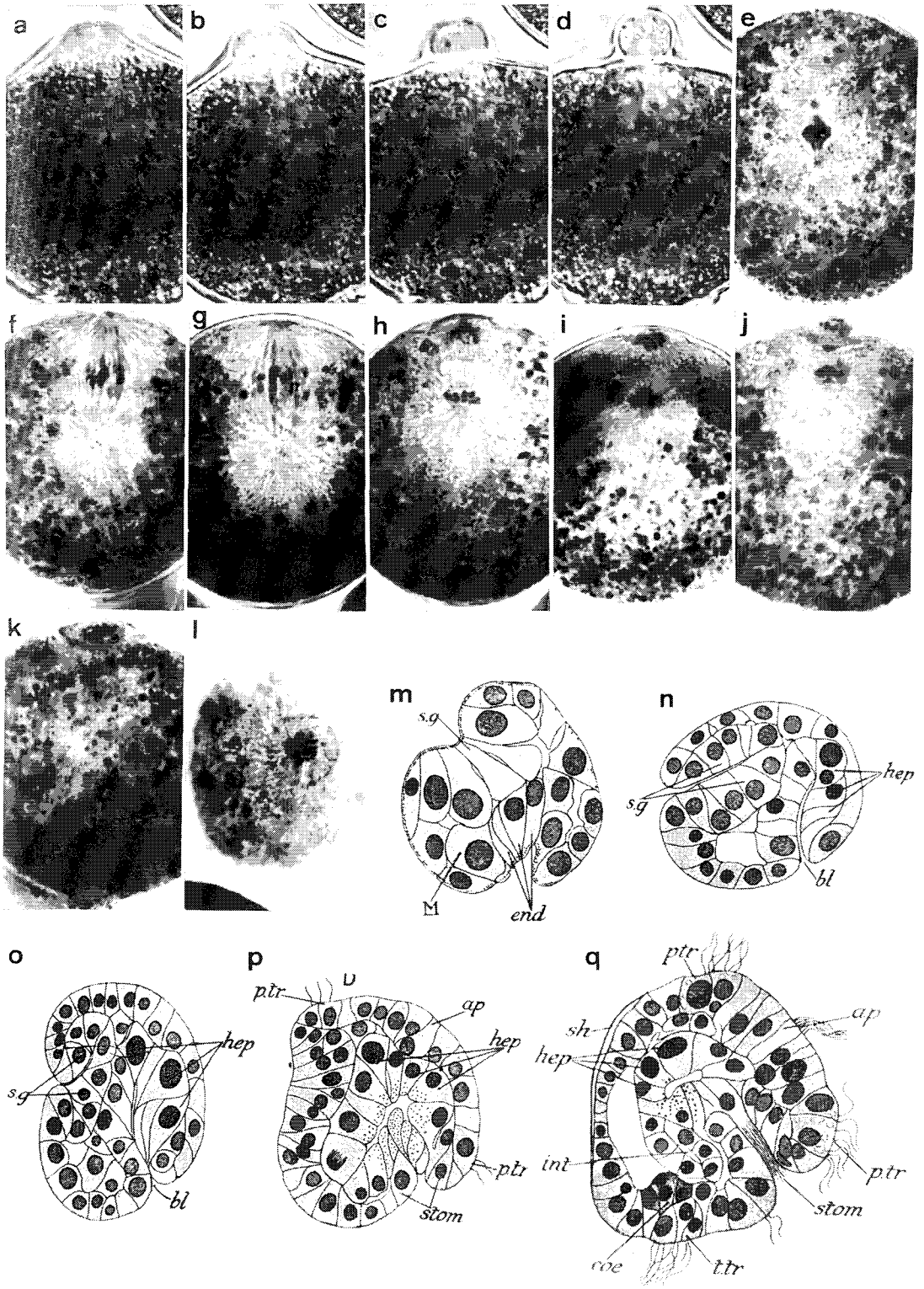

FIGURE 3 : a—l, Elimination of polar body in *Caecerra chinensis*.

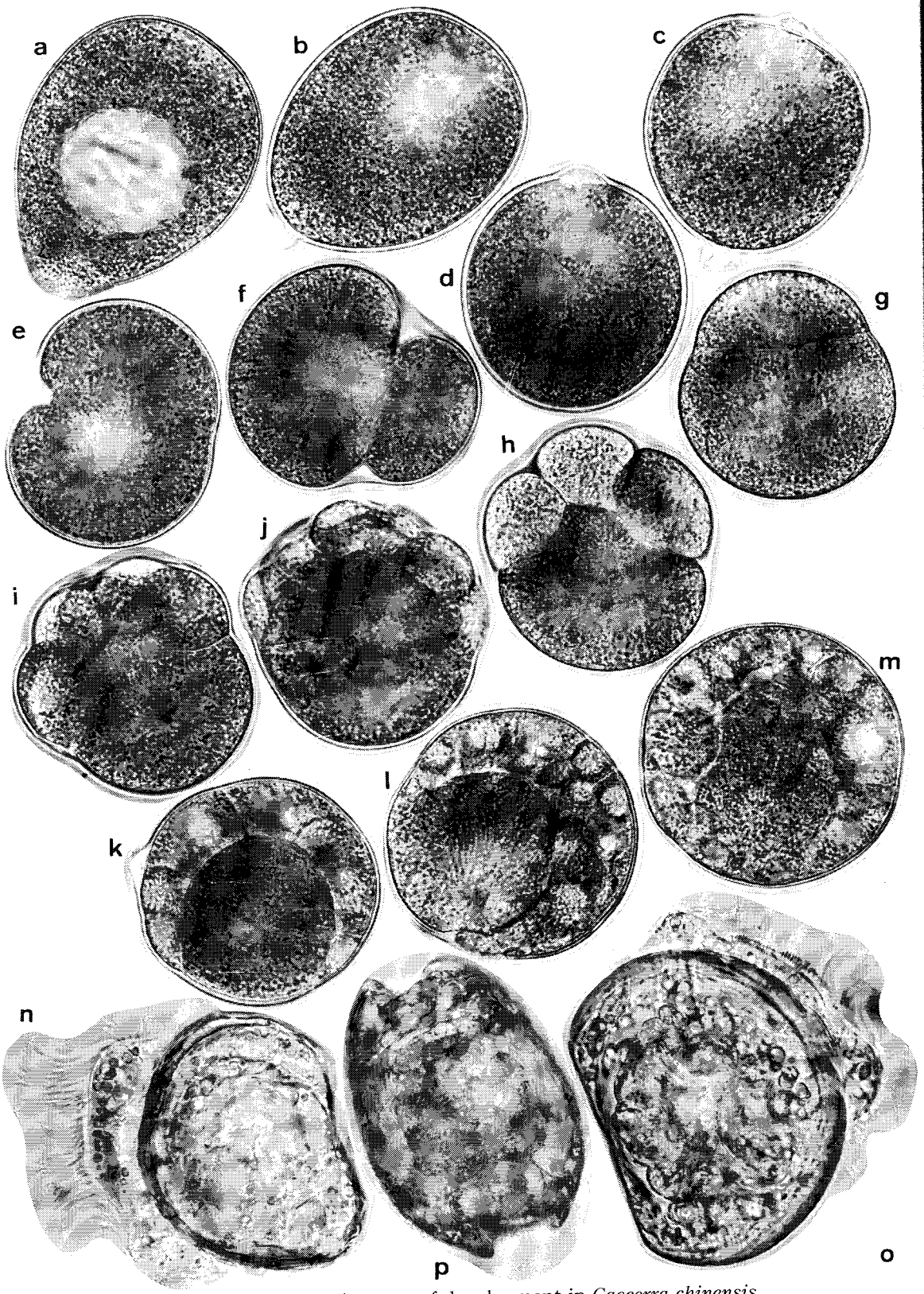

FIGURE 4 : Normal course of development in *Caecerra chinensis.*

molluscs, get into particularly difficult growing up more than the morula stage, because of the opacity and irregulaly arranged cell clasters. The primary trochoblast mentioned before then continues to divide, forming finally the antero-oral ciliary band of the trochophore larva.

According to Mac Bride (1914), using *Dreissensia* (Pelecypoda) found in brachish and fresh water environment, the blastomere 3D forms new daughter cells denominated as 4D and 4d. The 4d-blastomere is much larger than the 4D, and is situated slightly upward the ventral position of the larva. The newly formed 4d-blastomere is generally known as the second somatoblast or the mesoblast. It is usually written in the from of the abbreviation, M. As the results of the successive divisions, the M forms two mesodermal teloblasts. They further give rise to the organs and the tissues of the mesodermal origin (Raven, 1958). According to the 'Textbook of Embryology' written by Mac Bride, it was already described that the 4d-blastomere divided repeatedly sending a number of the mesodermal cells to the antrior position of the embryo. Until the development of this stage, it takes about four hours and a half. The origin of the three germ layers, ecto-, endo- and mesoderm, is thought to derive from the peculiar cell or certain cells in the morula (Fujita, 1929).

Gastrula to larval stage

In the writer's present experiment, by about the period five to six hours after insemination, gastrulation takes place as in a manner of the wrapping movement of the segmented upper micromeres to the underlying macromeres in the form of the loosely woven sheet of cells. (Fig. 4, k-m, showing development of *C. chinensis*). This movement of cells is called 'epiboly'. Epiboly has a important role for the formation of 'gastrula'. As the result of gastrulation, the macromeres 4A-4D located originally in the ventral side of the embryo, are completely covered by the ectoderm. During the advance of the gastrulation, the macromeres are pushed aside in the median ventral side of the gastrula. Therefore, the countour of the gastrula comes near to oval form. By the observation of the gastrula at this time, it can be noticed that the enomous number of the cilia encircles all over the gastrular surface.

By means of the ciliary movement, the embryo begins to move initially irregular, then turns round and round inside the fertilization membrane. Soon, the fertilization menbrane is dissolved by the secretion of the hatching emzyme from the body of the embryo. In the period immediatery before hatching, the ciliary girdle is formed on the upper half of the embryo. After hatching out, the trochophore begins to swim vividly by the ciliary movement of the fine hairs projecting like spokes of the wheel arround the whole circumference of the girdle. It is called as the prototroch or the ciliary circlet. The trochophore which follows from the gastrula is very characteristic larva found in the development of annelids and molluscs. The time required for the entrance on the trochophore stage takes about 7 to 8 hours in the case of *C. gigas*. The total size of the trochophore observed is almost the same bulks as that of the zygote. The external form of the trochophore of *C. gigas, C. chinensis* and the other, are almost spherical-shaped. They, at all tlmes, equip the apical tuft and two lines of ciliations, which encircle the anterior surface of the body as the girdles, lying perpendicular to the axis

of trochophore. The ciliation, locating in the frontal part, is called pre-oral ciliary girdle, and the rear, the post-oral one.

Turning our eyes to the larval composition at the same period of development, the primany digestive tract has been formed with the advance of gastrulation. At this time, the digestive tract is composed of the frontal and the hind parts. The fore-gut usually forms anteriorly the stomodaeum. Its tip later establishes an open connection to the out-side world, namely the mouth. The hind-gut later differentiates into the intestine. It later becomes looped and connects to the proctodaeum.

The normal course of development of the Pelecypoda has been reported by many investigators (Prytherch, 1929; Fujita, 1934; Erdman, 1935). According to them, it was described that the cells, constituting the roof of the trochophore, invaginated inward and differetiated into the rudiment of the larval shell gland. Owing to the secretion of mucoid substances, a couple of the larval shell initially very small in size, are formed at the lateral sides of the larva, then gradually grow up to downward direction.

Subsequent to the trochophore, the larva reaches new type of swimming larva termed veliger. The time required for the arrival of this larva, takes about 15 to 20 hours. The veliger is the very characteristic larval stage in the development of molluscs except cephalopods. The most remarkable structure in the veliger is the velum. This is the ciliated membrane derived from the conspicuous preoral ciliary girdle described before. The veliger, using the velum, can actively move in the sea. Besides, the ciliation also has a role for obtaining food particles into the stomodaeum. By the stimulus, the veliger withdraws the velum into the inner side of the shell. This suggests that the membranous velum has a contractile competency. With the advnce of development, the both sides of the body of the veliger, becomes completely to be covered by the growing larval shells, so that the larva by the loss of buoyancy, sinks down to the bottom of the sea. As to the changes occurring in the larval shells, a couple of shells are later connected by the specialized structure called 'hinge', a joint on which the tip of the two shells swings with each other. Subsequent to its formation, the aductor muscles are formed hence the two shells become to be linked with each other. In a little while, most of the larval body become to be kept under the inner sides of the growing shells. The ventral tip of the shell is composed of the straight line, and the other two sides as a whole, oval-shaped. Accordingly, the veliger reaching this stage, is designated as D-shaped larva. The pattern of the larval shell (Ranson, 1960) and the hinge constituents (Rees, 1950) are typical for the kind of every species of pelecypods. So they can be used for the recognition site of the classification.

As to the changes talking place during morphogenesis, there are a few investigations even in the cases of the familiar species, such as oyster, clam and scallop as far as the writer's awareness. Among them, the detailed studies occurring in the veliger, was done by using the American fresh or brachish water inhabiting pelecypod, *Dreissensid polymorpha* (Mac Bride, 1914). In the text book, he cited the excellent works, previously done by Meisenheimer (1901). Though this is merely the writer's personal opinion, it seems clear that the morphological changes described by them, are the typical of pelecypodian development. Therefore, the writers intend to refer their illustrations and the interpretations for the reader's under-

standing (Fig. 2, a–d and Fig. 1, m–q). In his text book, when the shell gland is formed at the later stage of the trochophore, the liver rudiment appears as a couple of a small vesicles on both sides of the larval body. At about the same period, the digestive tract pass through the body of the larva, initiating from the stomodaeum anteriory, and terminating to the proctodaeum. The mouth opens close to the position of the aductor muscles, and the anus opens near the mass of the mesenchyme. At this time, the trochophore reaches to grow into the early veliger larva. While the veliger is being established, the crystaline style, a semitransparent caecal organ, is formed at first as a small vesicle in the postero–ventral position of the stomach.

The crystaline style is known as a very special organ of the certain pelecypods, which is thought to contain much digestive enzyme or emzymes. They play a important role for the digestion. Almost nearly the same time, the foot gradually grows in a finger like form until its tip projects from the shells. The heart is formed at the anterior part of the intestine, forming a small tubular structure around the intestinal tract. In the beginnnng, the intestine is covered by a number of mesenchymal cells, which later develop two sheets of loosely woven connective tissue, namely the inner– and the outer–membranes. The internal space surrounded by two membranes becomes to be the rudiment of the heart. The kidney appears initially as a couple of the spherical cell masses on both sides of the intestine. Each of the cell mass then aquires a lumen, and finally form a couple of tubules. The one extremity of the tubule opens into the pericardial cavity, and the other one enters to the mantle cavity, thus forming the couple of the excretory duct. The waste substances, carried by the blood stream, are filtrated through the kidneys, and the excretion substaces are excluded outword.

It is evident from the above account that all major organs and organ systems are formed during the later veliger stage. Therefore, this period is called the 'period of organogenesis'.

As discussed previously, the active movement of the veliger using the velum, will dazzle the investigator's interest. However, it must be understood that the motive organs and musclar systems are concomitantly established during the larval development. When the larval metamorphosis takes place, the vividly beating cilia tend to disintegrate with the velar membrane. At this period, it is thought that the veliger comes into the full developed larval stage. From this period to the unboned stage, the veliger forms larval foot as a mass of cells, projecting along the dorso–ventral axis of the larval body. After a while the larval foot comes in contact with a solid matter. At that time, the larva withdraws the velum, and begins to crawl about on the sea botton for several period. This behavior of the larval shells is known as the 'setting'. According to Galtsoff (1964), a long lustrous bunch of the filament named byssus appears. It is formed by the secretion of conchioline like substances from the byssal gland, which terminates to an opening found at the base of the foot. This opening is thought to be serviceable for the larval shells, adhering to sea bottom by means of the secretion of the mucoid substances. Unfortunately, the formation of the byssus is not be able to ascertained in the writer's observation. Approximately at this time, rapid disintegration of the larval organs takes place. In these ways, the larva advances into the juvenile stage. The most striking chnages taking place during the metamorphosis of the veliger, are the rapid disintegration of the velum.

Concomitantly, the velar retractor muscles also regress and are absorbed into the body of the young shell.

According to Hori (1930), the juvenil shell begins to attach the solid matter on the sea bottom at right side of the larval shell. The attachment is thought to be accomplished by the secretion of cementing substance produced from the byssus gland. Subsequent to the attachiment, the gills are fromed from the mass of cells residing in the basement of the foot. After a while, the rudiment of the gill grows. Then, the larval gill are formed hanging down as sheets of curtains into the mantle cavity. The period of metamorphosis mentioned above is characterized by the rapid growth of larval boby and the maturation of the organ systems. In this manner, the larvae, by way of several young shell stages, gradually reach the adult form.

In the present expreriment, the writers devoted first to pursue the cell linage in the progress of development. In spite of the writer's expectation, it was difficult to pursue exactly, because of the opacity of the blastomeres and the irregularly arranged masses of cells. So they intended to review the development of the Pelecypoda, using their previous studies and the excellent discriptions having done by many investigators.

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EXPLANATION OF THE FIGURE

Fig. 1. (Page, 43) Normal development of the eggs of the oyster, *Crassostrea gigas*. a, unfertilized egg immediately after shedding; b, fertilizd egg five minutes after insemination; c, elimination of first polar body; d, early stage of polar lobe formation. Note the elimination of the second polar body; e, initiation of first cleavage; f, trefoil stage; g. progress of furrowing. Note the union of the polar lobe to one side of daughter blastomere CD; h, two cell stage; i, four cell stage; j, initiation of 8 cell stage; k, 22 cell stage; l, initiation of epiboly, side view; m, the same as l, polar view; n, trochophore;

Fig. 2. (Page, 44) a–d. Larval development of *Dreissensia polymorpha* from the veliger to the young shell. a, young veliger larva seen from the side; b, the same as a, seen from the ventral surface; c, older veliger larva seen from the side; d, side view of the young shell after the metamorphosis taken place. a, anus; add. a, anterior adductor muscle; a. p, apical plate; br, rudiment of gill-papillae; byss, byssus gland; add. p, posterior adductor muscle; coe, rudiment of coelon; c. p, cerebral pit; cr. s, crystalline sac; f, foot; H, heart; hep, lobes of

liver; int, intestine; k, rudiment of kidney; l.n, larval kidney; l.p, lateral palp; m. tr, metatroch; o, mouth; ot, otocyst; per, rudiment of pericardium; p. g, pedal ganglion; r. d, dorsal retractor muscle; r. f, retractor of foot; p. g, pedal ganglion; r. d, dorsal retractor muscle; sh, shell; t. tr, telotroph; r. g, visceral ganglion; (after Mac Bride, Text book of embryology. 1904)

e–f. Larval development of *Crassostrea gigas*. e, veliger larva; f, D–shaped larva;

Fig. 3 (Page, 45) . a–l. Succesive process of the elimination of first polar boby. To grasp the process of the maturation division readily understandable, the fertilized eggs of *Caecera chinensis* were exposed to DNP just after the breakdown of the germinal vesicle (Yamamoto, 1974). a–b, process of elimination of first polar body in living cells; e–l, the same as a–d. Sectioned and stained preparations. (with Heidenhein's iron hematoxylin); l, Completion of the elimination of the first polar body, polar view;

m–g, The process of gastrulation and the formation of the shell gland. Sagittal section of the embryo of *Dreissensia polymorpha* (after Mac Bride 1914). ap, apical plate; bl, blastopore; end, endoderm; h. p, hepatic cells which will eventually form the liver; M, primary mesoderm cell; p. tr, prototroch; s. g, shell gland; stom, stomodaeum.

Fig. 4. (Page, 46) Normal development of the eggs of the bivalve, *Caecera chinensis*. a, unfertilized egg immediately after shedding; b–d, process of maturation division; d, egg preceding syngamy. Both pronuclei are seen just under the second polar body; e–g, process of first cleavage; h, four cell stage; i, 8 cell stage; j–m, process of epiboly; n, veliger larva; o, more later stage; p, D–shaped larva;