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EMBRYONIC DEVELOPMENT OF *BIOMPHALARIA GLABRATA* (SAY, 1818) (MOLLUSCA, GASTROPODA, PLANORBIDAE): A PRACTICAL GUIDE TO THE MAIN STAGES

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ABSTRACT

The morphology of the gastropod mollusk *Biomphalaria glabrata* (Say, 1818) from the stage proceeding the first egg cleavage to the hippo stage is presented. The morphogenetic changes along its development were followed by *in vivo* observation and by cell lineage studies, with description of the origin and function of the main structures that characterize the embryonic and larval phases.

Key Words: *Biomphalaria glabrata,* Planorbidae, cleavage, embryonic, development.

INTRODUCTION

The studies of cell lineages in mollusks are of great interest for research in experimental embryology. The first ones date back to the end of the last century, when different species were investigated by many authors, such as *Neritina fluviatilis* by Blochmann (1882), *Umbrella mediterranea* by Heymons (1893), *Ischnochiton* sp. by Heath (1899), *Planorbis trivolvis* by Holmes (1900), *Physa fontinalis* by Wierzejski (1905), *Littorina obtusata* by Delsman (1912, 1914), *Limnaea stagnalis* by Raven (1946, 1958) and Verdonk (1965) and *Dentalium* sp. by Dongen (1976). Camey & Verdonk (1970) carried out studies on cell lineage of the gastropod *Biomphalaria glabrata* (Say, 1818); they analyzed its embryonic stages, with emphasis on the cephalic region, from the first egg cleavages to the veliger stage.

This guide was prepared in order to describe the various stages of the embryonic and larval development of *B. glabrata* and was also especially planned for those initiating research activities or attending or giving lectures on embryology. It covers the stages between the first egg cleavages, which are characterized by the spiral (helicoidal) type, and the trochophore and veliger stages, all of them occurring within the egg capsule.

MATERIAL AND METHODS

Egg masses of *Biomphalaria glabrata* (Say, 1818) were picked up in Belo Horizonte, State of Minas Gerais, Brazil, and maintained for several years in the laboratory, at 25°C, in a climatic chamber.

The developmental stages were followed and photographed *in vivo* by using a Zeiss photomicroscope. From the first to the fourth cleavages, the stages were photographed at intervals of 1 to 30 minutes. From the fourth cleavage on, the time intervals were of 5 hours. From the early trochophore stage on, the larvae were immobilized with ethyl ether for photography. These time intervals were decided upon based on data obtained in previous observations of the embryonic development of this species.

Stage 1 (Fig. 1B) was considered as the starting point for the determination of the embryo age due to the variation of the time intervals elapsing between egg laying and first egg cleavage, as pointed out by Raven (1946). Due to the asynchrony of egg division in the same egg-mass, zero time was considered to be the time when 50% of the eggs were in stage 1.

For the cell lineage study, the embryos were prepared according to the technique described by Verdonk (1965), which basically consists of the following steps: the embryos were decapsulated, fixed with 0.75% $AgNO_3$ for a few seconds until the cell contours became sharp, dehydrated in an alcohol series, cleared with xylene, and mounted with Permount between a slide and a coverslip. Embryos and larvae were drawn using a Zeiss camera lucida, and the nomenclature adopted was that of Conklin (1897).

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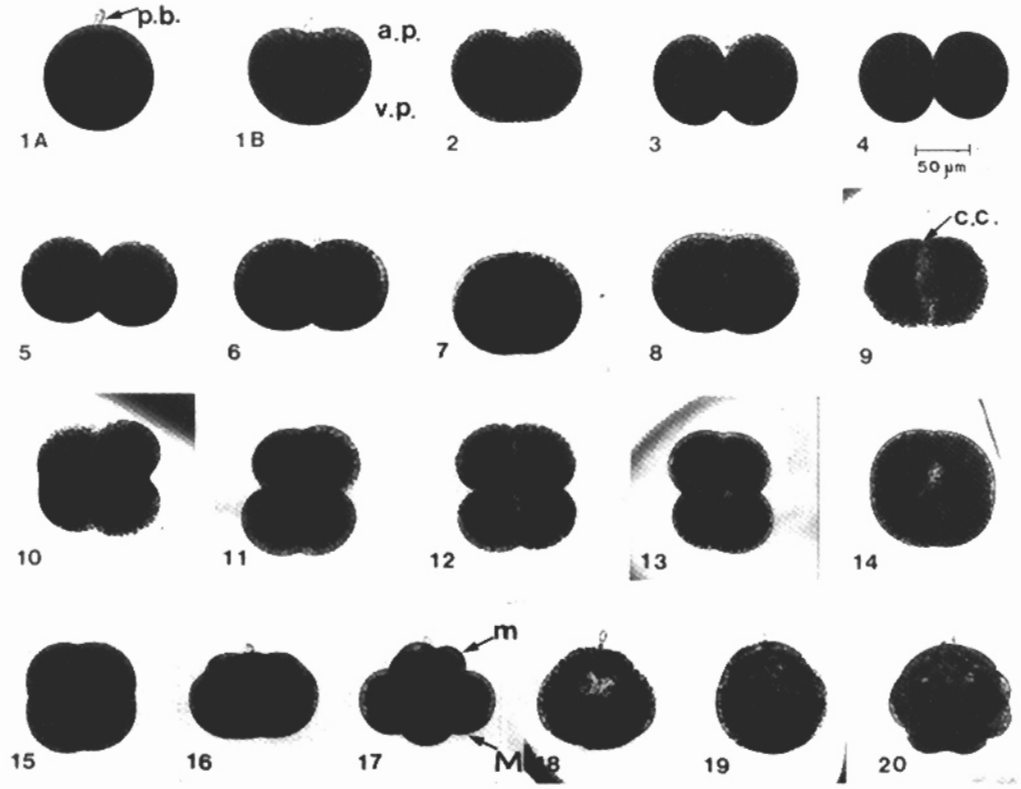


FIG. 1. Early development of *Biomphalaria glabrata*. (1A) Undivided egg with two polar bodies. (1B) Beginning of first cleavage, with appearance of the cleavage furrow in the animal pole (0 hr). (2) Appearance of the cleavage furrow, after 3 min. (3) After 4 min. (4) Two blastomeres attached to each other by a small cytoplasmic area, after 10 min. (5), (6) and (7) the blastomeres present a gradually larger contact surface: after 21, 26 and 38 min., respectively, (8) Emergence of the cleavage cavity after 45 min. (9) Cleavage cavity appearing more clearly, after 75 min. (10) Second cleavage, after 80 min. (11) Four blastomere stage, after 91 min. (12) Beginning of the appearence of the cleavage cavity, at 98 min. (13) After 103 min. (14) After 123 min. (15) Almost rounded surface of the blastomeres, at 134 min. (16) Third cleavage with formation of the first quartette of micromeres, at 160 min. (17) At 165 min. (18) At 173 min. (19) At 181 min. (20) Fourth cleavage, at 230 min. p.b. = polar body, a.p. = animal pole, v.p. = vegetative pole, c.c. = cleavage cavity, m = micromere, M = macromere.

RESULTS

Table 1 shows the main stages of the embryonic development of *B. glabrata,* from the beginning of the first cleavage to the hippo stage.

The morphological features of *B. glabrata* during the main stages of its embryonic and larval development are illustrated in Figures 1-3. The embryonic and larval cells are schematically presented in Figure 4.

Figure 1 presents a sequence of morphogenetic alterations covering the period of time between the zygote (or fertilized egg) and the fourth cleavage stage. Stage 1A shows the zygote before the first cleavage; two polar bodies (p.b.) can be seen at the upper apical region, exactly in the animal pole. The diameter of a viable egg is approximately 100 µm long. Stages from 1 to 19 are equivalent to those described for *Limnaea stagnalis* by Raven (1946). The first cleavage furrow appears during stage 1B in the animal pole. This phase corresponds to the beginning of the first cleavage, which is total and passes through the animal and vegetative poles. Stages 2 and 3 occur approximately 3 or 4 minutes after stage 1B, respectively, with

TABLE 1. Main stages of the embryonic development of *B. glabrata* at 25°C.

Embryonic stage	Time interval between observations	Number of stage in figures
Beginning of the 1st cleavage	0	1B
2nd cleavage	80 min.	10
3rd cleavage	160 min.	16
4th cleavage	230 min.	20
blastula	15 hrs.	21
gastrula	26 hrs.	23
early trochophore	43 hrs.	25
late trochophore	66 hrs.	27
early veliger	96 hrs.	28
late veliger	120 hrs.	29
hippo stage	144 hrs.	30

cleavage furrow becoming gradually more predominant; the cleavage of the animal pole region is more rapid than that of the vegetative one. At stage 4 the cleavage furrow almost completely divides the zygote into two blastomeres, both of them presenting a well-rounded surface and linked to each other by only a small cytoplasmic bridge. This phase occurs approximately 10 minutes after stage 1B (Fig. 1, stage 4; Fig. 4, stage 31). After stage 5, at approximately 21 minutes from the beginning of zero time, the two blastomeres approach each other, increasing their contact surface, that is, they flatten against each other, with the formation of a separating blastomeric membrane (Fig. 1, stage 5). During stages 6 and 7 (Fig. 1), the boundaries of the blastomeres can hardly be defined; these stages occur approximately 26 and 38 minutes after stage 1B, respectively.

The cleavage cavity, the function of which is osmotic regulation, can be seen between the two blastomeres at stage 8, approximately 45 minutes after stage 1B (Fig. 1). This cavity increases in size, and both blastomeres have an egg shape (Fig. 1, stages 8 and 9).

The beginning of the second cleavage (Fig. 1, stage 10) occurs 80 minutes after the first cleavage. Because the blastomeres do not divide synchronously, the cleavage furrow of one of the blastomeres appears before the other, so that this stage presents an asymmetrical shape (Fig. 1, stage 10). This cleavage is meridional and total.

During stage 11 (Fig. 1), after approximately 91 minutes, the blastomeres show a rounded shape but are not on the same plane when observed obliquely; that is, blastomeres A and C (Fig. 4, stage 32) are linked to each other by the furrow in the animal pole, and blastomeres B and D by the furrow in the vegetative pole.

At stage 12 (Fig. 1), after approximately 98 minutes, the cleavage furrow linking the alternate blastomeres in the animal and vegetative poles of the egg can be seen more clearly (Fig. 1, stage 12; Fig. 4, stage 32). At stages 13 (after 103 min) and 14 (after 123 min) (Fig. 1), the blastomeres are already more closely joined, and the cleavage cavity between them begins to appear. Material from the cleavage cavity then starts coming out, with contraction of the whole egg causing a visible reduction in diameter (Fig. 1, stage 15).

The third cleavage is laeotropic and occurs in the subequatorial plane of the egg, with the formation of the first micromere quartette (1a to 1d), approximately 160 minutes after the beginning of the first cleavage (Fig. 1, stage 16; Fig. 4, stage 33). A gradual increase of the cleavage cavity is observed during stages 17 (after 165 min), 18 (after 173 min) and 19 (Fig. 1).

Stage 20 (Fig. 1) illustrates the egg at the fourth cleavage stage, which is dexiotropic. The fourth cleavage is subequatorial and occurs approximately 230 minutes after stage 1B (Fig. 4, stage 34), originating the second micromere quartette (2a to 2d).

The embryo reaches the blastula stage approximately 10 to 23 hours after the 1st cleavage (Figs. 21, 22).

Stages 35 and 36 (Fig. 4) show the embryo with about 64 and 130 cells, respectively, and presenting a set of cells forming a cross-like figure ($1a^{11}$ to $1d^{11}$, $1a^{12}$ to $1d^{12}$ and $2a^{11}$ to $2d^{11}$ cell lineages) in the animal pole, which gives origin to almost the whole head region.

Gastrulation occurs about 24 to 39 hours after the first cleavage (Fig. 2, stages 23, 24A,

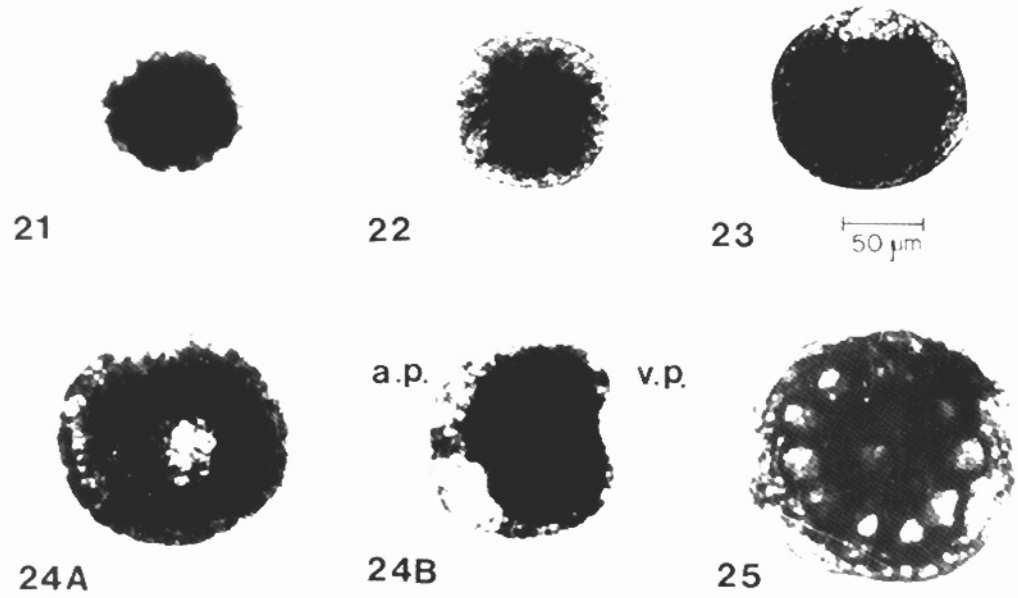


FIG. 2. Different stages of development of *Biomphalaria glabrata*. (21) Blastula, 15 hrs. after 1st cleavage. (22) Blastula, 21 hrs. and 30 min. after 1st cleavage (23) Gastrula, 26 hrs. after 1st cleavage. (24A) Late gastrula as seen from the vegetative pole after 34 hrs. (24B) Lateral view of the 34 hrs. gastrula stage. (25) Early trochophore, after 43 hrs. a.p. = animal pole, v.p. = vegetative pole.

24B; Fig. 4, stages 37 and 38). Flattening of the vegetative pole region towards the animal pole then occurs, followed by the invagination of this region, with the formation of a spherical opening, the blastopore, which then becomes gradually smaller.

The early trochophore stage appears approximately 40 to 65 hours after the first cleavage, and is characterized by the first larval movements (Fig. 2, stage 25; Fig. 3, stage 26) by means of the ciliated cells of the prototroch, which divides the larva in the pretrochal region, characterized by the presence of an apical plate, head vesicle and cephalic plates, and the posttrochal region, characterized by the presence of a shell gland, stomodeum and foot (Fig. 4, stage 39). The prototroch shows a double row of cells, an upper one formed by $2d^{111}$, $2b^{112}$, $1a^{21}$, $1a^{22}$, $1b^{21}$ and $1b^{22}$ cells, and a lower ciliated one consisting of $2b^{211}$ and $2b^{121}$ cells and of cells descending from $2b^{122}$ and $2b^{212}$ cells (Fig. 4, stage 39).

The late trochophore stage occurs approximately 65 to 80 hours after the first cleavage, with the larva having a slightly elongated, kidney-like shape. In this phase, cells responsible for the formation of the head and foot can be seen in its anterior region, and in the dorsal region one can see the shell gland (Fig. 3, stage 27). Stage 40 (Fig. 4) shows a greater development of the prototroch, leading to more active larval movements. The pretrochal region, located above the prototroch, is formed by a set of cells that gives rise to such larval structures as the apical plate ($1a^{111}$ to $1d^{111}$, $1a^{1121}$, $1b^{1121}$ and $1b^{1211}$), cephalic plates (cells located laterally to the apical plate), and the head vesicle ($1c^{21}$, $1d^{21}$, $1c^{22}$, $1d^{22}$, $1a^{122}$, $1c^{122}$, $1d^{122}$, $1d^{1211}$, $1d^{1212}$, $2a^{11}$, $2c^{11}$, $2d^{11}$), which in turn will form the head. In the posttrochal region, below the prototroch, are the stomodeum (M), which will give origin to the mouth, and cells that will form the foot and the shell gland.

The time interval between 80 and 100 hours after the first cleavage corresponds to the early veliger stage. The shell and foot are then more developed (Fig. 3, stage 28). The prototroch has evolved into the velum (Fig. 4, stage 41). In this stage, the shell gland shifts towards the right side.

The veliger stage occurs about 120 hours after the first egg cleavage. The eyes become more visible, and the elevation of the tentacle regions can be observed, as well as the mouth, foot and shell (Fig. 3, stage 29). Stage

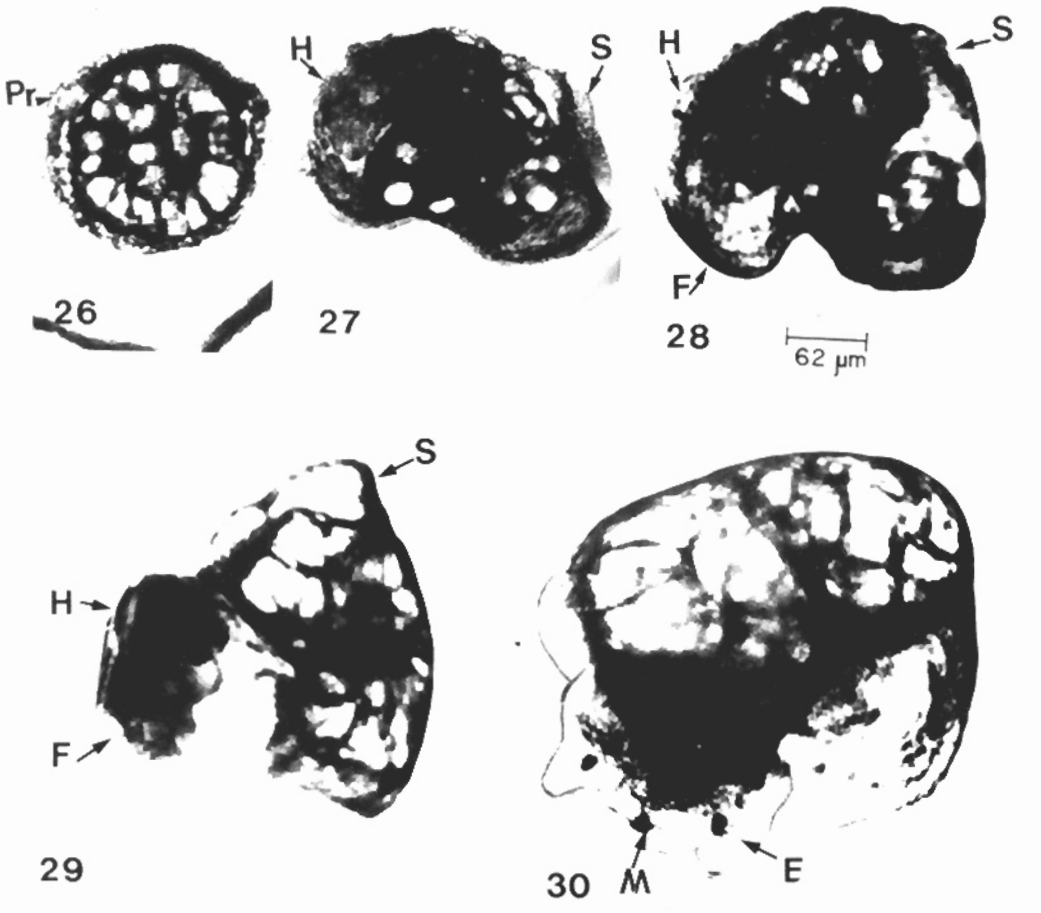


FIG. 3. Different stages of development of *Biomphalaria glabrata*. (26) Early trochophore, 55 hrs. after 1st cleavage. (27) Trochophore, after 66 hrs. (28) Early veliger, after 96 hrs. (29) Veliger, after 120 hrs. (30) Hippo stage, 144 hrs. after first cleavage. E = eyes, F = foot, H = head, M = mouth, S = shell, Pr = prototroch.

42 (Fig. 4) shows that the apical plates and head do not undergo any changes, while the cephalic plates go on dividing to form the tentacles and eyes.

The hippo stage is a phase of larval development that occurs within the egg capsule approximately 144 hours after the first cleavage. At this stage, the eyes and tentacles in the pretrochal region are already well developed, and in the posttrochal region the foot has grown in size and is much more differentiated. The shell starts coiling and covers almost the whole body (Fig. 3, stage 30). At 25°C, the young snail hatches from the egg capsule between the sixth and ninth day after the first cleavage.

DISCUSSION

Due to their semi-transparence and easy maintenance in the laboratory, the eggs of *B. glabrata* represent a suitable material for studies on embryology, allowing a good *in vivo* observation. At egg-laying, its egg-masses already contain fertilized eggs and, after approximately 30 minutes (at 25°C), the emission of the first polar body occurs, as a result of the first meiotic division. Approximately 60 minutes later, the second polar body emerges. Both polar bodies remain in the animal pole during the first cleavages. Within 60 minutes after the emission of the second polar body, the fusion of the male and

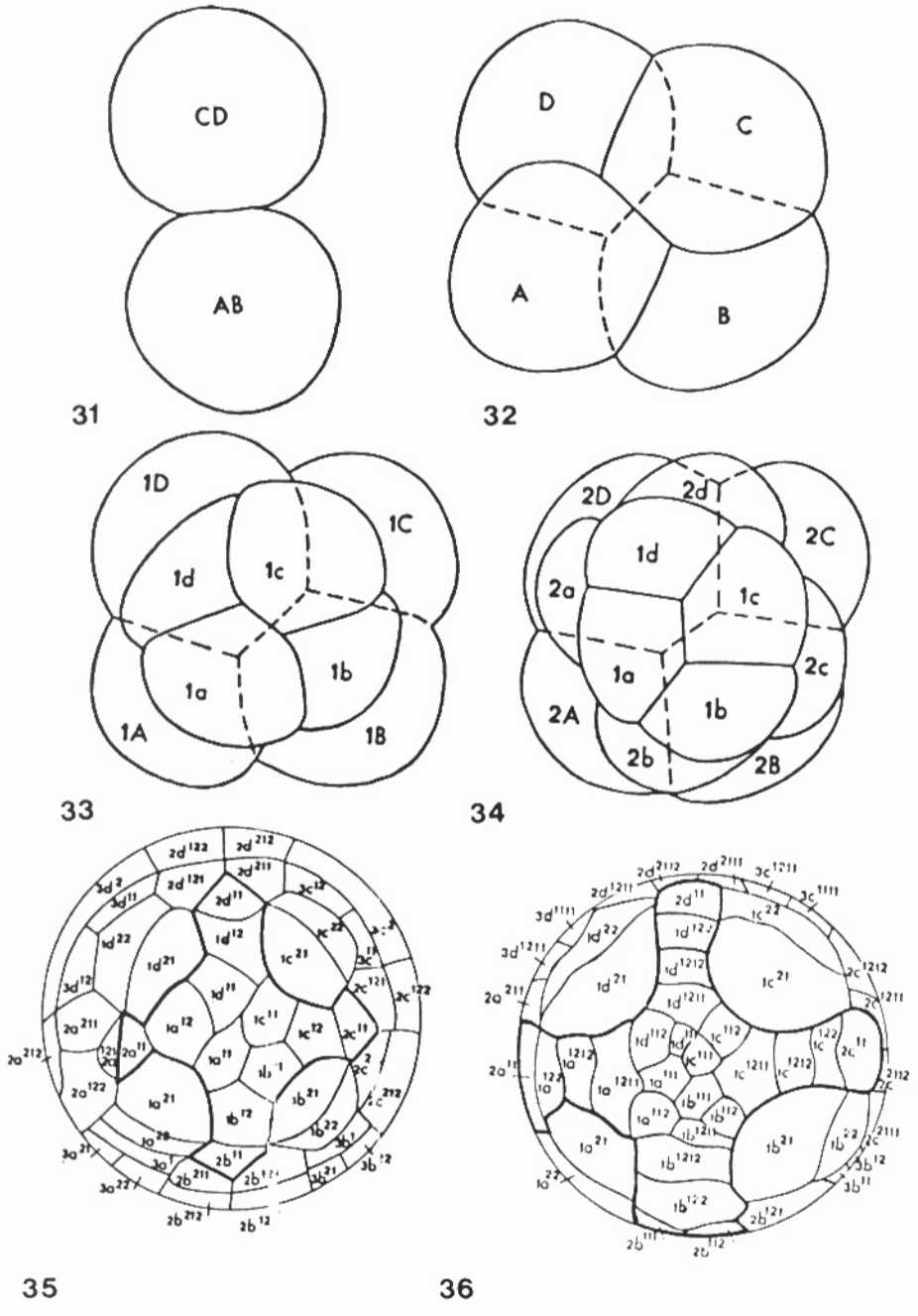
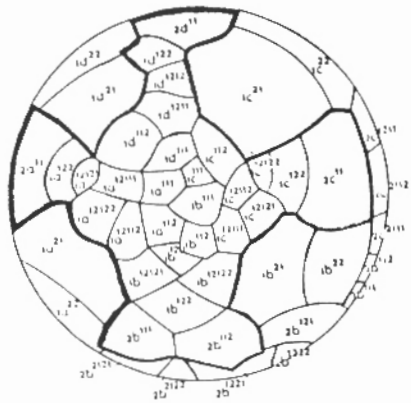


FIG. 4. Outline of cell boundaries on developing eggs of *Biomphalaria glabrata*. (31) 1st cleavage with 2 blastomeres. (32) 2nd cleavage with 4 blastomeres, 80 min. after 1st cleavage. (33) 3rd cleavage with 1st quartette of micromeres (1a to 1d) and with 8 blastomeres, after 160 min. (34) 4th cleavage with 2nd quartette of micromeres (2a to 2d) and 12 blastomeres, after 230 min. (35) Blastula stage with 64 blastomeres, showing the cross figure at the animal pole after 15 hrs. (36). Blastula with 130 cells (animal pole) after 21 hrs. and 30 min. (37). Gastrula, after 26 hrs. (38) Gastrula after 34 hrs. (39) Early trochophore after 34 hrs. (40) Trochophora after 66 hrs. (41) Early veliger after 96 hrs. (42) Veliger stage after 120 hrs. M = mouth, A.P. = apical plate, H.V. = head vesicle, C.P. = cephalic plate, V = velum, T = Tentacle, E = eyes, F = foot, Pr. = prototroch.



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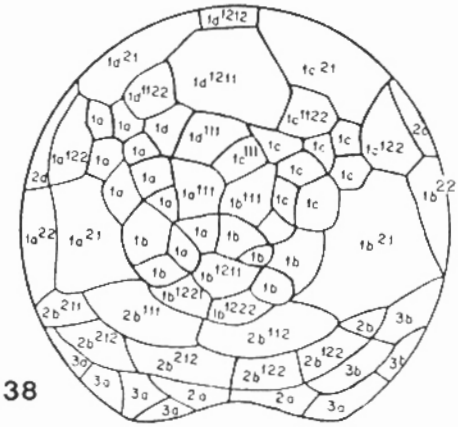

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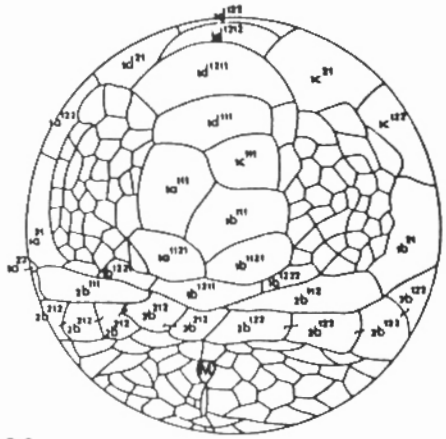

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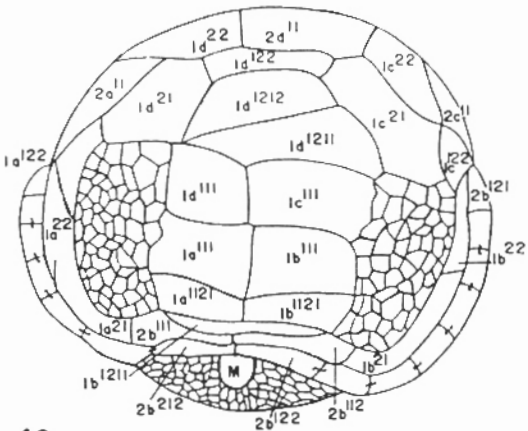

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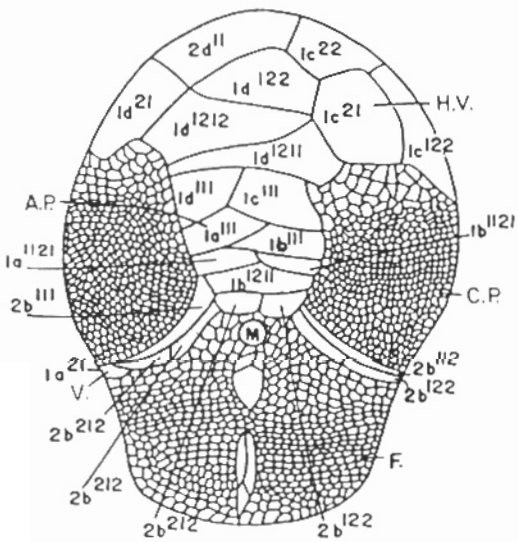

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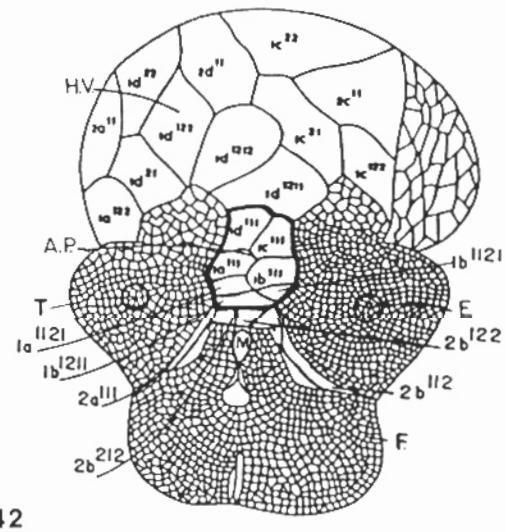

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female pronuclei occurs, the egg now being ready for the first cleavage (Camey, 1968).

Except for cephalopods, the cleavage pattern of mollusks is of the spiral type; that is, blastomere cleavage is oblique in relation to its axis and is of the determinative type, because the different egg regions are going to form the future organs (Raven, 1958).

The developmental stages described here are similar to those of *Limnaea stagnalis*, with some differences. Cleavage is laeotropic or reverse in *B. glabrata* (Camey & Verdonk, 1970) and dexiotropic in *L. stagnalis* (Verdonk, 1965). The differentiation of the type of cleavage occurs according to the orientation of the division spindle. When this is oblique in relation to the axis of the egg in clockwise direction, cleavage is dexiotropic, and when the spindle is oblique but in a counterclockwise direction, cleavage is laeotropic. The first indication of the orientation of the cleavage spindle in *B. glabrata* occurs during the third cleavage (Camey & Verdonk, 1970).

In the trochophore stage, the shell gland is shifted to the right side in *B. glabrata* (Camey, 1968) and to the left side in *L. stagnalis* (Verdonk, 1965).

When the helicoidal shell of an adult of *L. stagnalis* is placed with the apex turned up, it can be seen that the opening is towards the right side. In *B. glabrata*, with a planispiral shell, it is difficult to determine the direction of the shell opening, except for its shift in a direction opposite to that of *L. stagnalis* during larval development. It may be concluded, therefore, that the cleavage pattern and shell opening of *B. glabrata* are laeotropic.

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