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Computer Simulation of Early Embryonic Development

J. J. BEZEM AND CHR. P. RAVEN

Zoological Laboratory, University of Utrecht, Utrecht 2506, *The Netherlands*

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A simple model, formulated in terms of elementary geometry, is presented, describing the early development of *Lymnaea stagnalis*. It includes the main morphogenetic processes active at this stage: cell division, cell flattening and differentiation. Though the model has been designed primarily to fit the data of *Lymnaea*, it appears to have a more general validity: without altering its essential features it may be adapted to other species simply by changing the values of certain parameters. As an example, some results on *Podarke obscura* are given at the end of the paper.

1. Introduction

Early development in *Lymnaea stagnalis* is a process of remarkable regularity, in particular the so-called radialized development induced by treating the egg with a solution of lithium chloride. Though the latter process finally produces an embryo with abnormal properties, it will be discussed first. Its rules are few and simple, and up to a certain stage they remain valid in the normal development of untreated eggs. The next section, therefore, deals with the facts concerning cell division, cell flattening and differentiation observed in the radially symmetric embryos arising from lithium treated eggs. The points on which normal development differs from the abnormal will be discussed later on.

The information available on both types of development is contained mainly in series of drawings, showing views from different sides of embryos in various stages of development. There is, therefore, little choice beyond a geometrical model as a basis for a simulation program generating comparable structures. A model of this kind cannot have a high degree of abstraction: it has to keep rather close to the real thing and will retain a lot of detail. Nevertheless, it may be useful in several respects.

Execution of the program repeatedly requires the input of certain data, to be discussed in the paragraph on "external parameters". Since most of these data are not available in a sufficiently precise numerical form, the model furnishes a list of desiderata for future empirical research. Moreover, the existence of these external parameters as such must be considered as an

imperfection of the model. In reality, embryonic development in *Lymnaea* is, to a high degree, an autonomous process, requiring little or no outside information. Thus, the present model may suggest, on what points theoretical considerations should concentrate in order to eliminate the need for external input. The final aim should be a program that computes all relevant data itself and thus attains the same autonomy as the real process.

2. Radialized Development

(A) CELL DIVISION

Cell division (or cleavage) in lithium treated eggs is governed by the following rules.

(1) The behaviour of each cell seems to be defined entirely by its internal state, which in its turn is a function only of the series of divisions by which the cell has been produced. No interaction of any kind between cells is noticeable up to the 68-cell stage, apart from the spatial relations to be discussed in the paragraph on cell flattening.

(2) From the four-cell stage onwards the cells are arranged in homologous quartets, each quartet consisting of two pairs of cells that occupy symmetrical positions with regard to the egg axis. As an example, the configuration in the four-cell stage is shown in Fig. 1. All cells of the same quartet divide (or in

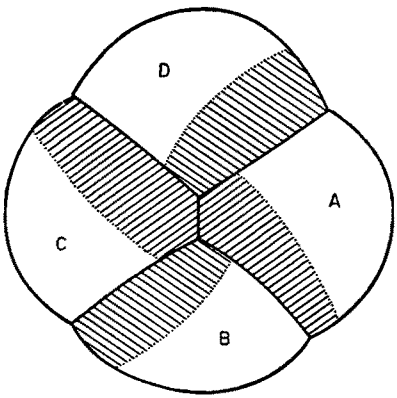

FIG. 1. Four-cell stage (real embryo) seen from the animal pole. The dotted lines indicate the intersections of the cleavage planes with the cell surfaces; the cleavage is dexiotropic. Hatched areas correspond with cell parts that after division will constitute the animal daughter cells. Cells in opposite quadrants are symmetrical with regard to the egg axis, which is perpendicular to the plane of drawing. Cells in quadrants A and C are located slightly higher than those in quadrants B and D. This difference in height is one of the parameters of the model.

later stages differentiate) at the same time and in the same way. Thus, the original symmetry is preserved throughout the period of development covered by the model, i.e. up to the 68-cell stage.

(3) The cleavage of a cell produces two daughter cells that are separated by a plane, called the "cleavage plane". From observations in later stages it is evident that all cleavage planes pass approximately through a fixed point on the egg axis, called the "cleavage centre".

(4) From the four-cell stage onwards there occur only two types of division, called "dexiotropic" and "laeotropic" respectively. They are distinguished from each other by the orientation of the cleavage plane, resulting in a relative displacement of the "animal" (=upper) and "vegetative" (=lower) daughter cells. Viewed from the animal pole, the animal cells turn clockwise in a dexiotropic and counterclockwise in a laeotropic division. Figure 1 gives a rough idea of the position of the plane in a dexiotropic division; a precise definition is given in Fig. 3.

(5) The two types of division alternate regularly in consecutive cell generations. The cleavage is dexiotropic in cells of even generation (assigning generation 0 to the uncleaved egg) and laeotropic in cells of uneven generation.

(B) CELL FLATTENING

The cleavage of each quartet is followed by a short period of cell movements. The newly formed cells, at first more or less spherical in shape, join the body of cells already present and assume the flattened appearance that may be observed in the pictures of real embryos shown in this paper [see e.g. Figs 1, 2(b) and 4(c)]. This process has nothing to do with the internal states of the cells; neither can it be attributed to active cell interactions in the strict sense. Presumably, it is a result of purely mechanical causes. At first, therefore, it was considered to fall outside the scope of the model, and a program was run without rules for cell flattening except for daughter cells of the same cell, where the process easily could be made part of the cleavage procedure.

Although the cell pattern generated by this program in the main region of interest, i.e. the part of the egg around the animal pole, resembled the pattern in the actual embryo fairly well, other features were less satisfactory. The cells show a tendency to drift apart, which is observable already in the early stages (cf. Fig. 4), and later on leads to a marked distortion in the vegetative part of the egg. It is clear that cell flattening has a great influence, not only on the shape of the cells, but also on their positions. From our point of view the shape of cells is not important but their positions are essential, since the main purpose of the model is, to serve as a basis for a computer

program that generates cell patterns resembling as closely as possible those that are observed in real embryos, not only around the animal pole but elsewhere as well. Thus, there is sufficient reason to include cell flattening in the model.

Though nothing is known about the mechanism of the process, its results in simple structures such as the two-cell and four-cell stages are sufficiently clear to furnish a basis for a good approximation (cf. Fig. 2). How this is incorporated in the program will be explained presently. This additional feature of the model is not characteristic for the radialized embryo, but remains exactly the same in normal development.

(c) DIFFERENTIATION

In a certain period of development, starting with the 36-cell stage, the regular sequence of cell divisions is interrupted. An increasing number of quartets stop dividing and, instead, differentiate to a few well-defined cell types, from which later on certain larval organs are derived. As in the case of cell flattening, the underlying mechanism is not known in sufficient detail, but fortunately the resulting pattern shows a regularity that is formalized easily. There are four types of cells, three of which are arranged in concentric zones around the animal pole, while the fourth occupies the vegetative part of the egg. The entire arrangement suggests the existence of some morphogenetic factor, the activity of which radiates from a fixed centre in all directions. This makes the fate of each cell dependent on its distance from that centre, a criterion that is incorporated easily in the model.

3. The Model

(a) REPRESENTATION OF CELLS

It has been argued in the introduction that for checking purposes a geometrical model is preferable. The first point to decide is then, how to represent cells in such a model. It is evidently impossible as well as useless to render the complicated shapes of cells exactly. Yet, the positional consequences of cell flattening should be retained. A representation of cells by spheres meets many requirements: spheres are easy to handle in computations, views of systems of spheres are readily constructed, and flattening of cells can be simulated by letting the spheres intersect each other as shown in Fig. 2.

Immediately after cleavage, the daughter cells are practically spherical in shape [Fig. 2(a)], but as cell flattening proceeds, the plane of contact increases until on the outer surface only a very shallow cleavage groove remains visible [Fig. 2(b)]. Neglecting this groove altogether introduces only

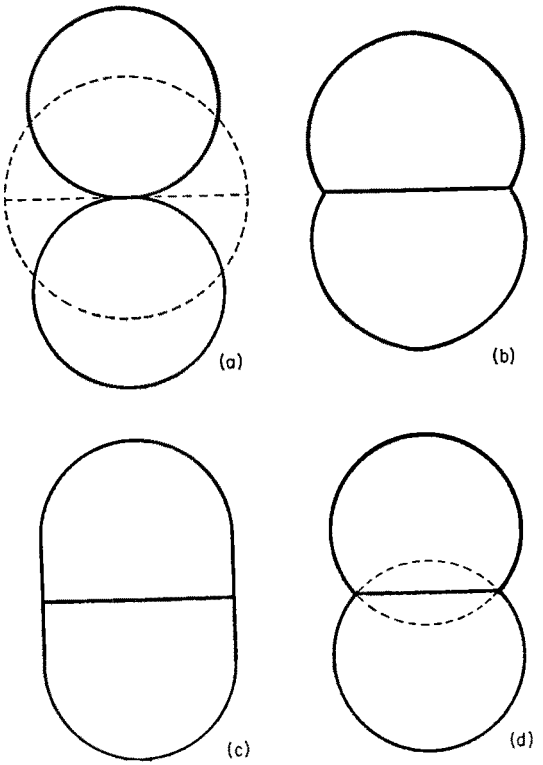

FIG. 2. Representation of cells by intersecting spheres. (a) Daughter cells of equal volume immediately after cleavage. Mother cell and cleavage plane are indicated by dotted lines. (b) The same cells after flattening. The picture shows the two-cell stage of a real embryo. (c) Approximation of this structure in the model. (d) Representation of the system used in pictures of simulated embryos.

a slight error in the distance between the cell centres, so that Fig. 2(c) may be considered as a sufficiently accurate approximation. It consists of two spherical parts connected by a cylindrical surface. In the case of daughter cells of unequal volume the connecting surface is conical. A simple calculation shows that, if r is the radius of the smaller and R the radius of the larger cell, the distance D between the centres is given by

$$D = (R\sqrt{R}+r\sqrt{r})^2/(R^2+Rr+r^2). \tag{1}$$

The distance D' of the centre of the smaller cell to the cleavage plane is

$$D' = \{(1+q)\sqrt[3]{1-q}-1\}r/q \tag{2}$$

and the corresponding distance D'' of the larger cell is

$$D'' = \{(1-q)\sqrt[3]{1+q}-1\}R/q \tag{3}$$

where q is given by

$$q = (R\sqrt{R}-r\sqrt{r})/(R\sqrt{R}+r\sqrt{r}). \tag{4}$$

Finally, the cells are replaced again by spheres that now intersect each other [Fig. 2(d)]. On the outer surface, the intersection takes the place of the original separating plane of Fig. 2(b). Its size is obviously too small, but this affects only the outward appearance. The distances between the cell centres, and with that the arrangement of the cells in the embryo, are approximately correct.

(B) CELL DIVISION

The way cell division is programmed in the model is explained in Fig. 3, the upper part of which shows a section of a dividing cell with centre P and radius PR. The plane of section is meridional and contains (with the egg axis, which does not appear in the picture) the cleavage centre O. The cleavage plane has to pass through O [rule (3) for cell division; cf. previous section], while dividing the cell in a given proportion p. All planes satisfying the latter condition are tangent planes to a sphere with a radius PQ that can be calculated from the cell radius PR and the value of p. From a starting position perpendicular to the meridional plane, the final position of the cleavage plane is reached by a rotation with the line OP as axis [rule (4) for cell division]. The ensuing displacement of the point Q is shown in projection in the lower part of Fig. 3. The clockwise rotation shown in the figure corresponds to a dexiotropic cleavage.

With the position of the cleavage plane, that of the centres of the two daughter cells is fixed as well. They are situated on the line PQ, which line corresponds to the axis of the cleavage spindle, and their distances from the point Q are given by the formulae (2) and (3). The fact that these formulae are applied means, that the mutual flattening of daughter cells of the same mother cell is already included in the cleavage procedure.

(C) CELL FLATTENING

Figure 4(a) shows the result of a program in which the process of cell flattening was restricted to daughter cells of the same mother cell. From this figure it is immediately clear that the matter cannot be left at that: cells in different quadrants are too far apart and leave a gap that, evidently, is not

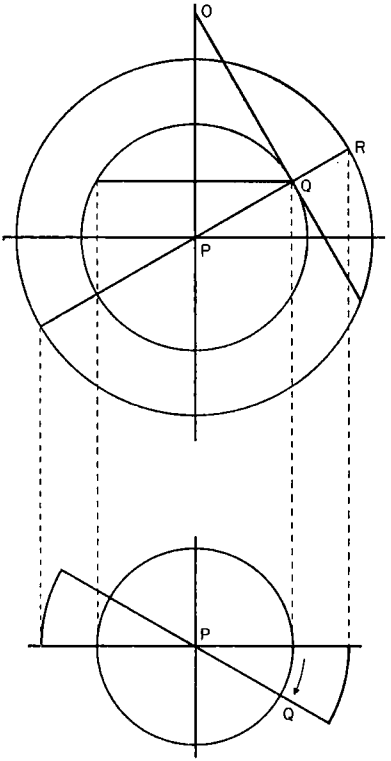

FIG. 3. Location of the cleavage plane in a dividing cell with centre P and radius PR. The point O is the cleavage centre. The cleavage plane is perpendicular to the plane of drawing. The line OQ is their intersection. The cell part to the right of OQ will produce the animal daughter cell. The lower part of the figure shows the sense of rotation in a dexiotropic cleavage.

present in the real embryo. The resulting distortion gets worse as development proceeds, especially in the vegetative region of the embryo.

To close this gap, the cells have to move inwards. The result of this procedure, the details of which will be given presently, is shown in Fig. 4(b). For comparison, a picture of the real embryo in the same stage is included in Fig. 4(c). As before in the two-cell stage of Fig. 2 cell shapes are rendered only roughly, all outlines at intersections being too small, but again cell positions are reproduced satisfactorily. The procedure is defined by the following algorithm.

(a) For each pair of intersecting ("neighbouring") cells the theoretical distance is calculated by means of formula (1).

(b) Each cell is allowed to move along a fixed line running parallel to the line OQ defined by the cleavage procedure. The shifts, necessary to bring neighbouring cells at theoretical distance, are computed and cell positions are adjusted accordingly.

(c) If two cells derived from different mother cells are at theoretical distance, their positions remain fixed until one of them divides. In that case rule (b) becomes operative once more.

(d) It may happen that cells that previously did not intersect each other do so after a first adjustment of positions. In that case another cycle is performed as long as still some freedom of cell movement is left.

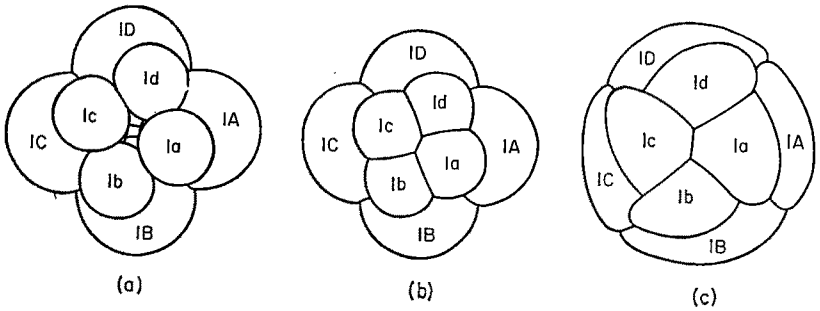

FIG. 4. Eight-cell stage of *Lymnaea*, seen from the animal pole. (a) Cell arrangement in simulated embryo after division of cells in the four-cell stage. Only daughter cells of the same mother cell are at the right distance. (b) Cell arrangement after the flattening process has been completed. (c) Eight-cell stage of real embryo (Verdonk, 1965).

These rules are only a pure formalism. They have no physiological basis whatever, but this is unavoidable in the absence of any precise knowledge of the mechanism of the process.

From Fig. 4 it may be concluded that flattening of cells is an essential part of the morphogenetic process. Including cell flattening in the program decidedly improves the agreement between simulated and real embryo as far as the relative positions of the cells are concerned. The agreement remains satisfactory also in later stages of development, of which Fig. 5 gives an example.

(D) DIFFERENTIATION

Differentiation may be included in the model by comparatively simple means if one accepts the idea of a gradient field of some type-determining

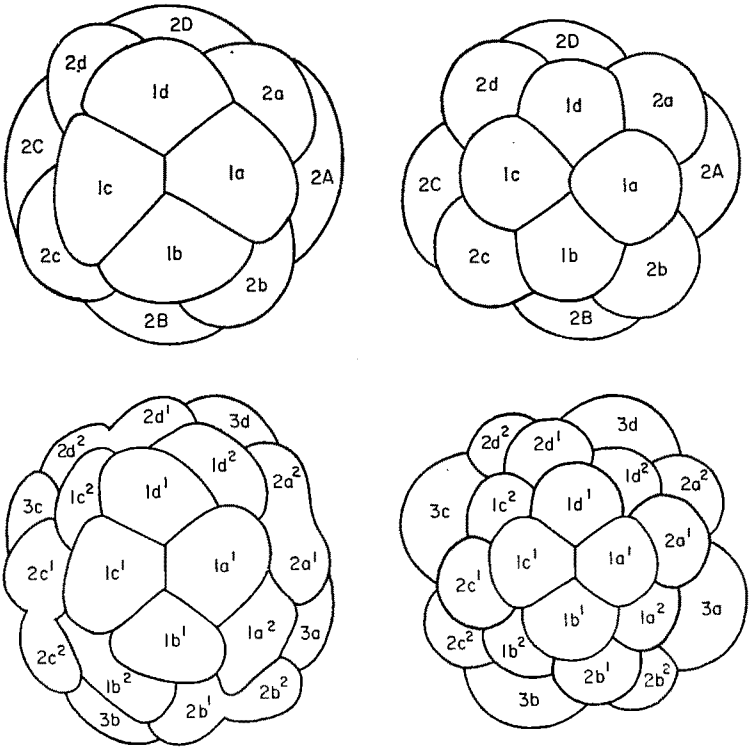

FIG. 5. 12-cell stage (above) and 24-cell stage (below) of *Lymnaea*, seen from the animal pole. Left: real embryo (Verdonk, 1965); right: simulated embryo.

factor, attaining its extreme value in a certain narrow region of the egg, called the "differentiation centre". At the 68-cell stage, four regions of different cell types can be distinguished in the embryo; from the animal pole downwards: the apical plate (A), cephalic plate (Uc), prototroch (P) and post-trochal region (Up). It is assumed that the type of each cell depends on its distance from this differentiation centre.

In geometrical terms this is equivalent to setting up a system of three surfaces, separating the four existing regions. Naturally, one would prefer a system that effects the desired separation with a minimum of programmatic involvement. The simplest system in this respect is a set of concentric spheres. The differentiation centre is then a single point, the common centre of the spheres. This possibility has been tried, but without success. The next best is a system of confocal ellipsoids, corresponding to a differentiation centre

consisting of two points, the common foci of the ellipsoids. In this system the type of a cell is defined by the sum of its distances from the two foci.

A separation of the four cell types may be obtained in this way if the focal points are situated on a line running parallel to the equatorial plane at some height above the cleavage centre. Two parameters are involved that can vary within rather narrow limits. If the radius of the egg is fixed arbitrarily at the value 100, the height above the cleavage centre is restricted to the range 20–36 and the focal distance to the range 21–31, so that the differentiation centre is confined to a small well-defined region of the egg. Table 1, corresponding to the values 28 for the height and 25 for the focal distance, shows the separating effect, the maximum distance of each type being less than the minimum distance of the next type.

TABLE 1

The separating effect

Cell type	Number of cells	Minimum distance	Maximum distance
A	12	74·1	82·4
Uc	8	87·0	92·7
P	20	95·2	125·3
Up	28	130·5	230·9

(E) EXTERNAL PARAMETERS

In the second section of this paper the assumption has been made [rule (1) for cell division] that the behaviour of a cell depends entirely on its internal state. It should be pointed out here that this assumption is only partly realized in the model. It is true that the model gives a definite meaning to the otherwise rather vague notion of the internal state of a cell by defining it as a set of five data: its generation, the radius and the three co-ordinates of its centre. Moreover, the data of the daughter cells are derived from those of the mother cell according to fixed rules. But the computation involves also certain quantities that are not related in any way to cell positions, but have to be established as outside information at various stages of the procedure as what may be called "external parameters". The following data are necessary.

(1) The cell positions at the four-cell stage (which serves as a starting point for the simulation program), in particular the difference in height between cells in quadrants A and C and those in quadrants B and D.

(2) The location of the cleavage centre.

(3) The two parameters defining the differentiation centre.

(4) The proportion in which each cell divides. There is no doubt that this quantity assumes different values for different quartets, especially in the early stages.

(5) The angle of rotation of the cleavage plane. Some runs were executed with a fixed value for this quantity, but decidedly better results are obtained if it is allowed to vary too.

(6) A specification, at each stage of development, which quartet is going to divide (or differentiate, as the case may be). The order in which the several quartets divide is important because the process of cell flattening makes the positions of new cells dependent on what other cells are present at the time of their formation.

With regard to the consequences for the model the first three items are rather harmless. They refer not to single quartets but to the egg as a whole. Their values are established once for all and may, therefore, be considered as part of the program. The other three, however, are more troublesome. They have to be specified anew at each stage of development and make the program dependent on outside information. A perfect model should contain rules enabling the program to compute these data itself. This ideal state of affairs will have to wait, however, pending further advances in the theory of embryonic development.

Observational data concerning the external parameters are very scarce. Only the order of division is known exactly, rough estimates of the cleavage ratios are available, but none of the other parameters have been measured in the actual embryo. Their values have to be determined by trial and error, carrying out several runs of the procedure with different values of the parameters and comparing the results each time with the pictures of real embryos. In this way the simulation program obtains practical significance as a means of determining relevant quantities that so far are inaccessible to experimental observation. Nevertheless, it would be nice to obtain a confirmation from actual measurements of the values finally accepted.

4. Normal Development

Normal development in *Lymnaea* differs in many respects from the process that gives rise to the radial pattern in lithium treated eggs discussed in the preceding sections. Up to the 32-cell stage the processes are identical, but after that more or less radical differences appear, that will be enumerated here, together with their consequences for the model.

(1) It is no longer true that all cells of the same quartet behave in the same way. Cell divisions are no longer strictly synchronous and may differ in

character as well. Furthermore, cells of the same quartet may differentiate to different types. In the simulation program, instructions will have to refer to single cells rather than to quartets.

(2) Beside dexiotropic and laeotropic cleavage two new kinds of-division occur: meridional (angle of rotation 0) and transverse (angle of rotation 90°). Since it has been acknowledged already that the angle of rotation even in radialized development preferably is to be treated as a variable, this fact offers no new problems.

(3) An entirely new phenomenon is the change of cell shape by a kind of growth process. So far, there seems to be no way to include this process in the model. Fortunately, in the period covered by the model, only a single cell is involved and, since the growth of this cell is restricted to the interior of the embryo, at the surface no serious displacement occurs.

(4) Finally, differentiation leads to an entirely different cell pattern. There are now five instead of four cell types and the symmetry of the pattern is bilateral instead of radial. The latter change is by far the most drastic of all. Presumably, it is caused by a process of cell interaction, called "induction", and initiated by the process of cell growth mentioned above, which establishes cell contacts that are absent in the radialized embryo.

This assumption suggests a method to cope with the new situation as far as differentiation is concerned. In fact, the position of the point of contact can be guessed at fairly well, and one can assign to this point, called the "induction centre", a role comparable to that of the differentiation centre. It has turned out that a combined action of both centres generates cell patterns resembling those observed in the normal embryo quite satisfactorily. Results are described in Raven & Bezem (1973).

The induction centre appears to have properties that are essentially different from those of the differentiation centre. While the latter can be located within narrow limits, the possible positions of the former occupy, surprisingly, an extensive region in space, both inside and outside the embryo. Any point within this region can act as induction centre and effect, in combination with the same differentiation centre, a separation of the five cell types encountered in the normal embryo. For a detailed discussion of this result and its developmental implications the reader is referred to Raven, Bezem & Baretta-Bekker (1973).

5. Some Results on *Podarke obscura*

Obviously, the usefulness of the model will increase greatly if its applicability is not restricted to the development of *Lymnaea*. An essential condition is, of course, that the procedures for cell division, cell flattening and

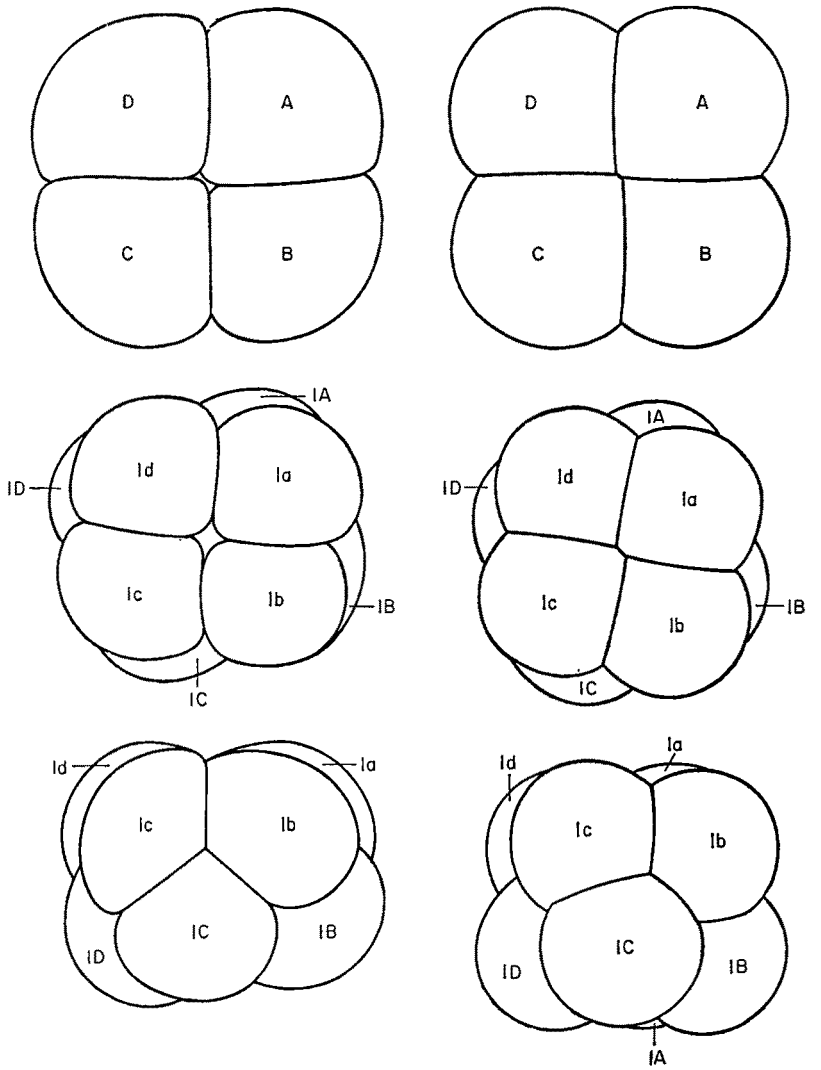

FIG. 6. Earliest stages of *Podarke*. Above: four-cell stage, seen from the animal pole. Middle: eight-cell stage, seen from the animal pole. Below: eight-cell stage, seen in the direction CA. Left: real embryo (Treadwell, 1901); right: simulated embryo.

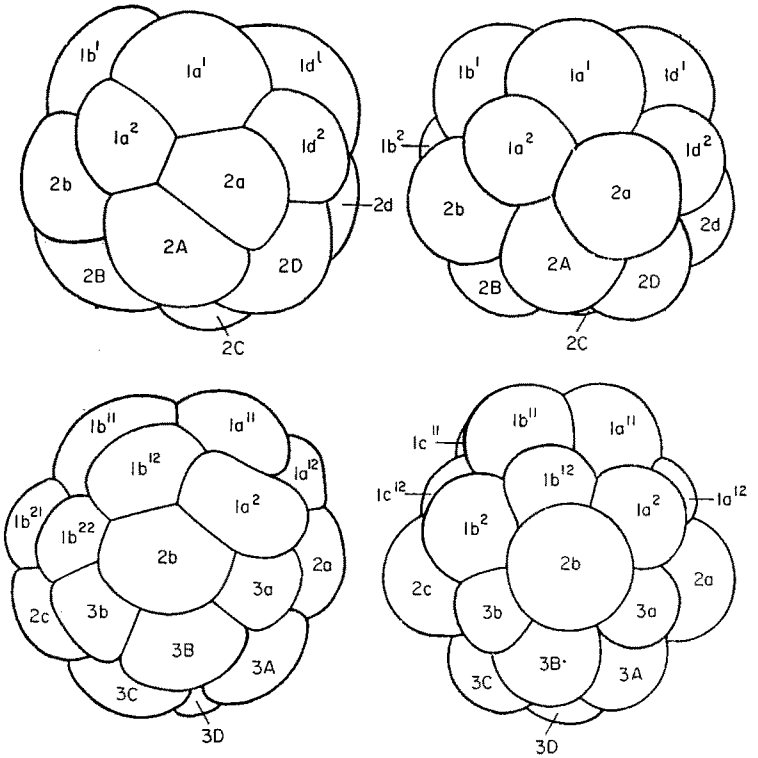

FIG. 7. Later stages of *Podarke*. Above: 16-cell stage, seen in the direction AC. Below: 24-cell stage, seen in the direction BD. Left: real embryo (Treadwell, 1901), right: simulated embryo. The real embryo is in transition from the 24- to the 28-cell stage. Cell $1b^2$ has divided, the division of cell $1a^2$ is in progress. In consequence, the cells $1c^{12}$ and $1c^{11}$ are no longer visible in the real embryo.

differentiation remain unaltered. Only a change in the values of the external parameters is acceptable. There are several species that may be considered for a trial; the first we have tried is *Podarke obscura*. A very detailed and extensive description of normal development in this species is given by Treadwell (1901). In the Figs 6 and 7 real and simulated embryos are compared. So far, only the cleavage and flattening procedures have been applied; what the results of the differentiation rules of the model will be, remains to be seen.

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