The Formation of the Second Maturation Spindle in the Eggs of Succinea, Physa, and Planorbis

by CHR. P. RAVEN

From the Zoological Laboratory, University of Utrecht

WITH TWO PLATES

In a previous communication (Raven, Escher, Herrebout, & Leussink, 1958) the maturation of the egg was described in Agriolimax reticulatus, Limax flavus, and Limnaea stagnalis, with special regard to the formation of the second maturation spindle. In the three species the second maturation spindle arises by direct transformation from the deep centrosphere of the first maturation amphiaster. In A. reticulatus and Limax flavus this centrosphere contains a pair of centrioles which move apart to opposite poles of the developing spindle. This is symmetrical and develops an aster at both ends, arising in the cytoplasm outside the original centrosphere.

In Limnaea stagnalis the centrosphere of the first maturation amphiaster contains only one centriole, which remains undivided. When the centrosphere elongates, the centriole moves as a whole to its outer pole. The spindle developing from the centrosphere is therefore asymmetrical and egg-shaped, and aster formation occurs at its pointed outer end only. The inner aster of the second maturation spindle is provided by the sperm aster, which fuses secondarily with the blunt inner end of the spindle.

In discussing this aberrant course of the second maturation division in L. stagnalis, it was concluded that its primary cause is the failure of the egg cytocentre to divide properly. This causes the lack of astral radiations at the deep end of the developing second maturation spindle, and enables the sperm aster to fuse with this end of the spindle. It was argued that a mutation bringing about a precocious inactivation of the egg cytocentre, in conjunction with the circumstance that a sperm aster, free to take over the task of the failing deep maturation aster, is present at the right moment, might have led to this course of events. As it appeared conceivable that a modification of development occurring at such an early stage might have a profound evolutionary significance, it seemed important to investigate the taxonomic distribution of this peculiar mode of formation of the second maturation spindle.

1 Author's address: Zoologisch Laboratorium der Rijksuniversiteit, Janskerkhof 3, Utrecht, Netherlands.

Since the completion of our previous paper eggs of various pulmonates, both Basommatophora and Stylommatophora, have therefore been fixed at various moments during the course of the maturation divisions. The study of these eggs in sections soon revealed that the above formulation of the problem is rather over-simplified. We had tacitly assumed that the mode of formation of the second maturation spindle as observed in _Limax_ and _Agriolimax_ is the ‘normal’ one in pulmonates, while the relationships found in _Limnaea stagnalis_ constitute a deviation of the process occurring in a limited number of species of this subclass. However, to our surprise it appeared that this early and quite fundamental process exhibits a bewildering variation among the species. Its study in as large a number of species as possible seems necessary before any generalizations can be framed. In view of the fundamental importance of all data relative to the processes of meiosis and mitosis, such a study may yield important results.

In this paper the formation of the second maturation spindle in four pulmonates will be described: the stylommatophore _Succinea putris_ L., and the basommatophore species _Physa acuta_ Drap., _Planorbis planorbis_ L., and _P. corneus_ L.

**MATERIAL AND METHODS**

The eggs of _Succinea putris, Planorbis planorbis,_ and _P. corneus_ were obtained from snails caught in the neighbourhood of Utrecht and kept for some time in the laboratory. Those of _Physa acuta_ were from specimens living in the aquaria of the Institute.

Since the formation of the second maturation spindle is a process which, especially in some species, takes place very soon after oviposition, it was important to be able to obtain the eggs at will at the desired moment. To that end, methods to stimulate oviposition in these snails have been worked out by Lucy P. M. Timmermans (1959).

The eggs of the land snail _Succinea putris_ are laid, preferentially within moist moss, in irregular masses, containing from 20 to 60 egg capsules. These capsules are each surrounded by a layer of jelly (Jura & George, 1958), by which they are cemented together into a loose aggregate. The spherical egg capsules have a diameter of about 1 to 1·3 mm. They have a thick, elastic, transparent wall, which is not encrusted with lime. The egg cells have a diameter of about 130 μ.

The egg-masses of _Physa acuta_ are gelatinous, oblong, and from 10 to 18 mm. in length. They may contain from 15 to 50 egg capsules, lying rather freely in the common jelly. The egg capsules are oblong and about 0·7 x 0·9 mm. in size.

The egg-masses of _Planorbis planorbis_ are flat and disk-shaped, and have a diameter of from 3 to 5 mm. The egg capsules lie side by side in one layer, close together. There are about 15 to 25 egg capsules, each about 0·5 mm. in diameter. The egg cells have a diameter of 110–30 μ. In the first 15 minutes after oviposition the egg masses are whitish and opaque; then they become transparent, with a yellowish tinge.
P. corneus also has flat, disk-shaped egg-masses, containing one layer of egg capsules (cf. Jura & George, 1958). The egg-masses are about 8 to 10 mm. in diameter, have a reddish tinge, and contain from 15 to 25 egg capsules. The outer jelly layer of the egg-mass is rather rigid. The egg capsules are transparent and 1.4-1.6 mm. in size. The diameter of the egg cells is about 140 μ.

A freshly laid egg-mass was observed under a microscope, and the time of formation of the first polar body in the majority of the eggs was noted. This time was taken as the starting-point of the subsequent observations; all indications of time mentioned below are counted from the moment of extrusion of the first polar body. Batches of eggs were fixed at regular intervals during the course of the second maturation division. Owing to the considerable variations in stage of development among eggs of the same batch, the maturation age of the eggs provides only a rough estimate of their stage of development.

The first polar body is formed in Succinea putris about one hour after oviposition; in Physa acuta 5-15 minutes, in Planorbis planorbis about 15 minutes, and in P. corneus 10-20 minutes after oviposition. The second polar body is extruded about 65 minutes after the first in Succinea putris; about 35 minutes in Physa acuta; 50 minutes in Planorbis planorbis, and 55 minutes in P. corneus (at an average temperature of 20° C.).

Before fixation the eggs must be decapsulated. In Succinea putris and Physa acuta this can be easily done in the same way as in Limnaea: the egg-mass is rolled out on a glass plate covered with moist filter-paper, so that the egg capsules come to lie apart. In Physa acuta care must be taken not to damage the egg capsules, as the capsule membrane is rather delicate in this species; in Succinea it is thick and resistant. The egg cells are isolated by pricking the capsule membrane; this is most easily done after the latter has become slightly wrinkled by drying. In Planorbis planorbis and P. corneus, decapsulation is only possible during the first 15-20 minutes after oviposition, before the outer envelope of the egg-mass has hardened. With watchmaker's forceps this envelope is removed; then the egg capsules are pricked under water.

RESULTS

Succinea putris

In the eggs of the first batch, fixed at 15 minutes, the first polar body has just been constricted off. It is still connected with the egg surface by a stalk or a 'mid-body'. The deep aster of the first maturation spindle lies beneath the surface at the animal pole, often slightly to one side of the attachment of the polar body and the spindle remnant. This seems to be a peculiarity of the Succinea egg, as it has never been observed in other species. The aster is provided with a dense centrosphere of moderate size. This is slightly flattened and elongated parallel to the surface. Two very minute, hardly visible centrioles are found near the ends of its longer axis. The dyads closely apply themselves against its outer
surface in a loose group; they even seem partly to have invaded the area of the centrosphere (Plate 1, fig. A). The aster and spindle remnant are surrounded by a dense area of the cytoplasm; many mitochondria have accumulated in this region.

In the next few minutes the spindle remnant disappears. The centrosphere with the dyads approaches the surface and comes to lie immediately beneath the egg cortex, flattening still more against it. The astral radiations of the old maturation aster become blurred and indistinct, but in some cases a new orientation of astral fibres is indicated near the ends of the elongated centrosphere where the centrioles are barely visible as ill-defined and somewhat darker dots.

Apparently this does not yet give rise to the asters of the second maturation spindle, however, for in subsequent batches all astral radiations have disappeared. As a matter of fact, in the eggs fixed at 30 minutes, the dyads are found in an area of clear protoplasm, lying immediately beneath the surface near the animal pole but often somewhat eccentric with respect to the first polar body (Plate 1, fig. B). This clear area, which may be rather irregular in outline, possibly represents the shrunken centrosphere. Subsequently it once more shifts its position and sinks somewhat below the surface but remains in the region of dense animal cytoplasm (Plate 1, fig. C). Here two small asters appear at opposite ends of the clear area, and become connected by a spindle. Part of the spindle fibres traverse the clear area and connect with the dyads, while other fibres run along its outer border (Plate 1, fig. D). The spindle, which may at first have a tangential or oblique position, then rises to the surface and places itself at right angles to it (Plate 1, fig. E). The dyads arrange themselves in the equatorial plane of the spindle, while the latter increases considerably in size. The aster at the outer end of the spindle flattens against the surface. The inner aster grows considerably in size and develops a large clear centrosphere, in the middle of which the centriole is now distinctly visible (Plate 1, fig. F).

During the early stages of the formation of the second maturation spindle no sperm aster is present in the egg. It appears for the first time when the second maturation spindle is in metaphase. It is situated at an arbitrary place in the cytoplasm, occasionally quite near to the second maturation spindle (Plate 1, fig. F), but mostly at a greater distance. During anaphase of the second maturation division it grows somewhat in size and develops a distinct centrosphere, which may often show a tendency to reduplicate. Immediately after the extrusion of the second polar body this centrosphere becomes strongly vacuolized, and the whole sperm aster rapidly disappears without leaving any traces.

*Physa acuta*

Early development in *Physa acuta* is much more rapid than in other species. The second polar body is extruded 35 minutes after the first. The formation of the second maturation spindle takes place in the first 20 minutes after the extrusion of the first polar body.
At telophase of the first maturation division the dyads reach the outer margin of the deep centrosphere. This is small, rather dense, and ellipsoid in shape (Plate 1, fig. G). No distinct centrioles are visible in it.

Very soon, this centrosphere transforms into the second maturation spindle. It elongates in a direction parallel to the surface and becomes spindle-shaped. At the same time a longitudinal striation appears in it. Figure H of Plate 1 shows an early stage of this transformation. The developing second maturation spindle, and the dyads situated against its outer side, are still connected with the egg surface by a remnant of the first maturation spindle. Where this reaches the surface there is a dark 'mid-body', with which the first polar body is connected (not in this section).

Figure J of Plate 1 shows a slightly further advanced stage. The developing second maturation spindle has become somewhat longer, and the spindle fibres are more distinct. At both its ends a small dark granule, presumably a centriole, has now become visible, and small asters are forming around them. But the spindle still lies in a superficial position parallel to the surface, and the dyads are situated against its outer side.

In the following few minutes the spindle begins to rotate and places itself at right angles to the surface. At the same time the dyads invade the spindle area and arrange themselves more or less irregularly in the middle region of the spindle. The asters grow in size and the outer aster becomes attached to the egg surface, where a small indentation may temporarily be formed (Plate 1, fig. K). The dyads arrange themselves into a metaphase plate; sometimes this may occur already when the spindle has not yet completed its rotation (Plate 1, fig. L), but in other cases it only takes place after the spindle has become attached to the surface (Plate 1, fig. K).

A sperm aster does not appear in the egg of P. acuta before the anaphase of the second maturation division. At first it is a very small structure, but soon it grows in size and develops a centrosphere. Immediately after the extrusion of the second polar body, however, a vacuolization occurs in its centre, and the sperm aster disappears. As early as 10 minutes after the formation of the second polar body no trace of the sperm aster remains.

Planorbis planorbis

The egg of Planorbis planorbis is very suitable for the study of its maturation divisions. It gives images of great clearness and beauty. This, together with its peculiar and quite unconventional mode of formation of the second maturation spindle, makes it an excellent object for studies relating to maturation, fertilization, and cell-division.

In the eggs of the first batch, fixed 15 minutes after the extrusion of the first polar body, the spindle remnant of the first maturation division has already disappeared. The deep aster that remained in the egg has risen to the surface and flattened itself against it. It has a large, round, disk-shaped, and rather dense
MATURATION SPINDLE 349

centrosphere; the boundary between centrosphere and astral rays is not very sharp. In the middle of the centrosphere lies a somewhat darker but rather vaguely delineated body, apparently a centriole. It is always single. The dyads are arranged in a more or less regular circle around the centrosphere, at the boundary between the latter and the astral rays (Plate 1, fig. M).

In addition to the maturation aster, a second large aster is present in each egg. It has a variable location, often near the centre of the egg, but sometimes at a short distance beneath the maturation aster. The fact that it is connected with the sperm tail in most cases (Plate 1, fig. P; Plate 2, fig. A) proves that it is the sperm aster. It is more or less reduplicated in all cases. Sometimes it has an elongate or biscuit-shaped centrosphere in which two rather large, but ill-defined, centrioles can often be distinguished (Plate 1, fig. N). In the middle, between the two centrioles, a longitudinal striation may appear, forming a short central spindle (Plate 1, fig. P). Finally, the centrioles may move farther apart, the central spindle elongates, and the aster divides, in this way forming a beautiful sperm amphiaster (Plate 2, fig. A). It must be emphasized that this is an achromatic spindle, as the sperm nucleus is still lying as a compact dark body somewhere beneath the egg cortex and remains so until after the extrusion of the second polar body, as it does in all pulmonates.

The mitochondria of the egg are accumulated around both the maturation aster and the sperm aster, often penetrating in rows between the astral rays.

Starting from this situation, which prevails shortly after the extrusion of the first polar body, the following processes lead to the formation of the second maturation spindle. The dyads begin to move centrifugally along the astral rays of the maturation aster, thereby widening their circle (Plate 1, figs. O, P). At the same time the sperm amphiaster rises to the animal pole and comes to lie close beneath the maturation aster. Now delicate fibres are ‘spun’ between the centre of the maturation aster and the sperm amphiaster, spanning the intervening stretch of cytoplasm. When the sperm amphiaster lies more or less horizontally and its two poles are at about the same distance from the centre of the maturation aster, these spindle fibres may at first run divergently from the latter to both sperm centrioles (Plate 1, fig. P). Later, two different possibilities may be distinguished. When the sperm aster is more or less horizontal and its centrioles lie near together, the fibres of the maturation spindle seem to end indiscriminately along the whole middle region of the sperm amphiaster (Plate 2, fig. B). When the poles of the sperm amphiaster are farther apart, however, and especially when it is situated obliquely or vertically, the maturation spindle is formed between the centre of the old maturation aster and the nearest pole of the sperm amphiaster (Plate 2, figs. A, C). So we get the uncommon situation of a spindle one of the poles of which is itself a (more or less well-developed) spindle.

The maturation spindle formed in this way is at first achromatic, but soon it becomes ‘colonized’ by the dyads. In their outward migration along the rays of
the maturation aster the dyads move at first immediately beneath the egg cortex, against which the aster remains closely applied (Plate 2, fig. A). But then the ends of the astral rays bend inwards, taking along the dyads (Plate 2, fig. B), and the latter reach the outer surface of the developing second maturation spindle, which has in the meantime increased in girth and has become more distinctively visible. In some eggs several of the dyads have reached the spindle (Plate 2, fig. C), whereas other dyads still lie in the peripheral parts of the maturation aster. Finally, all dyads have reached the outer surface of the maturation spindle, and begin to invade the spindle area. Then they arrange themselves into a metaphase plate.

Figure D of Plate 2 shows the second maturation spindle in early anaphase. Its inner pole is formed by the sperm amphiaster, which has two distinct centres connected by a central spindle. The latter at this stage begins to disintegrate, its spindle fibres becoming more or less blurred and apparently breaking up into smaller fragments. The maturation spindle is mainly centred towards the left pole of the sperm amphiaster. The outer aster of the maturation spindle has developed from the original deep aster of the first maturation spindle. As a matter of fact, during the later phases of the migration of the dyads the astral radiations have become somewhat less distinct, but at no stage have they disappeared altogether, and now they begin to become more pronounced again.

A late anaphase stage is shown in Plate 2, fig. E. The sperm amphiaster has two large clear centrospheres, each containing a dark rather ill-defined centriole, and lying obliquely one above the other. They are separated by the remnants of the central spindle, which run as a dark granular band between them. The maturation spindle is connected with the upper half of the sperm amphiaster.

In the next few minutes, while the second polar body is being formed, the two centrospheres of the sperm amphiaster unite to a single spherical clear area with a finely reticular structure, in which a band of coarse granules forms the last remnant of the former central spindle. On either side of this band a centriole is still seen; they have even become much more clearly defined than at previous stages, and present themselves as compact, rather large, and globular bodies. Both sperm centrioles are visible in Plate 2, fig. F, the second polar body having just been pinched off.

Immediately after the extrusion of the second polar body the egg chromosomes begin to swell into karyomeres. At the same time the sperm nucleus also swells. Temporarily it has the appearance of a closely packed group of karyomeres, forming a morula-like body; with further swelling these karyomeres unite to a polymorphic male pronucleus. This stage has been reached 20 minutes after the extrusion of the second polar body. The egg karyomeres are still separate vesicles at this time, but 10 minutes later they too have fused to a polymorphic pronucleus.

After the extrusion of the second polar body the deep aster of the maturation spindle (the former sperm amphiaster) moves somewhat below the surface. Its
astral radiations soon begin to disappear. Its centrosphere forms a large, clear, spherical area, finely reticulate in structure, but interspersed with coarse granules. Two centrioles may still be seen in it as distinct dark globules (Plate 2, fig. G). Simultaneously with the swelling of the sperm nucleus, the latter begins to migrate from its subcortical position towards the centre of the egg, where it comes to lie in the centrosphere of the sperm aster. This stage has been reached 20 minutes after the extrusion of the second polar body. Then the centrosphere with the male pronucleus migrates towards the animal pole, where it forms a clear crescent-shaped zone surrounding the two pronuclei. Twenty minutes later this clear zone has disappeared and is replaced by the dense animal pole plasm which has now accumulated at the animal pole.

*Planorbis corneus*

Although the formation of the second maturation spindle in *Planorbis corneus* occurs essentially in the same way as in *P. planorbis*, the former species is much less favourable material owing to different staining properties.

In all eggs fixed 10 minutes after the extrusion of the first polar body, the deep aster of the first maturation spindle lies closely beneath the surface at the animal pole. It is of moderate size and has, as a rule, no clear centrosphere, the astral rays continuing towards the centre of the aster. The dyads are situated in a narrow circle around this centre (Plate 2, fig. H).

In most eggs a small sperm aster is found. It has no distinct centrosphere. It has a variable location; sometimes it lies at a short distance beneath the maturation aster (Plate 2, fig. H), but as a rule it is situated in other parts of the egg and shows no topographic relations to the maturation aster. In most cases the sperm aster is a single structure; only in a few instances does it show a beginning reduplication of its centre.

In the next 10 minutes the dyads begin to move centrifugally along the rays of the maturation aster, arranging themselves in a wide circle in its periphery (Plate 2, fig. J). At the same time the sperm aster slowly approaches the maturation aster and comes to lie beneath it. Simultaneously, it grows in size, and a clear centrosphere appears in its centre (Plate 2, fig. J). The number of sperm asters showing reduplication increases; in extreme cases this may lead already at this stage to the formation of a small sperm amphiaster, consisting of two asters with distinct centrospheres and connected by a short achromatic central spindle.

When the sperm aster has approached the maturation aster to within a small distance, delicate spindle fibres begin to be 'spun' between the two. If the sperm aster is double, they are directed to one of its centres. At the same time the astral rays of the maturation aster bend inwards and the dyads, moving along them, reach the outer surface of the developing second maturation spindle. In the egg of Plate 2, fig. K, this has occurred with some of the dyads, but the majority of them still lie in the peripheral parts of the maturation aster.
In this way the second maturation spindle becomes 'colonized' by the dyads. This stage has been reached at about 25 minutes. The dyads, first lying at the surface of the spindle, then gradually penetrate into its substance and arrange themselves more or less irregularly along the spindle fibres (Plate 2, fig. L). At 40 minutes they begin to concentrate in the equatorial region (Plate 2, fig. M), and so the metaphase stage is reached. The outer aster of the spindle is the former deep aster of the first maturation spindle; its inner aster is formed by the sperm aster, which is now more or less reduplicated in all cases, ranging from a slight dumb-bell shape of its centrosphere to a well-developed sperm amphip- aster with central spindle.

DISCUSSION

It has already been remarked in the introduction to this paper that the present observations have brought to light a quite unexpected variability in the course of the maturation divisions within the group of the pulmonates. They show that some conclusions drawn from our previous investigations were premature, and necessitate a renewed consideration of some points discussed previously.

In a previous paper (Raven, Escher, Herrebout, & Leussink, 1958) it was concluded that in the pulmonates generally the second maturation spindle arises by a direct transformation from the centrosphere of the deep aster of the first maturation spindle. This was shown to be the case in Limax flavus, Agriolimax reticulatus, and Limnaea stagnalis. A similar mode of formation of the second maturation spindle seemed highly probable for other pulmonates from the data found in the literature. Especially the descriptions and illustrations given by Byrnes (1900) for Limax (Agriolimax) agrestis and by Lams (1910) for Arion empiricorum leave little doubt that the process in these species occurs in essentially the same way as in Limax flavus and Agriolimax reticulatus (cf. figs. 10, 15, & 16 of Byrnes, and figs. 42, 48, 56, & 57 of Lams with the photographs in the above-mentioned paper by Raven et al).

The present observations show, however, that we have been overhasty in extending this generalization to the pulmonates in general. It is true that the formation of the second maturation spindle in Physa acuta, though differing in some respects, belongs essentially to the same category as that of Limax, Agriolimax, and Arion. In Succinea putris this is much less obvious; only with some reservation can we adduce it to the same scheme. But as regards Planorbis there is no indication at all of a material continuity between the centrosphere of the first maturation aster and the developing second maturation spindle. Here, therefore, our generalization breaks down altogether; we have to reconcile ourselves with the thought that the second maturation spindle among the pulmonates may apparently be formed in at least two fundamentally different ways.

In Limax, Agriolimax, and Arion, the deep centrosphere of the first maturation spindle at a certain stage contains two centrioles. Some time after the
extrusion of the first polar body these centrioles begin to move apart within the area of the centrosphere. At the same time the latter elongates and becomes ellipsoidal, with its long axis coinciding with the line connecting the centrioles. Some connecting fibres between the centrioles may form the beginning of a central spindle. The main mass of the spindle arises by the appearance of a longitudinal striation in the substance of the centrosphere, occurring when the centrioles have reached the ends of its long axis.

The formation of the second maturation spindle in *Physa acuta* apparently occurs along the same lines. However, the process does not take place here with such diagrammatic clearness as in the above species. The centrosphere is small and dense, and rather ill-defined with respect to the surrounding aster and cytoplasm. The centrioles do not become visible until they have reached the poles of the developing spindle. Moreover, the whole process occurs very rapidly, the transformation of the centrosphere into the spindle already beginning when it is still connected with the surface by the remnant of the first maturation spindle. But on the whole there is no reason to doubt that the formation of the second maturation spindle in *P. acuta* essentially agrees with that in the slugs.

From the work of Kostanecki & Wierzejski (1896) we may draw the same conclusion with regard to *P. fontinalis*. If one compares figs. 7–12 of their paper with Plate 1, figs. G–L of the present paper, it is apparent that the formation of the second maturation spindle in the two species of *Physa* shows a great resemblance, though some structures, e.g. the centrioles, may appear with greater clearness in *P. fontinalis*.

In *Succinea putris* there are greater deviations from this general scheme. As a matter of fact, it does seem at first that the process will follow the same course. There is a distinct, elongate centrosphere, with minute centrioles near the ends of its longer axis; small asters may even begin to form around these centrioles. But this centrosphere rises to the surface and flattens itself against it. All astral radiations vanish. There remains an area of clear, presumably strongly vacuolated, cytoplasm, in which the dyads are situated. This may either represent the former centrosphere, or it is a new formation due to the accumulation of fluid around the dyads; in the latter case we must conclude that the centrosphere has disappeared altogether. After some time this clear area with the dyads sinks into the depth and gives rise to the spindle, two asters again appearing at opposite ends.

It apparently depends on our interpretation of the ‘clear area’ just mentioned, whether or not we can compare the formation of the second maturation spindle in *Succinea* with that in the slugs and in *Physa*. If the clear area represents the centrosphere, then there is a fundamental agreement. The first and last phases of the transformation of the centrosphere into the spindle take place more or less according to schedule; but the process is interrupted by a kind of ‘rest period’ immediately beneath the egg cortex, during which the asters temporarily disappear, and the centrosphere transforms by vacuolization into the ‘clear area’.
If, on the other hand, we assume that the centrosphere as such disappears and the clear area arises in another way, it follows that the formation of the second maturation spindle in *Succinea* is essentially different from that in the above-mentioned species. It is not possible at this moment to arrive at a decision between the two alternatives.

In a previous paper (Raven, Escher, Herrebout, & Leussink, 1958) we showed that the formation of the second maturation spindle in *Limnaea stagnalis* differs considerably from that found in slugs. The deep centrosphere of the first maturation spindle contains a single centriole. It lies at first near the centre of the centrosphere, but then it shifts towards the superficial pole of the elongating centrosphere. The formation of spindle fibres begins at this pole and extends only gradually towards the other end of the centrosphere. The spindle formed in this way is asymmetrical, its inner end being blunt and lacking an aster. The sperm aster fuses secondarily with this end and becomes the inner aster of the second maturation spindle.

The formation of the second maturation spindle in *Planorbis planorbis* and *P. corneus* has some points in common with that in *Limnaea stagnalis*, but differs essentially in other respects. As in *Limnaea*, the centriole of the deep aster of the first maturation spindle remains undivided. Moreover, both in *Limnaea* and in *Planorbis* the deep aster of the second maturation spindle is provided by the sperm aster. But there is a great difference in the formation of the spindle proper. While in *Limnaea stagnalis* this arises by a direct transformation from the substance of the centrosphere, there are no indications at all that the centrosphere has anything to do with spindle formation in *Planorbis*. The centrosphere here is disk-shaped, flattened against the surface, and remains in this position while the spindle is being formed. This occurs apparently by the 'spinning' of delicate protoplasmic fibres between the centriole of the maturation aster and one or both centrioles of the sperm aster. No indications have been found that the process begins at either one or both poles; it rather looks like an orientation of the protoplasmic micellae along the 'lines of force' in the 'field' between the poles, occurring simultaneously along their whole length.

In those cases where the two centrioles within the deep centrosphere of the first maturation spindle move to opposite poles of the developing second maturation spindle, new asters are formed at both ends in the cytoplasm outside the transforming centrosphere. This holds for *Limax, Agriolimax*, and *Arion*, and apparently also for *Succinea putris* and *Physa*.

In *Limnaea stagnalis* the aster at the outer pole of the developing second maturation spindle is formed in the same way around the centriole lying at this pole. The inner aster is supplied by the sperm aster which fuses secondarily with the spindle.

In *Planorbis* the inner aster of the second maturation spindle is also derived from the sperm aster. Either the whole sperm aster or one of the halves of the sperm amphiaster functions as the deep maturation aster. At the outer end of
the spindle a large aster is found; this is no other than the original deep aster of
the first maturation spindle, which is taken over as such by the second spindle.
As a matter of fact, the astral radiations may temporarily become somewhat
vague, so that it is possible that a kind of rejuvenation of the aster occurs; but
it is out of the question that the aster disappears entirely and is replaced by a
new one in between the two maturation divisions.

Apparently, therefore, variable as is the formation of the second maturation
spindle in the pulmonates, the origin of its asters is equally so.

In a previous paper (Raven, Escher, Herrebout, & Leussink, 1958) we have
summarized the variations in the development of the sperm aster in various
pulmonates. The present observations give new evidence of this variability.

In Physa acuta the sperm aster appears during anaphase of the second matura-
tion division. It remains always undivided, and disappears shortly after the
extrusion of the second polar body.

In Succinea putris the sperm aster becomes visible when the second matura-
tion spindle is in metaphase. It may show a tendency to reduplicate, but again it
disappears immediately after the extrusion of the second polar body.

In Planorbis corneus a small sperm aster without a centrosphere is already
present a short time after the extrusion of the first polar body. It grows rapidly in
size and develops a centrosphere; at the same time it becomes more or less
reduplicated, ranging from a slightly dumb-bell shaped centrosphere to a well-
developed sperm amphiaster. In the meantime it has approached the maturation
aster and participated in the formation of the second maturation spindle, whose
deep aster it becomes.

Finally, in P. planorbis the sperm aster apparently is formed already before
the extrusion of the first polar body. Fifteen minutes after first polar body forma-
tion it possesses a large centrosphere and is reduplicated in all cases; sometimes
it has even at this moment developed into a sperm amphiaster with central
spindle. It then approaches the maturation aster, initiates the formation of the
second maturation spindle, and supplies its deep aster. Its two centrospheres,
each containing a distinct centriole, fuse into a spherical clear area during the
extrusion of the second polar body. This area migrates to the animal pole,
temporarily forms a crescent-shaped clear zone around the pronuclei, and is
then replaced by the developing animal pole plasm.

It is evident that the four species, in the order mentioned, form a series of
increasing importance of the sperm aster. In P. planorbis it not only appears
very early, but reaches a stage of development unprecedented in any pulmonate
studied so far. Only the evolution of the sperm aster in Physa fontinalis, as
described by Kostanecki & Wierzejski (1896), shows some resemblance to that
in Planorbis. According to these authors, the sperm aster in Physa fontinalis
divides and forms an amphiaster with central spindle. This may occur at various
times, either before or after the extrusion of the second polar body, even as late
as the stage at which the male pronucleus approaches the female pronucleus at
the animal pole. Anyhow, the sperm amphiaster in all cases gives rise to the first cleavage spindle.

In a previous paper (Raven, Escher, Herrebout, & Leussink, 1958) and in my book (Raven, 1958) I have expressed some doubt on the accuracy of Kostanecki & Wierzejski's observations. However, in view of the great variability which prevails in the course of the maturation divisions in pulmonates, there seems at present to be no reason to think that these observations are incorrect. A re-investigation of the maturation divisions in *P. fontinalis* appears desirable.

Anyhow, the process as it occurs in *Planorbis planorbis* provides a strong argument in favour of the view that, in pulmonates, the poles of the cleavage spindle are also derived by division from the sperm cytocentre. As a matter of fact, the egg cytocentre in this species remains undivided, forms the outer pole of the second maturation spindle, and becomes presumably extruded with the second polar body. In the sperm amphiaster two distinct centrioles are visible. When the centrospheres of the sperm amphiaster have fused to a single spherical clear area after the extrusion of the second polar body, the two centrioles are still clearly visible in it. Presumably they shift together with the clear area and the sperm nucleus towards the animal pole, where the copulation of the two pronuclei occurs. However, at this stage it is impossible to distinguish the two centrioles from other basophil granules in this area. For about 45 minutes, therefore, the centrioles are invisible; then two small asters appear near the pronuclei and give rise to the cleavage spindle. One can hardly doubt that the poles of this spindle are formed by the sperm centrioles. This is the more remarkable as these two centrioles have had a different history, as a rule: for one of the two has formed for some time the deep pole of the second maturation spindle. In view of this circumstance it is conceivable that one of the two sperm centrioles dis-integrates, while the other divides once more and forms the poles of the cleavage spindle. Up till now no indications of such a process have been found, however.

A few words remain to be said about the behaviour of the dyads. In *Agriolimax reticulatus* and *Limax flavus* the dyads, after having reached the margin of the deep centrosphere at telophase of the first maturation division, remain as a tightly packed group against the outer surface of the developing second maturation spindle, as a rule near its equatorial region. They do not penetrate into the spindle area until the spindle has been fully formed and has begun its rotation.

The behaviour of the dyads in *Physa acuta* is quite similar to this. In *Succinea putris*, however, it appears that the dyads begin to penetrate into the centrosphere at an early stage. When the latter rises to the surface they come to lie entirely in the area of clear protoplasm which possibly represents the centrosphere. Later, when the second maturation spindle is formed, the spindle fibres penetrate secondarily into this area and connect with the dyads.

In *Limnaea stagnalis* the dyads, at first forming a compact group against the outer side of the centrosphere, become arranged into a more or less regular ring. When the centrosphere begins to elongate, the dyads form a crown around its
outer pole. This moves along the outer surface of the developing spindle until it has reached its equatorial region. Only after the sperm aster has fused with the deep end of the spindle do the dyads begin to invade the spindle area.

Finally, in _Planorbis planorbis_ and _P. corneus_ the dyads first form a narrow ring around the centre of the centrosphere. Then they begin to move centrifugally along the astral rays, in this way widening their circle. At first they move immediately beneath the surface, but then they are taken along by the astral rays bending inwards towards the spindle which has formed in the meantime. So they reach the outer surface of the spindle, after which they penetrate into it.

It is evident, therefore, that the course of the second maturation division in the pulmonates shows a great deal of variation. Various components of the process may vary more or less independently, of course, that they fit together in such a way as to ensure the normal completion of the maturation division. Every species shows a species-specific pattern, although smaller variations may occur within each species. This species-specificity is such that it would be possible to construct a key for the identification of the species here described merely on the basis of their second maturation divisions.

This is the more remarkable as we are here dealing with a process taking place at such an early stage of development. In general we are accustomed to the fact that developmental processes in related species show a greater similarity the earlier they take place, in accordance with v. Baer's principle. I know of no other example where such fundamental differences between developmental processes occur at such an early stage.

Of course it might be argued that the development of the new individual does not begin before the formation of the zygote nucleus, so that the maturation of the egg does not represent an early, but rather a very late, stage of development. But such a formal point of view does not discount the fact that differences in developmental processes during egg maturation might profoundly affect the course of subsequent development, e.g. by influencing the localization or the synthesis of cytoplasmic substances. In this way they might be of decisive importance for the determination of species-specific differences. Perhaps such a view might be substantiated by cytochemical investigations during maturation in pulmonates.

**SUMMARY**

1. Egg maturation has been studied in _Succinea putris, Physa acuta, Planorbis planorbis_, and _P. corneus_, with special regard to the formation of the second maturation spindle.

2. In _Physa acuta_ the second maturation spindle arises by direct transformation from the deep centrosphere of the first maturation amphistere. This process begins already when the centrosphere is still connected with the surface by the remnant of the first maturation spindle.

3. In _Succinea putris_ the deep centrosphere of the first maturation spindle
rises to the surface and flattens itself against it. The dyads come to lie in an area of clear cytoplasm, which subsequently sinks into the depth and gives rise to the spindle.

4. In *Planorbid planorbid* and *P. corneus* the centriole of the deep aster of the first maturation spindle remains undivided. This aster flattens against the surface and becomes the outer aster of the second maturation spindle. Its inner aster is provided by the sperm aster, while the second maturation spindle itself is formed by the 'spinning' of protoplasmic fibres between the centriole of the maturation aster and one or both centrioles of the sperm aster.

5. The sperm aster in *Physa acuta* is a rudimentary structure which appears late, disappears soon, and always remains undivided. In *Succinea putris* the sperm aster appears slightly earlier and may show a tendency to reduplicate. In *Planorbid corneus* the sperm aster appears when the first polar body is extruded. It grows rapidly in size, becomes more or less reduplicated, and participates in the formation of the second maturation spindle. In *P. planorbid* the sperm aster is formed before the extrusion of the first polar body. It reduplicates and forms a beautiful sperm amphiaster with central spindle. It participates in the formation of the second maturation spindle and becomes its deep aster. During the extrusion of the second polar body its two centrospheres, containing distinct centrioles, fuse to a spherical clear area. The sperm nucleus comes to lie in this area, then migrates together with it towards the animal pole. The sperm centrioles presumably form the poles of the first cleavage spindle.

6. In *Physa acuta* the dyads lie at first as a compact group on one side against the developing second maturation spindle, and penetrate only secondarily into the spindle when this is nearly completed. In *Succinea putris* the dyads presumably penetrate into the centrosphere at an early stage. In *Planorbid planorbid* and *P. corneus* the dyads first form a narrow ring around the centre of the maturation aster, then move centrifugally along its astral rays, and are conveyed by the latter bending inwards towards the outer surface of the second maturation spindle.

7. It is concluded that fundamental differences in the course of the second maturation division occur in the pulmonates.

REFERENCES


MATURATION SPINDLE


EXPLANATION OF PLATES

PLATE 1

FIGS. A–F. Formation of second maturation spindle in Succinea putris. ×910.
Fig. A. After 15 minutes. Telophase of first maturation division. Dyads against outer side of centrosphere.
Fig. B. After 20 minutes. Astral radiations have disappeared. Dyads in clear area beneath the surface at animal pole.
Fig. C. After 30 minutes. Clear area with dyads has shifted to deeper position; surrounded by dense animal cytoplasm.
Fig. D. After 40 minutes. Transformation of clear area into second maturation spindle.
Fig. E. After 55 minutes. Second maturation spindle has risen to the surface. Dyads still in irregular position in middle region of spindle.
Fig. F. After 80 minutes. Second maturation spindle in metaphase. Deep aster with clear centrosphere and distinct centriole. Sperm aster just beneath deep aster of maturation spindle.

FIGS. G–L. Formation of second maturation spindle in Physa acuta. ×910.
Fig. G. After 15 minutes. Telophase of first maturation division. Dyads against outer surface of centrosphere.
Fig. H. After 20 minutes. Centrosphere elongated parallel to surface. Dyads against its outer margin and still connected with ‘mid-body’ at surface by spindle remnant.
Fig. J. After 20 minutes. Centrosphere transformed into second maturation spindle. Centriole with small aster at either end. Dyads against outer side of spindle.
Fig. K. After 20 minutes. Second maturation spindle has placed itself perpendicular to surface. Dyads have penetrated into spindle, but are still irregularly situated. Indentation of egg surface at attachment of spindle.
Fig. L. After 20 minutes. Rotation of second maturation spindle not yet completed. Dyads arrange themselves in equatorial plane.

FIGS. M–P. Formation of second maturation spindle in Planorbis planorbis. ×910.
Fig. M. After 15 minutes. Tangential section. Maturation aster with dyads arranged in circle in circumference of centrosphere. Single centriole in middle of centrosphere.
Fig. N. After 25 minutes. Sperm aster with biscuit-shaped centrosphere containing two centrioles.
Fig. O. After 25 minutes. More or less tangential section. Beginning centrifugal movement of dyads along rays of maturation aster.
Fig. P. After 15 minutes. Maturation aster with single centriole and widening circle of dyads (above). Sperm amphiarister with two centrioles and short central spindle (middle) in connexion with sperm tail (bottom left). Beginning of spindle fibres, running divergently from centre of maturation aster to both sperm centrioles.

PLATE 2

FIGS. A–G. Formation of second maturation spindle and completion of second maturation division in Planorbis planorbis. ×910.
Fig. A. After 15 minutes. Maturation aster with dyads along peripheral part of astral rays, closely applied against egg cortex (above). Sperm amphiarister with well-developed central spindle (middle). The left half of the sperm amphiarister is connected with the maturation aster by the spindle fibres of the developing second maturation spindle, the right half with the sperm tail (bottom right, out of focus).
Fig. B. After 25 minutes. Maturation aster with single centriole (above), connected by second maturation spindle with sperm amphiaster possessing two centrioles (middle). Rays of maturation aster bending inwards, taking along dyads.

Fig. C. After 25 minutes. Second maturation spindle, connected with left half of reduplicated sperm aster containing two centrioles. Dyads have reached outer surface of maturation spindle.

Fig. D. After 20 minutes. Early anaphase of second maturation division. Sperm amphiaster with central spindle, showing beginning disintegration of spindle fibres. Deep end of second maturation spindle centred towards left pole of sperm amphiaster.

Fig. E. After 60 minutes. Late anaphase of second maturation division. Sperm amphiaster with two centrospheres, each containing a centriole, and separated by remnants of central spindle. Maturation spindle connected with upper half of sperm amphiaster.

Fig. F. After 60 minutes. Telophase of second maturation division. Centrospheres of sperm amphiaster fused to spherical clear area, containing remnants of central spindle. One sperm centriole just beneath telophase chromosomes, the other one in middle of lower half of clear area.

Fig. G. After 60 minutes. Remnant of sperm amphiaster as spherical clear area, still containing the two sperm centrioles (globular bodies in upper half of clear area).


Fig. H. After 10 minutes. Maturation aster with dyads near centre of aster (above). Small sperm aster without centrosphere (middle).

Fig. I. After 25 minutes. Maturation aster with dyads along peripheral part of astral rays (above). Sperm aster with centrosphere (middle).

Fig. J. After 15 minutes. Formation of second maturation spindle between maturation aster (above) and sperm aster (below). Some dyads have reached outer surface of maturation spindle (at left), others still in peripheral part of maturation aster (upper right).

Fig. K. After 25 minutes. Dyads have penetrated into maturation spindle. Partial reduplication of sperm aster.

Fig. L. After 40 minutes. Dyads begin to concentrate in equatorial region of maturation spindle.

(Manuscript received 3:ii:59)