THE GERM-RING IN THE EGG OF THE TOAD-FISH (BATRACHUS TAU).

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SINCE the Toad-fish has a number of characteristics peculiar to itself, it is natural to expect that it would differ from the ordinary teleost in its mode of development. The marked resemblance of the egg to that of Elasmobranchs has already been noted,[1] but the formation of the germ-ring has been left an open question. My observations were made during the summers of '95 and '96, under the direction of Dr. Whitman, at the Marine Biological Laboratory, Woods Holl, Mass.

It gives me great pleasure to express my indebtedness also to Dr. Cornelia M. Clapp for many helpful courtesies.

After the middle of June most of the material found in nests in Buzzards Bay was in an advanced condition, and it was necessary to resort to artificial fertilization. Eggs were fertilized by hundreds, covered with sea-water in shallow dishes, and studied from the earliest stages. The egg of Batrachus is 5 mm. in diameter, being much distended with yolk, and is, when deposited, attached to some foreign body by means of an adhesive disc. The blastoderm, in encompassing the yolk, is spread out into a cap of extreme tenuity, requiring delicate treatment. After repeated effort the paraffin method was given up and good results were obtained by use of the celloidin method with either Hermann's fluid or Flemming's fluid as fixing reagents.

Not until the fourth or fifth day after fertilization does a distinct axial thickening appear, with oftentimes a slight, marginal notch at the embryonic pole, and there is no marginal thickening around most of the blastoderm (Pl. II, Fig. 6). A median, longitudinal section is shown in Pl. III, Fig. 1. In

[1] Cornelia M. Clapp, "Some Points on the Development of the Toad-fish (Batrachus tau)." *Journ. of Morph.*, vol. v, No. 3.

the extra-embryonic region the ectoderm is two cells deep, with no peripheral thickening ; while in the embryonic region there is a centripetal growth of cells, thickest near the margin and thinning out anteriorly until some of the cells appear to be lying loose on the periblastic floor. A cross-section, passing through the axial thickening, shows that this tongue of cells also thins out laterally (Pl. III, Fig. 2). Very soon after the germ-ring attains its maximum development (Pl. II, Fig. 7) it begins to decrease. This decrease is shown in an enlarged view of a later stage, Pl. III, Fig. 9, in which the ring is narrow at the anterior pole, gradually broadening toward the posterior or embryonic pole. Cross-sections of the rim at the cardinal points reveal an interesting modification of the germ-ring in the ordinary teleostean egg. In a section at the anterior pole no invagination obtains, but rather a centripetal proliferation of cells from the ectoderm (Pl. III, Fig. 3). In Professor Wilson's paper on the Sea Bass[1] he says: "The peripheral part of the blastoderm, both where there is a large Randwulst and none at all, is an undifferentiated area, and the germ-ring consequently starts at some distance from the extreme edge of the blastoderm." If we follow this interpretation of terms, we have in Batrachus no germ-ring, as there is no under layer of cells differentiated from the rest of the blastoderm. A section through the lateral region more strongly expresses the fact that there is no invagination, the blastoderm being actually thinner at the periphery, as shown in Pl. III, Fig. 4. Here, also, we find no distinct under layer, but a few loose cells which are budded off centripetally from the slight peripheral thickening. As the occurrence of these loose cells is constant, might they not represent the distinct layer found in other forms? Passing to the posterior pole, a section is shown through the longitudinal axis of the embryo (Pl. III, Fig. 5). Here is a decidedly invaginated appearance, but no real invagination, so far as can be judged from a study of successive stages. The appearance may be due to a rapid proliferation of cells both centripetally and dorso-ventrally (*cf.* Pl. III, Fig. 1), and also to the growth

[1] Henry V. Wilson, "The Embryology of the Sea Bass (Serranus atrarius)." *Bulletin of the U. S. F. C.*, vol. ix. For 1889.

of the ectoderm over the yolk. The ectoderm is sharply differentiated from the ingrowing tongue of cells, especially at the periphery. From this time the ring becomes less and less pronounced. In surface views of the stage shown in Pl. II, Fig. 8, a little irregular thickening can be seen at the anterior pole, and in sections a few scattered cells are found lying beneath the thin, flattened ectoderm (Pl. III, Fig. 6). In some sections not even this much of the thickening remains, as the cells occur in patches. In the lateral region the reduction is not yet carried so far (Pl. III, Fig. 7). Sections through the rim of the stage shown in Pl. II, Fig. 9, have no thickening even in the lateral region, while the tongue of cells at the embryonic pole is steadily lengthening.

In Ctenolabrus Dr. Whitman finds that there is "a plain rolling under or involution as an initiatory step in the formation of the ring," but believes that the process is more correctly described as "an ingrowth due both to a rapid multiplication of the cells and also to the centrifugal expansion of the ectoderm." At the posterior margin "the inrolling portion presents a strongly voluted outline, while at the anterior border it is much more feebly expressed."[1] In Batrachus, around most of the margin there is found simply "the initiatory step," and even that lacks the voluted outline, except at the embryonic pole. The loose cells budded off from this small peripheral thickening represent, I believe, a true germ-ring. In the Sea Bass, Professor Wilson finds, at the embryonic pole, an apparent invagination caused by a centripetal growth of cells, and forming a Randwulst from which cells are proliferated centripetally to form a germ-ring. "Round the rest of the edge the ingrowth is likewise, at least in most places, preceded by the formation of a Randwulst, which, however, is inconspicuous."

From the stage given in Pl. II, Fig. 9, down to the closure of the blastopore at a distance behind the embryo, there is an apparent marginal thickening visible even in the living egg (Pl. II, Figs. 3–5). In specimens killed in Perenyi's fluid, a distinct

[1] Alexander Agassiz and C. O. Whitman, "On the Development of Some Pelagic Fish Eggs." Preliminary notice. *Proceedings of the American Academy of Arts and Sciences*, vol. xx.

opaque rim is noticeable, while in preparations with osmic acid, the rim becomes much darker than the rest of the blastoderm. By the study of surface mounts and from sections, this thickness was found to be due to the greater thickness of the periblast in that region, and also to the accumulation of huge periblastic nuclei. The presence of oil globules increases the effect, especially in the living egg.

Pl. II, Fig. 10, is a reproduction of Dr. Clapp's Fig. 1, d. She says: "In Fig. 1, d, this notch is seen at a little distance behind the embryo; a shadowy connection may be traced between the germ-ring and the embryo." While at this time only the *appearance* of a germ-ring exists, the "shadowy connection" between the "germ-ring" and the embryo has a more substantial basis. The same stage is given in Pl. II, Fig. 5. A longitudinal section through the embryo and the margin of the blastopore is shown in Pl. III, Fig. 8. A few cells, marked "m," are seen lying beneath the ectoderm and reaching from the posterior end of the embryo almost to the lip of the blastopore. Sections all around the blastopore show no thickening except of periblast.

Summary. — In the egg of Batrachus there is a centripetal growth of cells at the embryonic pole, the ingrowth having a voluted outline in sections. Around the remainder of the blastoderm there is not even the appearance of an invagination, but only a slight thickening due to an ingrowth of cells from the ectoderm, and a few loose cells which may represent a true germ-ring found as a layer in ordinary forms. The peripheral thickening gradually fades out, first at the anterior pole, until the last remnant is found in a few cells lying beneath the ectoderm, forming a linear streak from the posterior end of the embryo to the lip of the closing blastopore. In the gradual disappearance of the thickening, beginning at the anterior pole and continuing on either side toward the posterior pole, accompanied by the lengthening of the embryo, we see a highly modified form of concrescence.

Woods Holl, Mass.,
September, 1896.

EXPLANATION OF PLATE II.

FIG. 1. Ovum with blastoderm covering $\frac{1}{5}$ of yolk surface. × 9.

FIG. 2. Ovum with blastoderm covering $\frac{1}{4}$ of yolk surface. × 9.

FIG. 3. Ovum with blastoderm covering over $\frac{1}{2}$ of yolk surface. × 9.

FIG. 4. Ovum with blastoderm covering nearly $\frac{3}{4}$ of yolk surface. × 9.

FIG. 5. Ovum near the time of the closure of the blastopore. × 9. *a.d.* = adhesive disc; *l.* = lip of blastopore.

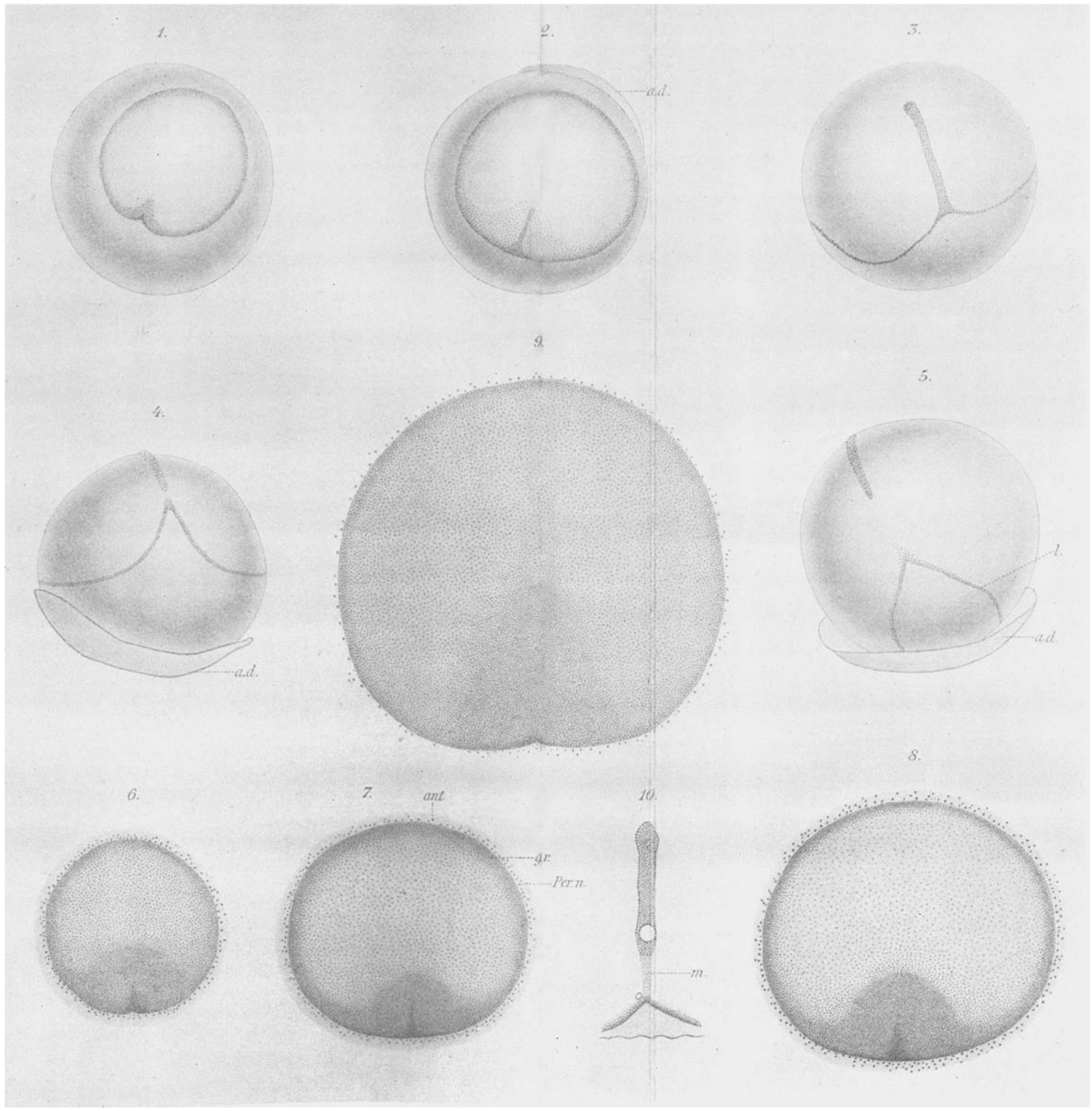
FIG. 6. Blastoderm of an earlier stage than Fig. 1.

FIG. 7. Blastoderm with maximum development of germ-ring. × 16. *g.r.* = germ-ring; *ant.* = anterior; *per.n.* = periblast nuclei.

FIG. 8. Blastoderm of later stage than Pl. III, Fig. 9. × 16.

FIG. 9. Blastoderm of a slightly earlier stage than that of Fig. 3.

FIG. 10. Reproduction of Dr. Clapp's figure showing "shadowy connection," *m.*, between "germ-ring" and embryo.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EXPLANATION OF PLATE III.

FIG. 1. Longitudinal median section of blastoderm shown in Pl. II, Fig. 6. × 100. *per.* = periblast.

FIG. 2. Cross-section of the same. × 100.

FIG. 3. Cross-section of rim at anterior pole of stage shown in Pl. III, Fig. 9. × 160.

FIG. 4. Cross-section of rim in lateral region of stage shown in Pl. III, Fig. 9. × 160.

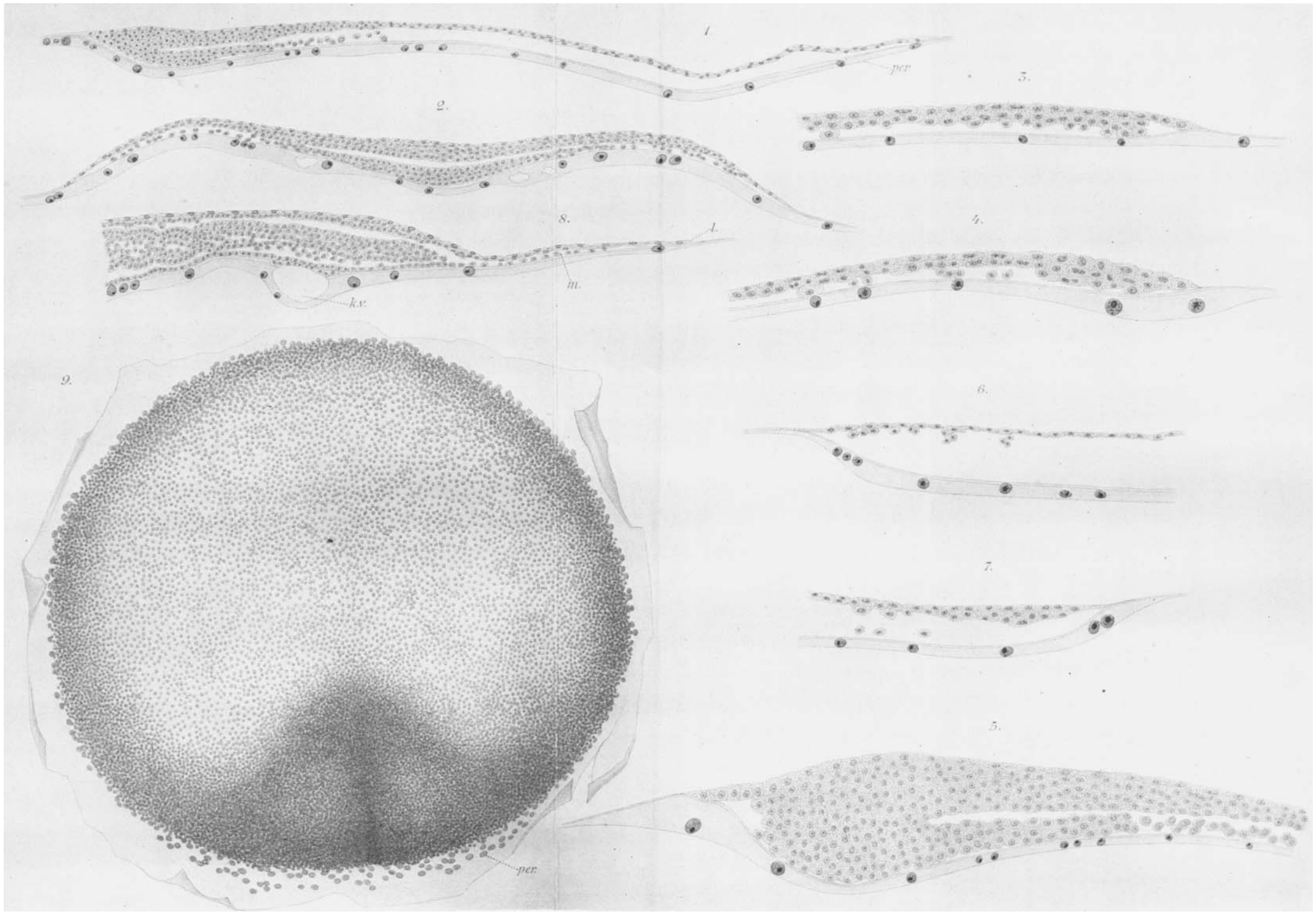
FIG. 5. Longitudinal section through axial thickening of stage shown in Pl. II, Fig. 9. × 160.

FIG. 6. Cross-section of rim at anterior pole of blastoderm shown in Pl. II, Fig. 8. × 160.

FIG. 7. Cross-section of rim in lateral region of blastoderm shown in Pl. II, Fig. 8. × 160.

FIG. 8. Longitudinal median section through embryo and lip of blastopore at stage shown in Pl. II, Fig. 5. × 160. *K.v.* = Kupffer's vesicle; *l.* = lip of blastopore.

FIG. 9. Enlarged view of blastoderm in which the germ-ring is beginning to disappear at the anterior pole. × 45. (Drawn by Mr. Hayashi.)



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