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Localization and induction in early development of *Xenopus*

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[Plates 1 and 2]

Our experimental results, as well as those of others, lead us to suggest the following steps in the dorsalization and axialization of the *Xenopus* egg and embryo: (1) the sperm aster determines the direction of rotation of the cortex relative to the deeper cytoplasm (endoplasm); (2) the rotation of the cortex activates latent dorsalizing–axializing agents in the vegetal hemisphere. The extent of rotation determines the amount of activation. The direction of rotation determines the location of the activated agents. (3) The activated agents determine the level of mesoderm-inducing activity of the vegetal cells cleaved from that cytoplasmic region. (4) The level of inducing activity determines at least the time at which marginal zone cells will begin gastrulation movements. (5) The time of its initiation of gastrulation may determine how anterior and dorsal a particular marginal zone cell can become.

1. Introduction

Early amphibian development is characterized by a long series of stage-specific morphogenetic processes by which the oöcyte is transformed into an egg, and that into an embryo. Each process seems to affect the temporal or spatial (or both) detail of a subsequent process, creating a chain of determinative events. This view of early development is hardly novel. If valid, it would imply that 'determinants' of development need not be thought of as long lasting instructions laid down in the oöcytes for the ultimate cytodifferentiation of the embryo, acting exclusively at the level of differential gene expression, but as transient cues recognized by the next developmental stage, and replaced by new cues for the subsequent stage. Thus, determinants might persist only briefly, have immediate effects, and act on any level of cellular activity crucial to the particular morphogenetic process of that stage. The following outline of *Xenopus* development illustrates these impressions.

2. Primary cytoplasmic localizations in the oöcytes and egg

The full grown oöcyte (1.3 mm in diameter) contains an animal hemisphere and a vegetal hemisphere, the former characterized by a large nucleus and abundant common organelles. The vegetal hemisphere consists mostly of a mass of large membrane-bounded yolk platelets, probably embedded in a meshwork of microfilaments (Colombo 1983). The animal–vegetal axis of rotational symmetry connects the poles of these hemispheres. The surface of the oöcyte is differentiated from the interior as a cortical layer of microvillus-associated microfilaments and of cytokeratin intermediate filaments (Franz *et al.* 1983), which excludes yolk platelets and most organelles. Melanin granules, however, occupy the cortex of the animal hemisphere, giving

it a distinctive blackness ending at the 'equator' of the oöcyte. The unfertilized egg preserves the organization of the oöcyte, with a few modifications related to the mixing of nuclear contents with the animal hemisphere cytoplasm, and with the preparation of the cortex for fertilization.

Oögenesis has many stages, as the 30 μm diameter oögonium differentiates into the 1300 μm oöcyte. There are the specific stages of meiotic prophase, of ribosomal DNA amplification, of 5S RNA synthesis, of mitochondrial proliferation and dispersal, of pigment synthesis, and of yolk uptake from the bloodstream and deposition in platelets. Many of these scheduled processes may be specializations of phases of the eukaryotic cell cycle. The hemispheric organization of the cytoplasmic materials of the oöcyte may be none other than an exaggeration of the basic nuclear–centriolar axis, and of the cortical–endoplasmic organization, typifying any metazoan cell. This speculation has not been submitted to detailed experimental scrutiny, but is in general supported by the finding of Wallace *et al.* (1981) that individual *Xenopus* oöcytes can differentiate their normal morphology when grown *in vitro* through the vitellogenic stages, in the absence of follicle cells or other tissues of the ovary.

On the subject of which localized cytoplasmic materials are crucial for the development of the regional differences of the embryo, Pasteels (1941) was able to obtain information by inverting the endoplasm of the newly fertilized egg relative to the cortex. Normally, the dense yolk mass of the vegetal hemisphere causes the fertilized egg to orient itself with the vegetal pole down and the animal pole up. Pasteels immobilized the egg and centrifuged it in an inverted position to drive the yolk mass into contact with the pigmented cortex. At the same time, the non-yolky cytoplasm was displaced into the non-pigmented half. Thus, the animal–vegetal differences of the endoplasm were inverted relative to the animal–vegetal differences of the cortex. Such eggs developed normally, and the hemisphere of yolky endoplasm gave rise to endodermal structures, regardless of the presence of pigmented cortex. Reciprocally, the hemisphere of non-yolky endoplasm gave ectodermal structures, despite the absence of the pigmented cortex. At the interface of the two regions, the blastopore appeared. These results have been repeated recently for *Xenopus* by Neff *et al.* (1983) and Black (unpublished). Thus, the yolky and non-yolky endoplasmic regions seem to constitute primary cytoplasmic localizations of the egg, determining the ectodermal and endodermal aspects of regional embryonic development, including the position of the blastopore, in the normal as well as inverted egg.

From this result, it cannot be said that the cortex never serves as a localization. At later stages, the cortex could of course carry regional differences, or generate them in deeper layers of the egg. The cortical layer of the *Xenopus* egg goes to form the continuous surface epithelium of the neurula, including the archenteron lining. It does not contribute to deep (mesodermal) tissues (Keller 1976). It has been suggested that the concentration of intermediate filaments in the cortex is a type of early localization used by the *Xenopus* egg for epithelialization of the embryonic surface (Franz *et al.* 1983).

3. Secondary cytoplasmic localizations

The single sperm entry point (s.e.p.), and the animal and vegetal poles, define the meridian on which the embryo's ventral mid-line will lie. Opposite this is another meridian on which the dorsal mid-line of the embryo will appear as the neural groove. These topographic relations obtain even though the sperm can enter anywhere in the animal hemisphere. Thus, the egg

can be said to have the potential to form dorsal or ventral structures at any meridian, and to determine the actual dorso–ventral aspect of the fate map in spatial relation to the point of sperm entry, as shown in figure 1. As a shorthand way to refer to the processes by which this regional determination is made, we shall speak of the 'dorsalization' or 'axialization' of the egg and embryo, even though posterior and ventral regions are also defined. The choice of dorsalization
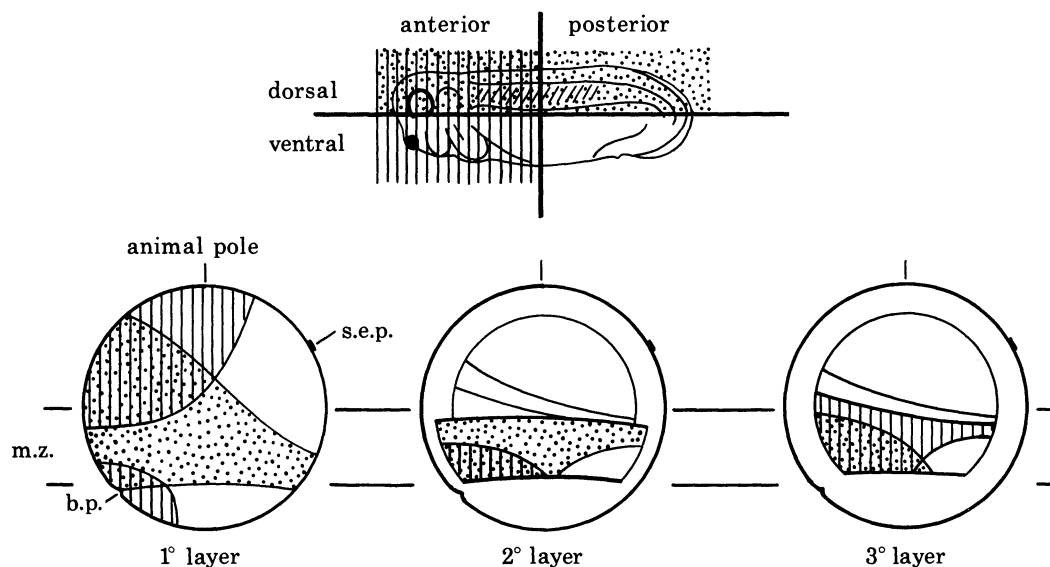

FIGURE 1. Anterior–posterior and dorsal–ventral quadrants of the fate map of the *Xenopus* gastrula. In the upper region of the figure, a post-neurula (stage 28) embryo is shown, divided into four quadrants based on the conventional anatomical planes. In the lower portion are shown three views of the gastrula fate map, redrawn from the data of Keller (1975, 1976). The early gastrula stage was chosen because the positions of cytoplasmic materials of the oöcyte are largely preserved, with the exception of the central region where the blastocoel has now formed. The drawings of the gastrulae are oriented with the animal pole uppermost, and the sperm entry point (s.e.p.) on the right side of the animal hemisphere. The marginal zone (m.z.) is contained between two horizontal parallel lines. This zone contains cells located above the blastopore (b.p.) but below the equator. The left most view is an external view. The ventral mid-line of the post neurula embryo will derive from the meridian through the animal pole, the vegetal pole, and the point where the s.e.p. had been. The neural groove of the neurula will derive from the opposite meridian. Notice that the anterior and dorsal regions of the epidermis, nervous system, and gut of the embryo tend to originate from egg and gastrula materials most distant from the s.e.p. The centre view of the gastrula shows marginal cells just under the surface layer (1° layer) of cells. These cells (2° layer) are arranged in a horizontal band. They are mesodermal precursors. Notice that within this band, dorsal–anterior embryonic structures derive from positions most distant from the s.e.p., although dorsal structures (posterior somites) derive even from positions near the s.e.p. On the lower right is shown the next deeper layer (3° layer) of cells of the marginal zone. These are also mesodermal precursors arranged in a horizontal band. Notice that dorsal precursors originate within the band at positions most distant from the s.e.p.

or axialization as a shorthand reference derives from the special importance of the embryonic regions containing cellular precursors of the dorsal axial mesoderm, since these provide the Spemann Organizer of neurulation, and the definitive notochord of the embryonic body axis.

Figure 2 summarizes the current information on morphogenetic processes occurring soon after sperm entry. These processes are scheduled within the first cell cycle, which is longer by almost threefold in comparison with the subsequent eleven cycles that occur before the mid-blastula transition (Newport & Kirschner 1982). Within the first 40 min after fertilization, the sperm aster enlarges and migrates centrally from the s.e.p. Animal hemisphere materials are relocated by the microtubular projections of the aster. At 40 min, a new cellular activity
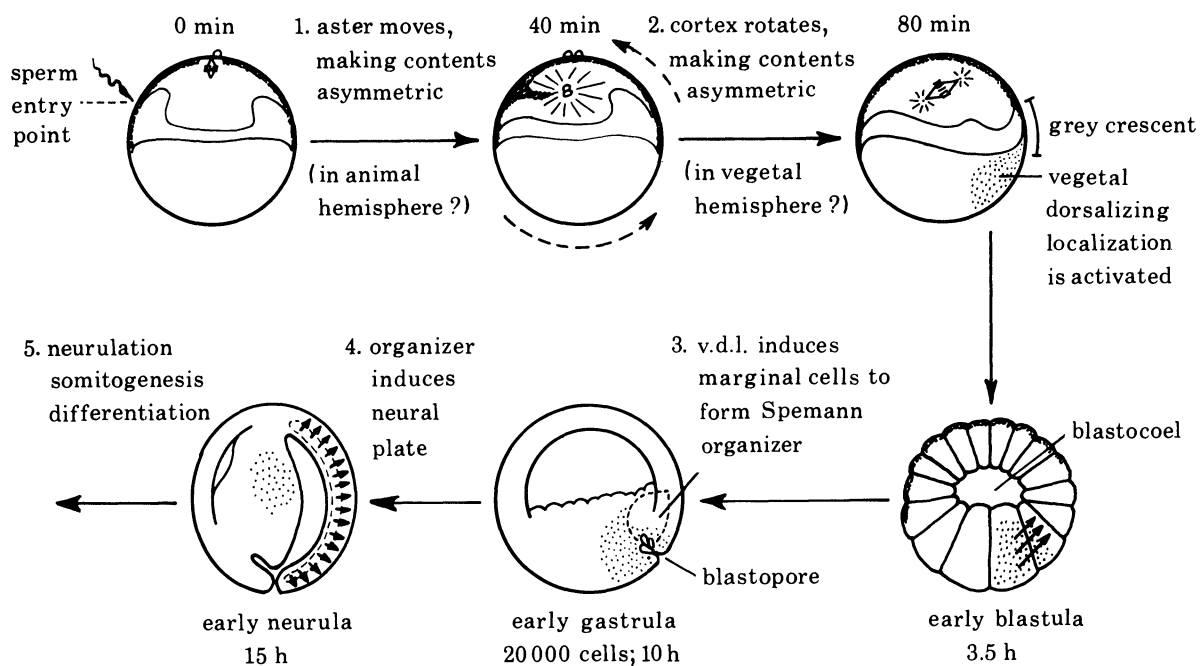
FIGURE 2. Schematic diagram of the sequence of events in embryonic axis-formation in anurans. The scheme is largely speculative, based on interpretations of several embryologists, as referenced in the text. In the upper left portion of the figure the radially symmetric unfertilized egg is shown at the time of fertilization, with the sperm entering in the animal hemisphere randomly at one point. In the 40 min period immediately after fertilization, the sperm MTOC forms a large aster and moves to the centre of the egg, coordinating the migration of the sperm and egg pronuclei, which complete DNA synthesis and meet at approximately 40 min. Cleavage will occur at 90 min, at 20 °C. It is suggested that aster formation and movement from one side of the egg cause a slight reorganization of the animal hemisphere contents and perhaps a slight regional difference in the build-up of the cortex. In the period from 40 to 80 min, the cortex shifts relative to the deep contents of the egg, by a rotation or directional contraction toward the side of sperm entry, as revealed by the emergence of the grey crescent. Deep contents adhering to the cortex are drawn up on the grey crescent side. It is suggested that this cortex-driven reorganization of the deep contents activates certain 'dorsal determinants' in the vegetal hemisphere, just on one side, to give a 'vegetal dorsalizing localization', abbreviated v.d.l. This region is inherited by vegetal cells arising in the next 12 cleavages, at which time the mid-blastula transition begins. Vegetal cells induce adjacent animal hemisphere cells to become prospective mesoderm, with the prospective dorsal mesoderm of the Spemann organizer region induced by the v.d.l. This induction of mesoderm may begin as early as the 64-cell stage. Then, during gastrulation, the prospective dorsal mesoderm migrates up the blastocoel wall and induces the neural plate, which in subsequent steps will form definitive dorsal organs of the nervous system.

seems to begin, namely, a rotation of the entire cortex relative to the yolk mass. In *Rana* eggs, this movement generates the grey crescent at the equatorial position farthest from the s.e.p. meridian, as pigmented cortex is withdrawn (Elinson 1980). In *Xenopus*, the grey crescent is difficult to see, perhaps because the pigmentation is less intense than in *Rana* eggs. However, this movement has been recorded recently by J.-P. Vincent in *Xenopus* eggs, the sub-cortical yolk mass of which can be stained with a hexagonal array of nile blue spots (figure 3, plate 1). The spots shift about 30° in relation to the immobilized cortex, and move toward the s.e.p. meridian. In other words, the yolk mass moves *away* from that meridian on which the dorsal mid-line will form. In some eggs, however, the movement is not directed exactly toward the s.e.p., but even 90° or more from it. In these cases, the s.e.p. turns out to be a poor predictor of the orientation of the fate map, whereas the direction of spot movement is an excellent predictor.

In normal cases, it is thought that the sperm aster determines the direction of cortical rotation, since in *Rana* eggs the grey crescent forms at the equatorial position opposite the site where an aster-forming sperm homogenate has been injected (Manes & Barbieri 1977). However, the aster is not essential for the rotation, since unfertilized *Xenopus* eggs that have been electrically activated also rotate the cortex on time, and artificially activated *Rana* eggs form a grey cresent, though at an unpredictable position. Thus, as a determinant, the aster would operate in the first 40 min after fertilization to provide a spatial cue to the independently scheduled process of cortical rotation. In the absence of this cue, the cortex seems to find some minor bias of the system and rotate according to it.

It has long been thought that the rotation creates a true and long lasting cytoplasmic localization, perhaps in the grey crescent cortex (Pasteels 1964). Since the grey crescent occupies approximately the position at which dorsal mesoderm (chorda mesoderm and head mesoderm) will arise, it is thought to contain 'dorsalizing' determinants. As discussed later, we agree with the notion that a dorsalizing or axializing localization exists near the grey crescent, but we think that it extends to polar positions of the vegetal hemisphere, and that it may reside in the endoplasm rather than the cortex (Gerhart *et al.* 1981).

What is the evidence that a dorsalizing or axializing localization is really formed at all in the course of this cortex-related movement? To begin with, we can assert that the embryo fails to develop dorsal axial structures if the rotation is prevented in the period before first cleavage. U.v.-irradiation of the vegetal surface can entirely block cortical rotation in *Xenopus* eggs (J. P. Vincent, unpublished), and block grey cresent formation in *Rana* eggs (Manes & Elinson 1980). The eggs cleave normally, but gastrulate late and symmetrically, and form a radial 'belly piece' resembling those embryonic forms obtained by Spemann (1902) from the ventral half of ligated eggs. These are the limit forms, which are not exceeded at still higher doses of irradiation. If irradiation of *Xenopus* eggs is done at 80 min, i.e. after the rotation has occurred, it has no effect on development. Cold shock and hydrostatic pressure, which probably affect very different targets than does u.v., have the same developmental effects as u.v., if applied before 80 min post-fertilization (Scharf & Gerhart 1983). Therefore, the effect is not treatment-specific, but response-specific. While discussing these effects, it is relevant to mention the intermediate grades of response. Low doses of u.v., cold, or pressure lead to reduced cortical movement (Vincent & Gerhart 1985; Scharf *et al.* 1984), and lead to incomplete development of the embryonic body axis (Malacinski *et al.* 1977; Scharf & Gerhart 1980, 1983). The axis is truncated at various levels from the head end. The tail is the last structure to be retained. Thus, the *amount* of movement seems related to the *completeness* of the axis, an intriguing relationship.

The second piece of evidence for the role of cortical rotation in the formation of a dorsalizing localization comes from rescue experiments (Scharf & Gerhart 1980, 1983). If the egg is held with its equator vertical, the yolk mass slowly slips to a new gravitational equilibrium, moving relative to the cortex. In this way, cortical rotation can be artificially restored to u.v.-irradiated, or cold-shocked, or pressure-treated eggs. These eggs, as it turns out, develop normally, and the dorsal mid-line of the embryo coincides with the meridian that had been uppermost in the gravitational field, that is, the region of the equatorial cortex *away* from which yolk had moved. Eggs can also be centrifuged in a fixed orientation, to obtain an artificial displacement of the endoplasm relative to the cortex, and the dorsal mid-line of the embryo coincides with the most centripetal meridian of the egg (Black & Gerhart 1984). The effects of gravity and centrifugal

force are independent of the position of the s.e.p. Intermediate extents of movement lead to intermediate degrees of rescue; the most anterior parts of the body axis are the last to be rescued. We suggest that the greater the displacement of the cortex and endoplasm relative to each other, the more extensive or intensive is the dorsalizing localization that is formed. We shall return to this quantitative relationship later.

What does it mean to say a 'localization' has been formed by cortical rotation? Are dorsalizing materials of the egg actually localized in one place, in the sense of collecting them from scattered positions? We favour a different possibility, namely, that dorsalizing agents are latent throughout the vegetal hemisphere, and are *activated* in only one quadrant. Our evidence is this. Twins can be formed easily when artificial movements of the cortex and endoplasm are carefully controlled by low-speed centrifugation of the egg, even of u.v.-irradiated eggs unable to form a single axis on their own (Black & Gerhart 1984a). It is necessary to perform two centrifugations, in opposite directions. The two axes of the twin form on the two centripetal meridians of the twice-centrifuged egg. It seems implausible to us that dorsalizing agents or 'determinants' would first collect in one vegetal quadrant during the first centrifugation, and then half of them would move to the opposite quadrant during the second centrifugation. More likely, the first centrifugation activates latent determinants of one region, and the second

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DESCRIPTION OF PLATE 1

FIGURE 3. The movement of vegetal dye marks at the time of grey crescent formation in *X. laevis*. A fertilized egg was dejellied and placed in a stainless steel cup perforated with hexagonally arranged holes (100 μm diameter). Nile blue (0.01 %) dissolved in chloride-free Ringer's solution, pH 7, was introduced beneath the grid, and staining was allowed to continue for 10 min. The egg was removed, washed, and embedded in a dish of 9 % molten gelatin (175 Bloom; 25 °C), which dehydrates the perivitelline space, and grips the egg surface firmly after it solidifies at 20 °C. The bottom of the egg was viewed with rhodamine fluorescence epi-illumination optics on a Zeiss microscope held in an inverted orientation. The dye marks appear bright because nile blue is fluorescent. Pictures were taken at regular intervals. In the lower right corner of each panel is given the time, represented as a decimal amount of the interval from fertilization (0.0) to first cleavage (1.0), which occurs approximately 90 min post-fertilization at 20 °C. Notice the progressive shifting of the dye marks toward the upper right. The distance of movement can be measured by reference to the line scratched on the bottom of the dish, at the right side. The closed and open circles indicate the original position of the mark, and its displacement of approximately 28° of latitude. From the direction of movement, it can be predicted with high reliability that the neural groove will appear at the meridian *away* from which the spots move. That is, the spots move toward the meridian which is prospective for the ventral mid-line of the tadpole. Notice that in this case, the s.e.p. position (indicated by S) would give a prediction of dorso–ventrality that would be incorrect by approximately 40°. Dorsal structures arose at the position marked D.

FIGURE 4. The ability of animal pole cells to develop as marginal zone cells, when transplanted at the 32-cell stage. At the 32 cell stage, 3 or 4 cells of the tier closest to the animal pole (tier 1) were removed from the s.e.p. side and were grafted to the marginal zone (tiers 2 and 3) of the second blastula, replacing cells of the grey crescent region. In (a) is shown the operated blastula, now at the 256 cell stage. The grafted blastomeres are easily recognizable by their dark pigmentation. In (b) is shown a fluorescence micrograph of a mid-trunk level transverse section of a transplantation control embryo. At the 32 cell stage, animal pole blastomeres of the type used for grafting were injected with fluoresein-lysine-dextran (FLDX), and left in their normal location. After development to stage 30 (tailbud), the embryo was fixed, sectioned, and examined for the positions of the progeny derived from the original fluorescent blastomeres. These progeny are found in the epidermis and in neural crest derivatives. Notice the absence of progeny in the notochord. (c) presents another control experiment in which marginal zone cells of the 32 cell stage, in the grey crescent region, were injected with FLDX. Their progeny at stage 30 occupy the notochord, somites, and neural tube, as shown in the mid-trunk transverse section. In (d) is shown a section of the experimental embryo, derived from a 32-cell stage blastula in which 3 or 4 marginal zone (grey crescent) blastomeres were replaced by FLDX-loaded animal pole blastomeres, as in (a). Notice in (d) that the animal pole blastomeres, after grafting, gave progeny which formed notochord, somite, and neural tube, just as did the marginal zone cells of the control of (c), and not as would be expected of animal pole cells left in position, as in (b). For details of the transplantation and cell-labelling methods, see Gimlich & Gerhart (1984).
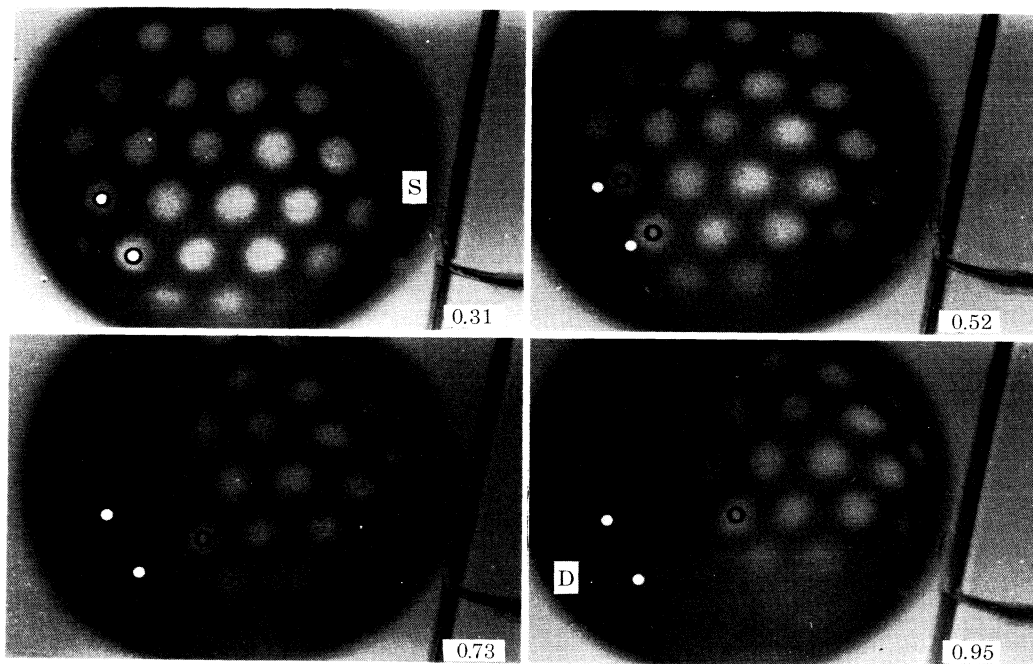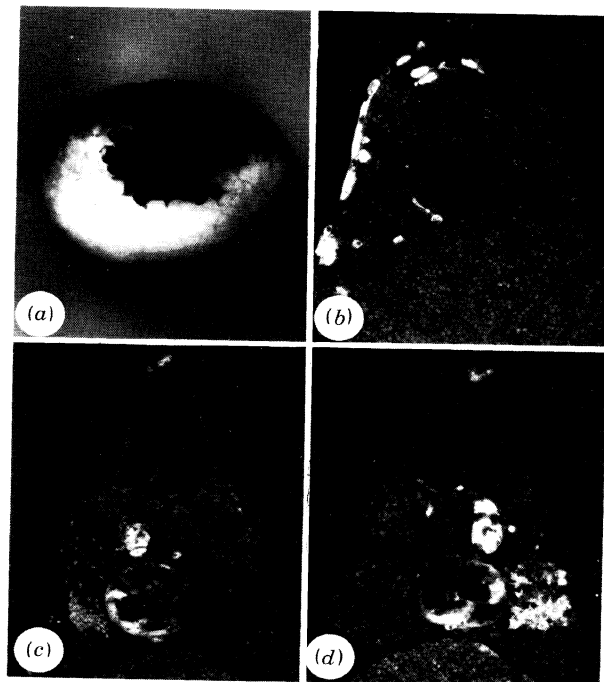
FIGURE 3. For description see opposite.

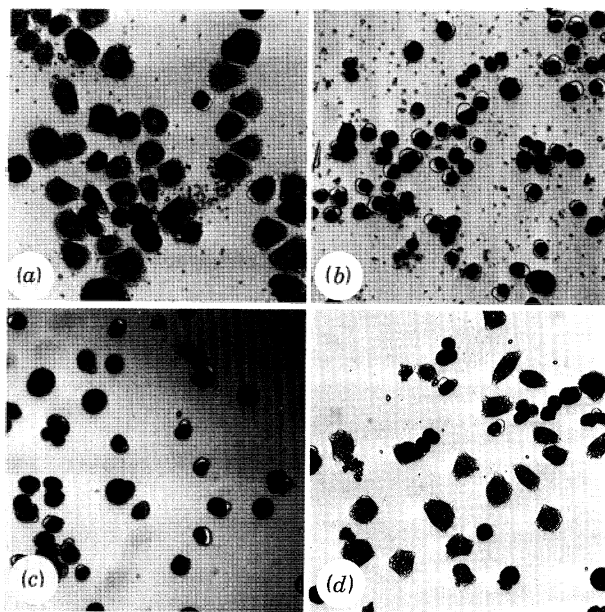FIGURE 4. For description see opposite.
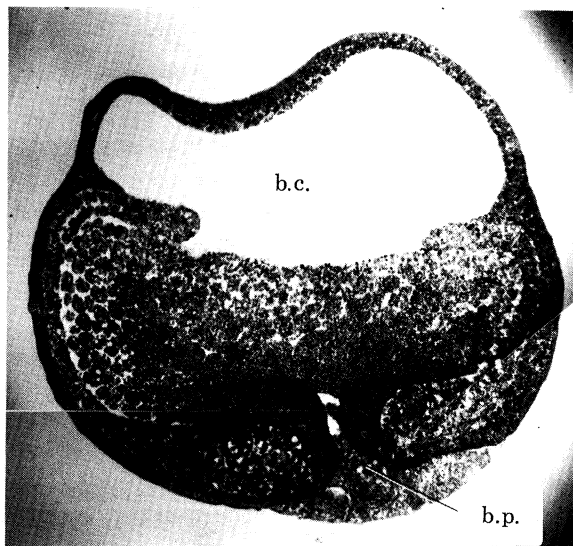
FIGURE 5. For description see opposite.

FIGURE 6. For description see opposite.

centrifugation activates others in the opposite region. Possibly a third and fourth centrifugation in the remaining opposed directions would activate the remaining quadrants, to produce a radially dorsalized egg.

As a last topic of this section, we can ask whether there is evidence that the dorsalizing localization is fully formed when the natural or artificial cortical rotation is completed, and whether the localization is long-lasting. Later we shall mention that the dorsalizing localization seems to control the inductive activity of the lineage of blastomeres cellularized from its region of vegetal cytoplasm. Is the localization ready, even at the time of first cleavage, to confer inductive activity on a cell, or are there additional steps needed before induction can begin? We have no answer to these questions, but should point out that other types of cytoplasmic movements start when the cortical rotation stops. The vegetal sub-cortical zone engages in new movements that, for example, concentrate dispersed germ plasm granules to the vegetal pole by the 4 cell stage (Whitington & Dixon 1975). Perhaps these movements create the real dorsalizing localization, oriented by a spatial cue left by the cortical rotation. That is, the effect of the cortical rotation may be no longer lasting than were the effects of the sperm aster, each in turn determining the spatial disposition of a next process. In summary, there is no evidence that the dorsalizing localization is actually complete in its inductive activity or spatial organization by the time of first cleavage, although it may be.

This reservation is in contrast with the classical view of the grey crescent as a cortical localization of a quantitatively graded morphogen (Dalcq & Pasteels 1937), a view that seems to imply that the patterning of subsequent development is finished in considerable detail in the uncleaved egg, with later stages merely expressing the pattern. We are suggesting that the pattern is far from complete in the uncleaved egg, but consists only of the primary localizations of animal and vegetal cytoplasm, and the secondary localization specializing one quadrant of the vegetal cytoplasm. Perhaps each early stage provides only enough 'completeness' of pattern for the next stage, and not a promorphology of the adult.

DESCRIPTION OF PLATE 2

FIGURE 5. Motile behaviour of marginal zone cells at the time of gastrulation. The blastocoel roof was removed and cells from the blastocoel wall, at the level of the floor, were removed and deposited on slides coated with collagen and human-serum fibronectin, at 22 °C. After 20 min, the cells were photographed. In (a) are shown cells from the marginal region closest to the blastopore of a stage 10+ gastrula. This region would correspond to the prospective head mesoderm and chordamesoderm. Notice the flattened appearance of most of the cells, with yolk-free lamellapodia at the periphery. Some cells show a leading edge, as they migrate. In (b) are shown cells of the marginal zone of a gastrula of the same age as in (a), but from a position opposite (180°) to the grey crescent, that is, well beyond the most lateral extension of the early blastopore. Notice that the cells have attached loosely to the substratum and are engaged in 'circus' or 'limicola' movements of the cell membrane, in which yolk-free protrusions rotate around the cell at approximately one r.p.m. There are no flattened cells. (c) Shows cells from the animal pole region of an early gastrula. These resemble the cells of (b) in showing limicola activity. In (d) are shown cells from the same position as in (b), but taken from a stage $11\frac{1}{2}$ gastrula (approximately 60 min later than stage 10+), when the blastopore has reached the opposite side from its starting point. Notice that the cells now show spreading activity and migration similar to that exhibited 60 min earlier by their opposite counterparts.

FIGURE 6. Section of an embryo, equal in age to stage 30 controls, that had been treated with 40 μM trypan blue at stage 10+. Trypan blue (400 μM stock, 30 nl) was injected in the blastocoel cavity. Notice the overall radial symmetry, persistent blastocoel (b.c.), and symmetrical blastopore lips (b.p.). This embryo lacks axial structures and resembles closely the grade 5 embryos derived from eggs treated before first cleavage with cold, pressure, or u.v.-irradiation.

4. Regionalization by inductive cell interactions

Nieuwkoop (1969*a*, *b*, 1973, 1977) and his colleagues established the importance of inductive interactions at the blastula stages for determining new specialized regions between and within the regions defined by the now-cellularized primary and secondary cytoplasmic localizations. The interactions concern particularly the formation of mesodermal precursors at the interface of the cellularized animal and vegetal hemispheres, as demonstrated in experimental 'recombinates' prepared at the midblastula stage from blastocoel roof cells and vegetal core cells. Neither of these types of cells would form mesoderm separately, but when they are juxtaposed by surgery, mesoderm originates from the animal hemisphere members. The origin of dorsal mesodermal precursors deserves special notice, since these cells constitute the Spemenn Organizer during gastrulation, and establish the site and pattern of neurulation. In the studies of Nieuwkoop, the dorsal mesodermal precursors arose from animal hemisphere cells juxtaposed by chance above the dorsal-most quadrant of the vegetal hemisphere. The vegetal cells of this quadrant have the capacity at the mid-blastula stage to induce their neighbours to become Spemann Organizer cells.

We have confirmed and extended these studies by working at early cleavage stages (32 or 64 cells) when blastomeres can be transplanted. R. Gimlich has tested for mesoderm-inducing activity in vegetal cells by grafting them into the vegetal tiers of 64 cell embryos from u.v.-irradiated eggs, which cannot develop dorsal mesoderm of their own. When two or three vegetal blastomeres are taken from the dorsal-most quadrant of the polar octet, and grafted into the same octet of an irradiated host, the host develops extensive dorsal mesoderm and a substantial body axis, in some cases including a complete rescue of even the anterior end (Gimlich & Gerhart 1984). When the grafted cells are marked with a lineage tracer (fluorescein–lysine–dextran; Gimlich & Braun 1984), it is found that their progeny cells occupy an entirely sub-blastoporal position at gastrulation, and give rise to gut derivatives, not axial ones. The host cells directly above the graft produce the notochord, head mesoderm, and neural tube. Thus, the rescue depends on the graft's ability to induce the host cells to form an axis. Furthermore, when these vegetal cells are grafted to the ventral-most quadrant of a normal 64-cell embryo, they cause a full-sized secondary axis to form at the otherwise ventral midline.

The inductive ability is strongest in the dorsal-most quadrant of the polar octet; in fact, rescue is not achieved with the lateral or ventral-most members (Gimlich & Gerhart 1984). From these results, we think that the vegetal dorsal-most cells do in fact harbour a dorsalizing or axializing localization, even though they originate beneath the grey crescent. They are specialized in their inductive ability, because of their possession of determinants received from the egg cytoplasm. We do not know at what time before the 64 cell stage the determinants arrive at the vegetal location, although it would be simplest to suggest they are activated there during the cortical rotation before first cleavage.

It is likely from Nieuwkoop's results that all animal hemisphere cells are able to respond to the vegetal induction, and to form dorsal mesoderm. We have tested this by taking animal pole blastomeres at the 32 or 64 cell stage of a normal embryo, and grafting them into the marginal zone, directly above the dorsalizing vegetal quadrant (figure 4, plate 1). Since the graft contains a lineage tracer, we can recognize the fates of the progeny cells. The grafted embryo develops normally and the progeny populate the notochord, somites, and archenteron roof, just as expected for marginal zone cells. Thus, even animal pole cells can respond to inductions from

their new neighbours, and can assume marginal zone fates. It seems likely that all animal hemisphere cells have this potentiality, perhaps as a result of their containing animal hemisphere endoplasm, one of the two primary localizations.

There is one respect in which the recent experiments of R. Gimlich could go beyond the results of Nieuwkoop, and this concerns the characteristics of normal marginal zone cells at the early cleavage stages. Since these cells originate from the border of the animal and vegetal hemispheres, they could be either inductive or responsive, it might seem. Nieuwkoop had to eliminate these cells from the midblastula by surgery, since by that stage they have the capacity to form mesoderm autonomously. R. Gimlich has tested marginal cells of the grey crescent area, for their ability to rescue u.v.-irradiated embryos when transplanted into their marginal zone at the 32 cell stage. Indeed, rescue is observed. In fact, the rescue is equivalent to that obtained with the dorsal-most vegetal cells. Lineage analysis shows that the grafted marginal blastomeres themselves contribute progeny to the notochord and somites of the rescued embryo. These are the expected fates of dorsal marginal cells, as shown in figure 4, and so we must conclude that the graft cells are at least able to *self-differentiate* as dorsal mesoderm. However, they also affect their marginal neighbours, inducing them to contribute progeny to somites and posterior notochord, structures not formed at all by u.v.-irradiated embryos in the absence of a graft. Thus, we may have to speak of the grafted marginal blastomeres as having mesoderm inducing activity, as well as themselves differentiating to mesoderm. On the other hand, a small amount of prospective sub-blastoporal material is transferred with the graft, and this contaminating material may be the inductive source. For simplicity, we suggest that the inductive activity of the marginal grafts is basically the same as that shown by vegetal grafts, and that the entire quadrant of the vegetal hemisphere, from the equator (and grey crescent) to the pole, contains dorsalizing determinants.

5. The effects of induction

We know nothing as yet about the mechanism of the induction, or of the precise time at which it occurs before the onset of gastrulation. None the less, we can make a few statements about the minimum effects of induction. These effects concern the time of onset of the region-specific motile behaviour of cells at gastrulation. The regional differences of morphogenetic activity represent what Spemann called the 'dynamic determination' of the early gastrula cells. Whereas we are sure this dynamic determination must exist by the time of gastrulation, we are not sure that the subsequent cytodifferentiation of gastrula cells has been determined by this time. For example, we should probably not speak of dorsal or ventral mesoderm cells at the gastrula stage, but only of early or late gastrulating cells of various regions.

According to Keller (1983), there are six or seven major regions differing in morphogenetic behaviour at gastrulation. These are arranged rather symmetrically along the animal–vegetal axis of the late blastula, and in layers from the surface to the interior (see figure 1). The marginal zone cells, located above the blastopore and below the geometric equator of the early gastrula, take the most active part in morphogenesis. It is likely that these regions are established by both localizations and inductive interactions. For example, the internal bands of prospective mesoderm, and the zone of involuting surface cells (prospective archenteron roof) in the marginal zone are probably established by a general inductive activity of the vegetal cells on the cells of the animal hemispheres (Nieuwkoop 1973, 1977). However, the formation of these major regions is probably not dependent on the special inductive activity of the dorsalizing

or axializing localization of the vegetal hemisphere, since they exist even in our 'ventralized' embryos produced from cold, pressure, or u.v.-irradiated eggs. These embryonic limit forms, which lack a dorsalizing localization, do manage to gastrulate, but do so in a completely symmetric manner.

What then could be the inductive effect of the dorsalizing localization? It is noteworthy that the main dorso–ventral differentiation of the gastrula stage concerns the *times* at which members of several of the six or seven regions begin their gastrulation movements. It is always the prospective dorsal members that gastrulate first. We shall therefore propose that the main inductive effect of the dorsalizing localization is simply to *advance the time* at which the responding cells will begin gastrulation.

To discuss this point of speculation, we must summarize the small amount of information about the timing of gastrulation. First, Kobayakawa & Kubota (1981) found that the initiation of gastrulation seems to depend on a maternal interval-meter not coupled to the timing of the midblastula transition (Newport & Kirschner 1982), nor to cell number, cell size, or replication cycles. It may depend on the progress of cytoplasmic processes initiated at the moment of egg activation, as has been suggested by researchers studying 'pseudogastrulation' in unfertilized *Rana* eggs (Holtfreter 1943; Smith & Ecker 1970). The 'real' intrinsic time of gastrulation is perhaps represented by the normal time at which the ventral lip of the blastopore is formed. In *Xenopus*, this occurs at approximately 11 h after fertilization. It is interesting that ventralized embryos from u.v.-irradiated or cold or pressure-treated eggs, form a complete circular lip abruptly at about this time (Gimlich & Scharf, unpublished). Thus, we propose that there is a basal rate of the embryo's advance toward gastrulation, set by an endogenous maternal meter.

Second, the relative times at which cells of the marginal zone begin gastrulation is rather well correlated with the dorso–ventral, and axial, fates of those cells. In *Xenopus*, the bottle cells of the dorsal lip appear approximately 60 min in advance of the bottle cells of the ventral lip, that is, one hour out of eleven. U.v.-irradiated eggs, when rescued by gravity or centrifugation, re-establish early bottle cells in the region of the blastopore lip that is prospective for the rescued dorsal mid-line. Furthermore, when u.v.-irradiated eggs are rescued at the 64 cell stage by the transplantation of normal vegetal blastomeres, the earliest bottle cells appear directly above the graft, and that region gives dorsal axial structures. In this case, it seems clear that the grafted cells induce their neighbours to 'bottle' earlier than they otherwise would. Finally, when eggs are treated with D_2O before first cleavage, they produce 'hyperdorsal' embryos which form a complete circular blastopore lip at the relatively early time, when dorsal bottle cells would normally form (Scharf 1985). Thus, bottle cells seem to follow a rough correlation of accelerated gastrulation activity and dorsal–anterior fate.

R. Gimlich has made a preliminary survey of the motile behaviour of deep marginal zone cells in gastrulae from normal and u.v.-irradiated eggs, to see if these cells also show a correlation of relative time of gastrulation and their fate. Cells were removed from the third layer (at the wall and floor of the blastocoel, see figure 1) and plated on fibronectin-coated collagen surfaces, upon which cells adhered and moved. As shown in figure 5, plate 2, the prospective dorsal marginal cells are the first ones to spread and move on the surface. Then, at later times, in sequence, the prospective lateral and ventral members begin to spread and move. Thus the deep cells of the marginal zone, like the bottle cells of the surface, begin gastrulation at different times.

It remains to be seen whether cells of each particular layer of the marginal zone initially

differ from one another *only* with regard to the time of onset of gastrulation. One could imagine that this is the case, since early and late bottle cells seem to have the same morphology, as do early and late deep marginal cells (figure 5). In this case, further differentiation of the cells would have to occur in the course of gastrulation. Perhaps the first cells to gastrulate have the longest time interval within which to acquire, in a sequence, various new behaviours related to eventual dorsal–anterior fates. Thus, dorsal cells would diverge most from ventral cells, the last ones to enter the series. In this way, the initial temporal differences could result in permanent qualitative differences among the cells of one region.

On the other hand, one could imagine that all pre-gastrula cells of a marginal region are changing their potentialities in a scheduled progression, and that early cells will not be identical to late cells, at the instant each starts gastrulation. In such a case, temporal differences would result immediately in regional qualitative differences of behaviour. None the less, by either scheme, temporal differentiation of cells in the marginal zone, because of inductive effects from the vegetal dorsalizing localization, would suffice for the generation of later dorso–ventral and anterior–posterior differences of cells.

In light of these proposals, it is interesting to note the effects on axialization, of agents that stop the morphogenetic movements of gastrulation (e.g. antibodies to fibronectin, Boucaut *et al.* 1984). M. Danilchik has found that the teratogen, trypan blue (Waddington & Perry 1956), terminates these movements almost immediately when it is introduced to the blastocoel during gastrulation, although neurulation and cytodifferentiation continue. If the agent is applied to the late blastula or early gastrula, the embryonic axis never forms (figure 6, plate 2), and the embryo resembles closely the 'ventralized' limit types produced by high doses of u.v., cold, or pressure applied to pre-cleavage-fertilized eggs. If trypan blue is applied at the midgastrula stage, the embryonic axis later contains tail and trunk, but no head. And if applied at the late gastrula stage, the eventual axis contains all structures except the most anterior ones. Kaneda & Hama (1979) have summarized evidence indicating that the prospective head and trunk mesoderm only gradually, in the course of gastrulation, gain their ability to induce anterior portions of the neural plate. This maturation process requires time, migration, and cell–cell interactions. In light of this evidence, we could explain the remarkable similarity of the anterior–posterior series of developmental defects in trypan blue arrested gastrulae, to the dose-dependent truncation series observed in the development of u.v., cold, and pressure treated eggs.

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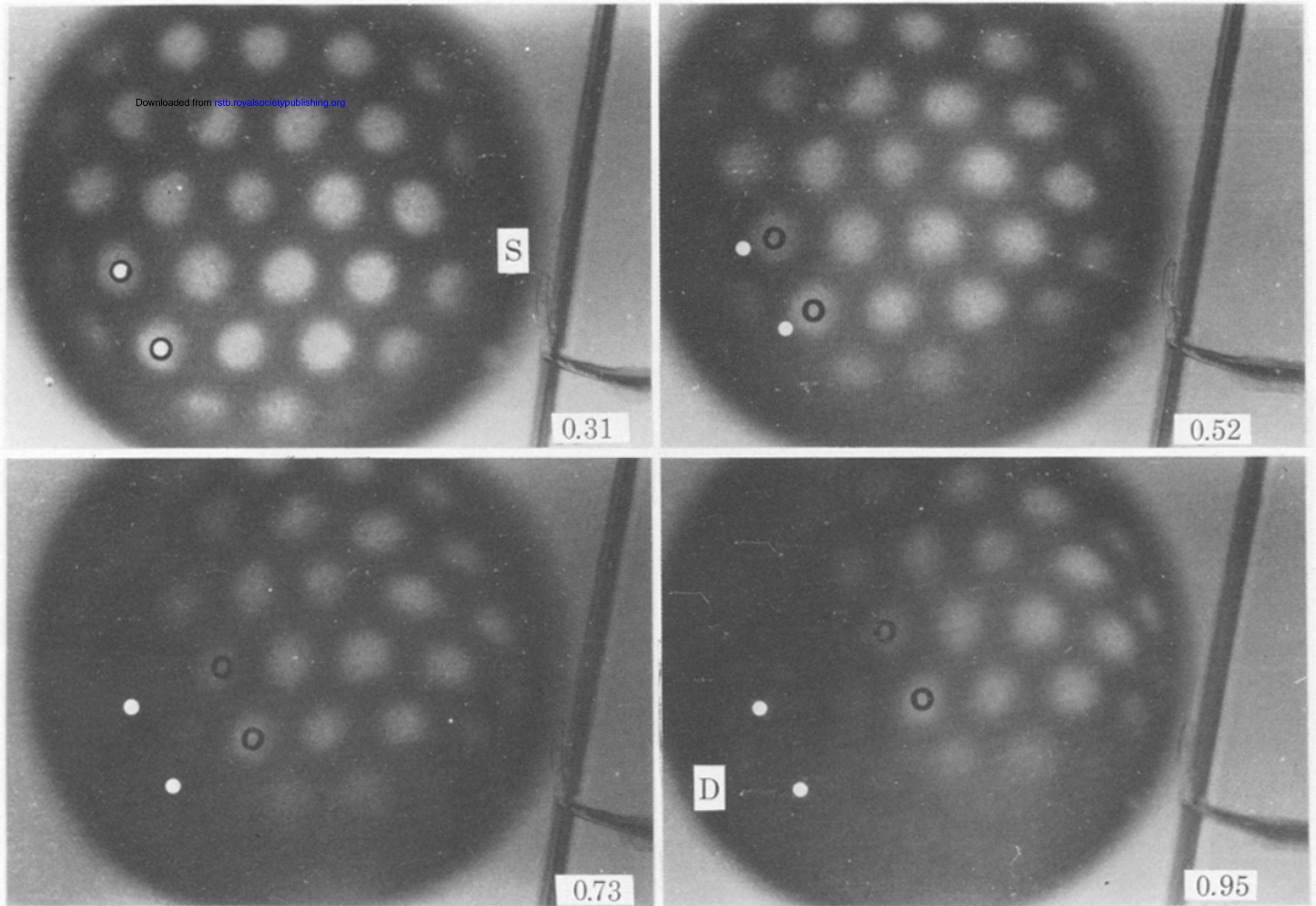
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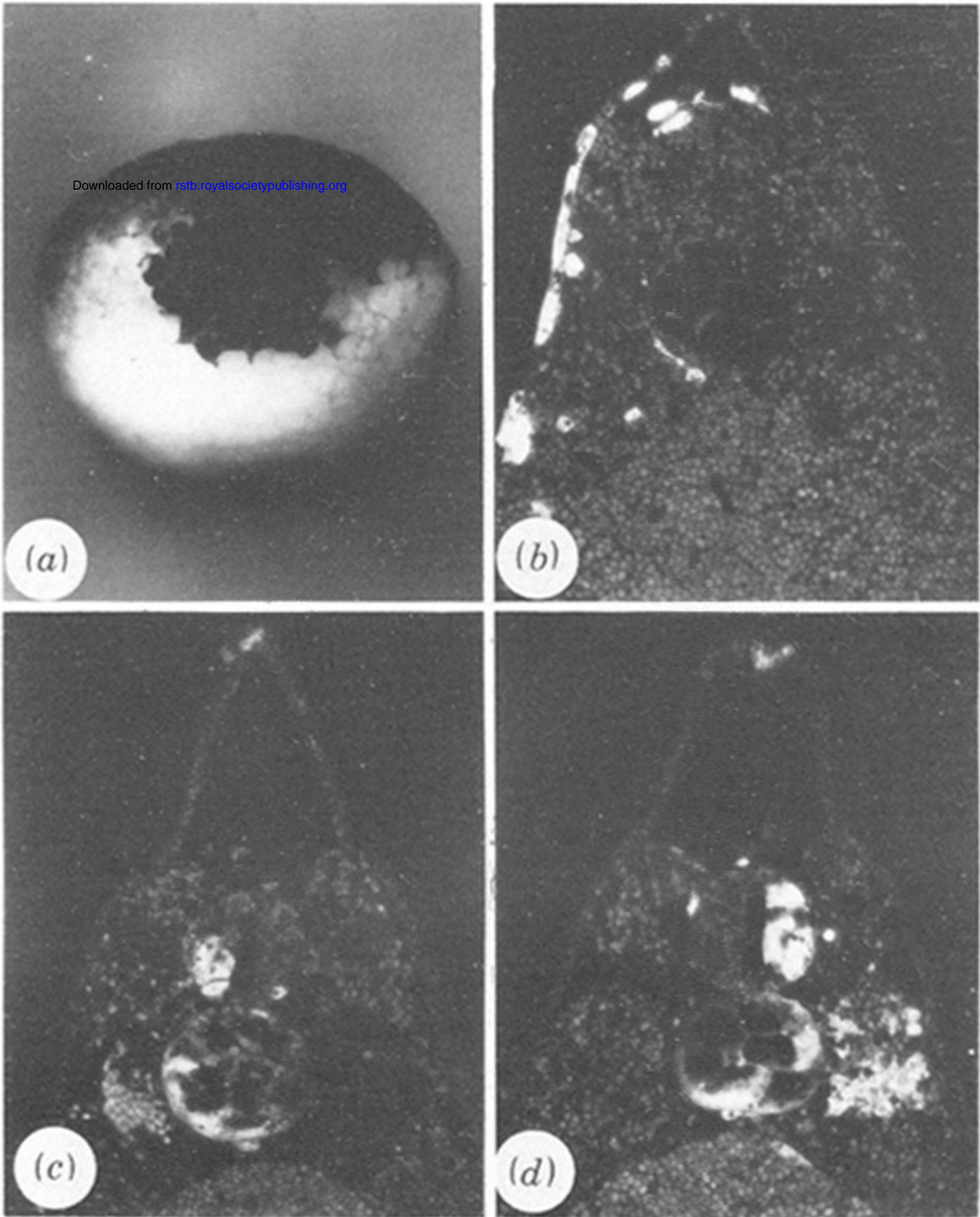FIGURE 3. For description see opposite.
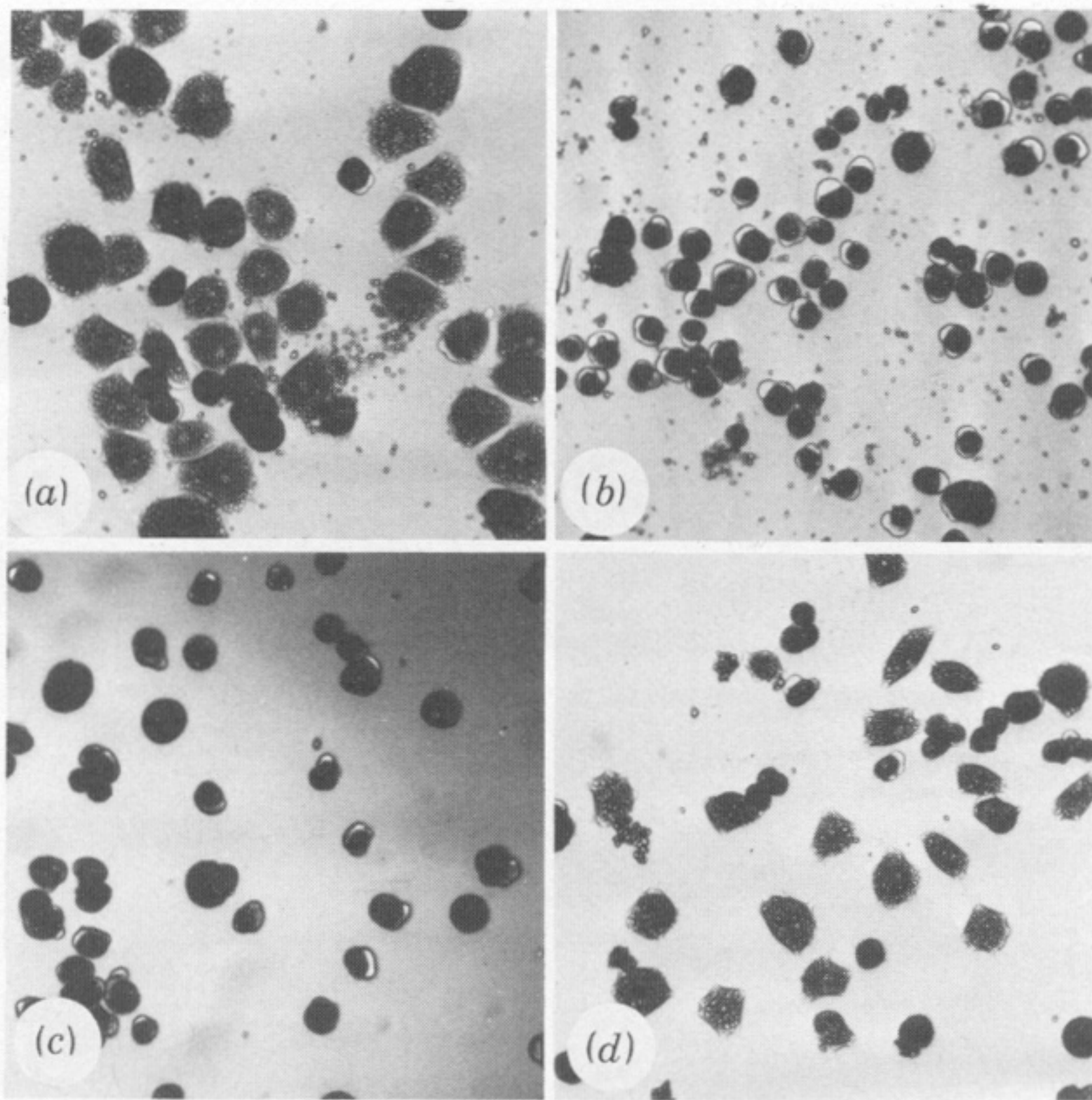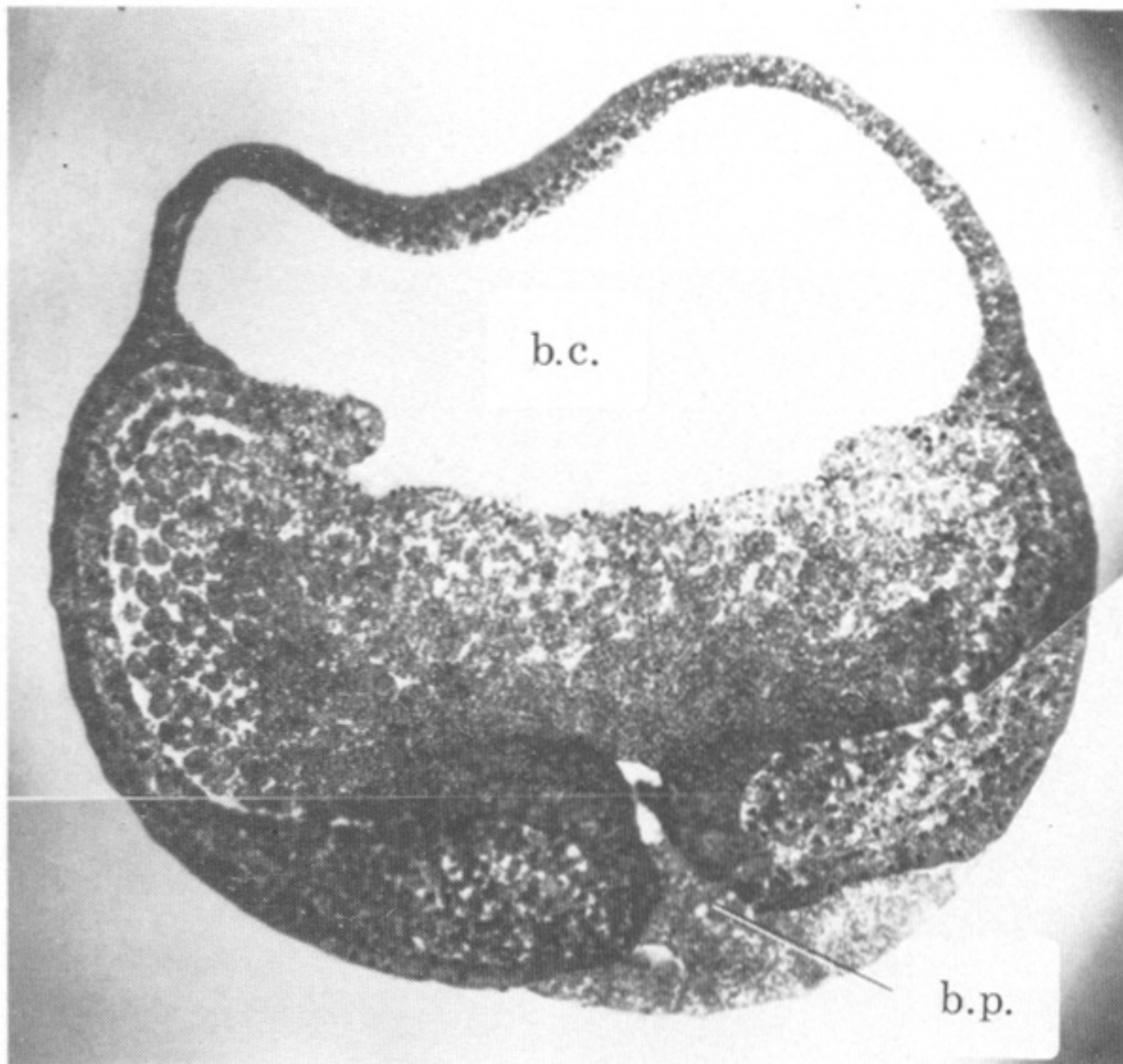
FIGURE 4. For description see opposite.

FIGURE 5. For description see opposite.

FIGURE 6. For description see opposite.