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Research Article

Mosaic and Regulation Phenomena during the Early Formation of the Chick Blastoderm

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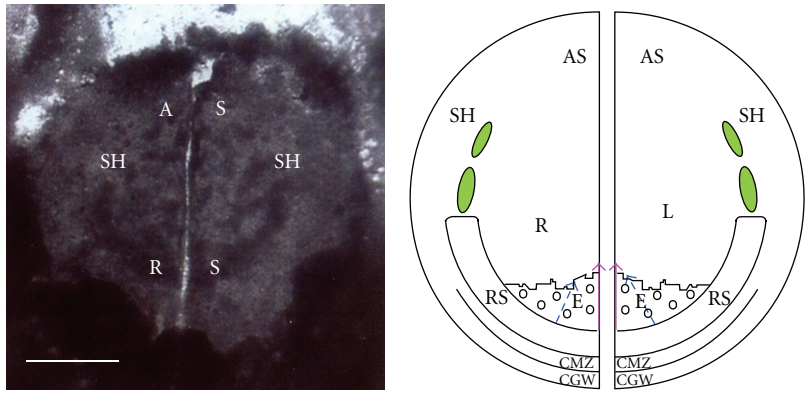
After culturing symmetrically hemisectioned unincubated chicken blastoderms, asymmetric hemiembryos developed (indicating mosaic development). In the present study, we observed that after prolonged culture, the further asymmetric development (way with no possible return) becomes profoundly disturbed, more particularly the Rauber's sickle-dependent phenomena: gastrulation and the formation of the coelomo-cardiovascular complex with absence of heart and pericard development. By contrast, the neural plate develops symmetrically. Asymmetrical ablation of Rauber's sickle and the neighboring upper layer results in the development of an apparently normal symmetrical embryo. Indeed, at the unoperated side, a normal half coelomo-cardiovascular system develops with a unilateral or bilateral heart tube and pericard formation (indicating regulation). Both regulation and mosaicism indicate that during normal early development, the interaction between the left and right sides of the caudal area centralis of the blastoderm is indispensable, depending on the spatial relationship between the elementary tissues (upper layer, Rauber's sickle, endophyll).

1. Introduction

Since the experimental studies in ovo of Lutz et al. [1, 2], Vakaet [3] and the in vitro studies of Spratt and Haas [4], it was generally accepted that the avian blastoderm always presents a highly regulative development, that is, that any isolated major part of it could develop into a normal symmetric embryo. However, we have shown that mosaicism can also be provoked in vitro, under certain circumstances, in unincubated avian blastoderm parts depending on the spatial distribution of Rauber's sickle [5] material and its relationship with the upper layer [6]. We use here the term mosaic development as originally defined by Conklin [7] in ascidian species; each region of the whole fertilized egg would be able to form more or less independently on its own. The development of the entire embryo was regarded as being the sum of the development of the interacting individual parts. In the present work, we studied in vitro the mosaic or regulation phenomena occurring during the early formation of the avian blastoderm and in later stages the development of the coelomo-cardiovascular system. We define the coelomo-cardiovascular system as the intimate association of blood islands (which will give rise to the cardiovascular system) with the more superficial coelomic vesicles (giving rise to the coelomic cavity) [8]. Both mosaic and regulation phenomena and the development of the coelomo-cardiovascular system are closely related since they are successively influenced by the localization of Rauber's sickle material (junctional endoblast). Until now, the earliest known localization of the cells of the prospective cardiovascular system in avian embryos has been determined in pregastrular blastoderms [9] and in the intermediate primitive streak stages [10, 11]. The latter authors localized heart and lateral plate precursor cells just lateral to/and parallel with the cranial part of the primitive streak. In the caudal blastoderm region, they found precursors of lateral plate and extraembryonic mesoderm. Explants from the caudal region of pregastrula chicken blastoderms give rise to blood tissue (haemoglobin) [12]. Caudal deep layer cells seem to play a role in cardiac myogenesis in pregastrular upper layer [13]. In these studies, however, no precise relationship with Rauber's sickle material was described since the fundamental inductive effect on gastrulation [14] and of the Rauber's sickle derived junctional endoblast on the formation of the coelomo-cardiovascular system was only more recently shown [8].



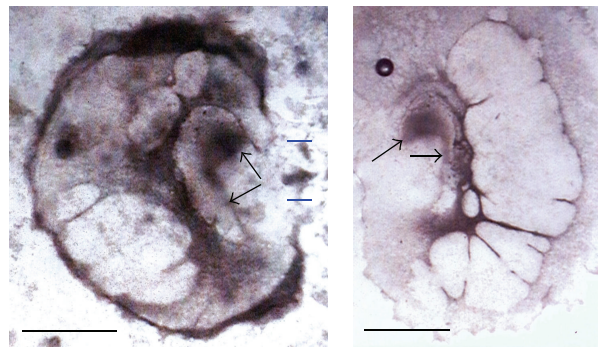
(a) (b)

Figure 1: (a): Ventral view of living symmetrical halves of an unincubated chicken blastoderm, after hemisectioning through the middle part of the Rauber's sickle (RS). AS, anti-sickle region where no Rauber's sickle material is present; SH, sickle horn. Bar = 1 mm. (b): Schematic representation of a symmetrically hemisectioned unincubated blastoderm as seen in Figure 1(a), to indicate its distinct components; AS, anti-sickle region; RS, half Rauber's sickle; SH, sickle horns. Tops are colored in green since they are often fragmentary and not always distinctly visible. Although containing Rauber's sickle material, they behave differently from the median part of Rauber's sickle. R, right hemi-blastoderm; L, left hemi-blastoderm; E, endophyll. The thick, red half-arrows represent the formation, after culture of hemi-primitive streaks at the cut edges of the blastoderm. CMZ, caudal marginal zone; CGW, caudal germ wall. The blue interrupted arrows indicate the localization of hypothetical symmetrical primitive streaks with reference to the half Rauber's sickles, if only regulation phenomena would occur, according to the median line, going through the three elementary tissues in one half blastoderm.

In the present experimental study, besides describing avian mosaic development in vitro we also studied regulation phenomena and compare them with the in ovo cleavage-with-traction experiments used by Vakaet [3]. In duck eggs, he performed cleavage experiments combined with traction either on the deep or the superficial blastoderm layer. He observed different developmental scenarios according to the kind of traction procedure, probably due to different reciprocal displacements of the deep layer with reference to the upper layer (more specifically at the rim of the blastoderm where endophyll-Rauber's sickle material is found). This incited us to perform in vitro analogous differential shifting experiments in more directly visible conditions by ablation or displacements of parts of one or more of the elementary tissues (upper layer, endophyll, Rauber's sickle). So we tried to find out why in terms of these elementary tissues, in one case regulation occurs while in another mosaicism takes place.

2. Materials and Methods

We used unincubated chicken (Gallus domesticus) eggs. We studied in culture the effect on general development or particularly the development of the coelom and associated cardiovascular system after ablation experiments in unincubated chicken blastoderms or parts of it. This was obtained by mediosagittal hemisectioning, or by removing half of the Rauber's sickle material in whole unincubated blastoderms or after oblique hemisectioning. Each experimental procedure is represented in a scheme accompanying the photomicrographs. The blastoderm parts were cultured according to the technique of Spratt [15]. The semisolid culture media allow microsurgery and further culture on the same substrate. Stereomicroscopic photographs were taken in the same direction at the beginning, during, and at the end of the culture period. After fixation, the blastoderms were stained with Unna in toto to visualize the localization of blood-containing structures in surface views [3]. Embedding in paraffin was performed as mentioned in earlier studies [8]. The blastoderms were sectioned perpendicularly to the visible or presumed axis. The deparaffinized sections were



(a) (b)

Figure 2: (a): Stereomicroscopic ventral view (alive) of the isolated right half of an unincubated chicken blastoderm after 1 day of culture; the hemi-embryo is localized close to the free cut edge (now bent). Two parts of the embryo are visible, the broad head region (upper arrow) and the trunk region with a narrow hemi-PS (indicated by lower arrow). Confirmed after sectioning and staining. (plane of sections, indicated by two parallel bars) Bar = 1 mm. (b): Stereomicroscopic ventral view (alive) of the isolated left half of an unincubated chicken blastoderm after 1 day of culture. It forms a mirror image with the right half embryo of Figure 2(a) with the same indications. Bar = 2 mm.

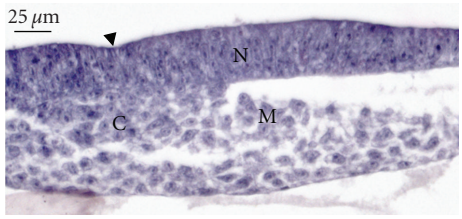


Figure 3: Section (plane indicated in Figure 2) through the neural plate anlage (N) with neural groove (arrowhead), developed in a half-chicken embryo after 1 day of culture. C, prechordal plate; M, cranial mesoblast. Harris hematoxylin eosin. Bar = 25 μm.

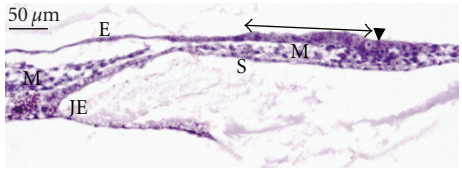


Figure 4: Section (plane indicated in Figure 2) through a chicken hemi-embryo cultured during 1 day. The arrowhead indicates a hemi-primitive groove in which upper layer cells ingress into the hemi-primitive streak, giving rise to a mesoblast mantle (M) which slides peripherally between the junctional endoblast (JE) and the epiblast (E); sickle endoblast (S); double-arrowed line indicates the surface of the thickened hemi-PS-forming upper layer. Hematoxylin eosin, bar = 50 μm.

stained with Harris's or Heidenhain's haematoxylin and eosin.

3. Results

3.1. Development after Culture of Isolated Symmetrically Hemisectioned Avian Unincubated Blastoderms ($n = 22$). A symmetrically hemisectioned unincubated chicken blastoderm is seen in Figure 1(a). The components of such half-embryos are schematically represented in Figure 1(b). After culture of the separated hemi-blastoderms (seen in Figure 1(a)) for one day, we obtained two mirror image hemiembryos disposed near the sectioned border of each (Figures 2(a) and 2(b)). The voluminous head region forms a medially directed angle with reference to the axis of the narrow trunk region. By comparing the stereomicroscopic aspect of the cultured half blastoderms with views after sectioning, it was obvious that a broad neural plate with neural groove and underlying cranial mesoblast, notochord and prechordal plate had symmetrically developed in each half blastoderm (Figure 3). In contrast, in the trunk region, a hemi-primitive streak, limited to a narrow asymmetric line lying near the cut-edge of the asymmetric half embryo was observed (Figures 2(a) and 2(b)). In sections, a hemi-primitive groove is seen in which unilaterally the thickened upper layer grows inwards, forming a unilateral mesoblast mantle. This mesoblast mantle extends between the sickle endoblast and upper layer medially and between the junctional endoblast and epiblast laterally (Figure 4). More laterally above the junctional endoblast, some Anlagen of blood

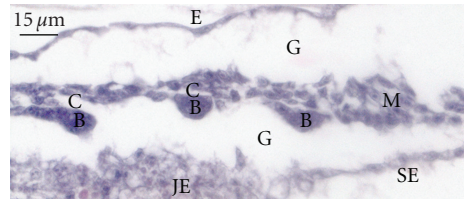


Figure 5: Section through the mesoblast mantle (M) and the more lateral coelomo-vascular area (area vasculosa) of a chicken hemi-embryo after one day of culture. E, epiblast; B, blood islands domed by rudimentary coelomic vesicles (C); JE, junctional endoblast; SE, sickle endoblast; G, intraembryonic cavity. Hematoxylin-eosin. Bar = 15 μm.

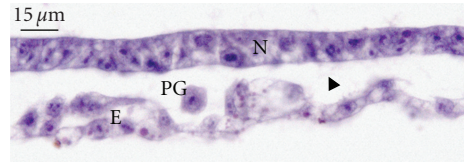


Figure 6: Section through the cranial part of a symmetrically hemisectioned chicken blastoderm after 28 hours of culture; neural plate (N); endophyll (E); PG, primordial germ cell in the neighborhood of the endophyll. Hematoxylin-eosin. Bar = 15 μm.

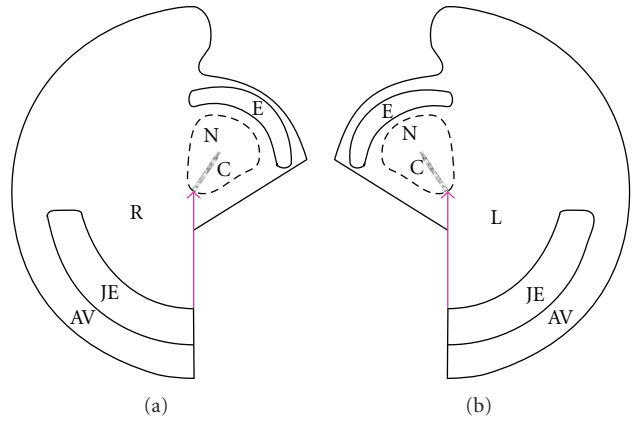


Figure 7: (a): Schematic representation of the different structures found in a right hemi-embryo after 1 day of culture. The head region is bent over the original midline and contains the endophyllic crescent (E) with associated primordial germ cells. A central notochord (C) and prechordal plate have formed below a symmetrical neural plate (N). A hemi-primitive streak (indicated by a red half arrow) is localized in the cut edge of the hemi-embryo. R, right side of the original blastoderm; JE, half junctional endoblast derived from the original half Rauber's sickle with parallel area vasculosa (AV). (b): Schematic representation of the structures found in a left hemi-embryo after 1 day of culture; the same indications as in Figure 7(a) but at the left side (L).

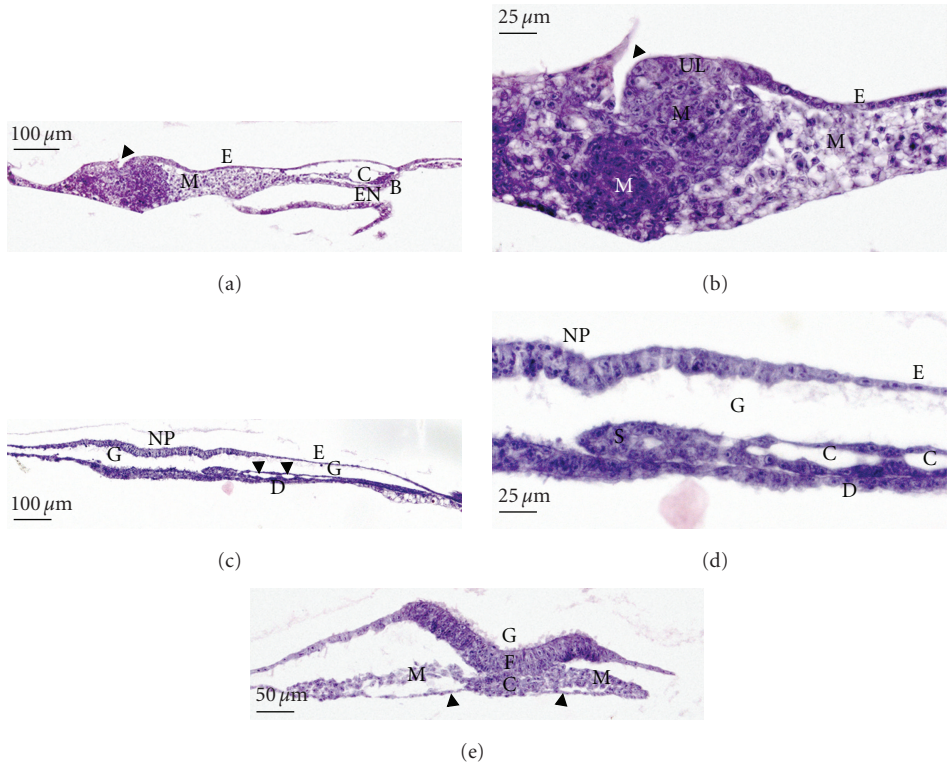


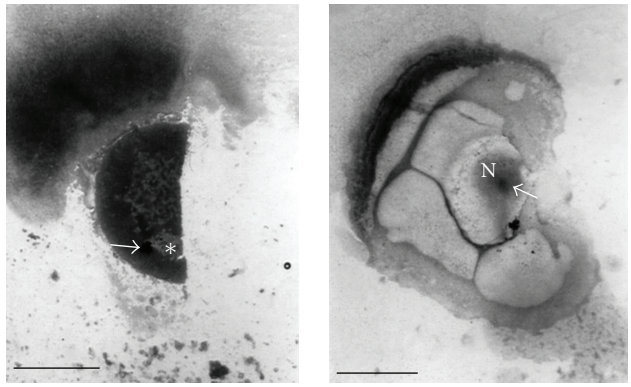
Figure 8: (a): Section through a hemiembryo after 2 days of culture. A hemi-primitive streak (with groove, indicated by arrowhead) is seen close to the cut edge; the unilateral mesoblast mantle (M) extends laterally (at the right of the figure) to form some coelomic vesicles (C) which form a dome over blood islands (B) in close association with the gut endoderm (EN). E, epiblast. Hematoxylin-eosin. Bar = 100 μm. (b): Magnification of the medial part of Figure 8(a), showing the hemi-primitive streak region in detail. The hemi-primitive groove (arrowhead) is formed by unilateral ingrowth of thickened upper layer cells (UL) forming mesoblast strands (M) in the depth; E, epiblast (note the different aspect of the thicker undifferentiated upper layer and the thinner skin forming epiblast). Hematoxylin-eosin staining. Bar = 25 μm. (c): Section through the cranial part (neural plate: NP) of a chicken hemi-embryo after 2 days of culture, at low magnification. Only a row of rudimentary coelomic vesicles (arrowheads) has developed in the unoperated side, forming the most cranial part of the coelomic cavity. Note the large intraembryonic cavity (G), empty at the operated side. No heart tube and no pericard are seen even in the unoperated side (right of the figure). Hematoxylin-eosin. Bar = 100 μm. (d): Higher magnification of median part of the unoperated side, seen in 8C. S, somite; C, coelomic vesicles between the superficial parietopleura and the deeper splanchnopleura (forming blood islands) and adherent to the deep layer (D); bar = 25 μm. (e): Section through the head region of a hemi-embryo after 2 days of culture, showing a neural groove (G) with median structures (derived from Hensen's node); floor plate (F) and accompanying notochord (C), definitive endoderm (arrowheads); M, cranial mesoblast; hematoxylin-eosin. Bar = 50 μm.

islands domed by coelomic vesicles appear (Figure 5). Most cranially, we see the preneural plate-inducing endophyll (derived from the half endophyll after hemisectioning of the blastoderm (Figure 6)). In the space between neural plate and endophyll wall, a few primordial germ cells, usually close to the endophyll (both containing δ ooplasm, [16]) can be observed. The neural plate expands beyond the surface area of the inducing endophyll [6] and this seems to be the reason for the median deviation, with accompanying prechordal plate and notochord, in the head region (see Figures 2(a) and 2(b)).

Figures 7(a) and 7(b) represent schematically the development of the different structures found in the right or left hemiembryos after approximately one day of culture (based on sectioning and staining).

After 2 days of culture, the hemi-blastoderms develop further, but their evolution becomes irreversibly disturbed and they no longer have the normal general aspect of a chicken embryo. However, in sections, the development of the two main regions (head and trunk) could be discerned. In the trunk region, the hemi-primitive streak and groove are still more prominent (Figures 8(a) and 8(b)).

The unilateral growth of the thickened upper layer into the half-primitive groove and the accumulation of parallel strands of mesoblast along the half-primitive groove are visible (Figure 8(b)). At the unoperated side, the mesoblast mantle develops coelomic vesicles forming a cap over the blood islands (Figure 8(a)). In the more cranial part of the embryo, unilaterally below the neural plate, somite-like structures form with laterally and peripherally extending coelomic vesicles (Figures 8(c) and 8(d)). The latter are localized between the superficial parietopleura and the deep splanchnopleura Anlagen. Neither primary heart tube nor pericard have developed. In the



(a) (b)

Figure 9: (a): Ventral view of living hemisectioned unincubated chicken blastoderm (right side), at the start of the culture period: the median part of the right Rauber's sickle half has been removed and the deep side of the upper layer is visible (*); graphite particle on the cross sectioned part of Rauber's sickle (indicated by white arrow). Bar = 2 mm. (b): The same hemi-embryo as in Figure 9(a), after 25 hours of culture: a rectilinear primitive streak, with tissue on both sides, starts from the cross-sectioned part of Rauber's sickle (graphite particle) in the direction of the nodus (indicated by white arrow) and neural plate (N). Bar = 2 mm.

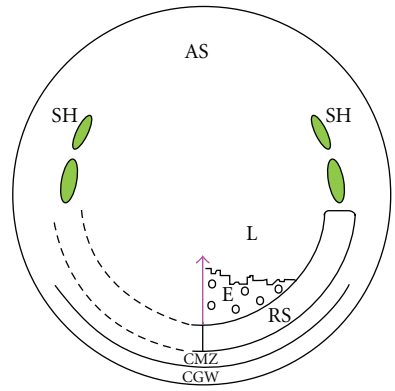
cranial region, a bilaterally symmetric neural plate with neural groove and floor plate, in close contact with a median notochord and flat definitive endoderm, is present (Figure 8(e)).

3.2. Development In Vitro of Isolated Symmetrically Hemi-Sectioned Avian Unincubated Blastoderms in Which the Median Part of Rauber's Sickle Was Scraped Away (n = 9). Such a half blastoderm is seen as the start of the culture (Figure 9(a)). Graphite particles were placed on the cross-sectioned part of the Rauber's sickle horn. After 25 hours of culture (Figure 9(b)), an area pellucida has developed in which a pyriform embryo is seen. The rectilinear primitive streak starts from the original cross-sectioned, graphite-labelled part of Rauber's sickle. Also a nodus and hemi-circular neural plate, cranially from this primitive streak are obvious. The composition of these structures can been recognized in sections.

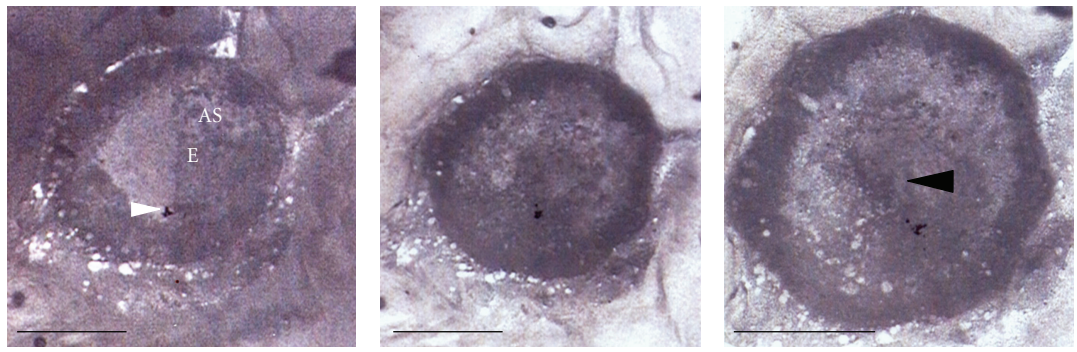
3.3. Development In Vitro of Unincubated Chicken Blastoderms after Removal of Half of Rauber's Sickle and Associated Endophyll (Schematically Represented in Figure 10(a)) (n = 9). Figure 10(b) depicts an unincubated chicken blastoderm in which the right half of the Rauber's sickle-endophyll complex was removed, at the start of the culture (as represented in Figure 10(a)). The sectioned middle part of Rauber's sickle is labelled with a graphite particle. Under the stereomicroscope, we can follow the evolution of the operated blastoderm in vitro. After 5-6 hours in vitro, a denser tissue grows into the median part of the operated side (Figure 10(c)). After 10 hours of incubation, usually a primitive streak, surrounded by a clear area, appears. The primitive streak starts growing from the sectioned middle part of Rauber's sickle and the primitive streak maintains its original expected orientation as if no ablation was performed (Figure 10(d)). In sections, at the operated side, the primitive streak-forming-upper layer is lower and extends less laterally then at the unoperated side (also seen under the stereomicroscope) (Figure 10(d)). It is important to note that the ingression of the upper layer into the primitive groove and streak occurs from both sides. Thus the neighboring upper layer from the operated side also is rapidly taken up and no hemi-primitive streak forms, in contrast to what happens after symmetrically hemisectioning of the blastoderm, indicating that also upper layer from the operated side is indispensable for normal development. After a culture period of 29 hours and after in toto staining with Unna, we can see under the stereomicroscope that an apparently symmetric normal embryo has developed (Figure 11(a)). In the operated side, no blood islands are observed. In contrast, the area vasculosa at the unoperated side contains many blood islands and blood vessels. This is also seen after histological sectioning. Most obvious is that the intra-embryonic cavity is laterally closed at the operated side since epiblast and deep layer adhere in the absence of Rauber's sickle-derived junctional endoblast (Figure 11(b)). The intra-embryonic cavity contains no mesoblast and no coelomic cavity. At the unoperated side, in contrast the intraembryonic cavity extends far peripherally and is there largely open. This side contains a normal mesoblast mantle which extends far laterally. Coelomic vesicles, at the origin of the coelomic cavity, and associated blood islands, in close contact with the endoderm layer, form laterally. It is in this region that the heart and pericard will develop. Indeed, after a somewhat longer period of incubation (34 hours), the Anlage of a hemi-pericard and associated primary heart tube were seen in the unoperated side (Figure 12). Somites and intermediary mesoderm (pronephros) are also seen.

Although mesoblast is present at both sides, neither hemi-pericardial cavity nor primary heart tube was observed in the operated side. Thus, only in the unoperated side, the Anlage of a primary heart tube is formed. At the operated side, the extent of the intraembryonic cavity is very limited by the adhesion of the epiblast with the deep layer (Figure 12). This is due to the absence of junctional endoblast over which the blood islands and coelomic vesicles-forming-mesoblast normally slide in a peripheral direction [16–18]. Asymmetry in heart formation is not seen in all the unilaterally operated blastoderms. This could be due to the fact that not all the Rauber's sickle material at the operated side could be seen and removed (particularly the sickle horn top, indicated in green in Figure 10(a)). To avoid this, we used a more radical oblique hemisectioning technique, between the two earlier described procedures, after which only one sickle horn region persisted (Figure 13(a)).

3.4. Oblique Hemisectioning of the Unincubated Chicken Blastoderm and Unilateral Removal of Rauber's Sickle Material and Endophyll (n = 7). The excision technique is



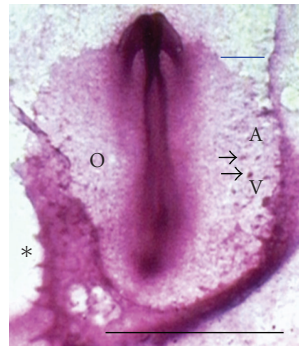
(a)



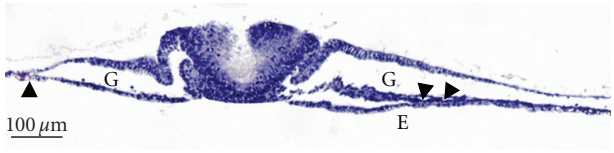
(b) (c) (d)

Figure 10: (a): Schematic representation of the surgical removal of the right half of the endophyll-Rauber's sickle (RS) complex of an unincubated chicken blastoderm. The top of the sickle horns (SH) is fragmentary (in green) and cannot always be distinguished. AS, anti-sickle region; CMZ, caudal marginal zone; CGW, caudal germ wall; L, left of the blastoderm; the red arrow indicates where the symmetrically formed primitive streak will appear after incubation. (b): Removal of the endophyll-Rauber's sickle complex at the right side as represented in Figure 10(a), at the start of the culture period. A graphite particle, indicated by white arrowhead, is placed in the middle region where Rauber's sickle is sectioned. E: half left endophyll sheet; Bar = 2 mm. (c): The same blastoderm as seen in Figure 10(b) after 5 hours of culture; a darker linear area has appeared at the operated side. Bar = 2 mm. (d): The same blastoderm as seen in Figure 10(c) after 9 hours of culture. A primitive streak (indicated by arrowhead) starting from the graphite particle is localized in a clearer area (a realization) and is directed to the unoperated side. Bar = 2 mm.

schematically represented in Figure 13(a) and performed alive in an unincubated blastoderm at the start of the culture period (Figure 13(b)). The top of the right sickle horn region is completely removed by oblique excision of half of the blastoderm. Moreover, the remaining right half of Rauber's sickle and endophyll are removed and discarded. At the left side, the half Rauber's sickle and accompanying endophyll are left intact. After seven hours of incubation, a centrally directed primitive streak (starting from the original middle of Rauber's sickle, as visualized by the application of a graphite particle) is seen (Figure 13(c)). The uptake of upper layer material from the operated side into a nearly symmetrically developing primitive streak is obvious. Thus, here also no hemi-primitive streak develops (as is also confirmed by histological processing). After prolonged culture (in casu 31 hours), a slightly asymmetric embryo proper has developed, bending with its convexity towards the unoperated side (here left side). In the cranial region of the unoperated side, a beating heart tube is observed (darker rounded aspect just rostrally from the anterior intestinal portal) (Figure 13(d)). After fixation and in toto staining with Unna (Figure 13(e)), the area vasculosa is seen to develop only at the unoperated side (mainly caudally). In sections through the cranial part of this region, one sees that only in one side (unoperated) a hemi-coelomic cavity (hemi-pericardial cavity) with accompanying epimyocard and endocard developed (Figure 13(f)). In the present embryo at the operated side, no pericardial cavity and no heart tube are found. However, in some embryos, bilaterally primary heart tubes developed with associated bilateral hemi-pericardial cavity (closed peripherally in the operated side) (Figure 14). This seems to indicate that in some cases the coelomo-cardiovascular system crosses cranially the midline and extends into the other side (horse shoe-shaped heart and pericard Anlagen). The primary heart tube(s) always develop in the region of a sickle horn and not in the caudal median region of the Rauber's sickle. This indicates some predisposed polarity along the Rauber's sickle.



(a)



(b)

Figure 11: (a): In toto Unna stained (after fixation) blastoderm, operated as represented in Figure 10(a), after 29 hours of culture. An apparently normal embryo proper has developed symmetrically. At the operated side (O), only a few blood islands in a narrow area vasculosa are seen, and in the area vitellina an empty region (*) is visible. In the unoperated side, a larger normal area vasculosa (AV) is seen with numerous blood islands (black arrows). Bar = 2 mm. (b): Section through the embryo of Figure 11(a) (plane of section is indicated in Figure 11(a) as a blue line). G, intraembryonic cavity closed at the operated side (indicated by the arrowhead directed upwards) contains no mesoblast. In the unoperated side, the intraembryonic cavity (G) extends far peripherally. It contains a mesoblast mantle which laterally forms blood islands close to the endoderm (E) which are domed by coelomic vesicles (indicated by 2 arrowheads directed downwards). Bar = 100 μm.

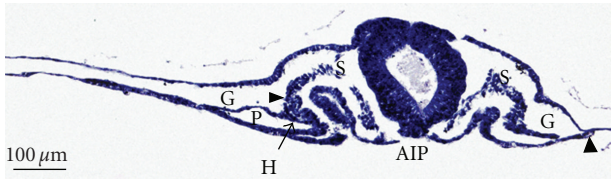


Figure 12: Section through a similar blastoderm as described in Figure 10(a), after 34 hours of culture. In the operated side (at the right of the figure), a mesoblast mantle is present in the intraembryonic cavity (G), which is closed as the result of the absence of junctional endoblast at that side. No blood islands and no coelomic cavity are formed. At the unoperated side, the intraembryonic cavity (G) is not interrupted laterally and contains blood islands close to the deep layer. Part of the coelomic cavity is visible (hemi-pericardial cavity: P) with unilateral primary heart anlage (H) in the neighbourhood of the infolding gut endoderm, laterally from the closing anterior intestinal portal (AIP). S, somites; arrowhead indicates intermediary mesoderm (nephrotome). Bar = 100 μm.

4. Discussion

Obtaining of hemi-primitive streaks and hemiembryos (right or left) in vitro by hemisectioning the avian unincubated blastoderm, in a plane going through the middle of its ooplasmic symmetry (Rauber's sickle material) (Figures 2(a) and 3(a)), is comparable with what was achieved in ascidians [7, 19] or in Rana [20, 21] by destroying one of the first two blastomeres. These results were considered as evidence for the preformation contra the epigenetic theory, where regulation was accepted [22]. We have demonstrated that, in the chicken blastoderm in culture, both mosaic or regulation phenomena can be obtained, only by the different geometric distribution of Rauber's sickle material in the isolated blastoderm fragment [6]. In birds, the bilateral ooplasmic symmetry (recognizable by the appearance of the Rauber's sickle) occurs much later than in anurans, already visible by the appearance of the grey crescent before the first cleavage takes place. The ascidian egg has been regarded as a typical mosaic egg which shows a highly determinate mode of development [23–25]. The localization and function of Rauber's sickle present a strong similarity with the localization and function of the also caudal sickle-shaped Wnt expressing gene from Halocynthica roretzi [26]. It is remarkable that in the case of twin formation in ovo by mediosagittal sectioning [1, 2, 27] or in vitro [4], it has not been observed that mosaic development also exists in birds. One of the reasons is that in their experimental procedure the exact orientation of the presumed caudocephalic axis was not precisely known, due to the external egg orientation according to Von Baer's rule [28]. Wolff and Lutz [27] found after incubation, in about half of the cases, incompletely developed embryonic formations after cleaving unincubated duck blastoderms in ovo. We think that part of these incomplete embryonic formations could be hemiembryos after occasionally sectioning through the very middle of Rauber's sickle. The comparison of the results of experiment 1, with those of experiments 3 and 4 indicates that a half Rauber's sickle-endophyll complex only induces a primitive streak and a whole embryo proper if upper layers from both sides (left and right) are disposable in the caudal area centralis. Both an equal quantity of upper layer (half the surface of a blastoderm) and an equal and identical quantity of Rauber's sickle material are present in experiments 1 and 4. A mediosagittal hemisectioning (in experiment 1) makes thus a world of difference for further embryonic development. That upper layer cells in the concavity of both left and right halves of Rauber's sickle are needed for normal primitive streak formation which can also been explained by the experiments of Lepori [29]. Indeed, he observed the existence in the upper layer of a centripetal, chiral, not-mirror symmetric counter clockwise movement, which results in an asymmetric ingression (left side earlier and more pronounced and directed to the right and the depth) during early gastrulation. The comparison of the results of experiments 1 and 2 indicates that a proportional lower quantity of Rauber's sickle material gives a more advanced development. In the first case (experiment 1), only a hemi-primitive streak and hemi-embryos formed indicating

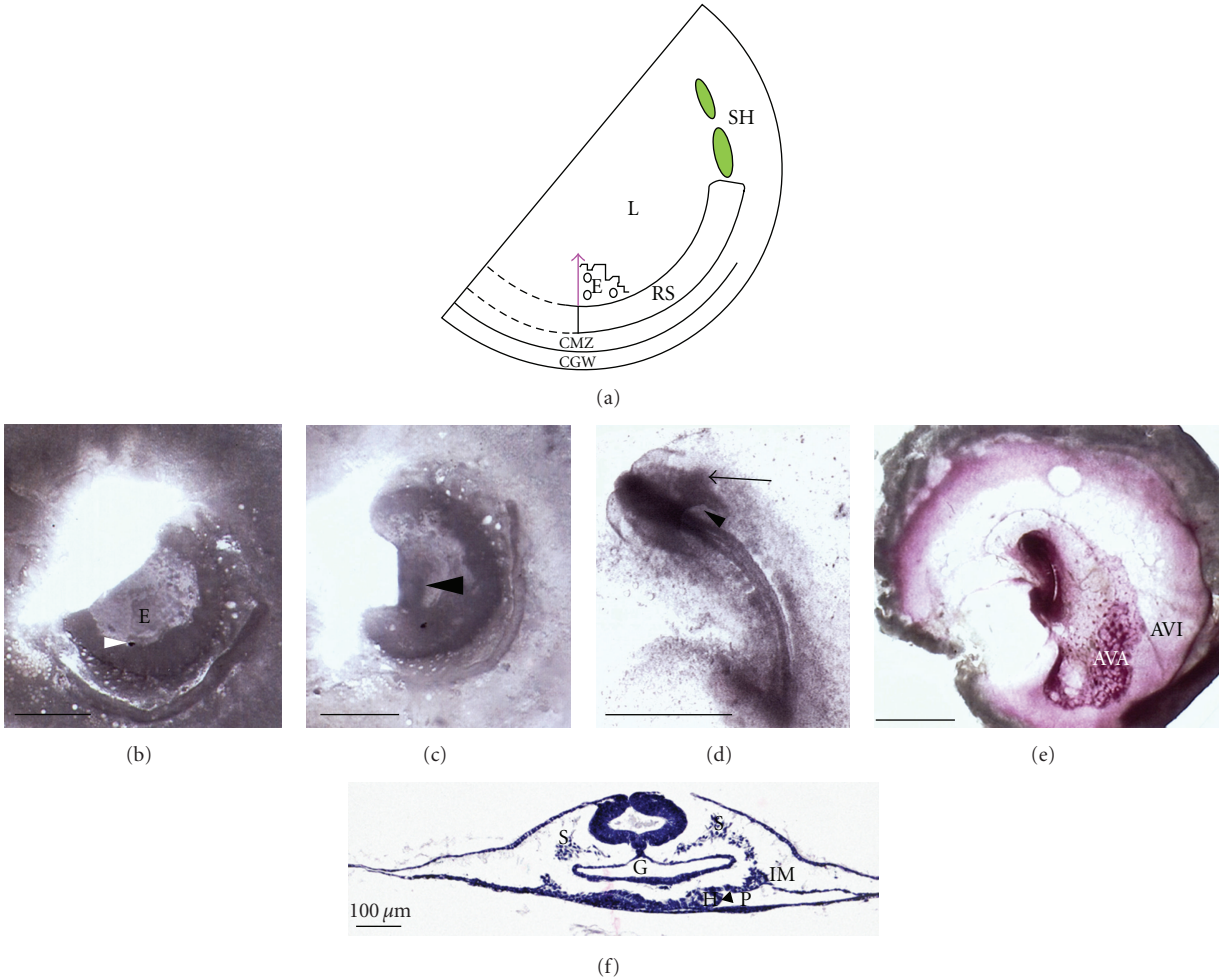


Figure 13: (a): Schematic representation of the oblique hemisectioning of an unincubated chicken blastoderm in which the sickle horn and half median part of the Rauber's sickle (indicated by double interrupted lines) and endophyll were surgically removed on the same side. SH, top of the remaining sickle horn region (in green) extending far cranially; RS, remaining Rauber's sickle half and E, remaining half endophyll; CMZ, caudal marginal zone; CGW, caudal germ wall; L, unoperated left side of the blastoderm. (b): Unincubated chicken blastoderm (operated according to the schematic representation in Figure 13(a)) at the start of the culture period; a graphite particle (arrowhead) is placed on the sectioned median part of Rauber's sickle. E, left half of endophyll sheet localized in the concavity of left half of Rauber's sickle of the unoperated side. Bar = 2 mm. (c): The same blastoderm after 7 hours of culture: a densification with primitive streak (arrowhead) formation parallel with the cut edge is observed. Bar = 2 mm. (d): Living somite embryo developed in the blastoderm of Figure 13(c), after 31 hours of culture; arrow indicates a beating denser round mass (corresponding to the left primary heart tube as visible in sections) just cranial from the anterior intestinal portal (arrowhead) at the left side; bar = 1 mm. (e): The same blastoderm after fixation and in toto staining with Unna; the area vasculosa (AVA) lies caudally and laterally from the embryo proper. AVI, area vitellina interna. At the right side no area vasculosa has formed due to the total absence of Rauber's sickle material in this side. Bar = 2 mm. (f): Section through the heart region of the embryo seen in Figure 13(d) shows unilaterally (at left side of the embryo) a hemi-pericardial cavity (P); arrowhead indicates the epimyocard; H, unilateral heart tube; G, foregut; S, somite-like material and intermediary mesoderm (IM) splitting into parietopleura and splanchnopleura. Harris hematoxylin-eosin. Bar = 100 μm.

mosaicism; in the second case (experiment 2), a pyriform embryo with bilateral primitive streak is formed, indicating regulation. In experiments 3 and 4, in which upper layer from both halves of the blastoderm is present, a more advanced embryonic development takes place (with uni- or bilateral heart tubes and pericardial cavities). Thus more or less pronounced regulation phenomena take place only in vitro when the cross-sectioned part of the remaining Rauber's sickle material (γ ooplasm containing) is surrounded with upper layer cells (β ooplasm containing). This can explain why in the cleavage experiments of Wolff and Lutz [27], Lutz [1], or Vakaet [3], only regulation phenomena were described. More particularly, the in ovo cleavage and/or traction experiments of Vakaet [3] giving rise to well-developed twins or even triplets can be explained by that local loosening of contact between Rauber's sickle material and upper layer, in the neighborhood of the incision rim, as is the case in our in vitro study. In any case, a primitive streak (regulation)

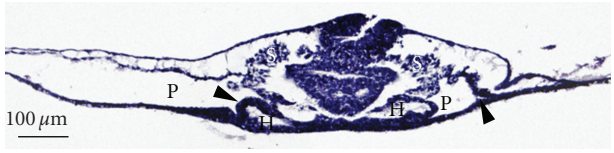


Figure 14: Section through heart region of an embryo after 40 hours of culture of an unincubated blastoderm operated on as represented in Figure 13(a). In the unoperated side (left side of the photograph), a normal far extending hemi-pericardial cavity (P) is seen with normal epimyocard (arrowhead). At the operated side, the hemi-pericardial cavity (P) is closed (upwards directed arrowhead) and no blood islands are formed peripherally. S, somite-like material; H, bilaterally formed primary heart tubes. Bar = 100 μm.

or half primitive streak (mosaicism) always starts from the incised part of Rauber's sickle. Also Levin and coauthors [30–32] concluded from their study of asymmetrically expressed genes that the left and right sides in the chicken blastoderm can be viewed as distinct and autonomous fields. Not only are both flanks (containing sickle horns as we describe here) required, suggesting that there is no single signalling source of left-right patterning, but the blastoderm also must be intact. Levin and Mercola [32] have observed that introducing discontinuities in the circumferential path by making peripheral slits in the chick blastoderm likewise affects left-right asymmetry. A pronounced asymmetric development is started from the beginning of incubation in the hemi-blastoderm under the strong influence of the half median part of Rauber's sickle-endophyll complex, but also by the absence of the upper layer cells of the contralateral removed side. The only possibility for the upper layer cells in the remaining half of the blastoderm is to slide medially over the basement membrane [33] into the direction of the cut edge of the half blastoderm, which results in the formation of a half primitive streak and mosaic development with an asymmetric embryo. We think that during normal development, at the start of incubation, both halves of the blastoderm develop more or less independently as parts of a mosaic under influence of the corresponding neighboring halves of Rauber's sickle. Somewhat later, the interaction between the left and right halves of the formed primitive streak becomes more pronounced and is indispensable for further normal embryonic development. The interaction between left and right halves of the blastoderm seems to be concentrated mainly in the midline region, at the contact zone between left and right lip of the top of the primitive streak. At a more advanced stage of development (3-4 HH), a medial band (resembling a hemi-primitive streak), a notochord, floor plate, and half neural tube were obtained by culture of lateral blastoderm isolates [34]. By contrast, in our study a complete bilateral neural plate was formed due to the presence of endophyll [6]. If only regulation phenomena would strictly exist in any part of the blastoderm (so-called totipotency), as propounded by Lutz et al. [1, 2], one could expect the development of a symmetrical primitive streak growing according to the median line, going through the whole of the three elementary tissues together, in one half blastoderm (see Figure 1(b)).

The production of avian hemiembryos can perhaps be useful for the early determination by laterality genes of left-right asymmetry since the left hemi-embryo is induced point by point, by positional information of the left half of Rauber's sickle and associated sickle horn [35] via the left half primitive streak. The same phenomena occur at the right side. By mediosagittal hemisectioning of the unincubated chicken blastoderm, some midline structures are destroyed in our study. This also could disturb normal morphogenetic development. Indeed it has been propounded that the midline structures play a role in the regulation of left-right sidedness [36]. The idea that the midline acts as a barrier during left-right specification was first proposed by Danos and Yost [37] to explain the results of experiments where the midline was experimentally manipulated or excised, as is the case in our mediosagittaly sectioned blastoderms (experiment 1).

In conclusion, in our present study we compared regulation and mosaic phenomena after in vitro ablation experiments of the three elementary avian tissues [16] with the formerly described studies of cleavage and/or traction performed in ovo. The very existence of mosaicism in birds indicates that interaction between both left and right sides of the blastoderm is indispensable for early development.

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