A study of the properties, morphogenetic potencies and prospective fate of outer and inner layers of ectodermal and chordamesodermal regions during gastrulation, in various Anuran amphibians

By T. A. DETTLAFF

From the N. K. Koltzov Institute of Developmental Biology, USSR Academy of Sciences, Moscow

SUMMARY

In both the ectodermal and the chordamesodermal regions of Anuran embryos, the outer layer of cells possesses epithelial properties and has the same restricted morphogenetic potencies. It is thus interchangeable between the regions, capable of epiboly and, when underlain by notochord material, of the formation of bottle-shaped cells as at the blastoporal groove, and invagination. When taken from the chordamesoderm region, this outer layer has no inducing effect on the ectoderm of the early gastrula. In normal development the outer layer of the neural plate takes an active part in forming the neural tube cavity. It gives rise to the neuroepithelial roof of the diencephalon and medulla oblongata and, when underlain by neuroblasts that develop from the inner cell layers, to ependymal cells of the brain wall. The outer layer of the notochord material is included in the epithelial layer underlying the roof of the gastrocoel – the hypochordal plate. The inner layers of these regions consist of loosely arranged cells and normally have no epithelial properties although, when taken from the ectoderm region, they may acquire such properties upon long-term contact with the environment. However they have wide morphogenetic potencies; the differences in these potencies between cells taken from the various presumptive regions being less than the differences between outer and inner cell layers in each region. Maps are provided which show the arrangement of presumptive rudiments in the ectoderm and chordamesoderm on sagittal sections through Bombina bombina embryos in early and late gastrulation.

INTRODUCTION

A study of the properties of ectoderm and chordamesoderm layers in late blastula and early gastrula in Anura may clarify the effects of contacts between cells in these two layers, which undergo different morphogenetic movements and possess different morphogenetic potencies. Early segregation of the entire material of the primary ectoderm in embryos of various Anurans into outer and inner layers, and different involvement of those layers in forming axial organs, was established back in the middle of the nineteenth century (Reichert, 1840;

1 Author's address: N. K. Koltzov Institute of Developmental Biology, USSR Academy of Sciences, Moscow, USSR.
Bambeke, 1868; Goette, 1875; and many others) and later confirmed for a number of species from the genera *Rana* and *Bufo* (Giersberg, 1924; Dettlaff, 1936–1948; Holtfreter, 1938; Schechtman, 1938; Lövtrup, 1965, 1966), and for *Xenopus laevis* (Nieuwkoop & Florschütz, 1950; Nieuwkoop & Faber, 1956).

Recently, segregation of the ectoderm and chordamesoderm into layers has been studied in greater detail using modern techniques. It was established in *Xenopus laevis* embryos (Perry, 1975; Keller & Schoenwolf, 1977) that the outer layer represents a monolayer of cells combined in the epithelium by circular apical tight junctions, whereas the inner layer consists of a large number of loosely arranged interdigitated cells interconnected by processes. There is no interlayer cell exchange during epiboly (Keller, 1978). In experiments involving separation and transplantation of ectoderm and chordamesoderm layers, evidence was obtained as to their morphogenetic potencies (Holtfreter, 1938, 1939; Schechtman, 1938; Dettlaff, 1936–1948; Rossi & Niste, 1963; Rossi, 1965a,b; Sudarwati & Nieuwkoop, 1971; Keller, 1981). However, the cellular properties of these layers have not been elucidated sufficiently.

The present paper offers information on cellular properties, morphogenetic potencies and prospective fates of the layers, and also shows fate maps of presumptive rudiments in the ectoderm and chordamesoderm layers in Anuran embryos at early and late gastrula stages, previously published incompletely (Dettlaff, 1936–1948).

**MATERIALS AND METHODS**

The subjects of investigation were *Rana temporaria, R. arvalis, R. ridibunda, R. esculenta, Bufo viridis, B. bufo, Pelobates fuscus* and *Bombina bombina* embryos, and in some experiments with xenoplastic transplantations, also *Ambystoma mexicanum* and *Triturus vulgaris* embryos.

The ectoderm and chordamesoderm layers were separated by means of a sharp ophthalmic knife. The embryos were maintained in a Holtfreter's solution. The outer layer was cut on three sides in the region examined and carefully lifted, if possible so as not to touch the inner layer cells; it was then bent aside to remove individual inner layer cells stuck to the inner side, using a hair loop, (Fig. 1). After that, the separated piece was cut on the fourth side and instantly, so that it could not curl up, transferred to a prepared site in a recipient embryo. The inner layer, already liberated from the outer layer, was then isolated and transplanted to another recipient embryo. Auto-, homo-, hetero- and xenoplastic transplantations were performed. Following the operation, the embryos were fixed after various intervals in Bouin's fluid, stained totally with boric carmine with additional overstaining of sections (10 µm) with Mallory or staining of sections with Delafield's haematoxylin.

To study the reversibility of polar segregation in the ectoderm, it was turned outer layer downwards and returned to its former place. To elucidate the ability
of the outer and inner ectoderm and chordamesoderm layers to differentiate, these regions were transplanted to various embryos at the late neurula or early tail-bud stages, via an incision in the wall into the pharynx cavity. In addition, layers of notochord material were transplanted either into a superficial position in place of a removed section of the presumptive epidermal outer layer or in the blastocoel and the ectoderm layers of late gastrulae, superficially in place of a removed section of the neural plate (onto an exposed chordamesoderm under-layer) in neurulae. To clarify the prospective significance of the outer and inner layers within the presumptive notochord and neural plate, we performed (a) exchange transplantation of the outer layer between embryos of various Anuran species and genera, (b) removal of the outer layer of the notochord, and neural plate material, and (c) their substitution for an outer layer taken from various regions and at different embryo stages to study the derivatives of both layers.

RESULTS

Reversal of ectoderm polarity

The result varied depending on the position of the inverted section, but the outer and inner layers behaved differently in all cases. The outer layer was separated from the inner layer and curled up; this was usually accompanied by the occurrence of large closed cavities lined with a flat epithelium (Fig. 2A). Only when the outer layer contacted the endoderm, along which it does not grow, did the endoderm comprise a part of these cavity walls. In cases where the epithelial vesicles adjoined the pericardium or were situated in the sucker region,
sucker cells producing mucin differentiated from the outer layer cells, the apical ends of which were invariably turned inside the epithelial cavities (Fig. 2B), i.e. the outer layer cells not only did not transform into inner layer cells, but retained their initial polarity.

Inner layer cells generally formed compact accumulations. These were situated between the original inner layer turned inside the embryo and the outer layer that had overgrown from the incision margins (Fig. 2C), or were without any connection whatsoever with cavities formed by the outer layer. In appearance, these accumulations resembled the inner layer cell accumulations in the neural plate; however, their final differentiation cannot be established from this experiment because of the early stages at which the embryos were fixed.

When the inverted region of the *B. viridis* ectoderm is transplanted to a newt embryo, the transplant outer layer also forms closed epithelial vesicles inside. Some of the inner layer cells that had stayed all the time on the surface are incorporated into the covering epithelium, and some of them differentiate into sucker glandular cells.

Thus, unlike outer layer cells, the inner layer cells of ectoderm can change their polarity, but only on long-term contact with the environment. When situated inside the embryo, their behaviour differs from that of the outer layer cells.

**Differentiation of ectoderm and chordamesoderm outer and inner layers following homoplastic transplantation to pharynx cavity**

In all the experiments, the implants were in the pharynx cavity for the first day; after that, the outer layer remained suspended in the pharynx cavity and the inner layer first fused with the pharynx wall and subsequently grew more or less completely into the body wall, where it was surrounded by mesenchymal cells. The differences described are indicative of different contact relationships between the outer and inner layer cells and the endoderm of the pharynx wall or, according to Holtfreter (1939), different 'affinitat'.

Table 1 shows the experimental results. By the time of fixation, some of the outer layer implants were absent, some were in a state of disintegration (such cases have not been included in Table 1), and only in half of the cases did the implants form lumps of usually unorganized epithelium and glandular cells, or even whole suckers (Fig. 3A, C). The sole difference between differentiation of the ectoderm and notochord outer layers was that individual vacuolized notochord cells were occasionally encountered in epithelial formations which had
Table 1. Differentiation of ectoderm and chordamesoderm outer- and inner layers (% of number of differentiated implants)

<table>
<thead>
<tr>
<th>Type of differentiation</th>
<th>Outer layer</th>
<th>Inner layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ecotderm</td>
<td>Notochord</td>
</tr>
<tr>
<td>Epithelium</td>
<td>68.4</td>
<td>58.8</td>
</tr>
<tr>
<td>Epithelium + sucker</td>
<td>21.1</td>
<td>41.2</td>
</tr>
<tr>
<td>Sucker</td>
<td>10.5</td>
<td>0</td>
</tr>
<tr>
<td>Epithelium + sucker</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ notochord cells</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epithelium + notochord</td>
<td>10.5</td>
<td>0</td>
</tr>
<tr>
<td>cells</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epithelium + sucker</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ neural tissue</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epithelium + neural</td>
<td>0</td>
<td>0</td>
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<tr>
<td>tissue</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Neural tissue</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Notochord + neural</td>
<td>0</td>
<td>0</td>
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<tr>
<td>tissue</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Notochord + muscles</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ neural tissue</td>
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<td>Muscles + neural</td>
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<td>Muscles</td>
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* A: R. temporaria and ridibunda; B: R. esculenta. In parentheses: number of differentiated implants.

developed from the outer layer of the notochord material. In cases when more inner layer cells were captured, they formed small accumulations of notochord cells in the body wall.

Unlike the outer layer of notochord material, the notochord inner layer formed neither epithelium nor sucker cells. Inside the body wall, at the pharynx level, there was a large notochord with or without neural tissue and muscles (Fig. 3B).

Cell differentiation of the ectoderm inner layer differed from that of both the ectoderm outer layer and the inner layer of notochord material. In all cases, the inner layer cells showed broader possibilities for differentiation than the outer layer cells. In R. temporaria and R. ridibunda, ectoderm inner layer cells developed into an epithelial monolayer if mesenchyme underlay them and they themselves bordered the pharynx cavity or (in gill slits) the outer environment; if mesenchyme did not underly them, they gave rise to unorganized epithelium
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Fig. 3. Differentiation of outer and inner layers of ectoderm and notochord material, taken at the early gastrula stage and implanted homoplastically in the pharynx cavity of *R. temporaria* embryos: (A) outer layer of notochord material remained in pharynx cavity to form a lump of unorganized epithelium, inside which four vacuolated notochord cells are seen; (B) inner layer of the notochord material is included into the body wall and differentiates into the notochord, muscles, renal tubules, and a small part of brain; (C) ectoderm outer layer has remained in pharynx cavity and transformed into a lump of unorganized epithelium with sucker cells on surface; (D) inner ectoderm layer has intergrown with pharynx wall to form a protrusion covered by an epithelial monolayer, inside which a brain region has differentiated among mesenchymal cells. Bar = 200 µm.
with sucker cells, or to suckers. In addition, brain regions differentiated from some of the inner layer cells that remained in the mesenchyme in the body wall at the level of the pharynx cavity (Fig. 3D); those sections consisted of grey and white material and in some cases also have ear vesicles. In *R. esculenta*, the implant was almost completely incorporated into the body wall, and the epithelium and sucker cells correspondingly occurred much less frequently than in *R. temporaria* and *R. ridibunda*; besides isolated neural tissue, in half of the cases they included notochord and, less often, muscle.

**Experiments with layers of presumptive notochord material**

After implantation of layers of presumptive notochord material into the blastocoel of early- and mid-gastrulae, 80 embryos were studied as sections. Of the 40 embryos which were implanted with outer layers, 32 (80%) had irregular epithelial masses, 11 of them also differentiating sucker cells. Only 8 of these 32 embryos had small volumes of graft-derived notochord and brain tissue at a distance from epithelial masses, and 9 had individual notochord cells without neural tissue. None of the 18 cases (45%) in which the implant differentiated only into an atypical epithelium and sucker cells showed additional neural tissue.

Of 40 embryos with an implanted inner layer of notochord material 36 (90%) developed an additional notochord, and 3 (7.5%) developed muscles; they had no additional epithelial differentiations whatsoever. Most of them (93%) had additional neural tissue of various types, from typical brain with eyes and ear vesicles to atypical accumulations of neural tissue. Thus, unlike the inner layer cells of the notochord, the notochord outer layer cells, when fully separated from the former, do not exert an inducing action on the early gastrula ectoderm, and themselves do not differentiate in the notochord cells.

After transplantation of the outer layer of presumptive notochord material to an area of presumptive epidermis in embryos at the early gastrula stage, the transplant did not invaginate in any of the 23 successful operations. The transplant areas increased; they changed their form, extended, and at the late neurula stage formed a typical covering epithelium on the ventral side. Only the most anterior region of the transplant, which in the donor was closest to the blastopore dorsal lip, formed a separate rod-like process not included in the epithelium, and the cells of this rod assumed the appearance of entodermal cells.

After replacement of the outer layer of presumptive notochord material with an outer ectoderm layer or the outer layer of presumptive notochord material

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Fig. 4. Changes in cells of outer and inner layers from ectoderm of early *R. esculenta* gastrula. The layers were transplanted homoplastically onto the exposed chordomesodermal underlayer of embryos at the late neurula stage: (A) the outer layer curls up to form a tube; its cells have become bottle shaped and penetrate inside; (B) the inner layer cells form a compact accumulation; they have retained their polygonal form and are overgrown from the outside by the recipient's epithelium. Bar = 100 μm.
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Fig. 4
from embryos of another species, the transplants were incorporated smoothly and invaginated fully. Such embryos proceeded to gastrulation and neurulation in a normal way. In *R. esculenta* and *R. ridibunda*, two incisions were made parallel to axial organs to separate the dorsal wall of the neurula, which was then bent aside to determine the positions of the vitally-marked transplants. These were found under the notochord within the hypochordal plate and had the form of more or less narrow strips.

Toad embryos were fixed at the early neurula and elongated tail-bud stages. In the former, the embryos had normal hypochordal plates, and in the latter, a small hypochorda under the notochord. However, we failed to distinguish donor cells from the recipient ones. In some of the operated embryos, the transplant invaginated incompletely and inhibited gastrulation. These either retained a big yolk plug or were found to have partially bifurcated axial structure (*spina bifida*).

After removal of the outer layer of presumptive notochord from *R. temporaria* embryos at the early gastrula stage, 17 out of 28 operated embryos developed *spina bifida*. They had non-regulated neural tube parts separated by endoderm and, beneath this, two half-size notochords lying directly on the endoderm with no hypochordal plates beneath. Partial exogastrulation occurred in four embryos, while seven developed normally and had a hypochordal plate under the notochord. Presumably this developed from a new outer layer of cells derived from the incision edges which had substituted for the removed outer layer section.

From the above experiments it follows that during gastrulation a hypochordal plate develops from the outer layer of the presumptive chordamesoderm; due to the epithelial properties of that layer, the plate, together with the ectoderm outer layer, co-ordinates the right and left halves of the embryo to ensure integrity during gastrulation and neurulation. The involvement of outer layer cells is apparently not essential for the gastrulational movements of inner layer cells of the notochord material, in agreement with the conclusion drawn by Keller (1981) on the basis of similar experiments.

*Transplantation of gastrula ectoderm layers onto the chordamesoderm underlayer found in embryos at late gastrula and early neurula stages*

Most of the transplantations were performed xenoplastically. In successful
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Fig. 5
cases, the ectoderm outer layer soon became involved in neurulation; it extended even before the neural folds closed, and deepened along the mid-dorsal line to form a dorsal groove, i.e. it integrated smoothly with the normal neural plate. The inner layer behaved differently: its outer surface was sometimes (not always) epithelized by the outer layer cells growing from the sides of the host. At fixation of the embryos, one could see (Fig. 4A, B) that the outer layer cells had become elongated and bottle shaped and were submerged inside, and that the transplant had curled up into a tube, whereas the inner layer cells did not change in shape and formed a compact mass of polygonal cells over the notochord. Thus, when isolated from each other, the outer and inner ectoderm layers respond differently to the inducing effect of the chordamesoderm, but in the same way as they do in situ at those stages.

To elucidate the histological differentiation of xenoplastic transplants, recipient embryos were fixed at later stages (37–38, after Glaesner, 1925). 20 newt embryos with a transplanted ectoderm inner layer, and 14 embryos with a transplanted ectoderm outer layer from B. viridis and R. arvalis early gastrulae were fixed in this way. In addition, two axolotl embryos with implanted outer and inner layers of R. esculenta ectoderm were also fixed. A more or less compact region of brain tissue, differentiated into grey and white matter (Fig. 5B, D) formed invariably from the ectoderm inner layer. In one case, an atypical eye occurred besides the brain, and in another an ear vesicle. In parallel experiments, the outer layer produced only an epithelial wall of the neural tube without neural cells and neural fibres or with a small number of neural cells. Fig. 5A and C show the results of those experiments. A brain with a cavity and grey and white matter developed from an outer layer transplanted onto the chordamesoderm underlayer after a recorded incomplete separation of the inner layer cells (Fig. 5E). These cases reproduce the results obtained by Schechtman (1938) after incomplete separation of the ectoderm layers and by Rossi (Rossi & Nista, 1963; Rossi, 1965a,b) after separation of early neural plate layers.

Replacement of the outer layer of the presumptive neural plate with the outer layer of the neural plate from another species

The experiments were performed in various combinations. Transplants taken from embryos slightly older than the recipients (from stage 11 to 10 or from stage 13 to 12) were found to graft and neurulate better. The transplants neurulated in 58 out of 200 operated embryos; however, the sections showed the grafts to have become harmoniously involved in the development of only a few embryos. During neurulation, the transplant cells in those embryos elongated, became bottle shaped, permeated between the inner layer cells (Fig. 6A), and surrounded the neural tube cavity; in other words, they behaved like outer layer cells in a normal intact embryo, described in detail in Xenopus laevis embryos by Schroeder (1970).

At the elongated tail-bud stage, transplant cells formed part of the epithelial
Fig. 6. Localization and differentiation of cells from ectoderm outer layer transplanted in place of the removed outer layer of the presumptive neural plate. (A) and (B): Transplantation from *Bufo viridis* to *R. esculenta*; recipients fixed at the stage of neural plate closure. (C) and (D): Transplantation from *B. bufo* to *B. viridis*; recipients fixed at the elongated tail-bud stage. (A) transplant cells are involved in neurulation and have become bottle shaped (bc). (B) transplant cells have remained on surface of neural tube, which has no cavity; Bar in (A) = 200 μm. (C) transplant has developed into epithelium in brain roof (ne), and ependymal cells in brain wall; (D) three ependymal cells (ec) which had differentiated from outer layer cells are clearly seen in brain wall. Bar in (C) = 100 μm.
roof of the medulla oblongata and the diencephalon, as well as of the basal plate, while in the neural tube wall they transformed into elongated ependymal cells, whose bodies extend from the inner to the outer surface of the wall (Fig. 6C, D).

Thus the cells of the neural plate outer layer, without the inner layer cells, differentiate into roof epithelium of the diencephalon and medulla oblongata and, in contact with the inner layer cells which form neuroblasts, into ependymal cells.

The embryos in which the transplant does not become involved in neurulation, and forms an epithelial cell layer over the nervous system, often have neural tubes without cavities (Fig. 6B).

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Fig. 7. Differentiation of the nervous system in R. esculenta embryos after removal of the neural plate outer layer at the slit-like blastopore stage: (A) embryo during removal of outer layer (ol); (B) and (C): medulla oblongata without cavity.
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The same result was obtained by removal of the neural plate outer layer region and by transplantation in its place of ventral epithelium from embryos at stages from late gastrula (slit-like blastopore) to open neural plate. The cephalic region of the neural plate was bent inwards and the outer layer of the surrounding epidermis overgrew it from the sides to cover the defect more or less completely. At the same time, the inner layer was further concentrated towards the mid-dorsal line. Defects in brain development showed in the absence of a cavity, or in an abnormal cavity. When the cavity was completely absent, it was replaced either by neural cells or neural fibres (Fig. 7B, C). In this case, the absence of an outer layer caused an apparent loss of polarity of neural fibre growth. The absence of a cavity or the presence of defects therein were more frequently observed at the level of the medulla oblongata and the anterior section of spinal cord, where the neural folds close more rapidly and there is relatively less time for replacement of the removed outer layer by that growing from the sides and for contact with the environment by the superficial inner layer cells of the neural plate. However, the absence of a cavity was observed in other brain parts, too, for example in the diencephalon, where it did not prevent the optic vesicles from pushing outwards and developing normally. Apart from differences in the structure of the nervous system at different body levels in the same embryo, there are also species-specific differences. After removal of the outer layer the defect was regulated more often in Pelobates and R. ridibunda embryos.

In place of a removed neural plate outer layer, regions of ventral epithelium of the neurula were transplanted in 73 embryos; these epithelial transplants were no longer able to take part in neurulation and remained on the surface. In 53 cases cavities were lacking in the nervous system under the transplants, while 18 Pelobates embryos had markedly atypical cavities. Thus, the neural plate outer layer not only lines the neural tube cavity, but takes active part in its formation.

**DISCUSSION**

The above data indicate that the outer and inner layers of the ectoderm and chordamesoderm differ sharply in their organization and the types of cell contact they make (see Introduction); in the Anuran species studied, they also differ in behaviour and in their relationship to other embryonic cells, in inducing action, and in morphogenetic potencies and prospective significance.

The differences in the properties of cells from the primary ectoderm inner layer in various presumptive regions are less than between the cells of the outer and inner layers in the same presumptive region.

Previously, Schechtman (1938), Rossi (Rossi & Nista, 1963; Rossi, 1965a,b) and Holtfreter (1938) arrived at an opposite conclusion. However, their experimental results allow for an interpretation that differs from the one they gave and is in good agreement with the evidence cited in the present work. Schechtman, who obtained the development of the nervous system from the ectoderm outer...
layer, nonetheless wrote that, in isolating the ectoderm outer layer, he simply reduced the number of inner layer cells. Rossi removed the outer layer from the entire neural plate at the early neurula stage, and also inner layer regions with a chordamesoderm underlayer, for cultivation in a saline solution. From the outer layer he obtained archencephalic differentiations and from the fragments of inner layer with underlayer brain regions corresponding to the sites from which they were taken, but often with abnormal cavities. However, Rossi did not mention whether he had liberated the outer layer from the adherent inner layer cells; and possibly in his experiments the neural tissue may have developed from these inner layer cells. Holtfreter maintained there were no differences in the properties of the ectoderm layers, since an epithelium differentiated from an inner layer cultivated in vitro, as it did from the outer layer cells. However, this fact does not eliminate the initial differences in the properties of the ectoderm layers and is simply indicative of changes under the effect of the environment. On the other hand, with more thorough separation of the *X. laevis* ectoderm layers in a Ca$^{2+}$-free Barth's solution and their subsequent incubation with the endoderm in a complete salt solution, Sudarwati & Nieuwkoop (1971) established that the outer layer possesses considerably less neural competence than the inner layer. This is in agreement with the evidence cited in the present work.

As for the outer layer of the chordamesoderm material, a number of investigators (Sudarwasti & Nieuwkoop, 1971; Keller, 1975, 1976), in the wake of Nieuwkoop & Florschütz (1950), correctly noting its continuous linkage with the outer layer of the neural plate and epidermis material, and its participation in the formation of the hypochordal plate, called it a suprablastopore endoderm. I cannot agree with this description. As was shown in the present work, the properties of the outer layer of the presumptive chordamesoderm do not differ from those of the outer layer of the presumptive neural plate and epidermis whereas, on the other hand, they do differ from the properties of the endoderm; hence, it would be more correct to term the outer layer of chordamesoderm material a presumptive hypochordal plate.

Flattening of embryonic cells and the formation between them of tight junctions during blastulation and gastrulation, occurring in normal development in outer layer cells and, in these experiments, also in inner layer cells, take place under the influence of the contact of those cells with the environment; Ca$^{2+}$ apparently plays a leading role in this cell transformation (see, for example, LeBlank & Brick, 1981a, b). It is important to note that changes in cell form and consolidation of the cells in the epithelial layer are accompanied by restrictions of their morphogenetic potencies and by their acquisition of the ability for specific morphogenetic movements, viz. epiboly and invagination.

Since the fate maps published by Vogt (1929a) and Pasteels (1936) for various Anuran species did not take into consideration the different prospective significance at the ectoderm and chordamesoderm outer and inner layers, in conclusion I present my previously published (Dettlaff, 1946) fate maps (Fig. 8) on
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sagittal sections of Bombinator igneus embryos at the stage of early and late gastrula; Vogt's maps (1929a, b) were used as initial sources for that species. In the maps presented, there is still need to specify the boundary between the presumptive neural plate and presumptive epidermis in the ectoderm inner

Fig. 8. Prospective significance of various regions of the outer and inner layers in ectoderm and notochord material at the early (A, B) and late (C, D) gastrula stages. 8A, C: after Vogt (1929a) and 8B, D after Dettlaff (1946b). Outer layer: 1 – epidermis; 2 – epithelial roof of diencephalon and medulla oblongata, some basal plate cells, and ependyma; 3 – hypochordal plate. Inner layer: 4 – sensible epidermis layer (placodes of sense organs, ganglia); 5 – nervous system; 6 – notochord. Bar = 200 μm.
layer, since it may be (see, for example, Giersberg, 1924; Dettlaff, 1936) that by the end of gastrulation some of the inner layer cells move from the presumptive epidermis region to the neural plate region.

Another method of visualization of rudiments in the material of ectoderm and chordamesoderm inner and outer layer was used by Løvtrop (1966) and Keller (1975, 1976) who gave fate maps for each of these layers separately. However, it is important to note that the prospective significance and the position of different areas on my map and that of Løvtrop coincide.

There follows a short note on comparative aspects of primary ectodermal structure and its significance for early embryogenesis. The early segregation of primary ectoderm into the outer and inner layers occurs in most Anamnia: in Chondrostei, Holostei, Teleostei, Protopterus and Lepidosiren of Dipnoi and Anura. Only in Selachii, Neoceratodus and Urodela the segregation of the ectoderm layers proceeds, as in Amniota, at later stages. These differences in the onset of the two-layered structure and the degree of restriction in the epithelial differentiation of the outer layer correlate with the peculiarities of early embryogenesis (Dettlaff, 1948). The animals where the outer layer of chordamesoderm invaginates during gastrulation, as in Anura, form gastrocoel and hypochordal plate. They include, besides Anura, Chondrostei, Holostei, Protopterus and Lepidosiren. On the other hand, Selachii, Neoceratodus and Urodela, which begin gastrulation with a single-layered chordamesoderm, have no hypochordal plate. Their notochord and somite material face the gastrocoel directly. In the Teleostei the outer layer of primary ectoderm is greatly specialized and does not invaginate but the cells of the inner layer fail to produce gastrocoel as they lack epithelial structure. So they have neither gastrocoel, nor hypochordal plate. In Teleostei and Holostei a specialized outer layer of ectoderm (periderm) does not participate in neurulation at all and, accordingly, their nervous systems initially have no cavity. This is reminiscent of the Rana embryos described above, where the outer layer of neural plate was replaced by ventral epithelium already incapable of taking part in neurulation, an 'artificial periderm', so to speak.

Thus, an early or late segregation of primary ectoderm into two layers, as well as a greater or lesser specialization of surface – located cells in embryos of different groups of Anamnia, is not only an example of heterochrony and a result of adaptive differentiation (Goette, 1875), but a cause of differences in the structure of the embryos between these animals.

REFERENCES


Gastrulation in various Anuran amphibians


DETTLAFF, T. A. (1946c). The topographic map of presumptive areas in Anura revised with reference to precision, (as above) 54, 277–280.


Reichert, X. (1840). *Das Entwickelungsleben im Wirbeltier-Reich*. Berlin:


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