Original Article

Effects of relaxation of mechanical tensions upon the early morphogenesis of *Xenopus laevis* embryos

LEV V. BELOUSSOV*, ANTON V. LAKIREV, IRINA I. NAUMIDI and VLADIMIR V. NOVOSELOV

Department of Embryology, Moscow State University and Institute of Mechanical Engineering, USSR Academy of Sciences, Moscow, USSR

ABSTRACT In *Xenopus laevis* embryos at the early gastrula stage, circumferential tensions of embryonic ectoderm were relaxed by making sagittal or transversal slits in the ventral parts of embryos and inserting into surgical cuts the sectors of homologous tissue from same-stage embryos. Changes in tensile patterns were controlled by measuring cell surface angles. Immediate decreases in surface cell wall tension as related to transversal wall tension were registered. Within minutes of the operation, the lobopodial activity of the inner ectodermal surface increased. The subsequent gastrulation movements were disturbed, germ layers partially mixed and archenteron reduced. The areas of extensive cell columnarization in the ectoderm of operated embryos were less regularly arranged and were extended much more ventrally than in intact embryos. Ventro-dorsal migration and latero-medial intercalation of mesodermal cells also were suppressed. As the operated embryos developed, we observed increases in the total amount of neural tissue, associated sometimes with duplication and even triplication of neural tubes, duplication of otic vesicles, partial fusion of axial rudiments, suppression of mesodermal segmentation and branching or bending of notochord. In the gravest cases the antero-posterior embryo polarity was disturbed. In some cases we observed the formation of axial rudiments in ventral implants. The role of tensions in determining the patterns of morphogenetic cell movements and in establishing the morphological order of normal development is discussed.

KEY WORDS: *mechanical tensions, morphogenesis, gastrulation, neurulation, Xenopus laevis*

Introduction

The fundamental mechanisms of pattern formation during embryonic development remain a matter of intense discussion. Two main concepts of patterning mechanisms – perhaps not entirely mutually exclusive – have dominated recent thinking on the subject. The first ascribes a leading role to the diffusible substances (morphogenes) and their concentrational gradients (Meinhardt, 1982), while the second points out the importance of mechanical stresses in creating and maintaining the regular arrangement of embryonic rudiments (Odell *et al.*, 1981; Harris *et al.*, 1984; Belintzev *et al.*, 1987). Recently (Beloussov *et al.*, 1988), it was demonstrated that pieces of early gastrula suprablastoporal regions taken from *Xenopus* embryos and explanted onto latex films require at least several minutes stretching in order to undergo integrated morphogenesis; in these experiments the axial organs oriented mostly along the applied tensions. On the other hand, if placed on a latex film not stretched at all, or stretched less than for 2-5 min, the explants dispersed into single actively moving cells. The difference in explant behavior on the stretched and non-stretched surfaces correlates with the rapid reinforcement of cell-cell contacts – normally rather rudimentary at that stage – in the stretched samples. Although these results seem to demonstrate, at least in *in vitro* conditions, the importance of tensions in maintaining the morphological integrity of embryonic tissues and in orienting morphogenetic movements, the question remains as to what extent this factor is involved in the normal development of entire embryos. In order to answer this question, we experimented with relaxing tissue tensions in entire embryos by a simple method first employed by Cooke (1975) for another purpose. This consisted in making a vertical slit in the ventro-vegetal part of the embryo and inserting a sector of homologous tissue from another same-stage embryo into a surgical cut. The operation led to a rapid relaxation of pre-existing circumferential (tangential) tensions of embryonic ectoderm, the existence of tensions having been detected in earlier experiments (Beloussov *et al.*, 1975). In this paper we describe both the short- and long-term effects of tension relaxation, and demonstrate that they correspond to the results of the above-mentioned *in vitro*

***Address for reprints**: Dept. of Embryology, Faculty of Biology, Moscow State University, Moscow 119899, USSR.

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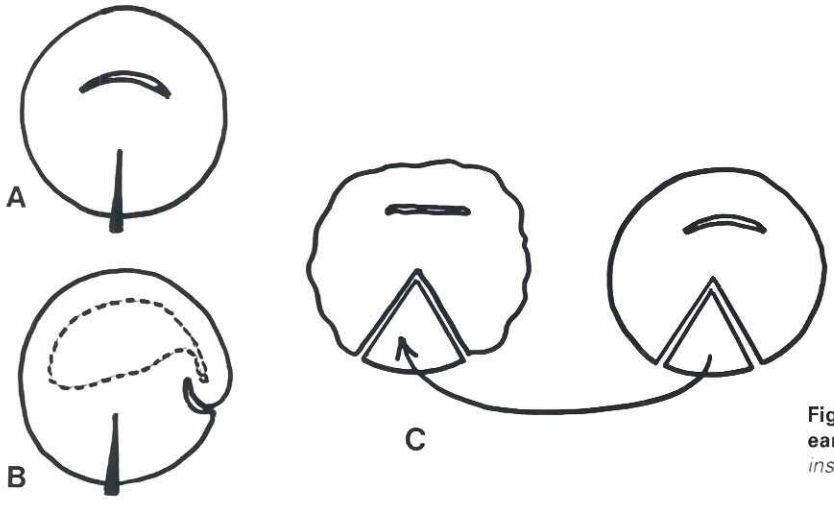

Fig. 1. Drawings of tension relaxation experiments at the early gastrula stage. (A) *Sagittal slit,* **(B)** *transversal slit,* **(C)** *insertion of implant from the same stage embryo into surgical cut.*

experiments (Beloussov *et al.*, 1988) as well to theoretical predictions (Belintzev *et al.*, 1987). We discuss the possible role of tensions in establishing morphogenetic order in entire embryos.

Results

Increase in lobopodial activity

During normal *Xenopus* development from the early blastula to early gastrula stages, the percentage of blastocele-exposed ectodermal cells with lobopodia gradually diminished (Fig. 2, light circles). At the early gastrula stage it became equal to that previously measured (Beloussov *et al.*, 1988) on the 10 min-stretched latex explants (Fig. 2, dark circle). Meanwhile, in the corresponding areas of operated embryos, already within 10 min after tension relaxation, the percentage of lobopodia-possessing cells had considerably increased, approaching that of explants incubated also for 10 min on a non-stretched latex film (Fig. 2, *cf.* empty and filled triangles). The percentage of lobopodia-possessing cells also remained very high in the mesoderm-exposed surface of the hypoectoderm within an hour after operation (Fig. 3C), and only later began to gradually decline. Generally, the increase in lobopodial activity on the internal surface of hypoectoderm appears to be one of the most distinct and rapid results of tension relaxation both in the latex-exposed explants and in entire embryos.

Dynamics of cell wall tensions

As seen from Table 1, both in the lateral and dorsal zones of intact 11-stage embryos (Fig. 3B, lat, dors), surface cell wall tensions were more than in 7 times greater than those in transversal cell walls. Moreover, in the intact 13-stage embryos, this difference even increased. On the other hand, in operated embryos a sharp drop in the F_S /F_t ratio took place, with only a small delay in the dorsal zone as compared with the lateral one. For at least an hour after operations, F_S and F_t appeared to be practically equal to each other, and only within 4 h after operations did F_S increase again. Hence, the operations performed led to a rapid overall decrease in surface cell wall tensions as related to transversal ones. The decrease in surface tensions is also made visible by extensive wrinkling of the surface of the embryo soon after operations (Fig. 3B; see also Fig. 4B, cf. 4A).

Disturbances in gastrulation movements and in ectodermal cells arrangement

Fig. 3A shows a sagittal section of an experimental embryo within 4 h after operation. The blastopore is abnormally widened, dorsal blastoporal lip folded and archenteron in the anterior area almost absent, due to abnormal fusion of endo- and chordomesodermal

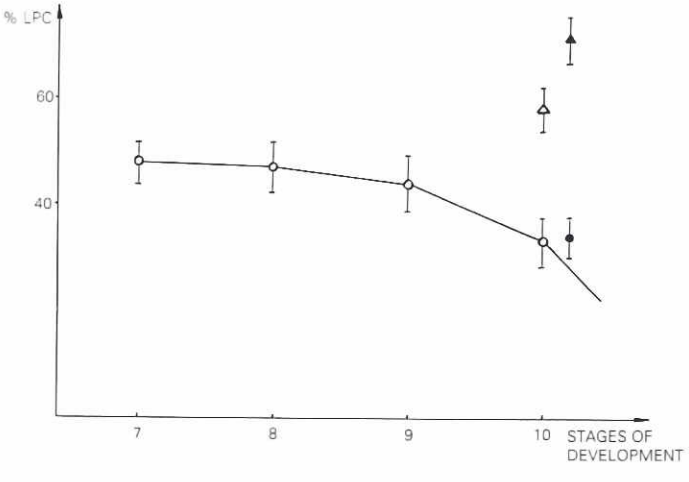

Fig. 2. Percentage of lobopodia-possessing cells exposed to the blastocele roof (% LPC) at successive developmental stages (7-10) of intact *Xenopus* embryos (empty circles): *in 10 min stretched explants of homologous tissue taken from stage-10 embryo (filled circle); in non-stretched explant counterparts (filled triangle); and in operated embryos (empty triangle), 10 min after making a transversal cut. The data concerning explants are taken from Beloussov* et al., *1988. Considerable increases in LPC % are seen both in non-stretched explants and relaxed entire embryos. About 2000 cells were traced for each measurement point.*

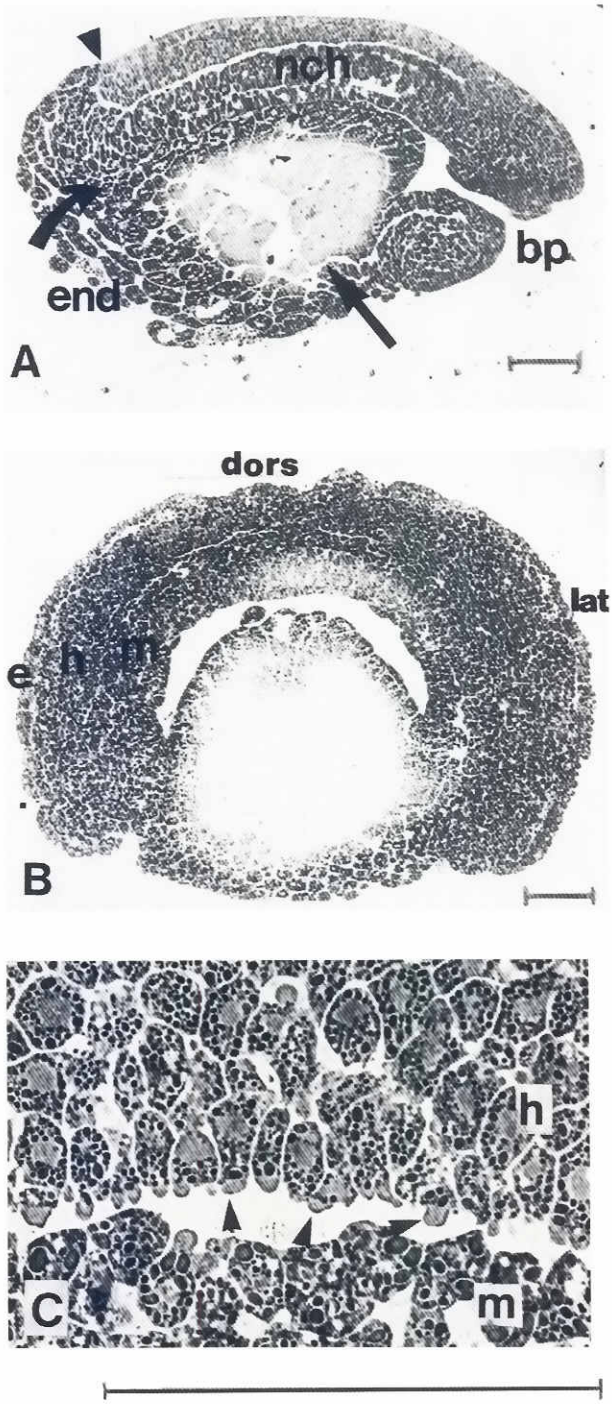

Fig. 3. *X. laevis* embryos soon after relaxation of tensions. (A) *Sagittal section, 4 h after making a transversal cut (cut location shown by straight arrow). No implants have been inserted. Note abnormally widened blastopore (bp), folded dorsal blastoporal lip, fusion of notochordal (nch) and endodermal (end) cells (bent arrow) and the abnormal crawling of endoderm upon ectoderm (pointer).* **(B)** *Transversal section, 1 h after making a transversal cut (out of a section plane). Ectodermal surface is wrinkled, meso-ectodermal border partly disappeared.* **(C)** *Detail of the mesoderm exposed hypoectodermal surface in the lateral area of (B) embryo. Numerous lobopodia (pointers) are seen. In (B, C) e - epiectoderm, h - hypoectoderm, m - mesoderm, lat - lateral zone, dors - dorsal zone. Bar = 200 μm.*

layers (bent arrow). Endodermal layer (end) looks like a disorganized freely moving cell mass crawling upon ectoderm (pointer). The rudiment of a notochord (nch) is also partially disorganized. The similar fusion of ecto-, meso- and endodermal layers is seen also in the ventral zones of experimental embryos an hour after operation (Fig. 3B). In the lateral zones of experimental embryos, very soon after operating the arrangement of hypoectodermal cells becomes highly irregular; at the same time epiectodermal cells become more columnar than normal (Fig. 3B, 4B, *cf.* 4A). Within 4 h after operations the order in the arrangement of ectodermal cells is somewhat restored, but both the hypo- and epiectodermal cells remain much more columnar than in intact embryos (Fig. 4D, *cf.* 4C).

Suppression of the ventro-dorsal movements of mesodermal cells in operated embryos

During gastrulation of the intact *Xenopus* embryos extensive convergent ventro-dorsal movements of mesodermal cells associated with cell intercalation have been traced (Keller and Danilchik, 1988). This is reflected by a considerable increase in R d/l from stage 11 to stage 14 (Fig. 5, solid line). Meanwhile, no such increase is seen in the operated embryos (Fig. 5, dotted line). In these embryos the number of mesodermal cell layers in the middorsal area is only slightly greater than in the lateral areas. We conclude from this that the relaxation of tensions suppresses the ventro-dorsal migration of mesoderm. Since such a suppression is revealed no earlier than an hour after operation, it cannot be related to ventral wound healing, which is fully completed at that time.

Extension of cell columnarization zones and irregularities in columnarization patterns

During normal development from stages 11 to 16, the ectodermal cell layer is gradually segregated to a dorso-medial domain of highly columnarized cells (IC>2), corresponding to neural plate and to the extended latero-ventral area of roughly isodiametrical cells (IC=1) (Fig. 6, solid lines). Cell columnarization started first in hypoectoderm

TABLE 1

CELL SURFACE ANGLES α AND CORRESPONDING F_s /F_t RATIOS

	Lat		Dors	
	α°	F_s /F_t	α°	F_s /F_t
1. IE_{11}	172±7	7.14	172±8	7.14
2. 1	126±9	1.11	162±6	3.12
3. 10	125±8	1.06	130±9	1.19
4. 30	115±6	0.92	137±9	1.35
5. 60	122±11	1.04	119±12	1.00
6. 240	166±8	4.10	170±7	5.90
7. IE_{13}	178±4	33.30	178±5	33.30

Cell surface angles α and corresponding F_s /F_t ratios (see Materials and Methods) in the lateral (Lat) and dorsal (Dors) zones of epiectoderm in intact stage 11 and stage 13 embryos (IE_{11} and IE_{13}) and in experimental embryos (lines 2-6). Minutes after operation are shown.

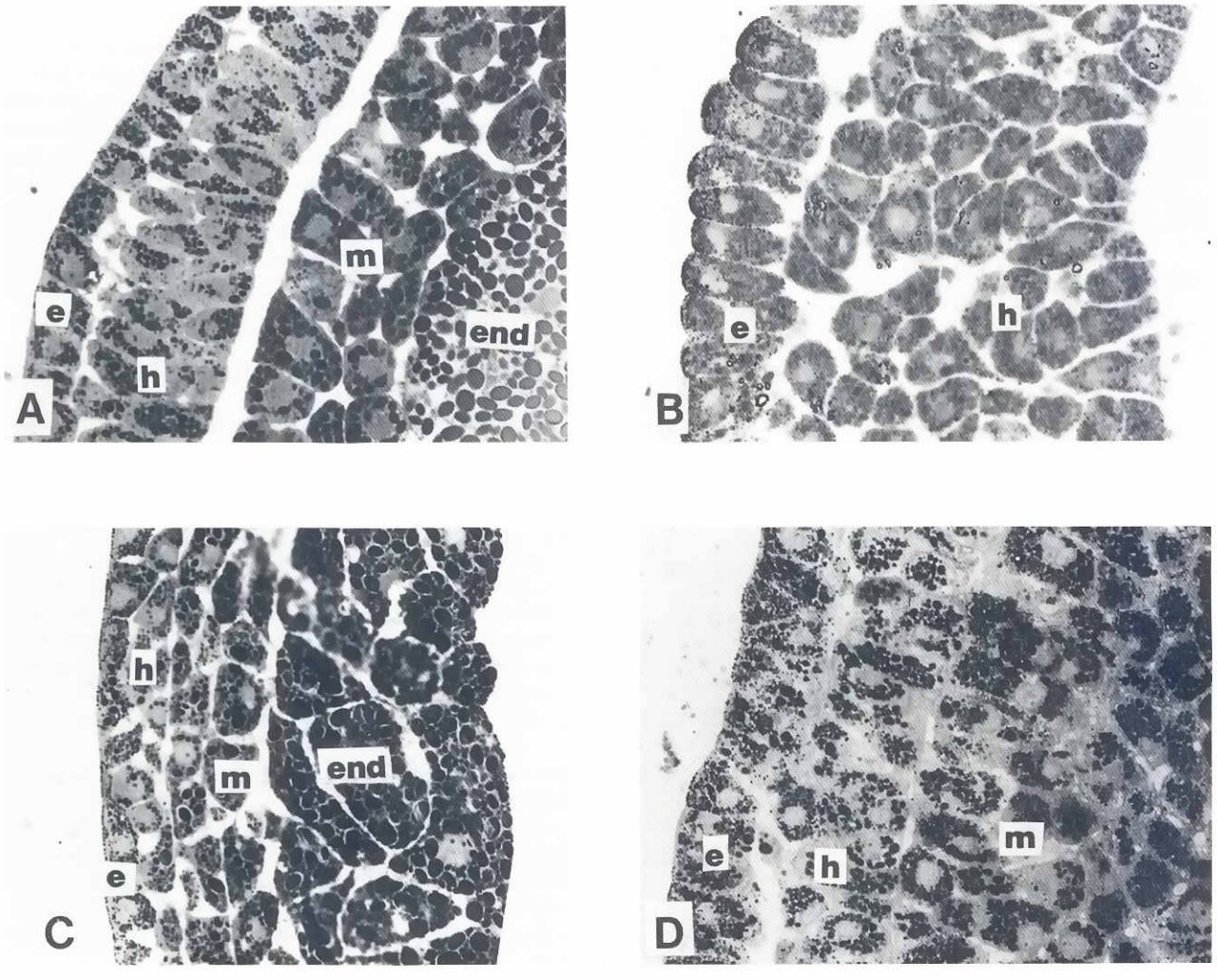

Fig. 4. Lateral zones of experimental embryos, *1 h* **(B)** *and 4 h* **(D)** *after operations as compared with the corresponding-stage intact embryos* **(A and C)**. *Extensive columnarization of epiectodermal cells, decrease in their surface angles and disorganization of hypoectodermal cells are seen in (B). In (D) the structure of ectodermal layer is partly restored but hypoectodermal cells are much more columnarized than those of the same-stage intact embryos (C). Designations as in Fig. 3. Bar = 100 μm.*

(stages 12-13) and somewhat later in epiectoderm (stage 14). In hypoectoderm two symmetrical columnarized cell domains appeared separated by a mid-dorsal flattened zone (notoplate by Jacobson and Gordon, 1976), whereas in epiectoderm the dorsal columnarized cell domain was continuous.

In experimental embryos, extensive cell columnarization started much earlier than in their intact counterparts. First, already within about a minute after operation, due to relaxation of pre-existing tensions, the IC rose from about 1.0 to 1.5. Then a slower process of cell columnarization proceeded leading to the appearance of cells with IC>2 in epiectoderm within an hour and in hypoectoderm within 2 h after operations. The mostly columnarized cells situated themselves in the ventro-vegetal regions, so that the IC gradient appeared to be reversed in relation to the later established natural one (Fig. 6, dotted lines). Only within 8 h after operations did the IC gradient in epiectoderm take the normal orientation, although the ventro-lateral zone cells still retained abnormally high IC.

Two-dimensional maps of IC patterns showed that in experimental embryos, the zones occupied by columnarized cells were not only much more extensive than in the intact samples, but were also highly irregular and disrupted, containing some small «islands» of cells with different IC values, never observed normally (Fig. 7, *cf.* A and B).

Abnormalities in the structure and arrangement of the axial rudiments

From the very beginning of their development, the neural rudiments of experimental embryos appeared less sharply delimited in relation to surrounding tissues than did intact embryos (Fig. 8, *cf.* B and A). Later on abnormal contacts between neural rudiments and surrounding tossues were maintained (Fig. 8, *cf.* D and C; Fig. 9 A-D). These contacts were established between neural tubes and otic

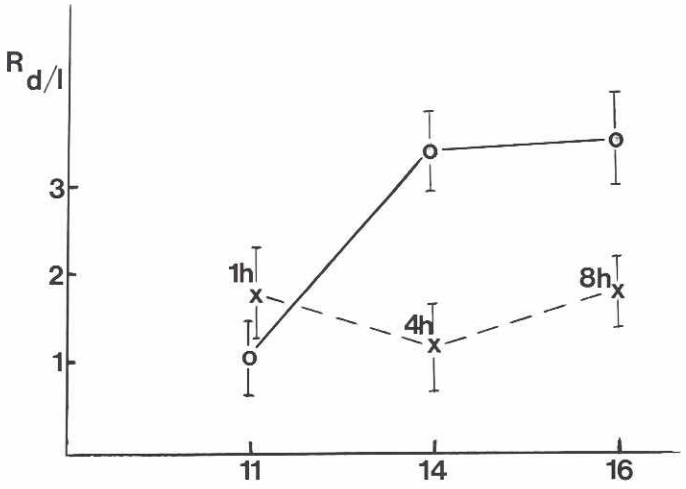

Fig. 5. The ratios of mesodermal layers (R d/l) in the middorsal to lateral areas of intact (solid line) and operated (dotted line) X. *laevis* embryos. *Horizontal axis: stages of development. Above the dotted line, hours after operations are shown.*

vesicles, the latter often being reduplicated (Fig. 9A), as well as between the neural tubes and somites (Fig. 9 C,D, pointers). Among other common anomalies, particularly worth mentioning are the abnormal ear-shaped lateral appendages of neural tubes (Fig. 9A,D,F), the duplication or triplication of neural tubes (Fig. 9 E,H) and the amorphous growth of neural tissue, mostly in anterior regions (Fig. 9G).

Measurements made on seven experimental embryos within 2 days after operation have shown that the total volumes of their neural tissues were significantly greater than those of the same-stage intact embryos (149 ± 31% of intact neural tissue volume, $P>0.95$).

In about a dozen cases, including those with transversal surgical cuts (relaxing mostly the sagittal circumferential tensions), the grave disturbances in antero-posterior embryo polarity and mesodermal segmentation were traced (Fig. 10, B-D). In extreme cases the anterior and posterior poles of embryos incubated for 2 days showed no differences at all. (Fig. 10C,D, *cf.* Fig.10A). Notochord was often bent or branched and mesodermal segmentation suppressed (Fig. 10B,D).

Formation of axial structures in ventral explants

A fully unexpected result was that in ten of the ventral implants inserted into surgical cuts (five of them studied in histological section), perfect axial structures developed, including notochord and neural tube (Fig. 11A-C). In some cases suckers also appeared oriented towards the anterior end of the host (Fig. 11A, s). No distinct contacts between the implant and host axial structures

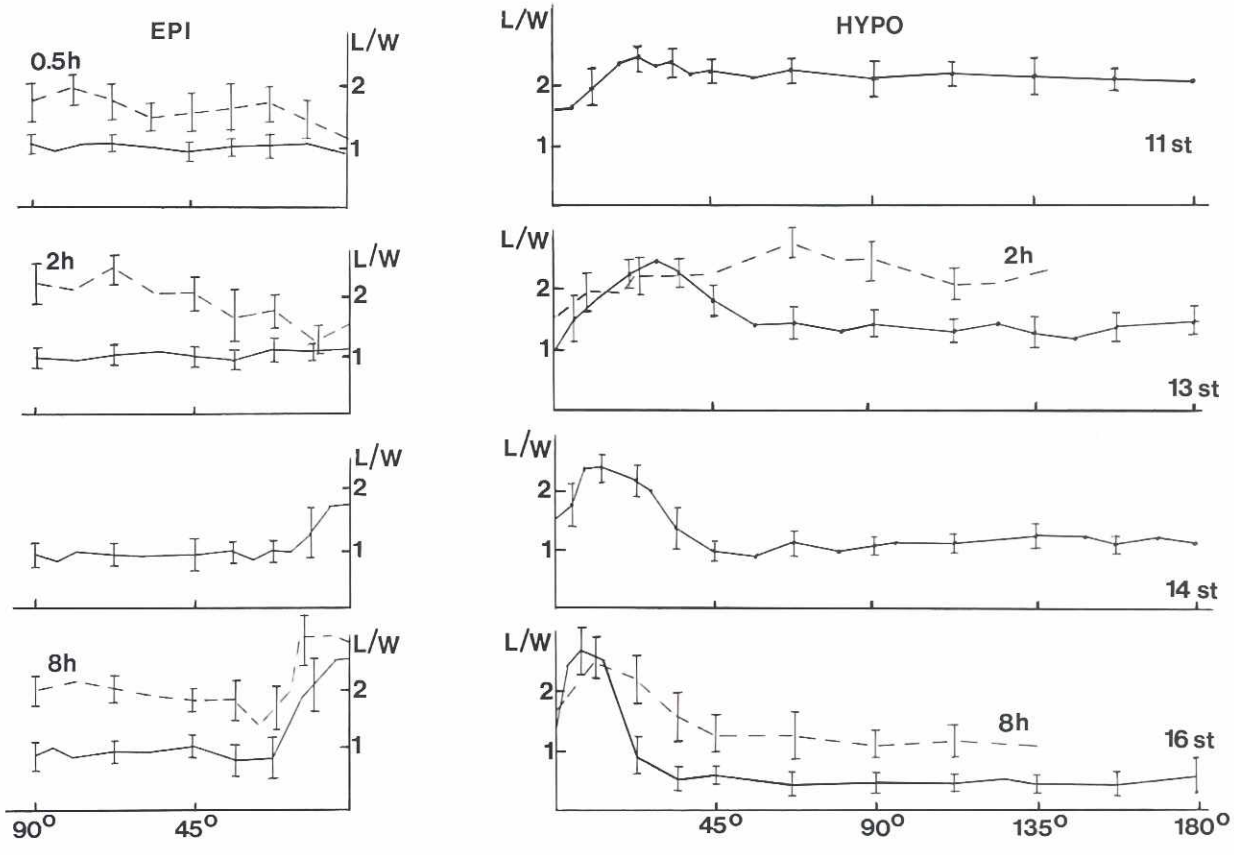

Fig. 6. Diagrams of cell columnarization in the ectoderm of *Xenopus* embryos (stage numbers shown at right). *Left column: epiectoderm* **(EPI)**, *right column: hypoectoderm* **(HYPO)**. *Horizontal axis: angular distances from the dorsomedial line taken as zero angle. Vertical axes: columnarization indexes (IC). Solid lines: intact embryos, dotted lines: same-stage experimental embryos (hours after operation are shown).*

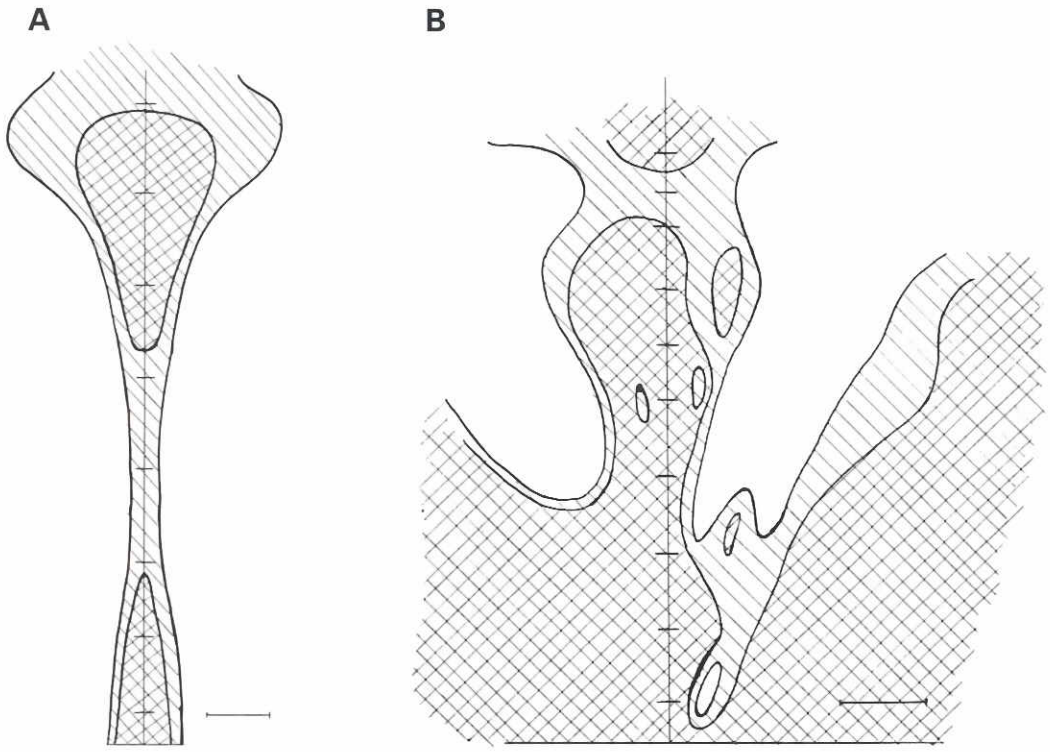

Fig. 7. Two-dimensional maps of cell columnarization in the epiectoderm of intact stage 16 *X. laevis* embryo (A) and of the same-stage (8 h after operation) experimental embryo (B). *Labels on the vertical axis (which coincides with embryo midline) indicate the levels of the separate IC measurements. Horizontal axis indicates the level of the blastopore. Cross-hatched area: IC>2. Hatched area: 1.5<IC<2. Empty area: IC<1.5. In the experimental embryos with relaxed tensions the pattern of cell columnarization is quite irregular. Bar = 200 μm.*

could be traced on the serial sections. As shown by Fig. 11C, the notochordal and neural tissues of the implants were closely associated with each other; their origin was probably due to the involution of ectodermal cells of the implanted piece (Fig. 11C, pointer). These cases are discussed below.

Discussion

As shown by measurements of cell surface angles (Table 1), epiectodermal cell wall surface tensions considerably decreased in comparison with transversal wall tensions immediately after operations, with the relaxation continuing for at least several hours. In intact embryos these tensions, easily detected by the deformations of dissected cell sheets (Beloussov *et al.*, 1975), are obviously created initially by osmotically driven blastocele (and later on archenteron) inflation (Warner, 1985) and are then reinforced by involution movements during gastrulation.

In order to relate the morphogenetic effects described in this paper only to tension relaxation, it is necessary to exclude the possibility that they are caused by some other damaging consequence of the operation. Our main arguments for excluding this possibility are as follows: 1.) The experimental embryos developed perfectly, without any non-specific destruction or considerable mortality. 2.) The effects observed took place as a rule in the dorsal region of host embryos – the area furthest removed from the operational areas. 3.) These effects express themselves mostly in excessive development of the axial rudiments rather than in their reduction. 4.) Even much more damaging operations, made at the same stages but not associated with the prolonged relaxation of tensions, do not lead to the same anomalies. Here we are referring to extirpations and *in vitro* culturing of isolated pieces of early gastrula dorso-medial areas (Golubeva, 1986; Keller and Danilchik, 1988). In these experiments the tensions relaxed surgically were rapidly restored due to curling of explants.

The main primary effects of the tension relaxation described in this paper are: (1) extensive formation of lobopodia on the free surfaces of hypoectoderm; (2) reduction of cell-cell contacts and irregular arrangement of cells, mostly in hypoectoderm, but also in meso- and endoderm; (3) partial fusion of cell layers; (4) suppression of the latero-medial convergence in axial mesoderm; (5) ventralwards extension of columnarized cell areas, both in epi- and in hypoectoderm, and loss of spatial regularity in the locations of cells with definite IC.

At least the first two categories of events fit perfectly with the behavioral properties of cells of early gastrula explants placed on non-stretched latex films, as opposed to those placed on stretched films (Beloussov *et al.*, 1988). Therefore, one may consider the extensive formation of lobopodia, loss of cell contacts and the regularity in cells arrangement as the primary universal consequences of tension relaxation. The influence of tissue stretching on direction and on the very existence of the convergent (intercalation) cell movements also seems to be supported by the results of studies cited above as well as the present work. As to extensive cell columnarization as a reaction to relaxation, this was theoretically predicted by the model of epithelial morphogenesis (Belintzev *et al.*, 1987). This model predicts also the IC gradient and irregular clustering of columnarized cells in relaxed tissues, as opposed to stretched ones. Which one of the typical relaxation reactions – whether loss of cell contacts or cell columnarization – will dominate seems to depend upon the coherency of the intact cell sheet and/

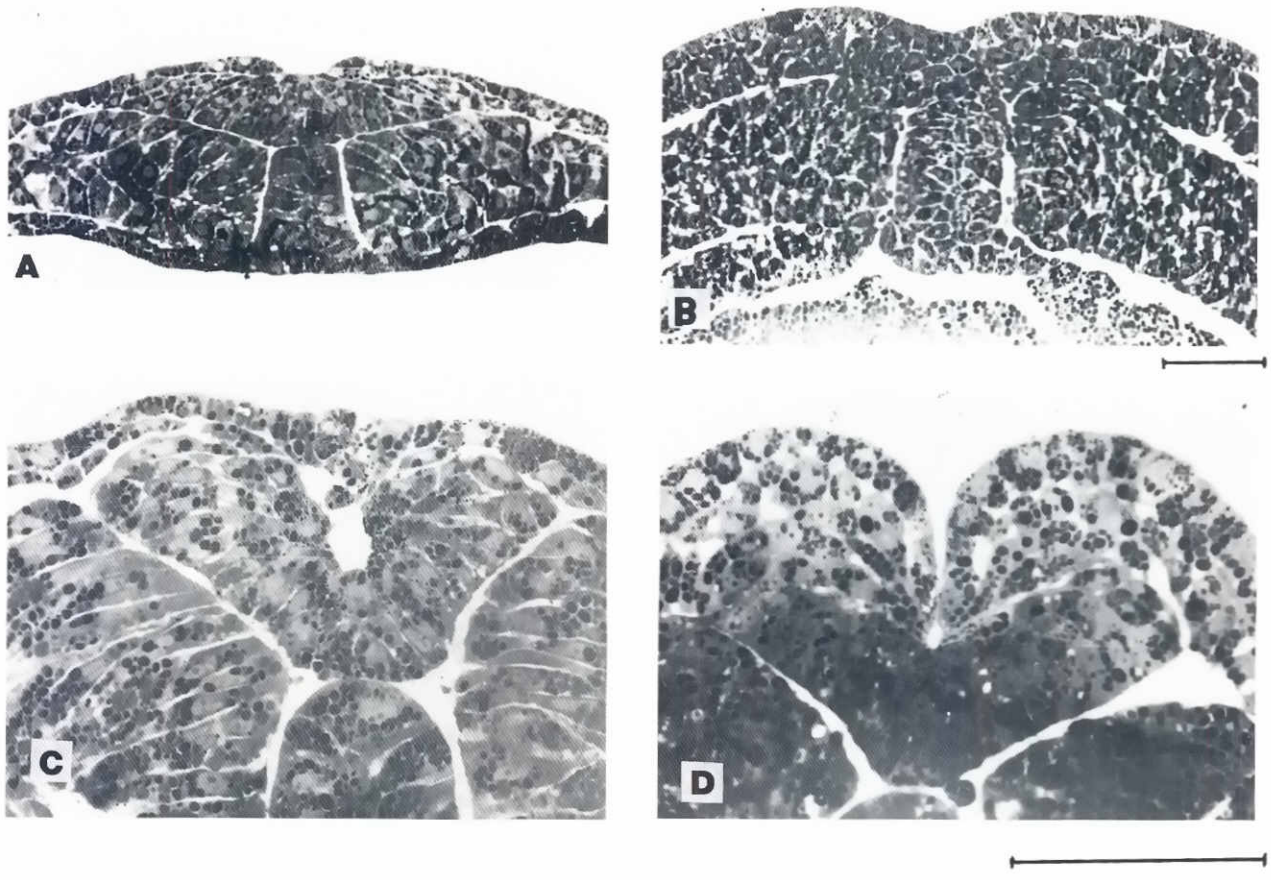

Fig. 8. Transversal sections of the dorsal areas of operated *X.laevis* embryos, 8 h (B) and 14 h (D) after operations as compared with the corresponding-stage intact embryos (A and C correspondingly). *In the experimental embryos the neuroectoderm smoothly passes to the lateral ectoderm and partly fuses with the notochord rudiment and somite tissues. Bar = 100 μm.*

or the presence of adhesive substrate for cell spreading. If such a substrate (latex film in artificial conditions, or the adjacent cell layer surface in intact embryos) is available, cells tend to lose their contacts. On the other hand, in a highly coherent epithelial sheet (epiectoderm of entire embryos) columnarization prevails. Hypoectoderm in entire embryos demonstrates an intermediate kind of behavior, first losing cell contacts and then demonstrating extensive columnarization.

Most of the subsequent morphogenetic anomalies, particularly those in neural rudiments (enlargement and multiplication of the rudiments, formation of lateral appendages and partial fusion with surrounding tissues, as well as the duplication of otic vesicles) may be considered the natural results of the extension of cell columnarization areas in the ventral direction and the lack of a complete separation of the different germ layer derivates. Most of these abnormalities, as well as the increase in the total amount of neural tissue in relaxed embryos, points to the involvement of the columnarized cells of the lateral hypoectoderm in neural rudiments. This may be associated or even identified with the homoiogenetic (lateral) induction of neuroectoderm from the adjacent lateral ectoderm (Yamazaki-Yamamoto and Sasaki, 1989). One cannot exclude the possibility that in these authors' experiments, lateral induction was also promoted by tension relaxation due to insertion of a transplant. On the other hand, an excessive columnarization of the lateral hypoectoderm may lead to the excessive development not only of neural rudiments, but of the placodes as well (duplication of otic vesicles, Fig. 9A). Hence, cell columnarization appears to be a not highly specific event promoting various trends of cell differentiation.

As to the disturbances in the structure of notochord, mesodermal segmentation and antero-posterior embryo polarity, they seem to be associated with the supression of the latero-medial convergence movements in relaxed embryos, these movements playing a leading role in the formation of axial rudiments (Keller and Tibbets, 1989).

One of the intriguing, and quite unexpected, results of our work was the formation of distinct axial structures in some explants inserted into surgical cuts. Since such a formation does not correspond to the normal morphogenetic potential of *Xenopus* ventro-vegetal tissues (Wylie *et al.*, 1987), the following possibilities remain: (1) the axial structures in these tissues develop autonomously, probably as a result of relaxation-induced involution movements and the associated temporary loss of the involuting cell contacts, as well as their subsequent columnarization (see Gordon and Brodland, 1987); (2) some of the host's chordomesodermal cells penetrated into implants and induced the local tissues to form axial structures; (3) a kind of homoiogenetic induction from host

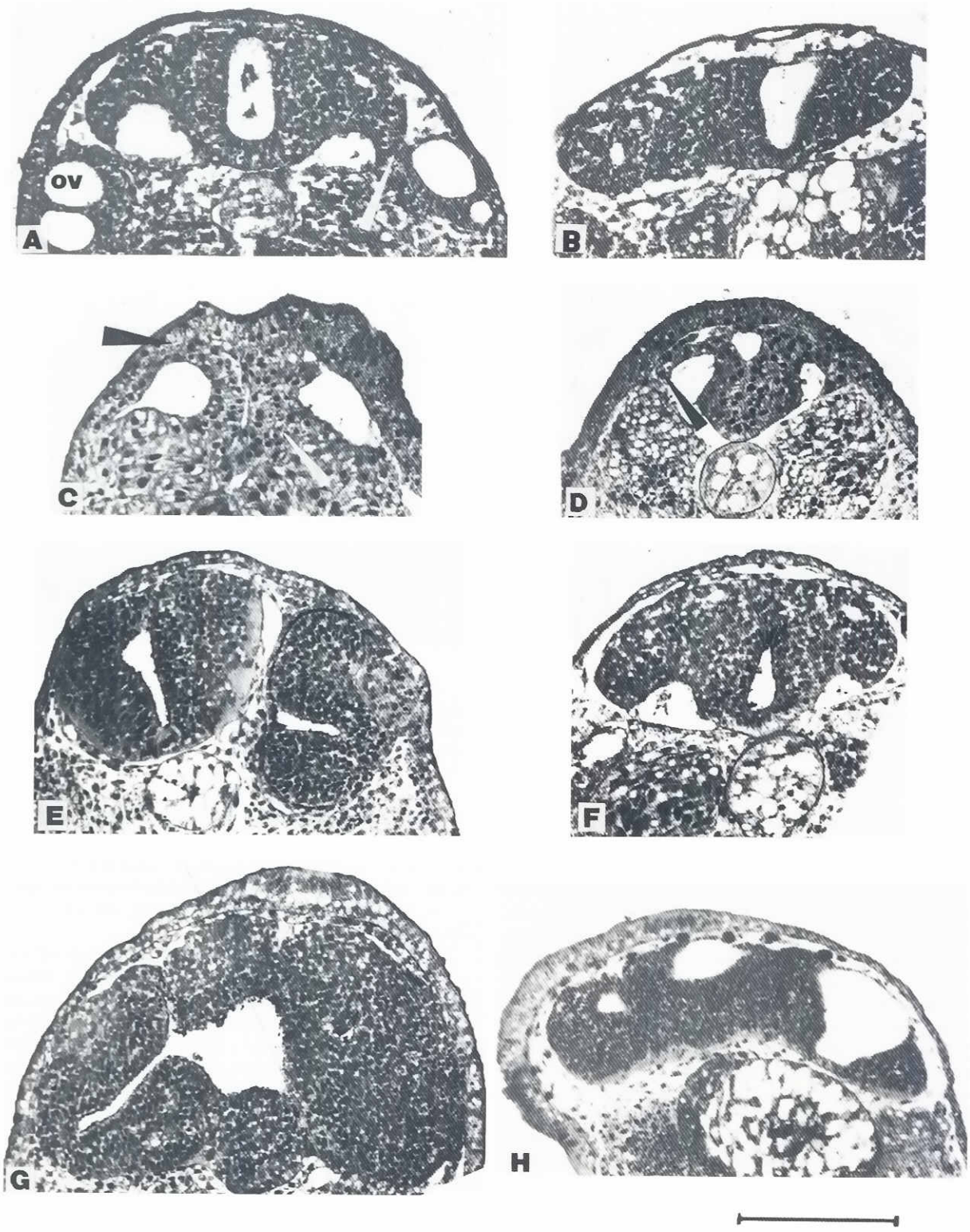

Fig. 9. Abnormalities in the neural and surrounding structures as seen on the transversal sections of experimental embryos, 2 days after operation. *Neural structures are abnormally fused with surrounding tissues* **(A-D)** *(see pointers in C,D) and often possess ear-shaped appendages* **(A,E)**. *An excess of neural tissue* **(G)**, *duplication* **(F)** *and even triplication* **(H)** *of neural tubes also takes place. In some cases otic vesicles are duplicated (A, ov) and notochord abnormally increased (H). Bar = 200 μm.*

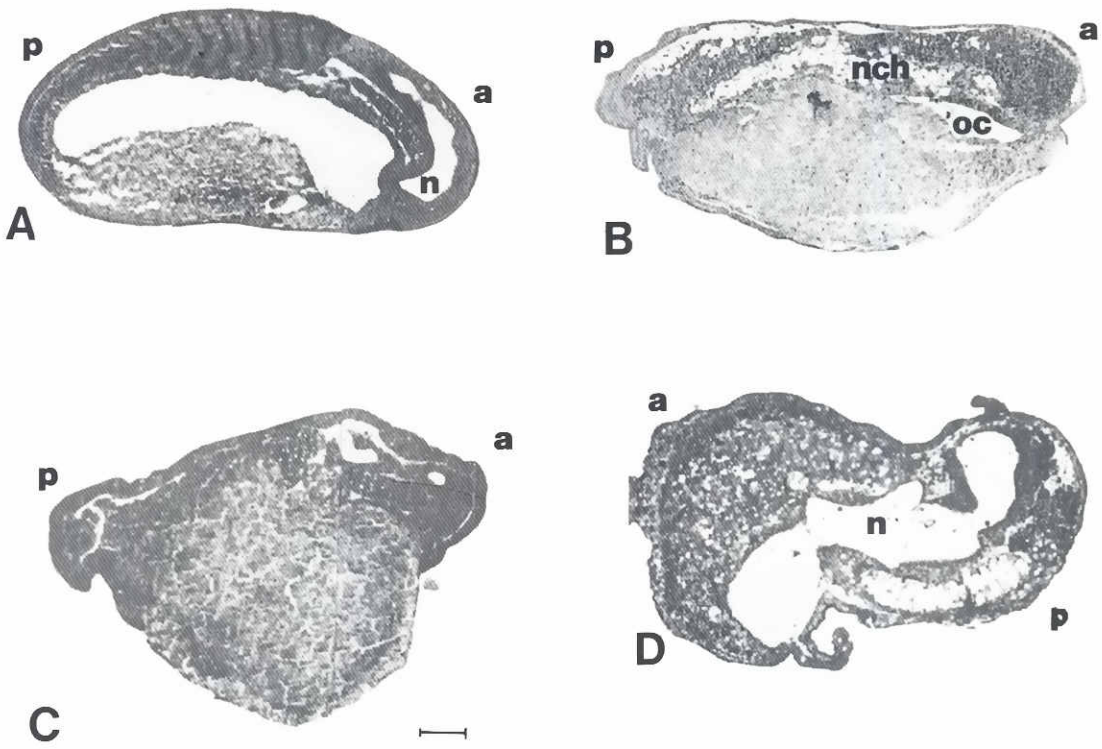

Fig. 10. Disturbances in antero-posterior polarity (ap), notochord structure and mesodermal segmentation in operated embryos *after 2 days incubation* **(B-D)**, *as compared with intact embryo* **(A)**, *sagittal sections. n - neurocoel, nch - notochord, oc oral cavity. Bar = 200 μm.*

axial structures via host ventral tissues to the implant tissues took place. At present, we cannot completely reject any of these possibilities, although the lack of continuous contact between the host and implant axial tissues (as seen in serial sections), as well as the close association of the new axial structures with implant involution movements, argues in favor of the first possibility. Meanwhile, to solve the problem unambiguously, tissue-labeling experiments are required and have been scheduled for the immediate future. Until now the interpretation of the present results may be questioned, but the fact that axial rudiments arise from ventral explant tissue now appears to have been histologically documented.

Materials and Methods

Operations and histology

Experiments were made on *Xenopus laevis* embryos at developmental stages 10-10 1/2 (early gastrula) as described by Nieuwkoop and Faber (1956). Following removal of the vitelline membrane, vertical slits were made in the ventro-vegetal (endodermal) parts of embryos without penetrating into the blastocele. Slits were made either sagitally or transversely (Fig. 1 A, B). The orientation of slits did not considerably affect the results of operations. Within minutes of cutting, the surgical cut opened to about 40°-60° and then continued to open more slowly. During incubation periods of more than a few hours, we inserted into the wound gap a sector of homologous (ventro-vegetal) tissue taken from another same-stage embryo (Fig. 1C), in order to fix the wound opening and to prevent infection (although some of the embryos survived perfectly for several days and demonstrated similar results without implants). As a rule, host and donor tissues fused perfectly within an hour after operation. A total of about 150 embryos were subjected to the operation.

Experimental embryos were fixed either immediately (within a minute) after operation or incubated before fixation in Slack and Forman (1980) solution for: 10 and 30 min; 1, 2, 4, 8 and 14 h (3-4 samples per each incubation time); and for 2-3 days (about 100 samples). Embryos cultivated up to 14 h were fixed in 2.5% glutharaldehyde, postfixed in 1% osmium tetroxide, embedded in epoxy resine (Epon-812) and studied in the sagittal or transversal semi-thin sections stained by 1% toluidine blue. Embryos incubated for the longer time periods were fixed in Bouin fluid, embedded in paraffin-wax and studied in 5-7 μm serial sections stained by Ehrlich hæmatoxylin. Intact embryos were treated by the same techniques.

Measurements

Estimations of the relative tension values in the surface and transversal cell walls of the outer ectodermal (epiectodermal) layer (Fig. 12)

In order to evaluate the relative values of tensile forces in the surface and transversal cell walls (F_s and F_t, correspondingly), we have used the classical methods of measuring cell surface angles (Thompson, 1942). The measured angles were those formed by tangents to the adjacent cell surfaces at the apex points (Fig. 12, α). Assuming that: (1) three cell walls conjoin at each apex point; (2) the transversal cell wall is as a rule the symmetry axis of the whole apex figure; (3) all tensions applied to a cell apex are mutually equilibrated (their total vectorial sum being equal to zero), the F_s/F_t ratio should depend on the surface angle α in the following way:

$1/2\ F_t = F_s \sin (90° - \alpha/2)$

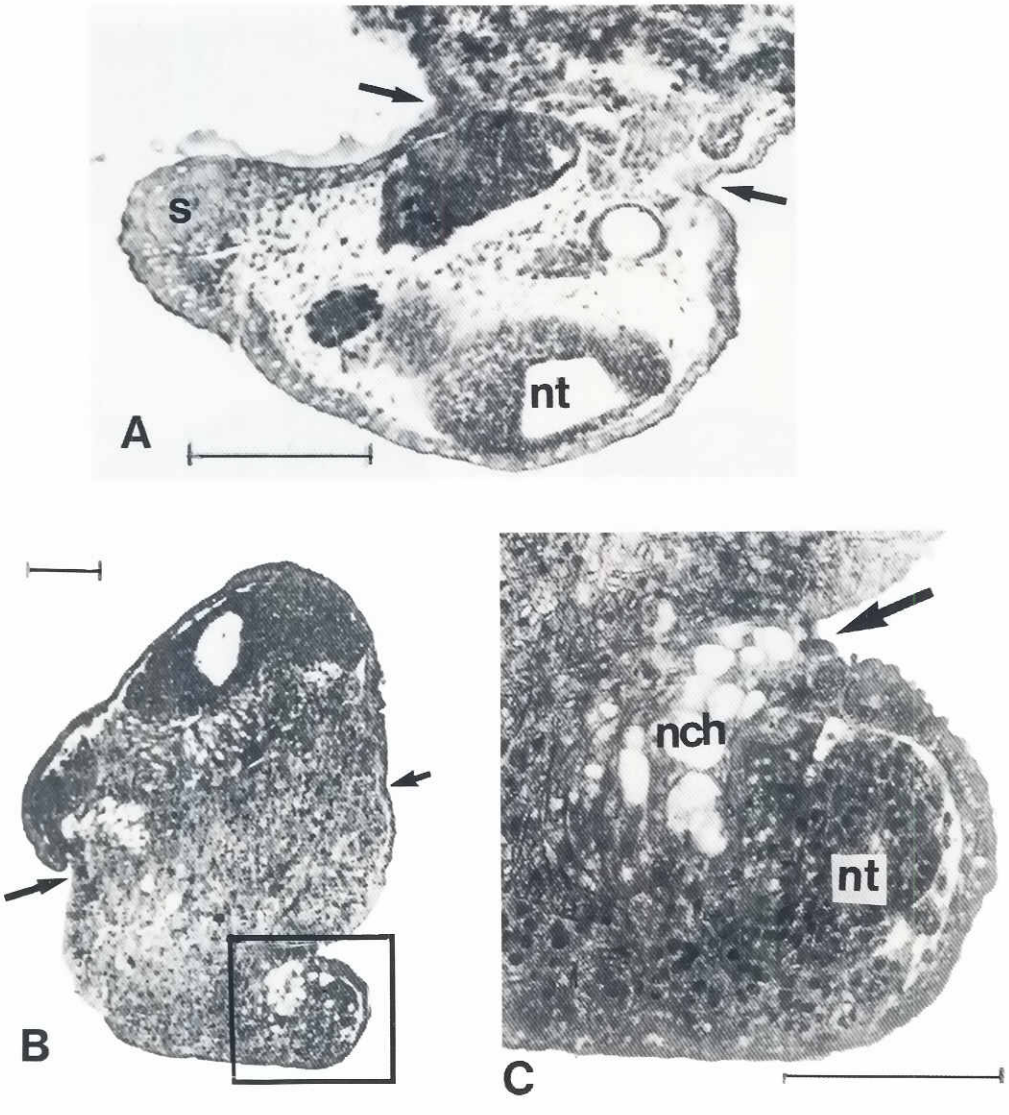

Fig. 11. Axial structures in ventral implants. *In* **(A,B)** *the implant-host border is marked by arrows (host tissues to the top).* **(C)** *a fragment framed in (B). Arrow points to implant ingression caused by involution movements. nch - notochord, nt - neural tissue (C) or neural tube (A), s - sucker. Bar = 200 μm.*

Roughly speaking, the greater the α angle, the greater is F_s / F_t and vice-versa. At α approaching 180°, F_s is infinitely large in relation to F_t, whereas at α=120°, the two values are equal. For each experimental figure presented in Table 1, we measured several dozens of α angles in the epiectodermal cells of the intact 11- and 13-stage embryos, and of the experimental embryos fixed either immediately or within 10, 30, 60 and 240 min after operation. The measurement areas were situated 250 μm in front of the blastopore.

Lobopodial activity

In the intact embryos of developmental stages 7-10 1/2, and in the experimental embryos10 min after operation, the percentage of lobopodia-possessing cells exposed to blastocele were counted and compared with those in tissue explants studied in previous experiments (Beloussov *et al.*, 1988).

Counting mesodermal cell layers

The number of cell layers inside the mesodermal sheet were counted on the transversal sections of 11-16 stage intact and operated embryos in the middorsal (d) and lateral (l) areas. The ratio of the number of cell layers in the two regions (R d/l), as measured in the same histological sections, was calculated.

Cell columnarization

Indexes of cell columnarization (IC) – that is, length/width ratios as detected on the medial sections of a given cell (Fig. 12, L/W) – were measured in the epi- and hypoectoderm of intact 11-16 stage embryos and in operated embryos of the corresponding age. Measurement areas included the whole ectodermal circumference 250 μm in front of the blastopore. Locations of measured cells were expressed in angular degrees of the embryo circumference (with 0 corresponding to the dorsal midline). Two-

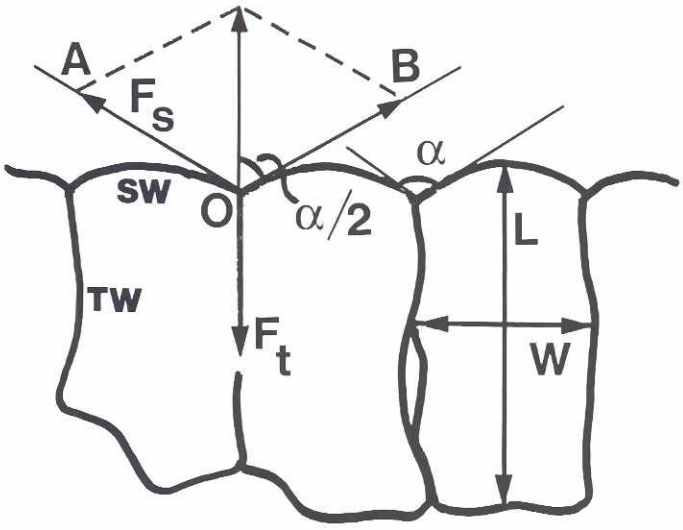

Fig. 12. Drawing of measurements taken of cell surface angles (α=AOB) and of columnarization indexes (IC=L/W). *OA and OB are the tangents to cell surfaces at apex point O. Forces of cell surface tension F_s oriented along the tangents to the cell surface are assumed to be equilibrated by force F_t of transversal cell wall tension. sw - surface wall, tw - transversal wall.*

dimensional maps of cell columnarization were also constructed for the dorsal epiectoderm of 16-stage intact and operated embryos.

Neural tissue volume

In seven operated embryos incubated for 2 days the total volume of neural tissue was measured by adding the squares occupied by this tissue on the successive serial transversal sections. The values obtained were compared with those of the intact same-stage embryos.

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