Int. J. Dev. Biol. 36: 527-535 (1992)

Original Article

Altering the position of the first horizontal cleavage furrow of the amphibian *(Xenopus)* egg reduces embryonic survival

HIROKI YOKOTA[1], ANTON W. NEFF[2] and GEORGE M. MALACINSKI[1]*

[1]*Department of Biology and* [2]*Medical Sciences Program, School of Medicine, Indiana University, Bloomington, Indiana, USA*

ABSTRACT The animal/vegetal cleavage ratio (AVCR), defined as the ratio of the height of the animal blastomere to the height of the *Xenopus* embryo at the 8 cell stage, can be shifted by placing embryos in novel gravitational fields: clinostating (microgravity simulation) increases AVCR, and centrifugation (hypergravity simulation) reduces AVCR. This report contributes to an understanding of the subcellular mechanism responsible for the furrow relocation and assesses its significance. Embryo inversion and D_2O immersion were found to increase AVCR, and cold shock was found to reduce AVCR. Based on the additive or antagonistic effects of combined treatments, it is postulated that the primary cause of AVCR changes is an alteration in the distribution of yolk platelets and the rearrangement of microtubule arrays. Embryos with a decreased AVCR exhibited reduced survival in early developmental stages, indicating serious difficulties in cleavage, blastulation and/or gastrulation. Cold-shocked embryos with a reduced AVCR could be rescued by D_2O pretreatment or clinostating, an observation which supports the notion that changes accompanying AVCR modifications represent the primary cause of the reduction in percent survival.

KEY WORDS: *Xenopus embryo, AVCR, cold shock, first horizontal cleavage furrow, reduced survival*

Introduction

The *Xenopus* embryo provides a favorable model system for investigating the effects of external forces such as gravity on early development. For example, various gravitational forces (reviewed in Malacinski and Neff, 1989) can be utilized for characterizing the extent to which pattern formation is altered by a uniform force field and, as well, as an experimental tool for producing embryos for analyzing the role that force-mediating cytoskeletal structures play in early embryogenesis.

According to Neff *et al.* (1992a,b), the location of the first horizontal cleavage furrow (third cleavage furrow) along the animal-vegetal axis can be shifted by placing eggs in gravitational fields of various strengths. Compared to controls under earth's gravitational field (1XG), embryos exposed to simulated weightlessness (μXG: microgravity) locate the first horizontal cleavage furrow closer to the vegetal pole, and embryos exposed to three times earth's gravity (3XG: hypergravity) locate the furrow closer to the animal pole.

Although both μXG and 3XG failed to generate catastrophic effects on embryonic development, tadpoles that had been exposed to novel gravitational forces showed slight dismorphogenesis of the dimensions of the head and eyes. Hatching tadpoles under μXG environment had a slightly enlarged eye diameter, and those under 3XG condition had a smaller eye diameter (Neff *et al.*, 1992).

In order to understand the subcellular mechanism responsible for furrow relocation and to assess the significance of altering the location of the first horizontal cleavage furrow, the following three questions were posed: (1) what treatments other than gravitational field strength manipulation alter the location of the first horizontal cleavage furrow (the «alternative treatment» question); (2) do treated embryos with substantial alterations in the furrow location exhibit developmental abnormalities (the «developmental consequences» question); and (3) can developmental abnormalities associated with furrow alterations be prevented by restoring the furrow position (the «prevention» question).

To address the first question regarding alteration of the furrow position, treatments that disturb cytoplasmic organization and affect cytoskeletal structures were employed. Three alternative treatments employed herein include: (1) embryo inversion, which has been demonstrated to rearrange internal cytoplasm (Neff *et al.*, 1984; Wakahara *et al.*, 1985; Cooke, 1986; Tencer and Goldfinger, 1992); (2) cold shock, which destabilizes microtubules (Tilney and Porter, 1967); and (3) D_2O, which stabilizes microtubules (Houston *et al.*, 1974; Scharf and Gerhart, 1983).

Abbreviations used in this paper: AVCR, Animal Vegetal Cleavage Ratio; μXG, clinostat-simulated weightlessness; 1XG, earth's gravitational acceleration (9.8 m/sec^2); 3XG, centrifuge-simulated three times earth's gravitational acceleration (29 m/sec^2); 5XG, centrifuge-simulated five times earth's gravitational acceleration (49 m/sec^2).

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0214-6282/92/$03.00

Printed in Spain

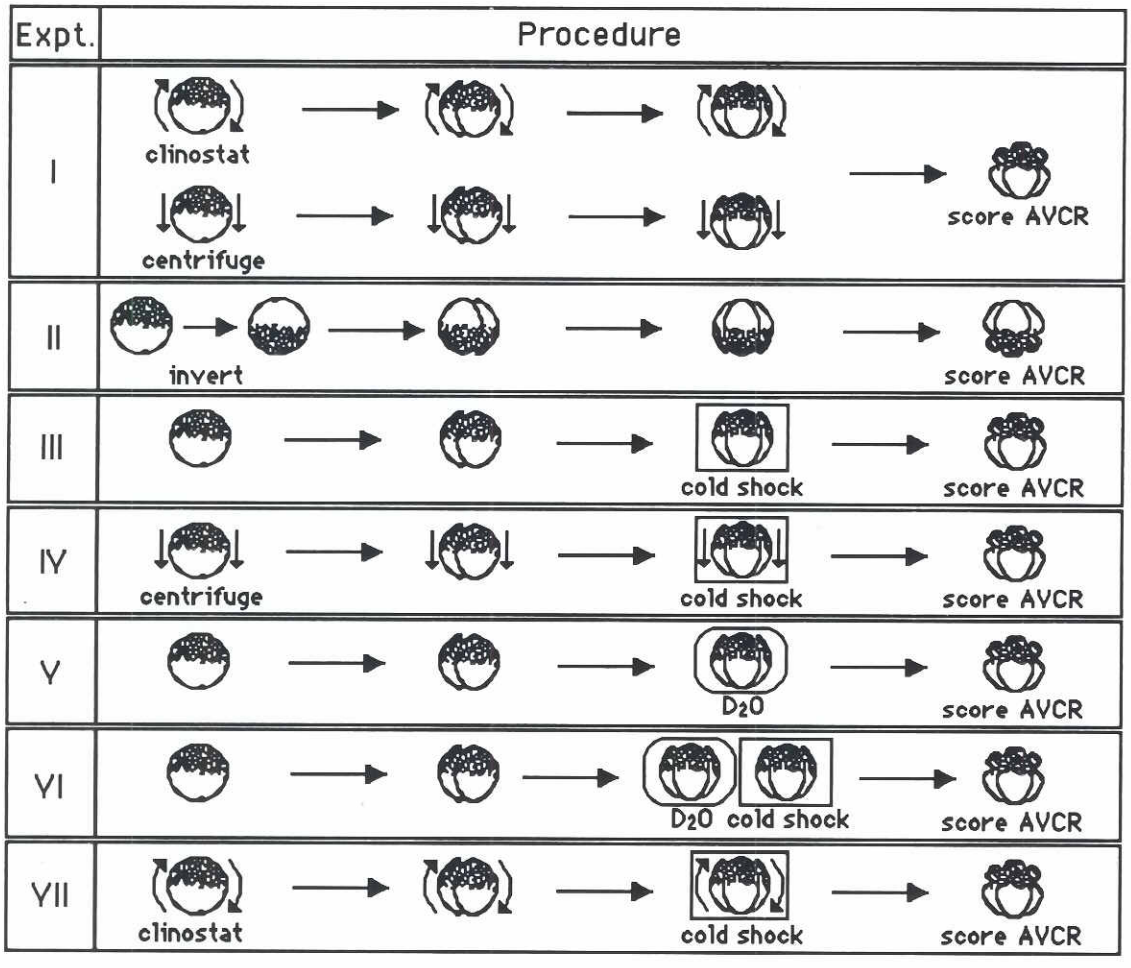


Fig. 1. Summary of experimental designs. *AVCR was scored at T_3 after exposure to a variety of treatments such as the clinostat or centrifuge (Expt. I), embryo inversion (Expt. II), cold shock (Expt. III), centrifuge and cold shock (Expt. IV), D_2O immersion (Expt. V), D_2O immersion and cold shock (Expt. VI), and clinostat and cold shock (Expt. VII).*

For the second question regarding developmental abnormalities, survival frequency was used as an index for «normality.» Although minor effects on morphogenesis could easily have escaped detection, this scoring system is both straightforward and quantitative.

To address the third question, regarding the causal relationship between altering the location of the first horizontal furrow and subsequent developmental abnormalities, a pair of rescue experiments was conducted. It will be demonstrated that the decrease in survival of cold-shocked embryos is restored by D_2O or μXG treatments that prevent cold-shock induced alterations in furrow position.

Results and Discussion

Alternative Treatments

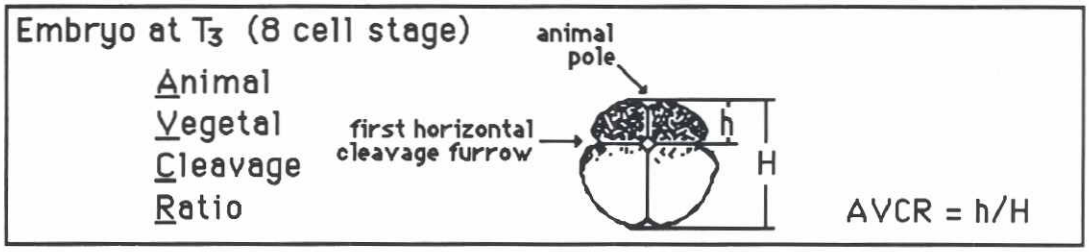
To answer the first «alternative treatment» question, concerning which treatments alter the location of the first horizontal cleavage furrow, a variety of methods were employed, including gravity manipulation, embryo inversion, cold shock and D_2O treatment. In some experiments those alterations were either reversed or amplified by a combination of treatments. The design of each experiment (Expts. I - VII) is included in Fig. 1. The following observations were made:

Substantial alterations in AVCR were achieved by gravity manipulation between T_1 (2 cell stage) and T_3 (8 cell stage)

In Expt. I, embryos were either clinostated to simulate microgravity (μXG) or centrifuged to simulate 3XG hypergravity for various times between T_0 (fertilization) and T_3. The data in Fig. 2B indicates that μXG treatment increased AVCR, and 3XG treatment decreased AVCR. Although a 40 min treatment between T_2 (4 cell stage) and T_3 was sufficient to change the AVCR, somewhat longer treatments such as T_0 - T_3 (200 min) and T_1 - T_3 (80 min) were more effective. Comparison of T_1 - T_3 with T_0 - T_3 reveals that maximal AVCR change can be achieved when treatment begins as late as T_1.

A probable cause of the gravity-driven shift of the third cleavage furrow is the rearrangement of yolk platelets and non-yolk cytoplasm. Preliminary examination of 4 μm cross sections of embryos

A. Definition of AVCR



B. Mean AVCR shifts in response to gravitational manipulation

G force	To - T3 (200 min)	T1 - T3 (80 min)	T2 - T3 (40 min)
μXG (clinostat)	0.45±0.03	0.44±0.03	0.40±0.03
1XG (control)	0.38±0.04	——	——
3XG (centrifuge)	0.29±0.04	0.29±0.04	0.32±0.09

C. Cross section at T3

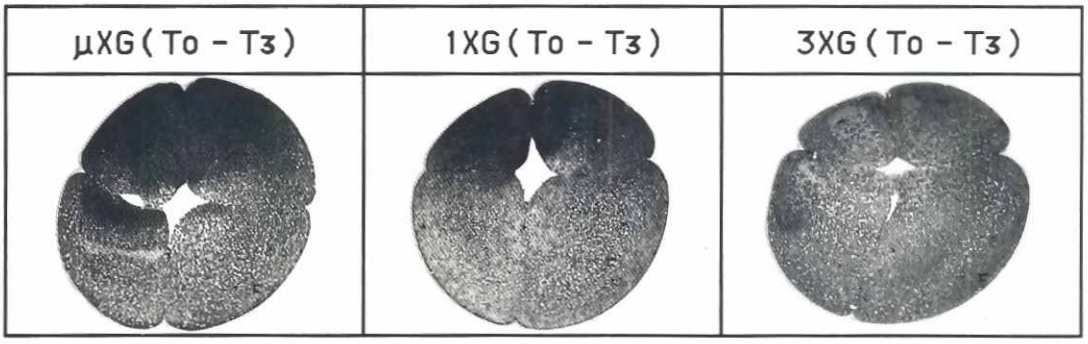


Fig. 2. Definition of AVCR and gravitational effects on AVCR and distribution of yolk platelets. (A) *AVCR is defined as the ratio of the height of the animal blastomere to the height of the embryo at the 8 cell stage.* **(B)** *Embryos were clinostated (μXG simulation) or centrifuged (3XG simulation) starting at T_0, T_1 or T_2, and mean AVCR was scored (6 spawnings, 671 embryos).* **(C)** *Cross sections of representative embryos at T_3 reveal that compared to control sections, animal blastomeres of a μXG treated embryo are more abundant in yolk platelets, and 3XG treated blastomeres are less abundant in yolk platelets.*

supports this idea. First, the number of yolk platelets whose longer axis is larger than 5 μm was counted in a 150 μm X 150 μm square area in animal blastomeres (2 spawnings, 9 embryos). Although there were spawning-to-spawning variations, compared to 1XG controls, the μXG counts were higher, whereas the 3.4XG counts were lower (μXG: 43, 1XG: 20, 3.4XG: 17 for spawning #1; and μXG: 95, 1XG: 80, 3.4XG: 60 for spawning #2). Second, with reference to Fig. 2C, where non-yolk cytoplasm was stained darker by toluidine blue than were yolk platelets, non-yolk cytoplasm of μXG treated embryos extended more vegetally than that of 1XG controls, while that of hypergravity treated embryos was localized more animally. The degree of rearrangement presumably depends on the size and shape of the embryo, the size, shape, and density of yolk platelets, and the local variations in egg cytoplasm viscosity. It is well known that the egg cytoplasm rearranges following egg activation (Gerhart *et al.*, 1981; Vincent and Gerhart, 1987), and that the apparent viscoelasticity of the cytoplasm varies from spawning to spawning and also changes over time (Elinson, 1985; Smith and Neff, 1986). Despite those dynamic features of the egg cytoplasm, the egg remains susceptible to gravity driven alterations in the third cleavage furrow even after the first cleavage forms.

Inversion of the fertile egg generates even more dramatic increases in AVCR

In Expt. II, embryos were inverted 180° at $T_{0.3}$, T_1 or T_2, and AVCRs were scored at T_3. Note that even when embryos were inverted, the

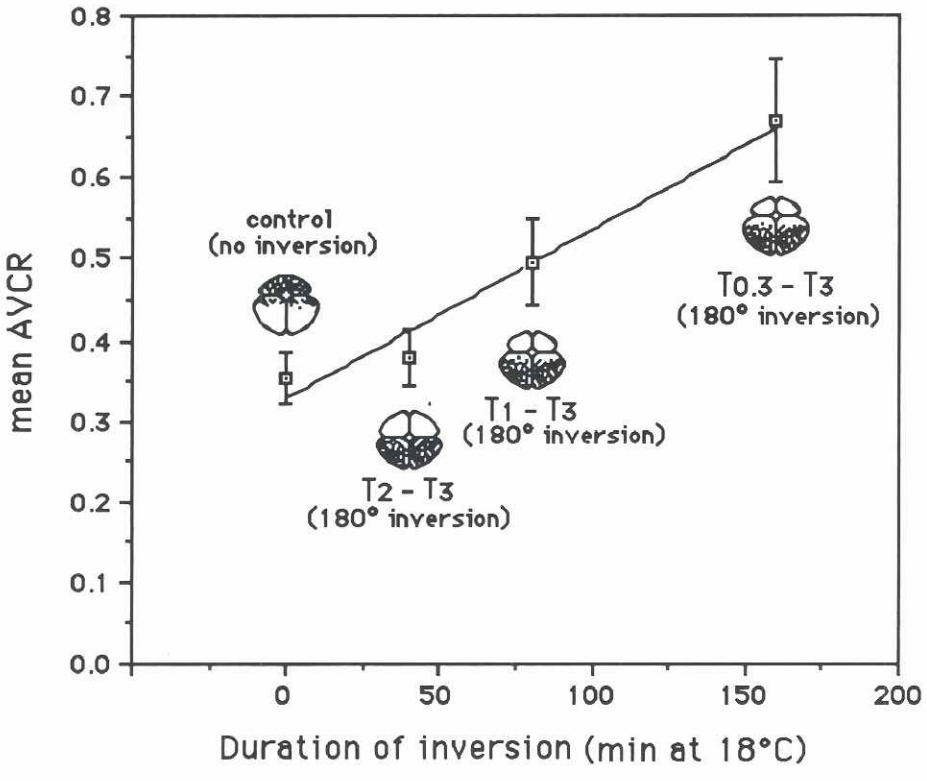


Fig. 3. A linear relationship between AVCR and duration of inversion. *Based on the pooled data (5 spawnings, 282 embryos), the best fit line is expressed as «(AVCR estimate)= 0.0021 X (duration of inversion in min) + 0.33» with r= 0.98. One standard deviation is shown.*

AVCR was defined based on the original animal/vegetal polarity. Fig. 3 demonstrates that longer inversion times produced the greatest changes (increases) in AVCR, and there is a linear relationship between duration of inversion time and mean AVCR with a correlation coefficient of r= 0.98.

Embryo inversion approximates (-1)XG in the sense that embryos experience the earth's gravity in an opposite direction, so that its effects on AVCR as well as on the redistribution pattern of cytoplasmic compartments (Neff *et al.*, 1984) and yolk platelets are in the same direction as μXG simulation, but with a more dramatic intensity.

Cold Shock also generates dramatic changes (decreases) in AVCR

In Expt. III, embryos were cold shocked (6°C) at T_2 for either 5 or 10 min. Fig. 4A demonstrates that a 5 min treatment decreased the AVCR, whereas 10 min generated an even more substantial decrease. Fig. 4B compares a representative cold-shocked embryo to a representative control embryo. Since cold shock depolymerizes microtubules (Tilney and Porter, 1967), the observation that the location of the third cleavage furrow arises along the animal/vegetal axis supports the notion that microtubules directly or indirectly affect the location of furrow formation.

Two possible interpretations of AVCR reduction in cold-shocked embryos are: (1) the rigidity of the internal cytoplasm has collapsed because cold shock depolymerizes microtubules (Tilney and Porter, 1967; and Neff *et al.*, 1989). The yolk platelets compact towards the vegetal hemisphere, as occurred in 3XG embryos, physically hindering the furrow formation in a tightly packed vegetal hemisphere; and/or (2) microtubules that are responsible for locating the mitotic asters are depolymerized by cold shock, thus blocking either the migration or anchoring of asters in the vegetal hemisphere. The first interpretation is consistent with the model based on Expts. I and II that the distribution of yolk platelets dictates the position of furrow formation. The second interpretation is based on the observation that the location of the cleavage furrow depends upon the position of asters (Rappaport, 1961), and the cleavage furrow appears between two opposing asters. Those interpretations are not mutually exclusive, because the migration of asters most likely depends upon such features as the integrity and organization of the cytoplasm. Those features of the amphibian egg cytoplasm are dependent to a large extent on the distribution of yolk platelets.

Hypergravity (3XG) and cold shock exert additive effects on AVCR

In Expt. IV, a brief cold shock (5 min pulse at 6°C) was administered at T_2 to embryos as they were being centrifuged. In Fig. 5A, the change (decrease) in AVCR usually observed after centrifugation was substantially enhanced by the combined treatment. As expected, since the data from Expt. III established that cold shock decreases AVCR, the additional compaction of yolk platelets provided by 3XG decreases AVCR even more. This observation is consistent with both interpretations described earlier that the location of the cleavage furrow reflects yolk platelet distribution and/or aster positioning.

D_2O treatment increases the AVCR

In Expt. V, embryos were immersed in 70% D_2O at T_2 for either 5 min or 10 min. Out of ten spawnings summarized in Fig. 5B, six spawnings exhibited increases in AVCRs. Among those spawnings

A. Mean AVCR of cold shocked embryos at T_2 (6°C)

control:		0.36±0.02
cold shock:	5 min	0.26±0.03
	10 min	0.19±0.03

B. Morphology of cold shocked embryos

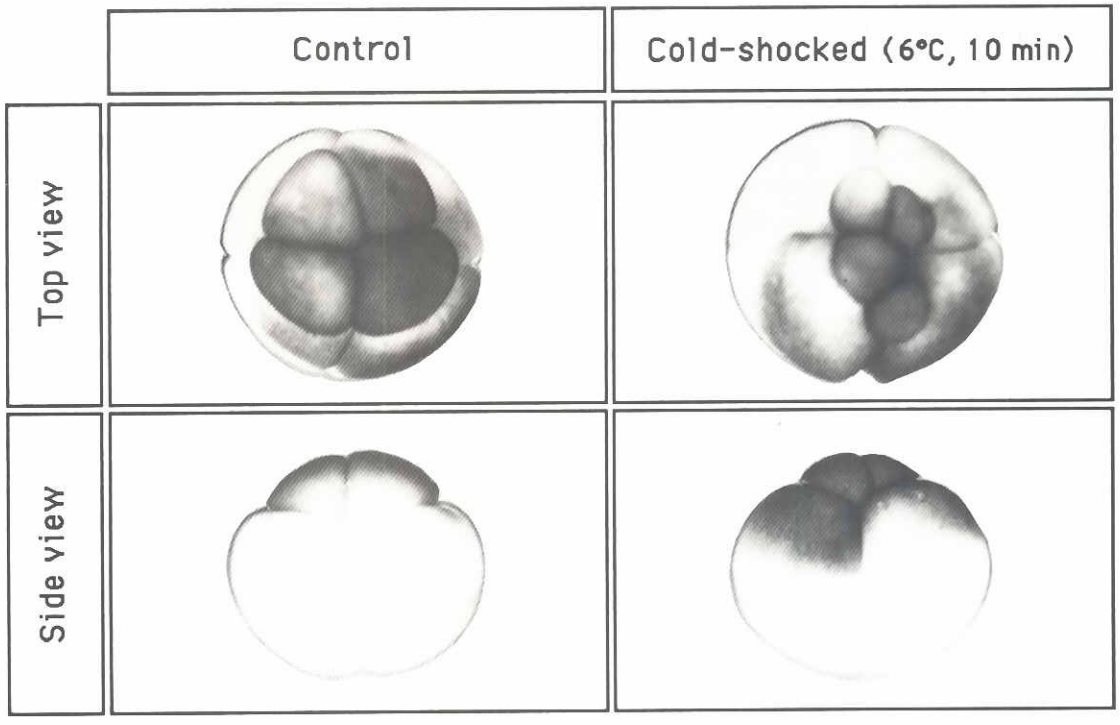


Fig. 4. Cold shocked embryo with reduced AVCR. **(A)** *Compared to control (10 spawnings, 130 embryos), mean AVCR was reduced by cold shock at T_2 from 18°C to 6°C for 5 min (6 spawnings, 60 embryos) or 10 min (5 spawnings, 80 embryos).* **(B)** *A typical 10 min cold-shocked embryo is illustrated.*

the increase was roughly proportional to the treatment time, although the sensitivity varied from spawning to spawning. Four other spawnings did not alter AVCR to a statistically significant extent (*t* Test at 5% significance). Since D_2O stabilizes microtubules (Houston *et al.*, 1974), D_2O is an antagonist of cold shock. The data in Expt. V are consistent with the data in Expts. III and IV: the treatment that depolymerizes microtubules shifts the furrow position towards the animal pole, whereas the treatment that stabilizes microtubules moves the furrow position towards the vegetal pole.

Pretreatment with D_2O protects embryos from the effects of cold shock on AVCR

In Expt. VI, embryos were treated with 70% D_2O for either 5 min or 10 min at T_2 prior to cold shock treatment. The data in Fig. 5C clearly demonstrate that the effects of cold shock, presumably mediated through the depolymerization of microtubules, can be prevented by pretreating embryos with D_2O, an antagonist of cold shock. The observation in Expt. VI is consistent with the data from Expts. III, IV and V.

Clinostating also protects embryos from the effects of cold shock on AVCR

In Expt. VII, embryos were clinostated from T_0 to T_3 to simulate a μXG environment, and an experimental group were given a brief cold shock (6°C, 5 min or 12 min at T_2). As the data in Fig. 5D indicate, almost complete protection of AVCR against cold shock was obtained by μXG simulation.

The observation in Expt. VII that μXG treatment is dominant over cold shock on AVCR implies that depolymerizing microtubules is not a direct cause of AVCR reduction, provided that μXG simulation does not stabilize microtubules. It is more likely that the direct cause of AVCR alteration is the redistribution of various components such as asters and/or yolk platelets.

Developmental consequences (survival)

To answer the second («developmental consequences») question concerning whether those embryos which exhibit a substantial alteration of the location of the third furrow also exhibit developmental abnormalities, survival frequency was scored and compared at four

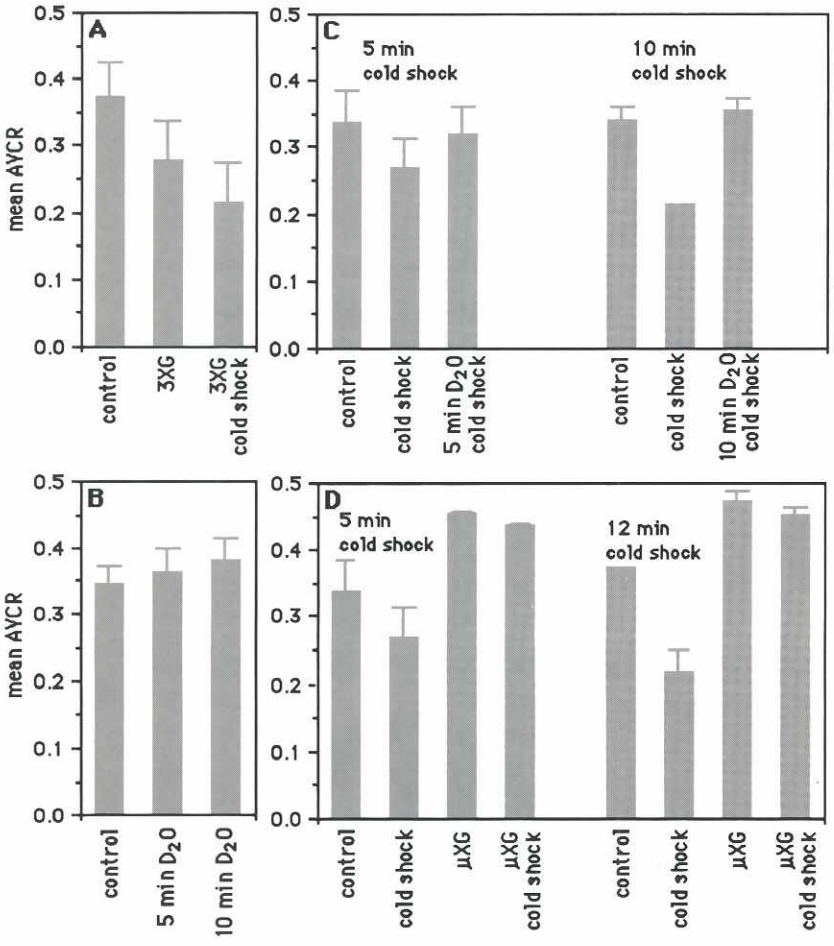


Fig. 5. Comparison of mean AVCR with various treatments. The mean AVCR with one standard deviation is shown. (A) *Enhanced decreases in AVCR were observed in centrifuged embryos treated with 5 min of cold shock at T_2 (3 spawnings, 180 embryos).* **(B)** *AVCR increased in 70% D_2O treated embryos for 5 min or 10 min cold shock (10 spawnings, 423 embryos).* **(C)** *Resistance to cold shock-induced AVCR changes was provided by D_2O pretreatment (4 spawnings, 222 embryos). D: Resistance to cold shock-induced AVCR changes was also observed in clinostated embryos (4 spawnings, 228 embryos).*

developmental stages (blastula, gastrula, neurula and hatching tadpole). Needless to say, minor effects on morphogenesis could easily have escaped detection, but this scoring system is straightforward and quantitative. Ten embryos from each control or experimental group were fixed at T_3 for estimating the mean AVCR of the population.

Embryos with increased AVCRs survive normally

Embryos were clinostated from T_0 to stage 8 (blastula stage), and survival frequency was scored. Fig. 6A includes percent survival of 1XG controls and μXG experimentals (5 spawnings, 835 embryos). The numbers in parenthesis represent the mean AVCRs. μXG treated embryos showed slightly reduced percent survival, with different spawning sensitivities, in all 5 spawnings, consistent with the previous study (Smith and Neff, 1986). In pooled data, 83% of control embryos with a mean AVCR of 0.38 survived to hatching tadpole stage, whereas 77% of μXG treated embryos with a mean AVCR of 0.46 survived. The decrease in percent survival due to μXG simulation was 6% (statistically different at $p<0.05$ in *t* Test) with 0.08 increase in mean AVCR. As will be demonstrated below, this reduction in percent survival is found to be 2- to 10-fold smaller than the reduction observed in hypergravity and cold shock treatment.

Embryos with reduced AVCRs have reduced survival

Embryos were centrifuged from T_0 to T_3 or stage 8 (blastula stage) to simulate either 3XG or 5XG hypergravity. In contrast to embryos with increased AVCRs in Fig. 6A, embryos with reduced AVCRs exhibited striking reductions in survival. Observation of centrifuged embryos in Fig. 6B (5 spawnings, 980 embryos) revealed that 3XG treated embryos with a mean AVCR reduction of 0.09 exhibit 13% decrease in survival. 5XG treated embryos with a mean AVCR reduction of 0.14 displayed a 59% decrease in percent survival. In both hypergravity conditions, major decreases took place until gastrula stage, indicating that major developmental problems occurred in either cleavage, blastulation or gastrulation.

Cold shock, which was demonstrated previously (in Expt. III) to reduce AVCR values, also diminishes survival frequency

Embryos were cold shocked at T_2 at 4-6°C for 10-12 min. According to the data in Fig. 6C (5 spawnings, 676 embryos), cold-shocked embryos with mean AVCR reduction of 0.16 showed 31% reduction in percent survival compared to control embryos. As was the case in the centrifugation experiment, some spawnings were more sensitive than others and a major drop in percent survival occurred before the gastrula stage.

The data for the second («developmental consequences») question are summarized in Fig. 7. That graph illustrates the relationship between percent survival at hatching tadpole stage and mean AVCR based on the individual spawning data summarized in Fig. 6A,B and C. The relationship is approximated by the second order polynomial

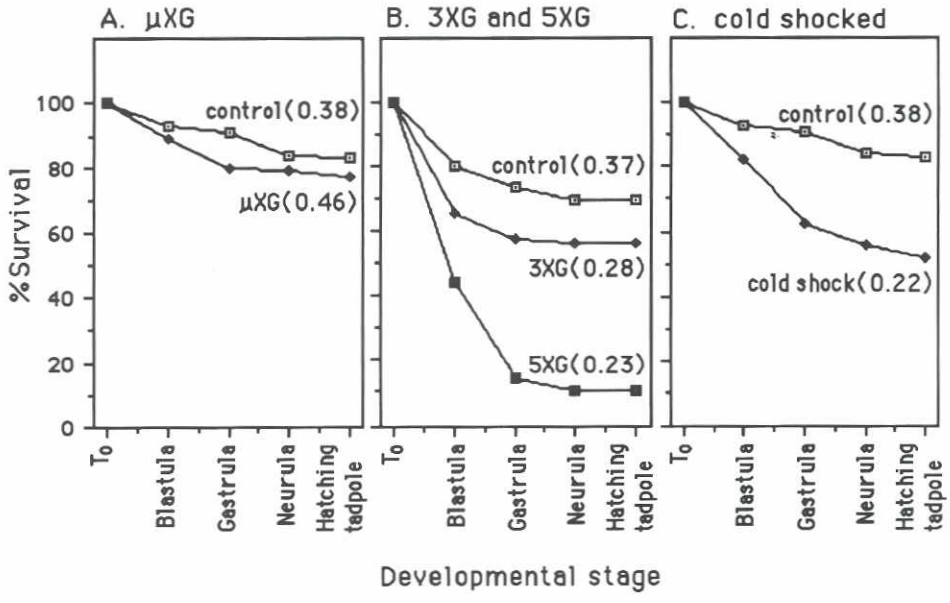


Fig. 6. Percent survival with various treatments. *Percent survival is plotted as a function of developmental stage. The value in parenthesis represents the mean AVCR.* **(A)** *Percent survival of µXG-treated embryos with increased AVCRs did not significantly diminish (5 spawnings, 835 embryos).* **(B)** *3XG-and 5XG-treated embryos with reduced AVCRs had reduced survival (5 spawnings, 980 embryos).* **(C)** *Cold shocked embryos with AVCR reductions showed a decrease in percent survival (5 spawnings, 676 embryos).*

with a correlation coefficient of r= 0.72, indicating that percent survival peaks approximately at the mean AVCR value of control embryos. AVCR increase does not significantly reduce percent survival, but AVCR decrease does reduce percent survival. In fact, the AVCR value of 0.25 represents an approximate «threshold» for maintaining 50% survival.

Prevention

The next two experiments were designed to answer the third («prevention») question concerning whether decreases in percent survival (at various developmental stages) due to furrow alterations can be prevented by restoring the furrow position.

D_2O, which protects the embryo from AVCR reduction, prevents cold shock-induced reduced survival

Embryos from two spawnings were cold-shocked at T_2 (4°C, 15 min) with and without 10 min 70% D_2O pretreatment. Without D_2O pretreatment, percent survival of cold-shocked embryos (0.15 mean AVCR reduction) at hatching tadpole stage was lower than percent survival of non-cold-shocked embryos by 32%. Fig. 8 clearly demonstrates that D_2O pretreatment is effective in diminishing AVCR reduction and preventing the decrease in percent survival. However, it is difficult to rule out the possibility that cold-shocked embryos were rescued by protecting microtubules that were critical for survival, and restoring AVCR was coincidental. To eliminate this possibility, the next clinostat-rescue experiment was performed.

Clinostating, which reverses decreases in AVCR, also prevents cold shock-induced reduced survival

Embryos from four spawnings were cold shocked at T_2 (4-6°C for 10-12 min) with and without µXG simulation, with two µXG treatment windows (T_1 - stage 8, and T_3 - stage 8). The µXG treatment starting at T1 prevents AVCR reduction by cold shock, but the µXG treatment starting at T3 does not prevent AVCR reduction. Fig. 9 shows: (1) compared to non-cold-shocked 1XG embryos, cold-shocked 1XG embryos exhibited 29% reduction (on average) in percent survival at hatching tadpole stage. Reduction in mean AVCR by cold shock was 0.15; (2) compared to non-cold shocked µXG embryos, cold-shocked µXG (T_1 - stage 8) embryos displayed a reduction in percent survival by 11% with 0.02 AVCR decrease; and (3) compared to non-cold-shocked µXG embryos, cold-shocked µXG (T_3 - stage 8) embryos showed 31% reduction in percent survival.

Comparison of (1) to (2) demonstrates that 29% reduction in percent survival was improved to 11% by µXG treatment, including a critical window of sensitivity at T_1 - T_3. Comparison of (2) to (3) reveals that 29% reduction became worse (to 31%) by µXG treatment, excluding a critical window of T_1 - T_3. Namely, the µXG treat-

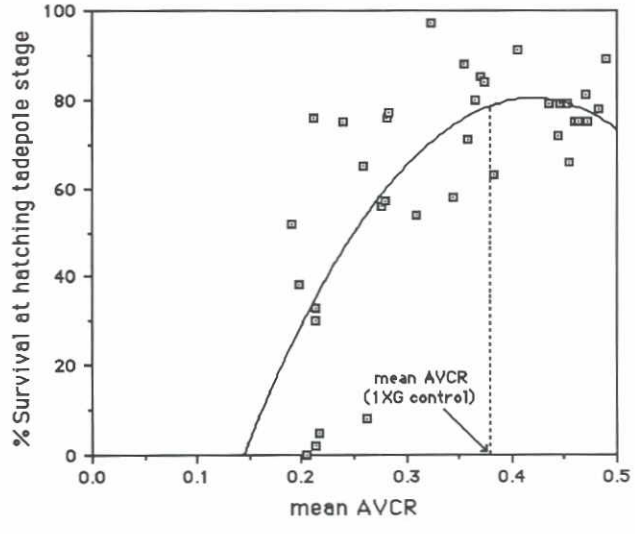


Fig. 7. The relationship between percent survival at hatching tadpole stage and mean AVCR. *The second order best fit polynomial is expressed as « % survival= -107+893 X (mean AVCR) -1060 X (mean AVCR)^2» with r= 0.72.*

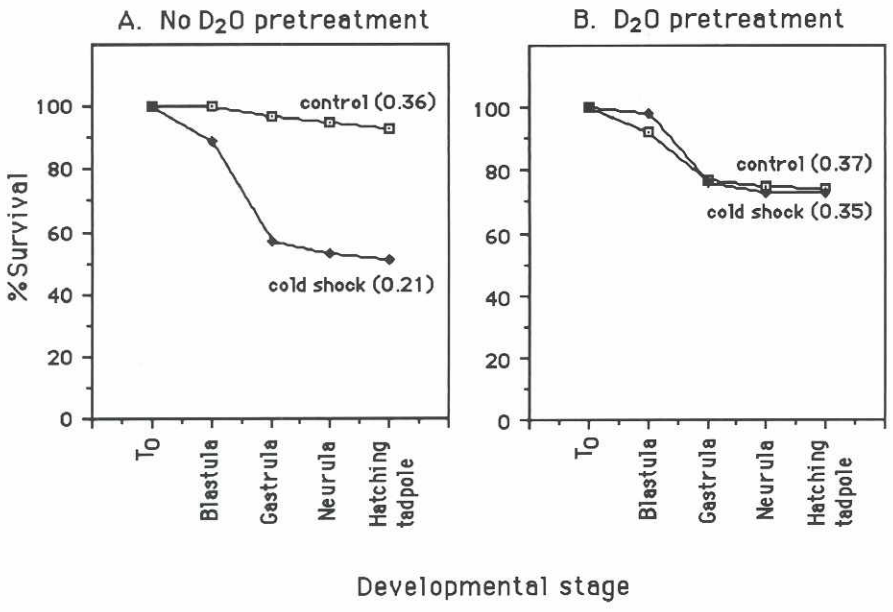


Fig. 8. Prevention of cold shock-induced survival reduction by D_2O. *Percent survival is plotted as a function of developmental stages with and without D_2O pretreatment. The value in parenthesis is mean AVCR.* **(A)** *Without D_2O pretreatment, cold shocked embryos had reduced survival (2 spawnings, 310 embryos).* **(B)** *With D_2O pretreatment, percent survival of cold shocked embryos was substantially improved (2 spawnings, 278 embryos).*

ment between T_1 and T_3 is critical for preventing the reduction in percent survival, and this rescue window is equivalent to the sensitive window of AVCR. Together with the results from D_2O rescue experiment, it is therefore likely that changes accompanying AVCR modifications represent the primary cause of the reduction in survival.

Materials and Methods

Source of Embryos

Xenopus laevis embryos were obtained by artificially fertilizing eggs from chorionic-gonadotropin-injected females by standard methods. Embryos were maintained at 18°C (except for the cold shock experiments) in 20% Steinberg's solution. The symbols T_0, T_1, T_2 and T_3 correspond to the time of fertilization (T=0), the 2 cell stage (T=1), the 4 cell stage (T=2) and the 8 cell stage (T=3). Embryos were staged according to Nieuwkoop and Faber (1956).

Animal/vegetal cleavage ratio (AVCR) measurements

With reference to Fig. 2A, the animal/vegetal cleavage ratio (AVCR) is defined at T_3 for scoring the location of the first horizontal cleavage furrow. Embryos were fixed in PBFG (2.5% glutaraldehyde, 4% formalin, 100% phosphate buffered saline (PBS), pH 7.4) overnight and washed in 100% Steinberg's solution. The AVCR was measured in 25% ethanol (75% PBS) using a dissecting microscope at 40X with a measurement resolution of 33 μm.

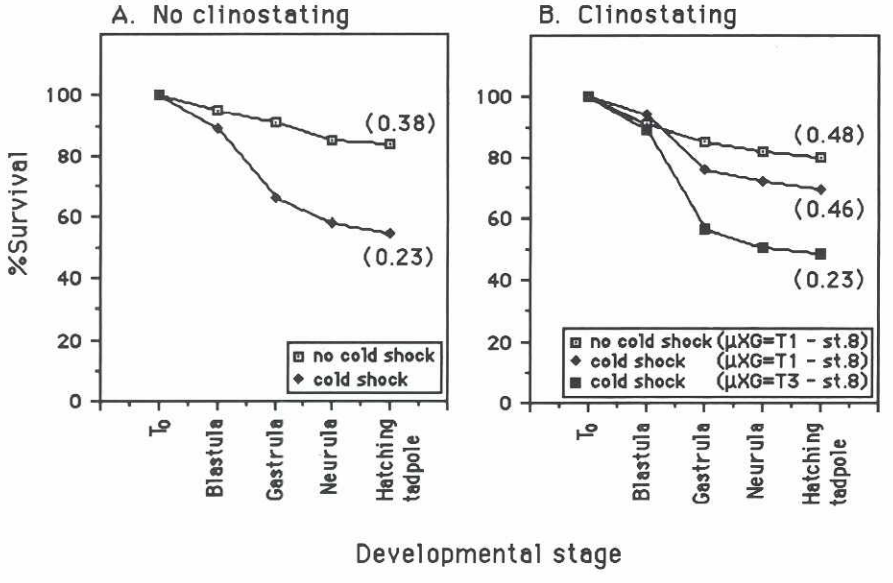


Fig. 9. Prevention of cold shock-induced survival reduction by clinostating. *Percent survival is plotted as a function of developmental stages with and without clinostating. The value in parenthesis represents mean AVCR.* **(A)** *Without clinostating, cold shocked embryos had reduced survival (4 spawnings, 633 embryos).* **(B)** *Clinostating between T_1 and stage 8 substantially improved percent survival of cold shocked embryos, but the same treatment between T_3 and stage 8 did not enhance percent survival (4 spawnings, 951 embryos).*

Microgravity and hypergravity simulations

Approximately 50 freshly fertilized eggs were placed in Cultusak™ 6"X9" 5-chamber units (Falcon) or 3.5X1.0 cm petri dishes (Falcon). Microgravity (μXG) was simulated by placing chambers or petri dishes on the horizontal clinostat with a 0.5 cm radius at 6 rpm (Neff *et al.*, 1985). Hypergravity (3XG: three times earth's gravity, and 5XG: five times earth's gravity) was simulated by placing fertilized eggs on a centrifuge with a 17.6 cm radius. The centrifuge was operated at 120 rpm for 3XG simulation and 158 rpm for 5XG simulation. Ground controls (1XG) were incubated on the desk top adjacent to the clinostats and centrifuges, or they were mounted on a vertical clinostat at 6 rpm. Preliminary results showed no significant difference between desk top and vertical clinostat controls.

Embryo inversion

Approximately 30 min after fertilization, when the darkly pigmented animal hemisphere faced upward by the natural gravity-driven rotation, fertilized eggs in 3.5X1.0 cm petri dishes were immobilized in 20% Ficoll (Sigma F-4375) solution. The petri dishes were then inverted by 180° at a designated time ($T_{0.3}$: 40 min after fertilization, T_1 or T_2). The Ficoll solution was drained off and replaced by 20% Steinberg's solution at stage 8 (blastula stage).

Cold shock

Fertilized eggs in 3.5X1.0 cm petri dishes were cold shocked at T_2 by flooding with ice-chilled 20% Steinberg's solution at a defined low temperature (4°C or 6°C) for 5-12 min. When a cold shock was administered as embryos were being clinostated or centrifuged, a clinostat or a centrifuge was turned off twice for approximately 1 min each to replace Steinberg's solution.

D_2O treatment

Fertilized eggs were dejellied by 3.5% cysteine (Sigma C-7880) solution 20 min after fertilization, and they were immersed in 70% D_2O (Sigma D-9764) at T_2 for 5 min or 10 min.

Survival studies

Survival frequency was scored at four developmental stages (blastula, gastrula, neurula and hatching tadpole) as an index for developmental consequences. One hundred percent survival means that all fertilized eggs survived.

Histology

PBFG fixed 8 cell stage embryos were embedded in methacrylate and cut in 4 μm sections. Sections were stained with either toluidine blue or a modified Heidenhain's Azan stain that stained yolk platelets yellow/orange and non-yolk cytoplasm and nuclei blue (Smith and Neff, 1985).

Acknowledgments

This study was supported by a grant from NASA (NAGW-1548). Thanks are expressed to Susan Duhon for helpful discussions, and to Betsy Osborne for histology and photography.

References

COOKE, J. (1986). Permanent distortion of positional system of *Xenopus* embryo by brief early perturbation in gravity. *Nature 319:* 60-63.

ELINSON, R.P. (1985). Changes in levels of polymeric tubulin associated with activation and dorsoventral polarization of the frog egg. *Dev. Biol. 109:* 224-233.

GERHART, J., UBBELS, G., BLACK, S., HARA, K. and KIRSCHNER, M. (1981). A reinvestigation of the role of the grey crescent in axis formation in *Xenopus laevis*. *Nature 292:* 511-516.

HOUSTON, L.L., ODELL, J., LEE, Y.C. and HIMES, R.H. (1974). Solvent isotope effects on microtubule polymerization and depolymerization. *J. Mol. Biol. 87:* 141-146.

MALACINSKI, G.M. and NEFF, A.W. (1989). An essay on redundancy within developmental processes. In *Cytoplasmic Organization Systems* (Ed. G.M. Malacinski). McGraw-Hill Publishing Co., New York, pp. 123-153.

NEFF, A.W., MALACINSKI, G.M. and CHUNG, H.-M. (1985). Microgravity simulation as a probe for understanding early *Xenopus* pattern specification. *J. Embryol. Exp. Morphol. 89:* 259-274.

NEFF, A.W., RITZENTHALER, J.D. and ROSENBAUM, J.F. (1989). Subcellular components of the amphibian egg: insights provided by gravitational studies. *Adv. Space Res. (Vol. 9) 11:* 177-186.

NEFF, A.W., WAKAHARA, M., JURAND, A. and MALACINSKI, G.M. (1984) Experimental analyses of cytoplasmic rearrangements which follow fertilization and accompany symmetrization of inverted *Xenopus* eggs. *J. Embryol. Exp. Morphol. 80:* 197-224.

NEFF, A., WAKAHARA, M., YOKOTA, H. and MALACINSKI, G.M. (1992a). Understanding the organization of the amphibian egg cytoplasm: gravitational force as a probe. *Adv. Space Res (12) 1:* 175-180.

NEFF, A.W., YOKOTA, H., CHUNG, H.-M., WAKAHARA, M. and MALACINSKI, G.M. (1992b). Early amphibian (anuran) morphogenesis is sensitive to novel gravitational fields. *Dev Biol. 154* (in press).

NIEUWKOOP, P.D. and FABER, J. (1956). *Normal Table of Xenopus laevis* (Daudin) North-Holland Publishing Company, Amsterdam.

RAPPAPORT, R. (1961). Experiments concerning cleavage stimulus in sand dollar eggs. *J. Exp. Zool. 148:* 81-89.

SCHARF, S.R. and GERHART, J.C. (1983). Axis determination in eggs of *Xenopus laevis*: A critical period before first cleavage, identified by the common effects of cold, pressure and ultraviolet irradiation. *Dev. Biol. 99:* 75-87.

SMITH, R.C. and NEFF, A.W. (1985). Use of the fluorochrome propidium iodide for the identification of *Xenopus* germ plasm during immunofluorescence studies. *Anat. Rec. 213:* 518-519.

SMITH, R.C. and NEFF, A.W. (1986). Organisation of *Xenopus* egg cytoplasm: response to simulated microgravity. *J. Exp. Zool. 239:* 365-378.

TENCER, R. and GOLDFINGER, M. (1992). Autonomous mesoderm formation in blastocoelic roof explants from inverted *Xenopus* embryos. *Int. J. Dev. Biol. 36:* 115-122.

TILNEY, L.G. and PORTER, K.R. (1967). Studies on the microtubules in heliozoa. II. The effect of low temperature on these structures in the formation and maintenance of the axopodia. *J. Cell. Biol. 34:* 327-344.

VINCENT, J.P. and GERHART, J.C. (1987). Subcortical rotation in *Xenopus* eggs: an early step in embryonic axis specification. *Dev. Biol. 123:* 526-539.

WAKAHARA, M., NEFF, A.W. and MALACINSKI, G.M. (1985). Development of delayed gastrulae and permanent blastulae from inverted *Xenopus laevis* eggs. *Acta Embryol. Morphol. Exp. 6:* 193-209.

Accepted for publication: September 1992