EXPERIMENTAL BIOLOGY

Cleavage of Deformed Embryos of Green Toad

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Variability of the first cleavage furrow is examined in intact and deformed embryos in deformed ova of green toad obtained by an original method: a short-term arrest of spawning. A relationship is revealed between the incidence of violations of the second law of Hertwig and the degree of deformation of the embryo. The orientation of the first cleavage furrow is evidently determined in oogenesis.

Key Words: *deformation; ovum; embryo; furrow*.

Methods of deforming embryos are used in experimental embryology to solve problems of determination and differentiation, specifically, to elucidate the role of some endogenous and exogenous factors in the process of cleavage [1,7].

Early cleavage is notably disordered in deformed amphibian embryos. However, these disorders sort themselves out in the course of subsequent embryogenesis, and the tadpoles develop [1,5,6]. On the other hand, there is evidence that the orientation of the planes of blastomere and cell division plays an important role in certain types of biological morphogenesis. Above all it should be stressed that the first cleavage furrow in normal embryogenesis of the majority of amphibians outlines the plane of bilateral symmetry of the organism [2,4,8]. Moreover, we know that the orientation of the cleavage furrow determines the localization of blastomeres in the embryo, their relative size, and the quality of the cytoplasm, determining the fate of each blastomere [3,10].

The important role of the orientation of the first cleavage furrow in the morphogenesis of the embryo prompted us to investigate the variability of its orientation in embryos of different shape.

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MATERIALS AND METHODS

Experiments were carried out with embryos of green toad found in the Vladikavkaz region. An original method was used to obtain deformed embryos: a toad starting to spawn was exposed to mild stress by being placed in a cellophane bag, after which it was brought to the laboratory. Spawning was interrupted for some time but resumed after 2-3 h in an aquarium with water. After fixation in 5% formalin, morphometry of the embryos was carried out under an MBS-9 microscope. Each embryo was measured along the first furrow plane and then in the transverse direction (along the putative long axis of the cleavage spindle).

RESULTS

The mild stress arrests only the last stage of the spawning process — the release of eggs into the water. Transport of ova into the oviducts continues, and their number there is even higher than normally. In the oviducts the ova are subjected to pressure from the membrane and from each other. If the gametes in a spawn are arranged in a single row, they become disk-shaped; if they are arranged in 2 or 3 rows, they are pressed from different sides and their

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shapes vary greatly. If the ova in the egg mass cord are situated at a distance from each other and are subjected to pressure only from the cord walls, the gametes deposited last in each spawn are egg-shaped. We would emphasize that none of the types of deformations we observed prevented cleavage, but the deformations of the embryo persisted till the end of cleavage.

We examined the first cleavage division of embryos of different shape from the same egg mass: round (gametes laid during normal spawning), disklike, and egg-shaped. The results are presented in Fig. 1.

One of the reasons that analysis of the morphology of amphibian embryos during the first cleavage division is important is because this is the stage laying the foundation for the main morphological axes of the individual. As a rule, the plane of the first cleavage furrow in the majority of examined species of amphibians outlines the plane of bilateral symmetry of the organism and, together with the gray falx, the principal morphological axes. The orientation of the first furrow, in turn, conforms to Hertwig's second law [3], according to which the cleavage spindle is located along the longest stretch of cytoplasm free of yolk. On the other hand, we know that the cleavage furrow runs perpendicular to this direction in the middle of the cleavage spindle.

Our results in fact do not confirm this law. Out of the 50 specimens of intact embryos, this rule was validated only in 3 during cleavage by the first furrow, cleavage in 2 contradicted it, and in 45 specimens the length of the first furrow and the long axis of the cleavage spindle was the same. Out of 50 disklike deformed embryos the first cleavage furrow was positioned according to the rule (along the circumference of the disk) in only 4, whereas in the rest it was positioned contrary to the law — across the disk. Out of the 50 egg-shaped embryos 12 divided in accordance with Hertwig's law (across the long axis of the embryo), whereas the remaining 38 violated this law.

Hence, deformation of ova appreciably altered the quantitative ratios of embryos dividing by the first furrow according to Hertwig's law in comparison with spherical embryos. The degree of deformation of the embryos correlated with the number of embryos violating Hertwig's law in the following series: spherical, egg-shaped, and disklike. Hence, deformation of the ovum is a significant factor destabilizing the cleavage of toad embryos.

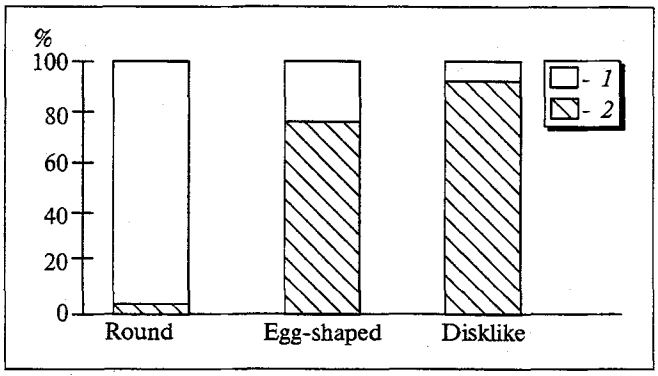

Fig. 1. Orientation of the first cleavage furrow in spherical and deformed embryos of green toad. *1*) the position of the first furrow conforms to Hertwig's law; *2*) the position of the first furrow contradicts Hertwig's law.

It should be noted that cleavage of some embryos culminated in 2 blastomeres of different size, a phenomenon observed in all the groups. This may result from displacement of the cleavage spindle under the pressure of the deforming yolk, or, in intact embryos, from variability of the yolk position in different ova.

These data suggest that the orientation of the first cleavage furrow in zygotes of green toad is not a function of cytoplasm length. It is possible that the orientation of the furrow is determined by processes in oogenesis and is connected with the bilateral symmetry of the ovum.

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