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Histochemical Detection of Biogenic Monoamines in Developing Amphibian Embryos in Health and during Exposure to a Static Magnetic Field

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UDC 591.31:593.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 12, pp. 652-654, December, 1993
Original article submitted May 17, 1993

Key Words: *biogenic monoamines; static magnetic field; amphibian*

The regulation of biological processes is still one of the greatest mysteries in biology. Embryologists long ago discovered a clear-cut relationship between certain successive morphological signs and processes unfolding with the appearance of these signs in the course of amphibian development. The following chain of events is considered: formation of an animal-vegetal axis in the ovum, site of spermatozoon penetration on the ovum surface, formation of a gray falx on the side of the ovum contralateral to this site, the plane of the first cleavage furrow determined by the above two structures, and bilateral symmetry and anteroposterior axis of the animals determined by the first cleavage furrow. It is evident that this chain of events has a direct bearing on the fundamental notion of the structural plan of an animal. The causes of these events, however, have not been adequately studied. The mechanism of orientation of the first cleavage furrow is one example. It is usually linked to the site where the sperm penetrates the ovum, that is, to a random event. At the same time, a magnetic field is known to have an effect on mitosis [2].

The first series of our experiments was devoted to assessment of the degree of randomness of the localization of the first cleavage furrow in zygotes of the Asia Minor frog (*Rana macrocnemius*) under different ecological conditions of the

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0007-4888/93/0012-1564$12.50

TABLE 1. Quantitative Characteristics of Various Directions of the First Cleavage Furrow in Three Populations of *Rana macrocnemius*

Site of observations	Number of embryos with specific furrow orientation			
	N-S	W-E	NE-SW	NW-SE
Kazbegi	390 (28.71%)	356 (26.17%)	318 (23.38%)	296 (21.76%)
Foothills	355 (22.05%)	362 (23.11%)	362 (23.11%)	488 (28.53%)
Kardzhin	86 (21.5%)	118 (29.51%)	92 (23.14%)	104 (26.10%)
Total, %	812 20.4%	936 20.74%	772 20.25%	888 20.59%

Note. N: North; E: East; S: South; W: West.

Northern slopes of the Central Caucasus. The incidence of cleavage furrow orientation in a certain direction in relation to the Earth's geomagnetic poles in a sample of zygotes was chosen as the criterion.

In the second series, using histochemical methods with toluidine blue, we detected in embryos of two amphibian species (*Rana ridibunda* and *Rana macrocnemius*) a specific metachromasia reaction indicating the presence of biogenic amines. γ-metachromasia predominated at the early developmental stages, β-chromasia to total monoamines at later stages.

According to current notions [1,3,4], biogenic monoamines appearing in mature unfertilized ova act as intracellular regulators at early stages and participate in morphogenetic processes at later ones. Use of the specific metachromasia reaction helped uncover new facts concerning the localization of biogenic amines and their functional role in the tested amphibians. It was of interest to check the suitability of histochemical methods for examining embryonal material and to refine, using these methods, the available data on the distribution of biogenic monoamines in Northern Caucasian amphibian embryos. The effects of a low-intensity (40-50 mT) static magnetic field were studied as well.

MATERIALS AND METHODS

Two amphibian species were used. Biogenic amines were detected using 0.1% toluidine blue at pH 5.6 and 6.0. The embryos were mounted on slides, their jelly coat was removed, and the pigment was bleached. A stain for the histochemical metachromasia reaction was then applied and the sample was incubated for 30 sec to 2 min at room tem-

TABLE 2. Metachromatic Reaction on Embryo Surface at Various Stages of Development of *Rana macrocnemius* collected at Kazbegi

№ of developmental stage	Whole embryo	Head portion	Branchial portion	Neural crests	Vitelline membrane
19	β	–	–	γ	γ
19	β	–	–	γ	γ
20	β	–	–	γ	γ
20	β	–	–	β	γ
20	β	–	–	β	γ
21	α–β	–	–	β	γ
21 1/2	β	γ	–	γ	γ
21 1/2	β	γ	–	γ	γ
21	β	β	–	γ	γ
21	β	–	–	γ	γ
21	β	–	–	–	γ
22	β–α	–	γ	γ	γ
22	β	–	γ	γ	γ
22	β–γ	–	γ	γ	γ
22	β	–	γ	γ	γ
22	β	–	γ	γ	γ
22	β–γ	–	γ	γ	γ
22	β	γ	–	γ	γ
22–24	β	γ	γ	γ	γ

TABLE 3. Metachromatic Reaction to Biogenic Monoamines in *Rana ridibunda* Embryos after exposure to a Hypogeomagnetic Field

Conditions	Developmental stage	Number of specimens	Metachromasia	Remarks
Hypogeomagnetic field	3	4	α–β	Bottom of furrows is of light blue
	4	21	α–β	Fine ectoplasm metachromasia
Dark control	3	9	α–β	Metachromasia of vegetal pole
	4	15	α–β	As above
Light control	3	6	α–β	Bottom of furrows is blue
	4	10	α–β	– animal hemisphere – vegetal hemisphere

perature. Control embryos were similarly treated but instead of the thiazine dyes they were stained with hematoxylin-eosin. The preparations were examined under an MBS-9 microscope, where the metachromasia reaction was assessed by the blue-crimson coloring.

A total of approximately 3400 zygotes of *R. macrocnemius* from three vertical topographic zones were examined. The embryos were examined under an MBS-1 microscope. For this purpose egg masses with embryos at the stage of the first cleavage furrow were selected and a wooden stick was inserted to show the directions of the North and South poles. Such an egg mass was examined under the microscope in a crystallizer. The results are summarized in Table 1.

The studies were carried out in the Caucasus at the Krestovii pass, in the vicinity of the villages of Kazbegi, Ursdon, Yuzhnii, and Kardzhin and the town of Vladikavkaz. These biotopes are located in various vertical zones of the northern slopes of the Central Caucasus that are characterized by different ecological conditions.

RESULTS

Preliminary analysis of our results indicated that the variability in cleavage furrow orientation did not correlate with the variability of ecological conditions in different vertical natural topographic zones. This suggested that cleavage furrow orientation was influenced by a factor that is not a main contributor to climate formation. We believe that the environmental magnetic field, consisting of the geomagnetic field and the local magnetic field, may be such a factor. Various rocks, soils, and natural solutions are known to generate their own magnetic fields, which are characterized by different magnetic features. There are, in addition, man-made magnetic fields. They may create different conditions in different biotopes, influencing the variability in the localization of the cleave furrow planes.

Histochemical studies have demonstrated an even distribution of biogenic monoamines in the ovum (Tables 2 and 3). Shifting of pigment to one of the poles results in a masking of a part of the specific metachromasia. The remainder of the ovum is characterized by bright metachromasia attesting to the presence of biogenic amines. Weak metachromasia is observed in some ova near the cleavage furrow. Two explanations of this phenomenon are possible: either the jelly coat is intact in the region of the cleavage furrow or biogenic monoamines have metabolized there as a result of the cleavage process.

Hence, a specific metachromasia reaction was observed at all examined stages of amphibian development, indicating the presence of biogenic monoamines.

Exposure to a static magnetic field of 40-50 mT induction for 3 h daily induced negligible changes in biogenic monoamine concentrations in amphibians at all stages of development. An exposure longer than 6 days led to an increase of the specific reaction to biogenic monoamines.

The reaction to biogenic monoamines may be explained by the amphibian organism's adaptation to new conditions in a magnetic field and may be interpreted as an adaptation syndrome.

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