EFFECTS OF MAGNETIC FIELDS ON EMBRYOGENIC DEVELOPMENT
OF THE NEWT Cynops pyrrhogaster

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Abstract

Embryogenesis of the newt Cynops pyrrhogaster was studied under very weak magnetic fields, eggs and embryos being placed in a magnetic shield room for few days at certain stage of development. They show high rate of abnormal development and death resulted from the change of magnetic environment. The gastrula-neurula stage is most sensitive to this change. For clear-cut space biology studies, magnetic environment should be taken into consideration of experimental configuration and operation.

1. Introduction

Many biological processes have evolved under the influence of surrounding environment. Space biology aims to clarify how deep biological phenomena are bound to their background. Space experiments are expected to be able to answer this problem from the universal standpoint. Major efforts of space biology have been focused on the effects of gravity and cosmic radiation on living organisms. This is based on the uniqueness of the two among other environmental factors of biosphere.

Since biological subjects usually react to many factors in complicated manners, design of control experiment is important to assure scientific significance of results. Magnetic fields is one of the factors which may dominate biological system. Effects of magnetic fields on life have not been much studied, even less being known than that of gravity. Steady geomagnetic force deflects heavy ionic components of cosmic radiation and form a shelter for a variety of organic substance and life evolved on earth. Strong magnetic fields more than 0.1 T have some biological effects (Refs. 1-10). Reproduction and growth of organisms are affected by reduced magnetic field (Refs. 6, 15-18). Some animals sense the earth magnetic field and utilize it as a compass for their movement (Refs. 11-13). In this study, effects of weak magnetic fields on embryogenic development of newt were investigated. Eggs and embryo of the newt Cynops pyrrhogaster were placed in a magnetic shield room for few days at certain stage of development. Ratio of abnormal development and survival rate after 14 days were compared to the control which was normally developed under geomagnetic field.

2. Materials and Method

Magnetic Shield Room

The magnetic shield room at Institute of Space and Astronautical Science, shown in Fig. 1, was used for this experiment. This facility was originally designed for the test of magnetic characteristics of spacecraft. It has a capability to shield geomagnetic field with an efficiency of $10^{-4}$ by three spherical layers of super permalloy. The room with 6 meters inner diameter has a flat floor to place experimental equipments. Strength of magnetic field strength was measured by a ring-core flux-gate type sensor (HM-10GR, Meisei Elec. Co. Ltd) which was placed close

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to experimental setup. Magnetic field strength was reported to be 5-7 nT (Ref. 19) at a wide range of position in the shield room. Access to the material during experiment was made through a small door. Penetration of magnetic flux through the door was measured to be less than 2 nT. Newt eggs and embryos were put into cases filled with water. These cases were stacked in two plastic containers which were placed at the center of the room and outside of it. Environmental factors such as ambient air, temperature and darkness for the two sets were kept identical except magnetic field.

**Newt**

Newt is a peculiar amphibian in the sense of fertilization of eggs. When a female newt copulates with a male, she stores the sperm in her body cavity. After oogenesis proceeds, matured eggs are fertilized with stored sperm. It is not necessary to mate for fertilization of egg. Even sperm stored in autumn can fertilize egg at next spring when adult female wakes from hibernation. This characteristic of newt provides an advantage of using newt as a subject in space experiment. Fertilized eggs of size about 2 mm can be obtained by a hormone treatment of female newts instead of mating. Mating behavior of animals is usually governed by many factors which are difficult to handle.

Development of fertilized egg proceeds through the several stages shown in Fig. 3. Number of days required from first cleavage of egg to tadpole stage is 20 days under typical conditions. Each stage of early development is characterized with the process of polarization and differentiation of cells, formation of organs and so on. These stages are the most dynamical ones in a whole life cycle. Because of high activity of organisms at early developmental stages, environmental factors affect biological processes to a great extent. Studies on early development will be one of good objects to understand interaction between biological phenomena and environment.
Experimental Procedures

Females of newt used in this experiment were collected from a river in Niigata prefecture just after mating season (May). For two days prior to experiment, gonadotropin with 50-100 IU was injected once a day to induce ovulation. Eggs and embryos produced were collected and divided into two identical sets of groups. Each group was specified according to the developmental stages. One set of groups was placed in the shield room and the other (control) on the floor outside. Eggs and embryos at several stages was exposed to weak magnetic field for 30 hours. These embryos were brought back to geomagnetic field after this exposure and were observed through a microscope for at least 15 days. In another experiment, hormone treated females were placed in the shield room. Eggs produced in the room were kept there for 7 days. Embryogenesis and survival rate were compared with control under geomagnetic field.

3. Results

A large number of embryos exposed to weak magnetic field for 30 hours at stages from gastrula (stage 12b) through neurula (stage 20) died because of abnormal development induced by the change of magnetic field. As shown in Table 1, a small number of embryos just before and after the stages also did not survive after exposure. No significant differences between experiment and control were observed for embryos at other stages up to just-before-hatching (stage 27).

In the second series of experiment, the great majority of embryos produced and cultured under weak magnetic field deteriorated during the course of development as shown in Table 2. Some of tadpoles grown after confinement in the shield room showed abnormal form of organs such as small leg and gill.
Table 1  Survival of Newt Embryos after Lying inside (Experimental) and outside (Control) a Magnetic Shield Room

<table>
<thead>
<tr>
<th>Group of Embryos</th>
<th>Percent Survival</th>
<th>Total Number Used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp./Cont.</td>
<td>Exp./Cont.</td>
</tr>
<tr>
<td><img src="image1" alt="Embryo" /></td>
<td>100/100</td>
<td>5/2</td>
</tr>
<tr>
<td><img src="image2" alt="Embryo" /></td>
<td>100/100</td>
<td>4/2</td>
</tr>
<tr>
<td><img src="image3" alt="Embryo" /></td>
<td>100/100</td>
<td>4/2</td>
</tr>
<tr>
<td><img src="image4" alt="Embryo" /></td>
<td>100/100</td>
<td>10/8</td>
</tr>
<tr>
<td><img src="image5" alt="Embryo" /></td>
<td>100/100</td>
<td>20/10</td>
</tr>
<tr>
<td><img src="image6" alt="Embryo" /></td>
<td>90/100</td>
<td>10/10</td>
</tr>
<tr>
<td><img src="image7" alt="Embryo" /></td>
<td>60/100</td>
<td>10/10</td>
</tr>
<tr>
<td><img src="image8" alt="Embryo" /></td>
<td>90/100</td>
<td>10/5</td>
</tr>
<tr>
<td><img src="image9" alt="Embryo" /></td>
<td>100/80</td>
<td>10/10</td>
</tr>
</tbody>
</table>

1) Embryos shown at left lay inside the magnetic shield room for 30 hours. In normal development, embryos grow to the stage shown at right after this period. 2) based on observation made at 15 days after the exposure.
Room temperature: ca. 23°C

Table 2  Survival of Embryos Produced by Newts inside (Experimental) and outside (Control) the Magnetic Shield Room

<table>
<thead>
<tr>
<th>Percent Survival</th>
<th>Total Number Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp./Cont.</td>
<td>Exp./Cont.</td>
</tr>
<tr>
<td>2nd day</td>
<td>11th day</td>
</tr>
<tr>
<td>12/63</td>
<td>12/35</td>
</tr>
<tr>
<td></td>
<td>58/80</td>
</tr>
</tbody>
</table>

1) Experimental embryos were produced by adult female newts laid inside the magnetic shield room during the period of confinement (141 hrs.), and control embryos by adults outside it during the same period. 2) based on observations made on the 2nd and 11th days after the end of the magnetic shield room confinement experiment.
Room temperature: ca. 23°C.
4. Discussion

Exposure to weak magnetic field of 5-7 nT disturbs embryogenesis of newt. Geomagnetic field regulates normal development of embryo. Abnormal development is caused by weak magnetic fields or change of field strength. The most severe damages occur at the stage of gastrula and neurula. These stages are characterized by biological activities to form primitive organs. During dynamical phase of processes such as active cell cycle or differentiation, biological system may perceive environmental factors even those changes are minor. Biological processes are based on biochemical reaction network in organisms. Small perturbations are amplified through a complicated sequence of reactions. Abnormality of embryogenesis in weak magnetic field is one of phenomena which should be understood in this context.

Interaction of biomolecules with magnetic field can be classified into several types. Relaxation processes which follow changes of environment may also relate to the phenomena observed. Magnetism is an essential physical property of molecules in these interaction. Radical forms of molecules could be formed as intermediate species in many biochemical reactions. Magnetic field affect energy states or other physiochemical properties and hence reaction rates and scheme of radicals. Anisotropy of magnetism originates conformation or orientation of molecules in magnetic fields which relate to biological processes. In most of cases, these interactions have been ignored to be small at a level of geomagnetic field strength. In other word, randomization by thermal energy hides the effects of orderly configuration induced by geomagnetic force. Those disorder and order states should be evaluated with proper characteristics of biomolecules and reaction fields for biosynthesis which concern to specific process in organisms.

Many environmental factors including gravity, magnetic field and radiation affect biological phenomena through their influences on elementary pathways. Each factor often interacts with others. There are conjugated interaction, competitive influences and more complicated fashion of interferences. From this fact, well designed control experiment is necessary to analyze biological effects of each environmental factor and mechanisms behind. Biological experiments carried on Spacelab or future space station adopt onboard control experiment of 1 G reference using centrifuges. With this concept, environment except gravity are assumed to be same for experiment and in-flight control. For biological systems which might be influenced by heavy cosmic radiation, tracks of HZE particles are monitored to gives informations about which cells are hit or not. Matrix of data, from (0 G - hit) to (1 G - no hit) in this case, indicates the effects of each factor and the interaction between the two. But configuration of onboard 1 G centrifuge for control experiment does not take biological effects of magnetic field into account. There are artificial magnetic field produced by electric motors and other sources. Orbital attitude of spacecraft may give different magnetic environment for onboard experiment, in-flight control on rotating centrifuges and ground control experiments, respectively.

In the case of newt embryogenesis, experimental results show the existence of some biological processes which are affected by magnetic fields. Other biological systems, especially in development biology, may have similar interaction with magnetic field. Possible effects of magnetic field on biological subjects should be considered in the design of control experiment. Ground experiments which simulate magnetic environment in space are strongly suggested to be performed for evaluation of its effects.

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References