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Effect of Weightlessness on the Development of the Nervous System and Peripheral Analyzers in *Xenopus laevis* Larvae

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The study was carried out within the framework of a Russian-Canadian experiment aboard the Bion-10 satellite. The volume and surface area of the gray and white matter and ventricles of the brain, retina, olfactory placodes, the VIII nerve ganglia, and vascular plexus were measured in *Xenopus laevis* which had been in a state of weightlessness for 2 weeks since their hatching. Zero gravity was found to stimulate the growth of nerve processes, to increase the surface of the vascular plexus, and to impede the development of the retina, olfactory placodes, and VIII nerve ganglia.

Key Words: *weightlessness; nervous system; peripheral analyzers; Xenopus laevis*

Human and animal organisms adapt to microgravitational conditions during long space flights. Adaptation involves primarily changes in the circulation and in mineral and tissue metabolism [1,3,6], but the adaptive changes of fully formed organisms differ radically from the adaptation of embryos, fetuses, and larvae of vertebrates. There are several reasons for this. Development is associated with active cell proliferation and the formation of rudiments of organs and systems. The instability of these processes is well known and is responsible for many congenital diseases and developmental abnormalities. On the other hand, mineral and tissue metabolism is more labile in a growing organism than in an adult. This imparts specific features to the mechanisms of adaptation to zero gravity.

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The informative value of experiments investigating the development of vertebrates under conditions of weightlessness is obvious, but poses quite a number of problems as well. Mammalian embryos are hardly fit for this purpose because of the intrauterine development of warmblooded animals. The effects of a maternal organism are hard to differentiate from the direct effects of weightlessness on the embryo. That is why autonomously developing amphibian embryos appear to be promising research objects, for the direct influence of weightlessness on their development can be detected.

The present research was carried out within the framework of the joint Russian-Canadian experiment *Development* devoted to a comprehensive study of the morphogenesis of *Xenopus laevis* larvae. In this study we investigated the effect of an 11-day stay under conditions of weightlessness on the development of the nervous system, peripheral analyzers, and vascular plexus of the brain. Hatch-

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ing of larvae and their development took place under conditions of microgravity.

MATERIALS AND METHODS

A total of 156 embryos of *Xenopus laevis* obtained after stimulation of adult animals with chorionic gonadotropin were used in the experiments. Fifty-three experimental embryos were placed in a 3-liter Plexiglas container half-filled with aerated water at 21.5°C; at this time the animals were at development stages 6.5-7 according to Nieuwkoop [5]. The container with the animals was taken on board the satellite, which was launched 40 h later. During the flight the animals were not fed, but consumed the microorganisms multiplying in the container. The duration of their stay on board the space ship was 11.5 days. During the last 2 days of the experiment, the temperature on board rose from 22 to 26°C. Directly after returning to Earth, the animals were sacrificed and fixed in 4% buffered neutral formaldehyde. A control group consisting of 53 animals similarly treated had been left on Earth. The material was embedded in paraffin, and serial slices were stained after Mallory or with hematoxylin-eosin. A microprojector was used to make graphic reconstructions from serial slices of larval head at ×250 magnification. Every other slice of the total brain was examined. Each slice was measured to estimate the volume and surface area of the examined structures. The volumes of the gray and white matter and ventricles of the brain, retina, olfactory placodes, VIII nerve ganglion, and vascular plexus were measured (Fig. 1). The areas of the surface of the brain and vascular plexus were estimated. Changes in the symmetry of the brain and peripheral analyzers were taken into account. Such an approach helped assess relatively small quantitative differences with an instrumental error of no more than 2-3%. Statistical analysis for paired data was performed using Wilcoxon's test [4].

RESULTS

After landing, 48 live larvae were found in the container; in the control, there were 52 larvae. Synchronous examination of these animals showed that the development of the brain and peripheral analyzer changes under conditions of weightlessness. The total volume of the brain, including the ventricles and gray and white matter was 7% greater in the animals which had undergone the space flight than in the control. Similar differences were observed as regards the surface area of the total brain. After the flight the surface area of the brain was only 5% greater than in the control. Hence, exposure to zero gravity causes no crucial changes in the rate of development of the brain. Nonetheless, we did find variations in the differentiation and development of the ventricles. The volume of gray matter - neuron bodies - was the same in both groups. The volume of white matter - neuron processes - was 30% greater in the group exposed to space flight. The increase in the volume of white matter seemed to be due to a decrease in the volume of the brain ventricles, which was somewhat smaller in the animals which had made the flight than in the control, although the differences were unreliable. Hence, we may conclude that nerve cell processes form more actively under conditions of weightlessness.

Special attention should be paid to the changes in the size of the vascular plexus resulting from the redistribution of the liquids and blood flow in the animal and human organism under conditions of weightlessness. The blood and plasma become redistributed in favor of the upper part of the body (head) at the expense of the lower limbs and pelvis. We may thus expect a pressure increase in the cerebral arteries to step up the rate of plasma filtration through the vascular plexus. In such a case, the surface area of the vascular plexus should be less in the experimental group than in the control. However, the volume of the vascular plexus differed unreliably, whereas its surface area measured inside the cerebral ventricles was 34% greater in the group exposed to the flight ($p<0.05$). These results are understandable if we consider the basic components regulating the flow of the cerebrospinal fluid (CSF).

Since the CSF exchange depends on the size of the vascular plexus, ventricles, and surface area of the meninges (brain surface), we examined the ratio between these structures. In the control the ratio of the surface area of the vascular plexus, ventricular volume, and brain surface is 2:1.5:82, whereas in the experimental (flight) group it is 2:1:66. These ratios suggest two possibilities. Either the permeability of the vascular plexus decreases or the rate of CSF discharge through the venous sinus increases, with the permeability remaining unchanged. No matter what the causes are, the increase of the surface of the cerebral vascular plexus may be regarded as an adaptation of the developing brain to weightlessness.

Quantitative assessment of the peripheral analyzers showed that in weightlessness the volumes of the retina and VIII nerve ganglion are 60 and 22% lower, respectively, than in the control. The dif-

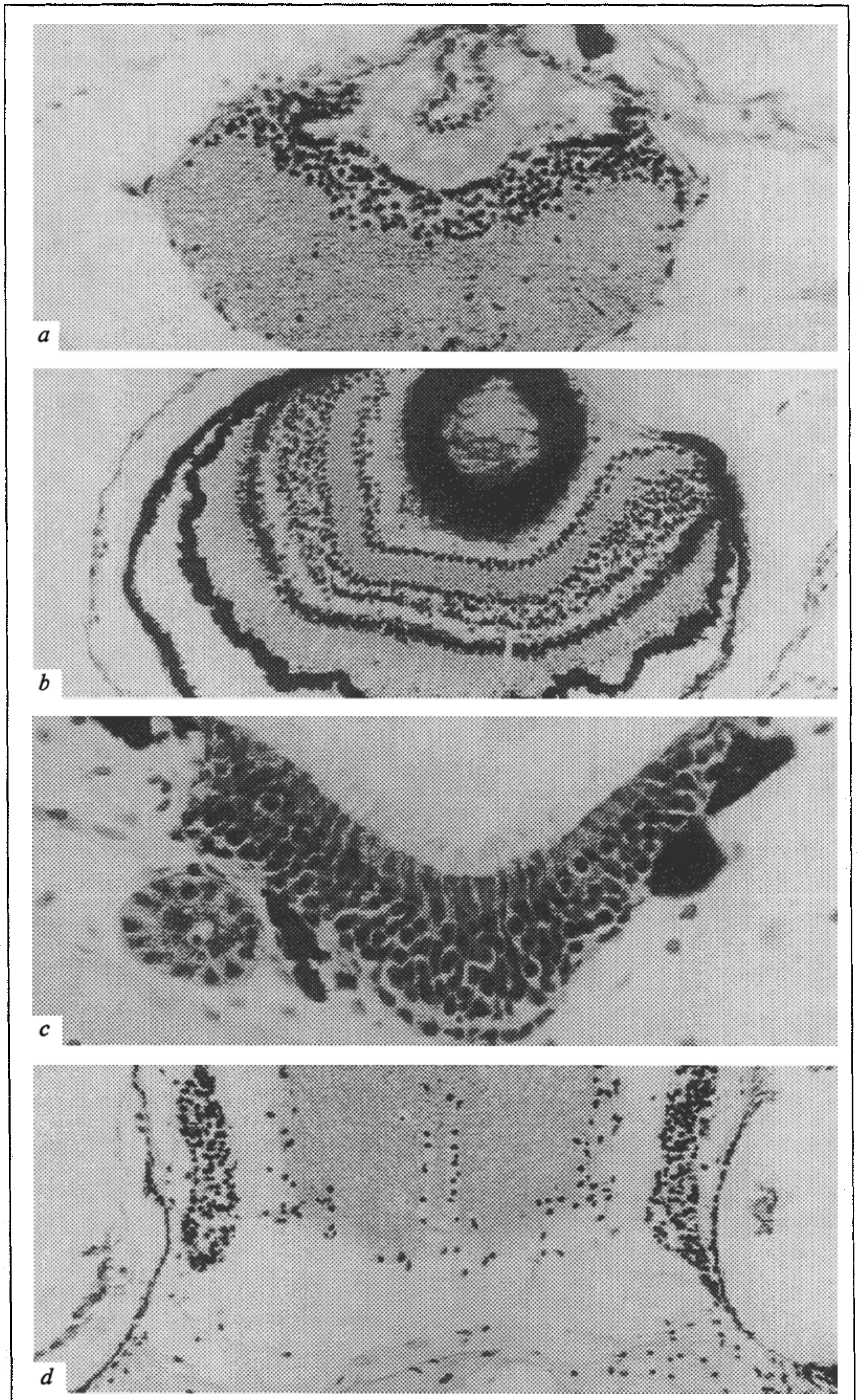

Fig. 1. Microphotographs of slices of *Xenopus laevis* larvae used to investigate quantitative changes in the development of the brain and peripheral analyzers under conditions of weightlessness. Staining after Mallory. ×180. *a*) frontal section through the diencephalon and vascular plexus; *b*) horizontal section through the retina; *c*) horizontal section of the olfactory organ; *d*) horizontal section through the VIII nerve ganglion.

ferences for the VIII nerve ganglion are easily explained. Since this ganglion is related to the vestibular and otolithic system of the inner ear, changes of afferent signals under conditions of weightlessness could not but have an effect on its morphological development. The volume of the olfactory placode decreased by 17% in comparison with the control. This value surpasses the individual variability 1.4 times. It is noteworthy that the individual variability of the olfactory system in flight is 1.3 times higher than in the control. The general reduction of the parameters of the olfactory system appears to be due to the formation of the vomeronasal organ. In weightlessness, the rudiments of the vomeronasal system develop atypically, without the characteristic cavity but with numerous epithelial processes. These data require further analysis, because the causes of the appreciable delay in the development of the eyes and olfactory placodes are not yet quite clear. After landing, the adenohypophysis retains a solid structure and consists of a dense mass of epithelial cells. Its size is within the normal range. No changes in the structure of the adenohypophysis and its interactions with the brain were detected in the experimental animals subjected to space flight.

The brain and peripheral analyzers were studied on the right and left sides of the body in both groups of larvae in order to elucidate the effect of gravity on the symmetry of development of the nervous system in amphibians. Our study failed to point up any appreciable differences between the control and experimental groups. In both groups the nervous system elements situated on the right side of the body were 2-9% larger in volume and surface area than those on the left side. These results indicate that exogenous gravitational effects on the brain (or the absence of such effects) do not influence the symmetry of nervous system development.

Hence, our study permits us to draw several conclusions. Neuronal differentiation is stimulated under conditions of weightlessness, this leading to an increase in the volume of axons and dendrites in the nervous system. No changes in the structure of the adenohypophysis or in its interactions with the brain were revealed. Individual variability of the nervous system increases under conditions of microgravity. The permeability of the vascular plexus decreases, this leading to an increase of its surface area; the development of the retina, olfactory placodes, and VIII nerve ganglion are delayed. Finally, endogenous regulation of the symmetry of brain development has been revealed which does not depend on the presence or absence of gravity.

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