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THE DEVELOPMENT OF THE VESTIBULAR APPARATUS UNDER CONDITIONS OF WEIGHTLESSNESS

Ya.A. Vinnikov, O.G. Gazenko, D.V. Lychakov, L.R. Pal'mbakh

Translation of "Razvitiye vestibulyarnogo apparata v usloviyakh nevesomosti", Zhurnal obshchey biologii Vol. 44, No. 2, 1983, pp 147-163.

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16. Abstract

A series of experiments has been carried out on the effect of space flight conditions on morphogenesis and the structure of the vestibular apparatus in amphibian and fish larvae. Larval development proceeded in weightlessness without serious morphological defects. The vestibular apparatus developed; its organization in the experimental animals did not differ qualitatively from that in the controls. The specific external stimulus (gravitation) appears not to be a necessary condition for the development of a gravitation receptor in ontogenesis although the appearance of the vestibular apparatus in phylogenesis was apparently related to this stimulus.

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THE DEVELOPMENT OF THE VESTIBULAR APPARATUS UNDER CONDITIONS OF WEIGHTLESSNESS

Ya.A. Vinnikov, O.G. Gazenko, D.V. Lychakov, L.R. Pal'mbakh

As is well known, the development of the structure and function /147* of the vestibular apparatus in vertebrates precedes the appearance of other sensory organs. We suggest that this is related to the fact that the embryo may very early occupy a determined place with respect to the earth's gravitational field. This phenomenon is demonstrated very clearly when we observe the disturbance of chick embryos at various stages of incubation. The question arises of whether the development of the vestibular apparatus is subject to the effect of the earth's gravitational field or whether it is genetically programmed and its morphogenic development unaffected by a specific stimulus. We should note, however, that in phylogenesis the organ may appear only in connection with the effect of a gravitational force. We obtained an answer to this question during an experimental study of the development of fish and amphibian embryos under the conditions of weightlessness on space vehicles. If we set up the fish and amphibian embryos at the stage of the gastrula, neurula or even tail bud (amphibians), then under conditions of weightlessness, according to our data, normal development of the embryos as a whole, and of their tissues and organs, including the vestibular apparatus, will occur. The experiments which we have conducted [Vinnikov et al., 1972, 1976, 1979, 1980; Vinnikov, 1974; Vinnikov et al., 1976], the results of which are shown in the table, allowed us to affirm that the development of this organ is not related to the effect of the earth's gravitational field. Stimulation with gravity is possible only when fish are returned to earth. However, in order to reach this conclusion, it was necessary for us to run a set of complex experiments and investigations, to the results of which this article is devoted.

* Numbers in the margin indicate pagination in the foreign text.

EXPERIMENTS DONE IN WEIGHTLESSNESS TO STUDY THE DEVELOPMENT OF THE VESTIBULAR APPARATUS IN FISH AND AMPHIBIANS

Vehicle	Year	Object	Length of development of embryos in weightlessness, days	Fixing site	Stage of development of embryos when the space vehicle starts
Soyuz-10	1971	Rana temporaria	2	In weightlessness	Blastula - early gastrula
Salyut-4 - Soyuz-17	1975	Xenopus laevis	16	"	tail bud
Salyut-6 - Soyuz-26	1977	the same	20		Early tail bud
Salyut-6 - Soyuz-36	1980	"	8	2nd day after landing	Middle neurula
Salyut-6 - Soyuz-39	1981	"	9	the same	Early blastula
Salyut-6 - Soyuz-40	1981	"	8	"	Tail bud
Soyuz-16	1974	Brachydanio rerio	6	In weightlessness	5 somites
Salyut-5 - Soyuz-21	1976	the same	9	"	Late gastrula
Soyuz-22	1976	"	8	2nd day after landing	Middle gastrula

The Development of the Vestibular Apparatus in Amphibians

As is well known, the vestibular apparatus (labyrinth) in amphibians is established in the ectoderm at the late neurula stage under the inducing effect of the underlying mesoderm and the presumptive medulla oblongata [Kogan, 1944; Ginzburg, 1946]. In experimental embryology it has already been known for a long time on the basis of experiments involving centrifuging of fertilized eggs that prolonged

and considerable acceleration exerts a negative effect on their development. Short and weak accelerations usually do not affect further embryogenesis. This in particular follows also from the experiments of Young and Tremor [Young, Tremor, 1968] in which the eggs of frog Rana pipiens were placed 12 hours after fertilization in the flight apparatus Bios-2. After brief acceleration during the ascent /148
of the apparatus and 44 hours under conditions of weightlessness, as in vivo observations and microscopic investigations showed, the eggs reached the neurula stage, the leaves primordium of which had a normal structure. From the embryos left alive mobile tadpoles and then frogs developed. However, since in these experiments the embryos were kept under the influence of weightlessness prior to organogenesis, the question of the effect of weightlessness on the formation of the presumptive foundations of the organs and their development as well as on further embryogenesis remains essentially open.

In order to study the development of the vestibular apparatus under conditions of weightlessness without the influence of any other additional factors, our first experiment on Soyuz-10 [Vinnikov et al., 1972] was planned in such a way that the accelerations during the ascent of the flight apparatus into space occurred at the stage of embryogenesis at which the foundation of the vestibular apparatus as well as of the other organs was still absent. As the object fertilized eggs of the frog Rana temporaria at the blastula and early gastrula stages were selected. In order to create the physiological conditions for the development of the eggs we constructed a special device, an EMKON container (Fig. 1) in which the eggs were kept throughout the experiment. At the same time this device made it possible to fix the developing eggs under conditions of weightlessness at any stage of their development. The embryos which developed under conditions of weightlessness were fixed at the end of the 2nd day of flight, on the 4th day after fertilization.

Examination of the material obtained showed that the embryos which developed under conditions of weightlessness as well as the controls were at the early tail bud stage at the time of fixation

Fig. 1. The general form of EMKON containers of various types.

(Fig. 2). In the head sections of the embryos in both the control and experimental groups we were able to detect with the light microscope the auditory vesicles on both sides of the medulla oblongata. They were filled with endolymph and had an oval shape. The auditory vesicles were at the stage of formation of the macula communis. /149 The latter is a thickening of the central part of the wall of the vesicle, a single embryo of all of the future receptor structures in the inner ear [Titova, 1968]. The embryo of the 8th ganglion is adjacent to the medial part of the auditory vesicle in the region of the macula communis (Fig. 3).

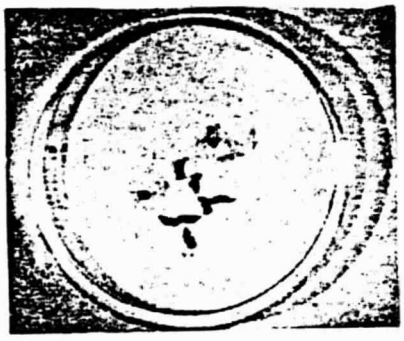

Fig. 2. Embryos of Rana temporaria at the tail bud stage which developed on board Soyuz-10.

Under the light microscope the cells of the wall of the auditory vesicle appeared undifferentiated, their cytoplasm, densely filled with yolk grains and pigment granules, forming a lumen only in the area of the nucleus. In the equatorial sections of the wall of the auditory vesicle in the area of the macula communis we detected a multinuclear epithelium, while the basic part of the wall of the vesicle consisted of a mononuclear epithelium (Fig. 3). The embryo of the 8th ganglion has the form of an oblong strand with its wide base adjacent to the base of the epithelium of the auditory vesicle and its narrow end in the direction of the developing brain. The cells of the ganglion are also rich in yolk and pigment granules but remain transparent in the area of the nucleus. The wall of the auditory vesicle is separated from the ganglion by a distinct basal membrane and a small lumen, which, as we see under the light microscope is free of any kind of fibers or cellular processes. Thus, under the light microscope at the stage studied we were not able to find substantial histological or cytological differences between the auditory vesicles in the

control and experimental embryos [Vinnikov et al., 1972].

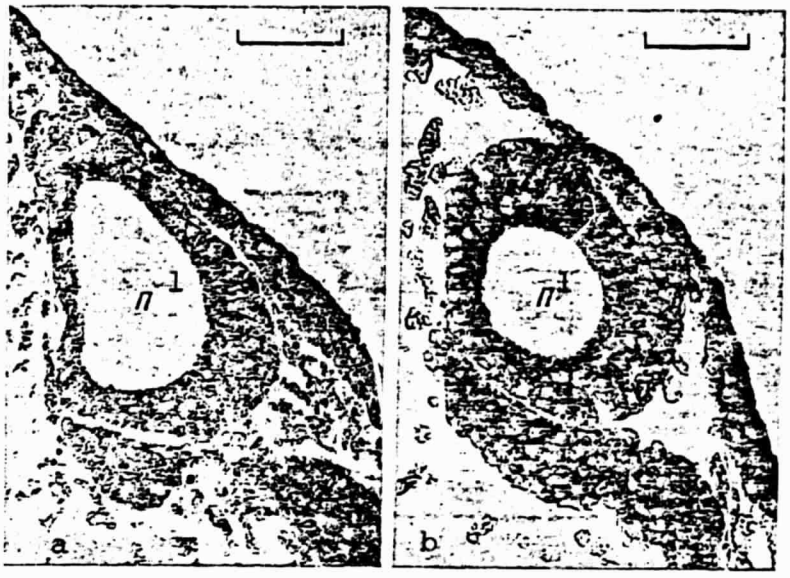

Fig. 3. The auditory vesicle in Rana temporaria at the tail bud stage. a-control, b-experiment. The cavity of the auditory vesicle (C) Scale 20 μm.
Key: 1-C.

In electron diffraction patterns the structural organization of the auditory vesicles in the control and experimental animals shows several analogous and essential details which naturally are not visible under the light microscope. The majority of cells in the auditory vesicle are distinguished by their large sizes and irregular cylindrical or pyramidal forms. They are filled with yolk grains of various sizes and irregular oval form with very high electron densities (Fig. 4). The grains of the yolk are more or less evenly distributed through the cell. Several yolk grains appear at the resorption stage. Between the yolk grains are numerous small pigment granules which also, as a rule, are distinguished by high electron density. However, some of them are paler; this evidently reflects the different stages of their development. The mitochondria are small and have round or oval forms with weak cristas and dark matrices. In some of the mitochondria we observed crystals with high electron

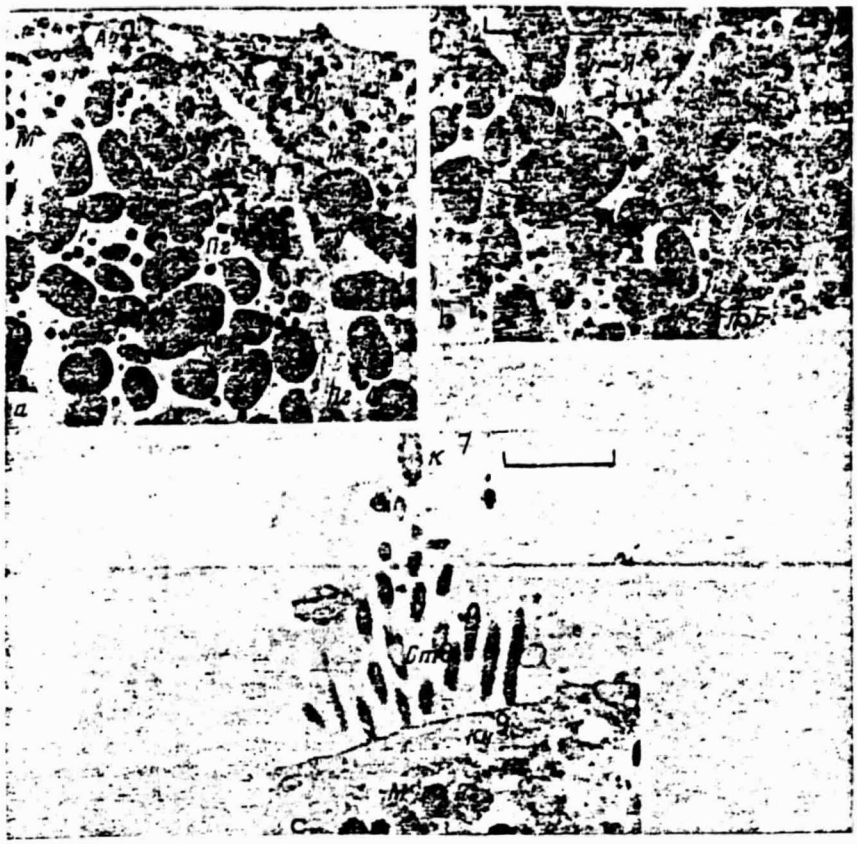

Fig. 4. Section of the epithelial wall of the auditory vesicle of Rana temporaria at the tail bud stage (experiment). a,b- undifferentiated embryonic cell. Scale 5 μm. apex of the undifferentiated embryonic cell with developing kinocilia (c). Scale 1 μm. The apical part of the cell (Ap), the basal part of the cell (Bp), yolk granules (Y), mitochondria (M), pigment granules (Pg), lysosomes (L), nucleus (N), kinocilium (K) stereocilia (St), cuticule (Cu).
Key: 1-Ap; 2-Bp; 3-Y; 4-Pg; 5-L; 6-N; 7-K; 8-St; 9-Cu.

densities. Single lysosomes may get into the loci. The embryonic cells are covered with a pronounced plasmatic membrane which at the points of contact with the neighboring elements form indistinct desmosomes which alternate with a zonula occludens and wide intercellular grooves. The membranes of the endoplasmatic net are indistinct. At all of the loci we see membrane fragments dotted with ribosomes and elements of the Golgi apparatus. The cell nucleus is usually of /150

moderate dimensions and has an irregular rounded form. It is covered by a double porous membrane, while the outer membrane may form a series of storages, converting the loci into cisterns of the endoplasmatic network. The karyoplasm is distinguished by a fine grained structure. In it we can see numerous large grains of chromatin which have a tendency to occupy the boundary position on the interior surface of the nuclear membrane. The large nucleolus occupies the central position and in its form recalls a mulberry with uneven electron density. Along with such essentially undifferentiated embryonic cells in the wall of the auditory vesicle in both the control and experimental animals in the area of the macula communis, we observe individual elements with clear signs of the beginning of cytological differentiation. Among these are evidently embryonic differentiating receptor cells (Fig. 4). Differentiation is expressed in the liberation of the apex of such a cell from the yolk grains and pigment granules. Instead of them, long, slightly coiled mitochondria with numerous cristas and a light matrix fill the cytoplasm. In this apical part of the cell is a centrosome, near to which we find a basal corpuscle with a stem. From the basal corpuscle to the interior of the cytoplasm may run a radicle with a clear period of striation or individual microtubules. The basal corpuscle, equipped with a stem with a radicle or microtubules, forms the beginning of the developing kinocilium which contains nine pairs of peripheral and two central fibrils. The central fibrils begin from the high basal membrane. The kinocilium is covered with the extension of the plasmatic membrane. Along with the embryonic cells in which one kinocilium has developed, we observe cells which are also distinguished by the beginning of the development of stereocilia. They develop from microvilli which are somewhat recessed from the kinocilium. Thus, the polar distribution of the beam of the stereocilia with respect to the kinocilium occurs at the moment of their differentiation. The stereocilia are covered by the extension of the plasmatic membrane of the cell. Inside the /151
stereocilia we observe thin fibrils which penetrate into the apical cytoplasm of the cell in the form of a constricted radicle. These fibrils, as it turned out, are actinic fibers which are connected in the cuticle to the myosin ring [Flock et al., 1981]. The apical

part of the cell reveals the beginning of the development of the cuticle which consists of a finely dispersed substance.

Thus, in the stages studied in both the control and the experiment in the area of the macula communis we observe the beginning of cytological differentiation of individual embryonic cells which, judging by the kinocilium and stereocilia which develop at their apex, are the future receptor cells of a definitive vestibular apparatus. The processes of differentiation begin in the apical part of the cell. In the nuclear-cytoplasmatic area of such cells no noticeable structural shifts were observed.

We must note that at these stages in the area of the macula communis in both the control and the experiment we observed a second type of differentiating cell which evidently forms the support elements [Vinnikov et al., 1972]. At first we see differentiation in these cells also at the apex. Differentiation is also manifested in the removal of the yolk and pigment granules in the depth of the cell body and the appearance in this area of the cytoplasm of numerous rounded mitochondria. However, the most characteristic feature of the differentiation of such cells is the appearance of a large number of basal corpuscles with developing cilia containing nine /152
pairs of peripheral and two central fibrils going from them into the chamber of the auditory vesicle. Along with the cilia we also observe numerous microvilli. Since flame cells are absent in the definitive vestibular apparatus of amphibians, and in the support cells only one kinocilium-like short rod is preserved, we may think that in the process of further development the remaining cilia of such a cell are subject to reduction.

Several words on the stage of development of the 8th ganglion. In both the control and experimental material by means of an electron microscope we were able to trace the beginning of large pear shaped cells covered by a clearly pronounced plasmatic membrane and filled with large yolk grains and small pigment granules (Fig. 5). In the cytoplasm of such cells we observed rounded or rod shaped

Fig. 5. The neuroblast of the 8th ganglion (experiment). The opposite poles of the neuroblast (P_1-P_2), the developing nerve process (N), yolk granules (Y) nucleus (Nu). Scale 5 μm.
Key: 1-P_1-P_2; 2-N; 3-Y; 4-Nu.

mitochondria. The nucleus is situated in the narrow part of the cell and has an irregular rounded form. It is covered with a double porous membrane and filled with small and large grained chromatin. At the opposite poles of such cells we observe plasmatic growths which are free of yolk and pigment and which evidently correspond to the dendrite and neurite. The dendritic process is pointed towards the basal membrane of the auditory vesicle in the area of the macula communis, and the neurite is pointed towards the medulla oblongata. As is well known the neurons of the 8th ganglion are bipolar and have mixed origin, developing both from the auditory vesicle and from the neuroblasts which migrate from the medulla oblongata. Consequently, at these stages in both the control and the experiment we observe only the beginning of differentiation of the bipolar neuroblasts of the 8th ganglion.

In the control embryos in one case in the basal area of the differentiating receptor cells between their base and basal membrane we observed fairly numerous nerve fibers of various sizes which intersect in both the longitudinal and transverse directions. These fibers are evidently the preliminary dendritic processes of the neuroblasts of the 8th ganglion which penetrate through the basal membrane into the wall of the auditory vesicle. The fibers are distinguished by a dark axoplasm which is filled with numerous mitochondria. There are no yolk grains or pigment granules in them. No synaptic structures between the embryonic receptor cells and the nerve fibers going to their base were found. Thus, at the examined stage of development of the auditory vesicle in Rana temporaria in one of the embryos we

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were able to see the beginning of the approach of the afferent fibers to the future receptor cells. We were not able to see this at the same stages in the experimental embryos. We should note that the approach of the nerve fibers to the basal part of the cell does not accelerate the process of its differentiation. This is demonstrated by the similarity of the description of the beginning of differentiation in the apical region of the embryonic receptor cells in the control and experimental animals.

The factual material obtained showed that under conditions in which a fertilized egg was kept for 2 days and then the embryo of the frog Rana temporaria was kept for 2 days under terrestrial conditions and then for two days in weightlessness, the development of the embryo in the EMKON container was not delayed. In its external appearance such an embryo practically is indistinguishable from the 4-day old control which remained for the entire time under conditions of the unchanging gravitational field of the earth. The embryo (control and experimental) reached the tail bud stage of development. The embryo passed through the first stages of division and the subsequent stages of the blastula and early gastrula under terrestrial conditions. Then, at the gastrula stage the embryo, which had undergone the effect of acceleration during the ascent, continued to develop for 40 hours under conditions of weightlessness, where it passed through the neurula stage and then into the tail bud stage, at which point it was fixed. Judging from the results obtained, the last two stages occurred under conditions of weightlessness as they did under conditions of the earth's gravitational field. If there were any deviations associated with the effect of short term acceleration and vibration, they were corrected in subsequent development under conditions of weightlessness. As is well known, at these stages amphibian embryos are distinguished by a broad range of regulation. Judging from the structure of the auditory vesicles which developed under conditions of weightlessness, they did not differ in any way from the controls; there is no doubt that their foundation, induction and formation which are related to immersion and subsequent shrinkage from the ectoderm under conditions of weightlessness, occurred as in the control.

In the set up of the experiment it was envisioned that the embryo be subjected to accelerations at the moment of launch of the space vehicle at the early gastrula stage when the foundation of the future vestibular apparatus is still lacking. This made it possible to prevent the acceleration from affecting the development of the auditory vesicle which, as our experiments on the definitive vestibular apparatus have shown, may affect the structural, cytochemical and functional organization of the receptor cells [Vinnikov, 1974].

Under conditions of weightlessness, not only the formation of the auditory vesicle, but also the beginning of the process of organ differentiation (the macula communis is established and the embryo of the 8th ganglion is formed) occurs. At the same time, in individual sections of the macula communis the processes of cytological differentiation begin; these are manifested in the beginning of the formation of individual receptor and support cells. It is basically the apices of these cells which differentiate; this precedes, as was already noted, the freeing of the apical part of the cell from yolk and pigment granules and the concentration of mitochondria at this locus. This indicates an increase in the energy processes in the differentiating parts of the cell. Evidently, at the later stages of embryogenesis, during division of the macula communis, these cells get into the loci of the future utricular, saccular and lagenal maculas and cristas of the ampoules of the semicircular canals and are converted /154 into the definitive receptor cells.

In the embryonic 8th ganglion in both the control and the experiment cytological differentiation is manifested at the beginning of the formation of pear shaped bipolar neuroblasts with two cellular processes in a polar arrangement. In one of the control embryos we noted the penetration of these processes, i.e. the future nerve fibers from the 8th ganglion, into the epithelium of the auditory vesicle. This fact may have several explanations. In the first place, in the other embryos we were not able to find them, which is highly possible when working with ultrathin sections. In the second place, which is more probable, this may be explained by the circumstance that, as is well

known, at the early stages of embryogenesis in embryos in a single clutch which develop under constant conditions we always observe some asynchronicity in development. Finally, in the third place, this may be explained by the fact that because of circumstances out of our control the control material was fixed 4 hours later than the experimental. In all it seems to us that this single deviation is not of fundamental importance. It does not hinder the basic conclusion of our investigation, that when frog embryos are kept beginning with the gastrula stage for 2 days in an EMKON container in weightlessness on a flight apparatus their development practically does not differ from that of control embryos [Vinnikov et al., 1972].

Thus, conditions of weightlessness practically do not affect the development of frog embryos at the neurula or tail bud stages, or organ formation or the beginning of cytological development, including that of the future vestibular apparatus, which accompany these stages.

In order to answer the question of the effect of conditions of weightlessness on later stages of embryonic development in amphibians and their transformation into tadpoles, we did a series of experiments on the orbital space stations Salyut-4 and Salyut-6 with eggs of the frog Xenopus laevis which had been fertilized on earth [Vinnikov et al., 1976, 1980]. Until the launch of the space vehicle the embryos were at the early blastula, middle neurula, early tail bud and tail bud stages (Table). Development occurred in EMKON biocontainers (Fig. 1) in a special BIOTERM-4 thermostat at 15°C. In some of the experiments fixation was done on board the space station during space flight while in other experiments the larvae were returned alive to earth and processed in the laboratory. We found that the development of the embryos and hatching of the larvae occurred on the whole normally under conditions of weightlessness and that the developing tadpoles moved actively in the EMKON containers both during the flight and after the space vehicle landed on earth. We should note, however, that the nature of their movement under

conditions of weightlessness differs somewhat from their usual behavior: the animals reveal an unusual "spinning" movement.

The vestibular apparatus of amphibians, as of other animals, has a complex spatial configuration. In order to decipher its structure, in an experiment on the orbital complexes Salyut-6 - Soyuz-26 and Salyut-6 - Soyuz-36 we prepared a series of frontal sections of the heads of the animals for light microscopy (the sections were 10 or 15 μm thick). From these ultrathin sections were cut for electron microscope analysis [Vinnikov et al., 1980]. When they were examined under the binocular microscope the experimental animals did not differ noticeably from the controls, no anomalies in general development were observed (Fig. 6). In the vestibular apparati of both the experimental and control tadpoles the utricular and saccular otoliths were visible through the transparent skin coverings (Fig. 6). A more detailed examination under a polarized microscope revealed a significant variation in the dimensions of the otoliths in both the experimental and control animals. No reliable differences were found between the experimental and control animals in this factor. Differences in the dimensions of the left and right utricular and saccular otoliths in a single animal were related primarily to individual otoconia being deposited from the otolithic membrane and being redistributed inside a single labyrinth. The reason for this deposit is evidently a disturbance in the connection of the

Fig. 6. Tadpoles of Xenopus laevis which developed under conditions of weightlessness for 20 days. On the right, the control; on the left, experiment. The saccular (S) and utricular (U) otoliths. Scale 2 mm. Key: 1-S; 2-U.

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otoconia with the yolk layer in the otolithic membrane which is caused by fixation of the material. The study of semi-thin sections also did not reveal substantial differences between the experimental and control material. The vestibular apparatus in Xenopus tadpoles showed utricular and saccular maculas with otolithic complexes consisting of numerous otoconia and cristas of the semicircular canals. The lagenal macula was not observed at these stages of development. The maculas were pronounced and differentiated according to the developmental stage of the animal (Fig. 7). The sac-

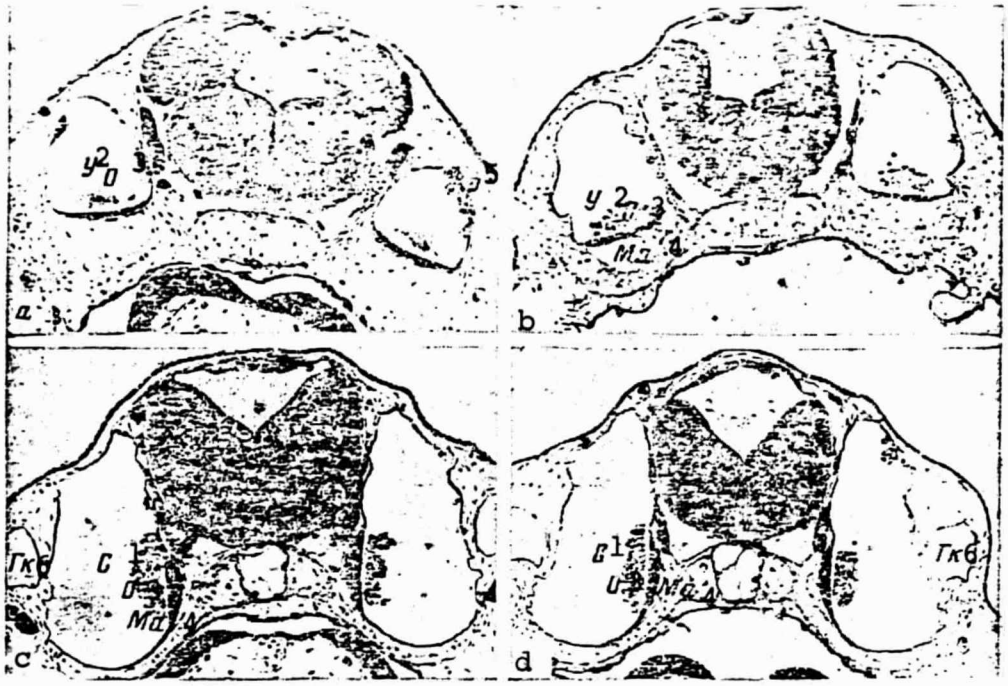

Fig. 7. Frontal sections of the heads of Xenopus laevis tadpoles which developed under conditions of weightlessness for 8 days. a,c - control; b,d - experiment. The saccule (S), utricle (U), otolith (O), macula (Ma), crista of the front vertical canal (Cr), chamber of the horizontal semicircular canal (Hc). Scale 200 μm.
Key: 1-S; 2-U; 3-O; 4-Ma; 5-Cr; 6-Hc.

cular macula has a crescent shape in the sections (Fig. 7). The thickness of the utricular macula is uniform throughout and only towards the edges do we see a reduction in the thickness of the cellular layer (Fig. 7). The thickness of the saccular macula in the central part is greater than that of the utricular macula. The

nuclei of the support and receptor cells in the utricular macula are arranged in a single row, while in the saccular macula the cell nuclei lie at various levels (Fig. 7). In the cellular nuclei one or more rarely two small dense nucleoli are clearly visible (Fig. 7). The cells within a single macula, particularly the saccular macula, vary in optical density. In the basal section we can see light, vacuolized formations. Similar vacuoles are found in the experimental and control animals; they are clearest in the saccular macula. The otoconia differ in dimensions, have polygonal forms and double refraction. In the utricle the otolithic membrane has fewer otoconia than in the saccule.

An examination of ultrathin sections showed that the receptor and support cells of the maculas of the otolithic organs are highly differentiated. On the surface of the receptor cells is a well developed cuticle from which comes a cluster of sensory hairs consisting of a large number of stereocilia and one kinocilium in a polar arrangement. The cytoplasm of the receptor cell is rich in ribo- /156
somes and polysomes and has a large number of mitochondria with matrices with high electron density. The Golgi apparatus and the endoplasmatic network are moderately well developed. The nerve endings go towards the base of the cell and they form synaptic contacts with the cell. In the area of contact with the afferent endings in the receptor cell is a spherical synaptic corpuscle with high electron density which is surrounded by synaptic vesicles (Fig. 8). In the basal sections of the receptor cell the synaptic vesicles are indistinct. An electron microscope analysis of the otoconia in the oto- /157
lithic organs showed that they consist of a thin fibrillar matrix with material of high electron density. Within the otolithic complex of a single receptor organ the type of inclusions of material of high electron density and the clarity of the fibrillar matrix differ in the otoconia.

Thus, a comparison of light and electron microscopic examinations of the otolithic organs and a light microscope examination of the crista of the semicircular canals in Xenopus which developed in

Fig. 8. The basal region of the cell of the utricular macula in Xenopus (20 day experiment). Receptor cell (Rc), support cell (Sc), afferent nerve ending (N). Scale 1 μm.
Key: 1-Rc; 2-Sc; 3-N.

weightlessness and on earth showed that there are no significant differences in the structure of these organs or anomalies in the development of the vestibular apparatus in either group of animals.

The Development of the Vestibular Apparatus in Bony Fish

The experiments which we did to examine the development of the vestibular apparatus in embryos of the fish Brachydanio rerio at the gastrula and five somite stage on space vehicles Soyuz-16, -21 and -22 (Table) showed that the general development under conditions of space flight proceeds normally. The embryos developed at 23.5°C. In the experimental larvae as well as in the control larvae which developed in weightlessness the vestibular apparatus was comprised of the utricle, saccule and embryos of the semicircular canals. The otolithic apparatus consisted of utricular and saccular otoliths (Fig. 9). After preparation the material was made into a semi-thin section of 10 mk in order to study the formation of the utricular otolith under the light microscope. The same sections were used for ultrastructural examinations. Under the light microscope we were not

. ig. 9. Larvae of Brachydanio rerio which developed under conditions of weightlessness for 9 days (experiment). Saccular (S) and utricular (U) otoliths. Scale 0.5 mm.
Key: 1-S; 2-U.

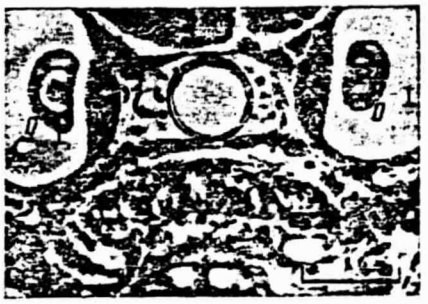

Fig. 10. Part of a frontal section of the head of Brachydanio rerio larvae which developed in weightlessness for 9 days. Saccular otolith (O). Scale 100 μm.
Key: 1-O.

able to detect any deviations in the general structure of the vestibular apparatus of the experimental and control larvae (Fig. 10). During an examination under the electron microscope special attention was paid to the structure of the receptor linings of the utricle and saccule and their otolithic complex. An examination of the receptor epithelium showed that in both the experimental and control larvae its central part consists of clearly differentiated receptor and support cells (Fig. 11). The receptor cells as a rule have cylindrical form and are cells of the 2nd type. The apical part of the cell ends in a cuticle from which one kinocilium and a cluster of stereocilia come. The kinocilia of all the receptor cells are always arranged in a polar configuration with respect to the cluster of stereocilia. They comprise nine pairs of peripheral and two central fibrils which are typical of all mobile cilia. The kinocilia of the receptor cells in both the experiment and the control may be close and even immediately adjacent to the otolith. The cluster of stereocilia is substantially shorter.

The cytoplasm of the receptor and support cells in both variants has similar ultrastructural features. Thus, the cytoplasm of the receptor cells is rich in mitochondria, a large number of which is /158 found under the nucleus in the base of the cell. The mitochondria have a dark matrix and densely arranged crista. Such a polar

Fig. 11. A section of the utricular macula and otolith of Brachydanio rerio which developed in weightlessness for 9 days. Otolith (O), nucleus of the otolith (No), receptor cell (Rc), cluster of stereocilia (St). Scale 10 μm.
Key: 1-O; 2-No; 3-Rc; 4-St.

arrangement of the mitochondria is explained by the fact that the nerve endings go towards the base of the receptor cell and form synapses with the receptor cells.

At this stage of development the nerve fibers are amyelinic and go towards the receptor cells from the basal membrane. The bud shaped nerve endings which are in contact with the receptor cells are light, having clearly defined neurofibrils and small mitochondria. Evidently these are afferent nerve endings. In the region of the synapses in both the experiment and the control, so-called synaptic corpuscles are often visible. This indicates that the receptor cells are in an active functional state. As distinct from the receptor cells, the cytoplasm of the support cells has a more highly developed endoplasmatic reticulum, secretory vesicles and vacuoles. The support cells are arranged with their bases on the basal membrane. On their apical ends they may have microvilli.

We should note the well developed system of specialized cellular contacts in the receptor linings which are represented by a large number of desmosomes which are arranged both on the apical surface of the receptor epithelium zone and through all its levels (Fig. 12). Such contacts may be formed both between the receptor cells and between the receptor and support cells. Thick clusters and long threads of tonofibrils which consist, in turn, of clusters of protofibrils, come from the desmosomes. Such a mechanical support system is seen to equal degree in the experiment and the control.

There were also no clear differences in the structure of the

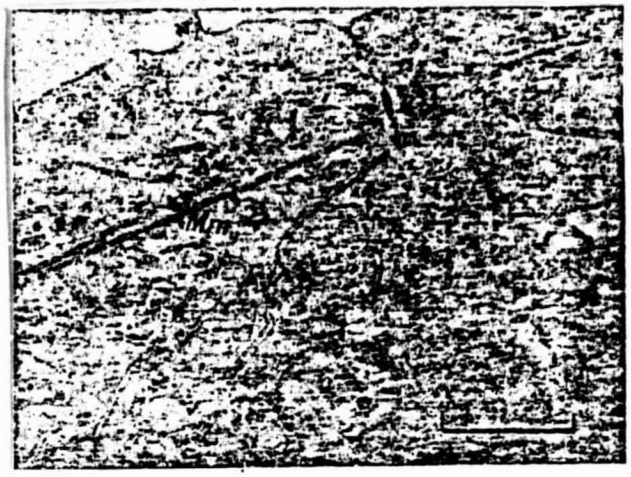

Fig. 12. The apical part of the utricular macula of Brachydanio rerio which developed in weightlessness for 9 days. The desmosomes (D) and microtubules (Mt) are clearly visible. Scale 0.5 μm.
Key: 1-D; 2-Mt.

Fig. 13. A section of the utricular otolith of Brachydanio rerio which developed for 9 days in weightlessness. The nucleus of the otolith (No) formed from individual globules with high electron density. Scale 2 μm.
Key: 1-No (illeg.)

otolithic apparatus (Fig. 10, 11, 13). Both the saccular and utricular otoliths in the experiment and the control had a characteristic beanlike form with clearly defined layers. A dense nucleus may be seen in the center of the otolith. It consists of individual globules which are as if cemented together into a single formation. These globules are the primary center of formation of the otolith, around which new layers of a mineral protein substance which comprises the structure of the otolith is gradually stratified. /159 Thus, analysis of the experimental and control materials did not reveal noticeable differences in the fine structure either in the receptor epithelium or in the otolithic apparatus. The results of the experiments which have been conducted show that the formation of the vestibular apparatus in Brachydanio rerio larvae and the cytological and ultrastructural differentiation of its parts may proceed normally under conditions of weightlessness to the larval stage.

Data obtained during an experiment on space vehicle Soyuz-22 are of special interest [Vinnikov et al., 1979]. This experiment

was undertaken for the purpose of detecting possible disturbances in ion exchange in the sensory organs of larvae of Brachydanio rerio which developed for 8 days in weightlessness. The distribution of K, Na, Ca, P and S in the vestibular apparatus and the eye was studied. After the vehicle landed the larvae were taken to the laboratory. In order to eliminate the possibility of loss and redistribution of the elements, the material was frozen in liquid propane at -160°C. The sections, which were prepared by a previously described method [Burovina et al., 1975], were analyzed on a JSM-U3 device. For the otoliths high concentrations of Ca, Na and S were characteristic. The hypothesized zonality in the distribution of Ca within the otoliths was not observed. The high concentrations of Ca and Na were seen previously in the otoconia of the frog and guinea pig [Allakhverdov et al., 1975; Vinnikov et al., 1981]. The presence of S corresponds to the hypothesis of the presence of sulfur containing acidic mucopolysaccharides and proteins in the otoliths. The cells of the macula are rich in K, P and S and contain little Na. The high concentration of these elements is also characteristic of other cells in the vestibular apparatus. The endolymph is rich in Na and K. The distribution of elements in the retina of the young fish corresponds to their localization in the retina of the frog [Burovina et al., 1972]. No noticeable differences were found in the distribution of elements between the experimental and control animals.

Of particular interest are data from investigations on the development of the vestibular apparatus of the fish Fundulus heteroclitus in weightlessness on board the orbital station Skylab and the Soviet satellite Kosmos-782 [Baumgarten et al., 1975; Sheld et al., 1979]. The eggs were placed in polyethylene packets which were filled with sterile prefiltered artificial sea water. At the time of launch of the space vehicle the embryos were at various stages: both when the vestibular labyrinth and its foundation were absent (32 hours after fertilization) and when the vestibular labyrinth was at various stages of development (42, 66, 88, 128, 216, 336 hours after fertilization). Under conditions of weightlessness development of the eggs and hatching of the young fish proceeded normally. After the launching /160

apparatus had splashed down part of the material was fixed for microscopic examinations: light, transmission and scanning electron microscopy. The authors were not able to detect perceptible differences in the dimensions, form or surface relief in the otoliths in the experimental and control fish [Sheld et al., 1979]. The ultrastructure of the otoliths and macular cells in the flight animals was normal. In addition to the vestibular apparatus the development of the central nervous system, the eye and the cardiovascular system was studied. No significant structural disturbances were observed here either. In addition to the morphological studies, behavioral studies were undertaken both on board Skylab and on earth after landing. It was found that under conditions of space flight there may be unusual movement of the young fish which is of cyclic nature. Furthermore, deviations in the geotaxial reaction were observed after 6 month adaptations on earth in fish which had been at the 32 hour stage at the time of the launch [Sheld et al., 1979]. On the whole, from the evidence of these authors we may say that fish which were in space are more sensitive to environmental conditions than are the control animals.

Conclusion

The question of the development of the vestibular apparatus under conditions of a constant gravitational field is of fundamental importance and was examined by us in the context of the general problem of differentiation of the receptor apparatus under the effect of an appropriate stimulus. It is well known, for example, that the photoreceptors of amphibians can undergo final differentiation either when the animals are in light or in complete darkness [Eakin, 1965].

In our laboratory L. K. Titova [1968] studied the embryology of the vestibular apparatus in vertebrates as well as in fish and amphibians. The basic ultrastructural, cytochemical and functional characteristics of differentiation of the receptor structures and their relation to the brain and the developing otolithic apparatus were traced. From these data we may suggest that differentiation of the receptor structures in the vestibular apparatus during embryogenesis

is related to the pressure of the developing otoliths on the surface /161
of the utricular and saccular maculas. If this is actually so, then under conditions of weightlessness we may expect disturbances in the normal differentiation of the receptor structures of the vestibular apparatus. The possibility cannot be excluded that disturbances in the normal formation of the otoliths themselves occur in weightlessness. For this reason, the establishment of the presence or absence of a causal link between the effect of the gravitational force and differentiation of the gravitation receptor was of considerable practical interest.

However, experiments which study the development of the vestibular system are significantly hindered by the fact that it is practimpossible to create conditions of complete deprivation - in all transferrals of the animal, whether on earth or under conditions of weightlessness, inertial forces arise which are adequate stimuli for one or another receptor organ in the inner ear. Nevertheless, the use of methods which simulate changes in the gravitational field such as klinostating, centrifuging and the creation of weightlessness, contribute substantially to the solution of this problem. However, we must note that we must proceed very carefully in approaching experiments which study the effect of klinostating as a model of weightlessness on morphogenesis of the vestibular apparatus, and in particular with respect to data on the appearance under "zero gravity" of vacuoles filled with necrobiotic mitochondria and bits of the endoplasmatic network in the basal part of the maculas. We have in mind the experiments of Neubert [Neubert, 1979] and Neubert and Briegleb [Neubert, Briegleb, 1980] and experiments which studied the effect of klinostating on the development of the vestibular apparatus in embryos of Rana temporaria. We must recall that from the moment that a highly sensitive specialized receptor appears, the gravitational forces arising during rotation of the klinostating chamber and the constantly acting force of gravity are adequate stimuli for the vestibular system and, consequently, that klinostating may be used as a model of weightlessness only at the very early stages of development of the labyrinth. It was precisely at these stages that Neubert

and Briegleb [Neubert, Briegleb, 1980] did not find any structural changes in the developing vestibular apparati of embryos in the klinostating chamber in comparison with the control.

The factual data presented above show that in fish and amphibian embryos which develop in weightlessness beginning at the stages at which the vestibular apparatus is still absent or is at various stages of morphogenesis, there is normal formation of the maculas, cristas and otolithic apparati [Vinnikov et al., 1972, 1979, 1980; Sheld et al., 1979]. No significant qualitative (and we emphasize qualitative) disturbances or delays in the formation of the ultrastructural organization of the receptor and support cells were observed. Calcification of the otoliths proceeds on the whole normally; this is shown by the presence of double refraction, high hardness of the otoliths and their high Ca concentration. Thus, a specific stimulus - the force of gravity - is evidently not a necessary condition for the formation of the structural organization of the vestibular apparatus at the early stages of ontogenesis. At the same time, we are alert to the fact that the unusual movement of the young animals (cyclic movement in the fish and spinning movement in the tadpoles) is maintained to one degree or another practically throughout the flight [Baumgarten et al., 1975; Sheld et al., 1979]. However, after landing in conditions of normal gravitation the larvae very quickly acquire the horizontal position and begin to move as the controls do. This indicates that the adaptation of the larve to the unusual environmental conditions should not be seen as fixed. This does not preclude the fact that the prolonged effect of weightlessness may exert a certain effect on the development of the structural functional organization of the vestibular apparatus. However, in experiments which studied the effect of prolonged exposure to hypergravitation (or centrifuging) on the /162
formation and development of the vestibular apparatus in rats, no important structural changes were observed [Lim et al., 1974]. We may only hope that future studies on long periods of flight, in which it will be possible to trace the development of the labyrinth as the larvae are transformed into adult animals, will answer this question.

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