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EVOLUTION OF THE GRAVIRECEPTOR AND ITS INVESTIGATION UNDER CONDITIONS OF ACCELERATION AND WEIGHTLESSNESS

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Translation of: "Evolyutsiya retseptora gravitatsii i yego issledovaniye v usloviyakh uskoreniya i nevesomosti." In: Arkhiv anatomii gistologii i embriologii, Vol. LXVI, No. 1, 1974, pp. 10-25.

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16. Abstract

The article reviews previous work conducted by the author and co-authors and by foreign researchers on the evolution of the mechanism of gravity reception in invertebrates and vertebrates. The structure of this mechanism in various organisms from protozoa to mollusks and crabs is described by means of numerous electron microscopic photographs. According to the author's analysis, this structure was retained in the vertebrates, and developed apparently in direct relation to the complexity of their muscular structure and interrelation with the earth's gravity. The effects of linear acceleration and weightlessness on its development are discussed; linear accelerations of 10 g for three minutes were created to stimulate utriculus receptor cells and those of the sacculus and semicircular canals, with considerable alterations in nuclear and cytoplasmic organization being observed. Experiments done on Soyuz-10 are described.

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lesser perfection in the process of evolution, but which nevertheless is constructed according to a unique principle. Essentially, the organ of equilibrium consists of two parts: the "testing mass," i.e., the otolith (sometimes of non-biological origin), which possesses freedom of movement within the limits of the organ (Fig. 1), and a system of receptors, which perceive the position or movement of this mass in the organ. A deviation in the position of the body is accompanied by displacement of the testing mass (otolith, otoconium, otolithic membrane) that excites the corresponding groups of receptors of the organ of equilibrium. The signal from these receptors is processed by the central nervous system, which also sends a command to the muscles which correct the placement of the body. In this sense one can speak of the fact that animals have special gravireceptors (or receptors of gravitation). In using this term, it is important not to forget that, in themselves, the cells of the organ of equilibrium do not perceive gravitation; they perceive only the amount and direction of the force with which the testing mass, under the influence of the earth's gravity, effects them. This report proposes to follow the evolution of the gravireceptor in the basic types of invertebrates and in vertebrate animals with the aid of electron-microscopic and histochemical methods and to /11 establish the basic structural and cytochemical changes which arise during the stimulation of this organ under conditions of a changed gravitational field (under the influence of linear and angular accelarations and weightlessness).

2. The principles of structural, cytochemical, and functional organization of the gravireceptor of Protista, invertebrates, and Primochordata.

In representatives of various types of invertebrates, beginning with the simplest and ending with echinoderms, we succeeded in following up how, in the process of natural selection, by a method of trial and error,the very perfect model of a gravireceptor suitable in its structural, cytochemical, and functional organization, and adequately perceiving the position of the organism in the gravitational field, is created.

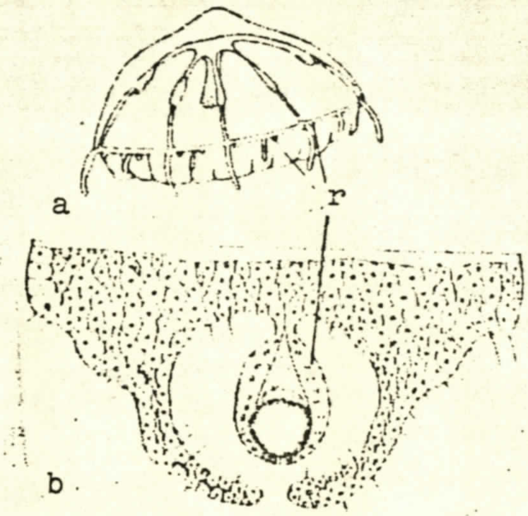


Fig. 2. Diagram of a rhopalium, the extremity organs of Phopalomena velatum (Hickman, 1967).
a.-the body of the medusa with the extremity organs; b - a rhopalium with one lithocyst; r - the rhopalium ;

In this respect, data on the statocyst-like organelle of Protista acquire special significance (V. A. Dogel', 1929; V. A. Dogel', Yu. I. Polyanskiy, and Ye. M. Kheisin, 1962). It may be thought that deviation of concretions in movement is perceived in the Protista by the cytoplasmic membrane of the wall of the organelle and then through the cytoplasm is transmitted to the cilia, the shortening of which regulates the position of the body. If it is ultimately proven that the statocyst-like organelle of the Protista really fulfills the role of a gravireceptor, then it will be possible to assume that during the process of evolution this function received a structural basis even in unicellular animals. The quest and solutions in the creation of a design for a model of a gravireceptor in Coelenterata and Ctenophora are most convincing. Actually, the Coelenterata have all varieties of growth of a gravireceptor, beginning with a small receptor field, covered with lithocysts (cells which contain a concretion in the cytoplasm to a lithostyle or rhopalium, which is an elastic pendulum, the basic mass of which is formed of lithocysts (Fig. 2). The submersion of the lithostyle in a hole which can close is often viewed formally as the formation of a statocyst, although in reality statocysts are organized in a much more complex manner. Any deviation in the position of the body of an animal in the gravitational field causes a deviation of the lithostyle which is loaded with lithocysts. This deviation is perceived either by the surrounding receptor cells, or by the receptor cells located in the lithostyle itself. The first method of reception is, apparently, more successful, but the existence of receptor cells around the lithostyle has not been conclusively proven.

Significant progress is, undoubtedly, revealed by the organization of the aboral organ of the Ctenophora (Fig. 3). In it we encounter for the first time, first, the presence of secondary-perceptive receptor cells, equipped with one movable flagellum containing 9 pairs of peripheral and 2 central fibrils and second, the presence of a free otolith, formed by special cells - otoconioblasts. The displacement of the otolith which accompanies a change in the positon of the body of the Ctenophora leads to a deviation of the flagellae, which are agglutinated in the form of springs, or balances (cf. Fig. 3), accompanied by excitation of the receptor cells and the transmission of this excitation, apparently, through the synapses to the nervous system. As is known, the Ctenophora have no other organs of perception, and the aboral organ, i.e., the gravireceptor, effects the integration through the nervous system of practically the entire life activity of these organisms.

We find several other relationships in the first representatives of the Deuterostoma - the Platyhelminthes. In the ciliary worms there is already a special organ - the statocyst - which is a vesicle filled with liquid. According to our data, it consists of one cell which contains a cavity. By means of a fine pedicle, a freely oscillating lithocyst is attached to this cell. Thus, in statocysts of Turbellaria worms there are no free otoliths, which are already present in the aboral organ of the Ctenophora. In them a lithocyst which appeared for the first time in the Coelenterata acts as the testing mass. In the Nemertinea, it is evident that statocysts have an analogous structure. The polychaeta have well-developed statocysts which can communicate with the external environment by means of a narrow canal. Otoconia in statocysts of polychaeta either are carried off from the external environment or arise due to the secretory function of the cells. Electron-microscopic data which we obtained show that it is precisely in the statocysts of the polychaeta that we first encounter typical proto-sensory cells, equipped with flagellae, which contain 9x2+2 fibrils (Fig. 4). It is evident that the deviation of these flagellae under the influence of change in position of the otolith leads to excitation of the receptor cell and transmission of this excitation to the complexly structured nervous system of the worm. /12

An improved form of organization of a statocyst, which is transformed into a true organ of equilibrium, is observed in the Arthropoda, and especially in the higher crustaceans (Fig. 5). The statocyst of the higher Crustacea is an invagination of integuments, openly communicating with the external environment by means of a crack. Only in the lower crustaceans do the stato-

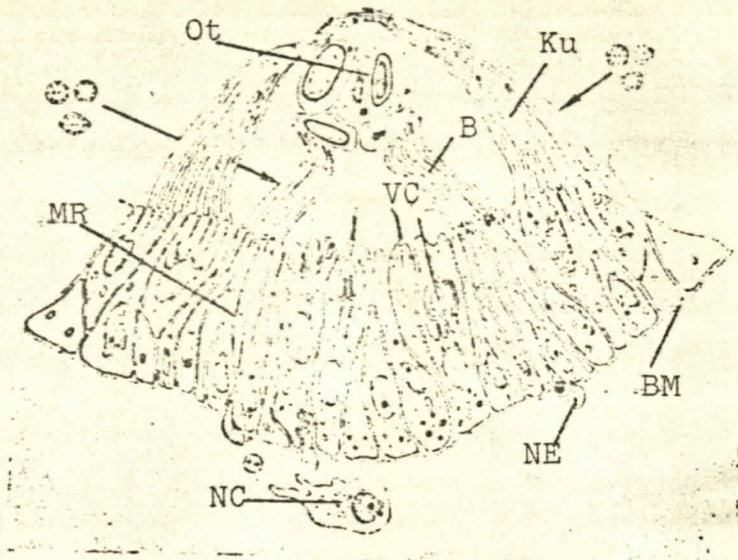


Fig. 3. Diagram of the ultrastructural organization of the gravireceptor (aboral organ) of the Ctenophora.
MR - mechanized receptor cells of the gravireceptor; VC - visual cells; B - balancers; Ku - cupola; Ot - Otolith; NC - nerve cells; NE - nerve endings; BM - basal membrane.

cysts completely shrink away from the integuments. But in both kinds, the chitinous cuticular lining of the statocyst changes with every molting; and then the bubble-like statocysts of the lower crustaceans also communicate with the external environment. During molting, the lining of the statocyst is lost together with the grit particles - otoliths which are contained in the statocyst. After molting, the animal introduces new grit particles either with the help of claws or by burying its head in the sand many times. As is known, the classic experiment of Kreiden was conducted on the statocysts of the crab (Kreidel, 1893); in it the animal, after molting, was offered iron shavings instead of sand to fill the statocyst, and then was acted upon by a magnet in imitation of the action of the gravitational field. The receptor cells of the statocyst of Crustacea are typical primary sensory cells. Their basic mass is concentrated in the foundation of the statocyst, which is located parallel to the earth's surface, i.e., horizontally. The axon of the receptor cell goes into the central nervous system, and the peripheral outgrowth represents, according to our data and the data of Schone and Steinbrecht (1968), a modified flagellum containing 9 pairs of peripheral fibrilla. This outgrowth enters into the chitinous cap, which has the form of hairs. The number of such chitinous hairs may run to 400-500. The tops of the chitinous hairs are attached /13 to the otolithic membrane. A deflection of the chitinous hairs at the moment of change in the position of the body of an animal in the gravitational field is perceived by the peripheral outgrowths of the receptor cells, which thus represent gravireceptors. Hairs which are not connected with the otolithic membrane are also located on the lower surface of the statocyst. These hairs are deflected under the influence of currents of the liquid and fulfill the function of a perceiver of angular accelerations. The presence in statocysts of Crustacea of hairs which perceive vibration was also established. Thus, in the higher Crustacea a very much perfected type of gravireceptor which represents an all-purpose organ of both static and dynamic equilibrium arises. The curious adaptation in statocysts is connected with the presence in the Crustacea of a chitinous lining. As a result of this, contact between the otolithic membrane and the peripheral outgrowths of the receptor cells of the statocysts of Crustacea is effected through an additional structure in the form of a chitinous hair-cap.

The mollusks have even more perfected statocysts (Fig. 6).

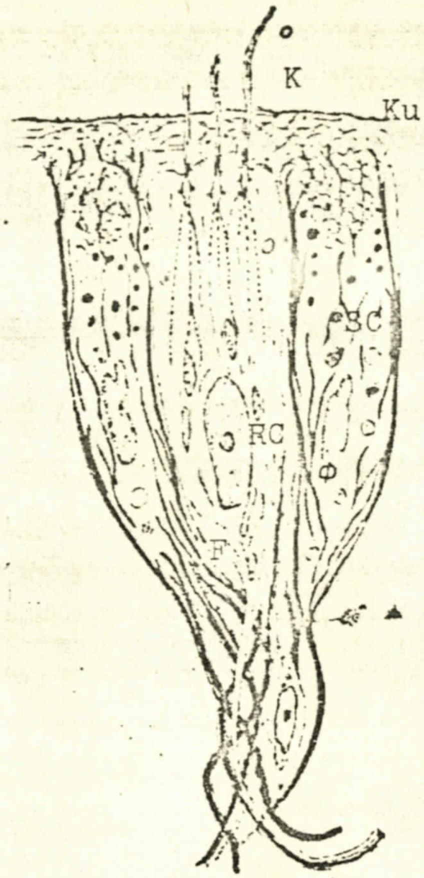


Fig. 4 - Diagram of a structure of receptor epithelium of a statocyst of Arenicola marina.
K - kinocilia; Ku - cuticle; RC - receptor cell; SC - support cell; F - fibrilla of the support cells.

They have been discovered in representatives of all their classes. In the Gastropoda the statocyst is a little round sac filled with endolymph and containing, apparently, a free otolith. In the Pterotrachea, a limited sector of receptor cells in the statocyst has been successfully distinguished, forming in combination a macula, the receptor cells of which are related to primary sensory ones and are supplied with hairs. Electron-microscopic studies of the fresh-water Gastropod mollusk Planorbis corneus showed that the hairs crowning the gigantic primary sensory cells of the statocyst of this mollusk are typical kinocilia. The receptor cells of the Bivalvia mollusks, whose statocysts, however, lack maculae, have a similar structure. The method of contact between the kinocilia and the otoliths (otoconia) in the statocysts of the Gastropoda and the Bivalvia mollusks is not yet clear to us. Are the otoliths attached to the distant ends of the kinocilia, or do they move around freely in the endolymph? One way or another, the deflection of the kinocilia of the receptor cells may be achieved only by menas of moving the otoliths during a change in the position of the body of the mollusk in the gravitational field. There is a basis for supposing that efferent innervation that regulates the function of the receptor cells already appears in the statocysts of the Gastropoda mollusks. The Cephalopoda mollusks achieved the highest organization among representatives of this type. The study of their gravireceptor acquires special interest in connection with the reactive method of their locomotion. In the octopus, statocysts possess not only an endolymph cavity, but also a perilymphatic space; in the squid, the perilymphatic space is absent (Fig. 7). The basic structures of the statocysts in both octopuses and squids are maculae with otoliths and cristae covered with cupolas that bend with the movement of the endolymph The macula of the octopus is located in the vertical plane. The chief macula of the squid lies in the same plane, but two additional maculae are located at a slight angle to it. The three cristae lie in three mutually perpendicular planes. In addition, in contrast to the vertebrates, two of them are horizontal and one is vertical. In the octopuses, one auticrista is introduced in the cavity of the statocyst, while in the more motile squids their number reaches 11, and the arrangement is a complex spatial system. It is proposed (Young, 1960, 1964) that the presence of

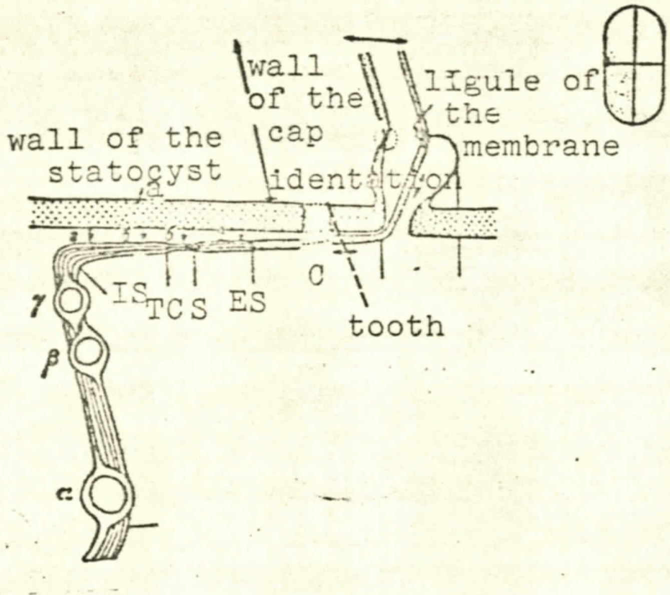


Fig. 5 - Diagram of the organization of receptor elements of a statocyst of the crustacean <u>Astacus fluviatilis</u> (Schone and Steinbrecht, 1968).
a, β, γ-- three receptor cells; IS, TCS, ES - correspondingly, the internal, ciliary, and external segments. C - the chord. Double arrows - directions of movement under stimulation.

the auticristae makes possible directed movement of the currents of the endolymph with changes in movement of the animal, analogous to the action of the system of semicircular canals in vertebrates. In a number of physiological studies it was established that the maculae of Cephalopoda mollusks are gravireceptors, i.e., /15 receptors which ensure static equilibrium, while the cristae are receptors of angular acclerations, i.e., they ensure dynamic equilibrium (Boycott, 1960; Kijkgraaf, 1961; Young, 1964). The function of the cristae was recently studied electrophysiologically (Wolff, 1972 - personal report). Both the cristae and the macula in the statocysts of Cephalopoda contain primary sensory cells with a very complex cytochemical and ultrastructural

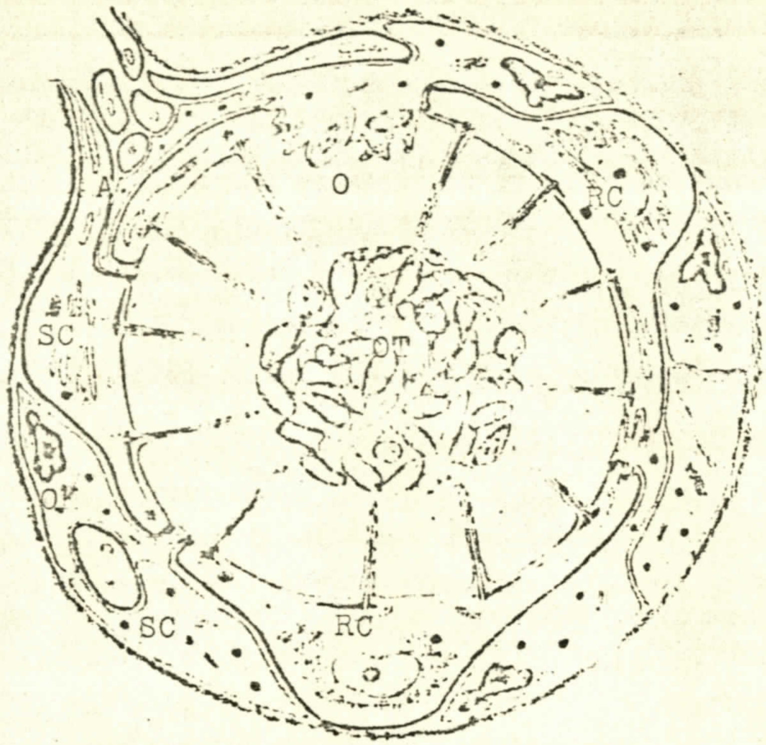


Fig. 6 - Diagram of the structure of a statocyst of a Gastropoda mollusk (<u>Clione limacina</u>).
RC - receptor cell; SC - support cell; A - axon; O - otolith; OT - otoconia.

organization (Fig. 8, a, b). These cells are crowned with kinocilia, containing 9X2+2 fibrils. The distal ends of the kinocilia, evidently are connected with the otolithic membrane or cupule. The bases of the kinocilia are surrounded by 2 to 5 microvillae, which by analogy with the vertebrates we call stereocilia-like. It must be pointed out, however, that the slanted apical surface and the hairs of the receptor cells of the macula and the cristae, as a rule have a strictly defined direction of slope, just as the pedicles of the basal bodies of the kinocilia. This, apparently,

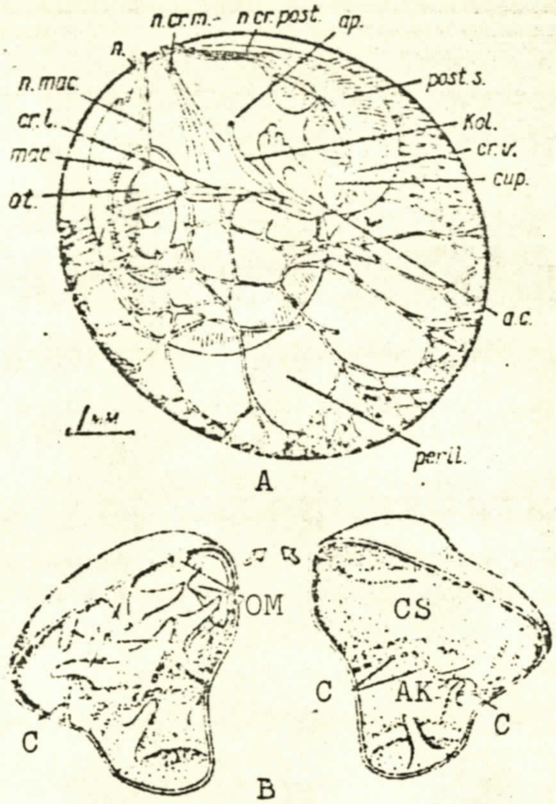


Fig. 7 - Diagram of the structure of statocysts of Cephalopoda mollusks. A - octopus (<u>Octopus sp.</u>) and B - squid (<u>Ommatostrephes sloanei</u>) (Joung, 1960).
For A: n - nerve; n. cr. m. - nerve of the medial crista; n. cr. post, - nerve of the rear crista; ap - top of the canal of Kelliker; post. s - rear sinus; Kol - canal of Kelliker; cr. v. - ventral crista; cup. - cupola; ac. - auticrista; peril. - perilymph; cr. l. - lateral crista; OM - otolith of the macula;, n. mac. - nerve of the macula.

For B: Ak - auticristae; OM - otoliths of the maculae; CS - cavity of the statocyst; C - cristae.

determines not only the strict orientation of the tops of the receptor cells, but also their functional polarization. It is interesting that among invertebrates other than the mollusks there are no other representatives whose gravireceptors have a similar polarization; the Arthropoda, it is true, have been studied insufficiently in this regard. Meanwhile, in all the vertebrates, as will be evident from the further exposition, the tops of the receptor cells of the organs of equilibrium are also strictly oriented, although this polarization is resolved in a different manner in them. Unipolar neurons with a complex ultrastructural and cytochemical organization are located in the statocysts of the Cephalopoda under the receptor cells of the maculae and the cristae. It is possible that similar neurons, absent in the statocysts of other mollusks, fulfill an integrative function, ensuring a connection between groups of receptors. But there are no factual data on this. Both the bodies and the axons of these neurons can attain gigantic dimensions and enter the layer of nerve fibers, where they are located next to the central outgrowths of the receptor cells. As electron-microscopic observations and cytochemical data show, in this layer there are also fibers filled with numerous synaptic vesicles, which reveal a high degree of acetylcholiesterase activity. Synaptic contacts are often observed between the fibers described, testifying to the possibility of a functional connection between the axons of the neurons and the central outgrowths of the receptor cells, and also the cholinergic fibers, and causing the layer of nerve fibers of the maculae and the cristae to be considered a neuropile. Fibers which contain numerous synaptic vesicles and which reveal high acetylcholiesterase activity can also form synapses on the bases of the side surfaces of the receptor cells. All this leads to the conclusion that the cholinergic fibers are efferent. Thus, just as in the vertebrates, the functions of the receptor cells of the receptor cells of the statocysts of the Cephalopoda mollusks are apparently regulated with the help of reverse communication. All the above permits the statocysts of Cephalopoda of

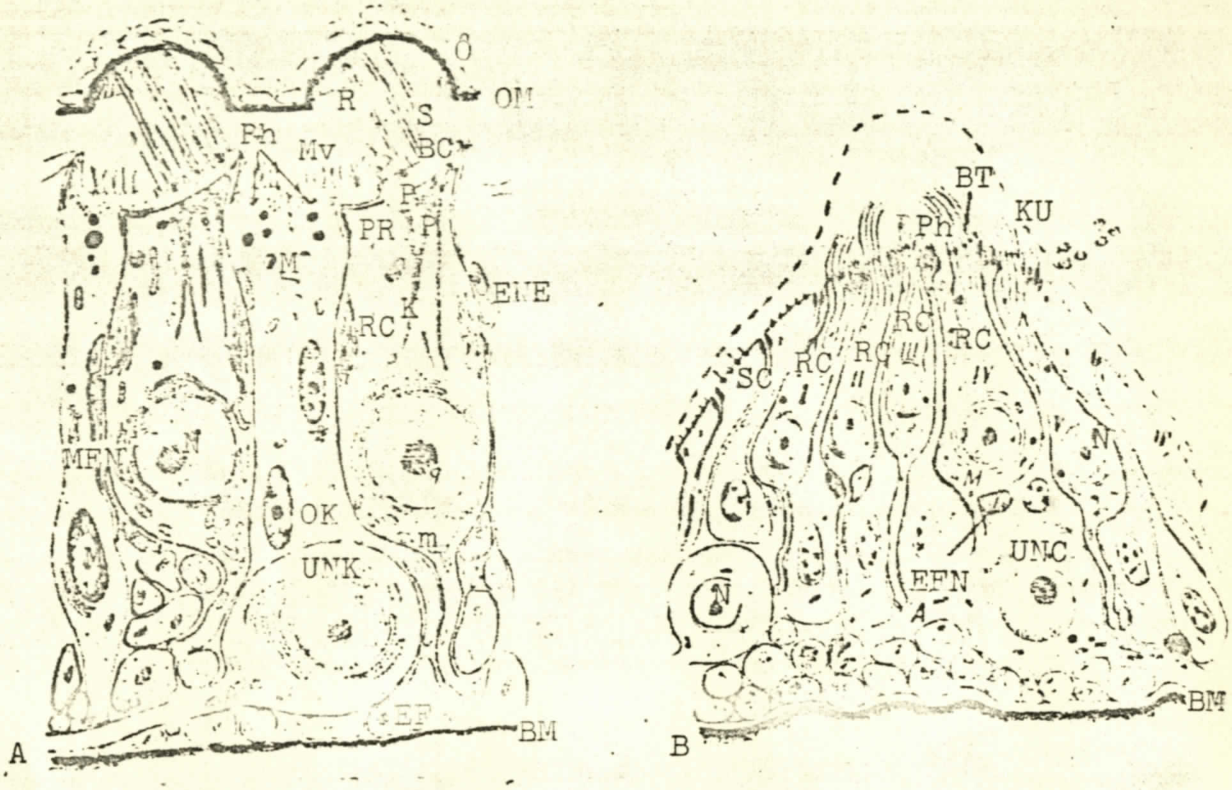
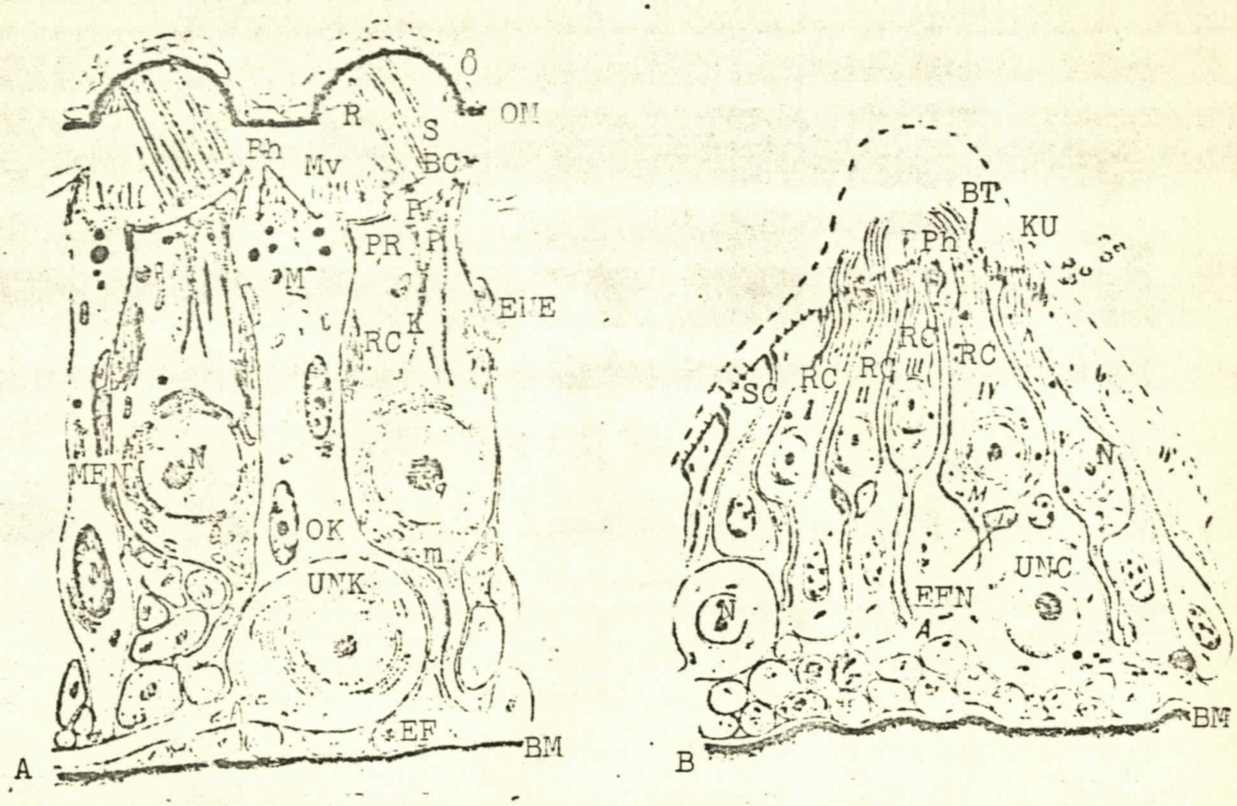


Fig. 8 - Diagram of the organization of maculae (A) and cristae (B) of Cephalopoda mollusks. /16

O-otoconia; OM - otolithic membrane;K - Kinocilia; S - stereocilia-like microvillae; BC - basal corpuscle; P - pedicles; PR - plexus of rootlets; R - rootlets; RC - receptor cells of I, II, III, IV, V orders of cells of the crista; ENE -efferent nerve endings; M - mitochondria; MEN - menbrane of the endoplasmic network; N - nucleus: A - axon, UNC - unipolar nerve cells; EF - efferent fibers; SC - support cells; Mv - Microvillae; Ph - phalanges; BM - basal membrane; Ku - Cupola of the crista.



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mollusks to be considered extremely perfected instruments - organs of equilibrium, which in many ways (including their connections with the central nervous system) are not inferior to the organs of equilibrium of the vertebrates.

We are not dwelling in detail on the statocysts of echinoderms or caterpillars, which, unfortunately, have as yet been little studied. Although they are also representatives of Deuterostoma, they have primitive multicellular or unicellular gravireceptors like the lithostyle of the Coelenterata or the Turbellaria worms. Therefore, the gravireceptors of the Primachordates may in no way be considered an initial form for the development of organs of equilibrium of the vertebrates.

Thus, an analysis of the evolution of the gravireceptor of one-celled invertebrates and Primachordates shows how by trial and error natural selection in the final analysis led to a more rational design of the gravireceptor, which arises independently in all phylum lines; practically the same elements are used: a primary-sensory cell with kinocilia, and a testing mass in the form of an otolith or an otoconium.

Using the example of the evolution of the gravireceptor of invertebrates, one can see that with the development of motor activity of animals, this receptor is transformed from an organ beginning as an organ of purely static equilibrium into an organ which possesses the capacity not only of regulating the position of an organism in the gravitational field, but also of coordinating its movements in three-dimensional space (this second function attains special development with the vertebrates).

3. THE PRINCIPLES OF STRUCTURAL, CYTOCHEMICAL, AND FUCTIONAL ORGANIZATION OF THE GRAVIRECEPTOR OF VERTEBRATES

The gravireceptor in all vertebrates (from Cyclostomata to /17 mammals) consists of utricular and saccular maculae, and also of semicircular canals. At the basis of the organization of receptor structures of the labyrith, including the utriculus of the lower vertebrates (fish and amphibia) lie the so-called Type II cells, to which in the higher animals, beginning with Reptilia, cells of Type I are united (Fig. 9). Individual

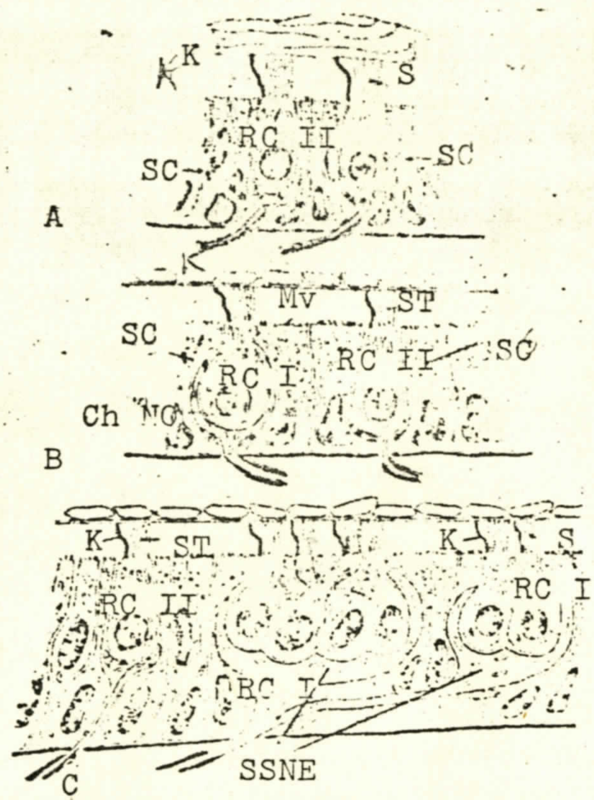

Fig. 9 - Diagram of the evolution of the structural organization of the utriculus of vertebrates.
A- fish; B - mammals; C - birds; RCI and RCII receptor cells of the Types I and II; SC - support cells; K - kinocilia; S - Stereocilia; SSNE - saucer-shaped nerve ending; Mv - Microvillae.

bud-shaped nerve endings are similar to Type II cells; Type I cells are submerged in special nerve bowls. Both of these are capped by a cluster of hairs which consists of several tens of stereocilia and one kinocilium oriented in a polar direction relative to them. Evidently, Type I cells appear for the first time in the higher vertebrates in connection with their more active movements in the gravitational field, which required more all-purpose and reliable transfer into the central nervous system of information received by the receptor cells. Meanwhile, the evolutionary transformations on the cellular level did not at all affect the organization of the hairs and their connections with the otolithic membrane, which in cells of Type I differs in no way from the phylogenetically initial cells of Type II. This fact is confirmed by the circumstance that the preliminary physical transformations connected with the reception of gravitation were apparently resolved by natural selection at the dawn of the evolution of multicellular animals. Later, with the creation of new models of a gravireceptor in the newly arising types of animals, including the vertebrates, the same construction was inevitably created to adequately perceive the action of the gravitational field. It must be pointed out that many researchers have often called attention to the fact that successful morphological, physiological, or biochemical solutions of some biological process, reached by natural selection as early as the one-celled animals, were later retained in the process of evolution of the multicellular animals and were only more precisely defined and carefully adapted to some definite function (Ye. M. Kreps and N. A. Verzhbinskaya, 1959).

In all vertebrates, with a change in the position of the body in the gravitational field, cutting motions of the otolithic membrane of the utriculus arise that deflects the bundle of hairs /18

l. Shearing

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Fig. 10. Utricular macula of the wall tortoise Testudo horsfieldi after the influence of accelerations of 10 g. over a period of 3 min. The deviation of the stereocilia toward the kinocilia. Magnification - 10,000.
RE - receptor cell; SC - support cell; M - mitochondria; K - kinocilia; S - stereocilia; OM -otolithic membrane; ZO - zonula occludens.

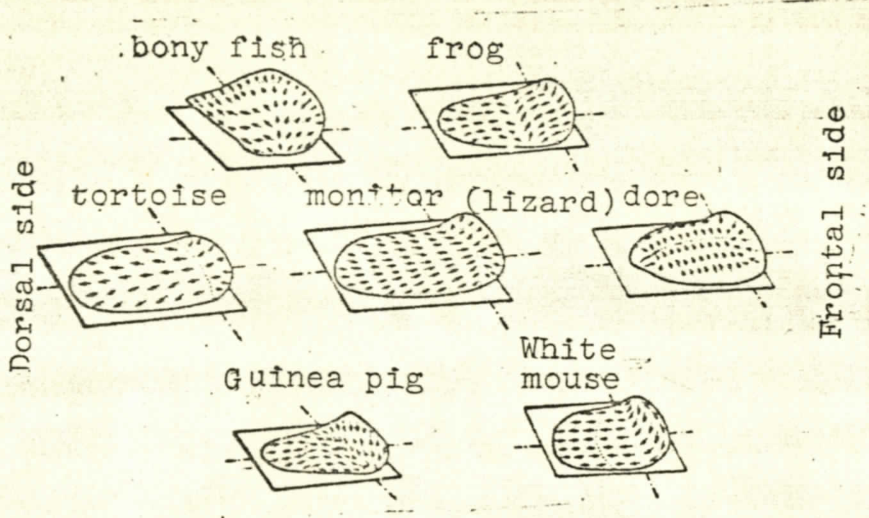

Fig. 11. Diagram of morphological polarizaton of kinocilia (needles) in relationship to the bundles of stereocilia in the receptor cells of the utriculus of vertebrates (fish, amphibians, reptiles, birds, mammals)

The clearest areas of polarization are outlined by dotted lines.

of the receptor cells. The deflection of the stereocilia toward the kinocilium is accompanied by depolarization of the cellular membranes and deviation to the opposite side - their hyperpolarization (Lowenstein and Wersall, 1959). In the first case, the impulse activity in the efferent fibers increases, in the second it decreases (Lowenstein and Sand, 1940; Trinker, 1957). While the stereocilia form a gradually increasing bundle, not connected with the exterior surface of the otolithic membrane, and can only, like the bristles of a brush, stick as a result of a movement of this membrane in its cracks, then, according to our data, the kinocilium with its own distal end penetrates deeply into the crossbars of the otolithic membrane, and in all probability attaches itself to it (Fig. 10). A shortening of the kinocilia, caused by molecular interaction of adenosinetriphosphoric acid

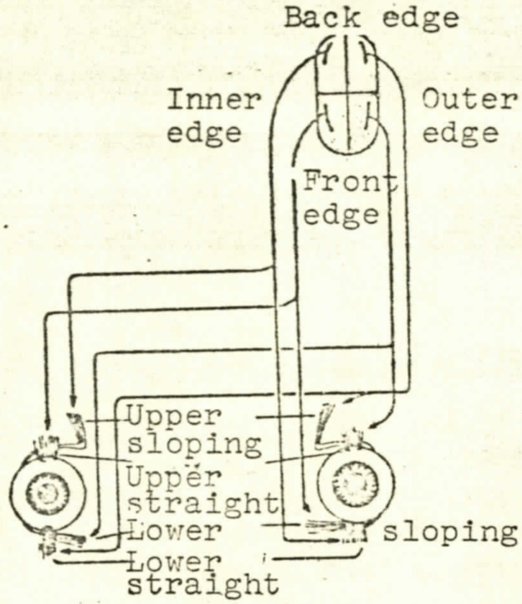

Fig. 12. Projection of the eye muscles in the utricular macula (view from above) (Sentagotai, 1967).
The points are sections of the maculae where the thickest fibers end.

with adenosinetriphosphatase contained in its fibrils, apparently regulates the degree of displacement, and there may even be a return to the initial positions of the otolithic membrane. It is somewhat more difficult to imagine the mechanochemical molecular processes which proceed in the stereocilia. Apparently, the role of the stereocilia in the receptor cells of the maculae and the cristae on the molecular level will be explained with the further progress of cytochemical and electron-microscopic methods. But now one may already speak of the great functional significance of the stereocilia. Thus, according to the calculations of Dohlman (1960a, 1960b), the total area of the membranes of the sterocilia is about 2000-4000 square nanometers, while the remaining protoplasmic membrane of the cell consists of only 200-300 square nanometers.

In contrast to the cristae of the semicircular canals, all the kinocilia of which are oriented in the same manner in the vertebrates (in the horizontal ampulla utriculopetally, and in the two vertical ones - utriculofugally), the receptor cells in the utricular macula with similar polarization are connected in complexes which in their entirety form a mosaic of four zones. Due to this mosaic, the parts of which differ in representatives of various vertebrate animals (Fig. 11), only certain groups of cells are stimulated by the cutting sliding of the otolithic membrane. From the physiological data obtained at /9
one time by Magnus (1924) and especially be Sentagotai and others (1967), who also divided the utriculus into four quadrants, it appears that a certain group of muscles corresponds to every part of this mosaic or quadrant; these take part in the adjustment of the body and the regulating functions of the utricular maculae (Fig. 12). At one time it was suggested (Ya. A. Vinnikov, 1966) that the difference in the character of the organization of the mosaic of distribution of receptor cells in the maculae of one or another representative of the vertebrates reflects the development and rise of the musculature in connection with the features of their position in the gravitational field. Evidently, this finds its confirmation in the comparison of the mosaic of the utricular macula. In birds it is distinguished by greater complexity than in mammals; which fact, it must be thought, is connected with the peculiarities of the development of the flying musculature. On the other hand, in reptiles, especially in tortoises, this mosaic is significantly simplified, which must reflect the reduction in body musculature. In amphibians, the mosaic of orientation of the receptor cells

of the utriculus, although it is similar to that of fishes, is nevertheless somewhat more complex, which, of course, is connected with the appearance in them of walking extremities and with the emergence from the water onto the land. Thus, the character of the mosaic discussed in the orientation of the receptor cells of the maculae in accordance with the position of the kinocilium reflects ecological peculiarities connected with the change in the position of animals in the gravitational field of the earth.

4. CHANGE IN THE STRUCTURE OF THE LABYRINTH UNDER THE CONDITIONS OF A CHANGED GRAVITATIONAL FIELD.

A. Linear accelerations. The receptor cells of the labyrinth of vertebrates differ in the unusual complexity of their structural and cytochemical organization, which ensures not only support of their life activity in a condition of relative calm, but also work under conditions of adequate stimulation. As we succeeded at one time in establishing (Ya. A. Vinnikov et al, 1963), with stimulation by linear acceleration of an intensity of 10 g. in the course of 3 minutes in the receptor cells of the utriculus and also the sacculus and the semicircular canals of the labyrinth, together with a deviation of the bundle of sensory hairs (cf. Fig. 10), substantial shifts take place in subcellular and cytochemical organization of the nucleus and the cytoplasm. They are expressed in a reduction in the contents of cytoplasmic ribonucleic acid, in a shift in the nucleus toward the nuclear membrane, and in the transition of ribosomal ribonucleic acid from the nucleolus to the cytoplasm, where they induce the formation of a limited sector of the endoplasmatic network. These processes were discovered in all the vertebrates we have studied, but are esepecially noticeable in guinea pigs (Fig. 13). Apparently the directed exit of the ribose of the nucleoli can also occur spontaneously, without experimental influence, reflecting some kind of rhythmic entrance of ribonucleic /20

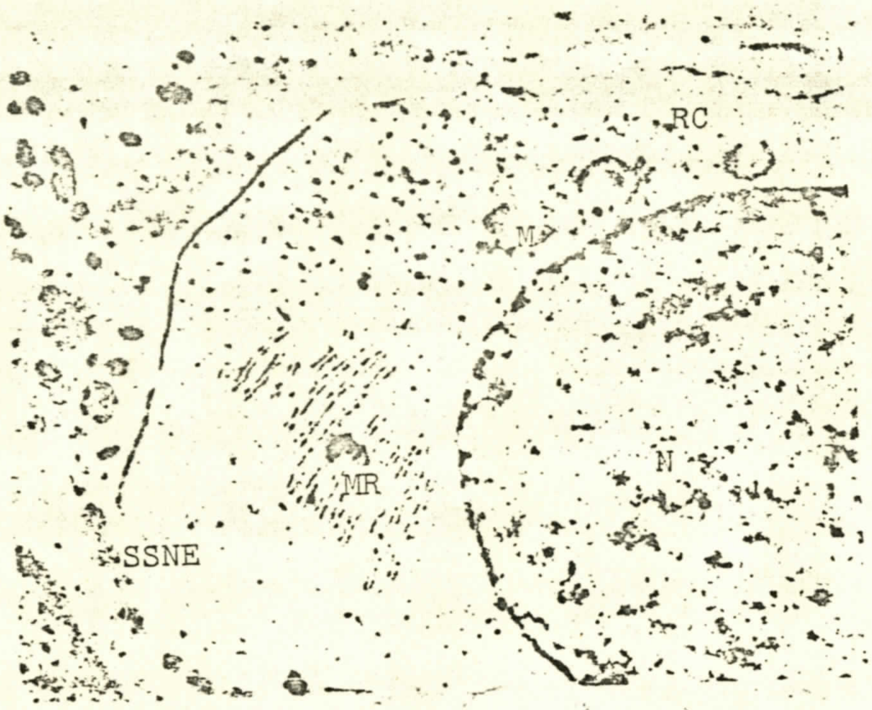

Fig. 13. Formation of the spiral-shaped sector of the rough endoplasmic network in the receptor cell of the utriculus of a guinea pig subjected to rotation at a speed of 10 g. over a period of 3 min. Magnification: 18,000.
RC - receptor cell; N - nucleus; M - mitochondria; MR - membranes of the rough endoplasmic network; SSNE - saucer-shaped nerve ending.

Fig. 14. Change in the optical density of products of reaction to proteins in the basal parts of receptor cells (according to data from cytophotometry) after single (continuous line) and repeated (dotted line) rotations.

Along the axis of the abcissa - the time passed after rotation; along the ordinate axis - conditional units of optical density (arithmetic means).

acid from the nucleus into the cytoplasm (A. A. Neifakh, 1965). By means of it, there occurs a protein reparation in connection with significant output of structural and functional proteins (enzymes) by the excited receptor cells during their intensive work under the influence of accelerations.

As it turned out, after the reestablishment of cytoplasmic ribonucleic acid in the receptor cells of the utriculus, there begins a process of increasing protein synthesis which, after reaching a certain maximum, decreases a great deal, almost to the norm, and again increases, forming a multipeak curve with the highest point 2 days after a single manipulation (Fig. 14). Repeated manipulations after 1, 2, and 8 hours and after 12 days, apparently, have an unfavorable influence, causing the destruction or even the curtailment of protein synthesis.

The study of the utriculus of vertebrate animals both in a state of relative quiet and under overloads showed that the mitochondria of the receptor cells can enter into the closest contact, i.e., can adjoin the presynaptic membrane. As a rule, a shift in the mitochondria is accompanied by their swelling both in the saucer-shaped and in the bud-shaped nerve endings. The phenomenon of approach of the mitochondria to the presynaptic membrane in the receptor cells of the utriculus has been traced fairly accurately. It may be regularly caused or strengthened by corresponding experimental manipulations, which, in our opinion, is convincing morphological proof of the energy participation of the mitochondrial apparatus in the function of conduction of the nerve impulse, in particular, in the receptor structures of the gravireceptor. This conclusion is confirmed by the results we obtained in cytochemical study of the distribution in the utriculus of vertebrates of oxidizing enzymes, the activity of which in the primary endings of this organ change in regular fashion at various stages of their functioning.

The electron-microscopic and cytochemical data which we obtained indicate that the acetylcholinesterase of the labyrinth participates in the transfer of afferent impulses from the receptor cells to the sensory nerve fiber of the neuron, located in the Scarpa's ganglion. These neurons also reveal enzymatic activity of acetylcholinesterase, especially in bony fishes (L. K. Titova and Ya. A. Vinnikov, 1964; Ya. A. Vinnikov and others, 1965). Both according to our data and according to the literature, the enzymatic activity of acetycholinesterase is found also in the efferent nerve endings of the labyrinth (Dohlman et al., 1958; Dohlman, 1960a, 1960b, Rossi e Cortesina, 1962a, 1962b, 1963; Rossi et al., 1964, etc.). But the affirmation of some authors that only in the efferent endings can impulsation be transmitted with the aid of the cholinergic system seems /21 strange. Moreover, the possible participation of the adrenergic systems in the transmission of a nerve impulse of the receptor labyrinth (Spoendlin, 1966) is not excluded.

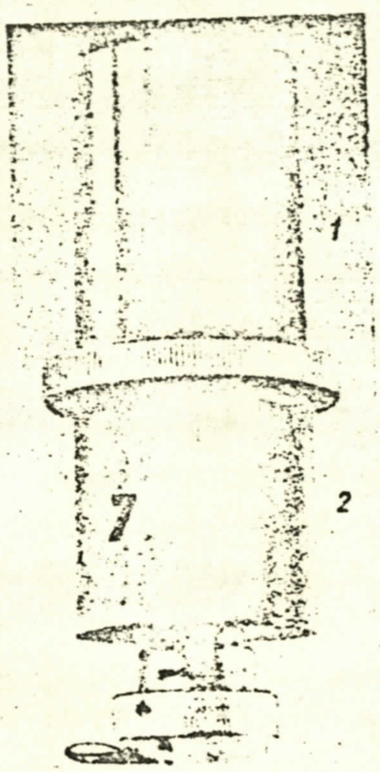

Fig. 15. General view of the EMKON container.
1 - case with embryos; 2 - tank with fixative.

Thus, there arises before us a more or less orderly picture of the structural, cytochemical, and functional organization of the receptor cell of the vestibular apparatus of vertebrates and of those displacements which occur in it both at cellular and subcellular levels. These displacements begin from the moment of deflection of the stereocilia and kinocilia under the influence of cutting forces that develop during the sliding of the

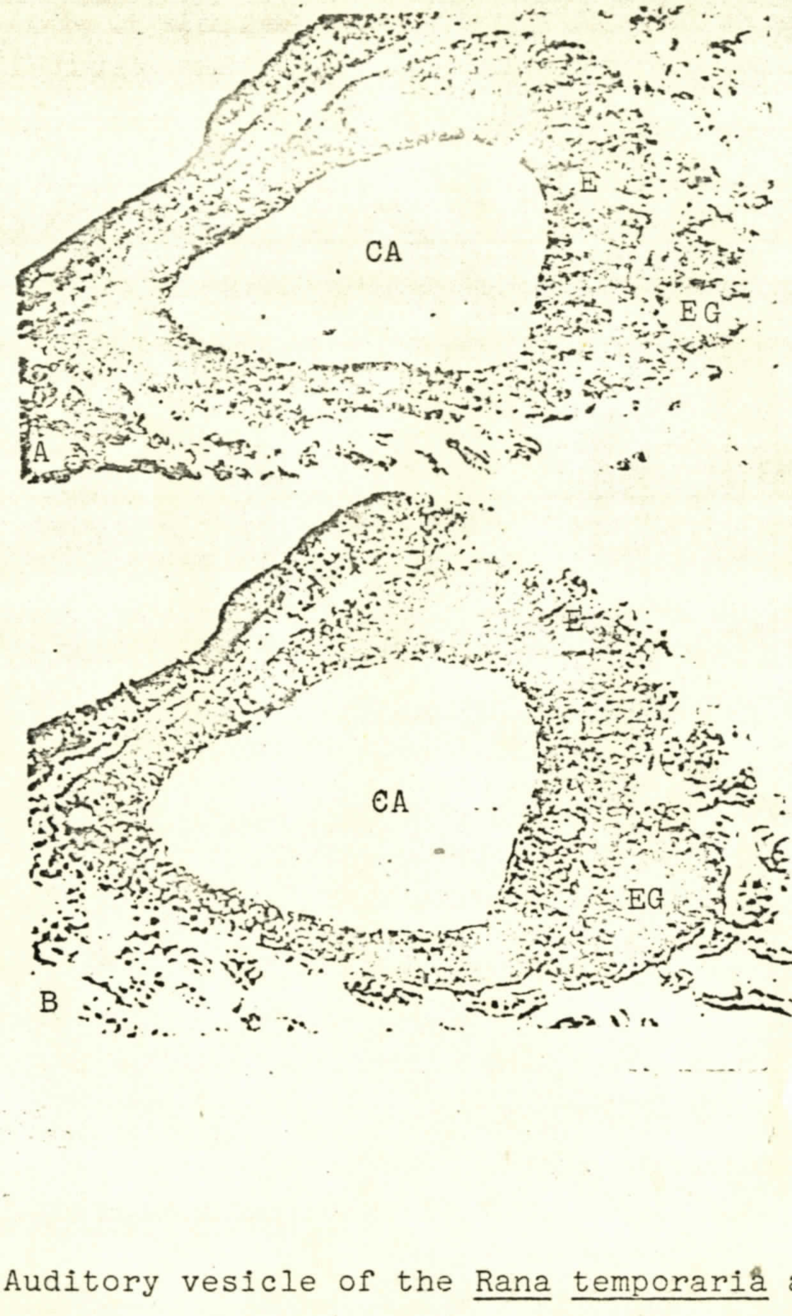

Fig. 16. Auditory vesicle of the Rana temporaria at the tail bud stage.
A - control; B - experiment. CA - cavity of the auditory vesicle;
E -epithelium of the auditory vesicle and macula communis;
EG - establishment of the 8th ganglion.

otolithic membrane in one or another direction and by its rotation caused by a change in the position of the organism in the gravitational field. It must be considered that this mechanism lies at the basis of the function of the gravireceptor of all vertebrates.

B. The development of the vestibular apparatus (labyrinth) of a frog under conditions of weightlessness. In the last section it was already indicated that the receptor cells of the vestibular apparatus of guinea pigs under accelerations of 10 g over the course of 3 minutes function very intensely. The shifts which occurred in ultrastructural organization and in the synthesis of nucleinic acids require at least 12 days for recovery. Consequently, the transition of animals and man after accelerations to conditions of weightlessness, which undoubtedly subjected the vestibular apparatus to new, still unclear functional requirements that the organ, apparently, cannot often fulfill, can be considered the reason for so-called motion sickness. The necessity of studying the influence of weightlessness alone on gravireceptors forced us to think up such a model for an experiment in which animals (the vestibular apparatus) would transfer to a condition of weightlessness without a preliminary stage of acceleration.

It is natural that such a model could be only an embryo in the stage of development when it is still lacks a vestibular apparatus. It was suggested that after overcoming of acceleration by the embryo, the vestibular apparatus would develop under conditions of weightlessness.

Thus, it was necessary to study the development of the vestibular apparatus under conditions of weightlessness and to compare its structural and functional organization with those of embryos

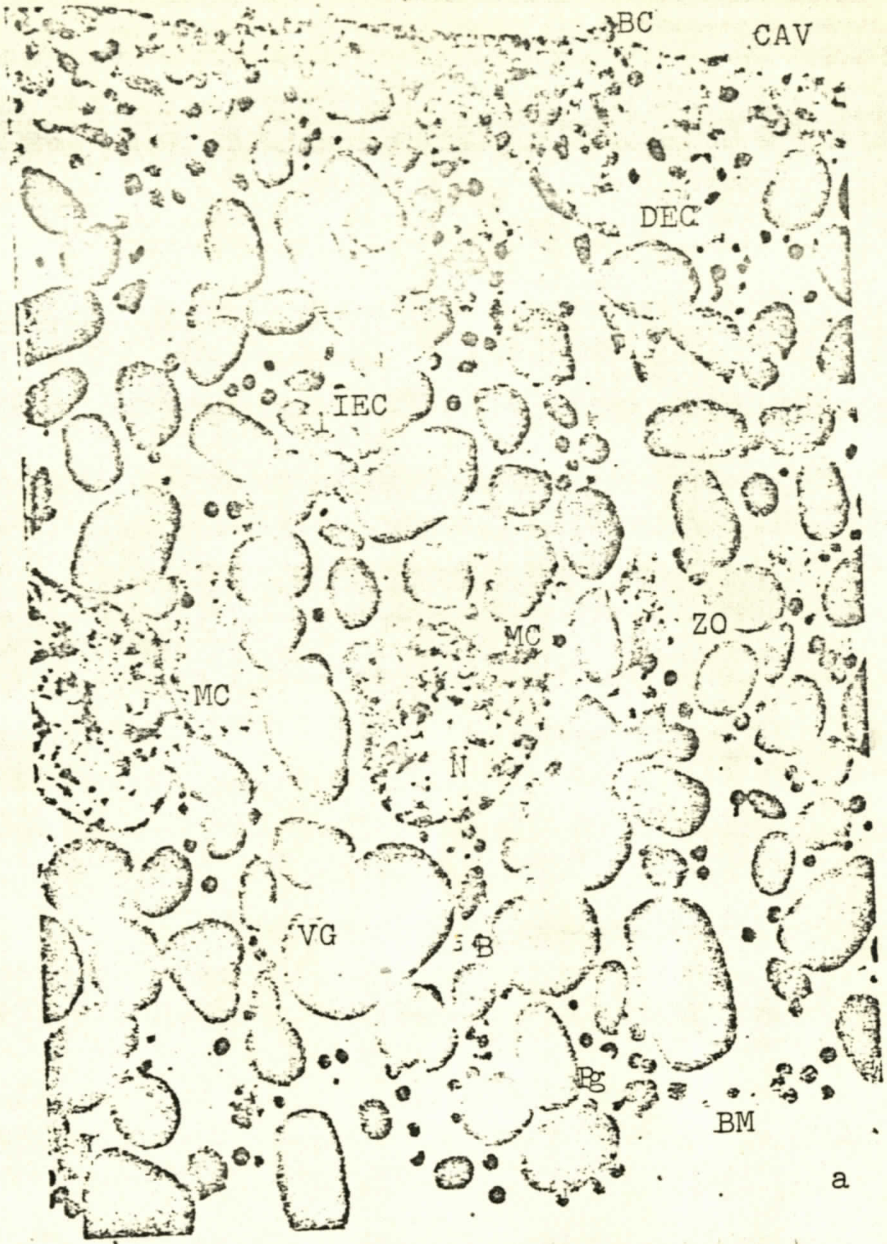

Fig. 17. Wall of the auditory vesicle of the Rana temporaria in the area of the macula communis (experiment).
a - general view in longitudinal section. Magnification 9500.
b - top of the differentiating receptor cell (magnification - 25,000); IEC - indifferent embryonic cell; DEC - differentiating embryonic receptor cell; A - apical part of the cell; B- basal part of the cell; CAV - cavity of auditory vesicle; BM - basal membrane; VG - vitelline granules; Pg - pigment; M - mitochondria; Mc - mitochondria containing crystals; N - nucleus; BC - basal corpuscle; MC - intercellular cracks; D - Desmosoma; ZO - zona occludens; K - kinocilium, S - stereocilia; CB - cuticular border.

and animals under the conditions of the gravitational field of the earth. The objects of the study were fertilized eggs of amphibians (Rana temporaria) (Ya. A. Vinnikov, and assistants, 1972.

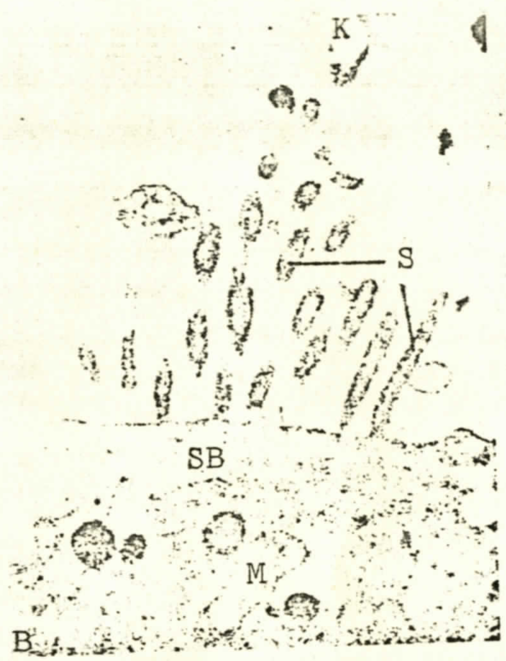

Fig. 17 (continued).

The task of studying the influence of weightlessness on the develoment of the vestibular apparatus of amphibians required the development of a special instrument for life support of the developing eggs and the subsequent fixation of the animals under the conditions of space flights (EMKON) (Fig. 15).

In the present report data are presented which were obtained in a flight experiment made by the crew of the "Soyuz 10" spaceship. The experiments were made on eggs which were artificially /2 fertilized under ground conditions 43 hours before the start and which at the moment of flight were in the late blastula and

and early gastrula stages. The embryos which developed under conditions of weightlessness were fixed at the end of 2 days of flight (40 hours), i.e., in the fourth day after fertilization.

The factual material obtained shows that under conditions of a 2-day stay of the fertilized egg, and later of the embryo of the frog Rana temporaria under ground conditions and a subsequent stay for two days in a condition of weightlessness, development of the embryo in the EMKON container did not cease. In external appearance such an embryo does not actually differ from the four-day-old control embryo which remained under the influence of the changed gravitational field of the earth during the entire time. The embryos, both control and experimental, reached the tail bud stage of development. The embryo proceeded through the first stages of division and the subsequent stages of blastula and early gastrula under conditions of the earth. Then, in the stage of gastrula the embryo, having been subjected to the action of accelerations in ascent, continued to develop further for 40 hours under conditions of weightlessness, when it transfered into the neurula stage, and then into the tail bud stage in which it was fixed. Judging by the results obtained, the last two stages proceeded in the same way under conditions of weightlessness as under the influence of the gravitational field of the earth. And if there were some deviations connected with the action of short-term acceleration and vibration, they were regulated in the subsequent development under conditions of weightlessness. As is known, in these stages the embryos of amphibians are still distinguished by a rather wide range of regulation (Geksli and de Ber, 1936). Judging by the structure of the auditory vesicles which developed under conditions of weightlessness, which in no way differ from the control ones, it cannot be doubted that their organization, induced by the medulla oblongata and the processes of formation connected with submergence and with subsequent constriction from the ectoderm under conditions of weightlessness, proceeded just as in the control. The experiment was set up so that the embryo in the early gastrula stage was subjected to accelerations at the moment of the takeoff of the space machine when the organization of the future vestibular apparatus was still absent. This allowed the elimination of the influence of acceleration on the development of the auditory vesicle, which, as our experiments on a definite vestibular apparatus showed, has a substantial influence on the structural, cytochemical, and functional organization of the receptor cells.

Under conditions of weightlessness there occurs not only the formation of an auditory vesicle (Fig. 16, a, b), but also the beginning of the process of organ differentiation; the *macula communis* is established and the rudiment of the 8th ganglion is formed. At the same time, in individual sectors of the *macula communis* processes of cytological differentiation begin, noticeable in the beginning of the formation of individual receptor and support cells (Figs. 17 a, b, 18). The tops of these cells are differentiated basically, preceded by the liberation of the apical part of the cell from the vitelline and pigmentary granules and the concentration at this spot of the mitochondria, which testifies to the strengthening of energy processes in the differentiating sectors of the cell. Evidently, in the last stages of embryogenesis, these cells in division of the *macula communis* enter the location of the future maculae of the utriculus, sacculus, lagenae, and crests of the ampullae of the semicircular canals and are transformed into definite receptor cells. The possibility of establishing their further development under conditions of weightlessness requires further experiments. /24

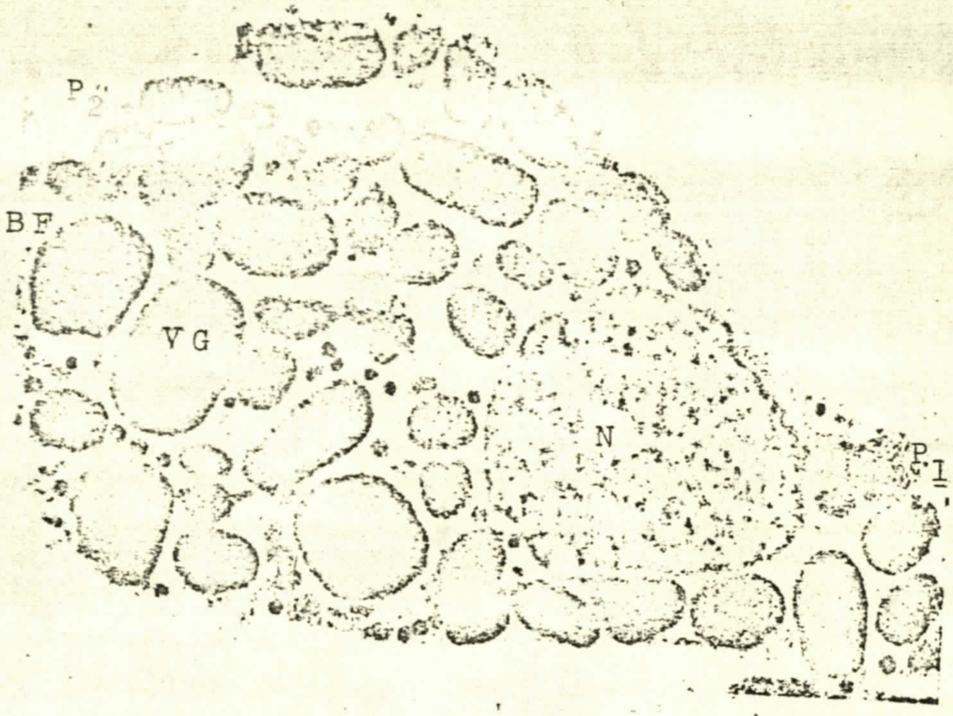

Fig. 18. Neuroblast from the 8th ganglion (experiment). Magnification - 11,000. P_1 - P_2 - opposite poles of the neuroblast:

BF - beginning of the formation of nerve outgrowths; N - nucleus; M - mitochondria; VG - vitelline granules.

In the embryonic 8th ganglion, both in the control and in the experiment, cytological differentiation is expressed in the beginning of the formation of bipolar neuroblasts of pear-shaped form with two cell outgrowths, located polarly (cf. Fig. 18), and also in the innervation of the differentiating cells in the macula communis. Thus, under conditions in which the frog embryos stayed for two days in the EMKON container in a condition of weightlessness, their development did not differ from the devleopment of the control embryos. The conditions of weightlessness have practically no effect on the development of frog embryos in the stage of neurala and tail bud and in the formation of organs which accompanies this stage and the beginning of cytological differentiation, including that of the future vestibular apparatus. Of course, only further studies with more lengthy periods of stay of the embryos in weighlessness will show to what extent this conclusion may be extended also to the later stages of development of the embryo and its transformation into a larvum and later into a grown animal. And it will also be possible to establish to what extent conditions of weighlessness influence the structural and functional organization of the vestibular apparatus.

Conclusion

Thus, a similar model of the gravireceptor is created in the process of the evolution of invertebrates and vertebrates. Despite the completely different origin and path of evolution, the statocysts and the vestibular apparatus of the vertebrates possess a surprising similarity both in the organic, the cellular, and subcellular levels of organization. The similarity in organization permits prediction of both the behavior of the gravireceptors as a whole and of the behavior of individual elements and transformations which are made in them under conditions of both the normal and changed gravitational field. In this regard, we have undertaken the first experiments so far on the vestibular apparatus of vertebrates. /25