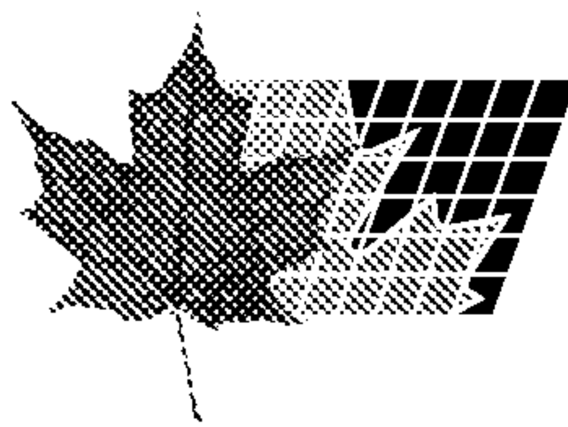

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(54) **UTILISATION DE PRINCIPES ACTIFS A EFFET BIOLOGIQUE POUR INFLUER SUR L'ESPACE EXTRACELLULAIRE DE CELLULES SENSORIELLES ET PROCEDE DE COMMANDE D'ADMINISTRATION DE PRINCIPE ACTIF ET DISPOSITIF APPROPRIE**

(54) **THE USE OF BIOLOGICALLY ACTIVE SUBSTANCES FOR INFLUENCING THE EXTRACELLULAR AREA OF SENSORY CELLS AND METHOD FOR CONTROLLING THE ADMINISTRATION OF ACTIVE SUBSTANCES AND DEVICE USED THEREIN**

(57) L'invention concerne l'utilisation d'un principe actif influant sur l'homéostasie du calcium de cellules pour traiter les dégénérescences de cellules sensorielles et de cellules adjacentes.

(57) The invention relates to the use of an active substance influencing the calcium homeostasis of cells to treat degeneration of sensory cells and adjacent cells.

ABSTRACT

This invention concerns the use of an active substance that influences calcium homeostasis of cells for treatment of degenerative diseases of sensory cells and nearby
5 cells.

THE USE OF BIOLOGICALLY ACTIVE SUBSTANCES FOR INFLUENCING THE EXTRACELLULAR AREA OF SENSORY CELLS AND METHOD FOR CONTROLLING THE ADMINSTRATION OF ACTIVE SUBSTANCES AND DEVICE USED THEREIN

This invention concerns the use of at least one bioactive substance for influencing the extracellular space in the vicinity of sensory cells of mammals, in particular humans with acquired or hereditary degeneration of sensory cells and/or adjacent epithelial cells, glia cells or supporting cells, for treatment of the intracellular milieu of the sensory cells, nearby epithelial cells, glia cells and/or supporting cells according to the definition of Patent Claims 1 and 2, as well as a method for controlling the distribution of administered active substances according to the definition of the method claim.

There are a number of known pathological conditions in which the normal visual, auditory, and/or vestibular functions of humans and mammals become permanently disturbed or lost due to genetically inherited diseases or due to acquired disorders such as infections, injuries, postoperative complications, unphysiological stress on sensory cells or as side effects of treatment with an active substance. For example, retinal degeneration may be inherited or caused by light in animals such as rodents, cats and dogs (Organisciak and Winkler, 1994). Examples of retinal degeneration in humans include: stationary night blindness, retinitis pigmentosa, rod-cone degeneration or dystrophy, cone-rod degeneration or dystrophy, macular degeneration or dystrophy, Stargardt disease, pattern dystrophy, fundus flavimaculatus, Sorsby's fundus dystrophy, myopic degeneration, Refsum disease, choroideremia and punctus albinopunctatus. In humans, vision may become seriously disturbed or even lost as a result of retinal infection, ischemic damage to the outer retina or surgical treatment of retinal detachment, and night vision may be lost after chemotherapy with vincristine. Usher syndrome in humans is characterized by defects in visual, auditory, and vestibular function. Humans with Bardet-Biedl syndrome and Leber's congenital amaurosis have disturbances in visual systems and other sensory systems. Numerous acquired

degenerative conditions in humans are also known in which sensory cells and nearby cells in the retina, the organ of Corti and/or the vestibular organ become damaged, e.g., as a result of a deficiency in physiological sensory stimulation, age-related sensory cell degeneration, unphysiological noise levels or as unphysiological whiplash. The normal function of the human pineal gland is not well understood. However, the pineal gland contains cells that are closely related to retinal photoreceptors (e.g., they synthesize the visual pigment opsin and the hormone melatonin) (Armstrong, 1998).

In recent years, scientists have made great advances in the understanding of photoreceptors and some of these forms of sensory cell degeneration.

The essential biochemical reactions of phototransduction are known for rod and cone photoreceptors, and many of the proteins that are involved have been isolated and identified (Pugh and Lamb, 1990, 1993; Molday, 1994; 1996; Azarian et al., 1995; Williams, 1995). The mechanism by which rod and cone photoreceptors renew their outer segments, the structure of the outer segments and the specific molecules that are renewed in this process are known to some extent. The role of the photoreceptor cytoskeleton in this renewal is partially understood, as is the role of the pigment epithelium in surrounding and digesting the outer segment fragments that are shed (Amos and Amos, 1991; Molday, 1994; Williams, 1995; Eckmiller, 1997).

Molecular genetic studies have identified several genetic defects that lead to retinitis pigmentosa and similar functional disorders, and in many cases the proteins encoded by these genes are also known (Bok et al., 1993; Milam, 1993; Kemp et al., 1994; Wong, 1994; Bird, 1995; Dryja and Berson, 1995; Papermaster and Windle, 1995; Weil et al., 1995; Papermaster, 1997; Steele, 1995; Travis, 1997).

It is known that in some cases of retinal degeneration (such as retinitis pigmentosa), the photoreceptors ultimately die by a process that is referred to as "programmed cell death" or apoptosis (Wong, 1944; Papermaster and Windle, 1995; Papermaster, 1997).

One disease that leads to retinal degeneration in humans, namely Refsum syndrome, has

been recognized as due to a dietary deficiency and is treated by increasing the administration of vitamin A. A study conducted recently by Berson et al. (1993) based on series of clinical trials in humans has shown that the rate of progression of retinal degeneration in patients with retinitis pigmentosa can be somewhat slowed by high
5 intake of vitamin A. Scientists have studied possible therapies for treating retinal degeneration in animals by transplantation of healthy retinal cells, by gene therapy, and by administration of survival factors or growth factors (Bok et al., 1993; Milam, 1993).

The rate of light-induced and inherited retinal degeneration in animals can be slightly slowed by administering certain survival factors or growth factors. This approach is also being investigated for humans.

5 For example, International Patent Application WO 93/15608 describes a method for reducing or preventing degeneration of retinal neurons in mammals that is caused by exposure to light or other environmental traumas, by administering therapeutically effective doses of the following substances before, during or following this exposure: a neurotrophic factor, preferably neurotrophin-3, neurotrophin-4, BNDF, CNTF, a
10 leukemia inhibiting factor, acid FGF, basic FGF with heparin, acid FGF with heparin, IL-1β, and TNF-α. The method mentioned above is also based on reducing or preventing degeneration of retinal neurons in mammals due to specific diseases, as explained in detail there in Claim 1. However, the object of the present invention as claimed here is neither described nor suggested there.

15

U.S. Patent No. 5,444,042 concerns a method for treating neurological degeneration of the brain in mammals that is caused by ischemia, for example due to a stroke, a heart attack, brain surgery, multiple infarct degeneration or a subarachnoid hemorrhage, where after a diagnosis of ischemia has been made, the patient is administered a
20 therapeutically effective dose of a calpain inhibitor, i.e., a peptide ketoamide compound or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable vehicle. However, the object of the present invention as claimed here is neither anticipated nor suggested by said publication.

25 International Patent Application WO 94/21817 concerns a method for assessing whether a host is susceptible to a reversible antigen-induced immunodeficiency, by first examining a lymphocyte specimen taken from the host for the presence of programmed cell death and then determining whether a calpain inhibitor can prevent the programmed cell death in the lymphocytes and thus restore lymphocyte function. As documented by
30 examples, the host is preferably and exclusively a human infected with HIV. In the remaining method for inhibition of calpain-mediated programmed cell death in mammalian cells, the cells are treated with a calpain inhibitor and said treatment is

performed both *in vivo* and *ex vivo*. Here again, this is done only for treatment of viral diseases, preferably HIV, so the object of the present invention as claimed here is neither anticipated nor suggested.

5 International Patent Application WO 90/06123 concerns a method for treating a patient who has ischemia or edema of the retina or the optic nerve, where the patient is treated with a therapeutically effective amount of a calcium entry blocker, preferably a calcium channel antagonist. These are preferably special dihydropyridine derivatives, diphenylpiperazines or benzothiazepines. In addition, this patent application also 10 concerns a corresponding prophylactic treatment. However, the treatment of other diseases according to the present invention is neither anticipated nor suggested by this treatment of ischemia or edema with these active substances.

The object of International Patent No. WO 92/17173 is the use of riboflavin (vitamin 15 B2) for production of a medication for prophylactic or therapeutic treatment of viral diseases such as diseases caused by HIV, herpes, malaria or retinitis pigmentosa. Since no vitamins are used as active substances according to the present invention, the object of the present invention as claimed here is neither anticipated nor suggested.

20 U.S. Patent No. 5,421,818 concerns a device for therapeutic treatment of the middle ear and the inner ear, by which active substances can be administered to the inner ear through a membrane. However, since the object and the method of achieving it are obviously different from those according to the present invention, the object of the present invention as claimed here is neither anticipated nor suggested.

25

International Patent Application WO 96/41638 discloses a method for stabilizing or improving vision of human eyes with macular degeneration, consisting of administering at least 500 ng of growth factor and the peptide transforming growth factor β (TGF-β) to humans. The aforementioned method preferably includes injection of at least a 30 portion of the active substance into the subretinal space and the remainder directly above the part of the retina to be treated. Since no growth factors are used as active substances according to the present invention, the object of the present invention is

neither anticipated nor suggested.

European Patent No. 681,840 A concerns the use of phosphate diesters of vitamins C and E for production of a pharmaceutical composition for prophylactic and therapeutic
5 treatment of diseases of the retina in humans. Since no vitamins are used as active substances according to the present invention, the object of the present invention as claimed here is neither anticipated nor suggested.

U.S. Patent No. 5,281,607 A concerns the treatment of degenerative neurological
10 diseases and/or trauma of the central nervous system by stimulating endogenous or *in vivo* recombinant expression of a nerve growth factor (NGF) in the central nervous system by administration to the central nervous system of a NGF in an effective quantity of a β-agonist, an α_1-agonist and/or an α_2-agonist. These include preferably dobutamine, prenaterol, clenbuterol, isoproterenol, epinephrine, fenoterol, albuterol,
15 terbutaline, metaproterenol, salbutamol, zinterol, rimiterol, tazolol, phenylephrine, methoxamine, circazoline, modafinine, yohimbine, folazoline, idaxozan, and atipamizol. Since no α_1-agonists, α_2-agonists or β-agonists are used as active substances according to the present invention, the object of the present invention as claimed here is neither anticipated nor suggested.

20

U.S. Patent No. 5,457,135 A concerns a method for treating age-related macular degeneration in a patient suffering from this condition, by administering at least 120 mg β-carotene to the patient each day, preferably systemically. Since no vitamins are used as active substances according to the present invention, the object of the present
25 invention as claimed here is neither anticipated nor suggested.

U.S. Patent No. 5, 596,011 A concerns a method for treating macular degeneration, i.e., an eye disease that occurs in humans of advanced age, by administering to the person suffering from this condition an effective dose of a glutathione-potentiating agent in
30 order to increase the intracellular glutathione content. The glutathione-potentiating agent is preferably administered together with an antioxidant, preferably a vitamin, or in conjunction with at least one anti-inflammatory agent, preferably interferon-α. The

preferred glutathione-potentiating agents specifically include N-acetylcysteine and similar cysteine derivatives, L-2-oxothiazoline-4-carboxylate and mercaptopropionone glycine. This class of substances is excluded from the scope of protection by a disclaimer. Furthermore, since the object of this U.S. patent exclusively concerns increasing the intracellular glutathione content and the resulting shrinkage of pathologically swollen glands in age-related macular degeneration, it does not anticipate the object of the present invention.

U.S. Patent No. A 5, 527,533 describes the use of astaxanthine for prophylactic or therapeutic treatment of injuries or degenerative diseases of the human central nervous system or the human eye, such as age-related macular degeneration, damage to due light, ischemia or inflammations. Since astaxanthine is not used as an active substance according to the present invention, the object of the present invention as claimed here is neither anticipated nor suggested.

A number of methods are known for administration of substances into the vitreous humor and the subretinal space of the human eye (Ogura and Kimura, 1995; Tasman and Jaeger, 1996).

The structure, function and regulation of "calpain" enzymes (i.e., calcium-activated proteases) are known for many types of cells. Various inhibitors of these two enzymes (type I and type II) have been prepared, which can inhibit these enzymes specifically and can in some cases even penetrate into the cells (Wang, 1990; Croall and Demartino, 1991; Mehdi, 1991; Michetti et al., 1995).

A number of measurement methods and analytical methods are known for spatial and temporal determination of functional and structural parameters of the retina and its components (Sabel et al., 1997; Tasman and Jaeger, 1996).

A number of compartment models are known for simulation of biological cells and their interactions (Hartline, 1989; Assimakopoulos et al., 1991; Pascoletti, 1991; Marder and Selverstone, 1992; Hymel et al., 1995).

A number of computer models and methods are known for controlling or regulating sensory guided processes (Gupta and Sinha, 1996).

Despite all efforts, a common cell biological mechanism underlying these sensory cell diseases has not yet been identified, and no satisfactory treatments for these diseases are known (Milan, 1993; Bok et al., 1993; Wong, 1994; Bird, 1995; Dryja and Berson, 1995; Papermaster, 1997; Steele, 1997; Travis, 1997). Transplantation of photoreceptor
5 cells and pigment epithelial cells has yielded only very limited success, and this approach is also made difficult by the small number of sources of donor tissue, as reported by Milam (1993).

The occurrence of inherited retinal degeneration has been prevented in individual
10 animals by genetic therapy during ontogenesis, e.g., by transfection of the healthy gene into the fertilized egg. Current animal research is also studying the possibility of treating retinal degeneration by intraocular administration of the healthy gene, although the genetic diversity of human retinal degenerations means that the different defective genes in different patients must first be identified and then replaced.

15

It is known from the article by Campochiaro et al. "Adenosine and its agonists cause retinal vasodilation and hemorrhages" in: *Arch. Ophthalmol.*, vol. 107, March 1989, no. 3, pp. 412-416, that adenosine and some adenosine agonists cause vascular dilation and blood effusions in the retina when these active substances are injected into the vitreous
20 humor of the eye. It has been proposed that these active substances could be used for treatment of degenerative eye diseases that are based on inadequate blood circulation.

The closest state of the art is known from the Russian Patent Application SU 1,297,862 Al, which proposes the use of a 1 % solution of sodium adenosine triphosphate (ATP)
25 for treatment of hereditary pigment degeneration of the retina. ATP is to be administered intramuscularly. The treatment also includes concomitant treatment with microwaves, oxygen baths and doses of vitamin B6. A directed influence on the intracellular milieu of the sensory cells would not be achieved through such nonspecific intramuscular administration of the active substance, and a directed topical
30 administration in the area of the damaged sensory cells is not proposed.

In summary, no treatments are presently known for preventing the onset of such

inherited or acquired sensory cell degenerations in humans or mammals, or for significantly delaying or stopping the progression of these degenerative conditions.

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The sensory cells of various sensory organs in vertebrates are closely related cells: their structure (a cilium is an important component of the receptor cell), their function (transduction of an external sensory stimulus is converted into a change in membrane potential), and their biochemical reactions have many similarities. The rod photoreceptors of the eye are the best known and the most extensively investigated type of sensory cell.

Rods are highly specialized cells, each having an outer segment in which phototransduction takes place. Outer segments have a complex but well ordered structure. They consist of cytoplasmic compartments that are separated by many foldings of membrane, which contain protein molecules (such as rhodopsin). The protein molecules are held at suitable locations within the membranes by binding to components of an internal cytoskeleton, e.g., to supportive microtubules. A portion of each outer segment is renewed each day. Newly synthesized components are incorporated into membranes at the base of the outer segment, packets of older membranes are shed from the tip of the outer segment and are ingested and degraded (phagocytosed) by adjacent pigment epithelial cells. Numerous biochemical reactions of phototransduction are known. The state of light adaptation of the cell is regulated via the intracellular concentration of free calcium. The calcium concentration can thus continuously change, which modulates the binding of certain calcium-binding proteins to certain enzymes, whose activity is thereby modulated. For example, the activity of the protease calpain is inhibited by binding of calpastatin, by oxidation of its sulfhydryl groups, by a pH that is not neutral, and/or by a low calcium concentration. When these parameters are altered so that calpain is activated, calpain can cause hydrolytic degradation of certain proteins (e.g., of tubulin and the CNG (cyclic nucleotide gated) channel) or cause irreversible modulation of specific proteins such as rhodopsin and arrestin. These reactions of calpain are important for normal phototransduction, adaptation, and outer segment tip shedding during outer segment renewal.

In humans with some forms of hereditary retinal degeneration, such as retinitis pigmentosa, one of the first pathological changes is a disturbance in adaptation of rod cells, which is indicative of a disturbance in their calcium homeostasis. The calcium

concentrations may be too high or too low, or they may follow the change in light level either too slowly or too rapidly. Such disturbances in calcium concentration can have different results. Since calcium-induced activation of calpain is involved in the normal shedding of the outer segment tip, if the calcium concentration is too low there is too
5 little shedding, so the rod outer segments grow to an abnormally long length, become mechanically unstable and lose their normal structure and function. If the calcium concentration is too high there is increased shedding, so the rod outer segments become abnormally short, which reduces their light sensitivity. If the change in calcium concentration takes place too rapidly or too slowly, this can have a negative effect on
10 the time-dependent coupling of various biochemical reactions, e.g., it can slow the shedding process, which normally occurs without leaks, i.e., without undue membrane damage. The membranes can become temporarily opened, which would allow an uncontrolled transfer of cytoplasmic components to occur from the rod outer segments into the subretinal space. Calpain can thus be released and it can become activated in an
15 uncontrolled manner due to the relatively high calcium content in the subretinal space, which can have an abnormal extracellular proteolytic effect on nearby cells.

Although in many cases these hereditary forms of degeneration of the human retina are caused by a genetic defect in a single protein within the rod cells (but not within the
20 cone cells), after an initial phase of progressive degeneration of rods (that leads to night blindness) there is a phase of progressive degeneration of cones (that leads to complete blindness). This disease course confirms that the degenerating rod cells release into the subretinal space toxic factors, which then secondarily attack the cone cells and cause their degeneration. The pathological effects are caused by disturbed calcium
25 homeostasis in the region of the rod outer segments. Therefore, one object of the present invention is to treat sensory cell degenerations that are based on disturbed calcium homeostasis by using appropriate active substances.

This object is achieved by a use having the features of Claim 1.

30

Another object of the present invention is to provide for the use of a special bioactive substance for influencing the intracellular milieu of sensory cells that are at risk of

degeneration, the milieu of nearby epithelial cells, the milieu of nearby glia cells and/or the milieu of nearby supporting cells of mammals by treating the milieu of the common extracellular space in their vicinity for prophylactic and therapeutic purposes. An intracellular or extracellular milieu in the sense of the present invention is understood to
5 refer to the milieu of a volume filled with various ionic species and biomolecules in different concentrations, i.e., the totality of the biophysical and biochemical parameters describing the functional state of the respective space.

This object is achieved through the active substances that are administered and made to
10 act in a specific manner according to Claim 2.

For the case of the retina, this object is achieved in particular on the basis of measurement of the spatial and temporal distribution and concentrations of substances in different types of cells that are at risk of degeneration, through a demand-controlled
15 release of active substance(s) in time and space according to the characterizing part of the claim.

Therefore, the present invention concerns the use of at least one bioactive substance for treatment of the intracellular milieu of the sensory cells and/or nearby epithelial cells
20 and/or nearby glia cells and/or the supporting cells of mammals having acquired and/or hereditary degenerative conditions, characterized in that a physiologically active amount of a compound containing alkaline earth metal(s) or modifying the effect thereof and/or a nucleotide and/or an enzyme inhibitor and/or an enzyme activator and/or a protein and/or a peptide and/or a nitrogen oxide-modifying compound and/or a calcium chelator
25 or buffer and/or a cytoskeleton-modifying active substance and/or a calcium channel blocker or a calcium antagonist and/or a calmodulin antagonist and/or a cationophore is administered directly outside these cells and is made to act within these cells.

It is advantageous that various substances administered into the extracellular space in
30 the vicinity of sensory cells are capable of altering the extracellular milieu and penetrating from there through the partially permeable cell membranes into the intracellular spaces of the nearby sensory cells, epithelial cells, glia cells and/or

supporting cells, and can thus influence their respective intracellular milieus in a prophylactic or a therapeutic manner. It is also advantageous through the invention disclosed here that, by a comprehensive neutralization of the pathological disturbances in the milieu of the common extracellular space, the nearby normal sensory cells can be
5 protected from these pathological disturbances in the extracellular milieu that are caused in particular by pathologically impaired sensory cells, thereby halting the spread of the cellular degeneration. Furthermore, it is advantageous that through the invention disclosed here a number of different acquired and/or hereditary forms of degeneration can be similarly treated by modification of the extracellular milieu, for example without
10 precise knowledge of the respective genetic cause of the disease.

Furthermore, it is advantageous that it is possible to employ a common principle for treating hereditary degenerations in which a single genetic defect affects several types of sensory cells such as that observed in Usher syndrome, with degenerative impairment
15 of the sensory cells in the eye, the auditory organ, and the vestibular organ, in addition to other degenerative diseases that may also affect the pinealocytes of the pineal gland, which are similar to photoreceptors in terms of morphology, biophysics and biochemistry.

20 It is also advantageous that because of biophysical and biochemical interactions, through the invention disclosed here the intracellular milieu within the sensory cells, as well as the nearby epithelial cells, glia cells, and supporting cells, can be influenced in a prophylactic or a therapeutic manner by changing the milieu in their common extracellular space.

25

Furthermore, it is advantageous that the invention disclosed here is applicable to both humans and other mammals because of the great structural, functional and biochemical similarities between their respective cell types, which both expands its scope and facilitates the further development of therapeutic details.

Furthermore, it is advantageous that the bioactive substances used here are readily available and that many of these active substances are already being used in humans for other medical applications (e.g., calcium channel antagonists or calcium agonists that are used clinically to treat hypertension, angina pectoris and/or arrhythmias) or have been proposed for such applications (e.g., calpain inhibitors in neurodegenerative diseases of the brain that are caused by ischemia, e.g., after a heart attack, neurosurgery or head injuries).

It is also advantageous that when using implanted microcontainers in particular, the active substance can be released in a dose-controlled manner in both time and space in treatment of the retina in particular. Another advantage of the invention disclosed here is that a reduction in the pathological impairment of the cells thus affected, as well as protection of normal cells from impairment by pathological cells in their vicinity, can be achieved with a common treatment principle. It is also advantageous that using a sensory guided control principle, on the basis of the distribution of relevant cell types and substances or concentrations in time and space, and on the basis of suitable multidimensional mapping and analysis of these measured values by a control system or a regulatory circuit with sensory feedback, the release of the active substance can be controlled according to need, and thus it is possible to achieve a need-regulated distribution of active substance with minimal possible side effects.

Furthermore, it is advantageous that a great variety of modern diagnostic and analytical systems are available in ophthalmology, which allow for the most accurate possible determination of the relevant retinal parameters as a function of time and space; functional and structural parameters in defined cell layers of the retina *in vivo* can thereby be detected with good resolution, not only along the retinal surface but also at various retinal depths, which can be used to establish the optimum distribution of active substance for controlled application in the given individual.

Furthermore, it is advantageous that through the invention disclosed here for the treatment of retinal degeneration, in particular the pathologically altered photoreceptor outer segment renewal process is treated and/or its pathological effects on the

extracellular milieu are counteracted. Furthermore, it is also particularly advantageous that forms of retinitis pigmentosa or macular degeneration that are due to very different genetic causes can be treated according to a single principle, without having to identify the specific genetic defect, which in many cases cannot be identified at present.
5 Furthermore, it is advantageous that the treatment of photoreceptor degeneration disclosed here is initiated at a very early stage of the disease, in contrast with therapies that attempt to stop the process of apoptosis ("programmed cell death" that ultimately leads to death of receptors in retinitis pigmentosa), which occurs at the end of receptor degeneration. It is especially advantageous that through the treatment disclosed here, it
10 is possible to reverse, or to prevent, the degenerative process.

Furthermore, it is advantageous that control or regulation of the release of the active substance that is distributed over time and space is based on a multi-compartment model, in particular in the case of the retina, as a coupled differential equation system
15 for taking into account the concentrations and mass flows between the common compartment of the extracellular space and the compartments of the multiple adjacent intracellular spaces of photoreceptors, pigment epithelial cells, Müller glia cells and/or supporting cells, and is also based on a dynamic learning computer model for controlling or regulating the individual intracellular milieus by supplying an active
20 substance at a given location in the extracellular space, taking into account retinal measurement data distributed in time and place.

When melanin particles having a size of approximately 1 μm to 20 μm are administered into the extracellular space, they can remain there for a long time and serve as
25 adsorption agents for toxic factors, reducing these factors in the extracellular milieu.

Furthermore, it is advantageous that by influencing the milieu of the subretinal space, it is possible to optimize the function of the pigment epithelial cells, which make an important contribution to the nutrition of the photoreceptor cells and to the outer
30 segment renewal process by phagocytizing the tips that are shed from outer segments, and which are themselves to some extent at risk of degeneration (e.g., in macular degeneration). Thus, for example, the form and material of microcontainers implanted

in the subretinal space can be designed so that they can fulfill their function as dispensers of the active substance for a long time but are not subject to premature phagocytosis by the pigment epithelial cells and also do not interfere with the normal phagocytic process. For the specific purpose of treating pathologically altered pigment epithelial cells, it is advantageous that suitably designed microcontainers filled with active substances are introduced into the subretinal space, so that they become surrounded and/or phagocytized by pigment epithelial cells but they are able to treat the intracellular milieu of the pigment epithelial cells by delayed release of their active agent for a long time, before they are ultimately digested.

Furthermore, it is advantageous that through the treatment disclosed here the adjacent retinal Müller glia cells, which are themselves to some extent at risk of degeneration, are influenced so as to achieve and maintain the normal intracellular milieu of the photoreceptors.

Furthermore, it is advantageous that the intracellular milieu of pinealocytes, which have morphological, biophysical and biochemical similarities with retinal photoreceptors and are at risk of degeneration, and of nearby cells in pineal gland, are influenced in a prophylactic or therapeutic manner according to the disclosed treatment principle.

Furthermore, it is advantageous that the intracellular milieu of hair cells in the organ of Corti of the inner ear, which are at risk of degeneration, as well as that of the adjacent epithelial cells, glia cells and supporting cells, are modified for prophylactic or therapeutic purposes through the release of active substance into the surrounding perilymphatic space, which has a diffusion connection to the adjacent endolymphatic space and the extracellular space of the hair cells and the surrounding epithelial cells, glia cells and supporting cells.

Furthermore, it is advantageous that the intracellular milieu of hair cells in the vestibular system close to the inner ear, which are at risk of degeneration as well as that of the nearby epithelial cells, glia cells and supporting cells, of the crista ampularis of the semicircular canal organs or the macula utriculi or macula saccului of the vestibulostatic

organs are modified for prophylactic or therapeutic purposes by the release of active substance into the surrounding perilymphatic space (which communicates directly with the nearby perilymphatic space of the inner ear) that has a diffusion connection to the nearby endolymphatic space and extracellular space of the hair cells.

5

Furthermore, it is advantageous for treatment of sensory cells that endogenous cells or subcellular structures or biomolecules, taken from suitable locations in the human patient or from healthy humans or mammals, are modified *ex vivo* and then (re)introduced into the patient, i.e., into the extracellular space to have a positive
10 influence on the local milieu while avoiding possible immune responses. In particular, such endogenous structures can be "loaded" or "treated" with concentrations of active substance(s) that are definitely higher than would be possible or tolerable by direct injection into the extracellular space.

15 Finally, it is advantageous that with the invention disclosed here, the above-mentioned sensory degenerative diseases that could previously be neither prevented nor cured can now be treated both prophylactically and therapeutically. The common treatment principle disclosed here can be used to advantage because the diseases in cells of different sensory organs can have partly common causes, e.g., they can be due to
20 genetic factors.

An advantageous embodiment of the treatment of the intracellular milieu of sensory cells of mammals according to the present invention consists of minimizing the disturbing influence on the surrounding extracellular space by modifying the
25 extracellular milieu through controlled sensory-guided administration of calpain inhibitor.

According to a preferred embodiment of the present invention, the sensory cells and/or adjacent epithelial cells and/or glia cells and/or supporting cells include those of the
30 retina and/or the auditory organ and/or the vestibular organ and/or the pineal gland.

According to another preferred embodiment, the cells to be influenced through the

extracellular space in the case of the retina are photoreceptor cells and/or the nearby pigment epithelial cells and/or retinal Müller glia cells, which interact with the extracellular space and with one another.

5 According to another preferred embodiment, the mammal is a human or a domestic animal or a pet.

According to another preferred embodiment, the acquired and/or hereditary degeneration of sensory cells and/or epithelial cells and/or glia cells and/or supporting
10 cells in the case of retinal degeneration in humans may be, for example, stationary night blindness, retinitis pigmentosa, rod/cone degeneration or dystrophy, cone/rod degeneration or dystrophy, macular degeneration or dystrophy, Stargardt disease, pattern dystrophy, fundus flavimaculatus, Sorsby's fundus dystrophy, punctus albinopunctatus, myopic degeneration, Refsum syndrome, choroideremia, the result of
15 retinal infection or surgical treatment of retinal detachment and in the specific case of night blindness, after treatment with vincristine and/or vinblastine. Furthermore, auditory and vestibular sensory cell degeneration also occurs with Usher syndrome in humans. In addition, disturbances in the visual sensory system as well as other sensory systems also occur in humans with Bardet-Biedl syndrome and Leber's congenital
20 amaurosis.

Various sensory cell degenerations that are due to external effects, such as hearing loss due to extensive noise exposure or retinal detachment due to mechanical effects, can be treated by inducing and promoting normal cell growth in the affected regions.

25

In another preferred embodiment, the active substance includes an active amount of a compound containing cationic alkaline earth metals or modifying the effect thereof and/or containing a cationic ionophore and/or a calcium chelator or buffer and/or a calcium channel blocker and/or a calcium antagonist and/or a calcium activated neutral
30 cysteine protease (calpain) and/or a calpain activator and/or other enzyme activators and/or a calmodulin antagonist and/or an exogenous or endogenous calpain inhibitor or other enzyme inhibitors and/or a cytoskeleton-modifying active substance and/or a

peptide and/or a protein and/or a nucleotide and/or a nitrogen oxide modifying compound.

This may be, for example, a cation ionophore that increases membrane permeability for cations such as calcium, e.g., calcimycin, gramicydin, ionomycin, monensin, thapsigargin. Additional preferred active substances include calpain inhibitors (Wang, 1990; Croall and Demartino, 1991; Mendi, 1991), for example, aloxistatin, antipain, diethyl pyrocarbonate, benzyloxycarbonyldipeptidylaldehyde, calpain inhibitor peptides, calpain inhibitor I, calpain inhibitor II, ZLLY-CHN2, PD150606, AK275, AK295, E64, E64-c, E64-d, MDL-28170, leupeptin, SJA 6017, thiol-reactive agents (such as iodoacetamide, iodoacetic acid, *p*-chloromercuribenzoate, N-ethylmaleimide) and tripeptidyl chloromethyl ketone. Other substances that can also be mentioned include calcium chelators or buffers such as BAPTA, EDTA or EGTA. Also to mention are calcium channel blockers or calcium antagonists such as bepridil, 1-*cis*-diltiazem, nifedipine, semotiadil fumarate (SD-3211). In addition, calcium itself can also be included here, because it activates calpain I and calpain II and modifies the stability of microtubules. In addition, examples of calpain activators include calcium protease activating protein (Croall and Demartino, 1911) as well as isovalerylcarnitine. Another class of active substances are the calmodulin antagonists such as mastoparen, calmidazolium, trifluoperazine, melittin or the active substances W-5, W-7, W-12, and W-13. Another class of active substances includes the calcium-binding proteins such as calmodulin, which modify the binding of calcium to photoreceptor proteins. Proteases, in particular type I or type II calpain, an abbreviation for calcium-activated neutral protease, may be used as another class of active substances. Examples of endogenous calpain inhibitors include calpastatin or low-molecular-weight kininogens (Croall and Demartino, 1991). Microfilament destabilizers such as cytochalasin may also be used as another class of active substances. Microtubule destabilizers and/or cytostatics may also be used as another class of active substances that include *cis*-diaminodichloroplatinum, demecolcine, colchicine, nocodazole, tamoxifen, vinblastine or vincristine. Another group of active substances are the cell-permeable analogs of 3′,5′-cyclic adenosine monophosphate (cAMP) that modify the stability of microtubules, such as dibutyryl-cAMP or 8′-bromo-cAMP. Another group of active substances are the cell-permeable

analogs of 3′,5′-cyclic guanosine monophosphate (cGMP) that modify the stability of microtubules, such as dibutyryl-cGMP or 8′-bromo-cGMP or 8′-bromo-cGMP. Another group includes dithiothreitol, which cancels the effect of sodium nitroprusside, i.e., hydrolyzing it to nitrogen oxide. Another preferred class of active substances includes cations such as ionic compounds of barium, lithium, potassium, selenium, sodium, manganese, magnesium or zinc that competitively modify the effects of calcium. Another class of phosphodiesterase inhibitors include, for example, IBMX and papaverine as well as the active substance SQ 65442. In addition, paclitaxel, compounds that release taxol (protaxols) and doxetaxel may also be used as microtubule-stabilizing agents. Finally, sodium nitroprusside itself should be mentioned, since it forms nitrogen oxide, which can activate calpain. Moreover, the active substances mentioned specifically in the patent claims may also be used.

According to another preferred embodiment, the active substance(s) is/are administered enterally, parenterally, locally, especially by local injection, systemically at the site of action or through delayed release of the active substance, in particular by means of at least one implant or catheter. Thus, for example, there is systemic administration orally or by means of a subcutaneous, intravenous or intramuscular injection. Delayed release of the active substance may take place, for example, through a catheter or by means of an implant, where the implant may consist of a porous, nonporous, or hydrogelatinous material that optionally contains membranes or fibers, biodegradable polymers or a protein-based material.

In the case of administration into the retina, there may also be intraocular administration or topical administration in the eye or intraocular injection, e.g., into the area of the vitreous humor, or subretinal administration into the area between the photoreceptor cells and the pigment epithelial cells. It is preferable here to administer a local injection in the subretinal space and/or through the pigment epithelial cells and/or the retinal Müller glia cells or to have a delayed release of active substance through an implant in the form of at least one microcontainer placed in the subretinal space and/or the posterior chamber of the eye and/or through systemic administration at the site of action.

According to another preferred embodiment, the treatment is administered as a prophylactic and/or a therapeutic treatment, in particular to maintain the extracellular and intracellular physiological milieu, to improve the pathological milieu, and/or to
5 eliminate pathological disturbances.

According to another preferred embodiment, the administration that is controlled in time and space is based on measurements performed before and during administration, using measurement and analytical methods such as those available in ophthalmology and
10 otolaryngology, where the proportion of pathological sensory cells and the concentrations of substances in their vicinity are determined, and in particular they are mapped and analyzed. For example, standard computer-assisted optical, electrophysiological and ultrasound methods may be used as the above-mentioned methods of measurement and analysis. Mapping is done, for example, by plotting the
15 measurement data or analytical results in the form of two- or three-dimensional maps at various times during the measurements.

The control itself is accomplished, for example, by defining the release of quantities of the active substance, with or without sensory feedback, as a function of time and place,
20 on the basis of the measurement and analytical data so prepared.

According to a preferred embodiment of the present invention, for example, the above-mentioned method is performed by influencing such cells in the retina in such a way that the relevant functional and structural parameters of the photoreceptors, subretinal
25 space, glia cells and pigment epithelial cells are monitored, mapped and analyzed in particular by using focal electroretinography (ERG), scanning laser ophthalmoscopy (SLO), fundus reflectometry, confocal *in-vivo* microscopy and/or fluorescence microscopy with nontoxic fluorescence markers as a function of the retinal location, the state of light adaptation and/or time as well as the diurnal light-dark rhythm, and the
30 results of these measurements and analyses as a function of time and space are used to establish the optimum control of administration of the active substance, to select the active substance(s) to be administered, to monitor the course of treatment, and as sensor

information for sensor-based control and/or regulation of administration of active substance(s).

According to another preferred embodiment for treatment of the outer segments of the
5 retinal photoreceptors, the controlled administration of active substance(s) in time and space takes place in such a way that after the pathological and normal photoreceptor areas of the retina have been determined from measurements, the normal photoreceptors are protected by subretinal administration of an active substance specifically in the border area between normal and pathological cells, and this is done before they are
10 subjected to any negative pathological effects from the pathological photoreceptors in their vicinity, and that the normal renewal of the outer segments of these normal cells is continued and/or maintained without any pathological changes, in particular in terms of changes in the extracellular milieu, the length and membrane permeability of outer segments, and the coordinated processes of tip shedding and phagocytosis with the help
15 of pigment epithelial cells.

The present invention also concerns a method of controlling the distribution of the various above mentioned active substances to be administered in time and space in the extracellular space of the sensory cells, in particular the retinal photoreceptor cells,
20 characterized in that a suitable dynamic multi-compartment model for computer simulation of the mass flows in the area of the extracellular space of the photoreceptors, the pigment epithelial cells, the glia cells, and the supporting cells is developed on the basis of the measurement data obtained according to the method described above; furthermore, a suitable model is developed as a computer simulation for control and
25 feedback-supported regulation of intracellular parameters in the photoreceptor cells by administration of externally supplied substances. By using these models, the distribution of the substances to be administered in time and space is controlled by appropriate experts and/or by the patient himself or herself and/or by partially autonomous control circuits, optionally also through selective control of locally distributed, implanted
30 microcontainers to achieve a delayed release of the active substance with suitable substances of the aforementioned type, with the goal of therapeutic optimization of the intracellular photoreceptor milieu.

According to another preferred embodiment, the sensory cells are retinal photoreceptors, with the retinal pigment epithelial cells being influenced to indirectly improve the intracellular photoreceptor milieu by administration and/or implantation into the subretinal space of implanted microcontainers with suitable active substances with or without binding to a carrier matrix, for example of melanin, and phagocytic uptake of these microcontainers by pigment epithelial cells, so that over a long period of time the microcontainers can release specific active substances, inside the pigment epithelial cells and thence into the extracellular space, that, by binding or activating certain substances, are suitable for improving the intracellular milieu of photoreceptors. It is preferable here, for example, to use calpain inhibitors as the suitable active substances that can be released with some delay by the implanted active substance containers.

According to another preferred embodiment of the use according to the present invention, the sensory cells are photoreceptors, and retinal glia cells can be influenced by administration of the active substances of the aforementioned type to improve the intracellular photoreceptor milieu or to influence the extracellular space by administration of active substances of the aforementioned type into the subretinal space to influence the glia cell functions in a suitable manner.

According to another preferred embodiment of the use according to the present invention, the sensory cells and nearby cells are pinealocytes and surrounding cells, whereby disturbances in their intracellular milieu are minimized by injection of the active substance(s) into the cerebrospinal fluid in the vicinity of the third ventricle.

According to another preferred embodiment of the use according to the present invention, the sensory cells and nearby cells are hair cells, epithelial cells and glia cells and supporting cells in the organ of Corti of the inner ear, whereby disturbances in their intracellular milieu are minimized by injection of the active substance into the adjacent perilymphatic space, e.g., through the round window.

According to another preferred embodiment of the use according to the present invention, the sensory cells and nearby cells are hair cells and epithelial cells, glia cells and supporting cells in the vestibular organs, whereby disturbances in their intracellular milieu are minimized by injection of the active substance into the adjacent
5 perilymphatic space.

The present invention also concerns a modification of the use according to this invention of the aforementioned type to improve the intracellular sensory cell milieu and/or intracellular milieu of epithelial cells and/or glia cells and/or supporting cells,
10 whereby monocytes, macrophages or microglia cells or other suitable endogenous cells or subcellular structures or biomolecules are removed from suitable locations in the human patient or from healthy humans or mammals, are treated or modified *ex vivo* in a suitable manner, and then (re)introduced into the patient, i.e., (re)inserted into the extracellular space.
15

With a device according to this invention for administration of the active substance into the extracellular space in the vicinity of sensory cells having at least one implantable depot of the active substance, means are provided for the active substance depot to allow for controllable, preferably externally controllable, release of the active substance,
20 so the release of the active substance can be controlled locally and as a function of time.

Implantation and optionally explantation can be accomplished easily if multiple depots of the active substance(s) are provided, connected to one another by a device for pulling and/or connected to a common through bolt.
25

For need-oriented administration of the active substance, it is advantageous if the active substance depots have sensor or motor functions that can be carried out autonomously or in a coupled manner.

30 As measurement and analytical methods for determination of the parameters for treatment of the retina using essentially known diagnostic methods, it is preferable for the ongoing status of the local distribution of the photoreceptor degeneration to be

determined and used as the basis for controlling the distribution of active substance; relevant functional and structural parameters of photoreceptors, the subretinal space, glia cells and pigment epithelial cells are monitored, mapped and analyzed in particular by using focal electroretinography (ERG), scanning laser ophthalmoscopy (SLO), fundus reflectometry, confocal *in-vivo* microscopy and/or fluorescence microscopy with nontoxic fluorescence markers as a function of the retinal location, the state of light adaptation and/or the time as well as the diurnal light-dark rhythm in order to determine morphological, physiological and/or biochemical parameters, and the measurements and analytical results as a function of time and space are used to determine the optimum control of administration of the active substance(s), to select the active substance(s) to be administered, to monitor the course of treatment and to provide sensory information for control or regulation of a sensor-based active substance administration. In addition, for the most accurate possible dosage of the active substance, it is advantageous if the sensor and/or motor functions include local measurement of the parameter in the vicinity and local administration of the active substance(s).

Flexible use of the implant is possible over a wide functional range if an interface with an external control unit is provided for administration of the active substance, with the interface preferably designed to be wireless and to transmit sensor signals and control commands as well as energy.

The present invention is explained below with reference to the figures and with reference to examples of applications, although the present invention is in no way limited to these explanations. The figures show:

Figure 1: the subretinal extracellular space of the retina;

Figure 2: the structure of retinal photoreceptors;

Figure 3: the extracellular space of the pineal gland and a pinealocyte;

Figure 4:	the extracellular space of the inner ear;
Figure 5:	the extracellular space of the organ of Corti;
Figure 6:	the extracellular space of the vestibular organs;
Figure 7:	the sensory hair cells of the vestibular organs;
Figure 8:	a light microscopic section of a *Xenopus laevis* retina before injection of the active substance;
Figure 9:	a light microscopic section of a *Xenopus laevis* retina after injection of nocodazole;
Figure 10:	the number of rod outer segment phagosomes per 100 µm of pigment epithelium in the *Xenopus laevis* retina as a function of the intraocular concentration of the active substance;
Figure 11:	the effect of calpain inhibitor and a high concentration of calcium on the structure of rods in the *Xenopus laevis* retina;
Figure 12:	a sensory cell region of the retina with active substance depots implanted there, connected by connecting structures to an external through bolt;
Figure 13:	an example of implantation of these active substance depots between epithelial cells and the retina by means of a transport fluid and an injector;
Figure 14:	the internal structure of an active substance depot according to Figures 12 and 13 in a far-reaching embodiment; and

Figure 15: a schematic cross-section through a typical sensory cell region, showing the relative arrangement of the various cells.

The subretinal extracellular space 2 according to Figure 1 is mainly bordered by the
5 pigment epithelial cells 1 and the retinal Müller (glia) cells 5. In addition, the space is also bordered by rod photoreceptors 3 and cone photoreceptors 4.

Figure 2 shows schematically an electron microscopic section through a retinal rod photoreceptor cell 6, a rod photoreceptor 7 and a cone photoreceptor 8 of the human
10 retina as well as the enlargement of an outer segment (OS) 9 of a retinal rod photoreceptor. This shows the enclosed membranous discs 10 and a rod outer segment fragment 11 that has been shed from the tip of the outer segment.

Figure 3 shows the pineal gland 12, also known as the epiphysis or glandula pinealis, of
15 a mammal. The extracellular space 13 of this organ is directly connected to the third ventricle. This also shows a pinealocyte 14, i.e., a photoreceptor-like cell in the pineal gland, with the typical cilium 15 of a pinealocyte.

Figure 4 shows a schematic diagram of the inner ear, showing the cochlea with the
20 organ of Corti 16, the three semicircular channels 17 of the vestibular organ, the macula sacculi 18 of the vestibular organ, the macula utriculi of the vestibular organ 19 and the extracellular endolymphatic space 20 of the vestibular organ.

Figure 5 shows an enlargement of the organ of Corti and the sensory hair cells of the
25 organ of Corti 16 in the cochlear duct, the extracellular endolymphatic space 21 of the organ of Corti, the extracellular perilymphatic space of the scala tympani 22, the extracellular perilymphatic space of the scala vestibuli 23 and the sensory hair cells 24 of the organ of Corti (shown both on a normal scale and enlarged).

30 Figure 6 shows a diagram of the vestibular organs with the macula statica 25 that corresponds to the macula utriculi and the macula sacculi, the sensory hair cells 26 of the macula statica, the crista ampullaris 27 (normal and enlarged), the sensory hair cells

28 of the crista ampullaris, semicircular canals 29 with sensory hair cells and the extracellular perilymphatic space 30 of the semicircular canals.

Figure 7 shows a schematic electron micrograph of the sensory hair cells of the
5 vestibular organs, including the vestibular sensory hair cells 31 and the kinocilium 32 of a hair cell.

For the examples of applications of the use according to this invention in the form of an animal experiment as described below, carried out on the retina of the toad species
10 *Xenopus laevis*, nocodazole (methyl-(5-[2-thienyl-carbonyl]-1H-benzimidazol-2-yl) carbamate) was used as the biologically active substance to influence the extracellular space of the retina.

In a first embodiment, it shall be demonstrated that abnormally elongated outer
15 segments can be shortened and their further degeneration can be prevented by controlled treatment of the subretinal space with an active substance (nocodazole, as an example of a microtubule-destabilizing agent) that induces shedding of the outer segments of the rods.

In a second embodiment, it shall be shown that by treatment of the subretinal space with calpain inhibitors or calcium chelating agents, the abnormal proteolytic effect of calpain released from degenerated rod outer segments is reduced so that neighboring cells (cone photoreceptors, pigment epithelial cells, Müller glia cells and healthy rod photoreceptors) as well as the extracellular matrix in the subretinal space are not attacked, and the normal structure and function are maintained.

Embodiment 1 with Figures 8-10

(Injection of an active substance used according to this invention leads to shedding of rod outer segments):

Adult African clawed toads, *Xenopus laevis* (available, for example, from the African Xenopus Facility, P.O. Box 118, Noordhoek 7985, Republic of South Africa), were given intraocular injections of 0.4 μL solution per eye of various doses of nocodazole (a microtubule-destabilizing agent) into the vitreous humor of one eye in order to investigate whether this would lead to increased shedding of membrane fragments from the tips of rod outer segments. To this end, the number of phagosomes in pigment epithelium was determined in histological preparations after the injections in order to estimate the number of rod outer segment fragments shed (see Figures 8, 9). To do so, the animals were decapitated five hours after the injection, their eyes were removed and fixed, prepared for histology, cut into sections and observed under a light microscope.

Results: In comparison with the control retina (Figure 8) the retina shows a greater amount of shedding after injection of nocodazole. This proves that in the experimentally-treated retinas many rod outer segments have shed their tips. This means that nocodazole-induced destabilization of microtubules has caused shedding of the rod outer segments. In addition, the number of rod outer segment fragments shed varies with the dose of nocodazole.

Figure 8 shows a light microscopic section of a retina of the Xenopus toad before/without injection of nocodazole (for comparison purposes). No phagosomes can

be seen in the pigment epithelium (in the upper part of the figure), which shows clearly that almost no membranes have been shed from the tip of rod outer segments.

Figure 9, on the other hand, shows a light microscopic section of the retina of the Xenopus toad after injection of 1.0 ng nocodazole. The pigment epithelium here has many phagosomes (indicated by arrows), which shows that many outer segment membrane fragments have been shed from the tip of rod outer segments.

Figure 10 shows a graphic bar chart of the number of rod outer segment phagosomes per 100 μm pigment epithelium in Xenopus retinas without injection, in comparison to retinas injected with the solvent DMSO and with low, medium, and high concentrations of nocodazole (from left to right), as obtained according to Application Example 1. In addition, the standard deviation is shown above the bars. In this graph, a high concentration of injected nocodazole is understood to be approximately 2.5 mg nocodazole/ml DMSO (dimethyl sulfoxide, the solvent for nocodazole), a medium concentration is about 0.25 mg nocodazole/ml DMSO, a low concentration is about 0.025 mg nocodazole/ml DMSO, and DMSO (solvent) is a solution that does not contain any nocodazole. The following concentrations of nocodazole in the eye can be estimated from the concentrations given above:

Approximately 8.33 μg/ml eye volume for the high nocodazole concentration, approximately 0.833 μg/ml eye volume for the medium nocodazole concentration and approximately 0.0833 μg/ml eye volume for the low nocodazole concentration.

Embodiment 2 with Figure 11

(Effect of a high calcium concentration, with and without calpain inhibitor, on the structure of rod outer segments)

To determine whether the normal structure of rod outer segments can be disturbed by high calcium concentrations (which might indicate a microtubule-destabilizing effect of calcium), living photoreceptors in sections of the retina of *Xenopus laevis* toads were

perfused with various solutions in a flow-through chamber and observed under a light microscope for a long period of time, and in addition, the results were recorded with a video system for documentation purposes. Since the rod outer segment plasma membrane is impermeable to calcium ions during this light exposure, in the experiments a calcium ionophore (4 μM A23187 (calcimycin)) was used along with a high concentration of calcium (1.8 mM calcium chloride) in a suitable perfusion solution. The morphological changes in the rod outer segments that were observed after increasing the intracellular calcium concentration could have been caused by destabilization of rod outer segment microtubules by the enzyme calpain (a calcium-activated protease that is present in rod outer segments and can cleave tubulin). To determine whether this was the case, retinal sections were perfused with a perfusion solution having a high calcium concentration (1.8 mM calcium chloride), a calcium ionophore (4 μM calcimycin), and an additional calpain inhibitor (50 mg/ml E-64d) (see Figure 11).

Results: In comparison with control preparations, the experimental preparations showed definite changes in the form of the rod outer segments. During perfusion with high calcium concentration and a calcium ionophore, the rod outer segments first developed a furrow running parallel to the longitudinal axis in their distal half. The width of the furrow then increased, until the rod outer segments developed kinks at several points between the distal tip and the proximal end of the furrow. The kinks resulted in the original columnar shape of the rod outer segments changing into a hook-like shape. Thirty minutes after the start of perfusion of the extracellular space with a high calcium concentration and a calcium ionophore, almost all the rod outer segments showed this abnormal morphological change. In additional experiments it was shown that the destabilizing effect on the shape of the rod outer segments of high calcium concentrations and a calcium ionophore could be significantly delayed by using a calpain inhibitor. These findings indicate that the normal, well-ordered shape of rod outer segments can be disturbed by a high extracellular calcium concentration, which leads to a high intracellular calcium concentration in the photoreceptor outer segments, and in addition, that activation of calpain is involved in the destabilizing effect of calcium.

Figure 11 shows various retinal sections as vital preparations to illustrate the effect of a high calcium concentration and a high calpain concentration on the structure of the rods of the Xenopus retina.

5

Figure 11a shows a retinal section at the beginning of perfusion with a high calcium concentration (1.8 mM $CaCl_2$) and a calcium ionophore (4 µM A23187). The rod outer segments have a normal columnar shape.

10 Figure 11b shows the same retinal section as in Figure 11a after 30 minutes of perfusion with a high calcium concentration and a calcium ionophore: almost all the rod outer segments are bent and have an abnormal hook-like shape.

Figure 11c shows another retinal section after 30 minutes of perfusion with a high

15 calcium concentration (1.88 mM $CaCl_2$), a calcium ionophore (4 µm A23187) and in addition, a calpain inhibitor (50 mg/ml E-64d): a small percentage of the rod outer segments have a distal furrow or are bent at two points, but most of the rod outer segments still have the normal columnar shape.

20 Figure 11d shows the same retinal section as in Figure 11c after 60 minutes of perfusion with a high calcium concentration, a calcium ionophore, and in addition, a calpain inhibitor: some of the rod outer segments have an abnormal hook-like shape, but many other rod outer segments are just beginning to bend over.

25 Figure 12 shows a schematic diagram of a sensory cell region 50 of the retina in a top view, viewed along its normal direction of gaze. In the area of the retina, there are numerous depots 51 of active substance with which the extracellular milieu in the area of the sensory cells can be influenced locally. Active substance depots 51 are in turn connected to a common through bolt 53 by a threadlike device for pulling 52, which can

30 also be used for transport of active substance or for signal transmission. The through bolt 53 may also remain outside of the sensory cell region 50 and may, for example, be secured within the eyeball.

Figure 13 shows a symbolic cross-section through the sensory cell region 50 with a retina 55 and an epithelial cell layer 56. Active substance depots 51 are inserted into an area between the retina 55 and the epithelial cells 56 by being injected in a transport
5 fluid 58 between the retina 55 and the epithelial cells 56 with an injector 57. Active substance depots 51 are suspended in the transport fluid 58 and, on injection, are flushed into the intermediate space along with the transport fluid 58. In the case of the cochlea or the vestibular organs, the active substance depots are introduced in a similar manner into the fluid space surrounding the sensory epithelium. The devices for pulling 52 are
10 released and guided through an opening 59 in the injector 57. After placement of the active substance depot 51, they remain in the subretinal space between the retina 55 and the epithelial cells 56, while the intermediate space produced by the transport fluid 58 is sealed up again over a period of time after suction removal or absorption of the transport fluid 58. In addition to their use in placement of the active substance depots
15 51, the devices for pulling 52 and the through bolt 53 may also be used to remove the active substance depots 51 after they have been emptied or after conclusion of the treatment, or to explant the entire structure.

Figure 14 shows one embodiment of an active substance depot 51 with a complex
20 internal structure. Specifically, the active substance depot 51 contains a drive 61 for autonomous or controlled motion, a signal processing unit 62, an interface 63 for optical signal exchange, a detector 64 for detecting parameters to characterize the extracellular space, an active substance dispenser 65 with an inlet valve 66 and an outlet valve 67, as well as an interface 68 for exchange of material and signals with the device for pulling
25 52. The device for pulling 52 itself has a valve 69 and a signal line 70, as well as a tubing or pipe for liquids 71 through which the active substance can be conveyed across the interface 68 into the active substance dispenser 65.

Finally, Figure 15 shows an enlarged cross-section through a typical sensory cell region
30 of mammals, e.g., the retina in a top view corresponding to Figure 13 or a sensory cell region which has a similar structure in principle, such as that of the organ of Corti or the vestibular organs. The respective arrangement of the sensory cells 55, the epithelial cells

56 and the glia cells or supporting cells 57 is illustrated here. The preferred position of the active substance depots 51, in the immediate proximity to the sensory cells 55, is illustrated, from which the active substance is to be dispensed for influencing the extracellular milieu.

5

In practice, the active substance depots 51 are filled with an active substance or an active substance mixture that is suitable for modifying the extracellular milieu and thus also the intracellular milieu of the sensory cells 55, the epithelial cells 56, the glia cells and/or the support cells 57. The depots are then connected to one another or to a
10 common connecting structure, namely the through bolt 53 by means of devices for pulling 52 and are suspended in a transport fluid. The transport fluid is inserted with an injector between the epithelial cells and the retina, or at any rate into the vicinity of the cells to be treated, in such a way that the active substance depots are spatially distributed within the area of the retina 55 to be treated. They can release the active
15 substance there as needed in a manner that is controlled in space and time. The need is determined either externally by laser ophthalmoscopy or through other suitable local diagnostic methods, or the demand may be determined *in situ* by the active substance depots themselves or by sensors positioned together with the active substance depots. To do so, the detector 64 may be designed, for example, as a sensor of conductivity or
20 as a detector for the ion concentrations of calcium ions. When the need has been determined, a small amount of the active substance is released. This may be accomplished either by internal control 62 of the active substance depot 51 itself or by means of an externally supplied signal. In the case of the eye, an optical signal exchange over the interface 63 is suggested for this purpose.

When the active substance dispenser 65 has been completely emptied, it can be refilled through the tubing or pipe for liquids 71 of the device for pulling 52. In the simpler case of unrefillable active substance depots, the sum total of the active substance depots 51 may be explanted by pulling on the devices for pulling 52, removing them from the site
5 of implantation so they can be replaced with new active substance depots, which are positioned in the manner described previously.

An especially simple embodiment may provide for the active substance depots 51 to be designed with devices for pulling 52 as permanently stable balloon-like containers (or
10 without devices for pulling as medium-sized containers that can be phagocytized or degraded) with the active substance surrounded by an envelope that is impermeable at normal body temperature. The containers are linked together by the plastic or carbon fiber filaments of the devices for pulling 52, but the devices for pulling 52 do not have any internal structure. When there is a need for release of the active substance, the
15 balloon-shaped active substance containers are heated to slightly above body temperature by external exposure to a laser of a suitable wavelength and power, thus causing local release of the active substance and hence modification of the extracellular milieu.

20 Active substances are referred to with abbreviations as followed in the text and in the patent claims where:

AK 275 = L,L-isomer of Z-Leu-Abu-CONH-CH_2-CH_2

25 AK 295 295 = CBZ-Leu-Abu-CONH-$(CH_2)_3$

Calmodulin binding domain = Leu-Lys-Lys-Phe-Asn-Ala-Arg-Arg-Lys-Leu-Lys-Gly-Ala-Ile-Leu-Thr-Thr-Met-Leu-Ala

30 Calpain inhibitor I = N-acetyl-leu-leu-norleucinal

Calpain inhibitor II = N-acetyl-leu-leu-normethioninal

Calpain inhibitor peptide = Asp-Pro-Met-Ser-Ser-Ser-Thr-Tyr-Ile-Glu-Glu-Leu-Gly-Lsy-Arg-Glu-Val-Thr-Ile-Pro-Pro-Lys-Tyr-Arg-Glu-Leu-Leu-Ala

5

DY-9760e = 3-[2-[4-(3-chloro-2-methylphenyl)-1-piperanzinyl]ethyl]-5,6-dimethoxy-1-(4-imidazolylmethyl)-1H-indazole dihydrochloride 3,5-hydrate

E64 = *trans*-epoxysuccinyl-D-leucylamido-(4-guanidino)-butane

10

E-64c = (2S,3S)-*trans*-epoxysuccinyl-D-leucylamido-3-methylbutane

E-64d = (2S,3S)-*trans*-epoxysuccinyl-D-leucylamido-3-methylbutane ethyl ester

15 H-7 = 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine

H-8 = N-2-(methylamino)ethyl-5-isoquinolinesulfonamide

H-89 = N-[2-bromocinnamyl(amino)ethyl]-5-isoquinolinesulfonamide

20

H-9 = N-(2-aminoethyl)-5-isoquinolinesulfonamide

HA-1004 = N-(2-guanidinoethyl)-5-isoquinolinesulfonamide

25 IBMX = 3-isobutyl-1-methylxanthine

L-NAME = Nω-nitro-L-arginine methyl

L-NNA = Nω-nitro-L-arginine

30

MDL 28170 = carbobenzoxyl-val-phe-H

PD150606 = I-benzyl-CH=C(SH)COOH

PMA = phorbol 12-myristate 13-acetate

5 SD-3211 = semotiadil fumarate

W-12 = N-(4-aminobutyl)-1-naphthalenesulfonamide

W-13 = N-(4-aminobutyl)-5-chloro-1-naphthalenesulfonamide

10

W-5 = N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

W-7 = N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

15 ZLLY-CHN_2 = carbenzoxy-leu-leu-tyr-CHN_2

CLAIMS

1. A use of at least one active substance that influences the calcium homeostasis of cells for treatment of degenerative diseases of the sensory cells and the
5 surrounding cells.

2. A use of at least one active substance or a combination of active substances selected from the group of:

10
- compounds that contain alkaline earth metals or modify the effect thereof
- nucleotides
- nitrogen oxide modifying compounds
- calcium chelators and buffers
- active substances that modify the cytoskeleton

15
- calcium channel blockers and calcium antagonists
- calmodulin antagonists
- cationophores
- peptides, enzyme activators, enzyme inhibitors
- proteins

20

for treatment of at least one disease from the group consisting of:

- degenerative diseases of the sensory cells, or epithelial cells, glia cells, or supporting cells near the sensory cells.

25

3. A use according to Claim 1 or Claim 2, characterized in that the sensory cells, epithelial cells, glia cells or supporting cells are those in the retina, the pineal gland, the organ of Corti and/or the vestibular organ.

30 4. A use according to one of the preceding claims, characterized in that the cationic compounds that contain alkaline earth metals or modify the effect thereof contain the following:

magnesium, calcium, barium, lithium, sodium, potassium, selenium, manganese, zinc.

5 5. A use according to one of the preceding claims, characterized in that the nucleotides include the following:

guanosine compounds, in particular dibutyryl-cGMP, 8′-bromo-cGMP, cGMP, GMP, GDP, GTP, Sp-8-Br-PET-cGMPS, Rp-8-Br-cGMPS;

10

adenosine compounds, in particular dibutyryl-cAMP, 8′-bromo-cAMP, cAMP, AMP, ADP, ATP.

6. A use according to one of the preceding claims, characterized in that the
15 compounds modifying nitrogen oxide include the following:

dithiothreitol, sodium nitroprusside, L-NAME, L-NNA.

7. A use according to one of the preceding claims, characterized in that the calcium
20 chelators and buffers include the following:

BAPTA, EDTA, EGTA.

8. A use according to one of the preceding claims, characterized in that the
25 cytoskeleton-modifying active substances include the following:

- microfilament destabilizers, in particular cytochalasin;
- microtubule destabilizers and cytostatics, in particular *cis*-diaminodichloroplatinum, demecolcine, colchicine, nocodazole, tamoxifen,
30 vinblastine, vincristine;
- microtubule stabilizers, in particular paclitaxel, taxol-releasing compounds (protaxols), doxetaxel.

9. A use according to one of the preceding claims characterized in that the calcium channel blockers and calcium antagonists include the following:

5 bepridil, L-*cis*-diltiazem, nifedipine, SD-3211.

10. A use according to one of the preceding claims, characterized in that the calmodulin antagonists include the following:

10 calmidazolium, calmidazolium chloride, mastoparan, trifluoperazine, trifluoperazine dimaleate, melittin, calmodulin binding domain, chlorpromazine, fluphenazine N-2-chloroethane, ophiobolin A, pentamidine isethionate, phenoxybenzamine, W-5, W-7, W-12, W-13 and indazole derivatives, in particular DY-9760e.

15

11. A use according to one of the preceding claims, characterized in that the cationophores include the following:

calcimycin, gramicidin, ionomycin, monensin, thapsigargin.

20

12. A use according to one of the preceding claims, characterized in that the proteins, peptides, enzyme activators, and enzyme inhibitors include the following:

25
- phosphodiesterase inhibitors, in particular IBMX, papaverine, and SQ 65442;
- calpain inhibitors, in particular aloxistatin, antipain, benzyloxycarbonyldipeptidylaldehyde, calpain inhibitor peptides, calpain inhibitor I, calpain inhibitor II, ZLLY-CHN_2, PD150606, diethyl pyrocarbonate, MDL-28170, E64, E64-c, E64-d, leupeptin, SJA 6017, 30 tripeptidyl chloromethyl ketone, AK275, AK295, thiol-reactive agents such as iodoacetamide, *p*-chloromercuribenzoate, iodoacetic acid and N-ethylmaleimide;

- endogenous calpain inhibitors, in particular calpastatin and low-molecular-weight kininogens;
- protein kinase inhibitors, in particular H-7, H-8, H-9, H-89, HA-1004, bisindolylmaleimide and staurosporine;
- calpain activators, in particular the calcium protease activating protein and isovaleryl carnitine;
- proteases, in particular calpain type I and calpain type II;
- calcium binding proteins, in particular calmodulin,
- protein kinase activators, in particular phosphatidylserine and PMA.

13. A use according to one of the preceding claims, characterized in that the degenerative diseases of sensory cells or epithelial cells, glia cells, or supporting cells in the vicinity of the sensory cells in humans include the following:

- stationary night blindness;
- retinitis pigmentosa;
- rod/cone degeneration or dystrophy;
- cone/rod degeneration or dystrophy;
- macular degeneration or dystrophy;
- Stargardt disease;
- pattern dystrophy;
- fundus flavimaculatus;
- Sorsby's fundus dystrophy;
- punctus albinopunctatus;
- myopic degeneration;
- Refsum's syndrome;
- choroideremia,

and

- Usher syndrome

- Bardet-Biedl syndrome
- Leber's congenital amaurosis.

14. A use according to one of the preceding claims, characterized in that the acquired degenerative diseases of sensory cells or of the epithelial cells, glia cells or supporting cells in the vicinity of the sensory cells in humans include the following:

    - night blindness after treatment with vincristine or vinblastine,
    - the sequelae of treatment with thioridazine, chloroquine, quinine or other ototoxic substances,
    - sequelae of treatment after retinal detachment,
    - sequelae of infection of the retina,
    - sequelae of a deficiency of physiological sensory stimulation,
    - sequelae of age-related sensory cell degeneration,
    - sequelae of nonphysiological sound exposure,
    - sequelae of whiplash.

15. A use according to one of the preceding claims, characterized in that for treatment of the retina with photoreceptor cells, adjacent pigment epithelial cells, and retinal glia cells, the extracellular milieu of the subretinal space surrounding these cells is influenced in time and space through local injection and/or time-delayed release of active substance(s) so that the structure and function and the intracellular milieu of the photoreceptor cells and/or the pigment epithelial cells and/or the Müller glia cells remain normal for as long as possible or are made to approximate the normal physiological condition as closely as possible.

16. A use according to one of the preceding claims, characterized in that the retinal pigment epithelial cells are influenced to indirectly improve the intracellular photoreceptor milieu and/or to improve the pigment epithelial functions by administration and/or implantation in the subretinal space of implanted microcontainers with at least one active substance, with or without binding to a

carrier matrix, according to one of the preceding claims and that, after phagocytic uptake of the microcontainers by pigment epithelial cells, these substances are released over a long period of time from the microcontainers within the pigment epithelial cells and thus into the subretinal space.

5

17. A use according to one of the preceding claims, characterized in that for treatment of the pineal gland with photoreceptor-like pinealocyte sensory cells and adjacent epithelial cells and/or glia cells, the extracellular milieu surrounding them in the vicinity of the third ventricle of the brain is influenced
10 locally and in time by injection and/or by time-delayed release of active substance(s) into the cerebrospinal fluid so that the structure and function and the intracellular milieu of the pinealocytes remain normal for as long as possible or are made to approximate the normal physiological condition as closely as possible.

18. A use according to one of the preceding claims, characterized in that for treatment of the auditory organ with sensory hair cells, surrounding epithelial cells and/or glia cells and/or supporting cells in the organ of Corti of the inner ear, the milieu of the perilymphatic space of the scala tympani and/or the scala vestibuli and/or the milieu of the endolymphatic space close to the organ of Corti is/are influenced through local injection and/or time-delayed release of the active substance so that the structure and function of the intracellular milieu of the hair cells remain normal for as long as possible or are made to approximate the normal physiological condition as closely as possible, and the development and/or growth of additional hair cells is promoted.

19. A use according to one of the preceding claims, characterized in that for treatment of the vestibular organ with sensory hair cells, adjacent epithelial cells and/or glia cells and/or supporting cells in the crista ampularis of the semicircular canal organs and in the macula utriculi and in the macula sacculi of the vestibulostatic organs, the milieu of the surrounding perilymphatic space and/or the milieu of the surrounding endolymphatic space close to the hair cells is influenced in time and space by local injection and/or time-delayed release of active substances so that the structure and function and the intracellular milieu of the hair cells remain normal for the longest possible time or are made to approximate the normal physiological condition, and the development and/or accumulation of additional hair cells is/are promoted.

20. A method of normalization or improvement of the structure and function and the intracellular milieu of sensory cells and/or adjacent epithelial cells and/or glia cells and/or supporting cells, characterized in that melanin particles with a size of approximately 1 μm to 20 μm are administered into the extracellular space.

21. A method of normalizing or improving the structure and function and the intracellular milieu of sensory cells and/or adjacent epithelial cells and/or glia cells and/or supporting cells using an active substance or a combination of active substances according to the preceding claims, characterized in that monocytes,

macrophages, microglia cells or other suitable cells or subcellular structures or biomolecules are first removed from suitable locations in the human patient or from healthy humans or mammals, these are treated or modified *ex vivo* in a suitable manner, and they are then (re)inserted into the patient, i.e., are
5 (re)introduced into the appropriate extracellular space.

22. A method of administration of active substance in the extracellular space in the vicinity of sensory cells, characterized in that the parameters characterizing the extracellular space and/or the intracellular space of sensory cells and adjacent
10 epithelial cells, glia cells or supporting cells are first determined, then a need for administration of an active substance is determined, and finally the active substance is administered or released as a function of need.

23. A method according to one of the preceding claims, characterized in that the
15 administration of active substance and/or the determination of parameters are performed within the retina and/or between the photoreceptor cells and the epithelial cells of a human eye or a mammal eye.

24. A method according to one of the preceding claims, characterized in that the administration of the active substance is performed in a differentiated manner in time and/or space.

5 25. A method according to one of the preceding claims, characterized in that the parameter is an ion concentration, in particular the calcium, magnesium, sodium or potassium concentration, the electric conductivity, the redox status, or the calpain activity.

10 26. A method according to one of the preceding claims, characterized in that the active substance is administered as a function of external control signals, in particular by means of light signals and/or electromagnetic signals, directed into the eye.

15 27. A device for administration of active substance in the extracellular space in the vicinity of sensory cells with at least one implantable depot of active substance, with means provided for controllable, preferably externally controllable, dispensing of active substance from the depot of active substance.

20 28. A device according to one of the preceding claims, characterized in that it provides for several depots of the active substance, which are connected to one another by device(s) for pulling or connected to a common through bolt.

29. A device according to one of the preceding claims, characterized in that the
25 active substance depots have sensory and/or motor functions that can be carried out autonomously or in combination.

30. A device according to one of the preceding claims, characterized in that essentially known diagnostic methods are used as measurement and analytical
30 methods for determining the parameters for treating the retina, and the ongoing status of the local distribution of the photoreceptor degeneration is determined as the basis for controlling the distribution of active substance; for determination of

morphological, physiological and/or biochemical parameters, the relevant functional and structural parameters of photoreceptors, the subretinal space, glia cells, and pigment epithelial cells are monitored, mapped and analyzed, in particular by using focal electroretinography (ERG), scanning laser
5 ophthalmoscopy (SLO), fundus reflectometry, confocal *in vivo* microscopy and/or fluorescence microscopy with nontoxic fluorescent markers as a function of the retinal location, the state of light adaptation and/or the time as well as the diurnal light-dark rhythm; the measurement and analytical results as a function of time and space are used to determine the optimal control of active substance
10 administration, to select the active substance(s) to be administered, to monitor the course of treatment, and as sensor information for a sensor-based control or regulation of active substance administration.

31. Device according to one of the preceding claims, characterized in that the sensor
15 and/or motor functions include the local measurement of the parameter in the vicinity and the local administration of active substance.

32. Device according to one of the preceding claims, characterized in that an interface with an external control unit is provided for administration of active
20 substance.

33. Device according to one of the preceding claims, characterized in that the interface is designed to be wireless and to transmit sensor signals, as well as control commands and energy.

25

Fig. 1

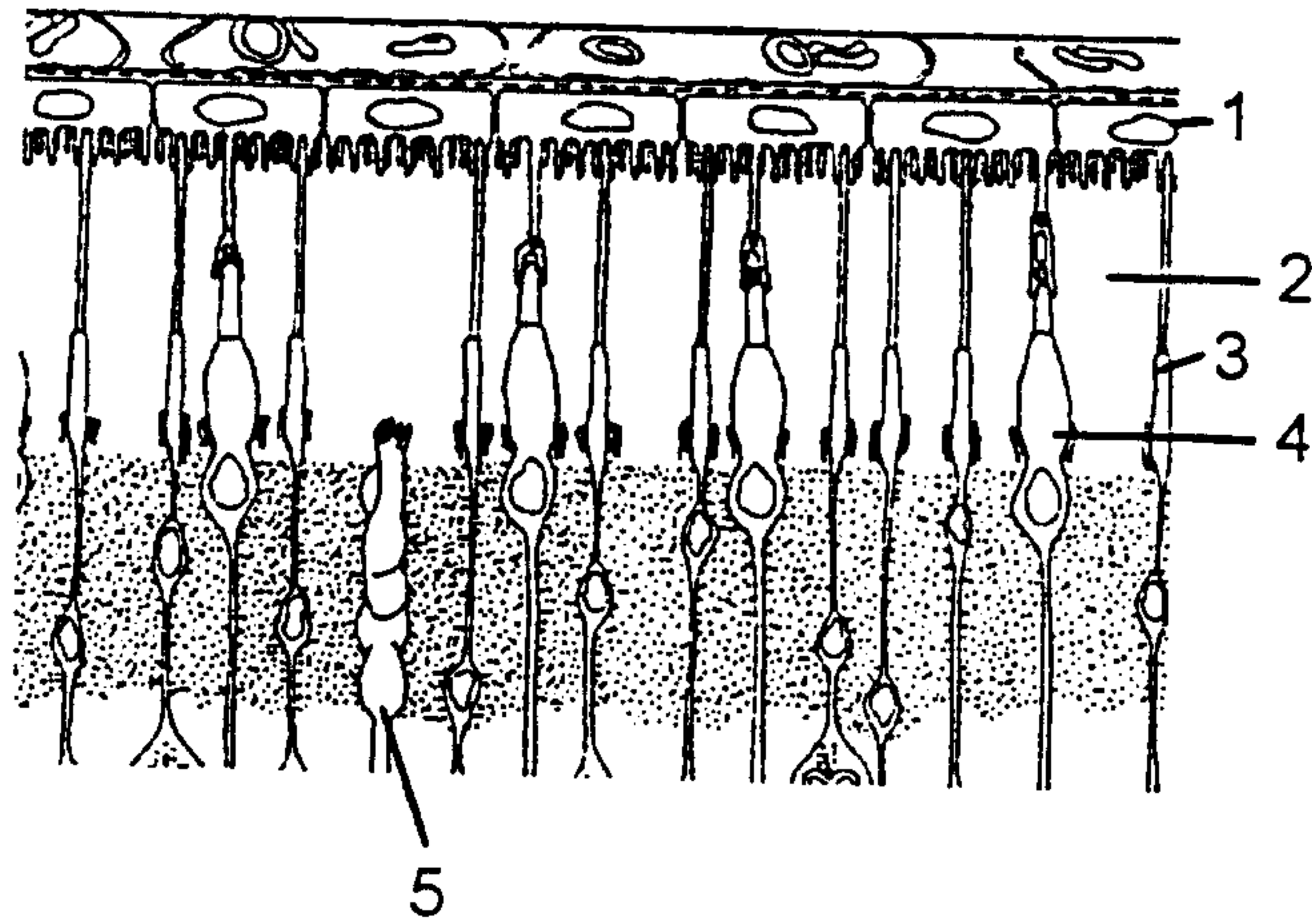


Fig. 2

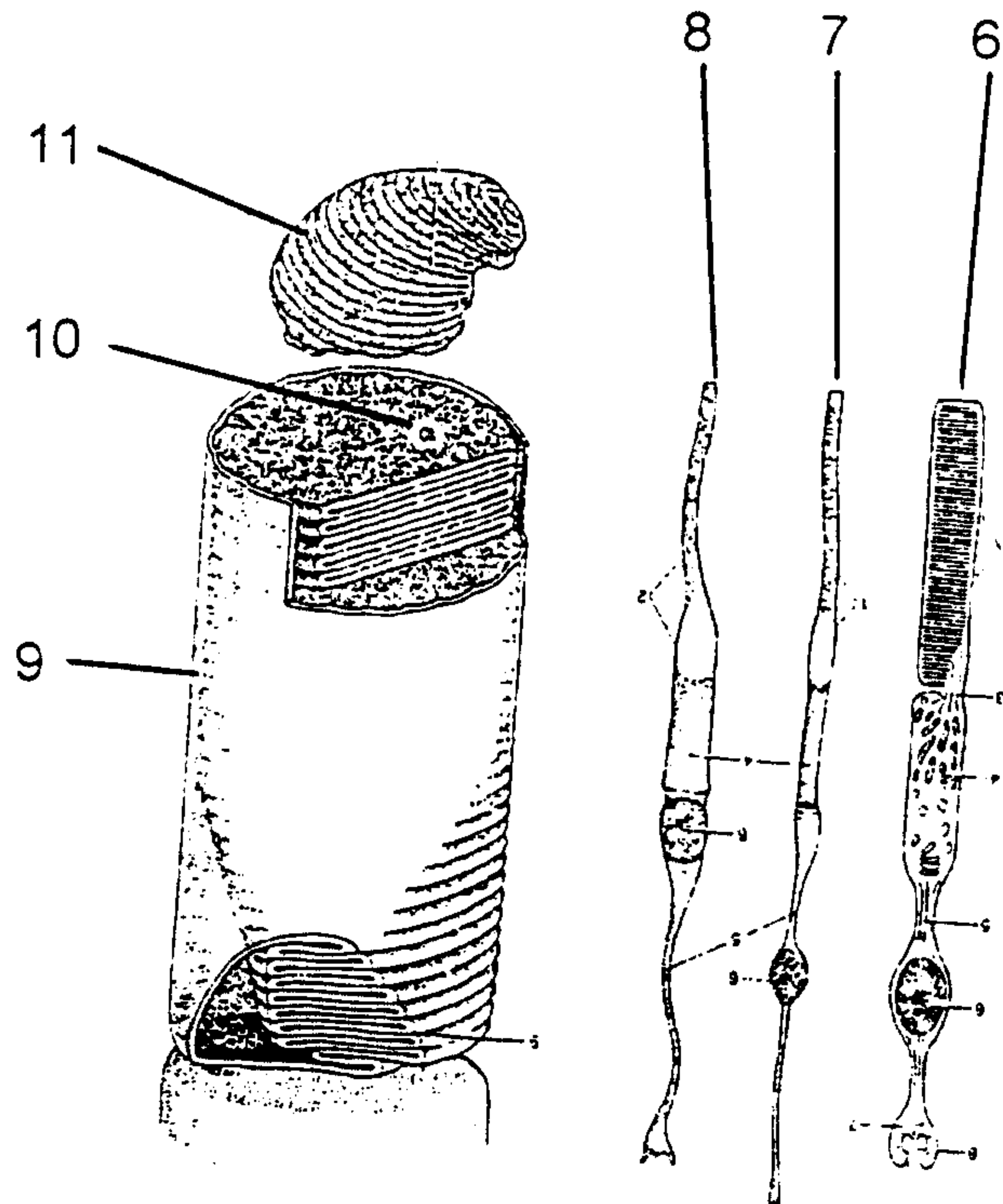


Fig. 3

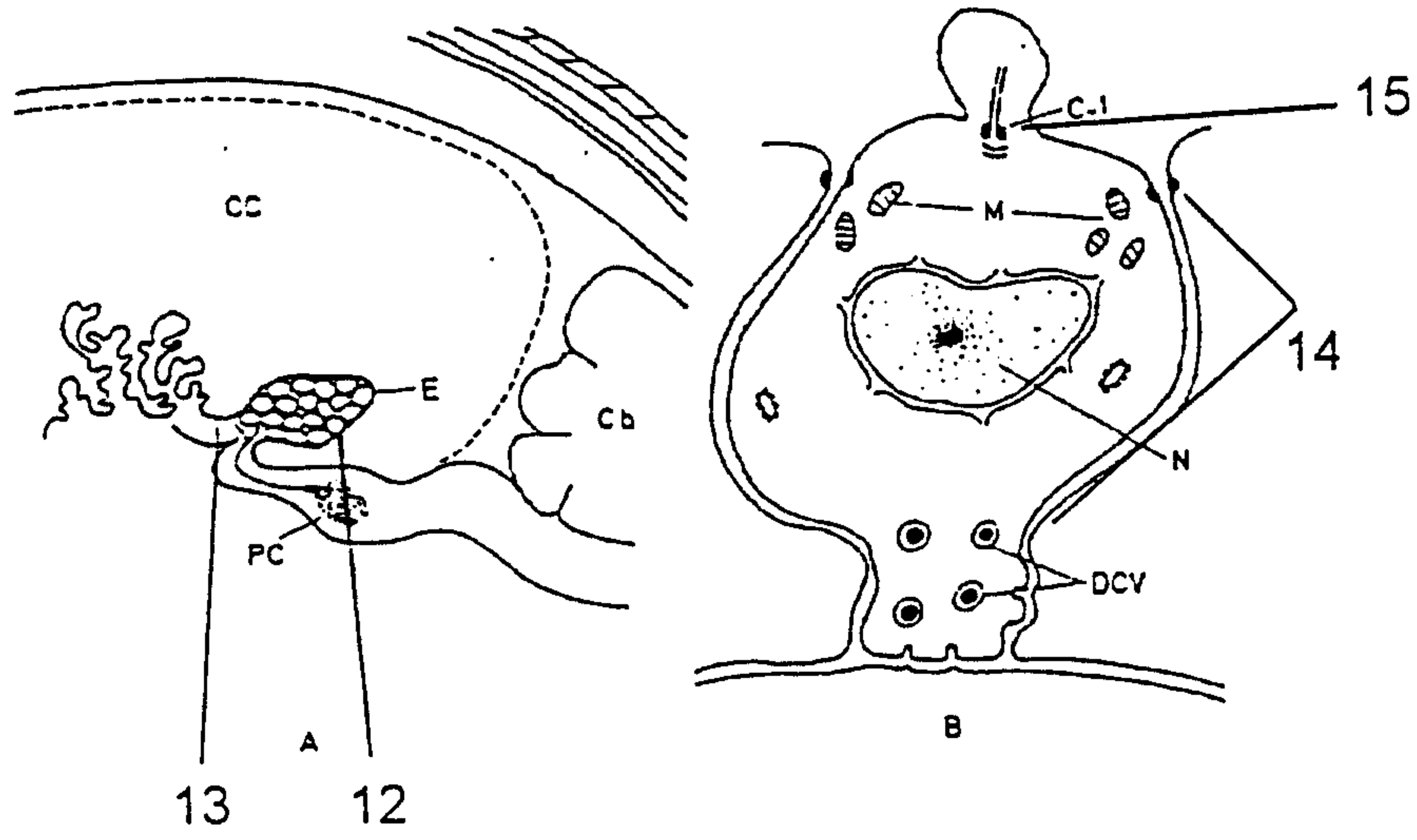


Fig. 4

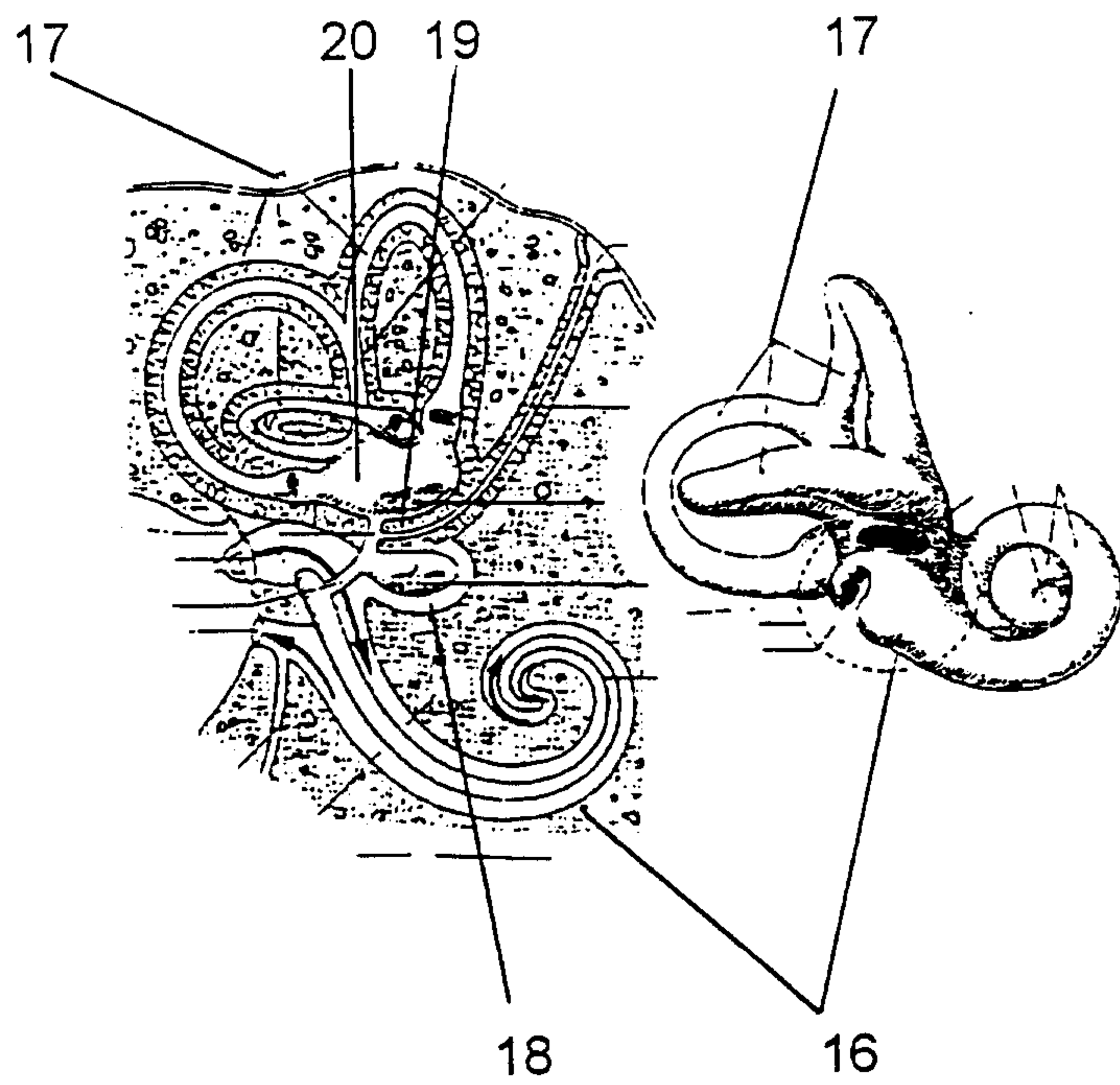


Fig. 5

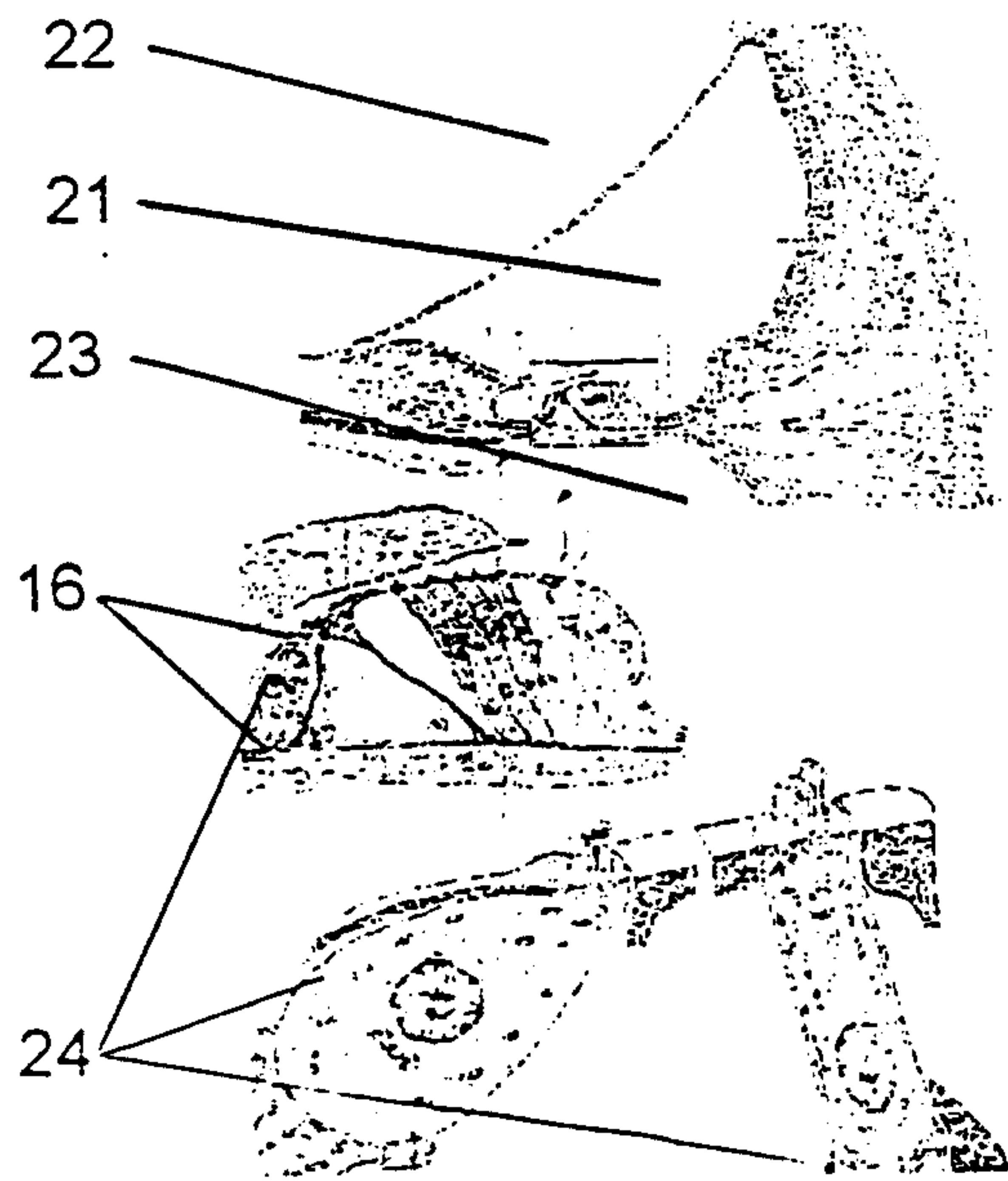


Fig. 6

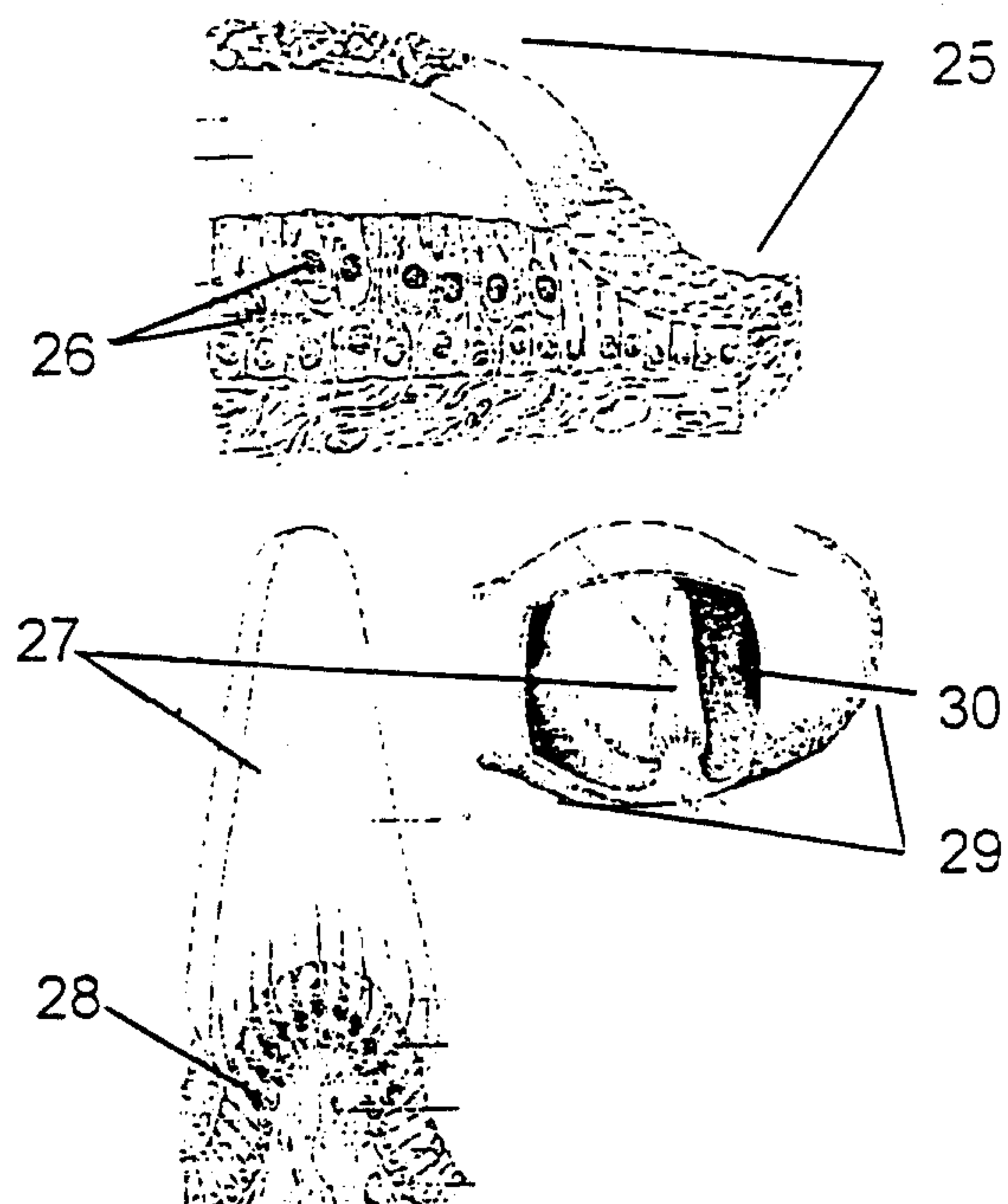


Fig. 7

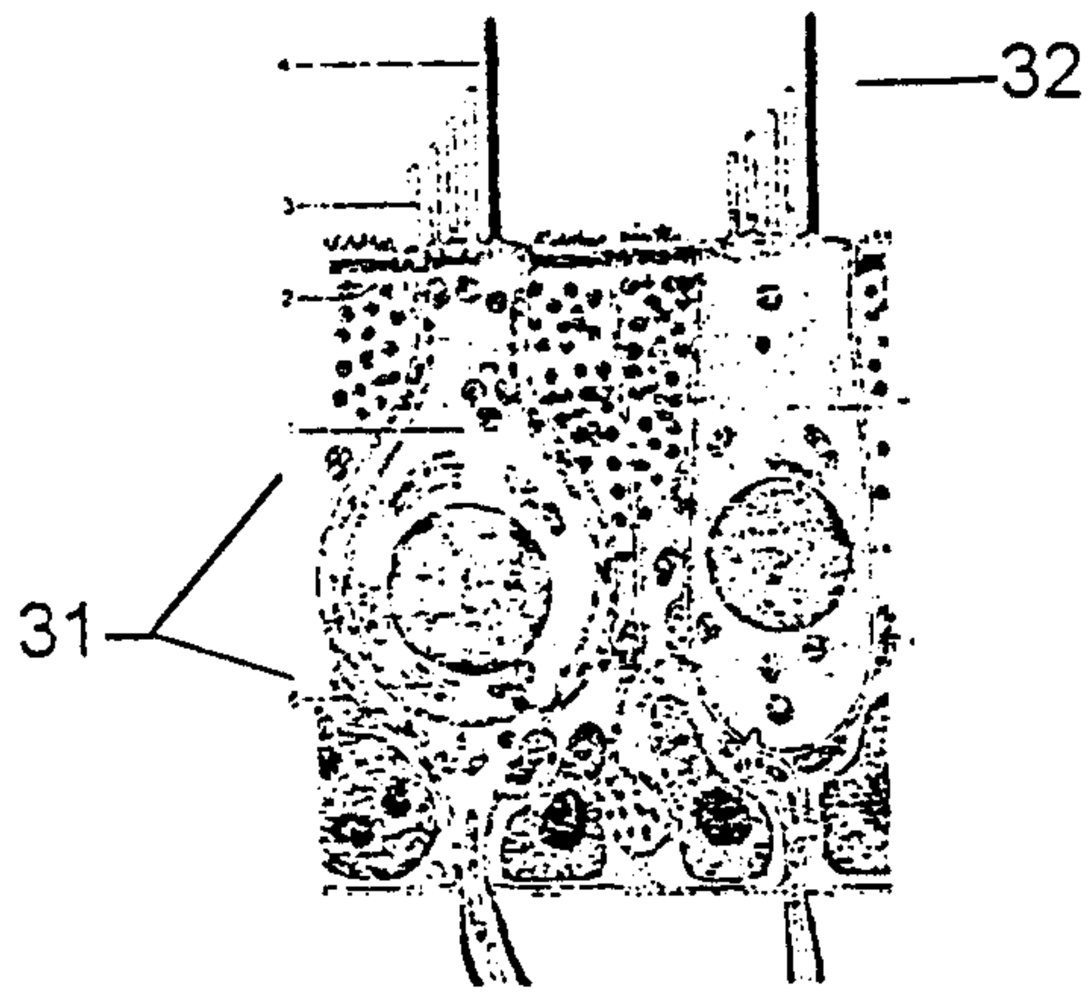


Fig. 8

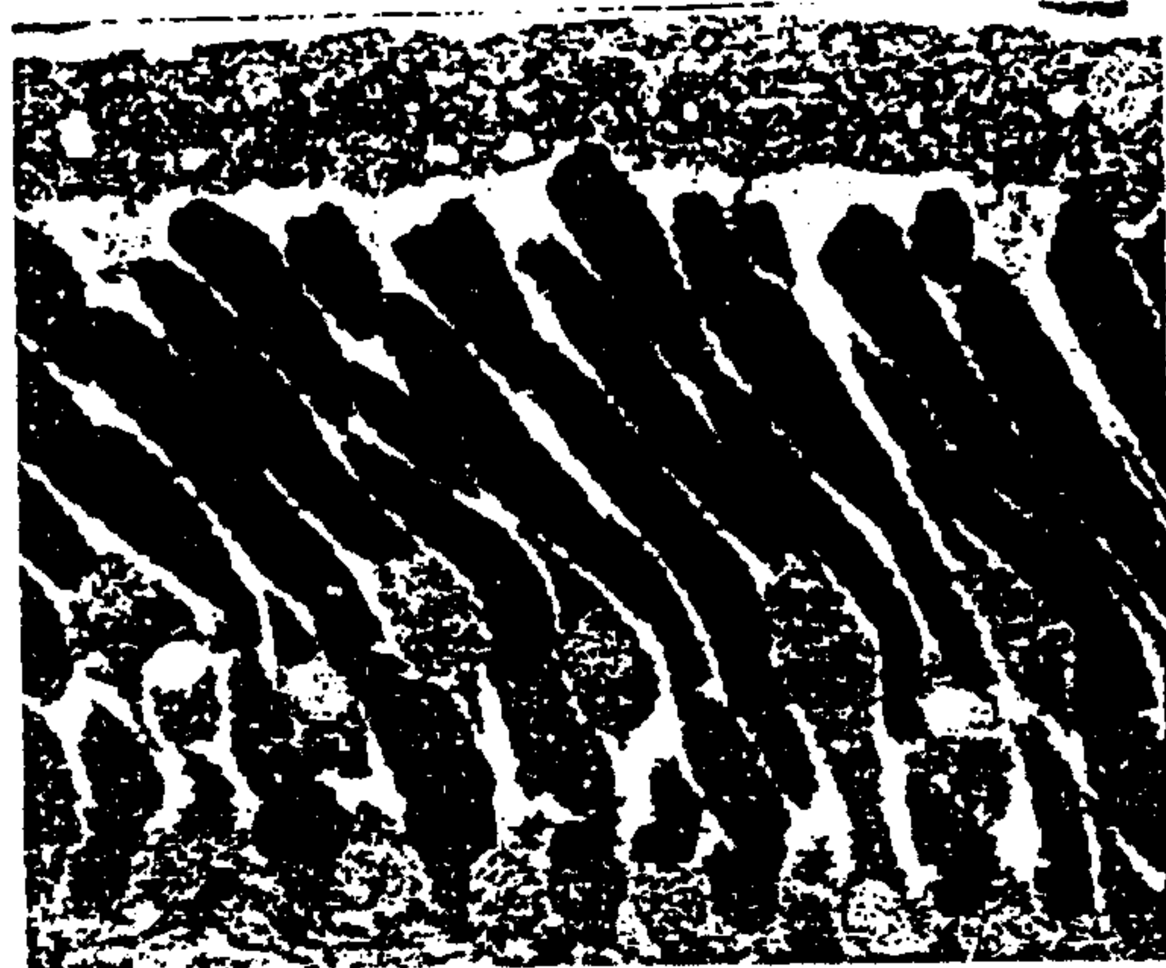


Fig. 9

Fig. 10

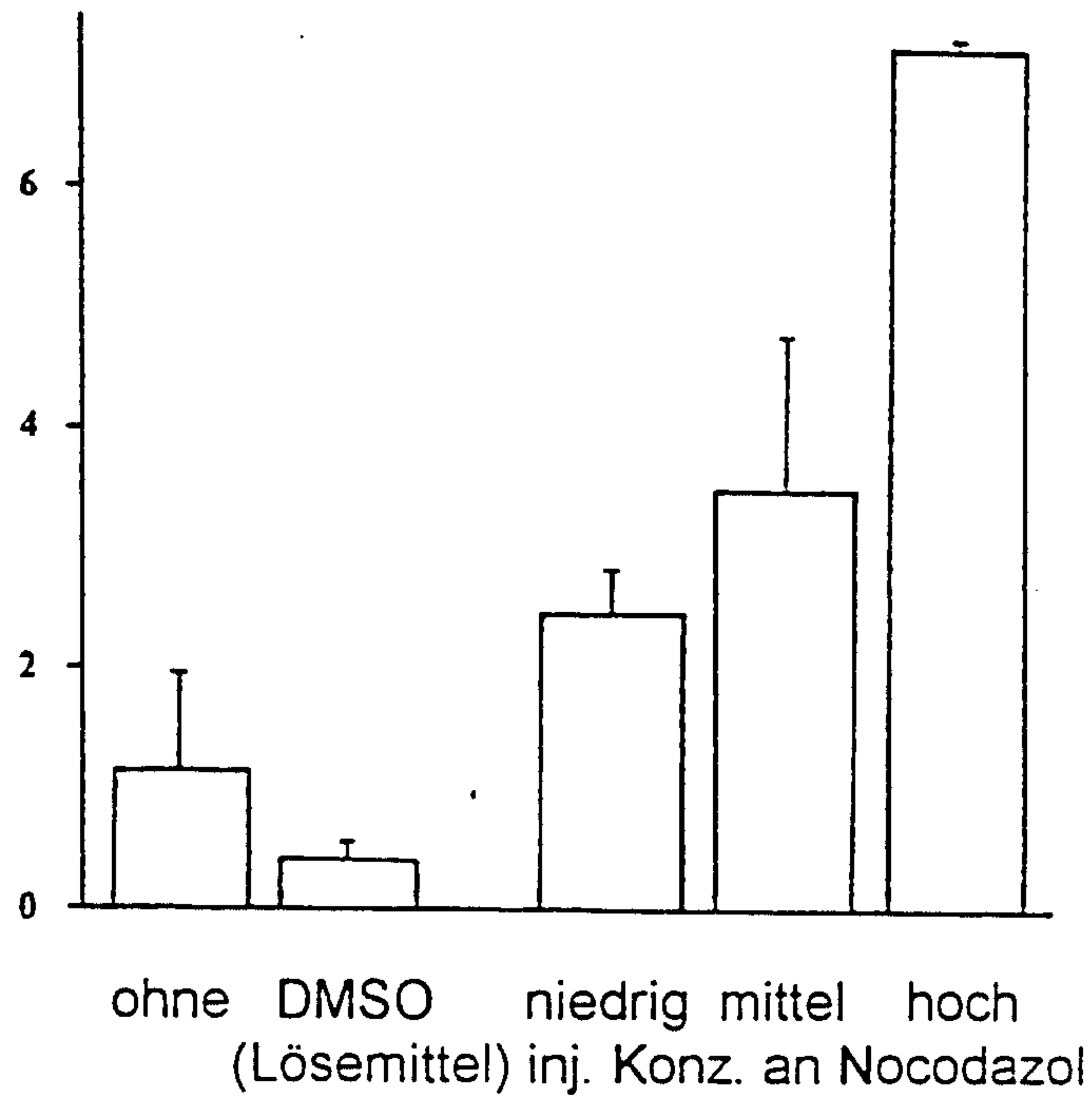
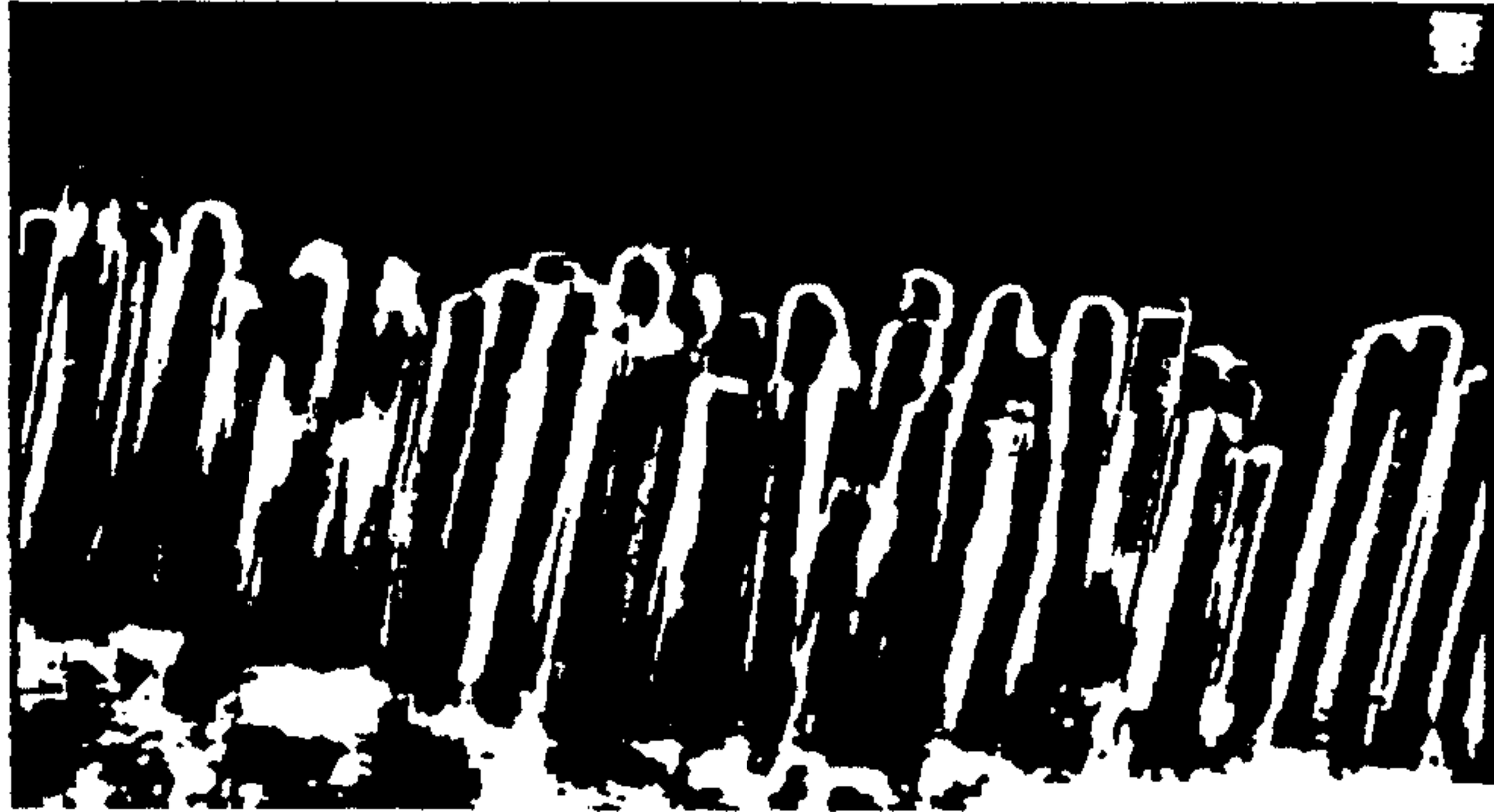


Fig. 11

a



b

c

d

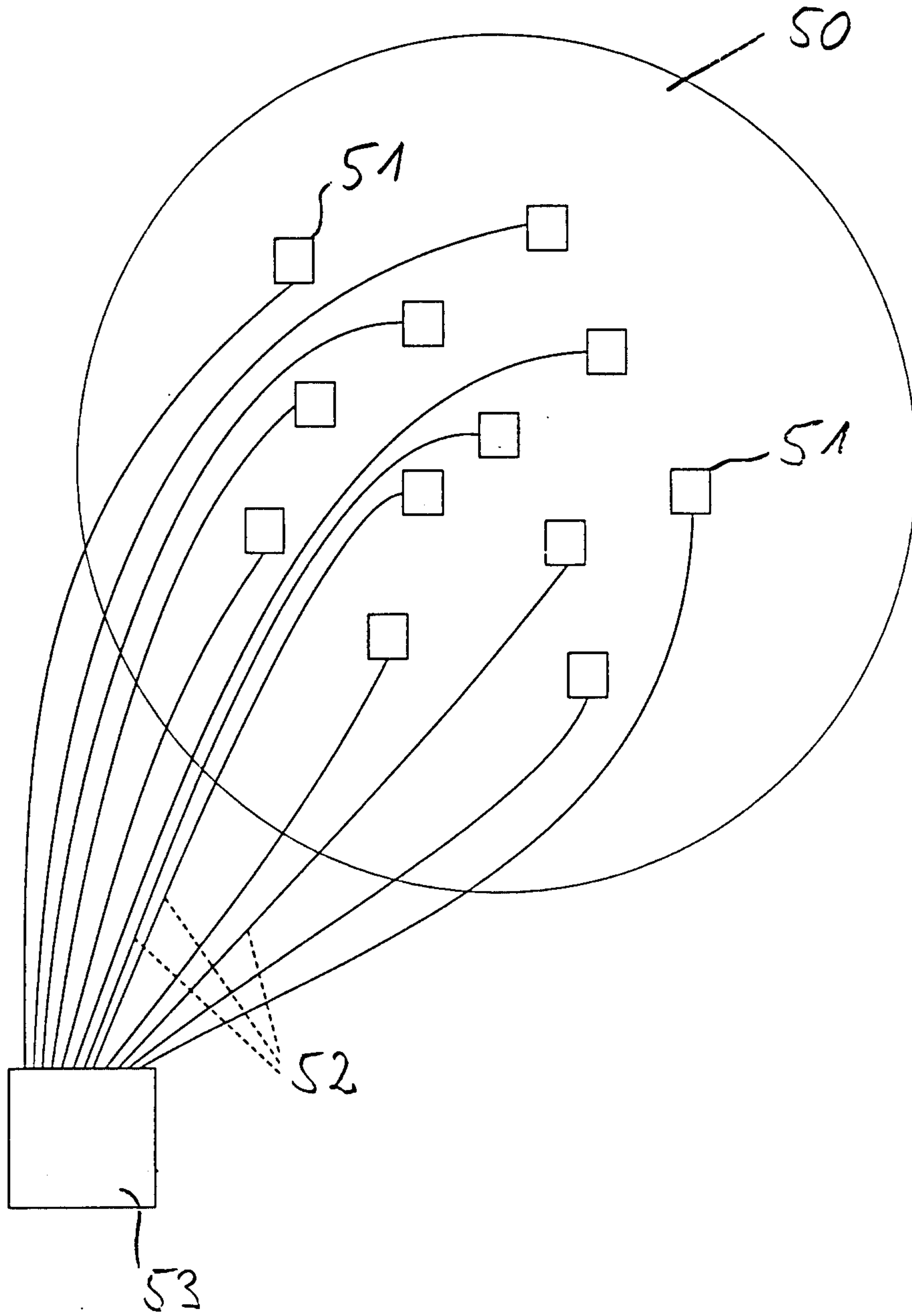


Fig. 12

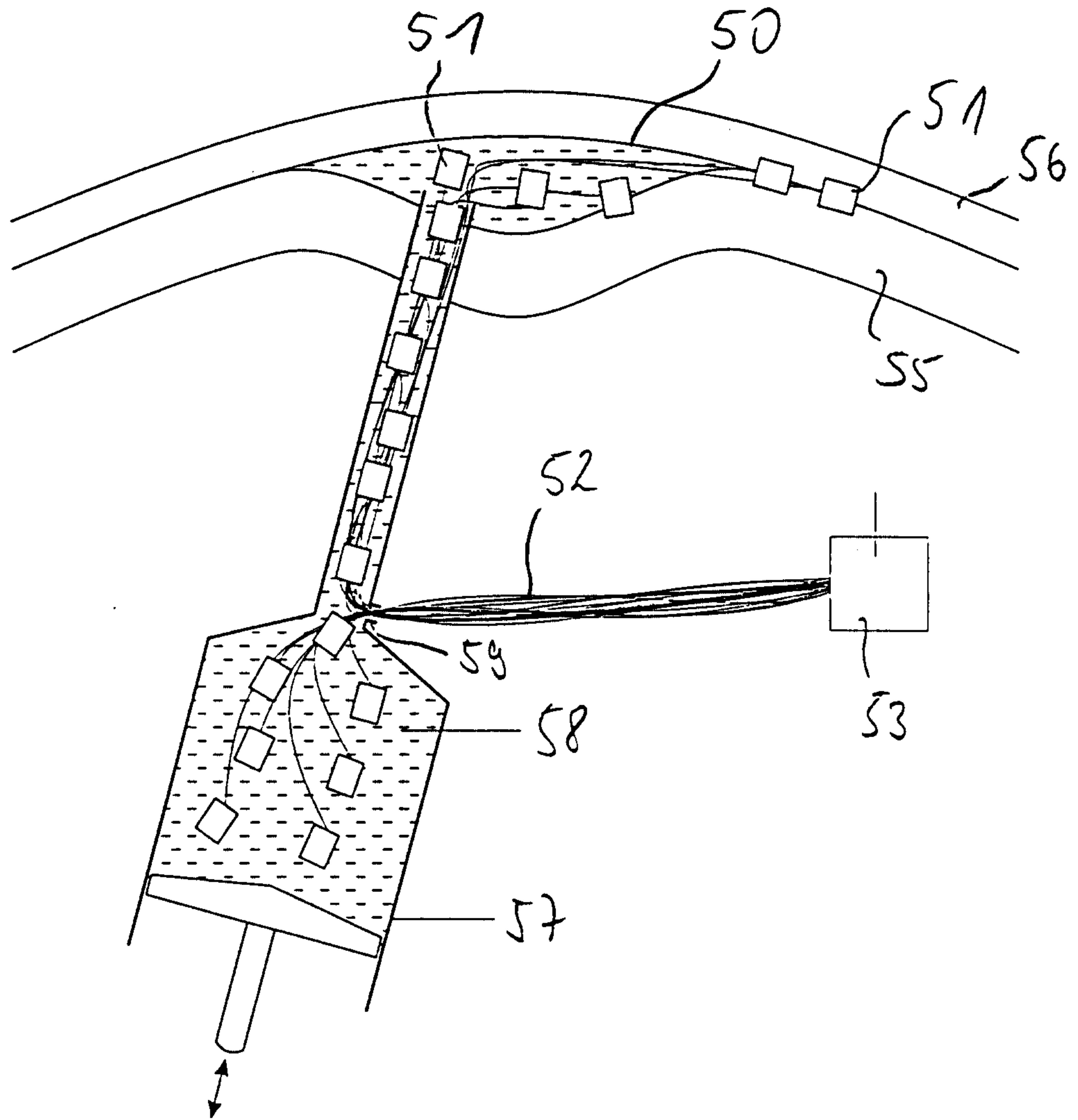


Fig. 13

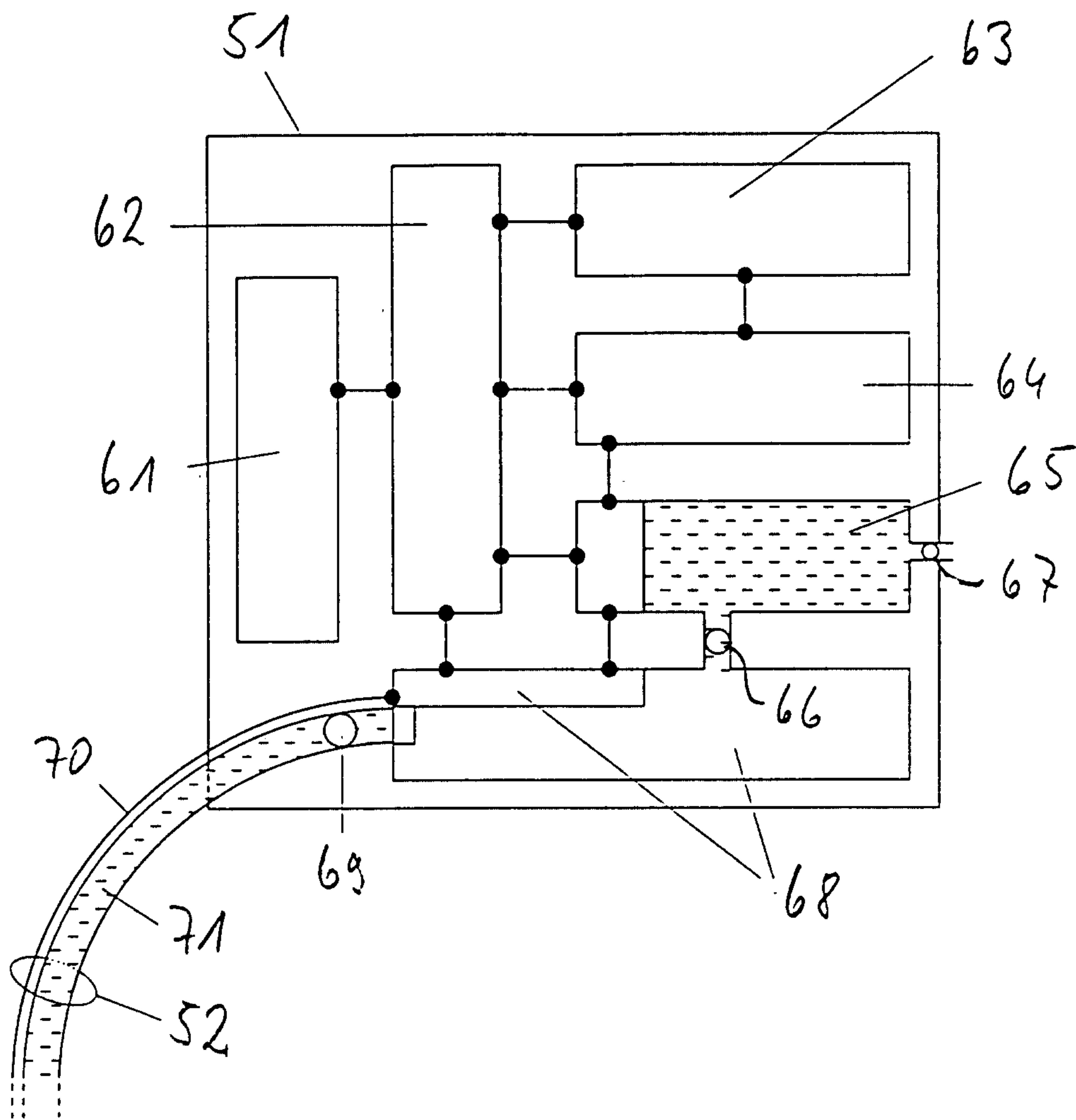


Fig. 14

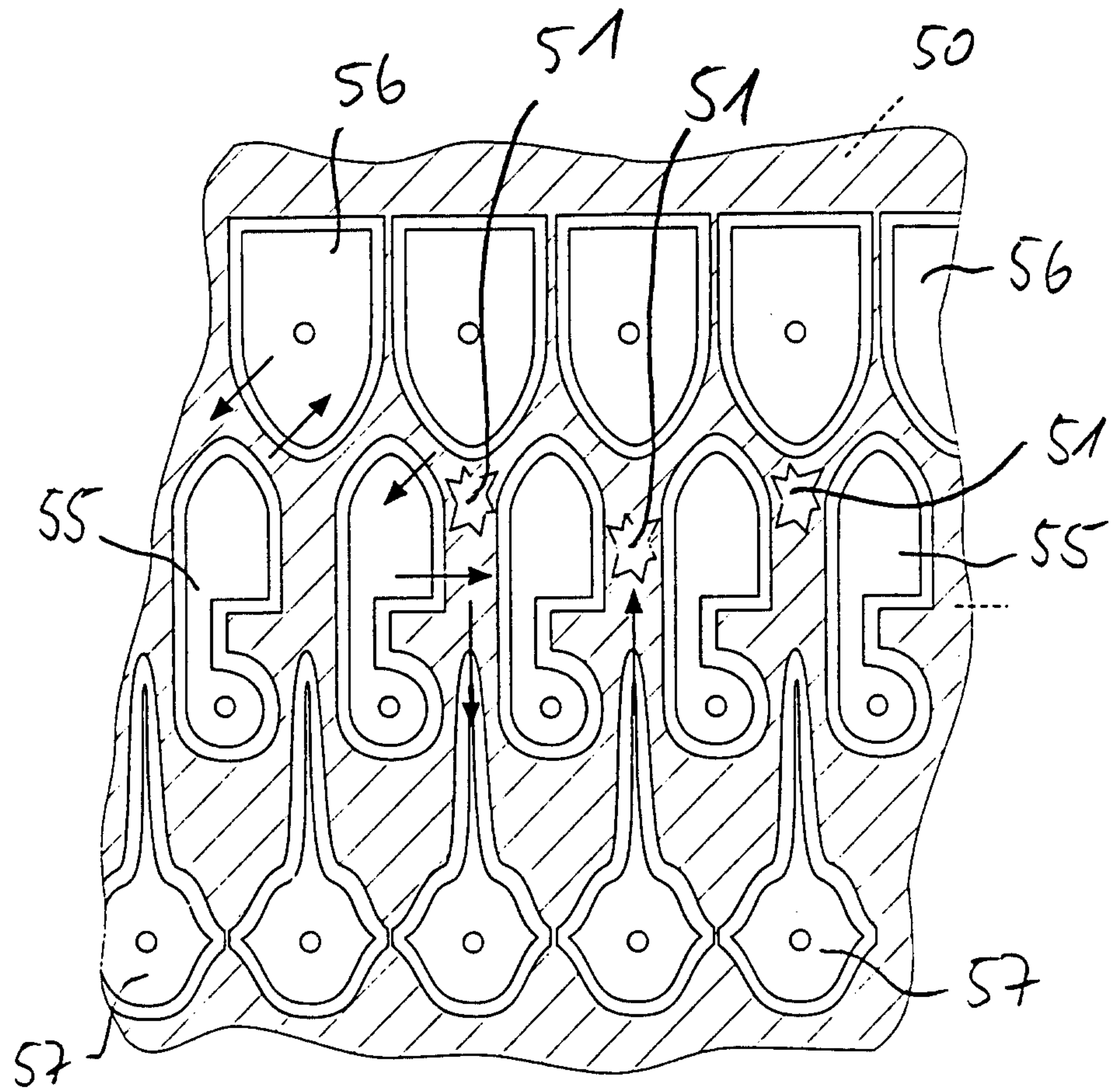


Fig. 15