The Shape and Function of the Cupula*

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IN a recent paper in this journal, Zalin (1967) has discussed the structure and attachments of the cupula and function of the hairs of the sensory epithelium in the inner ear. These discussions include some speculations which seem to be based largely on misconceptions which have appeared in the literature without being corrected. Such speculations therefore demand some further comment.

The mechanism of hair cell stimulation presents a very complex and difficult research problem which has not been solved in spite of enormous efforts and great experimental skill provided by a multitude of scientists all over the world. Contributions to an unbiased discussion are properly confined, however, to the exchange of arguments based on firm experimental evidence and to the correction of misconceptions which could impede progress.

The Structure of the Cupula

The author of the paper mentioned above starts with the assumption that the cupula is a "bag of jelly enclosed in a delicate membrane", without giving any experimental or histological evidence to support this assumption. The general structure of the cupula has been well established by the work of many authors.

Examination of the living vestibular apparatus shows that the cupula is a structure which always retains the general shape of a dividing partition stretching from the crista to the ceiling of the ampulla, filling the entire cross-section of the ampulla and creating a *nearly* water-tight partition which can move, within limits, as a "revolving door". It has been established that if the pressure of the endolymph applied to one side of the cupula rises above a certain value, a small leakage occurs between the upper limit of the cupular structure and the ampullary wall, but not at the crista end of the cupula, i.e. not through the "subcupular space". This has been demonstrated by several investigators and can easily be confirmed by anyone who will take the time to acquire the skill for such experiments.

It is important to note that the cupula fits equally well into the ampullary cross-section in the resting position and when deflected towards either the utricle or the canal. This indicates that the cupula *can*

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move in both directions without losing its function as an effective partition. Ledoux (personal communication) has also shown in a motion picture that a connection between the degree of cupular deviation and action potential frequency in the ampullary nerve could be established. The presumed cupular movements in response to normal physiological stimulations, however, still remain to be shown. These movements must be very small, since the force applied to the endolymph in physiological angular accelerations is exceedingly small, and this poses the great and most important obstacle to the production of a reasonable explanation of the mechanism of hair cell stimulation.

In routine histological sections the cupula always appears as a small shrunken structure above the crista, even in the best microscopic preparations. This is due not so much to the methods of fixation as to the action of the dehydrating agents used for the embedding. The fully extended form of the cupula has been shown in frozen dried sections (Vilstrup, 1950) and in gelatin embedded ears (Trincker, 1952), but these are difficult techniques and cannot be used for routine examinations of the cupula.

Iurato (1957) has examined the chemical constitution of the cupula and found that it contains a protein similar to epidermin and the protein of the neuroglia. He has also studied the cupula and the tectorial membrane in polarized light and has described a definite orientation within these structures. This structural problem can also be seen in every suitably orientated light microscopic section of the cupula and has been shown by every histologist who has examined the inner ear in the last 50 years except Bowen (1933), who in 1930 expressed the view that the whole cupula was a fixational artefact.

A cross-section through the ampulla (Fig. 1) shows the orientation of the "fibres" or "canals" in the cupula extending from the crista towards the vertex of the ampulla. It also shows the extent of the shrinkage due to dehydration. A closer view of this cupula (Fig. 2) shows that the structural orientation of the "canals" extends to its upper surface.

The "subcupular space" shows some characteristics worth mentioning, as seen in the section close to the crista (Fig. 3). The "canals" appear much more regular and "mature" at the upper end of the cupula (Fig. 2), than closer to the crista (Fig. 3). The small elevation at the middle of the crista (equivalent to the eminentia cruciata in lower animals), which contains no hair cells, shows a wide and long extension of the "subcupular space" lacking the longitudinal striation of the cupula over the hair cell regions of the crista. This supports the view that the "subcupular space" contains the secretion from cells on the crista and that the hairs constitute a mould from which the longitudinal canal-like striations of the cupular substance are cast. The assumption of a secretion from the surface of the hair cell area is further supported by observations on cupulae which have

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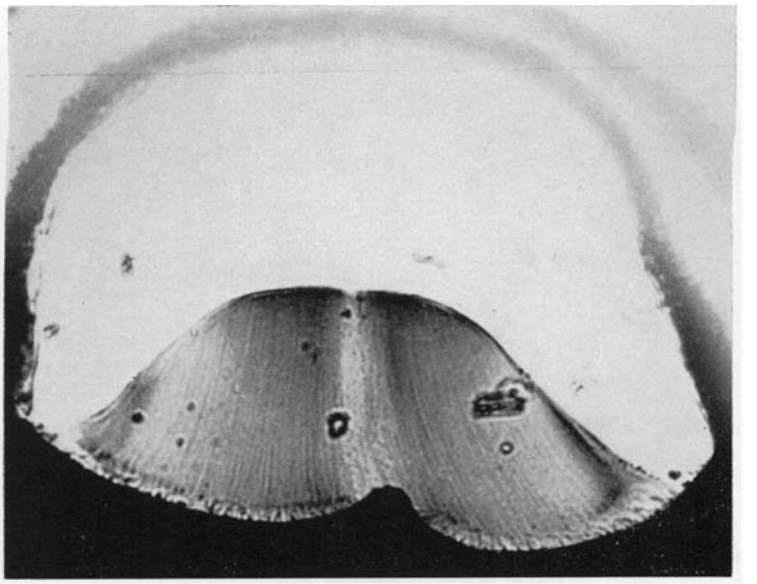


FIG. 1.

Cross-section of the anterior vertical ampulla from a cat. The cupula is shrunken as normal in histological procedures.

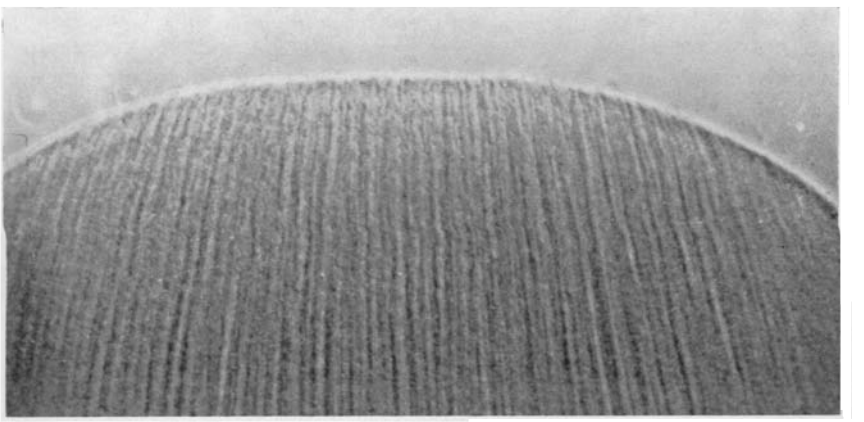


FIG. 2.

The upper part of the cupula shows the canalicular striation which extends to the uppermost surface of the cupula without any enclosing membrane and without any sign of attachment. Cat, formalin-osmic acid fixation, Epon embedding. Phase contrast.

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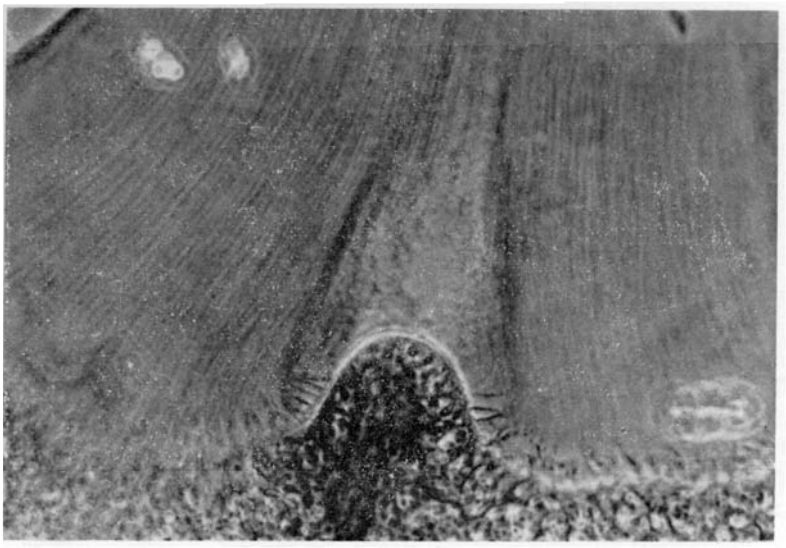


FIG. 3.
Section of crista and cupula from cat. Striation of the cupula over the areas with hair cells, but not over the "eminentia cruciata" where no hairs are found. Formalin-osmic acid fixation, Epon embedding. Phase contrast.

been destroyed with ultrasound. In this case the "subcupular space" (Fig. 4) frequently remains intact, as well as the part of the hairs passing through this "space". The observations that the "subcupular space" withstands the trauma of the ultrasound and that its contents coagulate in the form of globules in the fixation fluid indicate that this "space" is filled not with endolymph but with a secretion which presumably is much more viscous than endolymph, probably being the secretion supplying the material for a continuous growth of the cupula as it is worn out at its upper end. Thus, the cupula above the "subcupular space" is a meshwork of organized incomplete tubular structures, which have openings at its upper end as well as sideways, without an "enclosing membrane" as assumed by Zalin (Fig. 5). This is further proven in autoradiographic experiments which show that the secretion of endolymph from planum semilunatum in the side walls of the ampulla easily penetrates into the meshwork of the cupula (Dohlman, *et al.*, 1964).

The Interpretation of Cross Sections

The second misconception reported by Zalin (1967) is that the cupula hangs from the "dome" and that it could even be referred to as an appendage to the "roof epithelium". This is totally misleading, and stems from the misinterpretation of a microscopic section through the ampulla published by Belanger in 1961. The statement made by Belanger

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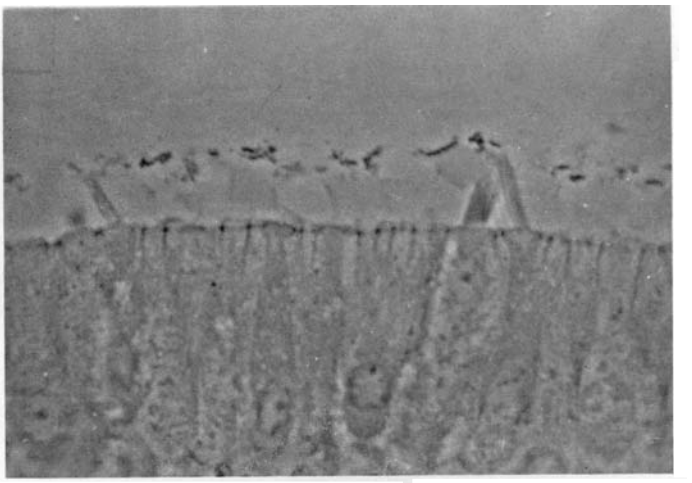


FIG. 4*a*.

Hair cell region after ultrasound treatment. Above the hair cell surface the hairs are preserved in the "subcupular space" but are shattered in the region of the cupula proper. The fluid in the "subcupular space" has coagulated in the fixation and appears as globules indicating a viscous fluid. Osmic acid fixation, Epon embedding. Section $\frac{1}{2}$ μ thick. Phase contrast $\times 1,200$.



FIG. 4*b*.

Normal crista from pigeon showing the cupula and the hairs of the hair cells. The hairs are erect in the "subcupular space" but are bent in the cupular substance in accordance with the direction of the shrinkage of the cupula in the process of dehydration. Osmic acid fixation, Epon embedding, $\frac{1}{2}$ μ sections. Phase contrast $\times 400$.

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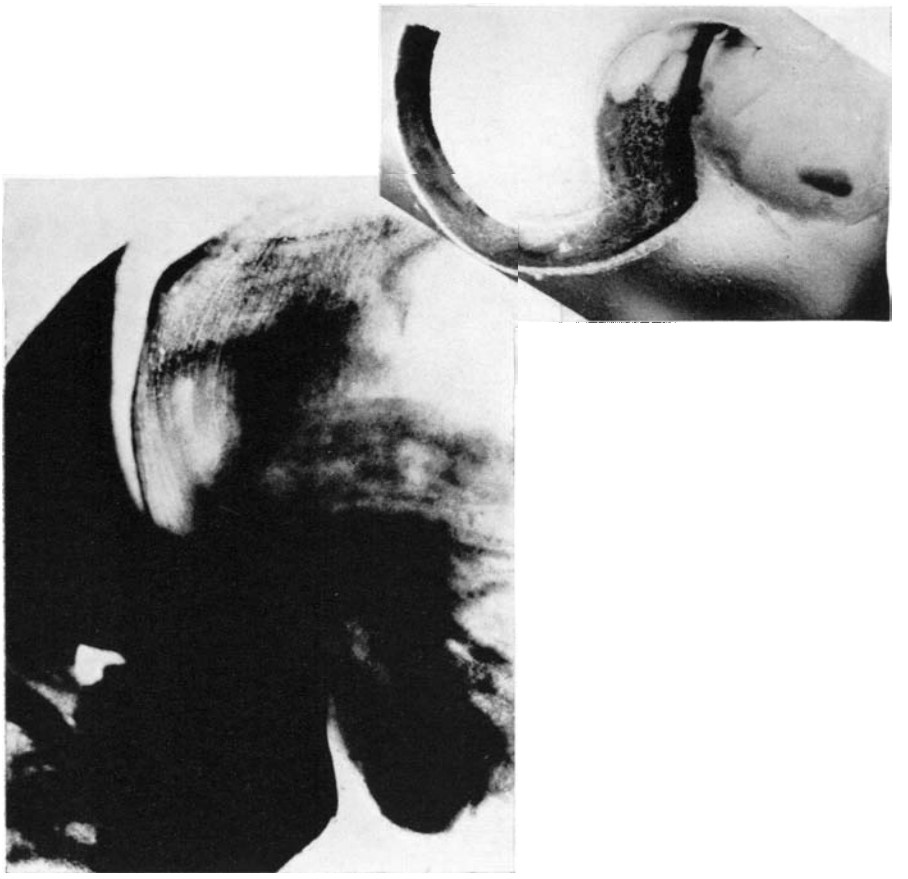


FIG. 5.

Injection of indian ink into the canal in a shark. (*a*). The indian ink has passed into the ampulla but stops at the canalicular surface of the cupula which has been slightly bent towards the utricle. The ampulla is viewed from its lateral side. A small leakage can be seen at the upper end of the cupula. (*b*). By tilting the specimen, a striated image of the cupula can be seen as a result of indian ink particles entering the "canals" of the cupula.

and cited by Zalin has since been quoted by several authors without contradiction or further explanation, and it therefore seems necessary to discuss the interpretation of microscopic sections through the ampulla in order to explain how such pictures as those of Belanger and others can be produced and what they actually show.

As every histologist acquainted with the anatomy of the inner ear knows, the orientation of the specimen is a most important part of the technical procedure of sectioning to obtain information about size, form and interrelations of structures in the complex labyrinth of the ear. Starting with the cross-section of the ampulla of the cat, as shown in

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Fig. 1 and represented at the top of Fig. 6, sections made in planes at right-angles to that of Fig. 1 are easily visualized. These are represented in Fig. 6 which shows the sections obtained in planes 1, 2 and 3, all perpendicular to the plane of cross-section of the ampulla.

The section in plane 2 gives the impression of an unusually high and well-preserved cupula, due to sectioning near the base of the cupula.

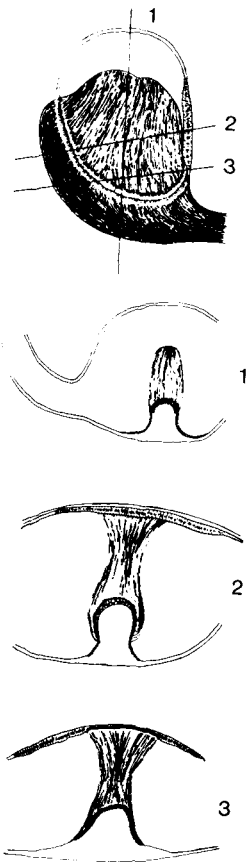


Fig. 6.

Effect on the appearance of the cupula due to the plane of sectioning.

The cupula may also appear to be without shrinkage and to be continuous to the "roof", as for instance in Fig. 4 (Igarishi, 1966), McLay *et al.* (1961). A low and flat epithelium, however, covers the proper vertex of the ampulla. Section 1 in plane 2 shows a much higher epithelium, and in good sections it is easy to recognize the secretory epithelium of the planum semilunatum on the lateral walls of the ampulla. Plane 3, corresponding to the section published by Belanger (1961), cuts the

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ampulla even closer to its base. Sections in this plane show the hair cells of the crista with its hairs and cupula fixed to the sides of the crista. As in plane 2 the cupula is cut continuously to the opposite side wall of the ampulla. Close to the crista, the "fibres" of the cupula usually extend more or less straight from the crista where they originate. Midway to the opposite ampullary wall they are sectioned more crosswise and appear irregular. Again, closer to the opposite ampullary wall the fibres can be seen to originate from this wall, which is in accordance with their fixation close to the area of the sensory epithelium which extends up on the lateral wall.

The epithelium on the lateral wall is composed of hair cells and cells of planum semilunatum, and not the flat epithelium of the ceiling of the ampulla at the vertex. This is demonstrated by the autoradiographs of Belanger (1961) where the labelled isotopes are shown to be active in the epithelium and in the secretion poured into the meshwork of the cupula. A section from an autoradiographic experiment on a pigeon, corresponding to the section of Belanger, is reproduced in Fig. 7 and shows that what Belanger calls "the high roof epithelium" is the hair cell region, surrounded on both sides by the planum semilunatum cells, of the lateral wall. This explains the misconception that the cupula is attached to the "roof epithelium", but is in accord with the view that the cupula is fixed to the crista with a *free* upper surface which in the living animal fits *nearly* watertight, as a "revolving door", to the walls of the ampulla.

The Function of the Hair Cells

In a reproduction from an electronmicrogram of one stereocilium from a hair cell, Zalin (1967) has interpreted the configuration of the tip of the hair as proof of fixation of the hairs to the cupula. I believe that this is contrary to the conception of most electron microscopists who are working on the histology of the inner ear. Further, in experiments removing the cupula (Dohlman and Boord, 1964), it could be evidenced that the resting action-potential frequency in the nerve remains unchanged. The ampulla which had been operated on no longer responded, however, to the usual physiological stimuli. This both confirms the importance of the lever action of the cupular structure to the dynamics of the transmission of the stimulating force in the vestibular system, and also shows that the hair cell-nerve ending function does not depend upon the presence or absence of the cupula. Moreover, as shown in Fig. 8 the sensory epithelium and the hairs remained intact. The hairs therefore are not firmly attached to the cupular substance, since, if they were, the removal of the cupula would detach the hairs and also kill the cell.

Zalin discusses the mechanism of hair cell stimulation further in

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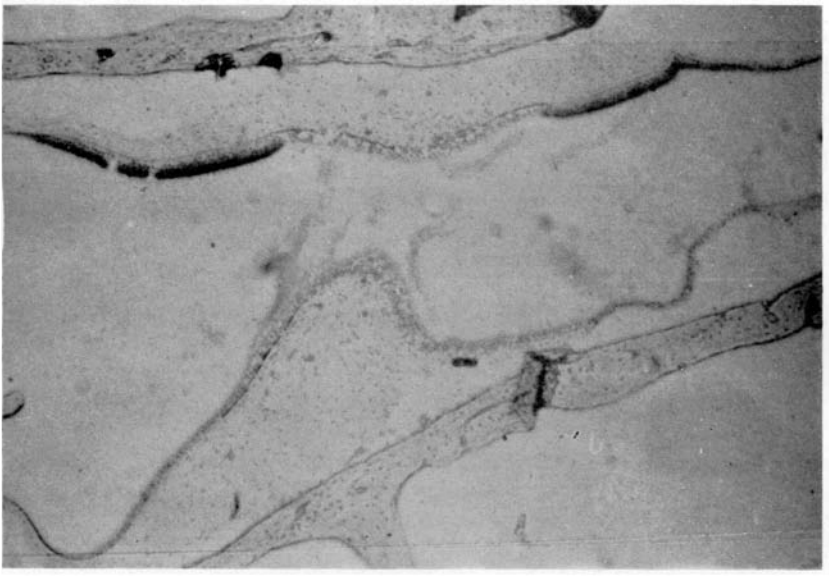


FIG. 7*a*.

Section of the horizontal ampulla in pigeon (corresponding to plane 3 of Fig. 5). The secretory cells of planum semilunatum on the upper wall show the uptake of sulphur autoradiographically by the blackening in the photographic emulsion over the section. The cupula is outlined roughly, due to the procedure used in autoradiography, but can be seen stretching from the crista (lower part of the picture) to the hair cell region between the sectioned parts of planum semilunatum.

terms of the concept of the "biased" cell, suggesting that the kinocilium tilts the stereocilia, thereby creating the resting activity in the nerve. This is a pure conjecture, unsupported by any known evidence. To explain the action of the kinocilium Zalin assumes that the kinocilium "provides a gentle tide of endolymph driven through the subcupular space". In spite of much careful observation no one has been able to show any ciliary movements in the region of the sensory area. Moreover, there is reason to believe that the "subcupular space" does not contain endolymph but a much denser secretional product, as has been mentioned earlier.

The absence of the kinocilium in the cochlea is explained on the assumption "that the brain does not need to know that the hair cell is swinging from one side to the other and hence biasing is unnecessary". This implies that every full cycle of a sinusoidal wave should produce two stimulations instead of one in the "biased" cell. It was shown by Davis (1958), however, that the hair cells in the cochlea give rise to an action potential only when the movement of the basilar membrane bends the stereocilia towards the missing kinocilium and not in the other direction. They behave in this situation in the same way as the "biased" cells in the vestibular apparatus, contrary to the assumptions made by Zalin.

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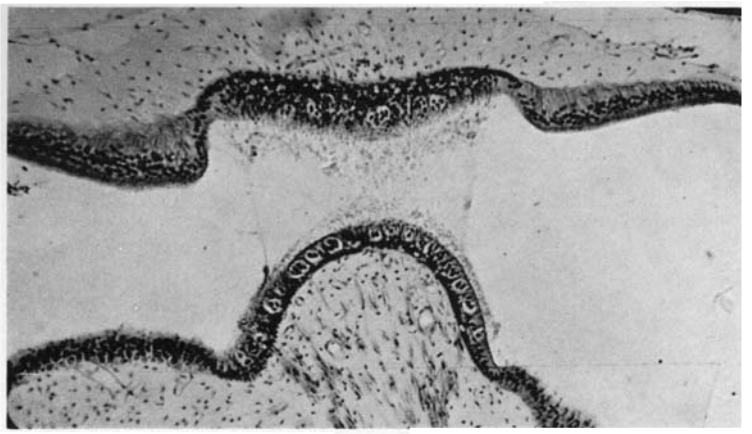


FIG. 7*b*.

Section similar to the section 7*a*, but in this the "roof" epithelium is clearly recognized as hair cell region. On either side of the "roof" epithelium the characteristic high epithelium of the planum semilunatum can be distinguished.

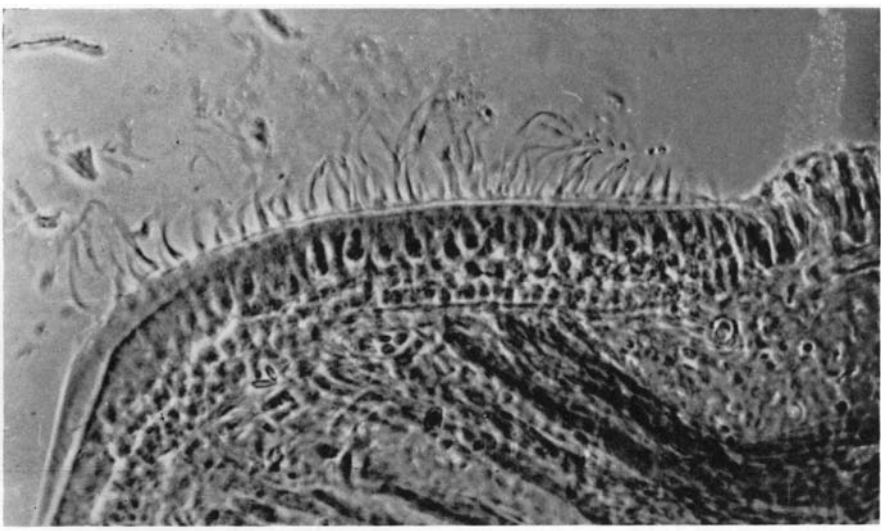


FIG. 8.

Cupula removed by suction. The hairs are floating free in the endolymph. Boeing fixation 5 μ paraffin sections. Phase contrast.

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The theoretical assumptions and predictions made in the article have been used further to explain some known clinical vestibular phenomena, with the addition of hypothetical changes in the "vital activity" of the stereocilia, "reduced activity of the kinocilia", etc. Such conjectural speculations contribute little of value to the solution of the intrinsically difficult problem of hair cell stimulation in the inner ear. For those who are not familiar with the results of physiological research in this field the article by Zalin might produce more confusion than clarification, and for those who have followed the progress made in vestibular physiology during the last decades, speculations like these are not enlightening.

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