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10. In contrast to the precursor 40*S* RNP in which one component of peak *c* may be missing, this physically produced 40*S* RNP may be deficient in two protein components as judged from the ratio of H^3 to C^{14}. A milder treatment of the 50*S* subunit with LiCl might be necessary to obtain the particle having the same protein components with the precursor 40*S* RNP.
11. Supported in part by PHS grant GM 12045 and a grant from Matsunaga Science Foundation.

23 March 1967

Otolithic Membranes of the Saccule and Utricle in Man

Abstract. *The otolithic membranes of the human saccule and utricle can be prepared as whole mounts or surface specimens for microscopic examination. They are not simple, homogeneous, gelatinous structures as heretofore described. Instead, each shows a definite and characteristic fibrillar design, which appears to be correlated with the known cytoarchitectural pattern of the underlying neuroepithelium.*

The problems posed by weightlessness and other unusual stimuli to the vestibular system that are met with in space flight have led to something of a revival of learning about the structure and activity of the vestibular end organs. The saccular and utricular maculae, in their updated role of "gravireceptors" (*1*) or "linear translation sensors" (*2*), have been receiving attention such as they have not enjoyed since the work of Magnus and his school on the physiology of posture (*3*). During the past few years the neuroepithelia of these structures have been intensively scrutinized under the electron microscope (*4*), and their responses, along with those of the ampullar cristae of the semicircular canals, have been analyzed in control-system studies aimed at the development of mathematical models to represent their function (see *2*).

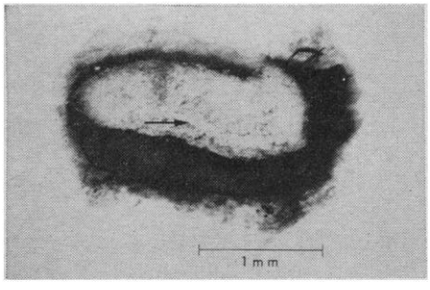

Fig. 1. Macula sacculi from an adult man (right ear). The white crystalline mass of otokonia still covers the otolithic membrane. The arrow indicates the "snowdrift" line, near the inferior margin, representing the heaviest deposit of otokonia. The surrounding membranes are stained with the OsO_4 fixative.

Although the otolithic membrane, the essential movable part of each macular end organ that overlies the neuroepithelium, was first pictured by Breschet (*5*) in 1836, it has remained, as in the time of Retzius (*6*), an ill-defined and vaguely apprehended "gelatinous layer," or, as Polyak has described it, "a jelly-like substance, of an almost homogeneous structure, in which the hairs of the haircells are embedded" (*7*). The reason for this vagueness lies in the unsatisfactory state of preservation of the otolithic membranes after routine histological treatment of the temporal bone for light microscopy, which, as reemphasized by Igarashi (*8*), involves shrinkage and distortion by powerful fixatives and acid decalcifying agents, and in the limited view of the membranes that is afforded by the usual stained cross sections. So far as we are able to determine from a painstaking search of the literature, the otolithic membranes, though always treated as familiar structures, have never been directly examined as separate entities comparable with the cupulae of the semicircular canals or the tectorial membrane of Corti's organ.

In a study of the normal and pathological anatomy of the human inner ear by direct microdissection in place of conventional microtomy, we have been able to prepare the otolithic membranes of the saccule and utricle for examination as whole-mounts or surface preparations. Their configuration is quite different from what has been universally assumed, and one may infer that it has important functional implications that are not taken into account by current vestibular theory.

Human temporal bones are obtained at routine autopsy by the standard method with the Stryker bone-plug cutter (*9*). The inner ear structures are fixed by perilymphatic perfusion through the oval and round windows with a buffered solution of 4 percent paraformaldehyde, stained in place for 1 to 2 hours with 0.5 to 1.0 percent solution of phosphate-buffered OsO_4 (Millonig), washed in physiological saline, and put through a graded series of alcohols up to 70 percent. Under the operating microscope the surrounding bone and the otic capsule are ground down to a thin shell with dental burrs and then removed. A final dissection of the membranous labyrinth is made with watchmaker's tweezers, iris scissors, and fine, electrolytically sharpened, tungsten-wire dissecting needles, as recently described (*10*).

This procedure affords an extraordinarily instructive view of the end organs, membranes, blood vessels, and nerve fibers of the inner ear.

The saccule and utricle are easily dissected out from the vestibule. The membranous portions surrounding the maculae are then removed. When the otokonia are still in place, the maculae (Figs. 1 and 2) look much the same as they have been shown for other species in Werner's (*11*) drawings and de Burlet and Hoffman's (*12*) stereophotographs. The characteristic raised

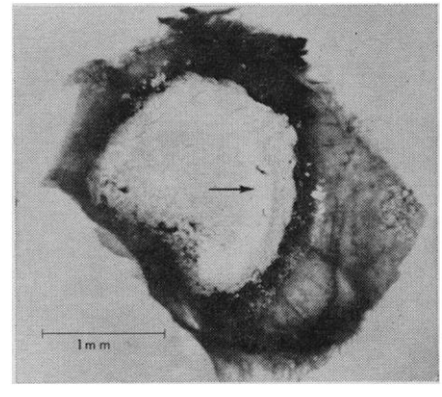

Fig. 2. Macula utriculi from an adult man (right ear) showing the horseshoe-shaped "snowdrift" line (arrow) along the lateral and anterior margins.

surface contour of the otokonial mass, with the "horseshoe-form" described by Odenius (*13*), is seen to correspond to Engström's (*14*) "snowdrift" and to the "striola" pointed out by Werner. The mass itself has the consistency of fine, damp, lightly packed sand. It is made up of elongated, rhombohedral crystals, varying in length from 1 to 20 μ. According to Carlström (*15*), the crystals consist of $CaCO_3$ in the form of calcite rather than aragonite, and they are held together by a thin, unspecified, organic gel. They tend to adhere to the upper surface of the otolithic membrane but are in no sense embedded in it.

If the alcohol in which the specimen is kept is slightly acidic, the otokonia often partly or completely dissolve. Otherwise, they can be carefully removed with a fine dissecting needle. The otolithic membrane, which still covers the macula and is loosely attached to it, can then be gently lifted off in one piece for detailed study by stereo- and phase-contrast microscopy.

The otolithic membrane of the saccule is a beautiful, feathery structure, reminiscent of frost-tracery on a window pane. Its complicated pattern is especially pronounced at the anterolateral end, with individual variations among the specimens we have examined (Fig. 3). The otolithic membrane of the utricle is thinner and finer, with a simpler, less emphatic design (Fig. 4). Both membranes show a definite thickening (striola), which forms the major dividing line of the fibrillar pattern.

Each membrane consists of flattened bundles of thicker radiating fibrils with an interlacing feltwork of finer transverse connecting fibrils, all embedded in what may still be regarded as a gelatinous ground substance, pending more precise chemical and electron-microscopic characterization. The margins have irregular projections, apparently for attachment to the epithelium immediately surrounding the haircell area (Fig. 5). Into an occasional opening in the underside of the membrane the long hairs of a sensory cell may be seen to protrude. The hairs of the majority of the haircells appear for the most part to be confined to the subotolithic space and may or may not enter the substance of the membrane. Fresh membranes possess great elasticity, perhaps even more than that of the tectorial membrane of Corti's organ, to which they are of course closely related in origin and composition, as well

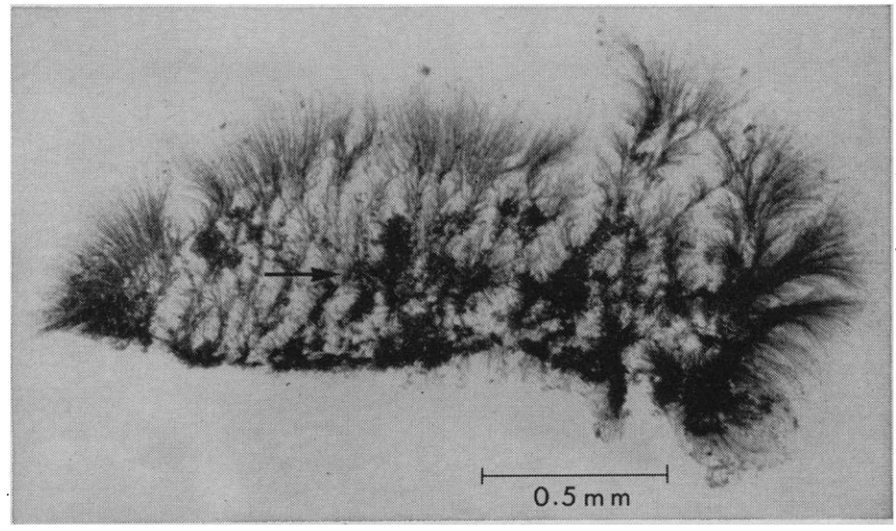

Fig. 3. Otolithic membrane of the right macula sacculi. Most of the otolithic mass has been removed, and the few remaining otokonia appear black with transillumination. The membrane shows a pronounced fibrillar pattern. The arrow points to the thickened portion of the membrane (striola) corresponding to the "snowdrift." Fixation and staining with OsO_4.

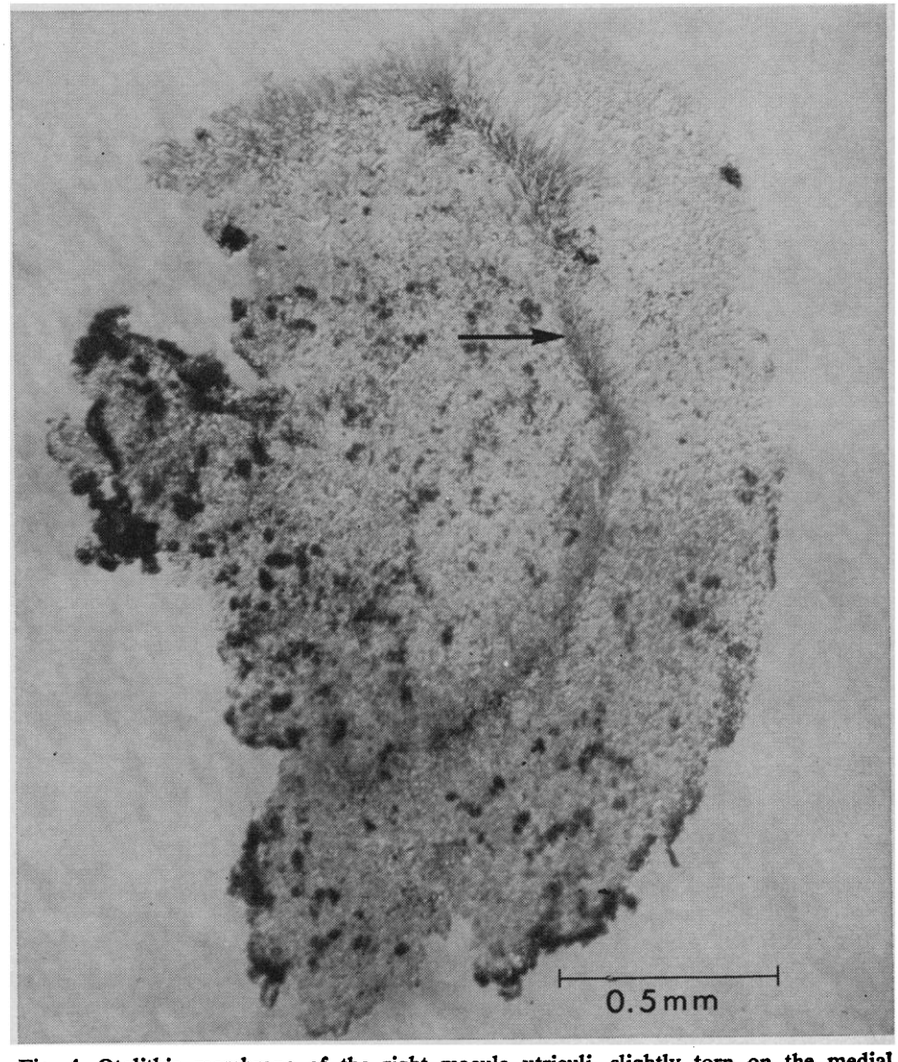

Fig. 4. Otolithic membrane of the right macula utriculi, slightly torn on the medial (left-hand) edge. Note the marked difference in texture and fibrillar pattern from that of the saccule. Dark spots represent remaining otokonia; the arrow indicates the striola.

Fig. 5. A portion of the otolithic membrane of the macula sacculi, showing bundles of coarser radiating fibrils and finer interlacing fibrils between them, all contained in a "gelatinous" ground substance. The margin is uneven, with finger-like projections for attachment to the epithelium. Near the point of the arrow are two small otokonia. Fixation with OsO_4; phase contrast.

as to the delicate, evanescent cupula of the ampullar crista. The configuration of the fresh membrane appears to be identical with that of the fixed, as is the case with the tectorial membrane (*16*).

The undersurface of the membranes is covered with fine, short fibrillae. In the subotolithic space between them and the surface of the neuroepithelium are membranous, transparent disks approximately 10 μ in diameter. Such disks are often seen in stained sections, especially in the subcupular space of the crista. They have generally been taken for either secretion products of the neuroepithelium or fixation artifacts. We can attest to their real presence in fresh specimens, but we know nothing of their chemical composition or their physiological significance.

Important differences among various areas of the maculae have been pointed out by several investigators. Lorente de Nó (*17*) first called attention to regional variation in mode and density of innervation of the neuroepithelium, corresponding to variation in size of the overlying otokonia. Werner (*11*) demonstrated a concentration of large haircells under the striola line, having individual nerve chalices and belonging to Wersäll's type I (*18*). Spoendlin (*19*) has explored the directional orientation of haircell groupings as indicated by the position of the lone kinocilium of each cell with respect to its accompanying bundle of stereocilia. These regional cytoarchitectural patterns now appear to be closely correlated with the unique textural patterns of the respective otolithic membranes.

The characteristic design of each macular apparatus, which includes the specialized forms of the otokonial mass, the otolithic membrane, the sensory epithelial mosaic, and the nerve supply, presumably creates a maximum differential and directional sensitivity to shearing forces (*20*) between the otolithic membrane and the hair cells in response to gravitational or linear acceleratory stimuli. The gravireceptors may be capable of transmitting to the vestibular nuclei sensory information of far greater precision than has customarily been credited to them in theoretical discussions of their function. The pitfalls in postulating an oversimplified macular structure for purposes of model-building are obvious.

The unexpected difference between the saccular and utricular otolithic membranes revives the possibility that the physiological roles of these two end organs may indeed be quite distinct, as many observers have suggested in the past. On the other hand, the coarser texture of the saccular membrane may merely provide for greater damping of its movements, perhaps to minimize stimulation by the vibrations of the stapes footplate in response to sound.

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21. Supported by PHS research grants NB 05065-04, NB 05491-03, and NB 05785-02. During the initial stages of this investigation Dr. Johnsson held a Fulbright Fellowship from the University Otolaryngological Hospital, Helsinki, Finland.

7 August 1967