DESTRUCTION OF THE OTOLITH APPARATUS BY IONIZING RADIATION. FIXATION OF APPLICATORS TO THE VESTIBULE

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One of the least extensively studied chapters of the physiology of the sense organs is the physiology of the sensory structures of the sacs of the vestibule and of the ampullae of the semicircular canals of the membranous labyrinth. The close anatomical association between the semicircular canals and the divisions of the vestibule constitutes an almost insuperable obstacle to a separate study of the sensory structures of the non-auditory portion of the labyrinth. Views on the function of the maculae and cristae of the labyrinth are often based on features of their anatomical structure. However such views cannot be considered reliable unless they are confirmed experimentally by complete elimination of function of one or other structure. Thus the anatomical resemblance in structure of the maculae of both utricle and saccule in vertebrates has led many investigators to consider the saccule and utricle together as organs of equilibrium [7]. However the first experiments on warm blooded animals, in which the saccule was successfully isolated, led to the altogether unexpected conclusion that the saccule exerted no influence on proprioceptive reflexes [1, 5, 9]. Because of the close anatomical association between the ampullae and the utricle, destruction of the macula utriculi and preservation of the ampullae could not be realized. Such attempts led to damage of the total endolymphatic region, affected the ampullary apparatus, and led to general destruction of the labyrinth.

Subjecting the animal to centrifugation was not a reliable method of determining the effect of isolated destruction of the otolith apparatus of the utricle. Firstly, after the otolith apparatus had been torn away the sensitive epithelium of the macula retained its function and gave information concerning linear accelerations [6]; secondly as a rule centrifugation damaged the cristae of the semicircular canals, and caused edema in them [5].

Successful elimination of function from the otolith apparatus was not achieved by Hasegawa [3, 4] who used intravenous injection of sodium bicarbonate to "dissolve the otoliths". After crystals of aragonite from rabbit otoliths have been washed, not only do the sensory elements of the acoustic maculae remain undamaged (as is also the case when the otoliths are torn off during centrifugation), but there remain also small spheres of the gummy substance of the otolith, which may take on the function of the otolith. This circumstance probably explains the fact that besides finding no response to shaking, Hasegawa found no differences from normal in the animals' behavior or movements as far as ability to maintain equilibrium was concerned.

Carefully avoiding doing anything which might damage the endolymphatic space of the utricle or the ampullae of the semicircular canals, Versteegh [9] tried to divide the utricular nerve in the membrane separating the upper and lower parts of the labyrinth. However he was unable to divide the whole of the nerve, because its proximal part supplying the proximal part of the macula utriculi was submerged in the bone. The method proposed by the Japanese authors [8] of dividing the utricular nerve of the rabbit at its exit from the bony labyrinth also fails to satisfy the condition of eliminationg utricular function alone, because in this case not only is the utricular nerve damaged but so also is the nerve supplying the ampulla of the horizontal semicircular canal.

We have attempted to damage the function of the otoliths alone by means of applicators giving soft β-radiation, for example with a radioisotope of promethium-147 (Pm^{147}). This substance gives off low-energy β-particles (0.22 keV); the energy is so low that at a distance of 250 μ from the surface of the source (a distance equal to the

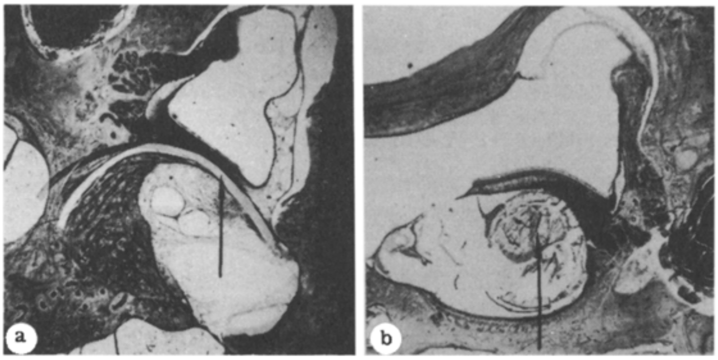

Fig. 1. Histological sections of the temporal bones; applicators introduced into the vestibule. a) Plate of polyethylene sulfate; b) sphere of ion-exchange resin. Magnification 3.5 × 7.

width of the sensitive layer of the macula) the dose decreases to about 1/250 of the initial amount. If the radioactive applicator is fixed in the immediate neighborhood of the sensitive maculae utriculi, it causes their damage or destruction, but at the same time the ampullary apparatus remains completely functional.

The main difficulty is to introduce the applicator onto the utricular macula. Not only must a means of fixation of the applicator be devised, but its size, shape, and the material of which it is made must be appropriately chosen. The size and shape must ensure its close contact with the sensory maculae utriculi. The applicator should be made of material which on the one hand will absorb the radioactive isotope, and on the other will not cause difficulties in histological treatment of the temporal bone. The most suitable substances in these respects is a plate of polyethylene sulfate measuring 1 × 2 mm, and a sphere of ion-exchange resin KY-2 1 mm in diameter.

The operation of introducing the plate onto the macula of the utricle is carried out under a binocular loupe. The rabbit is fixed on the belly, and the head is turned through a right angle to one side. The approach to the middle ear is through the external auditory meatus. The middle-ear cavity is opened sufficiently to allow access to the oval window and to the promontory jutting out into the cavity of the middle-ear. The promontory and the first turn of the cochlea are removed by means of a dental drill, and then a spherical recess is made; under the binocular loupe of the medial wall whitish cords can be seen which are the otolith of the sacculus. They are carefully removed, or destroyed by a fine, long, and slightly bent hook. The applicator is placed above this point, at the base of the utricle. For this purpose the plate is placed on a sharp slightly bent hook, and by this means is introduced into the vestibule by the thin end. The hook is removed, and the plate pushed in and carefully introduced into the base of the utricle beneath its macula.

By this means the applicator comes directly in contact with the sensitive macular utriculi, which has an area of about 1 mm^2. The macula sacculi is destroyed in the course of the operation (Fig. 1a). Despite the advantage of the operation just described, which is carried out under direct visual control, it has the disadvantage that the cavity of the inner ear is widely opened. Even when the operation is carried out under strictly sterile conditions, quite frequently it leads to infection of the inner ear, i.e., to unilateral labyrinthectomy.

We have also worked out another method of fixing the applicator in the utricle: a small sphere of ion-exchange resin is introduced through the oval window without damage to the bony walls of the inner ear. The first part of the operation and access to the middle-ear have been described already. After the middle-ear cavity has been exposed, the malleus and incus are removed, and the stapes is detached from the oval window by means of a sharp bent hook whose end is inserted into the loop formed by the anterior and posterior legs of the stapes. Then the oval window is freed from the primary membrane, it diameter is somewhat enlarged by application of a dental drill to the outer edges of the hole. A small glass sphere is then introduced into the middle-ear and inserted through the oval window into the perilymphatic space of the vestibule. Here is occupies a position in which it is in contact with both the macula utricli and the macula sacculi (Fig. 1b).

Radioactive applicators for soft β-radiation from isotope Pm^{147} are prepared as follows. Small plates or spheres are kept for 3 days in 5% hydrochloric acid, and then placed for 24 h in a solution of Pm^{147} nitrate at

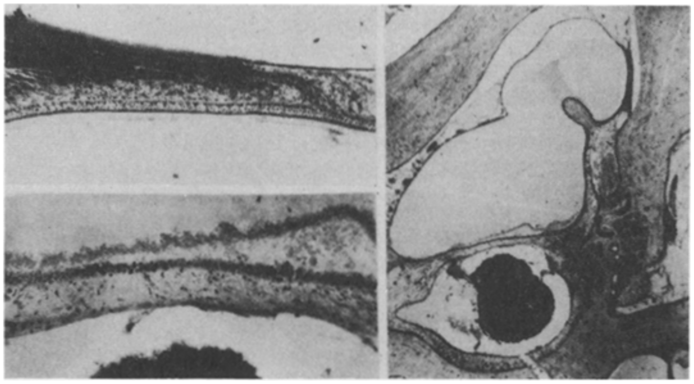

Fig. 2. Histological section of labyrinth with spherical radioactive applicator in vestibule. Magnification 3.5 × 7. The small pictures are parts of the macula of the utricle of the experimental animal (above) and of a healthy animal (below) at high magnification.

pH 4.0-6.0. The radioactive plates or spheres are dried after they have been removed from the solution and are washed in distilled water; they are then immersed two or three times in an alcoholic solution of BF-2 glue, and so covered with a thin layer of it.

The point at which the applicators are fixed in the vestibule and all the morphological changes in the tissues and organs of the labyrinth of the operated animals in whose vestibule the radioactive source has been placed are determined subsequently on histological preparations of the temporal bone treated by the usual methods (fixation in Vitmak's fluid, decalcification, dehydration in alcohols, and staining in hematoxylin-eosin).

As an example illustrating how an isolated destroyed otolith apparatus may be obtained by introduction of a radioactive applicator into the vestibule we show a photograph of a histological section of a rabbit labyrinth after a spherical radioactive source had been introduced into the perilymphatic space of the vestibule (Fig. 2). The continuous effect of the radiation produced a local destruction of the sensory otolith areas in the vestibule. The degree of tissue damage may be estimated by a comparison of the irradiated and control otolith organs shown at high magnification. Both the sensory cells and the nerve fibers of the maculae utriculi and sacculi have completely disintegrated and have been replaced by edematous connective tissue.

At the same time no morphological changes of the ampullary apparatus can be seen. The only exception is that there is some dilatation of the blood vessels of the cristae. In general all the tissues of the irradiated labyrinth show an increased vascularization.

Our method of fixation of a radioactive applicators producing soft β-radiation into the vestibule enables a strictly localized radiation of the tissues of the peripheral end of the vestibular analyzer to be produced; isolated damage of the otolith organ of equilibrium may be inflicted while the ampullary apparatus of the semicircular canals is preserved.

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