Autoradiographic Detection of S^{35} in the Membranes of the Inner Ear of the Rat[1]

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The cupula of the crista acoustica, the otolithic membrane of the macula, and the tectorial membrane of the organ of Corti are generally described as containing a gelatinous substance with other different constituents (*1–3*). Hardesty (*3, 4*) described the membrana tectoria as made of a "hyaline matrix probably keratin in gelatinous form, in which are embedded numerous fine fibers or threads of uniform size." Current experiments toward the understanding of the role of mucopolysaccharides in mineralization (*5*) have yielded autoradiographic images of S^{35} over the membranes of the inner ear, with unexpected variations. Rats of 4 days of age were injected with 5 microcuries-gram of S^{35} separated isotope and sacrificed at intervals of 1–2 hr, 1, 2, 4, and 8 days. After removal of surface tissues and brain, the head was bisected along the midline; one-half was fixed in neutralized alcohol-formaldehyde (*6*) and the other half in aqueous formaldehyde saturated with barium hydroxide, according to Dziewiatkowski (*7*). Autoradiographs were made by the inversion technique (*8, 9*), using melted Kodak high contrast positive film emulsion.

The early autoradiographs of animals sacrificed 1, 2, and 24 hr after the injection of tracer showed little penetration in the membranes of the inner ear whereas cartilage, bones, and teeth gave strong images (*5*). At 2 days, the membrana tectoria (Figs. 3 and 4) was positive in tissues fixed in the two different solutions; at 4 and 8 days, it seemed to contain comparable amounts of radiosulfur.

The cupula appeared strongly positive after fixation in formaldehyde containing barium hydroxide (Fig. 1) and more weakly so after alcohol-formaldehyde. In some instances, with both types of fixative, the crista showed no autoradiographic picture, an indication that the substance containing radiosulfur was irregularly retained by the technical process used.

The otolithic membrane showed only a weak reaction after both types of fixation (Fig. 3). The picture was uniformly diffuse.

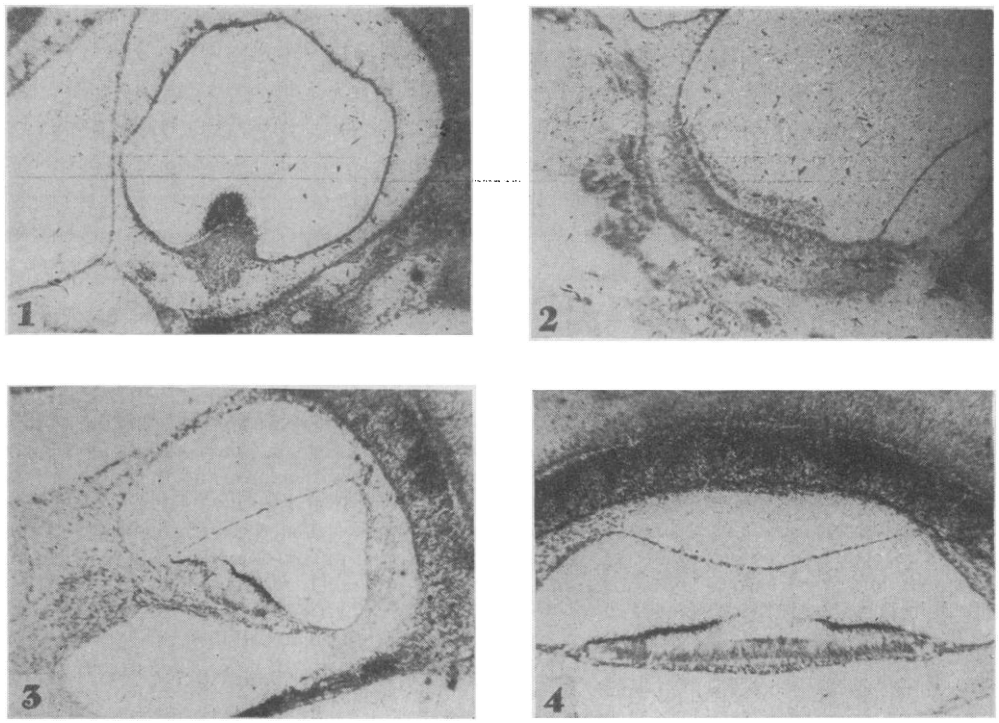

FIG. 1. Autoradiograph of crista acoustica from 8-day-old rat, injected with S^{35} at 4 days. Inversion technique (×100).

FIG. 2. Autoradiograph of macula utriculi from same animal as in Fig. 1. The reaction over the otolithic membrane is weak and diffuse (×100).

FIG. 3. Autoradiograph of the organ of Corti from 6-day-old rat injected at 4 days with S^{35}. Notice reactions over membrana tectoria and bony cochlea. Fixation alcohol-formaldehyde. Inversion technique (×100).

FIG. 4. Autoradiograph of the organ of Corti from 10-day-old rat injected at 4 days with S^{35}. Notice reactions over membrana tectoria and bony cochlea. Fixation formaldehyde-barium hydroxide. Inversion technique (×100).

[1] This work was supported by grants from the National Research Council of Canada through the Division of Medical Research and the Associate Committee on Dental Research. Radiosulfur was provided by Atomic Energy of Canada Ltd.

[2] The author is indebted to Mrs. Cécile Bélanger for skilled technique.

One animal injected at 10 days of age and sacrificed 2 days later showed variations from the above mentioned pattern. The reaction over the cupula was most intense at the outer border of the apparatus and also in the vicinity of the hair cells. Over the membrana tectoria, the autoradiographic picture indicated a concentration of S^{35} in the distal portion of the structure, away from the limbus spiralis.

When stained with toluidine blue, the tectorial membrane, the otolithic membrane, and the cupula exhibited metachromasia. The metachromatic reaction of the tectorial and otolithic membranes was persistent like that of mucus and mast cells after incubation with hyaluronidase for 6 hr at pH 5.8 and 37° C. No picture of cupula was available from sections treated in this fashion. The Chèvremont and Frédéricq reaction (*10*) for the sulfhydryl groups of keratin was negative with all three types of membranes.

In conclusion, it seems that all three membranes synthesize organic sulfur compounds. The cupula and the membrana tectoria show a larger concentration of S^{35} than the otolithic membrane. The sulfur compounds formed do not have a rapid turnover. The histochemical tests do not reveal the presence of keratin in the inner ear but point strongly to the existence of sulfo-mucopolysaccharides in all three types of membranes. In the tectorial and otolithic membranes at least part of these were found to be hyaluronidase resistant.

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Manuscript received June 18, 1953.

The Existence of "Beta" Ice

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Seljakov (*1–3*) has reported the existence of a low-temperature modification of ordinary ice (ice I) prepared at atmospheric pressure, which he has termed "beta" ice to distinguish it from normal hexagonal or "alpha" ice. He reported that beta ice gave a Laue pattern with rhombohedral symmetry different from the sixfold symmetry of the hexagonal Laue pattern of ordinary ice. The beta ice pattern was observed with crystals grown from water at temperatures (ambient air) in the range -5 to $-16°$ C. Seljakov further assumed that a strong (0002) reflection characterized the beta structure and that beta ice was transformed into the alpha structure on grinding.

The existence of this beta ice form has not been confirmed by subsequent workers. It is, however, frequently referred to in the literature as a distinct crystal form.[1] During the course of an ice physics investigation conducted for the U.S. Air Force,[2] we were led to consider the validity of Seljakov's conclusions as part of a laboratory investigation of nucleation and crystallization of supercooled water.

It was difficult to follow Seljakov's experimental procedure in detail as it was not explicitly described. Following his procedures as closely as possible, however, it was found that ice crystals appeared at water temperatures of approximately -2.5 to $-3°$ C, when the ambient air temperature varied between -5 and $-16°$ C. The water was kept in a metal pan and temperature measurements were made with a thermocouple immersed in the supercooled water.

These experiments cast considerable doubt on the assumption implicit in Seljakov's reports that the ambient air temperature is equivalent to, or an indication of, the temperature to which the supercooled water was cooled before freezing occurred. Further, Seljakov has not observed a critical temperature above which beta ice is never found. Thus beta ice was observed (*3*) (once out of 3 trials) when water was frozen at an ambient air temperature of only $-1°$ C. Seljakov also reported the presence of beta ice on the surface of lake water.

In a review article (*4*) Owston has noted that Seljakov did not make efforts to test for preferred orientation in his powder specimens, the probable cause of the absence of the (0002) reflection in some photographs, and that he took only one rotation photograph. Further, in an earlier paper Owston and Lonsdale (*5*) noted that "It is possible to get an apparently pseudotrigonal Laue picture from ordinary hexagonal ice when it is misset by a few degrees from the hexagonal axis."

At our request Dr. Barbara W. Low (*6*) recently examined ice crystals grown at an ambient air temperature of $-15°$ C. Her x-ray crystallographic studies confirm and elaborate those of Owston and Lonsdale; a single crystal gave a pseudotrigonal Laue pattern when tilted so that the sixfold axis made an angle of approximately 15° with the x-ray beam. Ordinary ice I (alpha) gives a pseudotrigonal pattern in this position because of the numerical relationship between the lengths of the a and c axes. The Laue pattern arises from two equivalent and one nonequivalent zones. The Seljakov pattern (*1*) itself shows this same feature—two identical and one dissimilar elipses. The characteristic beta Laue pattern of ice, therefore, may be obtained from ordinary alpha ice when it is misset in the manner described.

[1] See, for example, *Handbook of Chemistry and Physics,* 32nd ed., p. 2225. New York: Chemical Rubber Pub., 1950–51.

[2] U.S. Air Force Contract, AF-33(308)-18687.