basal enzyme activities and on the kinetics of the resulting variations in TKT activity was essentially the same for cells in both growth phases, the only observable difference being the decrease in the magnitude of the stimulation by dexamethasone. The effects on the basal enzyme activities, on the kinetics of the increase and decrease after addition or removal of dexamethasone, and on the inhibitors of RNA or protein synthesis were the same for cells in both growth phases, the only noticeable difference being the decrease in the magnitude of the increase. Nebert and Gelboin (*15*) described a similar phenomenon during the induction of aryl hydroxylase in cells in culture, and attributed that effect to a decreased rate synthesis of DNA, RNA, and protein in contact-inhibited cultures, as had been previously described (*16*). Although these latter findings may very well explain our results, the observed decrease in dexamethasone sensitivity may also be the result of an increase in the proportion of cells in early G_1 in a random cell population; the activity of TKT cannot be enhanced by dexamethasone in synchronized hepatoma cells in culture during the part of the cell cycle between the beginning of G_2 and the early part of G_1 (*17*).

It is important to maintain careful control of cell density in any study involving hormonal effects in cultured cells; recent experiments have also demonstrated that, whereas the enzyme pyruvate kinase is insensitive to insulin in contact-inhibited RLC cells, it is readily inducible in these cells during their logarithmic growth phase (*18*).

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Scanning Electron Microscopy of the Organ of Corti

Abstract. *With the scanning electron microscope we have examined normal cochlear sensory epithelium of the guinea pig and cat and that damaged by noise. The studies demonstrate how the regular surface architecture of the organ of Corti is altered after exposure to noise. The changes include loss of sensory hairs, formation of giant hairs, and complete degeneration of circumscribed areas of the organ of Corti. Our method greatly reduces the artifacts.*

The application of scanning electron microscopy to the study of biological materials, including, for example, pollen, protozoa, enamel, and respiratory epithelia, has been reviewed by Hayes *et al.* (*1*), Pease and Hayes (*2*), Barber and Boyde (*3*), and Small and Marzalek (*4*). Scanning electron microscopy has very recently been applied to the inner ear, first by Barber and Boyde (*3*), and since by Lim and Lane (*5*), Engström *et al.* (*6*), and Marovitz *et al.* (*7*).

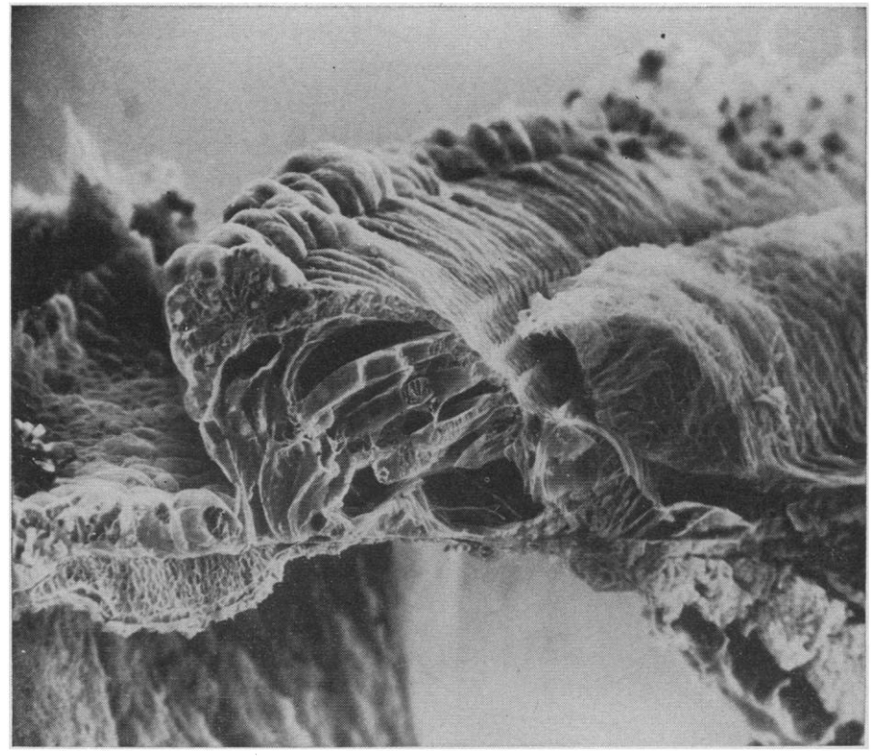

Fig. 1. Fracture through the organ of Corti of the guinea pig, showing the overall picture of the acoustic papilla. This specimen, prepared by fracturing the freeze-dried specimen, shows the possibility of studying the interior structures of the sensory epithelium (× 370).

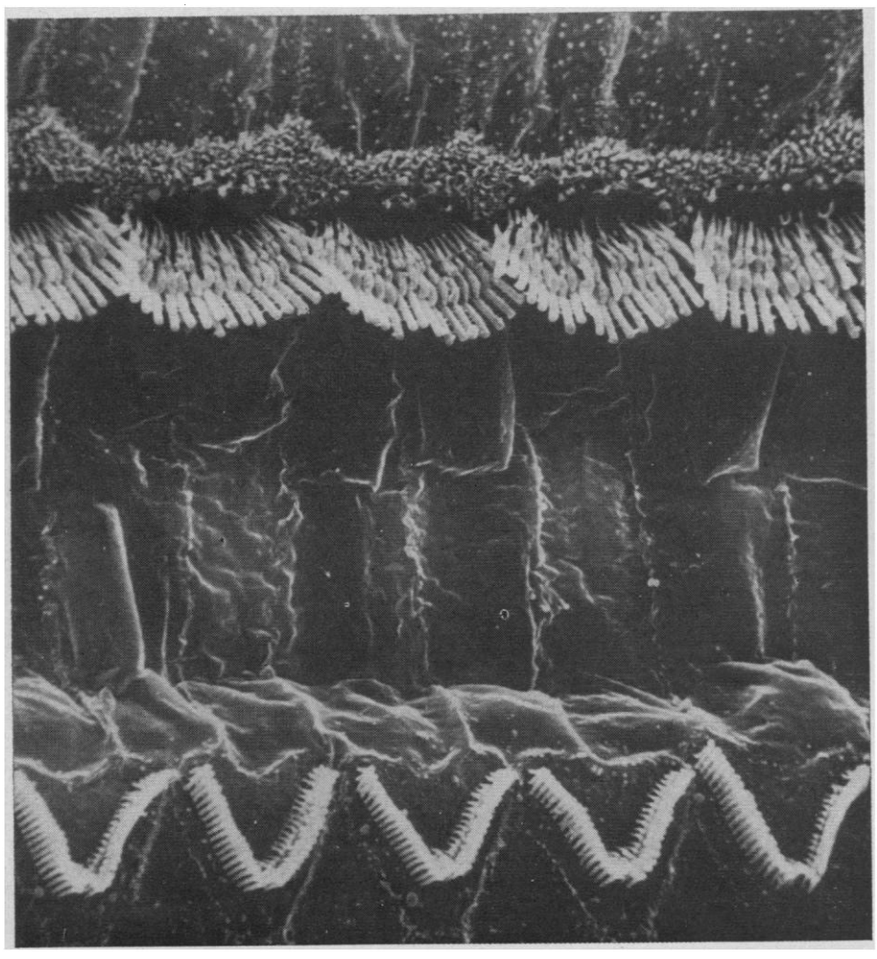

Fig. 2. There is one row of inner hair cells (upper part of the picture) as opposed to three rows of outer hair cells, of which only the innermost is shown (× 4325).

In our study of the inner ear by means of the scanning electron microscope we used a method modified from that described by Small and Marzalek (*4*). We report here results from normal and pathologically altered organs of Corti, free of some of the artifacts which have beset earlier studies.

The experimental animals (guinea pig and cat) were anesthetized with Diabutal and decapitated, and the temporal bones were removed. The perilymphatic cochlear spaces were perfused with a fixative modified after Parducz (*8*), consisting of six parts of 2 percent phosphate-buffered osmium tetroxide [Millonig (*9*)] and one part of saturated $HgCl_2$. The specimens were kept in the fixative for 1 to 2 hours at +1° to +4°C; they were then rinsed in glass-distilled water and dissected under a preparation microscope. The bony capsule of the guinea pig cochlea was easily opened with watchmakers' forceps; however, in the cat the capsule had to be thinned down by the use of dental diamond burrs before it could be opened. The spiral ligament was removed, and the osseous spiral lamina together with the associated organ of Corti was dissected free in half coils or smaller segments and placed in a drop of distilled water on a thin aluminum plate. The specimens were frozen by rapidly dipping the plate into isopentane kept at −150° to −160°C (just above its freezing point) by means of liquid nitrogen.

The aluminum plates with the frozen specimens were placed on a copper block, cooled in liquid nitrogen, and transferred to a Pearse tissue dryer, where the freeze sublimation occurred at −50° to −60°C at a vacuum of 5×10^{-2} torr. The drying procedure took 2 to 4 hours, after which time the specimens were allowed to attain room temperature very slowly. The aluminum plate with the specimens was then attached to specimen stubs with cement. The specimens were then coated with equal parts of gold and palladium (ap-

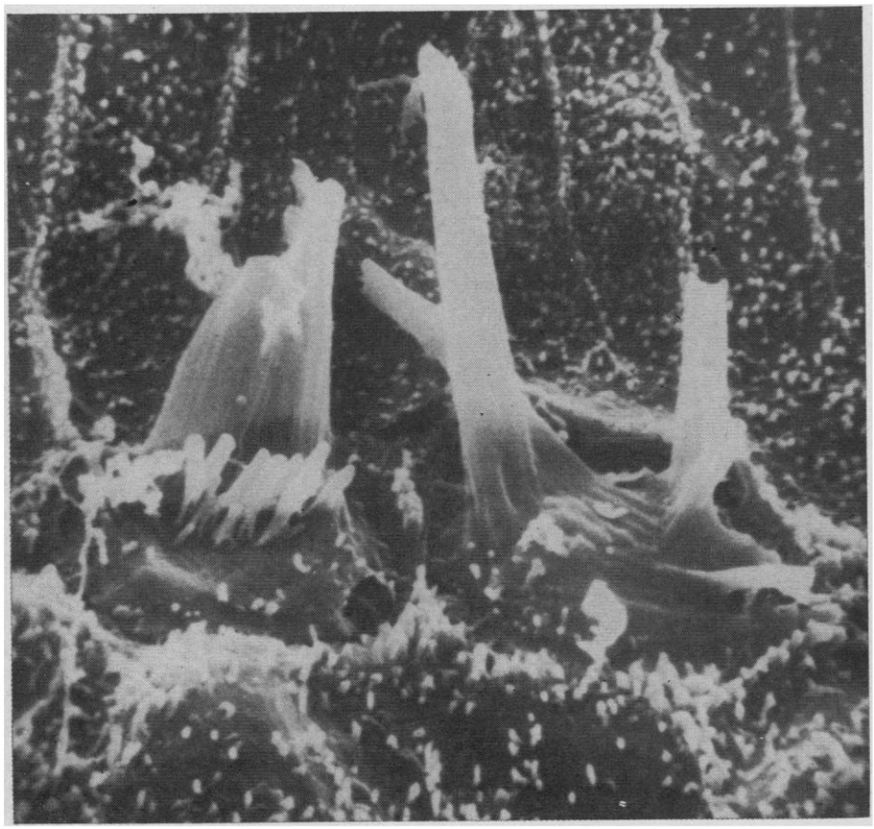

Fig. 3. Second coil of cochlea from cat exposed to pure tone (125 hz at 152.5 db for 4 hours). Note the fusion of stereocilia and formation of giant hairs on two inner hair cells (× 7700).

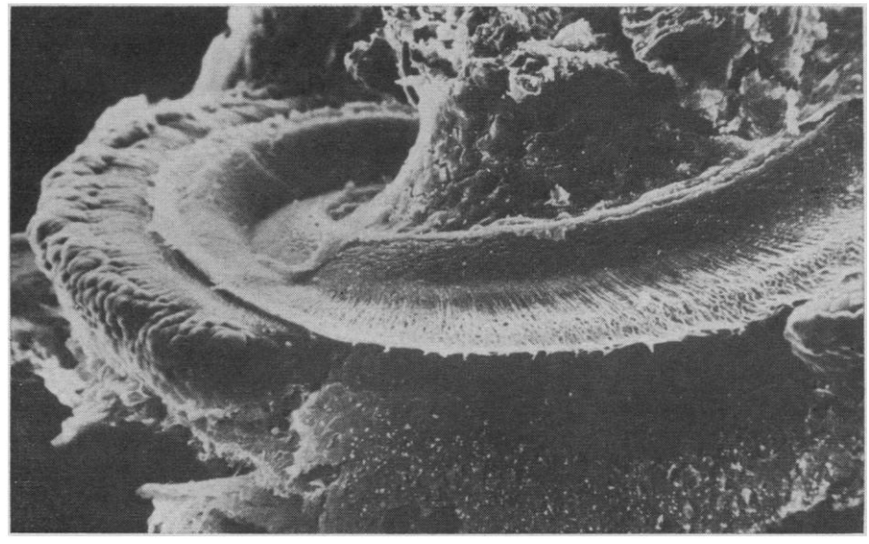

Fig. 4. Apical coil from a guinea pig cochlea showing an area of complete degeneration of the organ of Corti due to exposure to a pure tone (125 hz at 148 db for 4 hours). Note that the organ of Corti has its normal shape on both sides of the lesion. The tectorial membrane is hanging free over the area of the lesion (× 144).

proximately 100 to 200 Å thick). The specimen stubs were placed on a rotating stage in a vacuum evaporator in order to allow all parts of the specimens to become coated. The stubs with the specimens were then stored in a chemical desiccator until they were studied in a Cambridge Stereoscan scanning electron microscope under primary magnifications of 100 to 15,000 times.

Dissection of the fixed specimens or fracturing of the frozen specimens (Fig. 1) makes it possible to see the interior of the structure and to study the complex cytoarchitecture of supporting cells and sensory cells with associated endings and nerve fibers. Present knowledge of the innervation of the organ of Corti is still inadequate. In light microscopy the unmyelinated nerve fibers are very difficult to follow and several methods of impregnation have proven unreliable. Transmission electron microscopy and subsequent reconstruction have indeed added to our knowledge, but this method is very laborious and does not permit one to perceive the whole picture of the innervation. It is hoped that scanning electron microscopy, in conjunction with various methods of dissection, will yield new information concerning the complex pattern of innervation in the organ of Corti.

The morphology of the normal organ of Corti was examined. The surface pattern of the sensory epithelium is regular and geometric, with the hairs (stereocilia) of the sensory cells arranged in the characteristic W-pattern (Fig. 2). The scanning electron microscope with its great depth of field gives a good three-dimensional view of the structures. True stereoscopic pictures are easily obtained by tilting the specimens between the recording of two separate micrographs.

Scanning electron microscopy can provide new information in the study of the pathologically altered hearing organ. Investigations with phase contrast microscopy by Engström and Ades (*10*) showed that one of the early signs of damage to a sensory cell exposed to acoustic trauma is a disarrangement of the stereocilia. The changes, studied in detail, reveal several new aspects of the effect of exposure to noise. Some sensory hairs are structurally changed, disarranged or completely degenerated due to exposure to a pure tone of high intensity, whereas other hairs on the same cell appear normal. In the apical turns of the cochlea in both guinea pig and cat, remarkable changes are seen, including fusion of several sterocilia and the formation of giant hairs (Fig. 3), often many times longer and thicker than the normal hairs. In addition, the cuticular plates of the cells are frequently peculiarly deformed. To what extent these sensory cells are still functional is of primary importance in this research.

Exposure to high-intensity noise of different frequencies may also result in complete degeneration of circumscribed areas of the organ of Corti. A dramatic portrayal of a lesion (Fig. 4) shows clearly that the edges of the lesion may be very sharp. It demonstrates further that the lesion cannot shorten the organ of Corti because it is attached to the bone of the osseous spiral lamina. This is important in the estimation of the extent of the lesion, as it is studied by the surface preparation method (*11*).

Earlier scanning electron microscopy of the inner ear has shown considerable artifacts due to shrinkage and compression. The use of the freeze-drying technique greatly reduces the artifacts so that a more normal shape of the structures is preserved. The scanning electron microscope will no doubt become a useful tool in the study of the organ of Corti, and be of special value for the understanding of spatial relations and the pattern of innervation inside the organ. The effects of noise and ototoxic drugs on the organ of Corti and other degenerative changes will provide additional areas of study.

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