
Hair Cell Regeneration in the Avian Cochlea: If It Works in Birds, Why Not in Man?

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Abstract

Hearing loss caused by cochlear hair cell loss is the most common process afflicting the hearing impaired. Recent studies in the avian cochlea following ototoxic drug and noise damage have demonstrated a remarkable capacity for anatomical and functional recovery. Hair cell regeneration has been shown to play a major role in this recovery process. Future studies may one day make hair cell regeneration or transplantation possible in man.

Introduction

Sensorineural hearing loss resulting from noise induced cochlear hair cell injury is the most common form of hearing loss seen by Otolaryngologists today. Consequently, the relationship between hair cell injury and hearing loss continues to be a very active area of research. Both mammals¹ and birds² normally only produce sensory hair cells in the cochlea during early embryogenesis. Thus the neonate has the full adult complement of cochlear hair cells by birth. Noise, ototoxic drugs, infection, and age have all been shown to cause damage and destruction of cochlear hair cells. While hair cell damage may be reversible and cause only a temporary hearing loss (i.e., temporary threshold shift), hair cell destruction was held to be irreversible and associated with a permanent sensorineural hearing loss.

Nonetheless, the literature does contain reports of hearing recovery following aminoglycoside ototoxicity. Moffat and Ramsden³ reported partial hearing recovery in one patient with a gentamicin associated sensorineural hearing loss. Fee⁴ reported 55% of patients with an aminoglycoside induced hearing loss demonstrated some improvement in

hearing one week to six months following cessation of the drug therapy.

Recent studies in the avian auditory system may provide insight into the possible mechanisms of this recovery process. The avian cochlea has been shown to respond to both noise and ototoxic drug induced hair cell loss with post embryonic hair cell production. This process of hair cell regeneration results in the near complete anatomical and functional recovery of the cochlea. As one might predict, this has stimulated considerable interest and ongoing research. The following review will attempt to summarize our current level of understanding of the hair cell regeneration process followed by a discussion of some of the possible clinical implications and applications.

Background

We have known for years that some vertebrates are capable of post embryonic cochlear hair cell production. Corwin⁵⁻⁷ has shown that the auditory organ (macula neglecta) of sharks and rays and the saccule of amphibians undergo progressive enlargement throughout life. This occurs by the continual production and addition of hair cells to the periphery of the organ. This increase in hair cell number is associated with a significant increase (by 500-fold) in hearing sensitivity.⁶ In addition, further studies have shown these animals to be capable of producing new hair cells in response to traumatic hair cell loss.⁸ It was assumed that mammals and, until recently, birds had lost this regenerative capability during the evolution of a more complex inner ear apparatus.

Normal Avian Cochlear Anatomy

In the last several years the chick auditory system has become a popular model for the study of central and peripheral auditory function. The chick, like the mammal, has a highly specialized inner ear but offers certain anatomical advantages for auditory research. The sensory epithelium is curvilinear in shape and is much easier to

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examine histologically than the spiral-shaped mammalian cochlea. The chick cochlea has a tonotopic organization^{9,10} very similar to the mammalian cochlea. The sensory hair cells of the chick cochlea also have similar responses to injury.¹⁰ In addition, the central brainstem connections and auditory nuclei have been extensively studied anatomically and physiologically.

The chick cochlear sensory epithelium consists of a continuous sheet of hair cells (Fig. 1). Two hair cell types have been described, tall hair cells (THC) and short hair cells (SHC). Tall hair cells are located along the superior (neural) edge and have both afferent and efferent innervation much like the mammalian inner hair cell.¹¹ Short hair cells are located inferiorly (abneural edge) with predominantly efferent innervation like the mammalian outer hair cell. The chick hair cells are separated from the basilar membrane (BM) by a single layer of supporting cells (SC). Adjacent to the inferior-most hair cells are the hyaline and cuboidal epithelial cells (HEC) whose function remains unclear but may play a role in hair cell regeneration.

Indirect Evidence for Hair Cell Regeneration

The effects of noise and ototoxic drugs on the chick cochlea have been studied for over a decade. However, it was not until 1987 that two studies reported incidental findings which suggested the possibility of cochlear hair cell regeneration.

Cruz et al.¹² were examining the temporal pattern of chick cochlear hair cell loss following a 10 day course of gentamicin (an aminoglycoside antibiotic commonly used for gram negative bacterial infections but with known ototoxic side effects). Cochlear hair cell numbers were sampled from base to apex and compared to age matched controls for survival times ranging from one day to 32 days. There was an initial loss of hair cells isolated to only the basal one-third of the cochlea (low frequency region). However, over the ensuing week after gentamicin was discontinued, hair cell loss steadily progressed in an apical direction to involve the basal two-thirds of the cochlea. Unexpected was the finding of partial recovery of the hair cell numbers throughout the basal cochlea in the weeks following completion of the gentamicin regime. Total hair cell numbers in the mid-cochlea recovered from a maximal loss of 64% at one week post gentamicin, to a loss of only 27% at three weeks post gentamicin. The authors postulated the possible production of new (regenerated) hair cells replacing lost hair cells to account for this significant degree of recovery.

Cotanche¹³ independently reported similar findings while

using scanning electron microscopy (SEM) to study the response of the chick cochlear sensory epithelium to noise damage over recovery periods of 24 hours to ten days. Following exposure to a high intensity pure tone for 48 hours, an isolated region of hair cell loss was identified. Forty-eight hours following completion of the noise exposure, small embryonic-appearing stereocillia were seen only in the region of the damage. These small stereociliary bundles matured over time in a sequence similar to embryonic maturation until remarkably, by ten days after the noise exposure, the entire sensory epithelium appeared almost normal. Cotanche felt this most likely represented hair cell regeneration in response to acoustic overstimulation.

In a parallel study Cotanche¹⁴ also showed the tectorial membrane to be destroyed in the same region as hair cell loss following noise exposure. The tectorial membrane was found to regenerate over time along with hair cells. Transmission electron microscopy (TEM) suggested the surviving supporting cells in the region of damage were actively involved in this process of tectorial membrane regeneration.

While the studies by Cruz and Cotanche were both suggestive of hair cell regeneration in the chick cochlea following trauma, definitive evidence was still lacking.

Direct Evidence for Hair Cell Regeneration

Conclusive documentation of cochlear hair cell regeneration following trauma required proof of post-embryonic cellular mitosis in the cochlea with resultant production of new hair cells. This was achieved using tritiated (3H) thymidine as a cellular label and a well established technique known as autoradiography.¹⁵ Thymidine is a nucleic acid DNA precursor which is only taken up by a cell during the production of new DNA in preparation for cellular division (mitosis). Thus when radioactive thymidine is present it becomes incorporated into the nuclear DNA of any cell undergoing mitosis. Following mitosis the nuclear DNA of both daughter cells will contain the radioactive thymidine which can later be detected using a radiosensitive emulsion to coat the histologic slide. Following a prolonged exposure time, the slides are developed resulting in the deposition of silver grains over the cell nucleus. The labeled cells can then readily be identified under the light microscope.

Corwin and Cotanche¹⁶ exposed neonatal chicks to a high intensity pure tone to induce a region of cochlear hair cell damage. The chicks were then given injections of 3H thymidine for 10 days, after which the cochlea were

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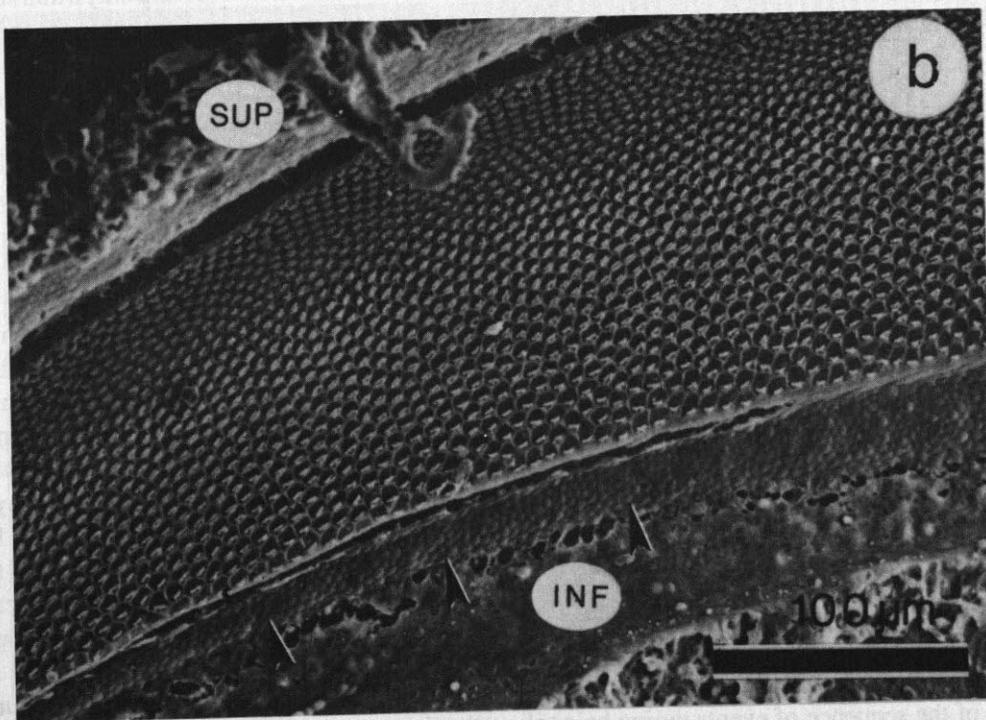
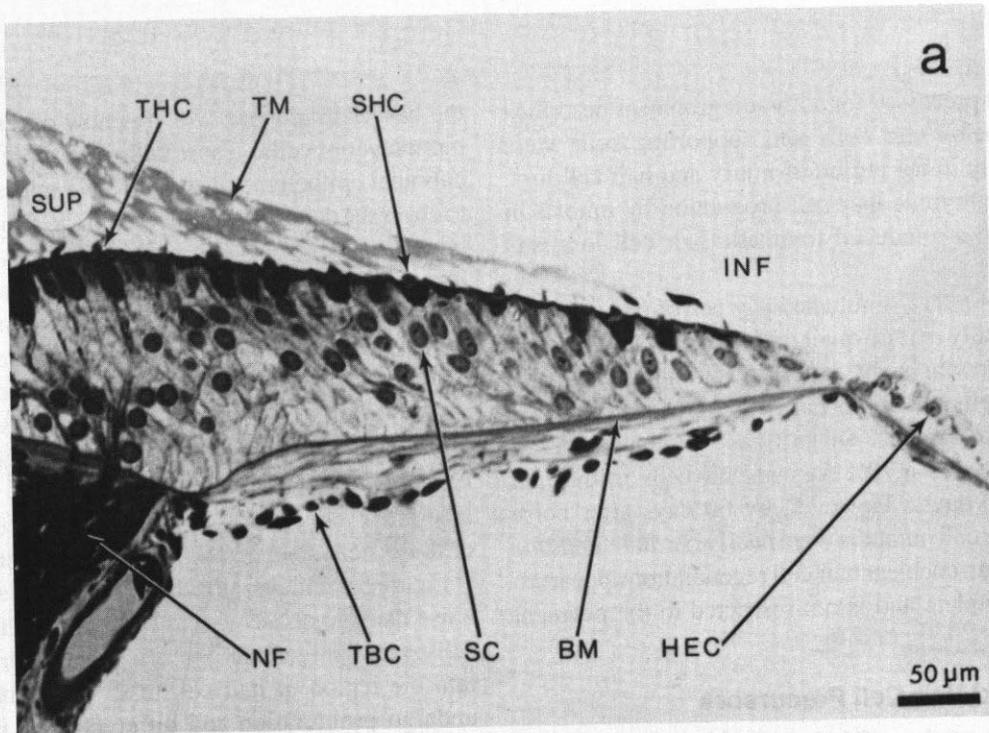


Fig. 1. Mid (1500 Hz) region of the normal chick cochlea. (a) Transverse light microscopic (LM) section. superior edge (SUP), inferior edge (INF), tall hair cells (THC), tectorial membrane (TM), short hair cells (SHC), cochlear nerve fibers (NF), tympenic border cells (TBC), supporting cells (SC), basilar membrane (BM), and hyaline and cuboidal epithelial cells (HEC).

(b) Scanning electron micrograph (SEM) of the sensory epithelial surface with the tectorial membrane removed. Superior edge (SUP) and inferior edge (INF). Hyaline and cuboidal epithelial cell region (arrows). (From Girod et al., 1989.)

removed and processed for autoradiography as described below. Labeled hair cells and supporting cells were identified only in the region of injury and hair cell loss. Thus post-embryonic hair cell production by mitosis in response to noise-induced traumatic hair cell loss was confirmed.

Rubel and Ryals¹⁷ simultaneously performed a similar study in sexually mature quail with very similar findings. Ten days following noise exposure autoradiographically labeled hair cells and supporting cells were identified only in the region of damage. Quantification of the number of hair cells revealed a 70% loss of hair cells in the mid cochlea at 10 days. However, by 60 days after noise exposure, hair cell numbers were nearly normal. Thus the process of avian cochlear hair cell regeneration appears to be nearly complete and is not restricted to the neonatal period.

Regenerated Hair Cell Precursors

The documentation of hair cell and supporting cell regeneration in the avian cochlea following acoustic trauma has resulted in speculation as to the source of these new cells.¹⁶ Potential candidates for precursor populations include hair cells, supporting cells, and yet unidentified latent stem cell population. There is precedence in the literature for both supporting cells and stem cell populations playing a major role in the production of receptor cells and supporting cells in other sensory systems during development and in response to injury.^{5,8,18}

Potential regenerating hair cell precursors were identified in the chick cochlea using autoradiography and SEM.¹⁹ Following an intense 1500 Hz pure tone 18 hour noise exposure, chicks were given 3H thymidine over survival periods of 6, 15 or 24 hours or 3 days. An additional group received 3H thymidine for only the first 3 days of a 30 day survival period. One cochlea from each animal was processed for autoradiography and the other for scanning electron microscopy (SEM). Control animals were not exposed to noise, but received 3H thymidine along with the experimental animals.

This design offered a cross-sectional sampling of the regenerative process following noise damage over recovery periods from 6 hours to 30 days. This allowed reconstruction of the sequence of events during hair cell regeneration and identification of a probable stem cell population.

Initially there was a discrete region of severe damage with inferior hair cell and supporting hair cell loss as seen in Figure 2. The sensory epithelium has pulled away from

the basilar membrane which is now covered with a thin monolayer of cells. These cells may represent hyaline or cuboidal epithelial cells which have migrated superiorly to cover the denuded basilar membrane although these cell types have not been well characterized. By 15 hours after noise exposure, these same cells undergo proliferation and eventual stratification by 24 hours. The first labeled and identifiable regenerated hair cells were seen after three days with a very characteristic appearance by AR and SEM (Fig. 3). By 30 days after noise exposure all labeled hair cells appeared completely mature and minimal residual damage was seen by SEM (Fig. 4). There was no labeling of hair cells or support cells in any control animal or in the nondamaged regions of experimental animals.

Figure 5 summarizes the sequence of events following noise damage as reconstructed by this method. Inferior cells in the hyaline/cuboidal cell region migrate superiorly into the region of hair cell loss. There they appear to undergo proliferation and differentiation into both hair cells and support cells.

Some animals in this study demonstrated a less severe form of hair cell loss involving only a thin strip of hair cells more superiorly in the transition zone between the tall hair cells and short hair cells. In these cochleae no hyaline or cuboidal cells were labeled despite cellular proliferation and hair cell regeneration. The precursors population in this setting could not be identified but may be a subpopulation of supporting cells as suggested by Corwin and Cotanche.¹⁶

Anatomical Regenerative Capacity

Multiple studies have documented the near complete gross anatomical recovery of the avian cochlea following noise damage. Ryals and Rubel¹⁷ documented the recovery of hair cell numbers by light microscopic quantification 60 days after noise damage in sexually mature quail. Henry et al.²⁰ reported complete recovery of hair cell density in the region of noise damage by 14 days using SEM. However, they did comment on the increased variability of hair cell apical surface area and on the relative disorganization of the stereocilia bundle orientation.

Using SEM, Marsh et al.²¹ attempted to further quantify the degree to which hair cell regeneration contributes to the gross recovery from noise damage. Immediately following noise exposure they found a 32% decrease in hair cell numbers in the region of damage. By 15 days, only 22% of the lost hair cells had been replaced. Thus, overall hair cell density was decreased, unlike the findings

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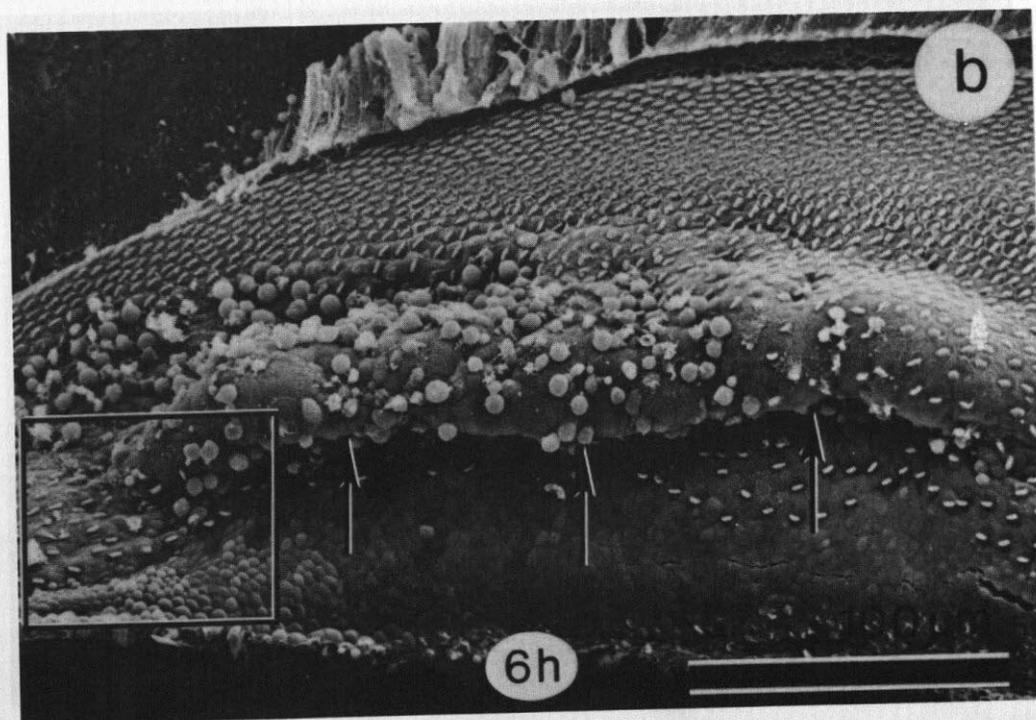
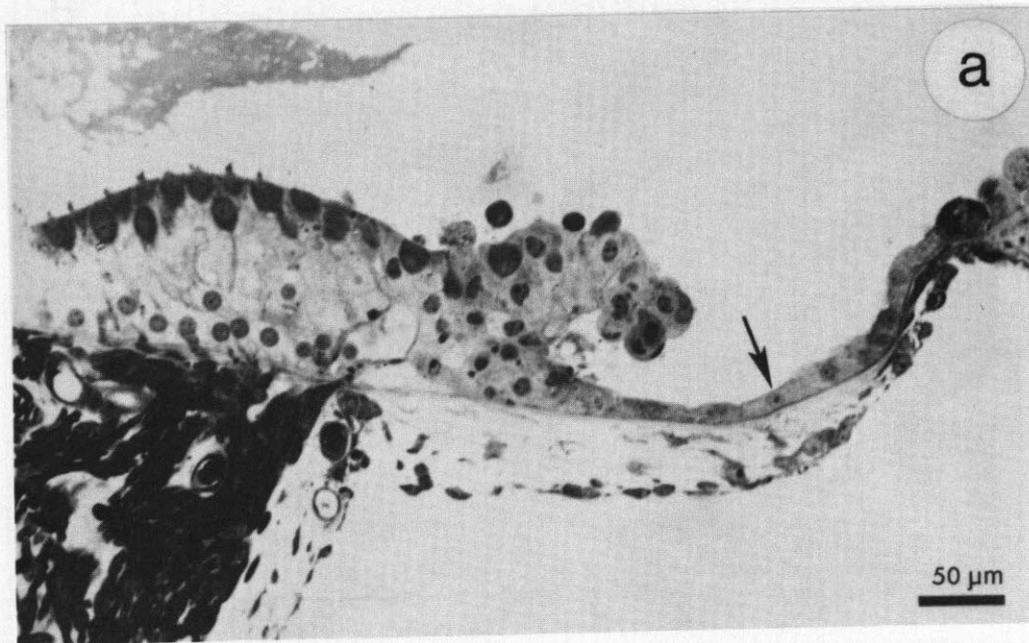


Fig. 2. Mid region of the chick cochlea 6 hours following an intense pure tone noise exposure. (a) Transverse LM section demonstrating extensive hair cell and supporting cell loss at the inferior edge of the sensory epithelium. Note the thin monolayer of cells spreading to cover the basilar membrane (arrow). (b) SEM demonstrating the discrete region of inferior hair cell and supporting cell loss and injury (arrows). (From Girod et al., 1989.)

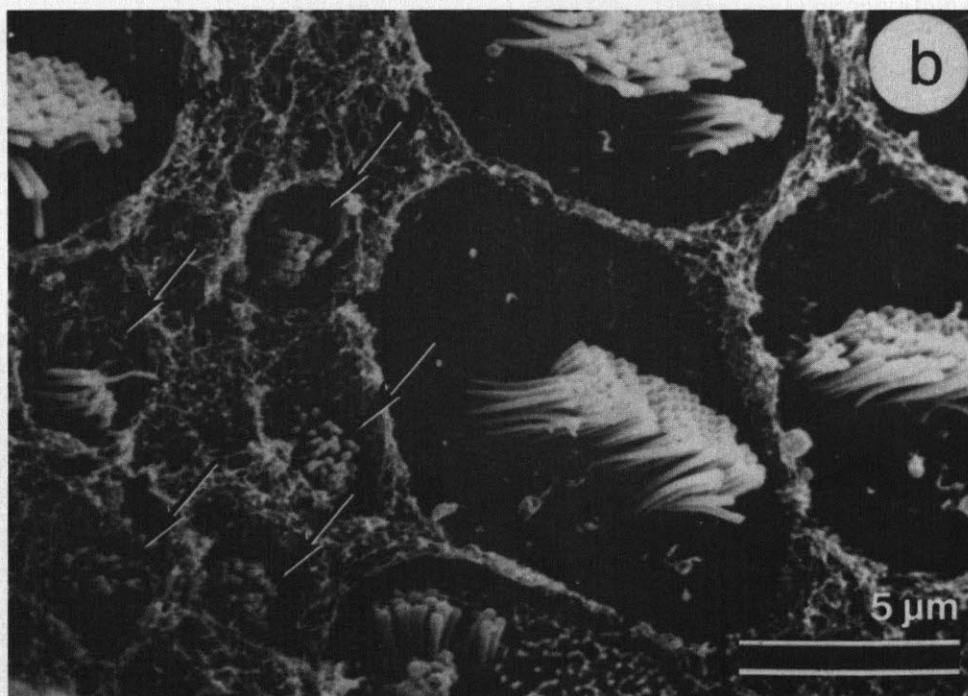
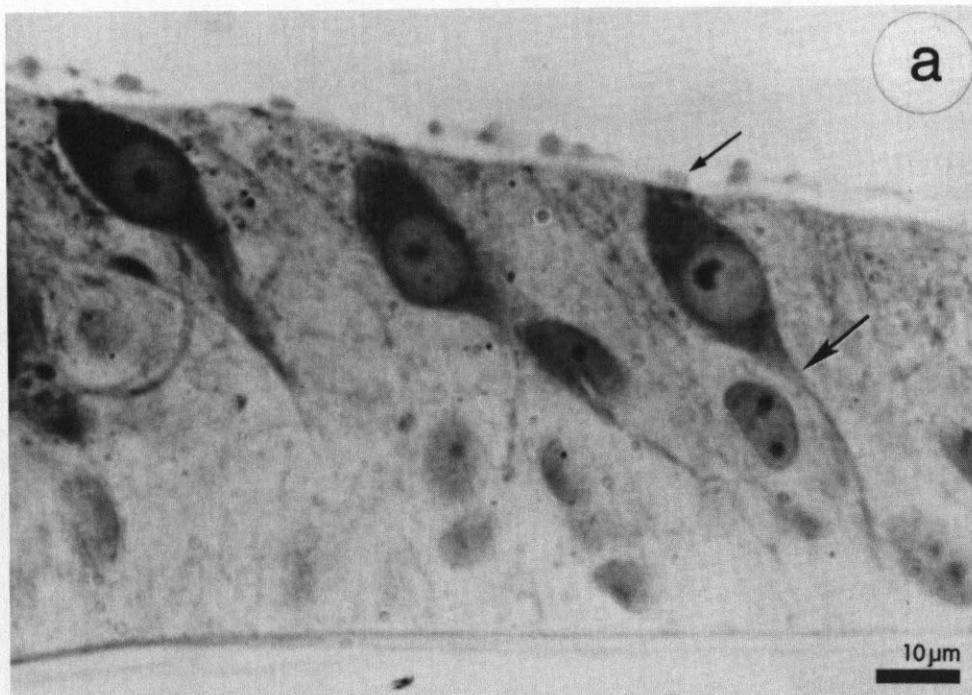


Fig. 3. Immature regenerated hair cells in the mid region of the chick cochlea three days following noise exposure. (a) Transverse LM section showing that regenerated cells have a unique appearance including a tall spindle-shaped cell body with lightly staining cytoplasm, a large round nucleus and very short stereocilia (small arrows). Processes seen at the cell base may represent innervating axons or trailing cytoplasm from cell migration to the lumen (large arrows). (b) SEM demonstrating the immature stereocilia of regenerated hair cells (arrows) contrasted to adjacent surviving hair cells (From Girod et al., 1989.)

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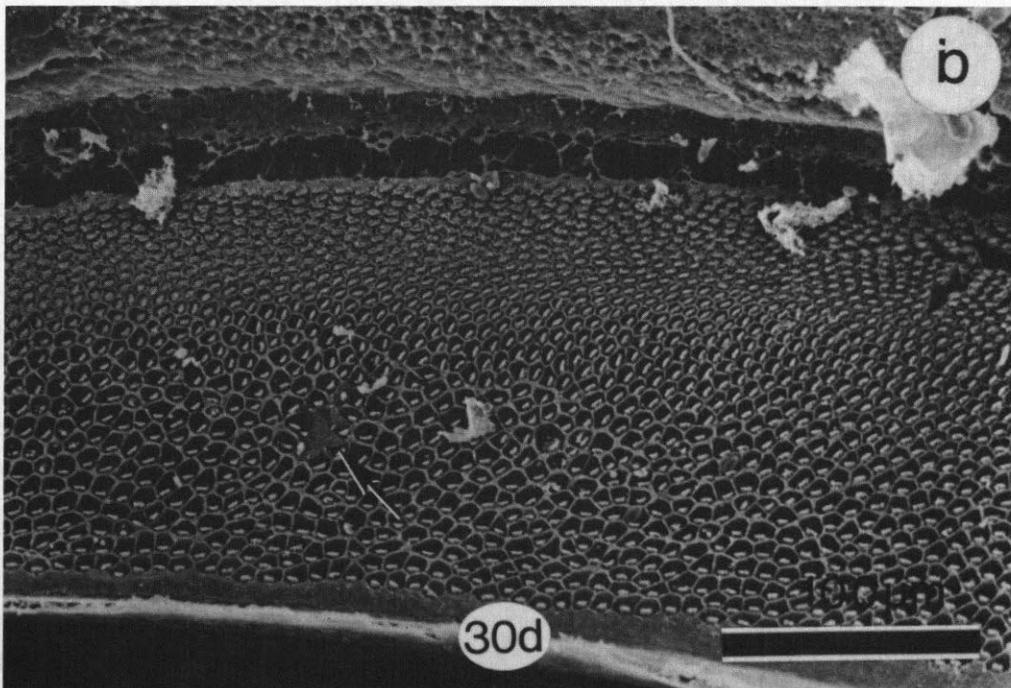
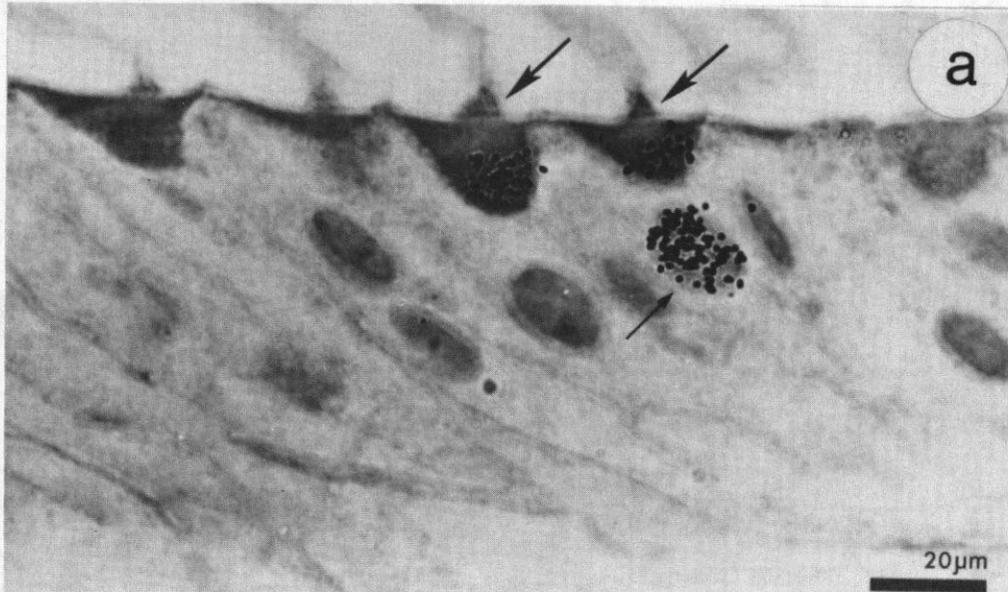


Fig. 4. Mid region of the chick cochlea 30 days following noise exposure.

(a) Transverse LM section showing regenerated short hair cells (small arrows) with overlying silver grain labeling by AR. Labeled cells are indistinguishable from adjacent non-labeled hair cells. Labeled mature supporting cell (large arrow) underneath labeled hair cells. There is no evidence of residual damage.

(b) SEM demonstrating near complete anatomical recovery of the damaged region. A degree of cellular disorganization and a small scar (arrow) persist. (From Girod et al., 1989.)

FIGURE 5

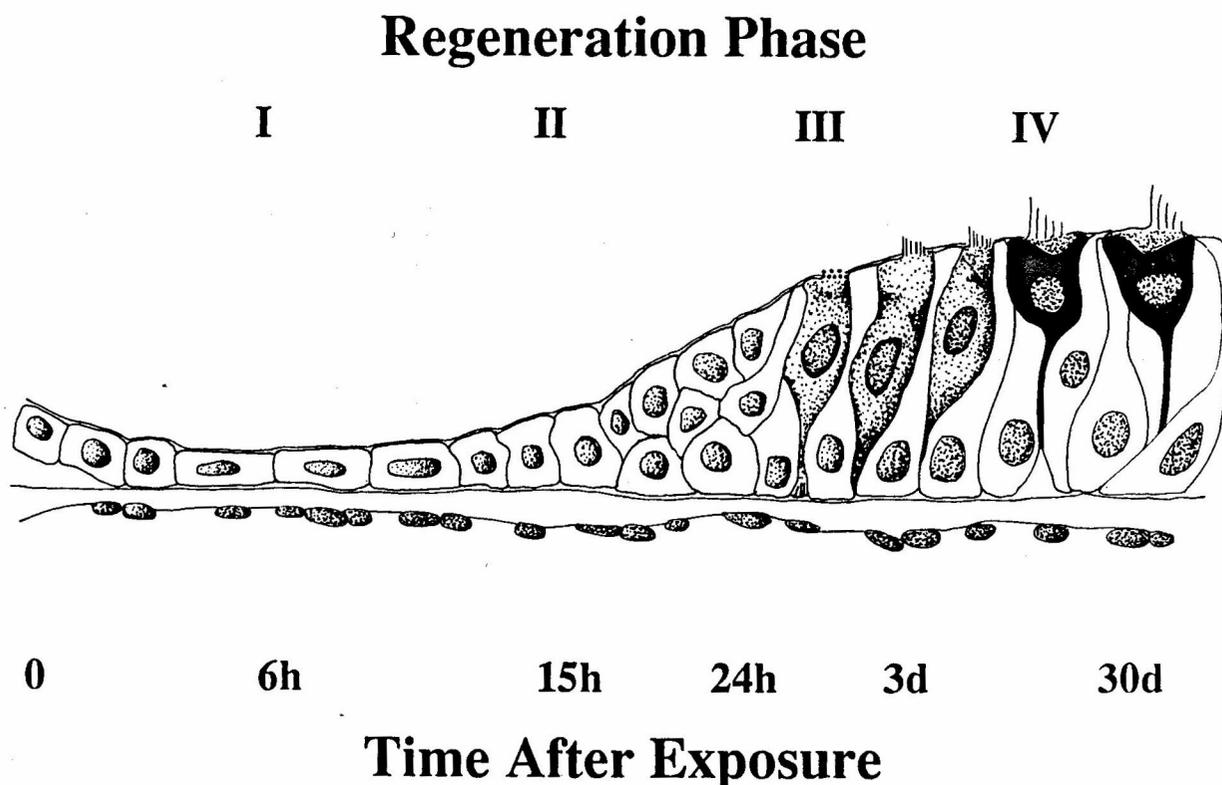


Fig. 5. Schematic representation of the proposed phases of regeneration of the inferior sensory epithelium following noise exposure. Normal (pre-exposure) hyaline and cuboidal epithelial cells are shown on the inferior basilar membrane at the left. The superior normal sensory epithelium is to the right. Time after noise exposure is on the x axis. Phase I: *Migration* of hyaline or cuboidal cells to cover basilar membrane exposed by lost hair cells and supporting cells. Phase II: *Proliferation* of hyaline or cuboidal cells to increase cell number. Phase III: *Differentiation* of proliferating cells into hair cells and supporting cells. Phase IV: *Maturation* of the regenerated sensory epithelium. (From Girod et al., 1989.)

reported by Henry et al.²⁰ The surviving hair cells in the lesion area appeared to have enlarged apical surfaces to fill in the gaps left by missing hair cells, resulting in a grossly normal sensory surface. No animals were studied at longer survival times to see if hair cell numbers eventually returned to normal as reported by Ryals and Rubel.¹⁶

In birds, as in mammals, the time course and pattern for hair cell loss following gentamicin ototoxicity appears to be very different from that seen with noise damage. As previously discussed, Cruz et al. treated chicks for 10 days with gentamicin and found an immediate basal hair cell loss which progressed in an apical direction over the next week. There was evidence of partial recovery of hair cell numbers by two weeks after the gentamicin was completed.

More recently, Girod et al.²² demonstrated an even more dramatic progression of injury over time following gentamicin exposure. Neonatal chicks given 10 days of

gentamicin underwent cochlear examination using SEM after five days of gentamicin or one, three, four or 20 weeks after 10 days of gentamicin.

Hair cell counts sampled at five locations demonstrated near total loss of hair cells at the basal tip after only five days of gentamicin treatment. Over the four weeks following cessation of the gentamicin, hair cell loss spread in an apical direction to involve all but the apical most portion of the cochlea. However, by 20 weeks after gentamicin, hair cell numbers were essentially normal. The basal most hair cells remained fairly immature and disorganized even after 20 weeks. This correlated well with the functional testing performed in these same animals by Tucci and Rubel which will be discussed in the next section.

Duckert and Rubel²³ studied the basal region of the chick cochlea following gentamicin with transmission electron microscopy (TEM). They found regenerated hair

cells had formed immature afferent synapses after only five days of gentamicin and in some cases even before the cell reached the apical surface of the sensory epithelium. Afferent and eventually efferent synapses increased in number during the four weeks following gentamicin, but longer survival times were not examined.

Functional Regenerative Capacity

The anatomical studies described thus far would predict some, if not complete, recovery of hearing in the avian cochlea following noise or ototoxic drug injury as a result of hair cell regeneration.

McFadden and Saunders²⁴ tested cochlear nucleus evoked potential thresholds in neonatal chicks following an intense pure tone noise exposure. They found an immediate broad band 60 dB threshold shift when compared to controls. There was rapid recovery with only an averaged 9.4 dB threshold shift by three days after noise exposure and nearly complete recovery by 15 days post exposure.

Regenerated hair cells are not seen in the most immature form until two to four days after noise exposure. Thus the recovery of thresholds seen by McFadden was not due to regenerated hair cells. Rather it was postulated to be the result of repair and regeneration of the damaged tectorial membrane as described by Cotanche.¹⁴

The tectorial membrane damage and the presence of surviving hair cells at the site of injury following noise exposure clearly interfere with the measurement of functional recovery due to regenerated hair cells. These problems were partially avoided by utilizing the gentamicin ototoxicity model of injury to the cochlea.

Tucci and Rubel²⁵ examined cochlear nucleus evoked potential thresholds of neonatal chicks after five days of gentamicin or one week to 20 weeks after 10 days of gentamicin. They found an immediate isolated high frequency threshold shift with progression over the ensuing five weeks to involve the entire frequency range tested (.25 - 5 kHz). However by 16-20 weeks after the gentamicin treatment, the evoked potential thresholds were essentially normal with only a slight residual high frequency hearing loss.

These electrophysiological findings correlated well with the anatomical changes seen in these same animals and described in the previous section.²² Immediate basal hair cell loss produced a high frequency threshold shift. Progression of hair cell loss in an apical direction over the ensuing weeks resulted in a threshold shift across all frequencies. Long term recovery results in normal hair

cell numbers and near normal thresholds. The slight residual high frequency threshold shift was associated with residual regenerated hair cell immaturity and disorganization in the basal cochlea.

These studies strongly suggest that mature regenerated hair cells are functional and can result in recovery from a traumatic sensorineural hearing loss in birds.

Clinical Implications

The mammalian cochlea and its response to injury has been a focus of anatomical and physiological study for decades. To date, a definitive study to look for cellular mitosis and hair cell production in the post traumatic mammalian cochlea has not been performed. Nonetheless, one would anticipate some indirect evidence for hair cell regeneration in the mammal given the volumes of literature on the subject. Thus, if hair cell regeneration does occur, it most likely does so infrequently or to a very small degree.

This does not necessarily imply that the mammalian cochlea has lost the capability for hair cell regeneration. Rather, cellular proliferation may be actively suppressed or require a stimulus not commonly present in the post traumatic mammalian cochlea. It is this prospect that holds promise for possible clinical applications of the avian studies.

In depth study of hair cell regeneration in the avian will allow detailed analysis of the cellular triggers and messengers involved in this process. This may ultimately allow the direct stimulation of hair cell production in the mammalian cochlea following trauma. Cell culture methodology may one day enable us to transplant living cultured hair cells into a damaged cochlea to replace lost hair cells and return to function. In addition, research in this field may offer insight into and allow exploitation of possible protective mechanisms to prevent hair cell loss.

Conclusion

Until recently, cochlear trauma resulting in hair cell loss and the associated hearing loss was thought to be irreversible in both mammals and birds. Recent studies have shown that the neonatal and sexually mature avian cochlea is capable of hair cell regeneration and near complete anatomical recovery following injury by noise or ototoxic drugs. This anatomical recovery is accompanied by recovery of hearing. Further studies in this field may provide insight into hearing protection and, ultimately, hair cell transplantation or regeneration in the mammalian cochlea.

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ERRATUM

The following is the corrected version of Table 1 from the article "Congenital Head and Neck Masses in Infants and Children (Part I)" by J. Lindhe Guarisco, MD.

TABLE 1. Congenital Head and Neck Masses in Children

I. Common Masses	
A.	Thyroglossal duct cysts
B.	Branchial apparatus abnormalities
1.	Second abnormalities
a.	Fistulas
b.	Sinuses
c.	Cysts
2.	First abnormalities
a.	Fistulas
b.	Sinuses
c.	Cysts
3.	Third abnormalities (rare)
4.	Fourth Abnormalities (no complete abnormality reported)
C.	Lymphangiomas (Cystic hygroma)
D.	Subcutaneous blood vessel abnormalities
1.	Hemangiomas
2.	AVM
II. Uncommon Masses	
A.	Teratomas and dermoid cysts
III. Rare Masses	
A.	Ectopic thyroid
B.	Congenital goiter
C.	Fibromatosis colli
D.	Lipoma
E.	Laryngocele
F.	Thymic cysts
G.	Cervical bronchogenic cysts
H.	Others