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$$\frac{d^2y}{dx^2}\left[1+\left(\frac{dy}{dx}\right)^2\right]^{-3/2} - C_0 + C_2(y - y_0) - C_1(B - B_0) = 0$$

where x and y refer to the mean points in the stream, B_0 is the total ocean depth at the origin (x_0,y_0), C_0 is the curvature of the free inertial jet at the origin, C_1 is a constant that equals $10^{10}f_0T/M$ where f_0 is the inertial frequency, T is a bulk value for the integrated volume transport across unit depth of the stream near the bottom, and M is the integrated momentum transport across the section of the stream. C_2 is also a constant, $10^{12}\beta V/M$, where β equals df/dy at any particular latitude, f is the Coriolis parameter, and V is the integrated volume transport. The numerical method of calculation is in (*12*).
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Regeneration of Sensory Hair Cells After Acoustic Trauma

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Any loss of cochlear hair cells has been presumed to result in a permanent hearing deficit because the production of these cells normally ceases before birth. However, after acoustic trauma, injured sensory cells in the mature cochlea of the chicken are replaced. New cells appear to be produced by mitosis of supporting cells that survive at the lesion site and do not divide in the absence of trauma. This trauma-induced division of normally postmitotic cells may lead to recovery from profound hearing loss.

HAIR CELLS ARE THE MECHANORECEPTORS that transduce acoustic stimuli into electrical activity in the ear. Disease, exposure to loud sound, treatment with antibiotics, and processes associated with aging can cause hair cells to die, which results in a loss of hearing (*1*). The hair cells in the cochleae of birds and mammals are produced during the first two-thirds of embryogenesis (*2*), and it has been thought that any subsequent losses lead to permanent deficits. However, in some fish and amphibians hair cells are produced throughout life and may contribute to self-repair (*3*). Recently, we have found that sound-induced damage resulting in the complete loss of the stimulus-transducing stereocilia bundles of hair cells in chickens can be reversed by the growth of new stereocilia bundles (*4*); this observation has been confirmed by other investigators (*5*). Counts of hair cells in histological sections also have shown recovery after an aminoglycoside was administered in chickens (*6*). These findings suggest that regeneration dependent on the production of replacement hair cells may occur in the avian cochlea.

We have investigated the potential regeneration of hair cells by exposing small groups of 9- to 13-day-old chickens to a loud tone, sufficient to cause hair cell loss (*7*). Since damage varies greatly with small changes in sound intensity, one chicken from each group was killed and the cochleae were fixed immediately after the acoustic treatment; only groups with moderate damage were used. Other chickens from each group were allowed to survive for 10 days after the treatment and were administered [^{3}H]thymidine. This radioactive tracer is incorporated into replicating DNA, allowing cells that are mitotically active to be identified later by autoradiography (*7*).

The crescent-shaped sensory epithelium in the chicken cochlea contains a dense population of hair cells, which have surface stereocilia bundles that are stimulated by sound in a high to low pitch gradient from the proximal to the distal end. The other cells in this epithelium are supporting cells, which have microvilli-covered surfaces that are normally interposed as thin lines between the hair cells (Fig. 1A).

When we exposed chickens to a tone of 1.5 kHz at 115- to 120-dB sound pressure level (SPL) for 48 hours, lesions were produced at a consistent location in the proximal half of this epithelium (Fig. 1B). In ears fixed immediately after sound exposure, stereocilia bundles were missing in the lesioned area, and the surface was covered by scattered cells that appeared to have been extruded from the epithelium. Some cells possessed stereocilia bundles that identified them as hair cells. The positions that hair cell surfaces normally would have occupied were filled by the expanded surfaces of supporting cells. During the 10 days after the treatment, the lesioned area gradually returned to normal as stereocilia bundles reappeared, differentiated, and grew larger, in a manner resembling their embryogenesis (*8*), so that the site of the lesion became almost indistinguishable from the same site in control epithelium (*4*) (Fig. 1, C to F).

Autoradiographic localization of DNA that incorporated the radioactive thymidine demonstrated that the damage produced by the acoustic treatment had stimulated mitotic replication of cells. At the lesion site, the

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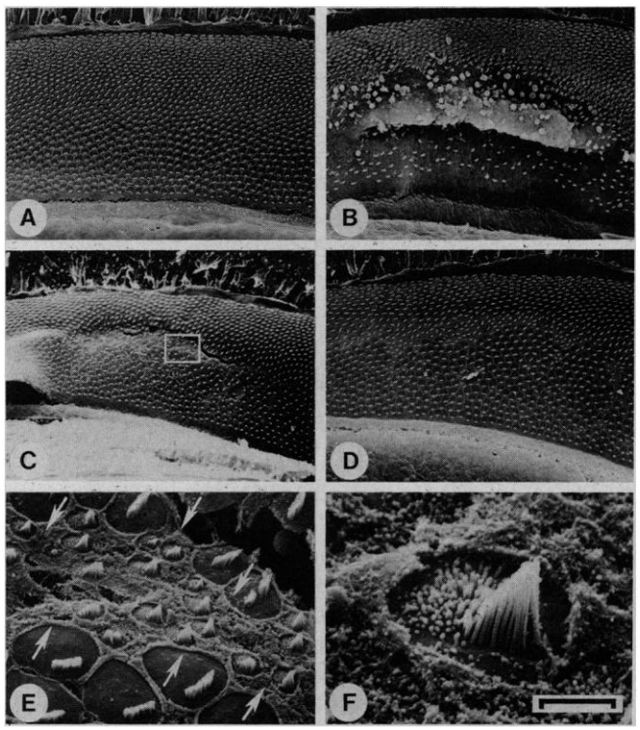

Fig. 1. Scanning electron micrographs of regeneration in the proximal part of the cochlear sensory epithelium of young chickens. (**A**) A control ear, in which sound-transducing stereocilia bundles of hair cells appear as white dots that are surrounded by thin rings of supporting cell microvilli. (**B**) A corresponding region located at a point approximately 30% of the distance along the epithelium from the proximal end in a cochlea fixed immediately after 48 hours of acoustic overstimulation at 1.5 kHz. Hair cell stereocilia bundles are missing in the area damaged by the sound. Normal stereocilia bundles surround the lesion, which is marked by a fold in the epithelium and covered by spherical extruded hair cell bodies. (**C**) An epithelium fixed 6 days after acoustic trauma. Small stereocilia bundles are present at the site of the recovering lesion. (**D**) An epithelium fixed 10 days after acoustic trauma; a nearly normal appearance has been restored. (**E**) Bundles of reduced height project from hair cells in the recovering lesion from the outlined area in (C). The expanded surfaces of supporting cells (between arrowheads) are flanked by normal cell surfaces above and below. (**F**) A single hair cell surface from (E). Hair cells such as this, with many microvilli adjacent to the stereocilia, normally are found only in embryos. Bar, 100 μm in (A) to (D), 10 μm in (E), and 2 μm in (F).

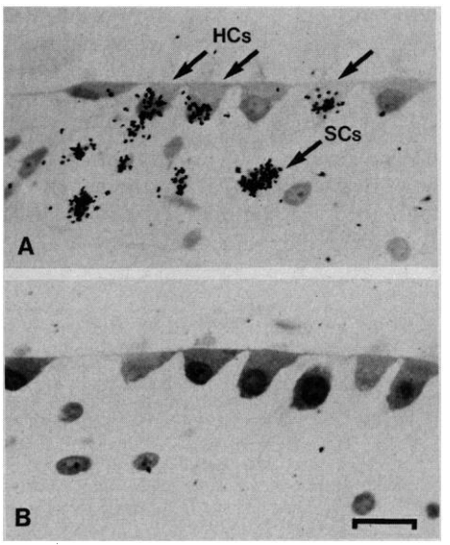

Fig. 2. Autoradiographs demonstrating new cell production in a chicken cochlea exposed to [^{3}H]thymidine and fixed after 10 days of recovery from acoustic overstimulation. (**A**) The nuclei of three hair cells (HCs) (upper row) and five supporting cells (SCs) (lower rows) are labeled by silver grains, indicating that DNA replication occurred at the level of this section through the acoustic lesion and that mitotic production of new hair cells underlies the regeneration seen in Fig. 1. (**B**) A section from an undamaged region of the same overstimulated cochlea. Here, and in controls, the cells remain unlabeled and appear postmitotic throughout normal postembryonic life. We conclude that the mitotic activity demonstrated in (A) is trauma induced in cells that would otherwise remain quiescent. Bar, 10 μm.

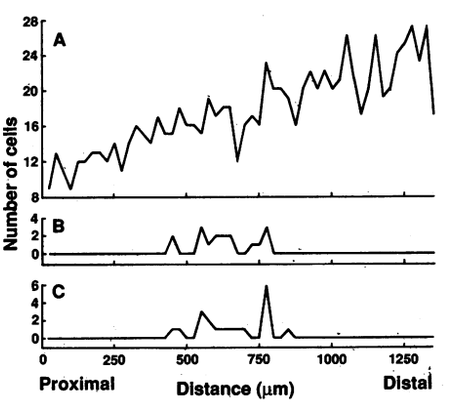

Fig. 3. The distributions of (**A**) all hair cells, (**B**) labeled hair cells, and (**C**) labeled supporting cells in transverse sections along a cochlea fixed 10 days after overstimulation. Labeled hair cells and supporting cells were present along 450 μm of the cochlea, but no epithelial cells were labeled more proximally or distally.

nuclei of both hair cells and supporting cells were labeled by [^{3}H]thymidine (Fig. 2A). In contrast, neither cell type was labeled in the undamaged regions of these experimental cochleae or in controls that received the isotope without the acoustic stimulation, even though thousands of those cells were exposed to [^{3}H]thymidine continually for 7 to 10 days (Fig. 2B).

Where did the new hair cells originate? All the cells in the sensory epithelium of the chicken cochlea become mitotically quiescent in the embryo, 3 weeks before the age at which we exposed structurally mature cochleae to acoustic trauma (*9*). However, some cells in the cochlea must retain a latent capacity for proliferation that can be activated by trauma. Transmission electron microscopy has shown that hair cells are often completely missing in these lesions immediately after trauma (*4*), and we suspect that the labeled hair cells observed during the 10 days after treatment originated from divisions of another type of cell and then differentiated as hair cells.

Supporting cells are known to proliferate during postembryonic life in fish and amphibians (*3*), and many supporting cells in chickens survive at the lesion site. Spatial correspondence in the peaks and valleys of labeling in supporting cells and hair cells (Fig. 3) is consistent with the hypothesis that regenerated hair cells originate from mitotic divisions of supporting cells or some unidentified latent stem cells that give rise to both cell types. These divisions might produce progeny that can differentiate either as supporting cells or as hair cells, when hair cells have been lost. Studies of lateral line hair cell epithelia suggested that supporting cells could give rise to hair cells by transdifferentiation of their progeny (*10*), and experiments in which microinjections of fluorescent dextran were used to trace specific cell clones supported this hypothesis (*11*). Furthermore, new hair cells can be generated in lateral line epithelia in which all previously existing hair cells have been killed, and these epithelia contain only supporting cells (*10, 12*).

Because hair cells attract growing neurites (*13*), it is expected that replacement cells will become innervated, but what is the likelihood that they will restore hearing? Compound action potentials have been recorded from the auditory nerve and used to measure hearing in young chickens after 48 hours of acoustic stimulation at 0.9 kHz and 111-dB SPL (*14*). A 50-dB loss of sensitivity at 0.9 kHz was measured after that treatment, but sensitivity gradually returned until normal hearing was restored at 10 days. This time course corresponds well with the time course of hair cell regeneration, so regeneration may underlie the measured recovery of hearing.

Ryals and Rubel have used cell counts and thymidine labeling in experiments showing that the ability to regenerate hair cells after trauma persists even in reproductively ma-

ture birds (*15*). The discovery of hair cell regeneration, with replacements originating from unidentified stem cells or cells that are ordinarily postmitotic, suggests that the possibility of self-repair after trauma should not be ruled out in mammalian ears and other neuroepithelial derivatives simply because their cells are mitotically quiescent in normal postembryonic life.

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Hair Cell Regeneration After Acoustic Trauma in Adult *Coturnix* Quail

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Recovery of hair cells was studied at various times after acoustic trauma in adult quail. An initial loss of hair cells recovered to within 5 percent of the original number of cells. Tritium-labeled thymidine was injected after this acoustic trauma to determine if mitosis played a role in recovery of hair cells. Within 10 days of acoustic trauma, incorporation of [^{3}H]thymidine was seen over the nuclei of hair cells and supporting cells in the region of initial hair cell loss. Thus, hair cell regeneration can occur after embryonic terminal mitosis.

PROLIFERATION, MIGRATION, AND differentiation of sensory hair cells occur embryonically or during early-postnatal development in mammals and birds. Once cell division ends, the capacity to produce new cells is thought to be lost for the duration of life, and hair cells that are lost as a result of toxic or traumatic events cannot be replaced. Certain bony fishes and amphibians, however, can produce hair cells throughout life and presumably replace any that are lost as a result of trauma or aging (*1*). In young birds, hair cell loss is reduced over time after ototoxicity and acoustic trauma (*2*), and stereocilia are replaced after acoustic trauma (*3*). This evidence suggests that the potential for hair cell regeneration after trauma may exist in young birds. In older birds (*4*) recovery of hair cell stereocilia does not occur, suggesting that the recovery process is age related and signifying that there may be a critical period during which recovery from acoustic trauma is possible. Another consideration, however, is that more extensive destruction of hair cells in the older birds renders the system incapable of significant repair (*5*).

In our study the following questions were addressed: (i) Is recovery from ototoxic agents or acoustic trauma related to the age at which acoustic overstimulation occurs or is it related to the extent of the trauma? (ii) How is the recovery taking place? Are new cells regenerated to replace lost hair cells? In the first experiment, we determined if recovery of lost hair cells can occur in adult birds. Sexually mature, adult quail (*Coturnix coturnix*) were continuously exposed for 12 hours to a 115-dB pure tone (*6*). Adult quail were used to determine if recovery stemmed from some process unique to early development, and 115-dB stimulation was used to optimize the probability of recovery. Survival time after acoustic trauma was varied from 0 to 60 days after exposure (*7*). At the appropriate time the birds were killed and their cochleae fixed, dissected free, osmicated, embedded in plastic, sectioned in the transverse plane, and stained. The number of hair cells extending across the basilar membrane was counted at each 100-μm interval from the basal to the apical end (*8*).

Ten days after the acoustic trauma the number of hair cells had decreased markedly (*9*) through the basal and middle portion of the cochlea (Fig. 1). In the middle of the cochlea as many as 70% of the hair cells were lost. In birds exposed to the same acoustic trauma but allowed to survive 30 days, hair cell loss was much less dramatic. Thirty days after acoustic trauma, the position of the maximum hair cell loss, at about the middle of the cochlea, was similar to that of the 10-day survival group, but the extent had decreased from 70% to only about 31%. For birds allowed to survive a longer time (60 days), there was evidence of a continued progression toward a return to the normal complement of hair cells. No

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