Otolaryngology-Head and Neck Surgery,

1990, 103: 443-450

Physiologic status of regenerated hair cells in the avian inner ear following aminoglycoside ototoxicity

DEBARA L. TUCCI, MD, and EDWIN W RUBEL, PhD, Charlottesville, Virginia, and Seattle, Washington

Regeneration of avian inner ear hair cells has been demonstrated after administration of aminoglycoside and after acoustic trauma. However, no published study to date has documented functional recovery of these regenerated sensory receptor cells. Newborn chicks were treated with gentamicin sulfate (50 mg/kg/day) for a total of either 5 (n = 10) or 10 (n = 76) days. Evoked potential thresholds were obtained one day after the 5-day treatment, or at intervals between one day and 20 weeks after the 10-day treatment course, and compared to thresholds of age-matched control animals. A significant hearing loss, predominantly in the high frequencies, was present after as few as 5 days of drug administration. The magnitude of hearing loss continued to increase, especially at lower frequencies, as survival increased from 1 day to 5 weeks after gentamicin treatment. Sixteen-to-20 weeks after treatment, partial recovery of thresholds was evident. These findings demonstrate that functional recovery does occur in the avian inner ear following aminoglycoside administration. Recovery occurs at all frequencies, but predominantly at low and middle frequencies, leaving significant residual high-frequency threshold elevation. Recovery lags 14 to 18 weeks behind anatomic evidence of hair cell regeneration, which was demonstrated in one study by 2 weeks after comparable administration of gentamicin. (OTOLARYNGOL HEAD NECK SURG 1990;103:443.)

In mammals, damage to inner ear sensory hair cells by ototoxic drugs, noise exposure, or as a consequence of advancing age is believed to be, with few exceptions, permanent. Structural damage is associated with permanent sensorineural hearing loss. However, the literature does contain reports of recovery from certain injuries.

From the Department of Otolaryngology–Head and Neck Surgery, University of Virginia Health Sciences Center (Dr. Tucci), and the Hearing Development Laboratories, Department of Otolaryngology–Head and Neck Surgery, University of Washington Health Sciences Center (Dr. Rubel).

Supported by NIH grant DC 00395 and by funds from the University of Virginia Department of Otolaryngology–Head and Neck Surgery.

Presented (in part) at the Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg, Fla., Feb. 1989, and at the Annual Meeting of the American Academy of Otolaryngology–Head and Neck Surgery, New Orleans, La., Sept. 24-28, 1989.

Received for publication Dec. 27, 1989; revision received July 7, 1990; accepted July 25, 1990.

Reprint requests: Edwin W Rubel, PhD, Hearing Development Laboratories, RL-30, University of Washington, Seattle, WA 98195.

23/1/23964

Moffat and Ramsden[1] reported partial recovery in one patient, beginning 3 weeks after gentamicin treatment. Continued improvement was demonstrated (by 45 dB in the lowest frequencies) at 8 months after therapy. Winkel et al.,[2] in a prospective study of 20 patients receiving gentamicin, demonstrated ototoxicity in half. These deficits, which were primarily cochlear, were fully reversible in four patients. In a large prospective study, Fee[3] reported recovery (amount not defined) in 55% of patients with aminoglycoside-associated hearing loss. Recovery occurred between 1 week and 6 months after cessation of therapy, and was noted to be more likely to occur in patients with an initial mild hearing loss.

Despite these reports in the literature, animal studies have failed to demonstrate recovery of auditory function after administration of aminoglycosides. A possible mechanism for recovery was suggested by Cruz et al.[4] They evaluated hair cell number in the basilar papilla, or cochlea, of the chick inner ear at various ages after administration of 50 mg/kg of gentamicin sulfate daily for a total of 10 days. Light microscopic examination of these basilar papillae revealed a 36% decrement in hair cell number, with most extensive loss in the basal 50% of the structure. Over time, there was progressive

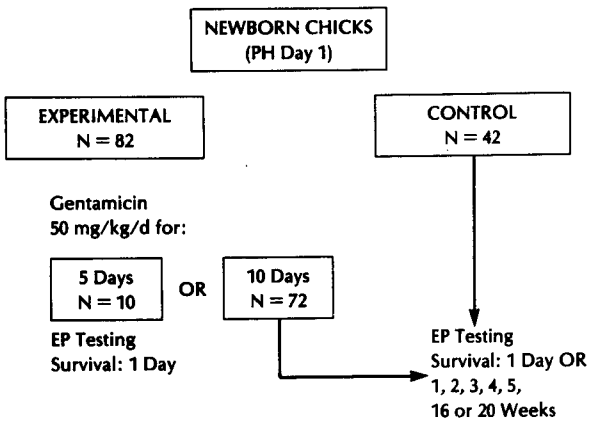

Fig. 1. Experimental design.

Table 1. Groups of subjects

No. of days gent. received/ time after gent. injection	No. of subjects	
	Experimental	Control
5/1 day	10	5
10/1 day	10	5
10/1 week	10	5
10/2 weeks	10	5
10/3 weeks	10	5
10/4 weeks	8	5
10/5 weeks	9	5
10/16 weeks	5	2
10/20 weeks	11	5

gent., gentamicin.

involvement of more apical cells. However, by 2 weeks after treatment, a significant *increase* in hair cell number was observed, with further improvement evident at 3 weeks.

Observation of the regenerative capacities of the chick inner ear after noise exposure were made by Cotanche[5,6] in a series of experiments using scanning electron microscopy (SEM). New or regenerated hair cells were identified in the epithelium within 48 hours after a 48-hour exposure period (1.5 kHz pure-tone at 20 dB SPL), and a nearly normal-appearing epithelium was seen by 10 days after exposure. Corwin and Cotanche[7] confirmed these findings and extended them, suggesting that supporting cells in the damaged region become mitotically active, producing newly regenerated hair cells. Similar findings in the adult quail were reported by Ryals and Rubel.[8]

The finding that the chick inner ear has the capability of producing new hair cells in response to injury naturally leads to the question of functional restoration of hearing. Complete restoration of hearing to normal sensitivity is dependent on several conditions. The regenerated hair cells must be physiologically mature, they must be innervated by appropriate axons, and the other elements of the peripheral and the central auditory system must be functional.

The present study was undertaken to answer two questions. *First,* does functional recovery occur in the chick auditory system after administration of gentamicin? *Second,* what is the time course of the recovery? On the basis of the study by Cruz et al.,[4] we expected that recovery would occur and that this would take place as regenerated hair cells appeared, 2 to 3 weeks after cessation of drug therapy.

METHODS

Subjects for this study were 124 newly hatched chickens (Hubbard × Hubbard). Experimental animals (n = 82) received one subcutaneous injection of gentamicin sulfate, 50 mg/kg daily for five (n = 10) or 10 (n = 72) days (Fig. 1). Control animals (n = 42) received no injections, and were raised and tested with experimental animals. In previous studies from our laboratory,[4] control animals receiving vehicle injections have shown no differences in hair cell counts from uninjected controls. No deaths attributable to the drug administration occurred, although body weight of the drug-treated chicks was substantially reduced (by almost 50%) as compared with age-matched controls by 5 weeks after injections.

The ten animals receiving only 5 days of gentamicin were all tested the day after drug treatment was completed. This experimental group (5–1 day) was included to determine how rapidly drug administration produces hearing loss. In the 72 animals that received the full 10 days of gentamicin, evoked potential hearing thresholds were measured at a variety of times after drug administration. Testing was carried out either 1 day after treatment (10–1 day), or 1, 2, 3, 4, 5, 16 or 20 weeks after gentamicin injections. Experimental groups generally contained eight to 11 animals each, while age-matched control groups typically contained five animals (Table 1). Evoked potential thresholds were measured for test frequencies ranging from 250 Hz to 4000 Hz, and for a 5000-Hz stimulus in the older animals. Tone bursts with 4-msec rise and decay times and 10-msec total duration were delivered at a rate of 5 per second. Stimuli were presented by a closed tube delivery system and calibrated before each experiment via a probe tube connection using a Knowles (BL 1830) microphone (Knowles, Franklin Park, Ill.) The entire system was calibrated using a Bruel and Kjaer 1/8-inch microphone (Bruel & Kjaer, Marlborough, Mass.) and a Hewlett-Packard (3561A) signal analyzer (Hewlett-Packard, Sunnyvale, Calif.), so that all threshold data were recorded in dB sound pressure level (SPL).

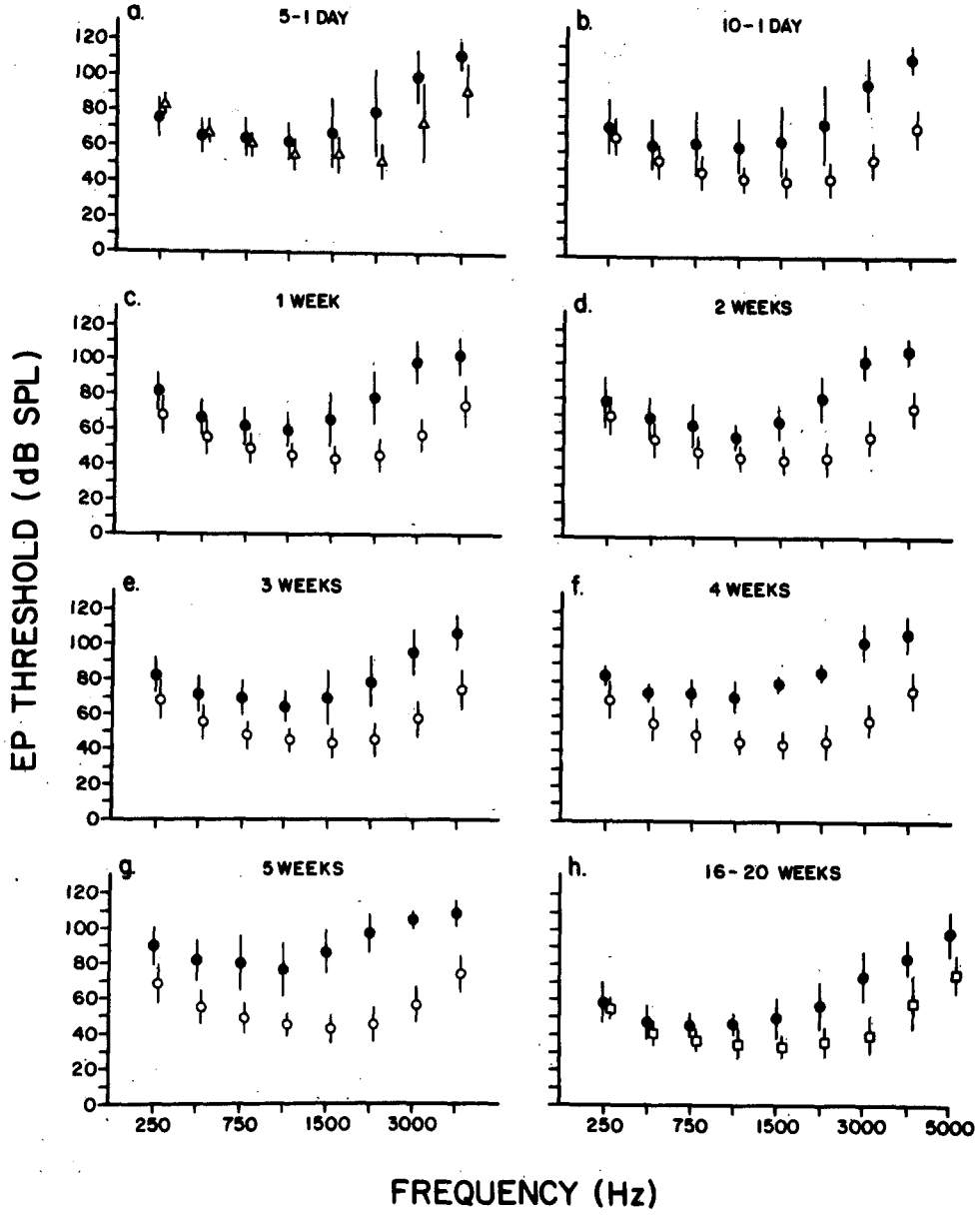

Fig. 2. Evoked potential thresholds in dB SPL, mean (one standard deviation) for experimental *(filled circles)* and control *(open symbols)* animals at survival intervals are shown. Control data are for five 1-day *(open triangles)*, combined ten 1-day to ten 5-week inclusive *(open circles)*, or for ten 16- to 20-week *(open squares)* groups.

Animals were prepared for testing after adequate anesthesia was induced by injection of Equithesin (1.5 mg/kg; intraperitoneally) and ketamine hydrochloride (80 mg/kg; intramuscularly). Anesthetics were supplemented throughout the recording session. Body temperature was maintained at 39° C. The cartilagenous portion of the external auditory canal on the test side (arbitrarily selected) was removed to facilitate placement of the sound delivery tube. The tympanic membrane was visualized under the microscope to ensure that it was intact. After stabilization of the head in a specially designed holder, pin electrodes (Grass Instrument Co., Quincy, Mass.) were implanted bilaterally through the skull at a level just above the brainstem auditory nuclei (active and reference electrodes) and into the thigh muscle (ground electrode). Responses were amplified, filtered (30- to 3000-Hz band pass), digitized at a rate of 10 kHz, and averaged over 200 to 500 stimulus presentations by a PDP 11-73 computer system.

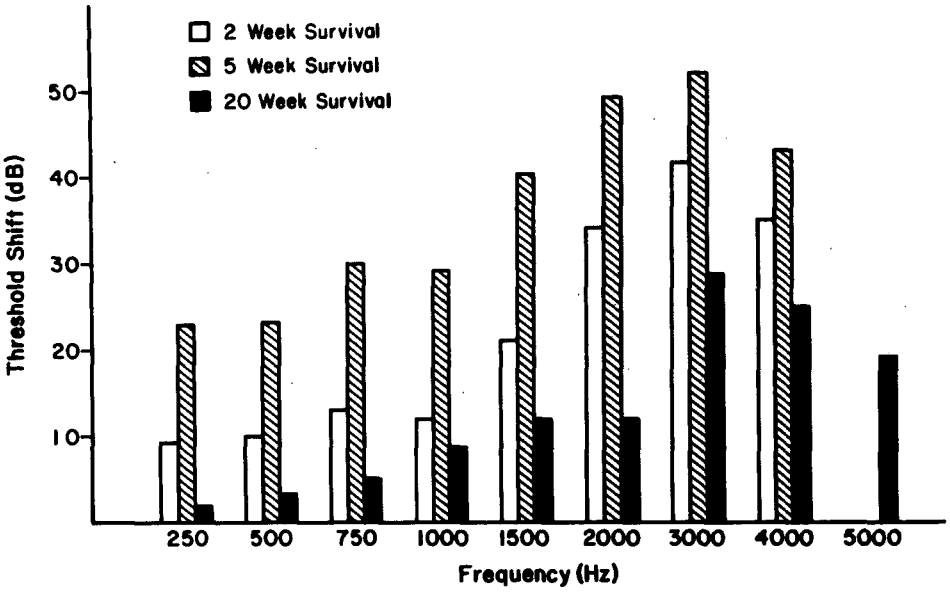

Fig. 3. *Bar graph* shows differences in mean threshold between experimental and age-matched control animals at three survival intervals.

Thresholds were obtained to the nearest 5 dB by identifying the lowest intensity of stimulation that evoked a response of at least twice the amplitude of the baseline variation. After thresholds had been measured at all test frequencies, the first frequency threshold measurement was repeated in order to assess reliability. On repeat, 66% of thresholds were identical, 31% were within 5 dB, and less than 3% demonstrated a 10-dB or greater change. Preparation and threshold measurement typically required 2 to 3 hours per animal.

Threshold data were analyzed by mixed design ANOVA using Statview 512. For each age group, threshold data from experimental animals are compared to appropriate control groups (see *Results*) with frequency analyzed as a within-subject variable. Individual comparisons were carried out by the method of Sheffe. In addition, data from control groups and experimental groups were analyzed in separate two-way ANOVA designs, with age as a between-subject variable and frequency as a within-subject parameter.

All experiments were carried out in strict adherence to the standards of animal care as specified by the National Institutes of Health (NIH publication No. 80-23, revised 1978).

RESULTS

Control animals. It has been reported[9] that hearing thresholds in the chick are adult-like at the time of hatching. However, thresholds measured from control animals at 6 days post-hatch (5–1 day) were on average 10 dB higher than those of animals tested at 11 days (10–1 day) to 5 weeks after treatment. Thresholds measured at 16 to 20 weeks were significantly better than those measured at 1 day to 5 weeks after injection ($p < 0.05$). Because of these differences, the youngest (5–1 day) and oldest (10–16 and 20 week) experimental groups are compared with their own age-matched controls. Thresholds of all other experimental groups (10–1 week through 10–5 week; see Table 1) are compared, for each frequency, to the mean threshold of these age-matched control groups combined.

Experimental animals. Figure 2 shows the mean evoked potential thresholds (± one standard deviation) for experimental and control animals at each age. Data shown in Fig. 2, *a* are for animals tested 1 day after a 5-day course of gentamicin (5–1 day), and are compared to age-matched controls. While a trend is apparent in increased thresholds at the higher frequencies tested, overall differences did not reach statistical significance. However, there was a significant interaction ($p < 0.001$) between age and frequency, indicating that this increase in high-frequency threshold is statistically reliable. Standard deviations are relatively large for this experimental group, perhaps reflecting variability in the onset of the drug-induced hearing loss.

A substantial hearing loss is evident 1 day after the 10-day course of gentamicin (10–1 day) and increases over the ensuing 5 weeks (Figs. 2, *b* through *g*). At

the earliest survival times, the hearing loss is most evident at the highest frequencies, but spreads to involve the low frequencies over time. By 4 weeks after treatment, a significant low frequency hearing loss is evident, and this increases by 5 weeks. ANOVA on each age group revealed a significant effect of treatment (experimental vs. control) for each group, except at 6 days (all p's < 0.001) and a significant frequency $\times$ treatment interaction was found in all groups (all p's < 0.001).

There were no reliable differences in thresholds between the 16- and 20-week groups; thus, those data were combined for comparison with age-matched controls (Fig. 2, *h*). In those groups, thresholds had decreased so that they were more nearly normal, particularly at the low frequencies. Residual hearing loss remains evident in the high frequencies. Threshold decreases between the 5-week and 16- to 20-week groups were statistically reliable ($F_{1,7} = 94.5$; $p < 0.001$).

Fig. 3 shows elements of the same data presented as threshold differences between three selected experimental groups (2-week, 5-week, and 20-week). In each case experimental groups are compared to age-matched controls. The pattern of hearing loss and recovery is quite evident. After 2 weeks *(white bar)* some hearing loss is evident in the low frequencies, but it is far more substantial in the high frequencies, measuring approximately 40 dB at 3000 Hz. Hearing loss at 5 weeks *(cross-hatch bar)* is of considerably greater magnitude at all frequencies tested, and reaches 50 dB at 3000 Hz. However, by 20 weeks after gentamicin treatment, little hearing loss remains. The residual impairment is less than 15 dB at frequencies below 3000 Hz. Interestingly, a significant impairment remains at 3000 Hz (30 dB), whereas at 4000 Hz and 5000 Hz relatively less hearing loss is evident.

DISCUSSION

Inner ear sensory cells of the cochlea are present in their full complement well before the time of birth in the mouse,[10] human,[11] and the chick.[12] While it is generally stated that sensory neuroepithelia in birds and mammals do not have the capacity to regenerate, there is evidence to the contrary. Regeneration of olfactory epithelium has been demonstrated after degeneration in a variety of animals, including the monkey.[13] Regeneration is most likely to occur when injury is followed by incomplete degenerative changes. Totally damaged epithelium is often not capable of repair.[14] Moreover, recently reported evidence suggests that the olfactory epithelium undergoes continual replacement in response to environmental stimulation as evidenced by the fact that turnover decreases in cases of sensory deprivation.[15] A stem-cell population has been identified that is capable of differentiation into several epithelial cell types, including neural elements.[13]

Postembryonic production of inner ear sensory cells has been demonstrated in fish and amphibians, and found to occur through much of the lifetime of these animals.[16-19] The newly supplemented sensory epithelium is innervated by acoustic-vestibular terminals in a topographic pattern in the skate and this arrangement is thought to account, at least in part, for the 500-fold increase in sensitivity over the first several of years of life. Newly produced hair cells appear to attract terminal branches of the ganglionic neurons, because more than 80% are directed toward areas of new hair cell production. The mechanism by which this trophic effect occurs, however, is not presently understood.[19]

Cotanche[5] studied recovery of chick inner ear hair cells after acoustic trauma. Chick cochleas were examined by scanning electron microscopy at intervals after a 48-hour period of high-intensity sound exposure. The damaged epithelium was noted to first show signs of repair by 48 hours after sound exposure, with evidence of newly formed hair cells. After 10 days the epithelium exhibited a nearly normal appearance. Cruz et al.[4] demonstrated by light microscopy an increase in the number of hair cells present in the chick cochlea after an initial decrement following gentamicin ototoxicity. This finding argues against the possible interpretation that hair cell redistribution or relocation is responsible for the reparative process noted in the cochlear epithelium.

Corwin and Cotanche[7] and Ryals and Rubel[8] confirmed that the restoration of the hair cell population resulted from proliferation and differentiation of new cells. Tritiated thymidine was injected into chicks or quail after noise exposure. Supporting cells, as well as hair cells, were found to be labelled, leading the authors to hypothesize that the supporting cells, or an unidentified stem-cell population, are induced to proliferate to replace damaged hair cells and supporting cells. The origin of these newly produced hair cells was investigated in the chick by Girod et al.[20] using DNA labelling with tritiated thymidine at time points ranging up to 30 days after noise exposure. On the basis of patterns of cell migration, proliferation, and differentiation observed in this study, the authors hypothesized that a population of histologically unique cells located at the periphery of the sensory epithelium is responsible for regeneration of both hair cells and supporting cells in the adjacent inferior portion of the papilla. However, the origin of the regenerated cells in the superior portion

of the papilla, which is removed from the proposed stem-cell population, is less clear. It was suggested that, in this region, supporting cells may serve as precursors for both hair cells and supporting cells. Balak and Corwin[21] also presented evidence that, after total hair cell ablation by laser in the salamander lateral line, remaining supporting cells gave rise to both hair cells and supporting cells.

Given that new hair cells are produced in the chick cochlea, the question addressed in the present study was: do these newly regenerated hair cells function normally? First, we will discuss general characteristics of the gentamicin-induced hearing loss, followed by a discussion of functional recovery.

Onset of hearing loss after administration of gentamicin was rapid, occurring in some animals after only 5 days of treatment, and in all animals after the 10-day treatment course. Hearing loss measured at the earliest survival times was predominantly at the highest frequencies tested. Over time, the magnitude of the hearing loss increased and lower-frequency involvement was more pronounced. These findings correlate well with the results reported by Cruz et al.[4] and others, that aminoglycoside damage is initially most prominent in the basal third of the cochlea, but spreads with time or increased dosage to involve more apical cells. These findings are also consistent with studies in human patients that report progressive aminoglycoside-related hearing loss.[22,23]

Functional recovery was identified in our experimental animals by 16 to 20 weeks after the end of gentamicin treatment. The length of time before recovery was demonstrated was longer than expected, given the evidence of an increase in hair cell number by 2 weeks after drug treatment.[4] This finding leads us to the assumption that the regenerated hair cells, although present soon after inner ear injury, are probably not functional for some time. Delay of recovery may be secondary to physiologic immaturity of the hair cells, or to a lack of sufficient or appropriate neural connectivity. Alternatively, it is possible that regenerated hair cells are contributing to hearing quite early in the recovery process, but their contribution is "masked" by the continuing deterioration of hearing because of the spread of ototoxicity. Experiments are currently underway to distinguish between these alternatives.[24]

It is important to note that, even at 20 weeks, functional recovery is not complete, particularly at the highest frequencies examined. It is possible that recovery would continue past the longest survival interval examined in this experiment, and that eventually complete recovery would occur. Alternatively, it is possible that persistent deficits may remain in the cochlea or central auditory structures. Debate exists in the literature as to whether neural elements are damaged primarily by aminoglycosides, or if all observed damage is secondary to sensory cell loss.[25] One recent review favors the latter hypothesis.[26] Additionally, second-order neurons in nucleus magnocellularis of the chick have been noted to undergo anatomic change after administration of aminoglycoside.[27,28] Another possibility is that extensive damage renders the epithelium incapable of total regeneration. This is supported by the finding that the olfactory epithelium regenerates only after incomplete injury.[14] Also, Cotanche[5] has observed that recovery does not occur in the chick basilar papilla after acoustic trauma of sufficient intensity to produce complete collapse of the sensory epithelium.

Data obtained from a scanning electron microscopic study of cochleas from our experimental animals (Girod et al.[29]) support the previously discussed findings. Extensive damage is seen in the basal or proximal portion of the cochlea in the chick immediately after a 5-day course of gentamicin, and after 10 days virtually all of the original hair cells in the basal portion have been eliminated. Even at these early stages, newly regenerated immature hair cells can be identified. Over time, as the epithelium is repaired at the proximal end, hair cell loss spreads distally. This correlates well with the finding of delayed low-frequency hearing loss. By 20 weeks after gentamicin treatment, cochleas from some animals appear grossly normal, while changes persist in others. These findings may help explain why hearing loss persists at the highest frequencies. Basal damage may be so extensive that the epithelium is incapable of complete repair.

Saunders and Tilney[30] and Saunders and Coppa[31] have reported changes in evoked potential thresholds in chicks after acoustic trauma. Audiograms were obtained after 1 hour and at intervals up to 10 days after exposure to noise. The majority of recovery is evident in the first 24 hours, with almost full recovery by 10 days. Because newly regenerated hair cells are present within this time frame, it is conceivable that return to baseline thresholds in this instance results from the newly regenerated hair cells. However, this time course appears inconsistent with the course of recovery in the present study. Furthermore, anatomic studies reveal that the new hair cells are extremely immature, and that there is disruption of the tectorial membrane in the immediate post-exposure period.[5,6] In these studies of acoustic trauma in the chick, hair cell loss is incomplete; even in the area of greatest damage, only one third to one half of the cells are lost.[32,33] We believe the rapid recovery seen in the first 24 to 72 hours after exposure to noise results because of recovery of existing hair cells and recon-

stitution of the tectorial membrane. This would, of course, be very different from the mechanism responsible for recovery from aminoglycoside ototoxicity, in which there is nearly complete loss of hair cells in the basal one fourth of the cochlea.

While the time course of recovery and the correlation with anatomic observations suggest that regenerating hair cells are largely responsible for the restoration of hearing seen between 5 and 20 weeks, other factors may also be contributing. Our recent data on evoked otoacoustic emissions[24] suggest that hair cell function precedes maturation of the receptoneural junctions necessary for transmission of information to the central nervous system. In addition, recovery of damaged but viable hair cells may be contributing to this process.

Many questions remain unanswered. It would be interesting to know if hearing recovery does become complete after survival periods longer than 20 weeks. Second, it has been suggested that although hearing thresholds may return to normal after cochlear insult, certain properties of the hair cells may be altered. McFadden et al.[34] suggest that hair cell tuning may be impaired after noise exposure. The capabilities of the auditory system for regeneration are probably limited, and it is conceivable that a more severe insult or repeated insults would not be followed by regeneration and functional recovery.

The most important question is: what is the mechanism by which a normally mitotically quiescent cell population is induced to proliferate? It is through further investigation into this question that hope exists for the reversal of the otherwise permanent hearing impairment that occurs in human beings as a consequence of a variety cochlear insults.

The authors wish to thank Drs. Jeffery Corwin, Susan Norton, and Douglas Girod for their helpful comments on an earlier draft of this article, and Dr. Robert Cantrell for his continual support and encouragement of this project.

REFERENCES

1. Moffat DA, Ramsden RT. Profound bilateral sensorineural hearing loss during gentamicin therapy. J Laryngol Otol 1977;91: 511-6.
2. Winkel O, Hansen M, Kaaber K, Rozarth K. A prospective study of gentamicin ototoxicity. Acta Otolaryngol 1978;86: 212-6.
3. Fee WE. Aminoglycoside ototoxicity in the human. Laryngoscope 1980;90:1-19.
4. Cruz RM, Lambert PR, Rubel EW. Light microscopic evidence of hair cell regeneration after gentamicin toxicity in chick cochlea. Arch Otoalryngol 1987;113:1058-62.
5. Cotanche DA. Regeneration of hair cell stereociliary bundles in the chick cochlea following severe acoustic trauma. Hearing Res 1987;30:181-94.
6. Cotanche DA. Regeneration of the tectorial membrane in the chick cochlea following severe acoustic trauma. Hearing Res 1987;30:197-206.
7. Corwin JT, Cotanche DA. Regeneration of sensory hair cells after acoustic trauma. Science 1988;240:1772-4.
8. Ryals BM, Rubel EW. Hair cell regeneration after acoustic trauma in adult *coturnix* quail. Science 1988;240:1774-6.
9. Saunders JC, Coles RB, Gates GR. The development of auditory evoked responses in the cochlea and cochlear nuclei of the chick. Brain Res 1973;63:59-74.
10. Ruben RJ. Development of the inner ear of the mouse: A radioautographic study of terminal mitoses. Acta Otolaryngol 1967;(Suppl)220:1-44.
11. Bredburg G. Cellular pattern and nerve supply of the human organ of Corti. Acta Otolaryngol 1968;(Suppl)236:1-135.
12. Tilney LG, Tilney MS. Observations on how actin filaments become organized in cells. J Cell Biol 1984;99:76S-82S.
13. Graziadei PPC, Monti Graziadei GA. Continuous nerve cell renewal in the olfactory system. In: Jacobsen M, ed. Handbook of sensory physiology. Vol. 9. Berlin: Springer-Verlag, 1978:55-83.
14. Takagi SF. Degeneration and regeneration of the olfactory epithelium. In: Beidler LA, ed. Handbook of sensory physiology. Vol. 4. Berlin: Springer-Verlag, 1971:75-94.
15. Farbman AI, Brunjes PC, Rentfro L, Michas J, Ritz A. The effect of unilateral naris occlusion on cell dynamics in the developing rat olfactory epithelium. J Neuroscience 1988;8: 3290-5.
16. Corwin JT. Postembryonic production and aging of inner ear hair cells in sharks. J Comp Neurol 1981;201:541-53.
17. Corwin JT. Postembryonic growth of the macula neglecta auditory detector in the ray, *raja clavata*: Continual increases in hair cell number, neural convergence, and physiological sensitivity. J Comp Neurol 1983;217:345-56.
18. Corwin JT. Perpetual production of hair cells and maturational changes in hair cell ultrastructure accompany postembryonic growth in amphibian ear. Proc Natl Acad Sci 1985;82:3911-5.
19. Corwin JT. Auditory neurons expand their terminal arbors throughout life and orient toward the site of post-embryonic hair cell production in the macula neglecta in elasmobranches. J Comp Neurol 1985;239:445-52.
20. Girod DA, Duckert LG, Rubel EW. Possible precursors of regenerated hair cells in the avian cochlea following acoustic trauma. Hearing Res 1989;42:175-94.
21. Balak KJ, Corwin JT. Hair cell regeneration from supporting cell progeny evoked by cell surgery using a laser microbeam. Presented at the Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg, Fla., February 1989.
22. Dayal VS, Whitehead GL, Smith EL. Gentamicin: progressive cochlear ototoxicity. Can J Otolaryngol 1975;4:348-51.
23. Nakai Y, Zuski K, Chang KC, Yogi H, Tokimoto T. An experimental study on the progressiveness of cochlear damage by aminoglycoside drug. Acta Otolaryngol 1981;91:199-206.
24. Norton SJ, Tucci DL, Rubel EW. Comparison of acoustic and neural responses from avian ears following gentamicin. Presented at the Midwinter meeting of the Association for Research in Otolaryngology, St. Petersburg, Fla., February 1990.
25. Webster M, Webster DB. Spiral ganglion neuron loss following organ of Corti loss: a quantitative study. Brain Res 1981;212: 17-30.
26. Huizing EH, deGroot JCMJ. Human cochlear pathology in aminoglycoside ototoxicity: a review. Acta Otolaryngol 1987; (Suppl)436:117-25.

27. Lippe W, Engling W, Schnute J. Effect of gentamicin on neural size in the cochlear nucleus of the chicken. Presented at the Midwinter Meeting of the Association for Research in Otolaryngology, Clearwater Beach, Fla., February 1988.
28. Saffran AJ, Dodson E, Lambert PR. Gentamicin-induced changes in the nucleus magnocellularis of the adult chicken. Presented before the Research Forum Meeting of the American Academy of Otolaryngology–Head and Neck Surgery, New Orleans, La., September 1989.
29. Girod DA, Tucci DL, Rubel EW. Anatomical correlates of functional recovery in the avian inner ear following aminoglycoside ototoxicity. Presented at the Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg, Fla., February 1990.
30. Saunders JC, Tilney LG. Species differences in susceptibility to noise. In: Hamernik KP, Henderson D, Salvi R, eds. New perspectives on noise-induced hearing loss. New York: Raven Press 1982:229-248.
31. Saunders JC, Coppa N. The contribution of stereocilia, rootlet, and cuticular plate injury to sensorineural hearing loss. In: Glattke TTJ, Collins MJ, Harker LA, eds. Sensorineural hearing loss: mechanisms, diagnosis, and treatment. Iowa City: University of Iowa Press, 1986:29-59.
32. Rubel EW, Ryals BM. Patterns of hair cell loss in chick basilar papilla after intense stimulation: exposure, duration, and survival time. Acta Otolaryngol 1982;93:31-41.
33. Ryals BM, Rubel EW. Patterns of hair cell loss in chick basilar papilla after intense auditory stimulation: frequency organization. Acta Otolaryngol 1982;93:205-10.
34. McFadden EA, Saunders JC. Recovery of auditory function following intense sound exposure in the neonatal chick. Hearing Res 1989;41:205-16.

EDITOR'S COMMENT

Drs. Tucci and Rubel's paper won an American Academy of Otolaryngology–Head and Neck Surgery Research Award in 1989. We are delighted to have the opportunity to publish this work.

It is a long way from restoring hearing in chicks to restoring hearing in patients; however, this is an exciting area of research for Otolaryngology. The results of this paper suggest that regenerated hair cells can form connections with the central nervous system that will allow hearing to be restored.