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Morphological correlates of aging in the chinchilla cochlea

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The inner ears from 80 chinchillas ranging in age from premature to 19.2 years were examined as plastic-embedded flat preparations to determine the morphological changes associated with aging. Three of the four forms of human presbycusis defined by Schuknecht were found in the chinchillas. All animals had losses of sensory cells or sensory presbycusis. Inner (IHCs) and outer hair cells (OHCs) degenerated at a rate of about 0.29% and 1.0% per year, respectively. Age-related degeneration of inner (IPs) and outer pillars (OPs) occurred at a much slower rate. In four animals (5%) the dendritic processes of some of the spiral ganglion cells had degenerated in areas where the loss of sensory cells was minimal. This pathological change is likely equivalent to neural presbycusis. Six animals (7.5%) had regions of degeneration of the stria vascularis or strial presbycusis. The other common finding in the aging cochleas was the presence of lipofuscin or age pigment. Lipofuscin deposits were found to accumulate in the subcuticular region of OHCs, IPs and OPs, near the endolymphatic surfaces of many of the supporting cells and in the epithelial cells of Reissner's membrane. The IHCs accumulated much less lipofuscin. The morphological changes seen in the ears of aging chinchillas were qualitatively similar to those seen in the temporal bones of aging humans although the magnitude of the changes was considerably less. These results suggest that some of the damage found in aging human cochleas may be due to aging plus exposure to one or more ototraumatic agents.

Aging; Presbycusis; Inner ear; Chinchilla

Introduction

Schuknecht (1974, 1989) has defined four forms of presbycusis on the basis of audiometric data and the histological findings in the temporal bones of aged humans. These forms have been termed sensory, neural, strial or metabolic, and cochlear conductive or mechanical presbycusis. Sensory presbycusis is characterized audiometrically by an abrupt high-tone hearing loss with speech discrimination ability dependent on the range of frequencies encompassed by the hearing loss. Histologically, these cochleas have a variable amount of degeneration of sensory and supporting cells at the basal end of the organ of Corti (OC) and usually a corresponding secondary degeneration of spiral ganglion cells (SGCs). Individuals with neural presbycusis have relatively poor speech discrimination compared to their pure-tone thresholds. Histologically, these cochleas have a moderate to severe loss of SGCs while sensory cell loss is usually minimal. Individuals with strial presbycusis have a flat audiometric pattern but good speech discrimination. Patchy atrophy or degeneration of the stria vascularis (SV) is the characteristic histological change in these ears. The diagnosis of cochlear conductive presbycusis is made when the loss of hair cells, SGCs and SV is insufficient to account for the degree of hearing loss. The hearing loss is manifested as a descending audiometric pattern with the speech discrimination score dependent on the slope of the threshold curve.

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The etiologies of the different forms of presbycusis are unknown. In order to develop prevention or treatment strategies, it is important to determine if presbycusis is a normal consequence of aging or if it is the result of a lifetime of exposure to 'micro-noise trauma' (Gravendeel and Plomp, 1960) or a variety of ototraumatic events (Nadol, 1980). Since detailed auditory histories of aged humans are generally not available, the role of microtraumatic events in the etiology of presbycusis is difficult to examine directly in humans.

One way in which to evaluate the role of microtrauma in aging of the auditory system is to identify a suitable animal model. Mice (Henry and Chole, 1980), rats (Keithley and Feldman, 1982), guinea pigs (Covell and Rogers, 1957; Coleman, 1976; Ulehlová, 1975) and gerbils (Mills and Schmiedt, 1989; Adams et al., 1989; Schulte and Adams, 1989) have been utilized most frequently for studies of aging of the auditory system. Because of their relatively short lifespans (i.e., 1.2–6 years), it is feasible to house these animals in a controlled laboratory environment for their entire lives. However, it has yet to be demonstrated that a short-lived animal provides a suitable model for human presbycusis.

The chinchilla is unique among rodents in that it has a lifespan of more than 20 years and the frequency range and sensitivity of its auditory system (Miller, 1970; Clark et al., 1974) is similar to that of humans (Sivian and White, 1933). These features make the chinchilla attractive as a model for aging studies. However, one drawback to the use of this species is the time and effort required to raise and house chinchillas until they reach old age.

Central Institute for the Deaf (CID) has maintained a breeding colony of chinchillas for more than 25 years. Another chinchilla breeding colony has been in existence at Washington University School of Medicine (WU) for 11 years. Because accurate birth records have been kept at both institutions, it was possible to obtain for histological examination a large number of chinchilla cochleas from animals of known ages. These specimens were examined in order to identify the histological correlates of aging in the chinchilla inner ear.

Materials and Methods

Subjects

Eighty chinchillas, ranging in age from premature to 19.2 years, with no known history of exposure to noise or ototoxic drugs were included in this study. These animals were processed over a period of 18 years from 1971 to 1989. For summary purposes, the animals were divided into seven age groups: 1) less than 0.50 year ($N = 12$); 2) 0.51 to 1.50 years ($N = 12$); 3) 1.51–3.00 years ($N = 12$); 4) 3.01–5.00 years ($N = 11$); 5) 5.01–8.00 years ($N = 12$); 6) 8.01–11.50 years ($N = 12$); 7) 11.51–19.20 years ($N = 9$).

The chinchillas were obtained from the sound-treated animal facilities at CID ($N = 31$) or at WU ($N = 20$) or from commercial chinchilla farms (COM) near St. Louis ($N = 29$). Most of these animals ($N = 58$) had been used as controls for noise or radiation studies involving measures of anatomy and/or physiology and hence were healthy at the time of processing. The remaining 22 animals were moderately to severely ill with conditions which should not have produced any cochlear pathology. A common chronic condition was excessive weight loss secondary to the development of tooth spurs which interfered with chewing. A few animals had acute episodes of what appeared to be bacterial or viral septicemia. The cochleas from the ill chinchillas were fixed either shortly before or immediately after death. In eleven animals, both cochleas were available for determination of cochlear pathology whereas in the other 69 animals, only one cochlea was available. Thus, the histological data reported here are based on the findings in 91 cochleas.

Histological preparation

All cochleas were prepared similarly for examination with a phase contrast microscope (Wild M-20). The specimens were fixed with 1% osmium tetroxide (OsO_4) in Dalton's buffer, dehydrated and embedded in araldite, and dissected so as to obtain plastic-embedded flat preparations of the cochlear duct (Bohne, 1972).

Histological analysis

Using a dissection microscope (Wild M5A) with a camera lucida attachment, simplified drawings were made of the dissected pieces of organ of Corti (OC) at a magnification of 55 ×. The length of the OC (in millimeters) was measured along the line of junction between the inner (IP) and outer pillar (OP) heads using a graphics tablet-computer system (Hewlett-Packard 85).

The technique used to prepare the cochleas for microscopic examination resulted in the destruction of the bodies and central processes of the SGCs. However, some idea about the health status of the SGCs could be inferred from the condition of their myelinated dendritic processes within the osseous spiral lamina (OSL). Loss of these myelinated nerve fibers (MNFs) was determined by noting regions in the OSL which were lightly stained by the OsO_4 fixative. The percentage of missing fibers was estimated by comparing the staining in the damaged area to that at a comparable location in an undamaged specimen of a similar age. It was also noted whether or not the MNF degeneration was associated with a significant loss of inner (IHCs) and/or outer hair cells (OHCs).

Stria vascularis (SV) degeneration appears as lightly stained or unstained areas along the lateral wall of the cochlear duct (Fried et al., 1976). Once identified, the length (in millimeters) of the degenerated region was measured and its location relative to percentage distance in the OC was determined (Bohne et al., 1985).

For each ear in this study, the loss of sensory cells was determined by counting the number of phalangeal scars which replaced the degenerated cells. A few sensory cells with normal or near normal apices, including stereocilia, had no plasma membrane below the reticular lamina and had either an enlarged pale-staining nucleus or a pyknotic one. These cells were judged to be in the process of degenerating and were included in the counts of missing cells (Bohne, 1976). The numbers of missing IPs and OPs were also counted. The percentages of missing IHCs and OHCs were calculated as previously described (Bohne et al., 1986). All specimens were inspected for focal losses of sensory cells. Based on our previous studies of noise-damaged ears, damage in the chinchilla OC may appear as follows:

1. Low-frequency lesions (LFLs) are defined as regions in the apical half (0–50%) of the OC in which IHC and/or OHC loss is equal to or greater than 50% over a distance of at least 0.03 mm or three IHCs (Bohne, unpublished data).

2. High-frequency lesions (HFLs) have a similar definition to LFLs except they are located in the basal half (50–100%) of the OC (Bohne and Clark, 1982).

The LFLs and HFLs were classified according to which hair cell(s) were missing in an amount equal to or greater than 50% (i.e., IHC LFL/HFL, OHC LFL/HFL or combined LFL/HFL) (Bohne et al., 1987).

3. 'OC wipeout' refers to regions anywhere along the basilar membrane in which 100% of the sensory and supporting cells have degenerated and the missing portion of the OC has been replaced by squamous epithelium (Bohne and Clark, 1982).

In addition to obtaining the quantitative data, the remaining sensory and supporting cells were examined in detail in order to identify alterations which may be indicative of impaired function or impending degeneration.

For animals in which both ears were available for analysis, the data from the two ears were averaged so that the graphs of sensory and supporting cell loss and the calculations of regional loss of hair cells as a function of age have only one entry per animal. This procedure was utilized in order to avoid biasing the results. Excellent correlation has been found with respect to sensory cell loss in the right and left ears of binaural animals which were used as aging controls or those which had been exposed to noise or ionizing radiation (Bohne et al., 1986). Thus, using data from both cochleas would increase the sample size without changing the interanimal variance. The statistical analyses of the percentage of missing hair cells and the incidence of LFLs, HFLs, SV degeneration and primary MNF degeneration are based on the number of involved animals rather than the number of ears. However, the tables of SV and primary neural degeneration include data from both ears of a particular animal, when available.

Results

In the 80 chinchillas, histological evidence was found for three of the four forms of presbycusis described by Schuknecht (1974). All chinchillas exhibited an age dependent loss of sensory cells (i.e. sensory presbycusis). Six chinchillas (7.5%) had areas of SV degeneration (i.e., strial presbycusis). Four animals (5%) had regions of degeneration of the MNFs within the OSL which were not associated with a significant loss of sensory cells (i.e., probably primary neural degeneration or neural presbycusis). The fourth form of presbycusis, termed cochlear conductive or mechanical, is speculative. It may be the result of atrophic changes in the spiral ligament, stiffening of the basilar membrane or a combination of changes which would alter the vibratory characteristics of the basilar membrane (Schuknecht, 1974; Nadol, 1980). These latter alterations cannot be identified with certainty in plastic-embedded, flat preparations of the cochlear duct.

Losses of sensory cells

In the premature chinchilla and three newborns, the total number of missing OHCs was 9, 21, 21 and 25, respectively. In all of Group 1 (0–0.5 years), the total number of missing OHCs ranged from 1 to 38 and averaged 21 (0.3%). With advancing age, the number of degenerated OHCs continued to increase, ranging from 584 to 2409 and averaging 1202 (16.5%) in Group 7 (11.5–19.2 years). Although some loss of OHCs occurred in all three rows, it was greatest in the third row for 65% of the ears; the first row for 18%; the second row for 8%; and equally large in two or more rows in 9% of the ears.

There were no missing IHCs in the four chinchillas which were less than one day old. In all of Group 1, the number of missing IHCs ranged from 0 to 5 and averaged one (0.08%). In Group 7, the number of missing IHCs ranged from 31 to 199 and averaged 88 (4.8%).

Graphs depicting the total percentage of missing OHCs and IHCs for individual chinchillas are presented in Figs. 1 and 2, respectively. Different symbols were used to illustrate the different sources of animals and their health status.

Linear, exponential, log and power mathematical models were fitted to the data. The best fits for

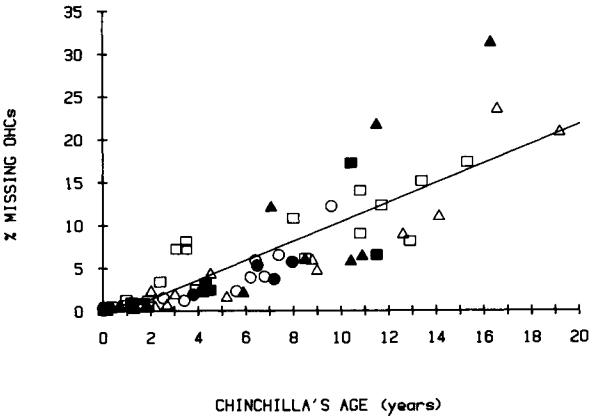

Fig. 1. Scatter plot of total percentage of missing OHCs as a function of age. Line is linear regression fit to data ($r_{xy} = 0.87$). Circles = WU-bred animals; triangles = CID-bred animals; squares = commercial-bred animals; open symbols = healthy animals; solid symbols = ill animals.

OHC and IHC loss with age (as indicated by the correlation coefficients) were obtained with a linear model and a power model, respectively. These equations are given below along with the correlation coefficients.

$$\text{Total \% of missing OHCs} = 1.13 \times \text{age (years)} - 0.878 \quad (r_{xy} = 0.87)$$

$$\text{Total \% of missing IHCs} = 0.29 \times \text{age (years)}^{0.96} \quad (r_{xy} = 0.92)$$

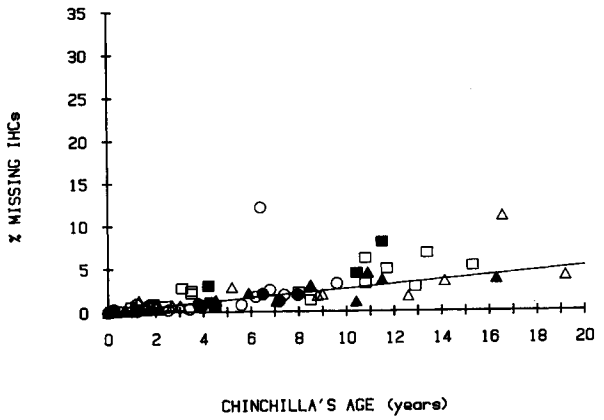

Fig. 2. Scatter plot of total percentage of missing IHCs as a function of age. Line is power curve fit to data ($r_{xy} = 0.92$). Symbols are the same as in Fig. 1.

Based on these equations, 'spontaneous' loss of OHCs occurs at a rate of about 1% per year or one cell every five days; loss of IHCs occurs at a rate of about 0.29% per year or one cell every 66 days.

For the seven age groups, the mean OHC and IHC loss ± one standard deviation (SD) in the whole OC and in four OC regions are listed in Table I. The mean losses of OHCs and IHCs clearly demonstrate the effect of aging. The increasing variance with advancing age indicates that susceptibility to aging was quite variable across animals. Examination of the losses in individual healthy and ill animals (Figs. 1 and 2) indicates that this variability was not related to the source of the animal or its health status. More ill animals had losses which are below the regression line than above the line. In addition, in a number of older animals, the loss of one type of hair cell was above the regression line while the loss of the other type was below the line. Thus, it is concluded that the variability in hair cell loss with age was mainly the result of biological variability.

In order to determine if there was a difference in susceptibility to aging in distinct portions of the OC, the percentages of missing OHCs and IHCs were calculated in 20.9% of the OC (approximately 3.8 mm) in the apex, base, centered at 2.86 kHz and centered at 5.7 kHz. These latter two regions were examined because they have been shown to be areas of maximum auditory sensitivity for the chinchilla by Miller (1970) and Clark (unpublished data), respectively. Regional comparisons of sensory cell loss were made in the different age groups using the Student's *t*-test (2-tailed) (Table I). For OHCs: apical loss was significantly different from that in the 2.86-kHz region and/or the 5.7-kHz region in four of the five youngest age groups (1–5) but was not different in Groups 6–7; basal loss was significantly different from that in the middle of the OC (i.e, 2.86 and/or 5.7 kHz) only in Groups 5–7; in only two

TABLE I

AVERAGE HAIR CELL LOSS IN WHOLE OC AND IN FOUR REGIONS AS A FUNCTION OF AGE

OHC Group	Whole OC	Apex [a]	2.86 kHz [b]	5.7 kHz [c]	Base [d]	Sig Dif [e]
1	0.3±0.16	0.5± 0.45	0.1±0.17	0.1± 0.07	0.2± 0.43	ab
2	0.7±0.24	1.4± 1.07	0.5±0.21	0.4± 0.24	0.5± 0.33	abc
3	1.2±0.85	1.1± 0.52	0.7±0.60	1.2± 1.96	1.7± 2.69	
4	3.8±2.38	5.9± 3.94	2.6±1.84	2.3± 1.86	3.1± 2.31	abc
5	5.4±3.13	8.5± 7.86	3.4±2.64	3.1± 1.60	5.7± 3.47	bde
6	9.7±5.22	13.4±11.56	6.9±4.78	6.6± 4.59	8.5± 4.44	e
7	16.5±7.21	17.5± 5.70	11.7±6.68	14.6±10.43	25.2±15.70	de
IHC Group	**Whole OC**	**Apex [a]**	**2.86 kHz [b]**	**5.7 kHz [c]**	**Base [d]**	**Sig Dif [e]**
1	0.1±0.09	0.0± 0.14	0.0±0.11	0.0± 0.08	0.1± 0.23	
2	0.4±0.35	0.5± 0.44	0.3±0.35	0.2± 0.15	0.3± 0.43	b
3	0.5±0.22	0.5± 0.53	0.4±0.43	0.7± 0.53	0.5± 0.59	f
4	1.5±0.88	2.7± 3.28	1.2±1.21	1.3± 1.17	1.1± 0.75	
5	2.7±2.89	5.0± 9.36	1.4±1.00	1.5± 1.06	1.8± 1.05	
6	3.5±1.94	4.7± 4.01	2.7±2.58	3.9± 5.02	3.4± 2.42	
7	4.8±2.57	5.9± 5.14	3.4±2.66	4.3± 2.08	6.5± 2.87	de

[a] 1.0–21.9% distance from apex.

[b] Area centered around 2.86 kHz (50.95–71.85% distance from apex).

[c] Area centered around 5.7 kHz (64.45–85.35% distance from apex).

[d] 79.1–100% distance from apex.

[e] *t*-tests (2-tailed) which are significant at 0.05 level: a = Apex verses 2.86 kHz; b = Apex verses 5.7 kHz; c = Apex verses base; d = Base verses 2.86 kHz; e = Base verses 5.7 kHz; f = 2.86 kHz verses 5.7 kHz.

of the seven groups were apical and basal losses significantly different; there was no significant difference between cell loss in the two regions in the middle of the OC. For IHCs: in Groups 1–6, there was no general trend for any region to have losses which were significantly different from the rest; in Group 7, basal loss was significantly different from that in the 2.86-kHz and 5.7-kHz regions.

LFLs, HFLs and OC wipeouts

Thirty-eight of the 80 chinchillas (47.5%) had focal loss of sensory cells at one or more locations within the OC. In the 91 cochleas in this study, there were 104 LFLs [95 (91%) - IHC; 6 (6%) - OHC; 3 (3%) - combined], 67 HFLs [41 (61%) - IHC; 14 (21%) - combined; 12 (18%) - OHC] and one OC wipeout. The high percentages of IHC LFLs/HFLs were unexpected in view of the finding that the percentage of missing IHCs exceeded that of OHCs in only seven animals.

There was a low incidence of LFLs and HFLs in animals below three years of age. The incidence of LFLs was significantly increased in Groups 4–7 over that in Groups 1–3. However, among Groups 4–7, the incidence of LFLs was not well correlated with age (Table II). The average size of the LFLs varied from 0.05 ± 0.02 mm to 0.15 ± 0.28 mm across age groups (Table III) and also was not well correlated with advancing age.

The incidence of HFLs was well correlated with advancing age, increasing from 27.3% in Group 4 to 77.8% in Group 7 (Table II). The average size of the HFLs varied from 0.04 ± 0.01 mm to 0.22 ± 0.46 mm in the different groups (Table III).

TABLE II

INCIDENCE OF LOW- AND HIGH-FREQUENCY LESIONS IN AGING CHINCHILLAS

Group	Age (years)	No. Animals	% with LFLs	% with HFLs
1	0 - 0.5	12	8.3	0
2	0.51- 1.5	12	0	8.3
3	1.51- 3.0	12	16.7	0
4	3.01- 5.0	11	63.6	27.3
5	5.01- 8.0	12	33.3	50.0
6	8.01-11.5	12	75.0	50.0
7	11.51-19.2	9	55.6	77.8

TABLE III

SIZE OF LFLs AND HFLs IN AGING CHINCHILLAS

Group	Age (years)	No. LFLs	Size (mm) [a]	No. HFLs	Size (mm) [a]
1	0 - 0.5	1	0.05	0	-
2	0.51- 1.5	0	-	3	0.04 ± 0.01
3	1.51- 3.0	2	0.05 ± 0.02	0	-
4	3.01- 5.0	15	0.06 ± 0.05	6	0.07 ± 0.02
5	5.01- 8.0	31	0.12 ± 0.21	6	0.16 ± 0.15
6	8.01-11.5	23	0.15 ± 0.28	17	0.08 ± 0.05
7	11.51-19.2	32	0.06 ± 0.05	35	0.22 ± 0.46

[a] Mean ± 1 SD.

Data from Bredberg (1968), Schuknecht (1974) and Johnsson and Hawkins (1972a) indicate that the basal tip of the human OC is especially vulnerable to degeneration. To determine if the same phenomenon exists in chinchillas, the number of ears with HFLs extending to the basal tip were counted and the average size of their lesions was determined. In Groups 1–4, only two of 47 animals (4%) had basal-tip HFLs which were both 0.06 mm in length. In Groups 5–6, three of 24 animals (12.5%) had basal-tip HFLs which averaged 0.25 ± 0.16 mm in length. In Group 7, four of nine animals (44%) had basal-tip HFLs which averaged 1.22 ± 0.79 mm in length. The cytocochleogram from the ear with the largest basal-tip lesion is shown in Fig. 3.

Among the 80 chinchillas, there was only one OC wipeout; a 13.4-year-old had a 0.09-mm OC wipeout within a 0.19-mm combined HFL which was located between 82.2 and 82.6%.

Sensory cell alterations other than loss

Except for the accumulation of lipofuscin (see section below), the majority of the remaining hair cells had normal appearances. Scattered IHCs and OHCs had disarrayed, absent or fused stereocilia; others had slightly shrunken, distorted or swollen bodies. These abnormal sensory cells occurred infrequently in young animals, somewhat more frequently in old animals, but were never very common.

Losses of supporting cells

Losses of OPs were rare below six weeks of age: one of eight animals was missing one OP.

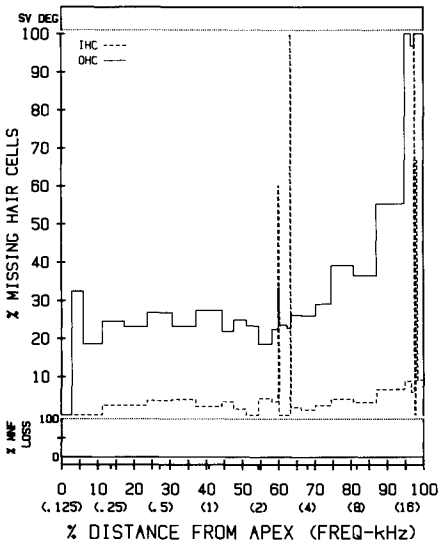

Fig. 3. Cytocochleogram of right ear of a 16.3-year-old chinchilla. This ear had three high-frequency lesions (HFLs): two IHC HFLs in the upper first turn which were 0.06 and 0.03 mm in extent, and a 2.55-mm combined HFL at the basal tip of the OC. The total percentages of missing OHCs and IHCs were 31.4% and 3.7%, respectively.

Likewise, loss of IPs was uncommon below three months of age: two of ten animals were missing one IP. In Group 1, the numbers of missing IPs and OPs ranged from 0 to 3 and 0 to 4 and averaged 0.4 and 0.8, respectively. For Group 7, the numbers of missing IPs and OPs ranged from 2 to 39 and 3 to 74 and averaged 14 and 29, respectively.

The total number of missing OPs and IPs for individual chinchillas is shown graphically in Figs. 4 and 5, respectively. The data show that there was no systematic relation between the source or the health status of the animal and its number of missing pillars.

Linear, exponential, log and power mathematical models were fitted to the data. The best fits for OP and IP loss with age were obtained with linear models. These equations are given below along with the correlation coefficients.

Total number of missing OPs

$$= 1.902 \times \text{age (years)} - 2.727 \quad (r_{xy} = 0.76)$$

Total number of missing IPs

$$= 0.938 \times \text{age (years)} - 0.824 \quad (r_{xy} = 0.70)$$

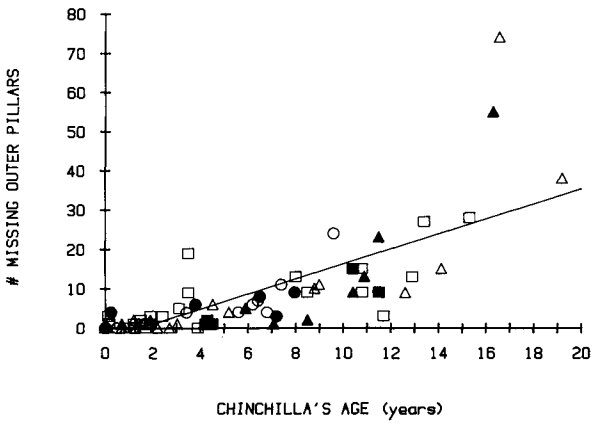

Fig. 4. Scatter plot of total number of missing OPs as a function of age. Line is linear regression fit to data ($r_{xy} = 0.76$). Circles = WU-bred animals; triangles = CID-bred animals; squares = commercial-bred animals; open symbols = healthy animals; solid symbols = ill animals.

Supporting cell alterations other than loss

In addition to the accumulation of lipofuscin (see section below), the other common alteration was the partial loss of cuticular-plate substance in the OP and IP heads and less frequently, in their bases. Affected pillar heads had a 'moth-eaten' appearance. This alteration was rarely found in young animals but was fairly common in old animals. A much less frequent alteration was the migration of the OP and IP nuclei from their normal position in the bases of the cells to the area immediately beneath their heads. Migration

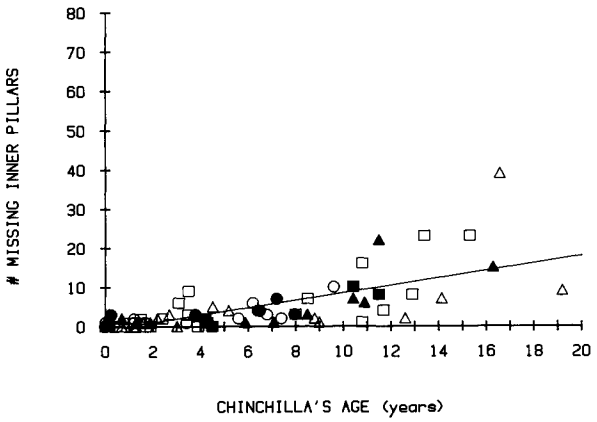

Fig. 5. Scatter plot of total number of missing IPs as a function of age. Line is linear regression fit to data ($r_{xy} = 0.70$). Symbols are the same as in Fig. 4.

of the pillar nuclei did not seem to increase in frequency with age.

Accumulation of lipofuscin

In addition to the loss of sensory and supporting cells, another hallmark of aging was the accumulation of lipofuscin or age pigment. This pigment appeared as deep brown deposits which occasionally contained large, pale spots. The deposits ranged in size from fine to large and in number from few to numerous. With advancing age, the deposits accumulated in the OHCs as well as many of the supporting cells, including IPs, OPs, Deiters', Hensen's, inner border, inner phalangeal, Claudius', inner sulcus and the epithelial cells of Reissner's membrane. Very little accumulation of lipofuscin was noted in the IHCs.

Chinchillas less than one year of age had little or no age pigment. When the pigment began to accumulate, it appeared as a few small deposits in the subcuticular region of the OHCs throughout the OC. With advancing age, the deposits became larger, more numerous and extended deeper into the cytoplasm of the OHCs. Lipofuscin also began to appear in the different supporting cells. Quantitative data on the accumulation of lipofuscin were not collected. However, inspection of the ears revealed that the size and quantity of the lipofuscin deposits were well correlated with the age of the animal.

Degeneration of the stria vascularis

Six of 44 chinchillas (13.6%) over three years of age had a total of 11 regions of SV degeneration. In the different ears, the total extent of the SV lesions ranged from 0.30 mm to 7.73 mm and averaged 3.13 ± 2.82 mm. There was no preferential location within the cochlear duct for the SV degeneration (Table IV) or for its proximity to focal OC lesions. One SV lesion was adjacent to an OHC LFL and another was adjacent to two IHC HFLs. However, in most instances, the SV lesions were adjacent to portions of the OC which had only scattered loss of sensory cells. The cytocochleogram from the ear with the largest amount of SV degeneration is presented in Fig. 6.

The regions of SV degeneration had a histological appearance which was similar to that seen in noise-damaged chinchilla cochleas (Fried et al., 1976). The SV capillaries along with the marginal, intermediate and basal cells had degenerated. Only an undifferentiated, squamous to cuboidal epithelium remained separating the spiral ligament from the endolymphatic space. In some of the

TABLE IV

SIZE AND LOCATION OF STRIA VASCULARIS DEGENERATION IN AGING CHINCHILLAS

Ear No.	Age (years)	Source	Size (mm)	% Location
616R	3.1	COM	0.70	22.7–25.0
			0.35	64.5–65.8
616L	3.1	COM	1.20	78.7–83.8
540L	3.5	COM	3.85	1.3–16.7
			2.10	36.1–43.8
			1.06	75.2–79.7
			0.72	83.1–86.0
728R	7.1	CID	1.70	3.2– 9.2
520R	8.5	CID	6.33	3.9–29.1
760L	16.6	CID	1.62	55.8–62.4
663R	19.2	CID	0.30	2.7– 3.9

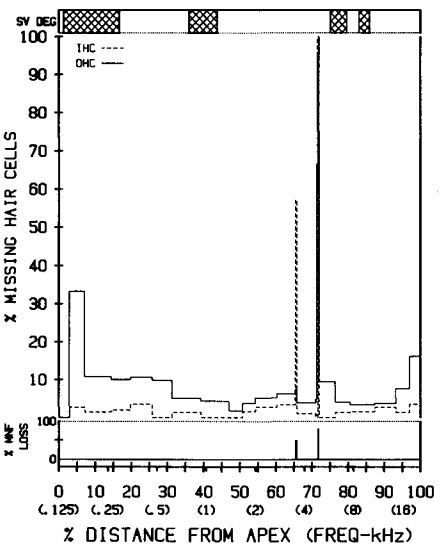

Fig. 6. Cytocochleogram of the left ear of a 3.5-year-old chinchilla. In the middle first turn, there was a 0.08-mm IHC high-frequency lesion (HFL) and a 0.11-mm combined HFL, both with associated secondary degeneration of myelinated nerve fibers (MNFs). This ear also had four regions of degeneration of the stria vascularis (SV), totaling 7.73 mm in extent. The location of degenerated SV (cross-hatched shading at top of graph) was plotted relative to location in OC. The total percentages of missing OHCs and IHCs were 8.1% and 2.1%, respectively.

TABLE V

SIZE AND LOCATION OF PRIMARY NEURAL DEGENERATION IN AGING CHINCHILLAS

Ear No.	Age (years)	Source	Size (mm)	% Location	% Loss [a]
401L	5.2	CID	0.27	0- 1.4	80
520R	8.5	CID	2.89	0–15.3	65–93
97R	9.0	CID	9.81	0–53.0	50–95
97L	9.0	CID	10.12	0–55.0	15–98
345R	11.5	COM	0.71	0- 3.7	20–85

[a] Estimated percentage of degenerated MNFs.

cochleas with degenerated SV, a large amount of cellular debris was seen in scala media adjacent to or just apical to the region of SV degeneration. In other cases no or very little debris could be located in the cochlear duct.

Degeneration of myelinated nerve fibers

At the apical and basal tips of the OC, the density of MNFs in the OSL was found to be somewhat variable. Some chinchillas had uniformly dense innervation while others had thready or sparse innervation. No relation was found between the age of an animal and its density of innervation. Thus, slight differences in innervation density at the apical and basal tips of the OC were considered an anatomical variation rather than a pathological alteration.

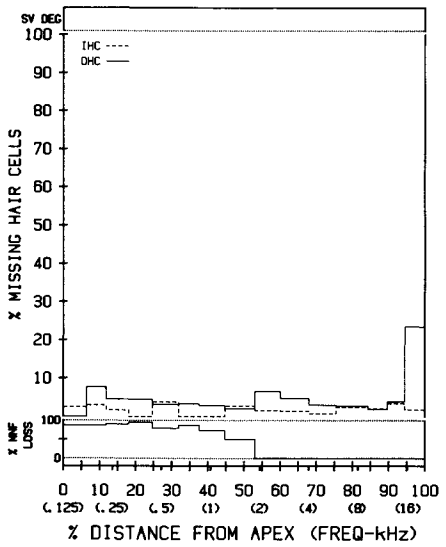

Fig. 7. Cytocochleogram of right ear of a 9-year-old chinchilla showing severe degeneration of myelinated nerve fibers (MNFs) over 9.81 mm in the apical half of cochlea. The location of MNF degeneration was plotted relative to location in OC. The total percentages of missing OHCs and IHCs were 4.6% and 1.8%, respectively.

The four animals with primary MNF degeneration were more than five years of age. The length of the individual lesions ranged from 0.27 mm to 10.12 mm and averaged 3.46 ± 3.89 mm (Table V). The cytocochleogram from the ear with the largest MNF lesion is shown in Fig. 7.

Secondary MNF degeneration (Fig. 6) was found in 19 of the 80 chinchillas (24%). It occurred in association with 15 of 104 LFLs (12 - IHC; 2 - OHC; 1 - combined) and 24 of 67 HFLs (14 - IHC; 9 - combined; 1 - OHC).

Vascular abnormalities

Except for the loss of strial capillaries in the regions of SV degeneration, no vascular abnormalities were apparent in any chinchilla cochlea. The vessels below the basilar membrane had the same dimensions, degree of filling etc. in the animals in Group 7 as those in Group 1. The intravascular strands and avascular channels which have been seen in the vessels of the spiral ligament of aging humans (Johnsson and Hawkins, 1972) and the micro-aneurysms of the SV vessels (Johnsson, 1973) were not seen in our chinchillas.

Discussion

A number of morphological changes have been described in the inner ear of the aging mouse (Henry and Chole, 1980), rat (Keithley and Feldman, 1982; Feldman, 1989), gerbil (Adams et al., 1989; Schulte and Adams, 1989), guinea pig (Covell and Rogers, 1957; Ulehlová, 1975; Coleman, 1976), chinchilla (Bhattacharyya and Dayal, 1985), rabbit (Bhattacharyya and Dayal, 1989); cat (Schuknecht, 1956), squirrel monkey (Dayal and Bhattacharyya, 1986), rhesus monkey (Hawkins et al., 1985) and human (e.g., Covell, 1952; Ishii et al., 1967; Bredberg, 1968; Krmpotić-Nemanić et al., 1972; Johnsson and Hawkins, 1972a,b; Johnsson, 1973; Schuknecht, 1974, 1989; Nadol, 1980). All species exhibited an age-dependent loss of IHCs and OHCs, with OHC loss exceeding that of IHCs. Some species (rat, gerbil,

guinea pig, chinchilla, rabbit, squirrel monkey, human) had increased loss of sensory cells at the apex and some species (mouse, rat, cat, rhesus monkey, human) had increased loss at the base.

Other age-related changes noted in the studies cited above were found in a smaller percentage of ears from these different species. These changes included alterations and/or loss of supporting cells of the OC [guinea pig (Covell and Rogers, 1957), cat, human (Bredberg, 1968; Johnsson and Hawkins, 1972a; Schuknecht, 1974; Nadol, 1980)]; loss of spiral ganglion cells or their peripheral processes in the OSL [rat (Keithley and Feldman, 1982; Feldman, 1989), guinea pig (Covell and Rogers, 1957), cat, rhesus monkey, human (Bredberg, 1968; Krmpotic-Nemanic et al., 1972; Johnsson and Hawkins, 1972a; Schuknecht, 1974 and 1989; Nadol, 1980)]; new bone formation in the fundus of the modiolus [rat (Feldman, 1989), human (Covell, 1952; Krmpotić-Nemanić et al., 1972)] or Rosenthal's canal [guinea pig (Covell and Rogers, 1957)]; accumulation of age pigment or lipofuscin in the epithelial cells lining the cochlear duct [rat (Feldman, 1989), guinea pig (Covell and Roger, 1957), rhesus monkey, human (Ishii et al., 1967; Nadol, 1980)]; rarefaction, atrophy or cystic degeneration of the spiral ligament [rat (Feldman, 1989), rhesus monkey, human (Schuknecht, 1974; Nadol, 1980)]; loss of mesothelial cells on the scala tympani side of the basilar membrane [guinea pig (Covell and Rogers, 1957)]; atrophy or degeneration of the stria vascularis [gerbil (Schulte and Adams, 1989), human (Johnsson and Hawkins, 1972b; Schuknecht, 1974, 1989; Nadol, 1980)].

As can be seen by this brief literature review, some age-related inner ear changes such as the loss of IHCs and OHCs are common to all species whereas others appear to be limited to only a few species. Some of the interspecies aging differences may be due to the fact that most animals used in these studies are short-lived. They may be dying before their ears have had sufficient time to age. This notion is supported by the recent finding of Feldman (1989) that the cochleas of rats with extended lifespans have an increased severity of aging changes compared to those with normal lifespans. It is also likely that some of the perceived interspecies aging differences are not real but are the result of deficiencies in experimental design. In some of the studies just cited, the sample size was too small to identify the changes which have a low incidence in the population (e.g., neural presbycusis). Some studies were designed to address only one or two aspects of aging such as the rate of loss of IHCs and OHCs. Finally, in some studies, animals with ages covering the full age spectrum of the species were not examined.

The present study was designed to overcome some of the limitations of the previous aging studies. A large number of chinchillas ($N = 80$) covering a wide age range ($>$ 19 years) were examined so that the probability of detecting changes with a low incidence in the population would be increased. During the evaluations, special attention was paid to those structures which show significant alterations in aging human temporal bones.

The loss of OHCs found in our chinchillas was somewhat less than that reported by Bhattacharyya and Dayal (1985) in 24 chinchillas ranging in age from one month to four years. In their 4-year-old animals, OHC and IHC losses averaged 7% and 1%, respectively. Our 4-year-old animals averaged 3.6% OHC loss and 1% IHC loss. We have no explanation for the discrepancy in the OHC data.

Although anatomical differences exist between the human cochlea (Bredberg, 1968) and chinchilla cochlea (Bohne et al., 1986) with respect to length of the OC and density of the sensory cells, there are a number of similarities. In both humans (Bredberg, 1968; Johnsson and Hawkins, 1972a) and chinchillas, the apical and basal tips of the OC have a somewhat irregular pattern of OHCs and a thinning of MNFs in the OSL. The variation in innervation density makes it difficult to identify beginning primary neuronal degeneration.

With respect to the morphological correlates of aging, there are many similarities between the human ear and the chinchilla ear. Histological evidence of sensory presbycusis is found in most human (Bredberg, 1968; Schuknecht, 1974) and chinchilla cochleas. However, the total percentage of missing sensory cells is less in old chinchillas than in old humans. Bredberg (1968) found that the total numbers of OHCs and IHCs were reduced by more than 50% and 25%, respectively, in humans beyond the eighth decade. Chinchillas in

their last few years of life average about 20% loss of OHCs and 7% loss of IHCs. Calculations from Bredberg's data indicate that the rate of 'spontaneous' loss of OHCs is approximately one cell every four days whereas IHCs are lost at a rate of about one cell every 24 days. In the chinchilla, the rate of 'spontaneous' loss of OHCs is about the same as in humans (i.e., 1 per 5 days) while the rate of loss of IHCs is about 2.5 times slower (i.e., 1 per 66 days).

In aging humans, the loss of sensory cells may be found in any turn of the cochlea but it is often greatest at the basal and apical ends of the OC (Bredberg, 1968; Schuknecht, 1974). Johnsson and Hawkins (1972a) reported that almost all humans have complete degeneration of both sensory cells and nerve fibers at the basal tip of the OC. This pattern of damage begins in childhood and slowly progresses apically with advancing age. Basal-tip damage is uncommon in chinchillas under five years of age; only two of 47 animals (4%) had significant sensory cell loss at the basal tip. On the other hand, seven of 33 animals (21%) five or more years of age had significant basal-tip damage. In contrast to humans, none of the 80 chinchillas had an OC wipeout at the basal tip.

Approximately 33% of the adult human cochleas examined by Bredberg (1968) had 'circumscribed loss' of sensory cells in areas other than the apical and basal tip of the OC. Based on Bredberg's description and published photomicrographs, his circumscribed lesions are similar to the LFLs and HFLs found in all regions of the OC in 47.5% of our aging chinchillas. Regions of total loss of the OC are occasionally found in aging humans (Bredberg, 1968; Johnsson and Hawkins, 1972a) but were found in only one of our aging chinchillas.

Comparison of the quantitative data from humans without a history of noise exposure or ear disease to our chinchilla data clearly demonstrates that the total amount of hair cell loss in aged humans is greater than that in chinchillas, although the patterns of damage are similar. These results may well indicate that the damage found in human ears is the result of aging plus exposure to one or more ototraumatic agents.

The incidence of neural presbycusis is fairly low in both humans and chinchillas, averaging 4% for a sample of 150 human temporal bones (Johnsson and Hawkins, 1972a) and 5% for the 80 chinchillas in the present study. In humans, primary MNF degeneration is often patchy and may be found in all cochlear turns (Schuknecht, 1989) while in chinchillas, it was confined to a variable extent of the OSL at the apical end of the cochlea.

Inner ear lesions, especially in aging cochleas, are usually bilaterally symmetrical in humans (Nadol, 1980) and chinchillas (Bohne et al., 1986). Also, the different types of presbycusis rarely are found in pure form in either humans (Johnsson and Hawkins, 1972a) or chinchillas.

The histological appearance of severely degenerated portions of the SV is quite similar in humans (Johnsson and Hawkins, 1972b) and chinchillas. All human temporal bones studied by Johnsson and Hawkins (1972b) that were more than 45–50 years of age were found to have some degree of SV degeneration in the hook, middle and apical turns. On the other hand, only 7.5% of the aging chinchillas had regions of SV degeneration.

In the chinchilla, the incidence of focal lesions (i.e., LFLs and HFLs) within the OC increased with age, especially beyond three years. The largest percentage of these lesions were IHC LFLs (91%) and IHC HFLs (61%). These data contrast sharply with the findings in noise-damaged chinchillas in which most of the focal OC lesions were OHC LFLs/HFLs or combined LFLs/HFLs (Bohne et al., 1987; Bohne, unpublished data) and suggest that the losses of sensory cells in our aging chinchillas were not the result of micro-noise trauma. The analysis of regional hair cell loss (Table I) provides additional evidence that micro-noise trauma is not the etiology of the sensory cell loss in these animals. The losses of sensory cells in the areas of maximum auditory sensitivity for the chinchilla (i.e., 2.86 and 5.7 kHz) lagged behind those in the apex and base. Additional work is currently in progress to examine the role of micro-noise trauma in the etiology of hair cell loss in the aging chinchilla ear.

Conclusions

- With respect to experimental studies involving chinchillas, the data presented here can be

used as a baseline for determining the approximate amount of sensory and supporting cell loss which should be attributed to aging in a particular experimental chinchilla.

- The cell loss which was found in chinchillas obtained from commercial furriers was similar to that found in animals born and raised in the sound-treated animal facilities at CID or WU. Thus, it is concluded that retired breeding stock from commercial chinchilla farms can provide a ready supply of elderly chinchillas for future aging studies.
- The only ototraumatic agents that our chinchillas were exposed to during their lives were animal sounds and the noise associated with the cleaning of their quarters. However, since the pattern of focal OC damage in our noise-exposed chinchillas differs markedly from that in our aging chinchillas, it is likely that the damage described in the present study represents pure presbycusis.
- The morphological changes found in the inner ears of the aging chinchillas were similar to those seen in the temporal bones of aged humans. However, the extent of cochlear damage in the old chinchillas was less than that in old humans. These results suggest that some of the damage found in the aging human cochleas may be the result of aging plus exposure to one or more ototraumatic agents.

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References

Adams, J.C., Tarnowski, B.I., Heape, S., Hellstrom, L.I. and Schmiedt, R.A. (1989) Cochlear histopathology and hearing loss in quiet reared aged gerbils. Abstr. Assoc. Res. Otolaryngol. pp. 46–47.

Bhattacharyya, T.K. and Dayal, V.S. (1985) Age-related cochlear hair cell loss in the chinchilla. Ann. Otol. Rhinol. Laryngol. 94, 75–80.

Bhattacharyya, T.K. and Dayal, V.S. (1989) Influence of age on hair cell loss in the rabbit cochlea. Hear. Res. 40, 179–184.

Bohne, B.A. (1972) Location of small cochlear lesions by phase contrast microscopy prior to thin sectioning. Laryngoscope 82, 1–16.

Bohne, B.A. (1976) Safe level for noise exposure? Ann. Otol. Rhinol. Laryngol. 85:711–724.

Bohne, B.A., Bozzay, D.G. and Harding, G.W. (1986) Interaural correlations in normal and traumatized cochleas: Length and sensory cell loss. J. Acoust. Soc. Am. 80, 1729–1736.

Bohne, B.A. and Clark, W.W. (1982) Growth of hearing loss and cochlear lesion with increasing duration of noise exposure. In: R.P. Hamernik, D. Henderson and R. Salvi (Eds.), New Perspectives on Noise-Induced Hearing Loss, Raven Press, New York, pp. 283–302.

Bohne, B.A., Marks, J.E., and Glasgow, G.P. (1985) Delayed effects of ionizing radiation on the ear. Laryngoscope 95, 818–828.

Bohne, B.A., Yohman, L. and Gruner, M.M. (1987) Cochlear damage following interrupted exposure to high-frequency noise. Hear. Res. 29, 251–264.

Bredberg, G. (1968) Cellular pattern and nerve supply of the human organ of Corti. Acta Otolaryngol. Suppl. 236, pp. 1–135.

Clark, W.W., Clark, C.S., Moody, D.B. and Stebbins, W.C. (1974) Noise-induced hearing loss in the chinchilla, as determined by a positive-reinforcement technique. J. Acoust. Soc. Am. 56, 1202–1209.

Coleman, J.W. (1976) Hair cell loss as a function of age in the normal cochlea of the guinea pig. Acta Otolaryngol. 82, 33–40.

Covell, W.P. (1952) Histological changes in the aging cochlea. J. Gerontol. 7, 173–177.

Covell, W.P. and Rogers, J.R. (1957) Pathological changes in the inner ears of senile guinea pigs. Laryngoscope 67, 118–129.

Dayal, V.S. and Bhattacharyya, T.K. (1986) Comparative study of age-related cochlear hair cell loss. Ann. Otol. Rhinol. Laryngol. 95, 510–513.

Feldman, M.L. (1989) Aging of the auditory system in normally aged rats and those with extended lifespans. Abstr. Inter. Conf. The Aging Ear, St. Louis, MO, May 31–June 3, 1989.

Fried, M.P., Dudek, S.E. and Bohne, B.A. (1976) Basal turn cochlear lesions following exposure to low-frequency noise. Trans. Am. Acad. Ophthalmol. Otolaryngol. 82, 285–298.

Gravendeel, D.W. and Plomp, R. (1960) Micro-noise trauma. Arch. Otol. 71, 556–663.

Hawkins, J.E. Jr., Miller, J.M., Rouse, R.C., Davis, J.A. and Rarey, K. (1985) Inner ear histopathology in aging rhesus monkeys (Macaca mulatta). In: R.T. Davis and C.W. Leathers (Eds.), Behavior and Pathology of Aging in Rhesus Monkeys. Alan R. Liss, Inc., New York, NY, pp. 137–154.

Henry, K.R. and Chole, R.A. (1980) Genotypic differences in behavioral, physiological and anatomical expressions of age-related hearing loss in the laboratory mouse. Audiology 19, 369–383.

Ishii, T., Murakami, Y., Kimura, R.S. and Balogh, K. Jr. (1967) Electron microscopic and histochemical identification of lipofuscin in the human inner ear. Acta Oto. Laryngol. 64, 17–29.

Johnsson, L.-G. (1973) Vascular pathology in the human inner ear. Adv. Oto. Rhino. Laryngol. 20, 197–220.

Johnsson, L.-G. and Hawkins, J.E., Jr. (1972a) Sensory and neural degeneration with aging, as seen in microdissections of the human inner ear. Ann. Otol. Rhinol. Laryngol. 81, 179–193.

Johnsson, L.-G. and Hawkins, J.E., Jr. (1972b) Vascular changes in the human inner ear associated with aging. Ann. Otol. Rhinol. Laryngol. 81, 364–376.

Keithley, E.M. and Feldman, M.L. (1982) Hair cell counts in an age-graded series of rat cochleas. Hear. Res. 8, 249–262.

Krmpotić-Nemanić, J., Nemanić, D. and Kostović, I. (1972) Macroscopical and microscopical changes in the bottom of the internal auditory meatus. Acta Otolaryngol. 73, 254–258.

Miller, J.D. (1970) Audibility curve of the chinchilla. J. Acoust. Soc. Am. 48, 513–523.

Mills, J.H., Schmiedt, R.A. and Kulish, L.F. (1989) Age-related hearing loss in quiet-reared Mongolian gerbils. Abstr. Assoc. Res. Otolaryngol., pp. 44.

Nadol, J.B. Jr. (1980) The aging peripheral hearing mechanism. In: D.S. Beasley and G.A. Davis (Eds.), Aging Communication Processes and Disorders, Grune and Stratton, Inc., Orlando, FL, pp. 63–85.

Schuknecht, H.F. (1956) Presbycusis. Laryngoscope 65, 402–419.

Schuknecht, H. (1974) Pathology of the Ear. Harvard University Press, Cambridge, MA, pp. 388–403.

Schuknecht, H.F. (1989) Pathology of presbycusis. In: J.C. Goldstein, H.K. Kashima and C.F. Koopmann, Jr. (Eds.), Geriatric Otorhinolaryngology, Am. Acad. Otolaryngol. Head Neck Surg., B.D. Decker, Inc., Toronto and Philadelphia, pp. 40–44.

Schulte, B.A. and Adams, J.C. (1989) Degeneration of the stria vascularis and stromal cells in the spiral ligament of aging gerbils as assessed by loss of Na^+, K^+-ATPase immunoreactivity. Abstr. Assoc. Res. Otolaryngol., p. 47.

Sivian, L.J. and White, S.D. (1933) On minimum audible sound fields. J. Acoust. Soc. Am. 5, 288–321.

Ulehlová, L. (1975) Ageing and the loss of auditory neuroepithelium in the guinea pig. In: V.J. Cristofalo and E. Holecková (Eds.), Advances in Experimental Medicine and Biology, Vol. 53, Cell impairment in aging and development, Plenum Press, NY, pp. 257–264.