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EXPERIMENTAL STUDIES OF VIBRATORY TRAUMA OF CORTI'S ORGAN. II. SCANNING ELECTRON MICROSCOPY

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Abstract. In the first part of this study, the authors presented the results of cochlear microphonics measurements in guinea pigs subjected to long-term whole-body vibration. In animals undergoing vibration over 30, 90, and 180 days, a statistically significant lowering of voltage over the range up to 2 kHz was seen. In accordance with cochlear-frequency position, this pointed to possible formation of sensory epithelium damages in the fourth and third turning.
In the present study an attempt was made to verify electrophysiological measurements with a scanning electron microscope. In all, 80 cochleae were examined, 20 in each group of animals: one control and three experimental groups. The healthy animals showed a correct picture of the sensory epithelium in each case, whereas its damage was ascertained in all the study groups. The damage advanced with a longer duration of the experiment and was most often seen in the outer hair cell region of the apex, where it gradually spread towards the base of the cochlea. The damage of cells decreased from the circumference to the modiolus, and the inner hair cells showed considerably greater resistance to vibration.
The results of the study demonstrate the harmful effect of mechanical vibration on the inner ear. According to the observed pattern of damage, one can expect an increasing hearing impairment in the low and medium frequency range in persons exposed to whole-body vibration.

Key words:
Whole-body vibration, Inner ear, Scanning Electron Microscopy, Guinea pig

INTRODUCTION

Since Dallos and Cheatham's experiments [1], it has been known that the source of cochlear microphonics (CM) are the outer hair cells (OHC), whose integrality is indispensable for the correct functioning of the inner ear [2]. According to Zenner and Plinkert [3], dislocations of the basilar membrane cause changes in the electric potential in the peri- and endolymph. The OHC are then in a variable electric field and react by contracting and producing their own biopotential. The frequencies of the tone and twitches of the basilar membrane should be identical with the frequency of the current. Simultaneously, the OHC twitches can interfere and strengthen significantly the influence of the traveling wave on the corresponding sections of the basilar membrane (cochlear amplifier). The immobile inner hair cells (IHC) are believed to be indeed the passive unit, which transforms mechanical movement to electric potential, releasing neurotransmitters. Widely accepted is the opinion that destruction of part of OHC leads to a lowering of hearing ability with changes located in a selected range and IHC (independent of the place of damage) at all the frequencies applied [4–8].
In the first part of this work [9] we presented the results of CM measurements on guinea pigs subjected to long-term, isolated whole-body vibration (vertical, sinusoidal shaking;

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10 Hz/5 mm/ 2g). In three groups of experimental animals, vibrated 5 h/day over 30, 90 and 180 days, we showed a statistically significant gradual lowering of CM voltage in the low and medium frequency range. According to the reception topography in the cochlea, the fall of CM over the range up to 2 kHz pointed to the possible formation of vibratory damage in its fourth and third turning [1,7,10].
The aim of the investigations was to determine the validity of electrophysiological measurements using morphological estimation of the state of the sensory inner ear epithelium.

MATERIALS AND METHODS

The study material comprised 80 cochleae (4 · 20), from animals of the control group (m0) and three experimental groups (m1, m3 and m6) subjected to vibration for 30, 90, and 180 days.
The sensory epithelia of the inner ears were prepared in the surviving animals. With a small hook and needle, the apex of the osseous cochlea was removed. The round window membrane was also perforated and perilymphatic perfusion, with a glass pipette (end section about 0.3 mm), for over 5 min was forced alternately into both openings. A 5% solution of glutaraldehyde in a phosphate buffer, pH 7.4, was used. The guinea pig was decapitated and the isolated temporal bone was at once dipped in a solution of aldehyde. Stapedectomy was carried out and the perilymphatic perfusion through the round and oval windows and the apex of the cochlea was repeated for another 5 min. Using a needle and hook, the bony capsule, stria vascularis, Reissner's membrane, and the tectorial membrane were removed. The specimen was fixed at 4°C for 48 h in a solution of glutaraldehyde. Later it was rinsed three times with a phosphate buffer and drained for 4 h each in alcoholic and then waterless acetone. Immediately after extraction from acetone, it was placed in the chamber of a critical point apparatus CPD 010 of the Balzer company. After drying in CO_2, the cochlea was mounted on a metal-notebook with double-sided sticking tape and covered with carbon, then with gold on a rotator table. Each specimen was examined and photographed in the scanning microscope DSM 950 (Zeiss) with an applied voltage of 20 kV.

RESULTS

Analysing the effect of whole-body vibration on Corti's organ we relied on an estimation of the state of the hair cells. Estimation was done each time following one-month rest after completing the experiment.

The control group

In all cases, the healthy control animals displayed a correct picture of the sensory epithelium of the inner ear. Only rare deviations from an ideal cochleogram were visible, but not judged as pathological (Fig. 1).

The experimental groups

In the experimental animals like in the control ones, single loss among correct hair cells or changes in their configuration could not be regarded as pathology. The vibration-related damage observed in all the experimental groups, varied in the frequency of occurrence (the number of damaged ears), range (the number of turnings of the cochlea), area (the number of cells), location (OHC 1, 2, 3 or IHC), and intensification (according to the scale of the morphological picture of a single cell damage) (Figs. 2 and 3).
In group m1, the changes were observed in nineteen cochleae, in groups m3 and m6, in all the twenty cochleae. The damage was seen most often in the neighborhood of

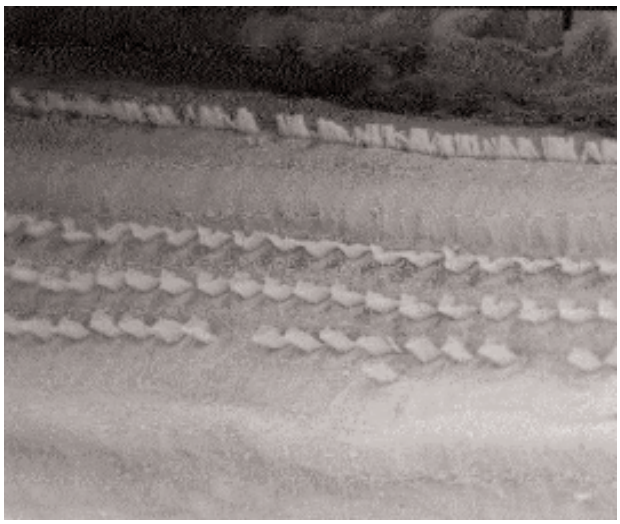

Fig. 1. Group m0, second turning of the cochlea. Two missing and one additional cell (OHC) are visible x 1200.

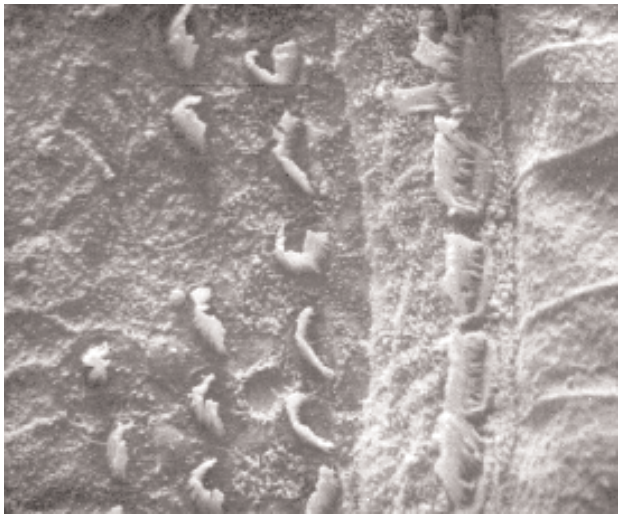

Fig. 2. Group m6, second turning of the cochlea. Lack of cells (OHC 1, 2, 3) and disarrangement of cilia x 3000.

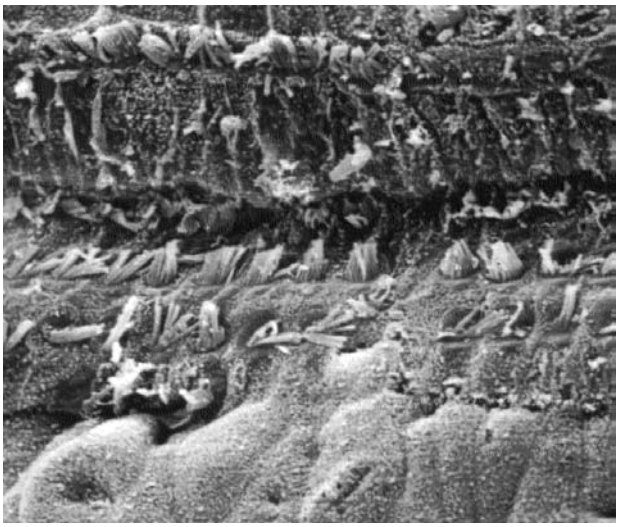

Fig. 3. Group m6, fourth turning of the cochlea. Loss and damage of outer and inner hair cells x 1250.

the apex, regardless of the duration of experiment. The extent of the damage grew towards the base with prolonged vibration exposure. In group m1, damage was found in the fourth or the fourth and third turnings, in group m3, in the fourth and third in all cases, and in group m6, it was found four times in the second turning of the cochlea. The highest susceptibility to vibratory trauma was found in OHC 3, followed by OHC 2 and OHC 1. The changes were diminishing from the circumference towards the modiolus. The IHC showed considerably greater resistance to vibration than the OHC. The IHC damage was more seldom, and its range and area were smaller. The animals of each experimental group frequently showed diverse changes as well. Cells which displayed the correct structure were mixed with others, presenting different forms of damage. The morphology of a single cell injury was also characterized by considerable variety, from light inclinations of the cilia to their entire lack. We observed all the well-known forms of the damage (Figs. 4–6).

To estimate the state of the sensory epithelium, the scale of three degrees was used: 1) fully correct ciliary appar-

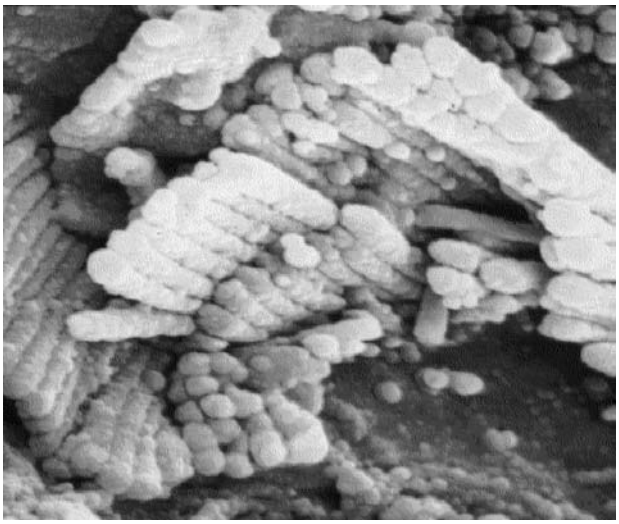

Fig. 4. Group m6, fourth turning, OHC 2. Disarrangement and lack of cilia starting from the inner side x 17,500.

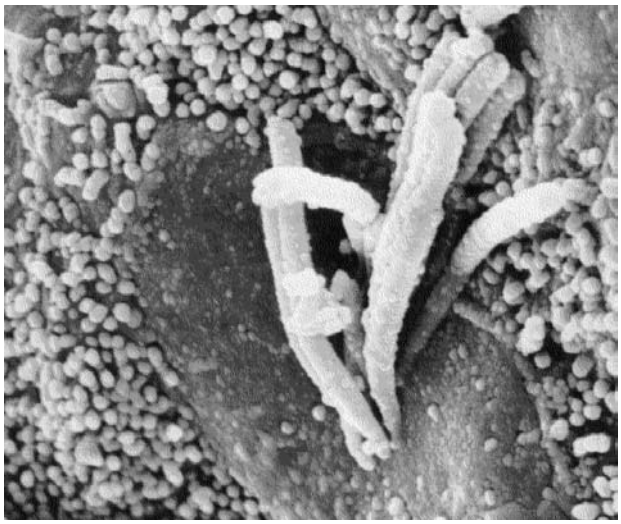

Fig. 5. Group m6, third turning. Almost complete lack of cilia x 10,000.

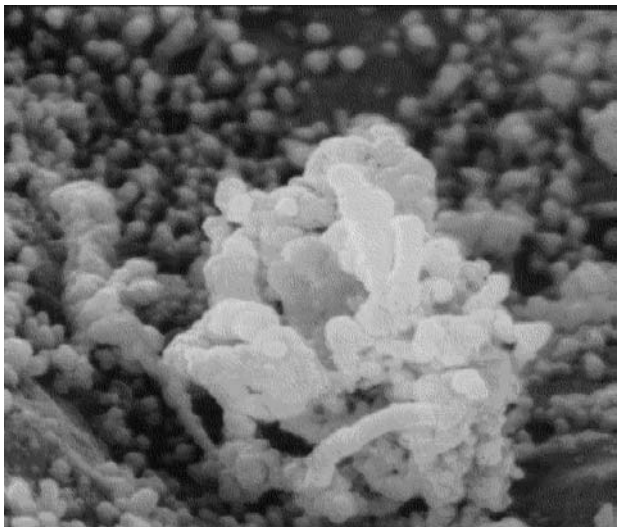

Fig. 6. Group m6, fourth turning. Sticking together cilia of OHC 3 x 16,100.

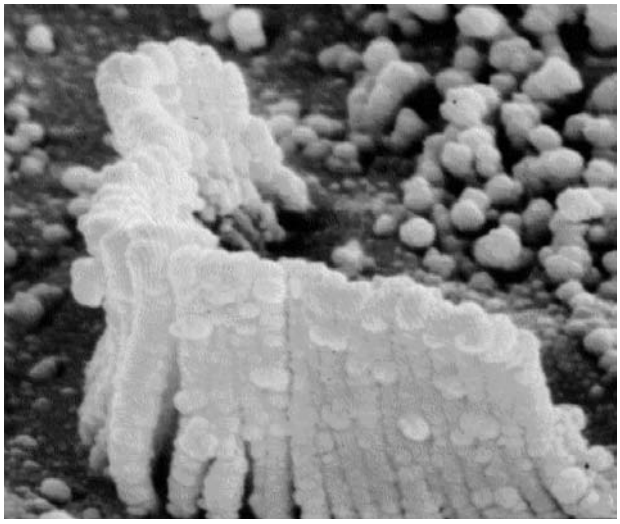

Fig. 7. Group m3, third turning, OHC 2. The rough surface of cilia along with and increase in the number and size of microvilli are visible x 19,000.

atus (with possible small changes in cilia settlement); 2) disorganization, deformation and partial lack of cilia, or cilia stuck together; and 3) entire lack of cilia. Data pertaining to the occurrence, range and degree of changes ascertained in the sensory epithelia of guinea pigs from all the experimental groups are shown in Table 1. A large magnification of the cilia often revealed their rough surface. Besides all the above mentioned changes, an increase in the number and size of the microvilli was also observed (Fig. 7).

Table 1. Localization and promotion of sensory epithelium changes in animals from the experimental groups

Hair cells	Degree of damage	Experimental groups						
		m1		m3		m6		
		turnings of the cochlea						
		3	4	3	4	2	3	4
IHC	1	20	19	20	17	20	18	12
	2	0	1	0	2	0	2	2
	3	0	0	0	1	0	0	6
OHC 1	1	18	10	14	10	20	11	8
	2	1	4	6	5	0	6	3
	3	1	6	0	5	0	3	9
OHC 2	1	17	8	13	4	17	9	4
	2	1	9	6	10	3	4	4
	3	2	3	1	6	0	7	12
OHC 3	1	16	4	7	2	16	4	0
	2	2	8	8	11	3	3	5
	3	2	8	5	7	1	13	15

DISCUSSION

The sensory epithelium of the inner ear is constructed of two kinds of hair cells and supporting cells lying on the basilar membrane. In mammals, the single row of IHC is found more internally and nearer to modiolus and the three parallel rows of OHC 1, 2 and 3 are placed more externally. Universally recognized is their regular arrangement and fixed number, though even healthy animals sometimes show deviations from the ideal pattern. In six-week-old guinea pigs, Úlehlová [11] accepts as admissible, in each turning and row, the decrease of up to 0.03% for IHC and from 0.31 to 2.2% for OHC. The quantity of cells (especially the OHC) decreases with age [12–15]. Apart from their decrease, atypical cell forms and changes in their position and orientation are also found [16]. These "standard" deviations from the ideal cochleogram were found in guinea pigs of both the control and experimental groups.

Besides entire or partial lack of cilia, the most often described morphological changes in the ciliary apparatus are (after being exposed to a harmful factor) disorganization, inclination, flaccidity, sticking together, or gigantic

cilia formation. All these damages were present in animals from the experimental groups. These changes are not specific and have been observed earlier, e.g. as a result of acoustic trauma [17], effects of ototoxic drugs [18], bacterial poisons [19], collagen [20], blending of cochlea liquids [21] and vitamin deficiencies [22].

Some information about the ability of sensory cell regeneration among mammals has appeared quite recently [23–25], and some of the changes resulting from acoustic trauma are believed to be reversible [26,27]. The changes in the settlement of the cilia are listed among them, while a partial or entire decrease in the number of cilia and their sticking together are now recognized as permanent changes [7,26,27].

In support of this, a three-degree scale, modifying similar earlier work (not taking into account occurrence of the cilia sticking together [18,28]), was used to estimate a single cell damage. Also, each animal was always given one month of rest following the experiment for possible regeneration.

Morphological analysis of the effect of whole-body vibration on the organ of hearing (using laboratory animals) have rarely been carried out, and those reported have been performed in different conditions, on species with varied resistance of the hearing organ [29–31], and thus produced different results. A few investigations focused on the combined effect of industrial noise and vibration on the inner ear. The first observations regarding this issue have been made in the former USSR. Temkin [32] reported that, already in the 1920's, the Soviet authors ascertained the damage of the sensory cells, especially in the upper turnings of the cochlea of guinea pigs and mice, dependent on the prolonged effect of both these factors. Enin [33] subjected mice and rats for one year (3 h/day) to vibrations of the vibroplate of a cement mixer (frequency 50 Hz, amplitude 0.9 mm) and noise (intensity 100 dB), and found degenerative changes in all the turnings of the cochlea, most distinct in the bottom region. Guseev et al. [34] examined rabbits placed in cages mounted 1.5 m from a working air-compressor for 15, 30 and 60 days. The authors revealed degenerative changes in Corti's organ, however, they did not specify the location, or the degree of their intensification. They believed that mechanical vibration makes hair cells more sensitive to the harmful effect of noise. Śliwińska-Kowalska et al. [35] reported the findings on the hearing state of guinea pigs subjected to the noise of a weaver's shop (thus also to vibration) for 3 months. Morphological changes were observed in the second, third and fourth turnings of the cochlea. OHC 1 and OHC 3 were damaged most frequently, whereas the IHC damage was rather sporadic.

Laboratory experiments with shaking devices also revealed different results. Among the Polish authors, Chodynicki and Hermanowicz were the first [36,37] to present the results of the study of the effect of whole-body vibration (frequency 40 Hz, amplitude 1 mm), exerted on guinea pigs exposed for the periods from two weeks to three months, 3 h/day; noise intensity of about 87 dB. After three months of experiment and a six-day rest, they observed reversible histochemical and morphological changes at 17–18 mm of the cochlea line, in OHC 1 area. Rogowski and Chodynicki [38] investigated the effects of whole-body vibration (63 Hz, 3 h/day, 30 days) and gentamycin on the inner ear of guinea pigs. Vibration alone damaged three rows of OHC (more seldom IHC) in the third and fourth turning of the cochlea. Chodynicki et al. [39] also assessed the state of hearing of guinea pigs subjected to vibration and noise during fetal life. The damage to the spiral organ cells was located in the fourth turning of the cochlea. Nekhoroshev [40] examined the influence of whole-body vibration (frequencies from 30 to 60 Hz) and white noise (85 dB) in the experiment on guinea pigs lasting up to 270 h. He found irreversible changes in the form of deformation of the nuclei with chromatin disintegration in almost all cells of the spiral organ. Hamernik et al. [41,42] subjected chinchillas for 1–10 h to whole-body vibration at a frequency of 30 Hz and intermittent (113 dB) or impulse (155 dB) noise. In the animals exclusively subjected to vibration, they did not reveal any changes that could be attributed to its effect.

In the above-quoted experimental studies, the use of shaking apparatus for sinusoidal vibration was always accompanied by noise of at least 75 dB, sometimes its level was not given. In our own experiment, a possible

harmful effect of noise (of the apparatus) was limited to the minimum (<35 dB(A)). It was not distinguishable from the background acoustics of the quiet isolated rooms in which the guinea pigs dwelt during exposure and rest. Thus, we could observe damage, which might be attributed exclusively to vibration. The time of rest after experiment made the permanence of the changes more likely.

It was found that the changes in the sensory epithelium, directed from the apex to the cochlea base, gradually advanced with the prolonged time of the experiment. In group m1, hair cell damage was most often observed exclusively in the fourth turning, whereas in group m6 it was also seen four times in the second turning of the cochlea. At the same time, we found that whole-body vibration affected mostly OHC 3. The damage was always less intensified in the direction from the circumference to the modiolus. The IHC-related changes were most seldom. The observed pattern distinctly differed from that resulting from the effect of noise only [7,26], where the damage usually began in the base of the cochlea.

The results provide evidence that the isolated whole-body vibration exerts a harmful effect on the inner ear. They also allow to presume its effect on humans. In view of the above, one can expect an increasing impairment of hearing, especially at low and then at medium frequencies in persons exposed to whole-body vibration.

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