in the cardiac muscles. In the smooth muscles, however, ATP after Mg produced almost maximum contraction and addition of Ca produced a little contraction, different from skeletal and cardiac muscles. No more contraction was observed by further addition of Ca in all preparations. Further addition of EGTA did not relax the smooth muscle, but gradually relaxed cardiac and skeletal muscles. These results are summarized in the table, A. Tension development of glycerinated smooth muscles was much weaker than that of glycerinated skeletal muscles. The ratio of the response in the absence of Ca (probably lower than 10^{-8} M of Ca ion) to the response in 3×10^{-6} M Ca ion were 2.2% and 37.2% for the skeletal and cardiac muscles respectively. The finding indicated that glycerinated cardiac muscles partially contracted without exogenous Ca ion. The values of glycerinated smooth muscles in rats were significantly higher than those of skeletal or cardiac muscles. The sensitivity to Ca ion among glycerinated skeletal, cardiac and smooth muscles in dogs (table, B) was similar to that in rats. The role of Ca ion in the contractile system of skeletal muscle was explained by Ebashi[10]. Binding of Ca ion to troponin removed the inhibition of troponin-tropomyosin to actin, so that actin filament interacted with myosin filament and a contraction produced. A similar mechnism have been assumed to exist in cardiac and smooth muscles[11]. Recently, it was reported that the regulation of the contraction via Ca ion was different in skeletal and in smooth muscles[12–15]. Under the conditions used in the present experiment, there are at least quantitative differences with respect to initiating contraction in the skeletal, cardiac and smooth muscles, respectively.

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Endolymphatic leakage in case of acute loss of cochlear microphonics[1]

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Summary. Rapid loss of cochlear microphonics in guinea-pigs previously exposed to high-energy impulse noise was shown to be related to the breakdown of the endolymphatic boundary. The cochlear duct was rendered leaky by deterioration of the reticular membrane, and damage of sensory and supporting cells.

Cochlear microphonics are considered indicative of the activity of cochlear sensory cells[2]. Accordingly, loss of cochlear microphonics (CM) following acoustic trauma was expected to parallel cellular damage at least in the basal turn of the cochlea. Light microscopic findings of less than 20% damaged hair cells are at variance with the almost 80% decrease of CM in guinea-pigs exposed to single impulses 2 h prior to sacrifice[3]. In the present paper we report on tracer studies providing evidence of the rapid breakdown of the endolymphatic barrier which is thought to impair hair cell function.

Material and methods. Anaesthetised young guinea-pigs subjected to resection of the tympanic bulla were individually exposed to 10 successive impulses of 164 dB SPL for 0.1 msec each produced by a spark-noise generator (built at the Zentralwerkstatt für Forschung und Entwicklung des Bereichs Medizin, FSU Jena). Previous to and immediately after impulse exposure CM were taken from the round window at frequencies from 500 to 10,000 Hz. About 40–50 min after exposure to impulse noise, perilymphatic perfusion with 6% horseradish peroxidase-Ringer pH 7.6 commenced for a period of 8 min. Following a 2-min rinse with Ringer-solution, the phosphate-buffered 2% glutaraldehyde-1.5% formaldehyde fixative was instilled for 30 min, and after removal the cochlea was fixed for another 180 min. Over-night the cochlea was incubated in phosphate buffer at 4 °C. Segments of the cochlear duct were micro-dissected free from the modiolus and, without removing the spiral ligament, the stria vascularis or Reissner's membrane, the specimens were subjected to the DAB-reaction for detection of peroxidase (for details, Geyer[4]). After completion of the histochemical procedure, the tissue was dehydrated and embedded in Durcupan.

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Mean amplitudes of cochlear microphonics registered from 11 guinea-pigs immediately following the exposure to 10 impulses of 164 dB SPL

Frequency (Hz)	500	1000	2000	3150	5000	8000	10,000
CM amplitude as % of the mean value previous to noise exposure ± SD	68 ± 2.7	70 ± 3.6	69 ± 4.0	67 ± 3.3	64 ± 2.5	62 ± 3.0	64 ± 4.1

According to the t-test, the amplitudes of CM before and after noise exposure differ from each other by a level of significance better than 1%.

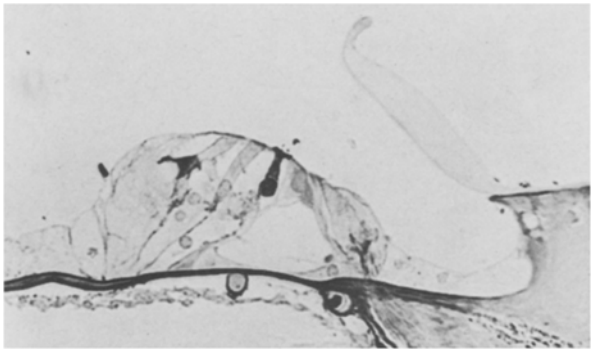

Fig. 1. Cross-section of the organ of Corti of a guinea-pig exposed to impulse noise and perilymphatically perfused with horseradish peroxidase. The tracer has penetrated a 1st-row outer hair cell. ×300.

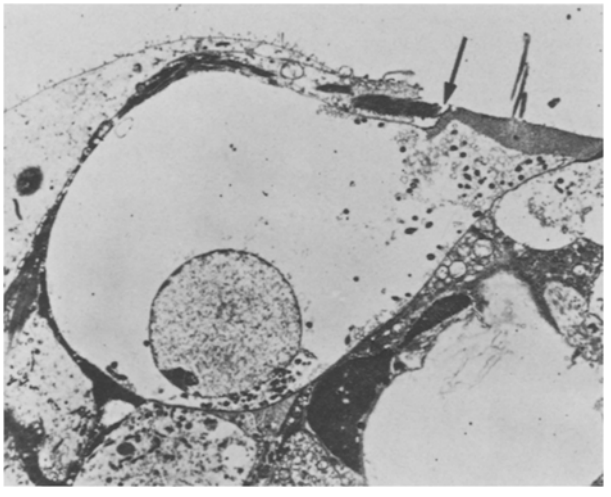

Fig. 2. Defective junctional complex (arrow) and a swollen 3rd-row outer hair cell of a guinea-pig impulse noise exposed. ×2000.

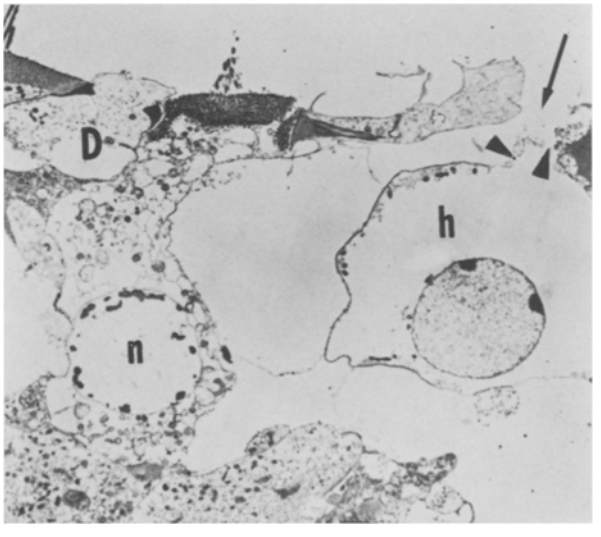

Fig. 3. Adjacent area of the same organ of Corti as shown in figure 2. A 1st-row hair cell (h) is swollen and its apical cell membrane became ruptured (arrowheads). The nearby reticular membrane is distorted and displays a cleft (arrow) connecting endo- and perilymphatic spaces. The middle-row hair cell is greatly altered, its nucleus (n) exhibits condensed chromatin. An adjacent cell of Deiters (D) also is deteriorated. ×2000.

Results. Immediately after exposure to 10 impulses of 164 dB SPL, the CM had decreased by 30–38% (table). Due to the tracer experimental procedure, CM of these animals were not registered 1 h after impulse trauma, i.e. the beginning of histological fixation. In similar experiments Biedermann and Meyer[5] could show a 50–60% loss of CM in guinea-pigs exposed to 10 impulses of 164 dB SPL 1 h before measurement. However, during a 2-h period, there was practically no change of the CM level in control animals.

Light microscopic examination of whole mount specimens and semithin sections of the impulse noise exposed organ of Corti revealed single and small groups of damaged hair cells, the vast majority of which were located in the basal and the lower half of the 2nd turn. Outer hair cells were dislocated and distorted. Local deterioration of the reticular membrane has been detected as well as both swollen and pycnotic nuclei. Single hair cells and some supporting cells were stained by the diaminobenzidine reaction, due to horseradish peroxidase which had penetrated them. 2 out of 9 organs of Corti exhibited a brown staining of the tectorial membrane.

In addition to light microscopic findings, adjacent ultrathin sections of damaged outer sensory cells showed swelling and clumping of hairs. The hair cell itself could swell considerably or even rupture and lose many of its constituents. Despite necrobiotic alterations, the cuticle of hair cells could have remained in situ, though the cell had lost part of its membrane by vesiculation. The uptake of peroxidase resulted in opaque staining of otherwise only slightly altered cells. Denuded sensory cilia were stained by irregularly bound peroxidase.

Morphological findings and the tracer distribution pattern provide evidence for multiple sites of breakdown of the permeability barrier of the cochlear duct. Although 1 h after exposure to impulse noise the alterations of the organ of Corti might not have come to an end, they had rendered the endolymphatic compartment leaky by a) rupture of the reticular membrane, b) necrosis of damaged sensory and supporting cells, and c) increased permeability to horseradish peroxidase (and, of course, to ions smaller in diameter) of the membrane of some cells. Endolymphatic leakage is associated with the rapid ionic exchange between endolymph and perilymph. The process is considered a short-circuit, invalidating the function of hair cells in a fashion comparable to the experimental replacement of perilymph by a high potassium medium[6].

In conclusion, the acute severe loss of CM following exposure to single impulses is due to both a) permanent or temporary damage of sensory cells, and b) degradation of ionic endo-/perilymphatic gradients. With regard to the small number of severely damaged hair cells, the decrease of ionic gradients must be more efficient in reducing CM than damage of sensory cells. This suggestion is consistent with the ubiquitous loss of CM at various low and high frequencies. It implies the partial restoration of cochlear function following the repair of the endolymphatic barrier.

It is not to be mistaken for a domain of cellular damage and loss of outer hair cells, which was shown to develop slowly during 1 week at the site of experimental rupture of Reissner's membrane[7].

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