are not uncommon. The preservation of remains of spongy mesophyll is remarkable, and no explanation or speculation with respect to the factors which were responsible is available at present. Experimental studies under laboratory conditions may throw light on the problem.

It is axiomatic to state that success in paleobotanical studies is determined by the quality of preservation of the material at hand. This is particularly true in investigations of fossil floras based upon leaf compressions. In general, the overall physiognomy of the leaf and the cuticular patterns, if preserved, provide the basic characteristics used in identifying fossil leaves. The patterns of mesophyll tissue, taken in conjunction with these characteristics, were found to be valuable as an additional aid in identification.

It is premature to amplify the significance of this extraordinary finding as a diagnostic taxonomic feature. Nevertheless, the discovery is reported here as an unusual example of excellent preservation in fossil plant remains.

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2. I thank Prof. W. N. Stewart, Department of Botany, University of Alberta, for his help in the preparation of this report.

9 December 1971; revised 31 May 1972

Cochlear Inner and Outer Hair Cells: Functional Differences

Abstract. *The cochlear microphonic response was measured with differential electrodes from the first and third cochlear turns of normal guinea pigs and those treated with the ototoxic drug kanamycin. Histological controls showed that the outer hair cells in treated animals were missing over the basal half of the damaged cochleas, while the inner hair cells were intact. Measurements are consistent with the hypothesis that the potentials produced by inner hair cells are proportional to the velocity of the basilar membrane, whereas potentials generated by outer hair cells (which dominate the response of normal cochleas) are proportional to the displacement of the basilar membrane.*

In all auditory systems the immediate stimulation of the hair cells is mediated by a relative motion between the cells and their stereocilia. The adequate stimulus is probably the bending of the hairs and the concomitant deformation of the apical pole of the hair cells. The hairs can be bent by several mechanisms, and there are apparently four anatomical forms that have evolved to subserve this function (*1*). The most common such form is a tectorial membrane that is attached to the tips of at least some of the cilia. The relative motion between the tectorial membrane and the reticular surface of the organ of Corti provides the bending or shearing force to the hairs. Other anatomical arrangements include the presence of sallets, lateral connections among cilia, and free-standing cilia (*2*). It has been assumed that only the first type of anatomical arrangement, namely stimulation by means of a tectorial membrane, is important in the mammalian cochlea. This assumption implies that at least some stereocilia (probably the tallest ones only) of both inner and outer hair cells contact the bottom surface of the tectorial membrane. Electron microscopic studies indicate, however, that whereas the cilia in the tallest row on the outer hair cells make intimate contact with the tectorial membrane, such contact is absent for any cilia of the inner hair cells (*3*). This observation then implies that the mammalian cochlea, like that of many lower vertebrates, is equipped with a dual stimulating system. According to Wever's classification (*2*), these are the tectorial system and free-standing cilia.

That the two types of hair cell would be stimulated by different mechanisms is not surprising, considering the profound morphological differences between them (*4*). What is important to consider, however, is that different mechanisms of stimulation imply different dynamic properties. Such differences in the dynamic behavior of inner and outer hair cells of the mammalian cochlea have been predicted by one of us (*5*). The mechanism of stimulation of the outer hair cells, because of their attached cilia, depends primarily on the relative displacement between tectorial membrane and reticular lamina (*6*), which in turn is directly proportional to basilar membrane displacement (*7*). Thus the outer hair cells are primarily displacement detectors. In contrast, the bending of the free-standing cilia of the inner hair cells must depend on viscous forces exerted on them by the movement of endolymph around them. Such forces ultimately depend on the velocity of endolymph flow, which in turn is determined by the rate of displacement of the basilar membrane. Thus, the inner hair cells are primarily velocity detectors. Because the outer hair cells also possess free-standing cilia in addition to the attached ones, they too are stimulated with a velocity component. However, the displacement stimulus is much more prominent than the velocity stimulus over most of the audio frequency range; consequently, a good first approximation is that the output of outer hair cells is proportional to basilar membrane displacement, whereas the output of inner hair cells is proportional to basilar membrane velocity (*5*).

We report an experimental verification of the above-stated hypothesis. Cochlear microphonic (CM) potentials in response to well-defined test stimuli were recorded with differential electrodes from both normal cochleas and those with damaged outer hair cells. The first premise of this method is that in a normal cochlea the recorded CM primarily reflects the output of the outer hair cells (*8*). Because the CM magnitude produced by the inner hair cells is approximately 30 to 40 db less than that generated by the outer hair cells (*9*), the CM recorded from a normal cochlea can be assumed to reflect the dynamic properties of outer hair cells. Ototoxic drugs, particularly kanamycin, selectively damage outer hair cells (*9*, *10*). With appropriately chosen doses of the drug one can generate patterns of hair cell destruction that yield virtually intact inner hair cells throughout the cochlea and virtually complete destruction of outer hair cells over the basal half of the cochlear spiral (*11*). In such cases a pair of differential electrodes placed in the basal turn of the cochlea would register the CM output of the inner hair cells in its vicinity. Thus by comparing the properties of the differentially recorded CM response from the basal turn of normal and kanamycin-treated animals, one can compare the microphonic output of outer and inner hair cells (*12*).

Guinea pigs in which hair cell damage was to be induced received subcutaneous injections of kanamycin (400 mg per kilogram of body weight)

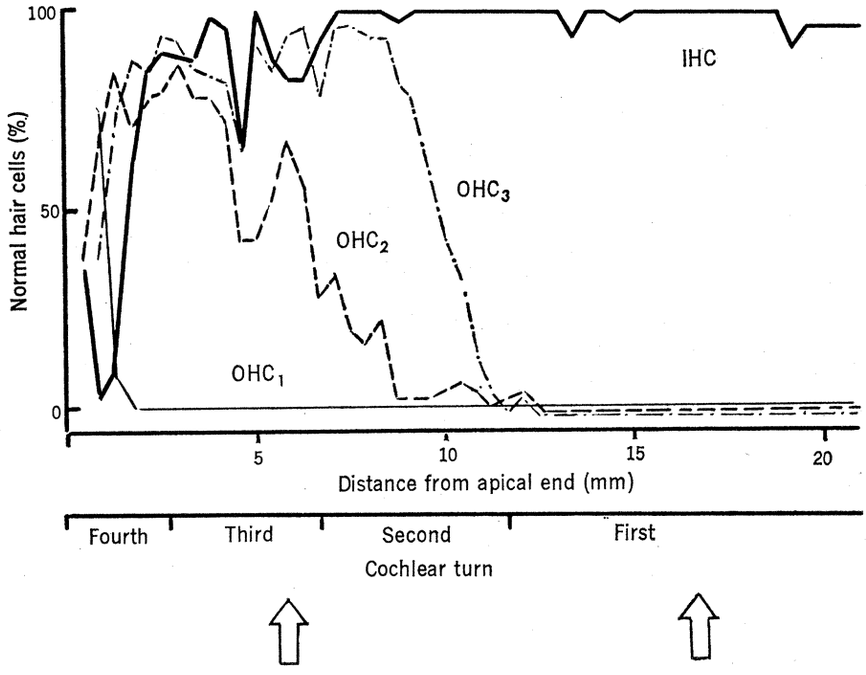

Fig. 1. Cochleogram of a representative guinea pig that received kanamycin injections. The ordinate gives the percentage of intact hair cells in the single row of inner hair cells (*IHC*) and the three rows of outer hair cells (OHC_1, OHC_2, OHC_3). The abscissa is the distance in millimeters from the apex of the cochlea. The extent of the various turns is also indicated, as well as the location of the differential recording electrodes (arrows).

for 8 to 10 consecutive days. A waiting period of at least 2 weeks between the last injection and the experiment permitted an appropriate degree of hair cell degeneration. After electrophysiological data were collected from the treated guinea pigs, their cochleas were perfused with osmium tetroxide solution, the animals were killed, and their cochleas were removed, further stained with the fixative, and then dehydrated with alcohol. Later the cochleas were dissected, and the organs of Corti were prepared as flat specimens for examination under the phase-contrast microscope with the surface preparation technique (*13*). The hair cells were counted and a cochleogram was prepared for each cochlea. A representative cochleogram is presented in Fig. 1. The inner hair cells are virtually intact, and there is a good complement of outer hair cells in the third turn of the cochlea where one electrode pair is placed. In contrast the outer hair cells are completely missing in the vicinity of the electrode pair in the first turn (*14*).

The electrophysiological techniques were the same for both normal and kanamycin-treated subjects. The animals were anesthetized with urethane; the auditory bulla was approached ventrolaterally and was opened; small holes were drilled through the bony cochlear wall over the scala tympani and scala vestibuli of both first and third turns. Glass-insulated tungsten wire electrodes were inserted into the holes and then secured to the edge of the bulla. A reference electrode was placed on the neck muscles. The difference in potential between the two electrodes of a pair (scala vestibuli minus scala tympani) was amplified and processed with an averaging computer.

The electrophysiological measurements were completed in two stages. First, pure tones were delivered to the animal's ear in a closed acoustic system and were monitored at the eardrum. With this type of stimulation the sensitivity of the animal, the adequacy of the electrode placement, and an estimate of the damage in drug-treated animals were determined. After these measurements were made the tympanic membrane was exposed, and a piezoelectric driver with an attached driving needle was brought in contact with the umbo. The movement of the drum (actually of the manubrium of the malleus) was monitored by measuring light reflection from it (*15*). The stimulus was a 73-hz triangular displacement of the malleus which produced a CM equivalent in size to what would have been produced by sound stimuli of 70 to 80 db (referred to 0.0002 dyne/cm²).

A triangular displacement of the ossicular chain results in a square-wave microphonic at all locations in a normal cochlea (*16*). This is seen in Fig. 2a; a square wave coincident with the triangular stimulus was recorded from the basal turn, and a similar square wave (but delayed about 0.9 msec) was obtained from the third turn. These results imply that the CM is proportional to

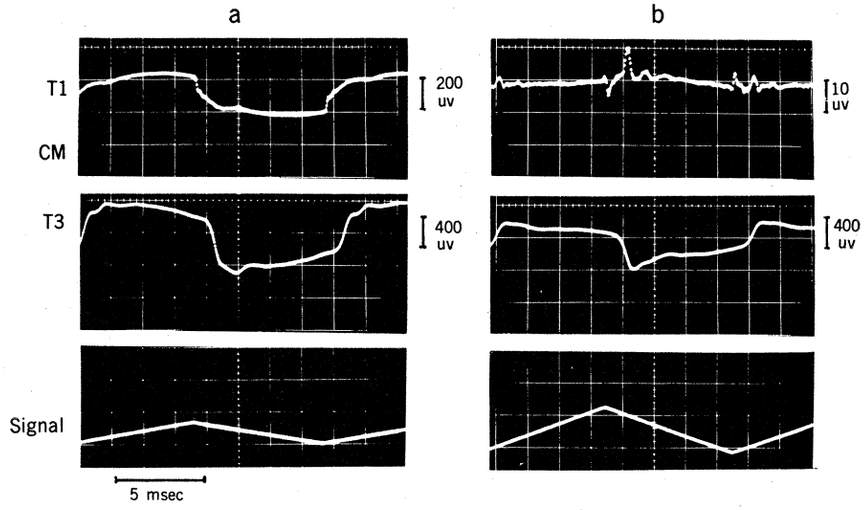

Fig. 2. Averaged CM responses from (a) a normal guinea pig and (b) a guinea pig treated with the ototoxic drug kanamycin. The two top traces show the averaged CM response obtained from electrodes in the first turn (*T1*) and third turn (*T3*). The bottom traces show the stimulus waveform (electrical signal across the transducer). We have demonstrated (*16*) that the displacement pattern of the manubrium of the malleus and the waveform of the driving signal are identical. In other words, the lowest traces are an accurate representation of the movement of the manubrium, and since at low frequencies the transfer function of the guinea pig's middle ear is flat, these traces represent stapes movement as well. Stimulus frequency is 73 hz and stimulus amplitude is measured as 0.3 μm (peak to peak).

the velocity of the stapes in the normal animal; or, to be more precise, that it is proportional to the displacement of the basilar membrane, which is proportional to the pressure at the oval window, which is, in turn, proportional to the velocity of the stapes. The time difference between the square waves in the first and third turns reflect the traveling-wave delay between these two points. In normal cochleas the CM reflects the output of the outer hair cells. Thus the output of the outer hair cells is proportional to the displacement of the basilar membrane, as we proposed earlier in this report.

In Fig. 2b are microphonic recordings from a cochlea of a kanamycin-treated animal. At the third turn—where both types of hair cell were in acceptable condition (Fig. 1)—the recording was a square wave having the appropriate time delay of approximately 0.9 msec relative to the stimulus peaks. This response is virtually indistinguishable from that in the normal cochlea. In contrast, the electrode pair at the first turn registered a pulse coincident with the peaks of the stimulus, and every second pulse was of opposite polarity. The additional, later peaks are uncanceled remote CM from the intact portions of the cochlea and are of no significance for our purposes *(17)*. When the responses from the normal and kanamycin-damaged first turns are compared, then the latter appears approximately proportional to the derivative of the former. Thus, inner hair cells produce a CM that is proportional to the rate of change of basilar membrane displacement, this in accord with our above-mentioned suggestions *(18)*.

In summary, comparison of CM output from inner and outer hair cells of the mammalian cochlea reveals that the two groups of hair cells possess different dynamic properties. The primary response of the outer hair cells is proportional to the displacement of the basilar membrane, and that of the inner hair cells is proportional to the velocity of the basilar membrane. The cause of these differences can be found in the relation between cilia and the tectorial membrane for the two groups. Apparently, the cilium of the inner hair cell is freestanding, and thus its stimulus is derived from viscous fluid drag; but some cilia of outer hair cells are attached, and their stimulus is the relative displacement of tectorial and reticular membranes. The existence of a dual system that is capable of providing information about both the signal and its first derivative can yield significant improvement in the data-processing capability of the peripheral auditory system.

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11. By "intact" inner hair cells we mean cells that appear normal under the phase-contrast microscope. Light microscopy can not reveal possible subtle morphological changes. Consequently, without electron microscopic verification, we must accept the possibility that some of the inner hair cells that appeared normal with light microscopic observation had actually sustained some damage.
12. In the classic experiments of von Békésy (*6*), in which he demonstrated that by directly stimulating the cochlear partition with a vibrating electrode an electrical output is obtained which is directly proportional to partition displacement, the electrical signal must now be interpreted as being produced by the dominant outer hair cells.
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14. To indicate the consistency of these histological results, we offer the following considerations. We have processed 32 cochleas from kanamycin-treated animals (not all were used in this experiment). If three animals that suffered severe damage to both hair cell populations are excluded, then in the remaining 29 preparations only 6.9 percent (mean) of outer hair cells in the basal half of the cochlea were intact, while in this same population 89.2 percent of the inner hair cells remained present. Thus in guinea pigs it is possible to generate selective destruction of outer hair cells in the basal half of the cochlea.
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17. The electrical output of the inner hair cells is at least 30 db less than that of the outer hair cells. Because of this, extreme care must be exercised in recording from a region of the cochlea containing only inner hair cells when there are intact outer hair cells in some other region. Under such circumstances a single intracochlear (or round-window) electrode would pick up potentials that are overwhelmingly derived from the remote normal regions of the cochlea. Such potentials would not reflect local conditions and would provide no information about the electrical output of the inner hair cells. A pair of differential electrodes carefully balanced to reject remote potentials, as used in this experiment, provides the only means of obtaining the characteristics of a small local potential in the presence of a relatively large remote response.
18. Experiments like those described in this report were performed on five guinea pigs treated with kanamycin. In two of these animals the displacement component was dominant even in the first turn. Subsequent histological study revealed that these animals had mild damage to outer hair cells, and thus the response was produced by outer hair cells remaining in the vicinity of the recording electrodes. In three animals that had lesions similar to that in Fig. 1, the electrophysiological results agreed with the description in this report. Steady-state measurements of cochlear microphonic phase were performed on an additional three kanamycin-treated guinea pigs, and the phase data from these animals is consistent with the dynamic measurements presented here.
19. Supported by various NIH grants. The piezoelectric transducer was supplied by R. Sachs of Industrial Research Products, Inc.

12 May 1972

Systemic Absorption of Intrauterine Copper

Abstract. *A comparison of the various effects, in rats, of intrauterine insertion of copper-64 or copper-67 wire with the effects of intraperitoneal injection of copper sulfate solutions has shown that copper ions, dissolved from the wire, are locally active contraceptively and, in part, systemically absorbed.*

The efficacy of intrauterine devices has been greatly increased by incorporating copper into their structure (*1*, *2*). Dissolved ions of the metal appear to be the actual contraceptive agent, since approximately 25 percent of a copper wire placed in a human uterus disappears within a year (*3*) and metals with low solubility, like platinum, or silver in the presence of chloride ions, are not very effective contraceptively (*2*).

The possibility that systemic toxicity may result from copper absorbed through the uterine mucosa must be considered in view of reports which indicate that 20 to 25 mg of copper are removed from these devices per year (*3*). To investigate this possibility, we