for example, in mice infected with the Gross or Rich strain, and in tissue cultures infected with the Moloney strain (*11*). These are RNA viruses, whereas the Epstein-Barr agent is a DNA virus. It has been suggested that different virus strains may be responsible for different clinical states (*11, 12*). The appearance of the same protein, phosphatase N, in different mammalian species and in diseases characterized by lymphocytic proliferation and viral infection suggests that this protein reflects changes in lymphocytes or lymphocyte precursors that are induced by viral infection. Efforts to distinguish between viral transformation associated with malignancy (acute murine leukemia and possibly human lymphatic leukemia) and that associated with benign lymphoproliferative disorders (IM) may lead to a better understanding of the etiology of human leukemias (*13*).

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1. In our designation of this heretofore unknown enzyme we used the symbol N for the Hebrew word *ne-elam* which means unknown. No reference to enzymatic activity toward a phosphoroamidate bond is intended.
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Cochlear Neurons: Frequency Selectivity Altered by Perilymph Removal

Abstract. *Removal of perilymph from the scala tympani of the guinea pig cochlea reversibly broadened the tuning curves of single spiral ganglion cells emanating from the basilar membrane. Thus, fluid continuity along the membrane is essential for normal cochlear function, in particular for sharp neural tuning curves. These data reveal a possible source of error in some estimates of basilar membrane motion and suggest a reappraisal of current concepts of the mechanism of sharp tuning in primary auditory nerve fibers.*

It has long been held that there is a serious discrepancy between the filtering properties of the basilar membrane and the frequency selectivity of single primary auditory neurons (*1–3*). Mechanical measurements, usually obtained at high sound pressures, indicate a broad tuning of the basilar membrane, whereas the tuning curves of primary neurons (obtained at threshold sound pressures), are much sharper. Rhode (*4*) and Rhode and Robles (*5*) have reported a nonlinearity in basilar membrane vibration which consists of a flattening of the input-output curves in the region of the peak of the membrane tuning curve. This nonlinearity results in a sharpening of the mechanical tuning curve at low sound pressures. Such a nonlinear behavior could be used to bring the neural and mechanical data into close agreement and thus eliminate discrepancies between basilar membrane and primary neural tuning (*6, 7*). There is, however, some conflict between these authors' findings and those of other workers. In particular, the failure to find a nonlinearity in the guinea pig cochlea (*3, 8, 9*) has lead some authors to state that the neural tuning curves cannot be explained solely on the basis of basilar membrane mechanics. Additional filtering processes have therefore been postulated (*2, 3, 10*). How much of this disagreement in mechanical measurements could be caused by species differences or by variations in technique is not clear. The results that are reported here show that removal of perilymph from the scala tympani could constitute a source of error in several of the mechanical measurements.

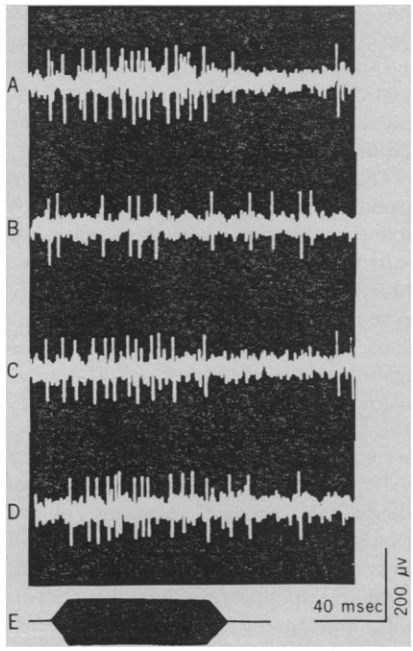

Fig. 1. Oscilloscope tracings of the response of a single ganglion cell in the guinea pig cochlea to a tone burst at the best frequency. The intensity of the tone burst was 10 db above threshold for the cell with the scala tympani full of perilymph. Responses were recorded (A) before draining perilymph, (B) with perilymph removed, (C) with perilymph only partially replaced, but covering basilar membrane, and (D) with scala tympani refilled. (E) Approximate position of tone burst.

The technique of recording from the bipolar cell bodies of the spiral ganglion has been described elsewhere (*7*). This procedure permits stable recording from single cells in a chosen and restricted frequency range in the basal cochlear spiral, while continuously monitoring the cochlea during experimental manipulations. The frequency selectivity of the ganglion cells was estimated by measuring the tuning curves (threshold versus frequency response curves) (*7, 11*).

As previously reported, sharp and sensitive tuning curves could be obtained from the ganglion cells after limited opening of the scala tympani (*7*). These curves are similar to those obtained from eighth nerve axons (*1, 2, 11*). From this, it is inferred that opening of the scala tympani without fluid removal does not substantially alter the neural tuning curves. On this point the results are in agreement with the findings of Evans (*12*), who recorded from the eighth nerve axons (the central processes of the spiral ganglion cells). However, in contrast

to Evans' results, the removal of perilymph bathing the basilar membrane caused drastic alterations in the sensitivity and frequency selectivity of single ganglion cells.

Preliminary experiments were performed by measuring tuning curves at a chosen location in the ganglion in the basal coil. After the recording microelectrode was withdrawn, a small cotton wick was used to gently suck out perilymph from the scala tympani, so that no large fluid bridges existed between the walls of the scala tympani and the basilar membrane. Care was taken not to touch the basilar membrane. The microelectrode was then reinserted at the same location and tuning curves of single cells were again measured. In all experiments (four animals and 15 cells), tuning curves obtained after fluid removal were about 20 db less sensitive and very much broader than those obtained when the scala tympani was full of perilymph. When the wick was removed and the perilymph allowed to refill the scala tympani, further cells encountered showed a partial recovery of both the sensitivity and frequency selectivity.

More conclusive results were obtained by continuously recording the response of the same cell before, during, and after perilymph drainage. When a single ganglion cell had been isolated and its tuning curve obtained, perilymph was sucked out of the scala tympani while the response of the cell was continuously monitored. As soon as the fluid bridge between the wall of the scala tympani and the basilar membrane was removed, the cell's sensitivity dropped by about 20 db. In five cells in three different animals it was possible to maintain contact with the cell throughout this procedure. The sensitivities at the best frequency fell by 18, 12, 21, 16, and 17 db. When the wick was removed and perilymph allowed to flood back into the scala, the response of the cells recovered (Fig. 1). The tuning curves of these five cells were drastically altered by perilymph removal. This change, which occurred together with the sensitivity change, consisted of a reduction in high-frequency slope and a dramatic fall in low-frequency slope so that the sharpness of the tuning curve was reduced (Fig. 2). The replacement of perilymph, as well as causing a return of sensitivity, resulted in a substantial recovery of the frequency selectivity. In one case the tuning curve recovered completely (Fig. 2), but in the other

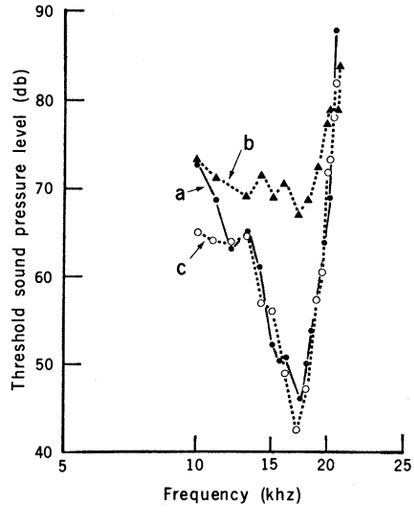

Fig. 2. Tuning curves of a single cell in the basal coil of the cochlea: (a) initial, (b) immediately after perilymph removal, (c) after refilling the scala tympani. The cell is not the same as the one in Fig. 1. The time between obtaining each of the curves was approximately 3 minutes.

cells the recovery was not perfect. In all cases the recovered tuning curves were much sharper than those when the perilymph was removed and the sensitivity returned to within 5 db of the original. The long low-frequency tail of the curves was not plotted below 10 khz so as to minimize the time required for the estimations.

The effects described above are probably not due to a disruption of neural processes, since histograms of both the mean rate and the interspike interval of the spontaneous activity were not altered by perilymph drainage. The rapid reversibility also argues against a deterioration of neural mechanisms. The effects appeared to be immediate when the final meniscus between the basilar membrane and the walls of the scala tympani was removed or replaced. Masking effects caused by wick placement or fluid movements are certainly not important, since the spontaneous activity, thresholds, and tuning curves were not affected by simply placing the cotton wick in the scala tympani. In Fig. 1C, the sensitivity had returned even though perilymph, now covering the basilar membrane, was still rising in the scala tympani.

These findings on the effect of fluid removal conflict with the results of Evans (*12*) who recorded from the eighth nerve axons in the internal auditory meatus. Evans' experiments did not allow recording from the same axon during the perilymph removal, nor did they permit such strict control of the cochlear location investigated. Direct visual observation of the cochlea was also probably difficult during neural recording in Evans' experiments. It is therefore possible that Evans, while removing the bulk of the perilymph from the scala tympani, did not remove that fluid lying directly over the basilar membrane.

One interpretation of the results reported here is that removal of fluid broadens the mechanical tuning of the basilar membrane and that this alteration is faithfully reflected in the neural tuning curves. This notion could resolve some of the contradictions in previous mechanical measurements. Rhode (*4*) found considerably sharper basilar membrane tuning in the squirrel monkey than that reported by Wilson and Johnstone (*3*) for the guinea pig. Rhode used a Mössbauer technique and took pains to leave perilymph in the scala tympani. Wilson and Johnstone used a capacitance probe and were obliged to completely remove perilymph to obtain their measurements. A further disagreement between these authors concerns the question of nonlinear basilar membrane motion. Rhode reported a nonlinearity in the region of the peak of basilar membrane displacement, which results in a sharpening of the membrane tuning at lower intensities, while Wilson and Johnstone found linearity down to 40 db. Although the possibility of a species difference cannot be ruled out, the present results suggest that Rhode's results reflect more reliably the true basilar membrane response. Results of laser measurements in the guinea pig show linearity, but these have only been reported in postmortem cochleas (*13*). Rhode (*14*) has shown that basilar membrane nonlinearity disappears after death. The results of Johnstone and colleagues are difficult to assess in this light, as these authors did not pay special attention to the level of perilymph. They did, however, state that drying the basilar membrane reversibly broadened its tuning (*8*) and they have recently reported the presence of a small nonlinearity in the guinea pig (*15*). It is thus possible, if these results are considered together, that the sharpness of neural tuning curves may be accounted for by the pattern of vibration of the basilar membrane in the living, fluid-filled cochlea.

A second interpretation is that the

broadening of neural tuning curves reflects, not an alteration in basilar membrane vibration, but a disruption of the functioning of a second filter of unknown nature, whose existence is considered necessary by some authors (*2, 3, 10*). The disruption could involve a structural change, perhaps a reversible displacement of the tectorial membrane (*16*), or an alteration in the flow of current in some important extracellular pathway. The lack of effect of fluid removal on spontaneous activities, however, implies that standing current levels through the hair cells are not altered, since it is known that polarization of the cochlear partition strongly affects rates of spontaneous activity in primary fibers (*17*). The results reported here do not make it possible to choose between these various hypotheses. However, by revealing a major source of error in some previous mechanical measurements, they again pose the question whether a second filter is necessary in addition to the basilar membrane to account for the sharpness of primary auditory frequency selectivity.

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Leukocyte Peroxidase Deficiency in a Family with a Dominant Form of Kuf's Disease

Abstract. *Use of a spectrophotometric assay of peroxidase with* p-*phenylenediamine as cosubstrate demonstrated deficient enzyme activity in leukocytes from two patients with a dominantly inherited form of ceroid lipofuscinosis (Kuf's disease) and a clinically healthy unaffected sibling. When the reaction was performed in the absence of added hydrogen peroxide, oxidation of the* p-*phenylenediamine cosubstrate (indicating the presence of endogenous peroxide) occurred only with enzyme samples from the three siblings but not with those from a large number of unrelated, unaffected controls. This demonstrates that the deficiency of peroxidase found previously in the recessively inherited infantile and juvenile forms of ceroid lipofuscinosis (Batten-Spielmeyer-Vogt disease) is also present in an adult form with dominant inheritance.*

In a previous report (*1*) we described a deficiency of leukocyte peroxidase in patients afflicted with the late infantile and juvenile forms of ceroid lipofuscinosis (Batten-Spielmeyer-Vogt disease). These disorders represent forms of neuronal storage disease characterized by progressive cerebral degeneration, accumulation of lipopigments in nerve cells, and recessive inheritance (*2*). Another form of ceroid lipofuscinosis, Kuf's disease, is characterized by onset during adulthood (*3*). Although most reports of cases of Kuf's disease suggest recessive inheritance, one pedigree showing inheritance of the disease as a dominant trait has been described (*4*). It was important, therefore, to determine whether the peroxidase deficiency found in the late infantile and juvenile forms of ceroid lipofuscinosis could also be demonstrated in the adult form and particularly in a pedigree in which the trait was dominant. Accordingly, we examined a peroxidase that acts on hydrogen peroxide with *p*-phenylenediamine as cosubstrate in leukocyte preparations from members of a pedigree in which ceroid lipofuscinosis is expressed as a dominant trait after the age of 30.

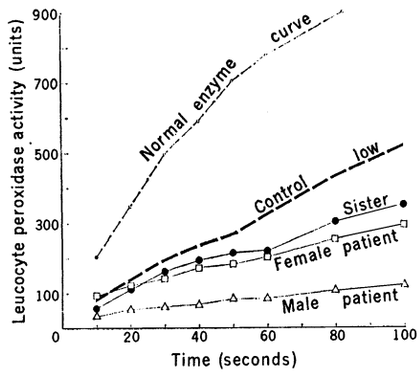

Fig. 1. Progress curves of peroxidase obtained from equivalent leukocyte samples. Enzyme was prepared from white blood cells as reported (*1*). The incubation was performed at 25°C and consisted of 0.15*M* phosphate buffer, *p*H 7.3, 0.28*M* *p*-phenylenediamine, and 1 m*M* hydrogen peroxide in a volume of 3.2 ml. Following addition of enzyme preparation derived from 5×10^4 cells, progress of the reaction was determined by recording, at 10-second intervals, the absorbance at 485 nm, which indicated appearance of the oxidized form of the hydrogen donor. Each curve is derived from a single incubation. The absorbance measurements were converted to units of enzyme activity by comparison with a standard curve prepared with horseradish peroxidase; these values were divided by the number of milligrams of total protein in each sample and plotted; patient IV/11, ●; patient IV/13, △; patient IV/15, □.

In Fig. 1 we have plotted the progress curves for equivalent samples of leukocyte enzyme from three siblings. The sibling designations have been used previously (*4*). Two of the siblings, a male of age 38 (IV/13) and a female of age 35 (IV/15), are patients with clinical manifestations of the disease, whereas the other, a female of age 41 (IV/11), is unaffected clinically. Also plotted are control data comprising the mean progress curve for 46 individuals who were neither affected with ceroid lipofuscinosis nor related to the affected kindred; the lowest values for a progress curve obtained from a control individual are also shown. The curves from the two siblings with clinical manifestations of the disease are well below that of the lowest control except at the earliest reading. The curve for the unaffected female sibling (IV/11) is below the lower control but above the curves for her two clinically affected siblings.

Further evidence of reduced capacity for removal of peroxide in the presence of a *p*-phenylenediamine cosubstrate was obtained as follows. When the enzyme reaction as described in Fig. 1 was performed in the absence of added hydrogen peroxide and the incubation