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Vulnerability and adaptation of distortion product otoacoustic emissions to endocochlear potential variation

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The endocochlear potential (EP) was reversibly decreased in adult gerbils by the intraperitoneal injection of furosemide, while cochlear functioning was monitored by measurement of distortion production otoacoustic emissions (DPE) at a range of stimulus intensities. Stimulus frequencies for DPEs were $f_1=6.8$ and $f_2=8$ kHz ($f_2/f_1=1.18$). Emissions monitored in the ear canal and scala media were $2f_1-f_2$, $3f_1-2f_2$, $2f_2-f_1$, and f_2-f_1. Typically, the EP decreased smoothly, reached a minimum one-half hour after injection, then recovered slowly over several hours. Emissions at $2f_1-f_2$ and $3f_1-2f_2$ *at low stimulus levels* were particularly vulnerable to the change in EP. These vulnerable emissions showed characteristic trajectories in which the amplitudes changed little with the initial EP decrease, then dropped sharply as the EP continued to decrease. However, the amplitudes then began to recover even before the EP reached minimum, and recovered completely while the EP remained subnormal. The trajectories of the other odd order emissions were similar, but lacked the abrupt decrease. The variation of the even order (f_2-f_1) component was completely different, but appeared related to the odd order trajectories in a complex fashion. During the initial decrease for the vulnerable components, the decrease in emission amplitude (in dB) was found to be proportional to the square of the change in EP (in mV). The recovery with a subnormal EP was interpreted as an adaptive effect with a time constant of about 15 min.

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INTRODUCTION

There is increasing evidence that proper functioning of the cochlea depends on metabolically active elements associated with outer hair cells (e.g., Dallos, 1992; Patuzzi and Robertson, 1988; Zweig, 1991). Motility of outer hair cells (OHC) due to modulation of the OHC membrane voltage by stereocilia deflection has been proposed as the cellular basis for this dependence (Ashmore, 1987). Because the transduction current into the OHC is proportional to the difference in voltage between the endocochlear potential (EP) and the OHC intracellular voltage, manipulation of the EP can be employed as a method of investigating these vulnerable aspects of cochlear functioning.

One method of manipulating the EP is the injection of furosemide. With intravenous (I.V.) injection, the EP drops within seconds to a dose-dependent minimum, usually recovering completely within an hour (e.g., Kusakari *et al.*, 1978). Simultaneous measurement of EP and single eighth nerve afferent response found that the effects on frequency tuning curves (FTC) apparently followed the changes in EP (Sewell, 1984a,b). The sensitive tip of the tuning curve was most affected, and the change in best frequency (BF) threshold appeared to closely follow the EP during the recovery phase. The low frequency tail of the tuning curve was found to be less affected by the furosemide injection. Direct measurements of basilar membrane (BM) motion with furosemide injection indicate that the most obvious tuning changes are due to changes in the mechanical response of the cochlea (Ruggero and Rich, 1990, 1991). At BF, the relationship between the stimulus and resulting BM motion became linear at maximum effect. This was due to a substantial reduction in response at low stimulus levels, with little change at high levels. At frequencies well below BF, the response at all stimulus levels was relatively unchanged.

While otoacoustic emissions have been considered primary evidence for the existence of an active process in the cochlea (Kemp, 1978; Patuzzi and Robertson, 1988), there have been no previous studies which have simultaneously measured the changes in EP and distortion product otoacoustic emissions (DPE) with furosemide injection. DPEs, evoked when two sinusoids (frequencies f_1 and f_2) are introduced in the ear canal, have been increasingly employed as a noninvasive probe of vulnerable cochlear function (e.g., Brown and Kemp, 1984; Martin *et al.*, 1987; Norton *et al.*, 1991; Probst *et al.*, 1991). Unfortunately, it has gradually become evident that the validity of DPEs as a measure of such function depends strongly upon the stimulus intensities (L_1, L_2) employed (e.g., Rubel and Norton, 1991; Schmiedt and Adams, 1981). Further, it has not been clear which ratios (L_1/L_2) of the two stimulus intensities would be the most sensitive to a degradation of cochlear performance; equal intensity amplitudes have most often been employed without any particular justification.

In the current study, the EP was monitored while DPEs were measured for a number of different stimulus pairs, $L_1 \times L_2$, with each stimulus amplitude varied independently over a wide range. In addition to the cubic dis-

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tortion tone (CDT,2 f_1-f_2), several other distortion frequency components were recorded and analyzed. To increase the time available for the parametric measurements, it was decided to employ intraperitoneal (I.P.) furosemide injection rather than I.V. injection.

It has not previously been shown that there is a detailed, second-by-second correlation between the amplitudes of specific distortion components and proper cochlear functioning. The existence of sensitive, sharp tuning is a reasonably direct, noncontroversial measure of cochlear functioning. Therefore, a parallel series of measurements was also conducted, using I.P. furosemide injection under the same conditions employed here, to simultaneously measure DPEs in the ear canal and FTCs in the anteroventral cochlear nucleus. The results of these measurements, which are reported separately (Mills *et al.*, 1993; Rubsamen *et al.*, 1992, 1993), demonstrate that there is a virtually perfect temporal correlation between BF thresholds and specific DPE amplitudes. These "vulnerable" DPEs include the CDT and fifth-order term ($3f_1-2f_2$), both *at low stimulus levels.* This makes it possible to interpret these DPEs in the present experiments directly as a measure of cochlear functioning.

I. METHODS

A. Animal preparation

Young adult gerbils (*Meriones unguiculates*) 60–90 days of age were obtained either from a commercial supplier (Tumblebrook Farms, Brookfield, MA) or from breeding pairs in our colony originally obtained from the same source. All animal preparation and recording was performed in an I.A.C. double-walled acoustic booth, maintained at 35 °C. This was found necessary to eliminate the precipitous drop in core temperature otherwise caused by anesthesia, and to moderate the differential cooling of the exposed cochlea. This latter effect was found to be a potential problem in preliminary experiments, when the cochlea was exposed ventrally to air at normal room temperature while the anesthetized animal was heated dorsally (Nuttall and La Rouere, 1980). Animals were initially anesthetized with an I.P. injection of a mixture of ketamine hydrochloride (Ketaset; 15 mg/kg) and xylazine (Rompun; 5 mg/kg). A surgical depth of anesthesia was maintained by subcutaneous injections of ketamine (5 mg/kg) and xylazine (2 mg/kg) as needed. Lactated Ringer's solution (40 cc/kg) was injected subcutaneously at 2-h intervals.

After making a sagittal incision in the scalp, two small screws were placed in the calvarium, and the animal was attached to a custom-designed headholder using denture lining material (Truliner). A thermocouple was inserted in the rectum, and the temperature of the entire sound room changed as necessary to maintain the animal's core temperature at 35–37 °C. A reference electrode (World Precision Instruments, MERE 2) was surgically placed in a thigh muscle. The pinna, surrounding skin and outer portion of the external ear canal were removed unilaterally. Using an operating microscope, the external ear canal was checked for debris and the tympanic membrane was inspected to insure that it was intact and clear. An Etymotic microphone/probe tube assembly (ER-10B) was coupled to the ear canal with a short (0.5 cm) rubber tube. The bulla on the same side was reached using a ventral approach. Specifically, an incision was made from mandible to sternum, a tracheal cannula inserted (artificial respiration was not needed) and tissue removed over the bulla. Custom-designed retractors attached to the headholder were used to pull aside the mandible and trachea to open a wide field to the bulla. After the ventral wall of the bulla was opened, a small hole was carefully drilled in the basal turn of the cochlea. A micropipette (filled with 0.1 to 3 M KCl, with resistance $\cong 5$ MΩ and a Ag–AgCl electrode) was inserted into the scala media. The location was approximately the same as the 3-mm basal location described by Schmiedt and Zwislocki (1977).

B. Stimulus generation and data recording

Acoustic stimuli, measurements, and data analysis involved a custom-designed system which included a host computer (Macintosh IIfx), a digital signal processing board (Spectral Innovations) and three channels for analog to digital conversion and two for digital to analog conversion. The system allowed the simultaneous real-time fast Fourier transform (FFT) analysis of two channels carrying significant frequency components to 40 kHz, plus a third low-frequency measurement channel. Two tones (frequencies f_1 and f_2) were generated by this system and delivered through a remote-controlled attenuator to two Etymotic ER-2 transducers. These were connected through small diameter (high-impedance) tubing to an Etymotic ER-10B microphone coupler, which was sealed directly to the ear canal. Signals from the microphone were fed back though a remote-controlled preamplifier and bandpass filter to one of the high-frequency channels. Closed cavity tests showed that the total instrumental distortion was at least 70 dB below the primaries in all cases.

Electrical signals from the micropipette inserted into the scala media were fed to an ac/dc preamplifier (Grass P16). The ac output (the cochlear microphonic, CM) from this preamplifier was amplified further and delivered to the other high-frequency channel, while the dc output (EP) went to the third analog-to-digital input.

A synchronous detection system was employed: The stimulus signals f_1 and f_2 were synthesized at frequencies which were (different) integer multiples of 12.2 Hz, a frequency corresponding to a period of 0.082 seconds. Taking samples of both return signals, DPE and CM, at the sample rate of 200 kHz filled two 16 384-point arrays in this time period. Because this time period was equal to a multiple of the period of the total stimulus waveform, and all waveforms of interest also had this same periodicity (i.e., the stimuli and all of the distortion products), the signals from the cochlea could be averaged for any length of time which was an exact multiple of 0.082 s. The noise floor could then be reduced by increasing the averaging time without any increase in storage space or time to accomplish the FFT.

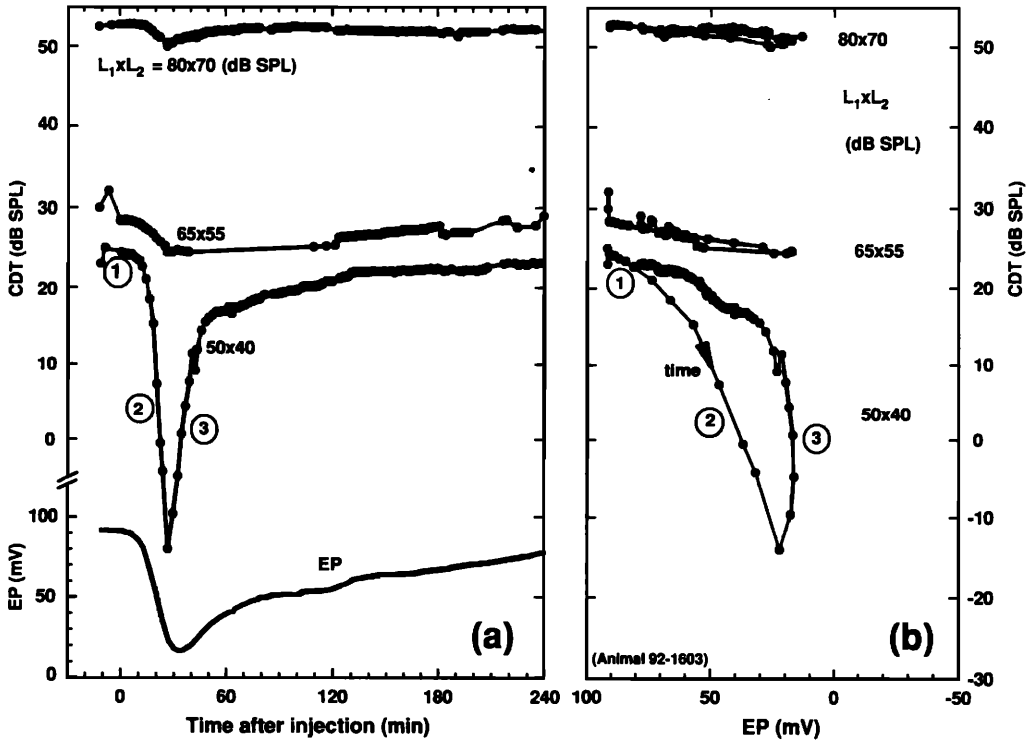

FIG. 1. Results for injection of 78 mg/kg I.P. furosemide. (a) Endocochlear potential (EP) as a function of time compared to cubic distortion tone (CDT) emission intensities observed for three different stimulus levels, for all of which stimulus amplitude L_1 was 10 dB higher than L_2. Stimulus frequencies were $f_1=6.8$ kHz, $f_2=8$ kHz for all data. (b) CDT intensities shown as a function of the simultaneously measured EP. The arrowhead denotes the direction of increasing time. Note that the time may be determined for any point in (b) by locating the corresponding point directly across in (a). See text for references to segments labeled 1–3.

C. Procedures and criteria for inclusion of data

Baseline DPEs were measured as soon as the microphone was coupled to the ear canal. Equal amplitude primaries with intensities stepped from 30 to 80 dB SPL in 5-dB steps were used, with $f_1=6.8$ kHz and $f_2=8$ kHz. After the core temperature was stabilized, if the amplitude of the CDT emission was not at least 10 dB SPL with 50-dB SPL stimuli, the preparation was discarded. The CDT levels were typically remeasured after each major surgical procedure. There was generally a small increase in emissions at all stimulus levels when the bulla was opened (Schmiedt and Zwislocki, 1977), and sometimes a slight decrease for low-stimulus levels after the hole was drilled in the cochlea. These changes did not disqualify the preparation unless the resulting CDT emission dropped below the 10-dB SPL criterion established for the 50-dB SPL equal level stimuli.

If the measured EP was not at least 70 mV immediately after insertion into the scala media, the preparation was discarded. After insertion of the electrode, it was required that both the DPEs and EP be stable for one-half hour before data collection began. A single I.P. injection of furosemide (Lyphomed) of dosage 75 to 100 mg/kg was then given. If this did not produce an EP decrease of at least 40 mV within one-half hour, an additional injection was usually made, but data from such multiple injection cases are not included in this report. The DPEs and EP were monitored for a minimum of four hours and in some cases up to 6 h following furosemide injection. If the EP had not recovered to within 30 mV of the preinjection value by 6 h, the preparation was discarded.

D. Stimulus parameters and emissions recorded

For all measurements, stimulus frequencies were $f_1=6.8$ kHz and $f_2=8$ kHz ($f_2/f_1=1.18$). The associated stimulus amplitudes L_1 and L_2 were typically varied independently over the range 30 to 80 dB SPL. The precise sequence of amplitudes varied across animals, with a total time for each sequence of 2 to 3 min. The averaging times were usually also preset, depending on stimulus levels, and typically ranged from 1 to 8 s. The resulting noise floor was -30 dB SPL in the acoustic channel at the longer times. The stimulus amplitudes were monitored on each presentation, and automatically recalibrated if either measured amplitude was more than 1 dB from that desired.

Amplitude and phase information in both acoustic and CM channels were recorded for each stimulus presentation for each of four different DPEs. These were: (1) $2f_1-f_2$, the third-order term usually known as the cubic distortion tone (CDT); (2) $3f_1-2f_2$, the fifth-order term having, like the CDT, an emission frequency below the two primaries; (3) $2f_2-f_1$, the third-order term with an emission frequency above both primaries; (4) f_2-f_1, the simple difference tone.

The EP was recorded at the same time as each stimu-

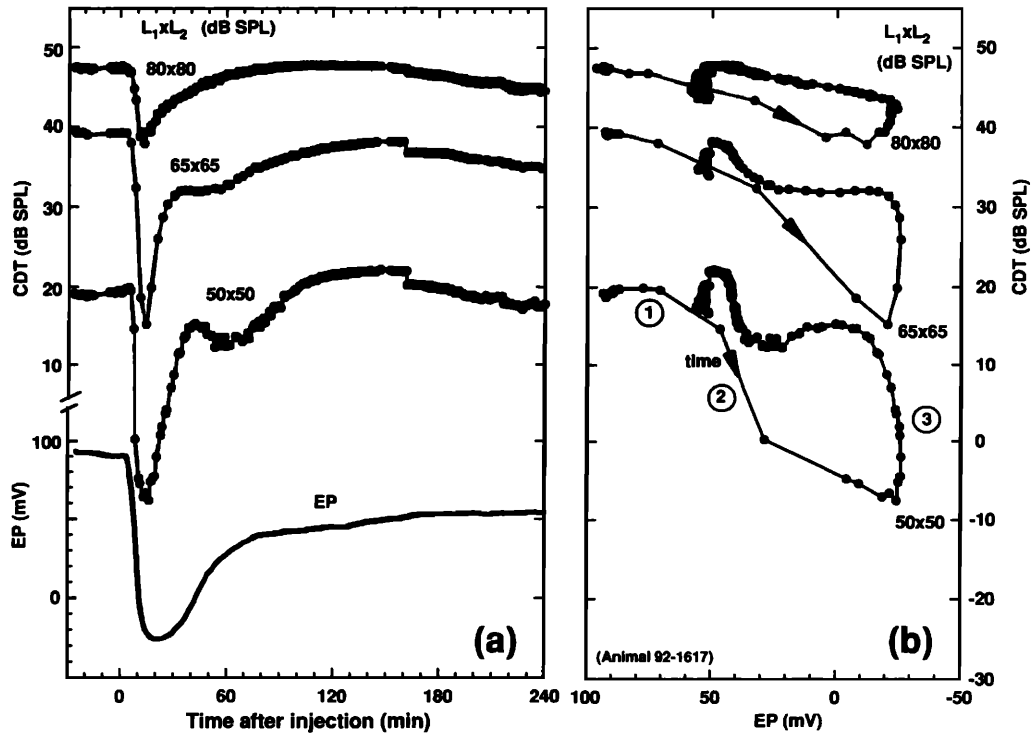

FIG. 2. Results for injection of 100 mg/kg I.P. furosemide. (a) Endocochlear potential (EP) and cubic distortion tone (CDT) emission intensities as a function of time for three different stimulus levels, for which stimulus amplitudes L_1 and L_2 were equal. (b) CDT intensities shown as a function of the measured EP. Arrowheads denote the direction of increasing time. See text for references to segments labeled 1–3.

lus presentation; it therefore includes the (usually quite small) dc shift known as the summating potential.

II. RESULTS

A. Overview: Variation of EP and CDT with time

There were five single-injection gerbil preparations which met all criteria for acceptable data. There were also many other partly successful preparations and successful multiple injection cases which support these results, but only data from these five cases are included here.

Characteristic results for two different animals are presented in Figs. 1 and 2. The variation of EP with time is shown in the bottom portion of Figs. 1(a) and 2(a), with the CDT intensities for three different stimulus levels above. It is clear that the CDTs resulting from lower stimulus levels responded most dramatically, with intensities decreasing 40 dB or more with a 70-mV drop in EP. Surprisingly, it was observed that these vulnerable DPEs began to recover even before the EP reached minimum, and generally recovered fully well before the EP did. This can be seen most clearly in Figs. 1(b) and 2(b), where the variation of CDT amplitude is plotted as a function of the EP measured at the same time. The arrowheads denote the direction of increasing time. Three different segments in the response trajectories can be noted. First the CDT emissions dropped very little or not at all as the EP began to decrease (segment 1). Then, the emissions dropped more sharply as the EP continued to fall (segment 2). Then, the CDT recovered nearly completely while the EP was near minimum (segment 3).

As shown in Figs. 1(a) and 2(a), the EP dropped more slowly with I.P. injection, and did not drop as much, compared with I.V. injection of the same amount as reported in previous investigations (compare Figs. 1 and 2 with Kusakari *et al.*, 1978). The effect of I.P. furosemide injection also was more variable than I.V. injection, and occasionally did not even produce a substantial drop in EP. With almost all successful I.P. injections, the EP returned to normal ranges *much* more slowly than with I.V. injection. An unintended benefit of the slow recovery was that it produced a clear separation of the response of the cochlea into distinct phases, and made it more obvious that the DPEs in response to low-level stimuli recovered well before the EP did.

B. Variation of distortion components in ear canal and scala media

The typical variation observed in amplitude and phase for different distortion frequencies measured in the ear canal and in scala media is shown in Fig. 3, for stimulus amplitudes $L_1 \times L_2$ equal to 55×45 dB SPL. As in the first two figures, the left sides of Fig. 3(a) and (b) display the variation with time, while the right sides plot the trajectories of the distortion component amplitudes versus the simultaneously measured EP. The amplitude response is given on both sides by the larger filled circles connected by solid lines, while the phase response is shown on the left side only, indicated by smaller dots. Upward phase changes indicate an increase in phase lead (a decrease in

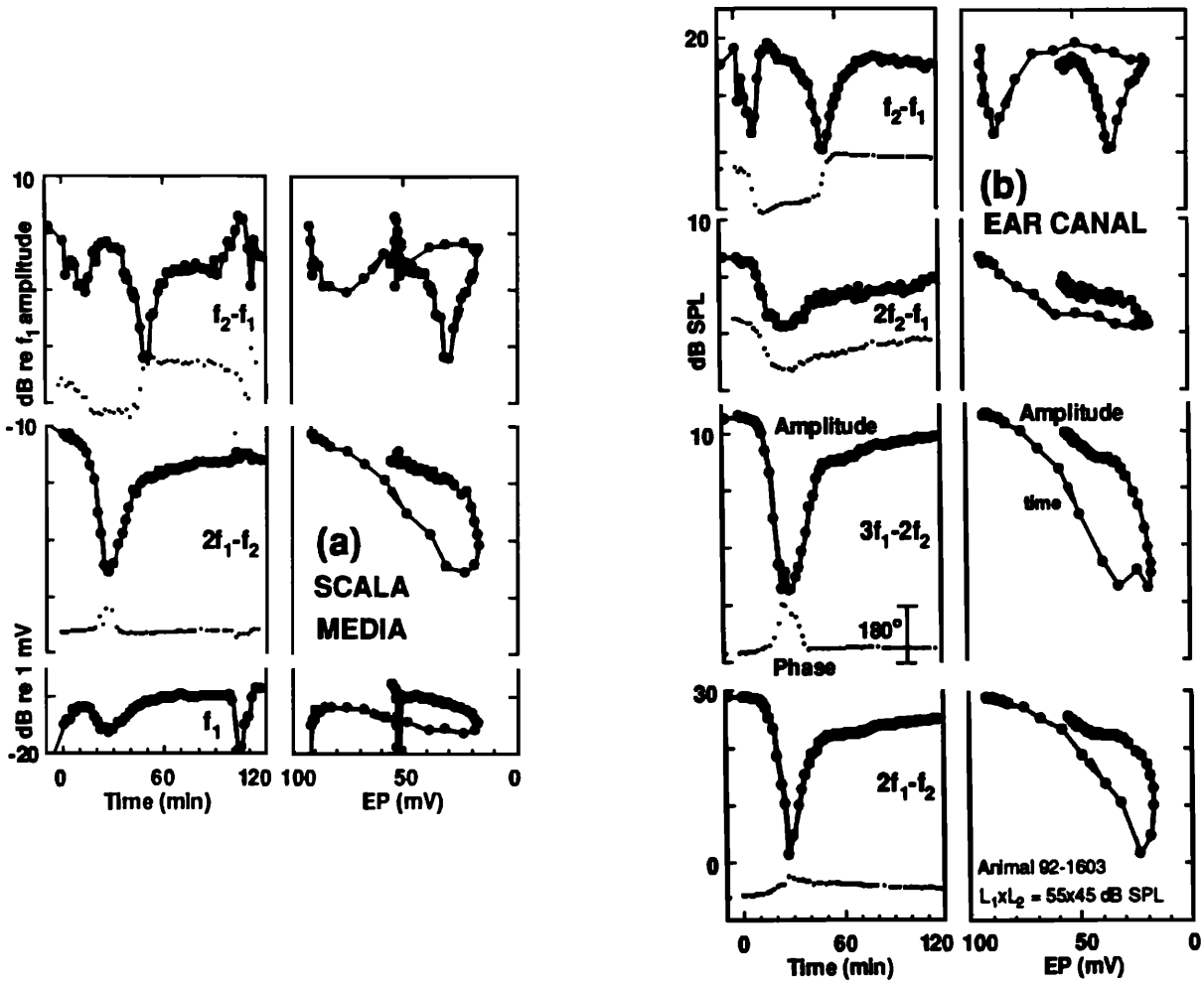

FIG. 3. Results for injection of 78 mg/kg I.P. furosemide for different distortion product frequencies measured in the ear canal and in the scala media. Stimulus amplitudes were $L_1 \times L_2 = 55 \times 45$ dB SPL. On the left side is plotted the time variation of the amplitude of each component, with lines connecting data points. The intervals on the vertical axis correspond to 10 dB in amplitude. The corresponding phase observed for each component is shown immediately below the amplitude, in smaller points without connecting lines. The phase angle reference level is arbitrary; a decrease in phase lag (an increase in lead) is represented by an upward direction on the figure. The scale bar indicates 180 deg. On the right is plotted the amplitude of each component versus the simultaneously measured EP. (See Fig. 1 for variation of EP with time.) Data are included for 2 h following injection. (a) The distortion components measured in the scala media (cochlear microphonic). Only frequency components which were sufficiently above the noise floor are shown. The amplitude of f_1 is given in dB *re*: 1 mV, the amplitudes of the distortion component frequencies are given *relative* to the measured f_1 amplitude. (b) Amplitude and phase variation for all distortion components recorded in the ear canal.

phase lag); the reference level is arbitrary. These data are from the same animal as in Fig. 1, and the EP variation with time was the same as in that figure.

The amplitude variation of the CDT [lowest section of Fig. 3(b)] was essentially the same as in Fig. 1, which was for slightly lower stimulus amplitudes. The phase angle increased slightly (the phase lag decreased) near the minimum amplitude. The behavior of the fifth-order term $(3f_1-2f_2)$ shown in the next section up was generally very similar to that of the CDT. The same three phases were observed in the amplitude trajectory, with recovery essentially complete with a subnormal EP. In this case, however, there was in addition a sharp phase increase of about 180 deg near the minimum, with an associated "bottoming out" of the amplitude decrease. Similar behavior was also occasionally observed in the CDT term depending on the stimulus levels and animal; for example, the bottoming out of the amplitude seen for the 50×50 level in Fig. 2 was also associated with a 180-deg increase in phase angle (not shown).

The variation of the other cubic term $(2f_2-f_1)$, which has its emission frequency above both primary frequencies, is shown in the next section up. The amplitude behavior was clearly related to the vulnerable emissions in the lower two sections, but lacked the sharp decrease seen in both of these. Unlike the vulnerable components, however, the phase angle changed substantially, but smoothly, over time. There was a smaller recovery of amplitude with a subnormal EP, in proportion to the smaller decrease originally caused by the injection.

The behavior of the simple difference tone (f_2-f_1) shown in the top section of Fig. 3(b) was completely different from all of the other components. The amplitude dipped sharply before there was a significant change in any of the other components, with an associated phase change of about 180 deg. At the time at which the vulnerable

components recovered to an initial plateau, the difference tone went through another apparent zero, i.e., the amplitude had a sharp minimum and the phase shifted abruptly about 180 deg. The trajectory plotted on the right side clearly shows the difference in this distortion product. At this stimulus level, the measured amplitude of the simple difference tone was generally second only to the CDT amplitude, of the distortion products recorded in the ear canal. Across animals, the first sharp minimum was not always obvious, but the second minimum was fairly consistently observed to occur at about the same time as the "overshoot" or plateau in the recovery of the vulnerable components.

The amplitude and phase of frequency components as measured by the electrode in scala media (CM) are shown in Fig. 3(a). The amplitude of the recorded f_1 primary signal is shown in the bottom section of Fig. 3(a). The slow variations (and occasional fast changes) shown were commonly observed in the recorded amplitudes for *both* f_1 and f_2; there were no corresponding changes in the recorded phases, which were quite stable. The origin of these recorded amplitude variations is not known. Above the f_1 signal are plotted the amplitudes of the distortion components *relative* to the amplitude of the f_1 signal recorded at the same time.

At this stimulus level, only two of the distortion frequency components were sufficiently above the noise floor in scala media to be tracked over the 2-h time: These were the simple difference tone (f_2-f_1) shown at the top of Fig. 3(a), and the cubic distortion tone ($2f_1-f_2$) immediately below. It can be seen that the amplitude and phase responses of both frequency components as measured in the scala media were very similar to the corresponding frequency components measured in the ear canal, when the variations due to overall fluctuations in the measured CM amplitude were subtracted.

C. CDT trajectories—group data

Figure 4 presents the CDT emission trajectories versus EP for all five animals meeting the criteria noted above, for stimulus amplitudes $L_1 \times L_2 = 50 \times 40$ dB SPL. Amplitudes are offset vertically at 30-dB intervals for clarity; the initial CDT amplitude is listed on the left ordinate in dB SPL. The lower four animals were all injected with approximately 75 mg/kg furosemide; the top one with 100 mg/kg. The time in minutes from injection to the minimum CDT amplitude is shown below each trajectory; the horizontal arrow indicates a time 15 min after this minimum.

It can be clearly seen that the response of the lower two animals was qualitatively different than the upper three. Even though they met minimum criteria, they just barely met the preinjection CDT amplitude criteria, and the preinjection emissions at this stimulus level were about 10 dB lower than the other cases. It seems likely that these two examples (which were the first in the series) illustrate the effect of small, but significant, damage to the cochlea due to surgery and insertion of the electrode. Even so, there was a small amount of recovery evident with a subnormal EP. Overall, however, the cases with presumed minor damage did not illustrate clearly the three phase response clearly noted in the top three cases, and the response was more reminiscent of the response at higher stimulus levels, as seen in Figs. 1 and 2.

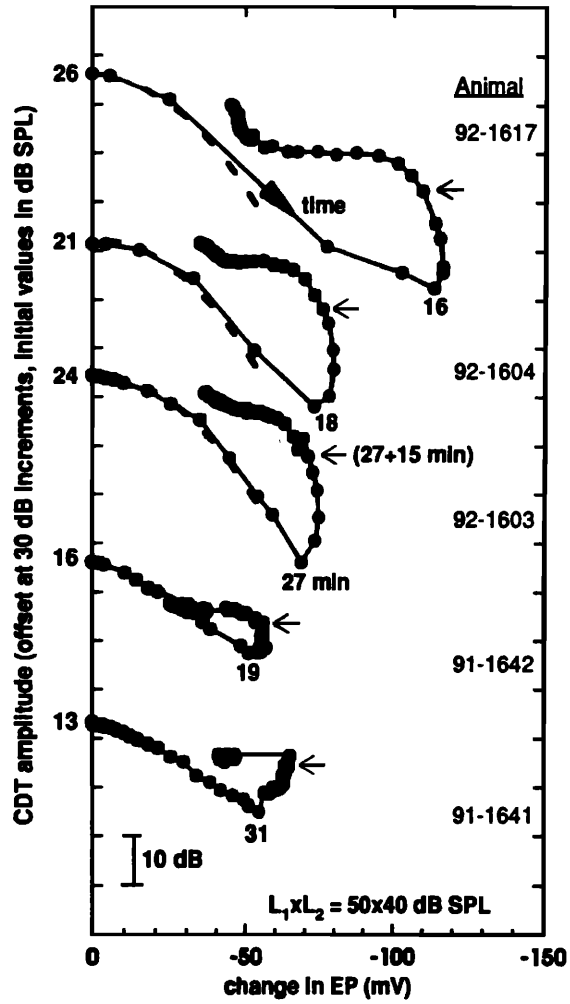

FIG. 4. Results for all animals for the cubic distortion tone for stimulus amplitudes $L_1 \times L_2 = 50 \times 40$ dB SPL. Data are shown for a 2-h interval from the time of injection. The change in CDT amplitude is plotted versus the simultaneously measured EP change. The initial CDT amplitude is shown on the left axis, and the time from injection to reach the minimum CDT amplitude at the bottom of each curve. The open arrowheads indicate the point 15 min after this time of minimum. The dashed lines give for comparison with the initial observed decline the parabolic trajectory given by Eq. (1).

For comparison to the data, the dashed lines shown in the top three curves give the parabolic trajectory

$$\Delta\mathrm{CDT(dB)} = -0.009[\Delta\mathrm{EP(mV)}]^2, \qquad (1)$$

i.e., the change in the CDT amplitude in dB is proportional to the square of the change in EP in mV. Note that the constant of proportionality is the same for each curve.

The shape of the CDT versus EP function was remarkably constant for the three most successful animals even though the amount of furosemide, time to the minimum, and minimum EP reached were different for different animals. The initial trajectories for all three fit Eq. (1) quite

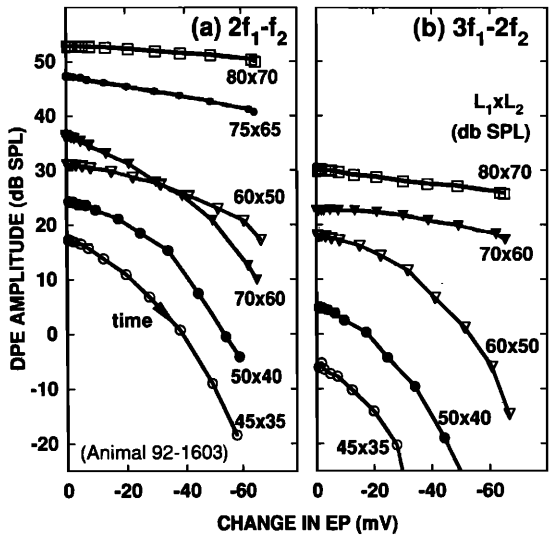

FIG. 5. Initial decrease of distortion product intensities with endocochlear potential (EP) for one animal (same case as Fig. 1) at different stimulus amplitudes $L_1 \times L_2$, all for $L_1 = 3.2L_2$. Data are included from the time of injection to the time that the EP reached 90% of its maximum decrease. (a) The amplitude of the cubic distortion tone $(2f_1 - f_2)$; (b) amplitude of the fifth-order term $(3f_1 - 2f_2)$.

well, and the degree of recovery 15 min past the time of minimum CDT amplitude was approximately the same for all three animals.

D. Initial DPE trajectories—different stimulus levels

Figure 5 displays the initial decrease in distortion product intensities as a function of stimulus level for the two vulnerable components, the CDT $(2f_1 - f_2)$ and the fifth-order term $(3f_1 - 2f_2)$. Data are shown from the time of injection until the time that the EP had reached 90% of its maximum decrease. It can be seen that the change at lower stimulus levels was parabolic in form, with a curvature that increased with a decrease in stimulus levels. The curvature also increased somewhat for the fifth-order term relative to the third, at least comparing the lower stimulus levels. This was also evident in Fig. 3, comparing the lowest two trajectories on the right side.

E. Variation of CDT input–output functions with time and EP

Figure 6 displays the measured CDT "input–output" functions, i.e., "growth" functions obtained by plotting the CDT amplitude over a range of stimulus levels with fixed L_1/L_2 ratio. Figure 6(a) shows results for the case $L_1 = L_2$, and Fig. 6(b) for L_1 10 dB higher than L_2 $(L_1 = 3.2L_2)$. Note that the emissions at high stimulus levels did not change much (in dB) even with massive changes in EP, while the CDT emissions at lower stimulus levels were sharply reduced with the drop in EP.

The contrast between the response for high and low stimulus levels was more dramatic for stimulus amplitudes $L_1 = 3.2L_2$ [Fig. 6(b)] than for equal amplitudes. At low stimulus levels, the preinjection emissions were higher for the unequal than for the equal amplitudes (comparing the

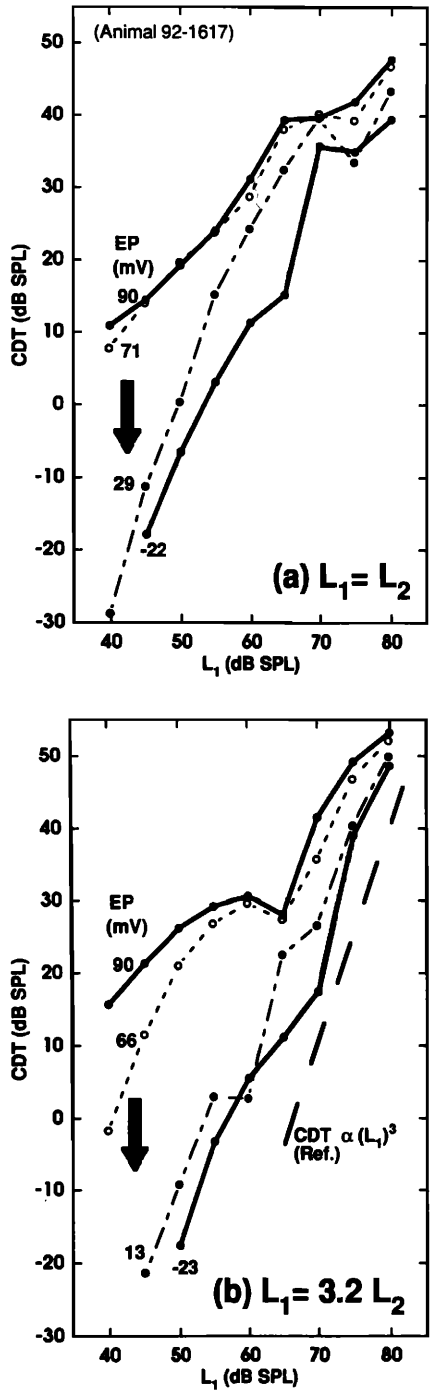

FIG. 6. Cubic distortion tone (CDT) intensity as function of input stimulus level ("growth functions") during the initial fall of endocochlear potential (EP) for (a) equal stimulus amplitudes, and (b) stimulus amplitude L_1 10 dB higher than L_2. The dashed line gives the slope expected in the purely passive case for which the CDT intensity is proportional to $(L_1)^3$—see Eq. (2). (Same case as Fig. 2.)

same L_1, even though L_2 was 10 dB less for the unequal amplitudes). In contrast, near the EP minimum the same emissions were lower for the unequal than for equal amplitudes. Note also the existence of a "dip" in the growth function in Fig. 6(b) at $L_1 = 65$ dB SPL at normal EP. For the stimulus frequencies used in these experiments, this dip or notch was always observed at about the same stimulus levels (i.e., for some L_1 in the range 65–70 dB SPL and for all $L_2 < 60$ dB SPL).

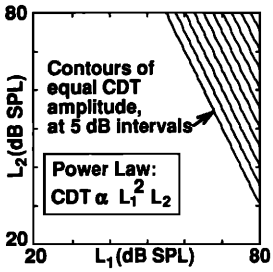

FIG. 7. Illustrates use of a contour map to display the response of a distortion product as a function of both inputs L_1 and L_2 at a given time. Displayed are the contours of equal CDT amplitude which would result if the emission followed the power law given by Eq. (2). Contours are only shown above an arbitrary reference level. This display also serves as a key to the next two figures, in which the lowest CDT contour shown is at 0 dB SPL, with subsequent lines at 5-dB increments above that level.

For a power law nonlinearity the emission from two sinusoids at frequencies f_1 and f_2 and amplitudes A_1 and A_2 at the frequency $|pf_1 \pm qf_2|$ is

$$\text{DPE amplitude} \propto A_1^p A_2^q. \tag{2}$$

For a system without two-tone suppression, the amplitudes A_1 and A_2 would be, respectively, proportional to the stimulus amplitudes L_1 and L_2. The CDT intensity, for example, would then be proportional to L_1^3 for fixed L_1/L_2 ratio. At the maximum effect of furosemide, the CDT emissions approached this relationship [dashed line in Fig. 6(b)].

F. CDT contour maps with time and EP

In addition to examining the behavior of emissions for different "cuts" through the two dimensional space $L_1 \times L_2$, it can be useful to look at the contours of equal emission intensities over the entire stimulus space. For reference, Fig. 7 illustrates the CDT contours which would be expected if the emission were given by Eq. (2). This figure also serves as a key to the sequence of contour maps shown in Fig. 8, which are for the same animal as in Fig. 2. For each contour map, the lowest contour is the 0 dB SPL CDT, and subsequent contours are shown at 5-dB intervals of CDT amplitude. The time relative to injection and measured EP associated with each contour map are indicated on each panel. For reference, the entire trajectory for inputs $L_1 \times L_2 = 50 \times 40$ dB SPL is given at the bottom of the figure, with corresponding points referring to contour maps above. A cross marks the 50×40 point in each contour map.

It can be seen that, in the normal preinjection case [Fig. 8(a)] the emissions to low level stimuli (lower left quadrant) were maximal along a ridge, which lay approximately along the line where L_1 was 10 dB higher than L_2. It was this ridge which was most affected by the drop in EP. At eleven minutes postinjection [Fig. 8(b)], with an EP of about -12 mV, the ridge had essentially disappeared, and the contours across the entire space of stimulus amplitudes were approximately given by the power law, Eq. (2).

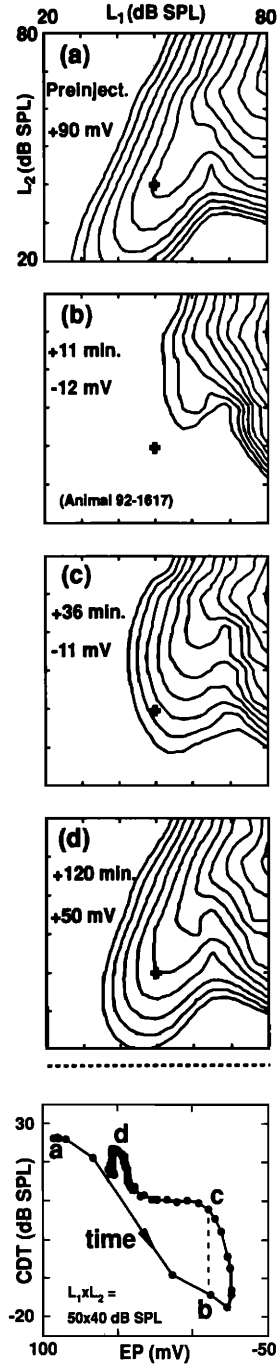

FIG. 8. Contours of equal intensity cubic distortion tone (CDT) as function of stimulus amplitudes L_1 and L_2, shown at four different times (a)–(d). Contours begin at 0 dB SPL and increase in 5-dB increments. The times relative to the time of injection and the measured EP are noted on each contour map. For reference, the bottom figure displays the measured variation of CDT amplitude with EP, noting the corresponding times for which contour maps are displayed. The stimulus amplitudes for this figure are $L_1 \times L_2 = 50 \times 40$ dB SPL; the location of this level is indicated by a cross on each of the contour maps above. Same animal as Figs. 2 and 6.

Figure 8(c) shows that 15 min later the emissions had substantially recovered throughout the $L_1 \times L_2$ space. The recovery occurred even though the EP had only returned to the same level as in Fig. 8(b). Finally, Fig. 8(d) shows that at 2 h postinjection, with the EP still only 50 mV, the

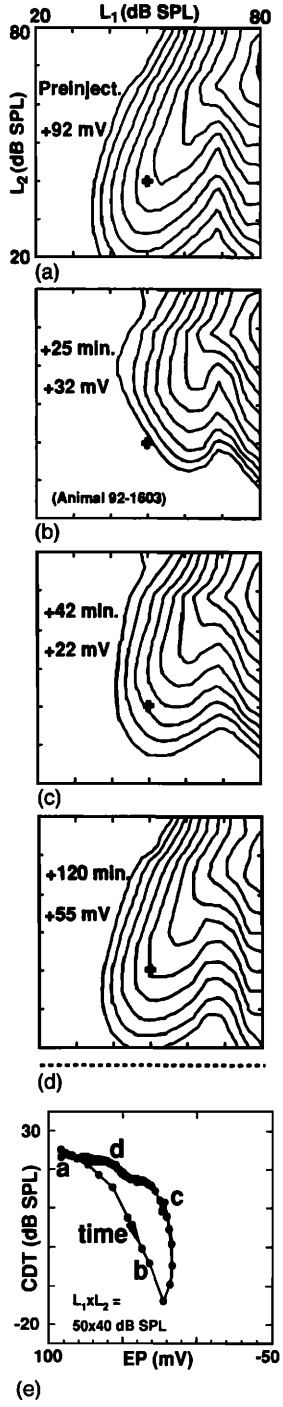

FIG. 9. Same display as Fig. 8, for case presented in Figs. 1 and 3.

CDT emission had recovered completely through most of the stimulus input space. The exception was the area where both stimulus amplitudes were very low, in the lower left of the contour map.

Figure 9 illustrates the variation in contour maps with time for an animal with a slightly lower, more characteristic injection level. In this case, the emissions generally did not drop as much, and at minimum [Fig. 9(b)] did not approximate a power law distribution. However, with the EP not decreasing to negative levels, the recovery was more complete. The emissions at two hours were essentially the same as the preinjection emissions over the entire $L_1 \times L_2$ space, even though the EP was only 55 mV. Notice again that the emissions in Fig. 9(c) recovered substantially compared to those near minimum [Fig. 9(b)] even though the EP was actually 10 mV less.

III. DISCUSSION

A. Primary site of action of furosemide

While it is possible that furosemide affects cochlear function by direct cellular effects on, for example, OHCs, the available evidence indicates that furosemide at the dosages employed in these experiments affects cochlear functioning primarily through the change in EP (Akiyoshi, 1981; Comis *et al.*, 1990; Forge and Brown, 1982; Kalinec and Kachar, 1992; Kusakari *et al.*, 1978; Ruggero and Rich, 1991; Ryback and Morizono, 1982; Rybak, 1986). The results of the present study provide some additional support for this hypothesis. The most important argument comes from the consistent trajectories observed in the variation in DPEs plotted as a function of the measured change in EP (Fig. 4). The consistency of the trajectories, considering differences in furosemide dosage, the extent of EP change, and the time scale of the change, argue that the EP—not a direct action on hair cells—is the major effect of furosemide injection. One would expect much more scatter of the data *plotted as a function of EP change* if the major effect was due to some other, more direct effect of furosemide on hair cell function.

Further, furosemide has been found to have no effect on EP *or* tuning in the pigeon (Schermuly *et al.*, 1990). In the current experiments, DPEs with the same parameters (except that f_2 was 2 kHz) were also measured in chickens. There were no significant changes observed in DPEs even with multiple I.P. injections of furosemide at dosages of 150 mg/kg. If the major effect of furosemide was a direct action on vertebrate hair cells which affected cochlear performance, some such effect might have been expected in birds even with no change in their EP.

B. Possible effects of EP change on cochlear functioning

Additional support for the hypothesis that the major effect on the cochlea is the EP change can be obtained by considering the likelihood that such a change could indeed affect cochlear functioning in the manner observed. A decrease in EP would obviously directly decrease the transduction current into the inner hair cells, resulting in a decrease in afferent output for a given BM motion. However, with typical EP decreases found in this study, this effect would account for threshold increases of only a few dB and would affect all frequencies and stimulus levels equally. It also would not change the BM motion at all. It therefore cannot account for the main effect, i.e., the selective decrease in the BM motion near BF with furosemide injection (Ruggero and Rich, 1990, 1991).

It seems much more likely that the main effect of furosemide is a change in the EP which affects the cochlear response through the OHC involvement in the "cochlear amplifier" (CA). The CA is the name given to a proposed

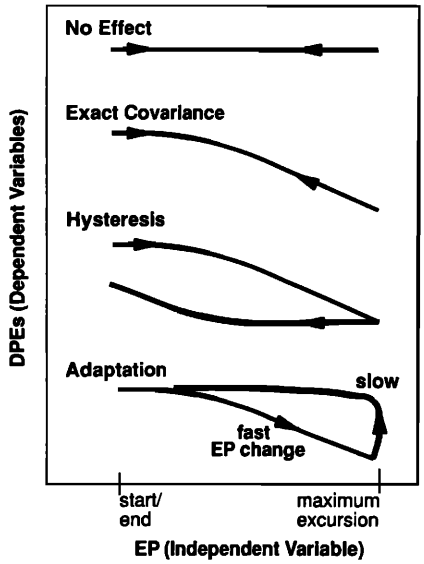

FIG. 10. Hypothetical trajectories of dependent variables versus independent variable. The independent variable is assumed to begin at the left, change to reach maximum excursion to the right, then return to its starting point. Arrows denote direction of increasing time. See text for discussion of trajectory labels.

mechanical feedback loop which acts to enhance the BM peak response (Davis, 1983; Gold and Pumphrey, 1948; Gold, 1948). One proposed mechanism for this feedback process involves OHC fast motility (Ashmore, 1987; Dallos *et al.*, 1991). The force produced by the OHC is thought to be proportional to the OHC membrane voltage change, which at a given frequency is proportional to the transduction current into the cell. A decrease of EP would therefore be expected to decrease the transduction current, and could thereby interfere with the operation of the cochlear amplifier.

Modeling studies (e.g., Neely and Kim, 1986) typically show that the cochlear amplifier affects BM motion substantially only near BF. Therefore, the observation that the change in EP affects cochlear functioning, and selectively affects the tip of the tuning curve more than the tail (Sewell, 1984a,b; Ruggero and Rich, 1990, 1991) is mutually compatible with the existence of a cochlear amplifier whose driving force depends on the EP. The observations made here that the functioning of this amplifier is not only vulnerable to a change in EP, but *recovers* with a subnormal EP has additional implications which will be considered next.

C. Interpretation: Evidence for adaptation?

If the major effect of furosemide on hair cell function is through the EP, then the typical trajectories found for the vulnerable DPEs as a function of EP provide some challenges for their interpretation. General categories of possible trajectories are illustrated in Fig. 10. The independent variable is assumed to be changed reversibly, going from its initial value on the left, to the maximum excursion on the right, and back to its initial value. The top curve illustrates the appearance when no effect is observed. The next trajectory illustrates "exact covariance"—there is a one-to-one relationship between the independent variable and the dependent, and no independent variation with any third variable, such as time, for example. The relationship between voltage and current in a resistor is an example of such covariance. In hysteresis, there is a *failure* of the dependent variable to completely return to its initial value when the independent variable is returned to its initial value. A typical example given is the failure of the magnetization in an iron-core transformer to return to its initial value after a current excursion. For hysteresis, the time course may or may not be a confounding variable; in biological systems, for example, there is often repair of damage, which is of course time dependent.

Finally, consider how the trajectories would appear if the dependent variable could *adapt* to the changes in the independent, as for example in a thermostatically controlled heating system. In this case, the observed trajectories depend strongly on the relative values of the time scale for change in the independent variable and the time scale for system adaptation, T_{sys}. If the independent variable is changed very slowly, the system adapts completely at each point and no change in the dependent variable is observed. If the independent variable is changed very quickly, returning to its initial value in a time short compared to T_{sys}, there is no time for adaptation. The observed trajectory looks like exact covariance. However, if there is any effect and the time variation of the independent variable is appropriate, then adaptive effects can be observed. In the example illustrated, the independent variable rapidly moves to its maximum excursion in a time less than T_{sys}, then slowly returns to its initial value, with the time for the return being greater than T_{sys}. This time variation is exactly that which will maximally show the effects of adaptation, and is, of course, very similar to that which was introduced by the I.P. injection of furosemide.

Therefore, the observations with I.P. injection reported here can be interpreted as evidence in favor of an adaptive process functioning in the cochlea. The adaptive process enables the cochlea to return to normal functioning with a subnormal EP. Using model fits to data, the time scale for the adaptive process was estimated to be 15 min. That the time scale must be about this value can be seen by direct inspection of the observed recoveries, such as in Fig. 4, where the time point 15 min past the CDT minimum amplitude is noted.

A possible objection to the interpretation of this data as reflecting adaptation arises from the fact that while the present observations were made for a range of stimulus amplitudes, only one set of stimulus frequencies was employed. The possibility exists that there was something unique about these parameters. Control experiments for the companion experiment using a range of f_2 values from 4 to 8 kHz, all with $f_2/f_1=1.18$, found this not to be the case (Rubsamen *et al.*, 1992, 1993). Further, a followup series of experiments has recently been completed, in which the effects of I.P. furosemide injection were monitored with DPEs for fewer stimulus amplitudes but for a wide range of stimulus frequencies (Mills and Rubel, un-

published). At a given stimulus level, the response was very consistent for a range of f_2 frequencies from 2 to 16 kHz and for frequency ratios f_2/f_1 from 1.1 to 1.6.

Finally, it is important to consider the implications of the proposed adaptive processes for detailed cochlear models. Cochlear models using active feedback require a precise set of feedback parameters to accomplish the effective amplification of traveling waves, in order to match observed tuning curves, etc. (e.g., Neely and Kim, 1986). The need for precise self-regulation of such feedback mechanisms was noted in the original suggestion by Gold (1948). Yet none of the current models have included mechanisms whereby the cochlea can attain and maintain these precise values. Adaptive processes are obvious candidates to provide such mechanisms.

Therefore, the most important function of the adaptive process discovered in this experiment is probably not simply to adapt to changes in the EP. Rather, it would be to provide a mechanism to vary the set point of the OHC feedback process so that it can be adjusted to that which gives the optimal amplification on the BM at the appropriate frequency—given that a number of other parameters are attained (e.g., in development) that cannot be so easily changed. The importance of the existence of an adaptive feedback process may not be the feedback process itself, but that it implies the existence of a set point, which can presumably be easily changed through a feedback system at a higher level of processing.

D. Comparison with other studies: Possible adaptive effects

In this section, the results obtained here will be compared to relevant results of other studies which measured cochlear function when the EP was changed. These include earlier studies which used furosemide to manipulate the EP, those which employed asphyxia or anoxia, and those which employed other ototoxic agents to decrease the EP. Attention will be focused on relevance of the results to questions of adaptation.

1. Other furosemide studies

As mentioned in the introduction, there have been previous measurements of EP and tuning in the eighth nerve (Sewell, 1984a,b). I.V. furosemide injections were used and the EP typically returned to normal ranges within several minutes after the minimum was reached. With this rapid a change in EP compared to a 15-min adaptation time, one would not expect adaptation to be observed, but rather covariation of tuning and EP. This was in fact what Sewell observed. The initial EP decrease was far too fast to determine if the change of tuning appeared to be at a relative maximum, i.e., was delayed compared to the start of the EP decrease. The fact that covariation of tuning and EP was observed with this manipulation—with such an abrupt change in EP—argues further that most of the change in cochlear function observed with furosemide injection is directly due to the change in EP.

There have also been direct measurements of basilar membrane motion with I.V. furosemide injection (Ruggero and Rich, 1990; Ruggero and Rich, 1991). In one such experiment (1991, Fig. 2) the input–output function of the cochlea had recovered to within about 5 dB of normal at 40 min after injection of 50 mg/kg, and completely at 2 h. Unfortunately, the EP was not simultaneously measured in this study, so a direct comparison is not possible. However, the EP typically recovered only to 55–60 mV at 40 min with I.V. injection of this dosage (Kusakari *et al.*, 1978). The direct measurements of BM motion therefore seem compatible with the results obtained here.

There have also been studies of the effect of furosemide injection on the emission of signals with electrical ac stimulation of the scala media (Hubbard *et al.*, 1986; Hubbard and Mountain, 1990). Using somewhat larger dosages (125–150 mg/kg) than used here, these authors typically observed a rebound and overshoot in the emitted signal at about 10–15 min past the minimum in EP. The CM signal also showed sudden dips similar to those recorded here.

2. Asphyxia or anoxia

While cessation of oxygen to the cochlea undoubtedly has many effects, one of the most immediate is a rapid drop of the EP to negative levels, followed by a gradual partial recovery (Schmiedt and Adams, 1981). Earlier experiments, usually employing cardiac arrest, for example, showed an immediate decrease of CDT for low and moderate levels of stimulation (Kemp and Brown, 1984; Lonsbury-Martin *et al.*, 1987; Rubel and Norton, 1991; Schmiedt and Adams, 1981; Whitehead *et al.*, 1992b) While the low-level CDT never returned above the noise floor, there were observed puzzling rebounds of emissions from moderate and higher level stimuli. Precise comparison cannot be made because in all these studies there were no simultaneous measurements of both EP and DPEs for low stimulus amplitudes. Recently, however, Rebillard and Lavigne-Rebillard (1992) presented data using reversible hypoxia. They found that the DPE did not begin to drop for 10–40 s following the start of the EP decline. There were rebounds in CDT amplitude found following brief (several minute) depressions of the EP. However, the magnitude and time scale of changes in EP induced in this experiment—and the resulting changes in CDT—were much smaller than in the present furosemide results, and it is difficult to compare them directly.

3. Other ototoxic agents which affect EP

Ethycrynic acid, a loop diuretic like furosemide, has also been employed to investigate the vulnerability of DPEs as a function of stimulus amplitudes (Whitehead *et al.*, 1992b). These authors also observed rebounds and overshoots at similar stimulus levels and time intervals as in the present experiment, but since the EP was not monitored the results cannot be directly compared.

4. Apparent adaptive effects with similar time scales employing other manipulations

During constant stimulation the difference tone (f_2-f_1) was found to decrease significantly over a period

of about 10 min (Brown, 1988; Whitehead *et al.*, 1991). Kemp (1986) found a brief increase in sensitivity of the cochlea following exposure to a low-frequency intense sound, with a time scale of 2–3 min for the first bounce. A similar rebound was found in spontaneous otoacoustic emissions amplitude following intense ipsilateral sound (Norton *et al.*, 1989). We are not contending that these effects are due to the same mechanisms which cause the adaptive effects observed here. However, it is worth noting that all of these phenomena operate on a time scale of minutes. Also, most of these observations demonstrate a characteristic of adaptive processes in general, which is a tendency for an overshoot or "bounce" to appear as the environmental variable suddenly increases, for example, after it has previously decreased and been adapted to. Such a bounce is clearly seen in Fig. 2 for the 50-dB stimuli at 40 min postinjection, where the EP had increased sharply.

In comparing the results of other studies to those obtained here, it must be emphasized that the effect of even small amounts of damage—amounts of damage which certainly would not make the cochlear response seem grossly dysfunctional—can apparently mask the effects of adaptation seen here (compare the lower two to the upper three cases in Fig. 4).

E. Dynamics of different DPE frequency components with EP change

1. Odd DPE frequency components

Figure 3 summarizes typical differences in responses of the different DPE frequency components at low stimulus levels. The CDT ($2f_1-f_2$) and fifth-order ($3f_1-2f_2$) terms were very alike in their response, both having a substantial relative decrease in amplitude at maximum effect. The frequencies of both, of course, are below both of the primary frequencies. The other cubic term ($2f_2-f_1$) was similar, but did not display the sharp decrease in amplitude. Its frequency is higher than both primaries. This behavior can be interpreted in the following way, using a linear approximation to the BM response to two input frequencies; i.e., assuming that at sufficiently low stimulus levels the BM response is approximately the linear sum of two traveling waves of frequency f_1 and f_2. The change in EP is expected to affect the BM motion only near the sharp peaks of these two traveling waves. Since the two vulnerable emissions are assumed to be generated in the overlap region between the two waves, at or near the f_2 peak, the amplitude of these emissions will be strongly affected by a reduction in the f_2 peak amplitude. However, the other cubic term cannot be generated near the f_2 peak, as its traveling wave cannot propagate on the BM at the place corresponding to the f_2 frequency. This term is presumably generated basal to the f_2 peak, near the place corresponding to its frequency (Martin *et al.*, 1987). Therefore, its generation is not affected as much by a reduction of the f_2 peak itself.

2. Even order term

The response of the even order f_2-f_1 component was obviously very different than the odd order terms. Across animals, the difference tone consistently appeared to go through a sharp minimum or a "zero" about 40–50 min postinjection. As seen in Fig. 3, this zero always occurred at about the same time that the CDT intensity at low stimulus levels went through a nearly zero slope, or a relative maximum. Another apparent zero was also frequently seen a few minutes postinjection (as in Fig. 3) but was not as consistently observed. Our data on the complex response of the even order harmonic is consistent with previous findings (Brown, 1988; Fahey and Allen, 1985; Humes, 1985; Kim *et al.*, 1980; Mountain, 1980; Schmiedt and Adams, 1981; Siegel and Kim, 1982; Whitehead *et al.*, 1991).

While intriguing, the interpretation of the present observations is not certain. A zero in the even order term would occur if the generating mechanism went through a point of locally odd symmetry in the input–output function. Given the complexities of the generation of the DPEs, it is not clear why this should occur for the low stimulus amplitudes just when the odd order terms pass through an apparent relative maximum in their recovery. These observations must nonetheless provide important clues to the generation of the DPEs and their relationships to cochlear mechanics.

3. Vulnerability of DPEs as a function of stimulus amplitudes

The growth functions (Fig. 6) and contour maps (Figs. 8 and 9) clearly show that the vulnerability of the CDT was strongly dependent on the stimulus amplitudes. The decrease was maximal for lower stimulus amplitudes, and for L_2 lower than L_1. At maximal effect [esp. see Fig. 8(b)] the emission was approximately that expected from a passive power law generator. This passive component also appeared to be present with little change in other contour maps, but overlaid by a vulnerable component which dominated at lower stimulus levels. The overall shape of the vulnerable component—a ridge with a center line about at $L_1=3.2L_2$—seems directly attributable to the effects of two-tone suppression. In other words, the amplitude of the emission generator is substantially reduced if either, but not both, of the stimulus tones are large. With L_1 10 dB greater than L_2 outside the cochlea, the "two traveling waves" are about the same amplitude at the point of interaction, near the f_2 peak, giving maximal emission (reasoning from the linear approximation).

The trajectories for the cubic and fifth-order ($3f_1-2f_2$) emissions at the highest stimulus levels seemed different qualitatively than those at the lower level responses (Figs. 1, 2, and 5). They appeared similar to those at all levels for the other cubic term ($2f_2-f_1$, Fig. 3) and for the CDT at lower stimulus levels with apparent minor damage (Fig. 4, lower two curves). One interpretation would be that components with similar responses probably originate in similar regions of the cochlea (i.e., similar regions with respect to the normal f_2 peak).

The observed normal distribution of emissions of the various DPEs for different L_1 and L_2, and their vulnerability, also agree with previous findings, where data exist for different ratios of L_1/L_2 (Brown, 1987; Brown and Gaskill, 1990b; Whitehead *et al.*, 1990; Whitehead *et al.*, 1992a,b; Zwicker, 1981) and for the odd order DPEs in addition to the CDT (Brown and Kemp, 1985; Brown, 1987; Brown and Gaskill, 1990a; Kemp and Brown, 1986; Lonsbury-Martin *et al.*, 1987; Martin *et al.*, 1987).

F. Recommendations for using DPEs to monitor cochlear function

There has been some controversy regarding the physiological vulnerability of DPEs (Brown *et al.*, 1989; Johnstone *et al.*, 1990; Kemp and Brown, 1984; Lonsbury-Martin *et al.*, 1987; Mills *et al.*, 1992; Norton and Rubel, 1990; Rubel and Norton, 1991; Schmiedt and Adams, 1981; Schmiedt, 1986; Whitehead *et al.*, 1992a; Wiederhold *et al.*, 1986). These previous studies can be reconciled if it is recognized that only specific DPEs at low stimulus intensities are adequate indicators of cochlear function, and that adaptation of the cochlea (e.g., to changes in EP) can cause apparently contradictory or confusing results if such adaptation is not taken into account.

From the current study, it can be concluded that the only emissions which are substantially *vulnerable* to the initial EP decrease are the CDT and fifth-order term (with emission below the stimulus frequencies) at low stimulus levels. Taken together with earlier data (Brown, 1987; Brown and Gaskill, 1990a; Kemp and Brown, 1986) and that from a companion study (Rubsamen *et al.*, 1992, 1993), these results may be generalized in the following way. The emissions which are vulnerable to cochlear insult, and which may safely be used in determining the health of the cochlea, are those odd order terms *at low stimulus levels* at the frequencies

$$f_{dp}=(k+1)f_1-kf_2, \tag{3}$$

where $k=1$, 2, etc. These emission frequencies are all below both stimulus frequencies. From the current study, input amplitudes of $L_1\times L_2=50\times 40$ dB SPL or lower are recommended. This estimate has been justified more quantitatively using a simultaneous determination of cochlear threshold sensitivity (Rubsamen *et al.*, 1992, 1993)

Note that because the emissions adapt to a decrease in EP, and because the emissions are correlated with the sharpness of tuning (Rubsamen *et al.*, 1992, 1993), these emissions are a direct measure of the ability of the cochlea to produce a sharp peak in BM motion for low stimulus levels. They are not, however, a useful independent measure of the EP level.

The present results further confirm that, for frequency ratios near 1.2, when single points or single "growth functions" are used to evaluate cochlear functioning it is advisable to choose values for which L_1 is 10 dB above L_2, rather than using equal amplitude primaries. Similar suggestions have been made by others (e.g., Gaskill and Brown, 1990; Whitehead *et al.*, 1990). It should be emphasized that it is not the absolute value of the emission that makes it useful for a measurement of cochlear functioning, but the amount of *change* in the emission when the cochlear functioning is disrupted. A reasonable normal value is, of course, required for a measurable signal. Figure 6 makes it clear that the emission from the unequal level was much more sensitive to cochlear insult than the equal level was.

An additional reason for using unequal primaries can be understood by reference to the normal emission contours [Figs. 8(a) and 9(a)]. Stimulus amplitudes of 50×40 dB SPL for example, locate the measurement point in a region of relative maximum. Small changes in middle ear transmission—even small frequency-dependent changes which may affect f_1 and f_2 transmission differently—will cause relatively little change in the observed emission. However, in contrast, consider locating on the "side" of the ridge at, say, 50×50 dB SPL stimulus levels. Here, such a small change in the stimulus amplitudes at the cochlea can cause a large change in the observed emissions, and can lead to unreliable results.

IV. SUMMARY

Distortion product otoacoustic emissions were measured in gerbils for a wide range of stimulus amplitudes while the endocochlear potential (EP) was reversibly decreased by an intraperitoneal injection of furosemide. Stimulus frequencies were $f_1=6.8$ and $f_2=8$ kHz ($f_2/f_1=1.18$). The observed variation of emissions with time can be classified as follows.

(1) Emissions at $2f_1-f_2$ and $3f_1-2f_2$ for low stimulus amplitudes. These "vulnerable" emissions were strongly decreased by the change in EP. There was a significantly greater decrease for unequal stimulus amplitudes (Fig. 6) with the best ratio for measurement being about $L_1/L_2=3.2$ (a 10-dB difference) The trajectories of these emissions against the measured EP change showed two main effects: First, the decrease of the amplitude of the vulnerable emission (in dB) was found proportional to the square of the change in EP (in mV) during the first segments (Fig. 4). The curvature of these trajectories increased with decreasing stimulus levels, and with DPE order (Fig. 5). At maximum decrease, the emissions approximated a distribution given by a simple power law generator [Figs. 6, 7, and 8(b)]. Second, these DPEs recovered substantially even before the EP reached minimum, and often recovered fully with the EP still at 50-mV levels (Figs. 1 and 2, segment 3; Figs. 8 and 9).

(2) All odd order emissions at high stimulus levels and $2f_2-f_1$ at all levels. These emissions were relatively unchanged by furosemide injection (Figs. 1–3, 5–9). If there was any change observed, the change was similar to the "vulnerable" emissions except that this group lacked the precipitous decrease found there, i.e., segment 2. When there was a decline, there was a similar recovery with subnormal EP, but, of course, smaller in proportion to the smaller decline.

(3) Even order term, f_2-f_1. The behavior of this term was completely different from all the odd order terms, however, it appeared to be *related* to their behavior. The

even order term consistently appeared to go through a zero at about the same time that the "vulnerable" components went through a relative maximum (Fig. 3), about 45 min postinjection.

Because of the close correspondence between tuning and vulnerable DPEs shown in a companion experiment (Rubsamen *et al.*, 1992, 1993) the recovery of the odd order DPEs can be interpreted as a recovery of sharp tuning. This recovery with a subnormal EP was interpreted as an adaptive effect with a time constant of about 15 min.

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