H. B. DE BREY — THE INFLUENCE OF COCHLEAR TEMPERATURE ON ELECTRIC TRAVELLING WAVES IN THE GUINEA PIG COCHLEA

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKS-UNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR MAGNIFICUS DR. A. E. COHEN, HOOG-LERAAR IN DE FACULTEIT DER LETTEREN, VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN TE VERDEDIGEN OP DINSDAG 29 JUNI 1976 TE KLOKKE 14.15 UUR

Door

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Dit proefschrift is bewerkt onder leiding van
Dr. J.J. Eggermont

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Mej. E. Stuurman typte het manuscript.
Het Heinsius-Houboltfonds stelde de 'gain phase meter' ter beschikking.

Aan Guusje
Aan Baerte en Hanske

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Wie altijd zoekend voorwaarts streeft,
mag op verlossing hopen!

(v. Goethe)

Chapter I

GENERAL SURVEY AND FORMULATION OF THE SUBJECT OF INVESTIGATION

I.1. SURVEY OF THE TRANSDUCER PHENOMENA IN THE EAR

Pressure fluctuations in the air are, within certain frequency and intensity limitations, perceived as sound. The fluctuating air pressure sets the eardrum into vibration and by this way activates the pressure transformer of the middle ear: the ossicular chain. The middle ear ossicles translate the air particle vibrations into pressure waves in the inner ear fluid. These fluid pressure waves set the membranous portion of the inner ear into vibration. The sensory cells of the hearing organ are excited by movement of the basilar membrane which results in an electric signal and initiation of a chemical process which causes a change in permeability of the neuron membrane resulting in a post synaptic potential that generates nerve spikes in the axon part of the neuron.
The arrangement of hair cells and supporting structures is called the organ of Corti. Every hair cell is innervated by several nerve fibers. The outer hair cells have a diffuse innervation pattern in contrast to the inner hair cells where one nerve fiber is related to one hair cell (*Spoendlin*, 1966). The scala media is lined with a cell-rich vascular layer of tissue on its outer wall, called the stria vascularis. In this stria the energy source that maintains the potential difference across the organ of Corti is situated (*Tasaki & Spiropoulos*, 1959). Parts of certain cells in the stria vascularis are packed with mitochondria. These cells are well furnished with free and membrane bound ribosomes, a Golgi complex and many vesicles. A very high concentration of enzymes participating in oxidative metabolism and a high incorporation of protein and RNA precursors have been established in histochemical studies of the stria vascularis. Also a high content of enzymes of oxidative and glycolytic metabolism has been histochemically established in the hair cells (Details in *Kuypers*, 1969).
This thesis deals mainly with the electrical output of the sensory cells of the hearing organ for various frequencies, especially in relation to the vibration of the basilar membrane. Therefore a detailed account will be given on the movement of the basilar membrane, the properties of the sensory cells and the generated cochlear microphonics. This will be followed by a presentation of one of the existing theories relating mathematical models to experimental data.

I.2. BASILAR MEMBRANE MOVEMENT

I.2.1. *Introduction*

Stimulation of the ear with sound induces pressure waves in the perilymph which cause movements of the basilar membrane in the form of a travelling wave. The varying compliance of the basilar membrane, changing a factor 100 from the stapes to the apex of the cochlea results in a certain degree of tuning to the sound

wave (*von Békésy*, 1960). It is known (*von Békésy*, 1960; *Johnstone & Boyle*, 1967; *Rhode*, 1971; *Wilson & Johnstone*, 1972; *Kohllöffel*, 1972) that the cochlea performs a frequency analysis in that a given position along the basilar membrane is maximally sensitive to a particular input frequency. For the higher frequencies this position is located near the stapes; for the lower frequencies it shifts to a more apical place on the basilar membrane. At any given frequency the amplitude of the vibrations builds up to a shallow maximum located at a particular distance from the stapes. Beyond this point the vibration decays relatively fast and the portions of the basilar membrane that are far apically from the maximum are at rest. The basilar membrane amplitude leads the stapes displacement with a phase difference of 90° which means that the basilar membrane amplitude is proportional to the derivative of the stapes displacement, i.e. to its velocity (*Dallos*, 1973a).

I.2.2. *Quantitative data*

A relatively large amount of quantitative data concerning the movements of the basilar membrane have been provided in the last few years. The results of three different methods for measurement of the basilar membrane movements are summarized below.

I.2.2.1. *Optical methods*

Von Békésy (1960) used a light microscope in combination with stroboscopic illumination to perform measurements on the apical part of the basilar membrane in cadaver cochleae. At the very high intensities of 130-140 dB Sound Pressure Level (SPL) that are necessary for visualization of the basilar membrane motion, it was noted that the amplitude of vibrations changed in proportion to the stimulus strength and thus non linearities contribute second-order effects at the most. He observed a continuous change of phase with frequency such that the apical part of the basilar membrane could be as much as 3π radians out of phase with the motion of the stapes. The phase difference accumulated well beyond the maximum of the basilar membrane displacement, supporting his hypothesis of travelling waves. In order to overcome the problem of large sound levels the use of a more sensitive method was felt necessary and found in the application of laser technique. Due to its coherence laser light scattered from neighbouring points on the basilar membrane, as on any other surface, interferes and thus gives a typically speckled appearance. When the basilar membrane is vibrating micro regions will be deformed. The specks will then lose their sharp definition and appear fuzzy. *Kohllöffel* (1972) used this method to determine the amplitude and the phase relations of the basilar membrane in the guinea pig. At a given stimulus, frequency fuzziness appears first over a relatively short length of the basilar membrane: the region of best response. In this way the frequency space coversion is obtained. As the stimulus strength is increased a greater part along the basilar membrane is driven above minimal amplitude and a longer membrane region will appear fuzzy both in the apical and the basal direction. From the amount of spatial spread as a function of increase in stimulus strength the values for the basal and apical slopes

of the amplitude envelope vibratory pattern are obtained. The slope values of the frequency response curve can be inferred from combination of the frequency/space conversion and the spatial data.

I.2.2.2. *The capacitive probe technique*

When a small metal plate, with a diameter of e.g. 0.15 mm, is brought in the proximity of a vibrating object the two can be considered as forming a capacitor whose capacitance changes with the varying distance between them. The variations in capacity give a measure of the vibratory amplitude. *Wilson & Johnstone* (1972) used this method to measure the vibratory amplitude of the basilar membrane in the scala tympani of the basal turn of the guinea pig cochlea after draining it. Linearity of the basilar membrane vibration amplitude was measured up to 110 dB SPL while sound intensities of 70-120 dB were used.

I.2.2.3. *The Mössbauer technique*

The Mössbauer effect is a Doppler phenomenon at the atomic nuclear level which permits the measurement of small velocities in the order of 0.2 mm/sec. Because velocity is related to displacement, the latter can be derived. In this technique a radioactive source is placed on the basilar membrane. During radiation the frequency of vibration of the radioactive nuclei is perfectly stable. Due to the phenomenon of nuclear resonance a nucleus in the vicinity of a radioactive nucleus in the source on the basilar membrane will vibrate with the same frequency as the radioactive one. When the emitter of gamma radiation moves towards or away from the absorbing nucleus a slight change is observed in the frequency of the gamma rays: the Doppler effect. Because of the extreme sharpness of nuclear resonance very small relative velocities between the emitting source and absorber can create enough of a frequency shift to destroy resonance. Thus the velocity of motions can be measured by noting changes in absorption between conditions of rest and motion. *Johnstone & Boyle* (1967), *Johnstone et al.* (1970) and *Rhode* (1971) observed the basilar membrane vibration using the Mössbauer technique. In vivo the absolute amplitude of vibration of the basilar membrane was measured in the first turn of the guinea pig and the squirrel monkey cochlea. Used intensities very from 70 to 120 dB SPL. The corresponding amplitudes were about 0.01 respectively 0.55 microns.

I.2.3. *Discussion of experimental data*

Due to the newly developed techniques as described above much quantitative information concerning the mechanical action of the basilar membrane is now available. In discussing the contradictions between the different measurements and establishing similarities it is possible, when old and new data mutually support each other, to achieve one concept for the basilar membrane movement.

I.2.3.1. *The tuning curve*

A tuning curve is an equal response curve as a function of stimulus frequency. All recent information refers to the basal high frequency end of the basilar membrane while *von Békésy*'s (1960) results were obtained from the apical low frequency end of the basilar membrane. The intermediate region may be bridged by interpolation (*Johnstone et al.*, 1970) but is not yet experimentally verified. *Von Békésy*'s (1960) low frequency slope amounts to 6 dB/octave which is entirely consistent with *Kohllöffel*'s (1972) post mortem experiments. *Johnstone & Boyle* (1967) measured 12 dB/octave. *Rhode* (1971) obtained 6 dB/octave increasing to 24 dB/octave just below the maximum. *Wilson & Johnstone* (1972) found 12 dB/octave. *Kohllöffel* (1972) measured 10 dB/octave. Only *Rhode*'s (1971) data show a bend in the low frequency slope. None of the other experiments show this. Combining the experimental data one can conclude that the low frequency slope of the frequency response curve is in the order of 10 dB/octave.
Von Békésy's (1960) data show a high frequency slope of 20 dB/octave which is inconsistent with recently obtained values. *Johnstone & Boyle* (1967) and *Johnstone et al.* (1970) obtained 95 dB/octave. *Rhode*'s (1971) data show 100 dB/octave. *Wilson & Johnstone* (1972) found 100 dB/octave as did *Kohllöffel* (1972). These experimental data are quite uniform in pointing to a high frequency slope of the frequency response curve of 100 dB/octave. *Tonndorf & Khanna* (1968) showed that the width of the tuning curve becomes less as the measurements move towards the stapes. The low frequency slope is largely independent on the location of measurements. The high frequency slope, however, becomes significantly steeper at basal positions.

I.2.3.2. *Phase relations*

According to *Siebert* (1962) and *Flanagan* (1962) *von Békésy*'s (1960) amplitude and phase plots are not internally consistent. *Von Békésy* (1960) did not take into account a constant 90° phase difference between the stapes and basilar membrane movements. At low frequencies *Johnstone & Taylor* (1970), *Rhode* (1971), and *Wilson & Johnstone* (1972) note a 90° phase difference between the ossicular chain and the basilar membrane movements. All investigators agree that as the frequency increases a gradually accumulating phase lag of the basilar membrane motion is observed. There is, however, disagreement about the amount of phase shift. From *von Békésy*'s (1960) observations it appears that at the point of maximum the phase lag is between π and 2π radians. From the data of *Johnstone & Taylor* (1970) this figure is in the order of π radians. *Rhode* (1971) obtained a figure in the order of 5π radians. From the data of *Wilson & Johnstone* (1972) this figure is in the order of 6 to 8π radians. This contradicts the possibility of a dominant resonance in the cochlea. Combining the above named data one must conclude that the phase difference between the ossicular chain and the basilar membrane is a linear function of frequency.

I.3 COCHLEAR MICROPHONICS

1.3.1 *Introduction*

Sound stimulation of the ear evokes an electric response in the cochlea that mimics the electrical signal obtained by a microphone from the same sound stimulus. Since *Wever and Bray* (1930) discovered this electric response it is called the cochlear microphonic (CM). To clarify the origin of the CM *Davis* (1965) formulated a "resistance modulation hypothesis". It is assumed that two biological batteries i.e. the endolymphatic resting potential of + 80 mV in the scala media (positive with respect to the perilymphe in the scala tympani) and the negative polarization in the hair cells of - 60 mV in a serial arrangement create a steady current flow across the organ of Corti. The oscillations of the basilar membrane are thought to bend the cilia of the hair cells resulting in a changing electric resistance of the hair bearing surface. *Strelioff et al.* (1972) found that this resistance change can be as large as 100 Ω (about 1% of the total impedance). Since the hair cells are in the path of the current flow a change in resistance is coupled with an inherent change in the total current. The variations of the resistance have the same frequency as the basilar membrane movement and therefore as the acoustic stimulus. Because the basilar membrane oscillates about its resting position an alternating current flows through the hair bearing surface and is called the CM. *Tasaki et al.* (1954) located the source of the CM in the hair bearing surface of the hair cells. By penetrating the cuticular plate (lamina reticularis) covering the upper surface of the hair cells they registered an abrupt phase change of 180°. In a normal cochlea the CM is proportional to the basilar membrane displacement (*von Békésy*, 1960). From experiments of *Dallos et al.* (1972) in kanamycin intoxicated guinea pigs it appears that the outer hair cells are primarily responsible for the generated CM because the CM of the inner hair cells is about 35 dB less sensitive. *Tasaki & Fernandez* (1952) and *Honrubia et al.* (1971) altered the endolymphatic potential in the first turn of the guinea pig cochlea by application of electric current. They found a linear relationship between the endolymphatic potential and the CM. From the above cited evidence one must conclude that the mechanical movements of the basilar membrane are converted into an electric voltage by the hair cells.

I.3.2. *Methods of measurement*

Single electrode recording produces a problem concerning the origin of the recorded electrical signal. *Wever & Bray* (1930) demonstrated that CM can be recorded from almost anywhere in the cochlea or from the cochlear surface. One must conclude that CM measurements with a single electrode do not provide much information about the electric response evoked in the direct environment. A round window electrode for example, is located close to a segment of the basilar membrane which is maximally sensitive to high frequencies but which also responds to low frequencies. The contribution of adjacent hair cells to the recorded CM is relatively strong compared to hair cells farther away. In addition, when a travelling wave passes along the basilar membrane the output of several hair cells will be in counter phase

and thus no CM response will be recorded from these particular cells. The round window electrode will record mainly from the direct environment, however, at low frequency stimulation it will also pick up electrical signal from higher turns. In the differential electrode recording technique (*Tasaki et al.*, 1952) two electrodes are placed opposite each other, one in the scala vestibuli and one in the scala tympani of one turn, in a plane perpendicular to the basilar membrane. The theoretical considerations that explain the differential electrode recording technique have been provided by *Dallos* (1969). Because of the different sign of the CM in the scala vestibuli and in the scala tympani a source of CM can be considered to represent a dipole with an electric field. In Figure I.1 this dipole is expressed by the opposing positive and negative charges, arranged perpendicularly at the basilar membrane. Here r is the

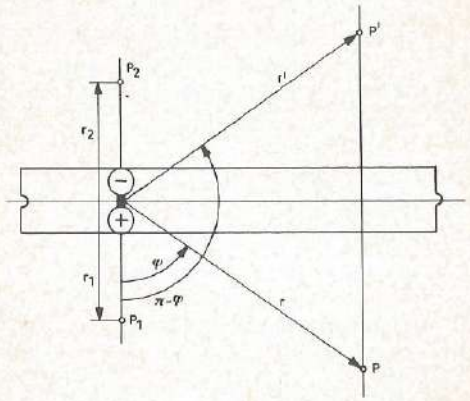

Fig. I.1 Schematic representation of a dipole source (after *Dallos*, 1969)

distance from the dipole center to any point (P). φ is the angle between the axis of the dipole and the line r. In P the magnitude of the electric field due to the dipole is $E = K \cos\varphi/r^2$, where K is a constant dependent on the strength of the dipole. An electrode at point P_1 will record $E_1 = K/r_1^2$ because here $\cos\varphi = \cos 0^\circ = 1$. An electrode at point P_2 will record $E_2 = -K/r_2^2$ because here $\cos\varphi = \cos 180^\circ = -1$. When $r_1 = r_2$ then $E_1 = -E_2$. At all points that are symmetrically situated on both sides of the line $\varphi = 90^\circ$ (i.e. the basilar membrane) the electrodes will record the same potential magnitude with opposite sign as will be shown below. Comparing the potentials at the points P and P', $r = r'$, then $E = (K \cos\varphi)/r^2$ and $E' = [K \cos(\pi - \varphi)]/r^2$. Since $\cos(\pi - \varphi) = -\cos\varphi$ it follows that $E = -E'$. Amplifying both potentials with a differential amplifier one obtains $E_{out} = A(E-E') = A.2E$ where A is the amplification factor. It follows that differential recorded signals from the cochlea are due to all generators situated in a plane equidistant from the two electrodes at the points P and P'. The contribution of a remote dipole source to the recorded signal is small if r is large because it is inversely proportional to the square of the distance and because $\cos\varphi$ approaches 0. Also because of the curvature of the basilar membrane the differential electrode pair will subtend

approximately the same angle from the remote dipole source and consequently tend to be cancelled in the differential amplification (details in *Dallos*, 1969). From the above stated arguments one must conclude that cancellation of remote generated CM is not an inherent property of the differential electrode recording technique but a combination of the angle effect and this technique. Because the basilar membrane curvature is relatively mild in the first cochlear turn, the angle effect for the CM sources in this region will be less pronounced compared to electrode placements in the higher turns. Consequently in the higher turns the electrical response originates from a narrower cochlear segment. The action potential (A.P.) is a remote source seen at the same angle and with the same magnitude and sign by both electrodes of any pair. Amplification of the potentials recorded from a differential electrode pair with a differential amplifier will cancel the AP from the response while adding the potentials cancels the CM. It is concluded that with a well balanced electrode pair (i.e. both electrodes recording potentials of the same magnitude and with 180° phase difference) the recording is more selective than with a single electrode i.e. the CM is measured from a region of about 2 mm along the basilar membrane (*Tasaki et al.*, 1952).

I.3.3. *Cochlear microphonics in relation to basilar membrane displacement*

I.3.3.1. *Frequency space relations*

Differentially recorded CM from several cochlear turns in the guinea pig show that the CM amplitude at any given recording location varies with frequency (*Tasaki et al.*, 1952). *Honrubia & Ward* (1968) recorded from the scala media with KCL filled pipettes in four turns of the guinea pig cochlea and also found a spatial preference. *Tasaki & Fernandez* (1952) in recording from the guinea pig cochlea with differential electrodes, selectively suppressed the CM response in certain cochlear regions by introduction of KCL solution into the perilymphatic space or by passing a direct current through the cochlear partition. This experiment showed that low frequency CM response could be recorded from the basal turn without alteration when the apical response was diminished. It follows that the first turn does generate CM at all frequencies. No CM could be recorded from the higher turns in response to high frequency stimuli. *Misrahy et al.* (1958) obtained comparable results recording from the scala media of the first turn of the guinea pig cochlea. Destroying the cochlea from the fourth turn down did not affect the CM recorded from the basal turn. *Dallos* (1969) produced electrical polarization in the two lower cochlear turns in the guinea pig with glass pipettes filled with Ringer solution. Differential recorded CM from the third turn indicated that at high frequencies the local contribution to the CM is negligible. *Whitfield & Ross* (1965) inferred from CM measurements that with increasing stimulus intensity the point of maximal stimulation on the basilar membrane shifted towards the base. According to *Dallos* (1973a) this is much more pronounced than what might occur in the mechanical process and is most likely a consequence of the non-linearity of the CM input-output function. *Whitfield & Ross* (1965) observed that the maximum CM response moved towards the stapes with increasing frequency in accordance with

the observations of *von Békésy* (1960) concerning the mechanical movement of the basilar membrane. In conformity with the shape of the travelling wave envelope the basal side of the distribution curves of the CM is much shallower than the apical side. Both slopes become steeper with increasing frequency. *Dallos* (1969) compared the mechanical tuning curves discussed in section I.2.3.1. with his differential recorded 1 μV-isopotential curves in the guinea pig. He found a discrepancy in the location of the "best" frequency. The CM functions peak at lower frequencies than the mechanical ones. The main reason (*Dallos*, 1973a) for this is that his results incorporate the filter effect of both the middle ear and cochlea. Correction for the middle ear effect showed good agreement with the corresponding mechanical tuning characteristic for the cochlea. It is concluded from the above cited evidence concerning the longitudinal distribution of the CM that the spatial patterns are at least in qualitative agreement with the travelling wave envelope. The microphonic tuning curves, irrespective of recording method (i.e. differential or scala media) are not narrower than the mechanical tuning curves. Thus, at least as reflected in the gross microphonic potential there is no sharpening of spatial patterns between the mechanical motion and the first electrical sign of this motion, the CM.

I.3.3.2. *Cochlear microphonic phase*

Tasaki et al. (1952) registered in the four cochlear turns of the guinea pig the phase difference between the differential recorded CM and a remote position in the basal turn. The phase differences appeared to be frequency dependent and reached the 0° value at a frequency well above zero. Subtraction of the absolute phase difference measured from the third and the basal turn reveals the phase difference between the electrode location in the first and the third turn. Apparently it takes several cycles of the travelling wave to bridge the distance between the first and the third turn. According to the *Tasaki et al.* (1952) experiments this phase difference can amount to as much as 5π.
Dallos & Cheatham (1971) compared the interscala phase difference between the CM recorded from electrodes in the scala vestibuli and the scala tympani in the second and the third cochlear turn in the guinea pig with the "absolute" phase difference between differential recorded CM from these electrodes and the sound pressure at the eardrum. When the output of a given electrode pair is dominated by local CM sources the phase differences between the electrodes is approximately 180°. When the output is predominantly remote CM both electrodes see the same phase and there is no measurable phase difference. A high cut off frequency for a given electrode pair was shown to exist beyond which the interscala phase difference assumes zero value. From these experimental results one must conclude, as the authors do, that beyond this frequency the CM data recorded from these electrodes cannot be used for quantitative purposes because it is not due to local response but to remote contributions. Thus the reliability of differential electrode recorded CM is closely related to the 180° phase difference. In order to obtain well balanced experimental electrodes it is always necessary to check if the phase difference is approximately 180° together, of course, with equality in the CM

amplitude recorded from both electrodes. *Dallos et al.* (1971) showed the existence of a transitional zone, where the local and remote responses are approximately equal, where they registered radical and rapid phase changes between 180° and 0° in the interscala phase difference. In this transition zone the stimulus intensity was found to exert a significant effect upon this interscala phase difference. The absolute phase difference in the *Dallos & Cheatham* (1971) experiments shows at very low frequencies a CM lead of the sound by approximately 90°. Then a steadily accumulating phase lag of the CM appears with increasing frequency. Beyond a certain frequency there is a clear change in the course of the phase difference coinciding with the zero value of the interscala phase difference. This means that when the absolute phase difference is measured, the local response, which reflects the basilar membrane movement in the vicinity of the electrodes, dominates the measurements up to the "break" frequency but the phase of remote CM response is measured beyond this "break" frequency.Thus the high frequency segment of the absolute phase function does not reflect the actual travelling wave activity.

I.3.3.3. *Travel time and cochlear microphonics*

Several authors concluded that the cochlea is a dispersive medium (*Tasaki et al.*, 1952; *von Békésy*, 1960; *Teas et al.*, 1962; *Nordmark et al.*, 1969). Especially in the high frequencies the travelling wave velocity appeared to be frequency dependent. From the above cited CM phase measurements it is known that the CM potentials are proportional to the basilar membrane displacement at a given point along the cochlea. This however holds only for the differential electrode recordings in the range below the "break" frequency corresponding to a given electrode location. Also CM travel time recordings only provide information about the propagation time of the travelling wave in the same region i.e. in the third turn below 1500 Hz and in the second turn below 5000 Hz (*Dallos & Cheatham*, 1971). Experimental data from *Dallos & Cheatham* (op. cit.) indicate that in the cochlea different frequency components propagate with the same velocity within the above cited limited region. Because the travelling wave velocity is frequency independent, over a limited frequency region for a given electrode location, the cochlea can be treated as a nondispersive medium and consequently the long wave approximation of the wave equation is permissible.

I.3.4. *Limitations of gross cochlear microphonic recordings*

The study of the excitation pattern along the basilar membrane by way of the CM distribution along the cochlear duct is basically an indirect method. CM recorded with round window or intracochlear electrodes does not, like the direct measurement of the basilar membrane motion, reflect the disturbance at a given point along the basilar membrane. CM thus recorded is not a representative for the output waveform of a given individual hair cell (*Whitfield & Ross*, 1965). Instead a diffuse version of the original excitation pattern is recorded over a wide area of the basilar

membrane. According to *Kohllöffel* (1971) this is a weighted average over the individual hair cells. The difference in frequency selectivity between response curves from CM and the response curve of the basilar membrane is considerable. With a single electrode in the scala tympani a high frequency slope for the basilar membrane response of about 10 dB/octave is obtained. A well-balanced differential electrode pair measurement results in a high frequency slope determination of 30 dB/octave. As mentioned before, with direct measurement of the basilar membrane displacement a high frequency slope of about 100 dB/octave was registered. On theoretical grounds *Kohllöffel* (1971) calculated the contribution of a single hair cell to the overall response recorded from the first turn of the scala tympani, this single hair cell contribution reflecting the basilar membrane displacement at that particular point. He obtained a low frequency slope of 10 dB/octave and a high frequency slope of 110 dB/octave in excellent agreement with direct displacement observations. It is thus clear that gross responses obtained from the round window and even from intracochlear recordings are diffuse. Because a differential electrode pair records from a restricted basilar membrane region (2 mm) this method remains usable to record gross changes in frequency selectivity in spite of its limitations. A more sensitive registration can be obtained by also recording the CM phase from differential electrodes because, as was cited above, the reliability of the CM recorded is closely related to the 180° phase difference.

I.4. THEORETICAL DESCRIPTION OF THE BASILAR MEMBRANE MOVEMENTS ACCORDING TO ZWISLOCKI

I.4.1. *Derivation of the wave equation*

An analytical expression for the vibratory pattern of the cochlear partition has been derived by *Zwislocki* (1953) and extensively described by *Dallos* (1973a). An abridged version will be presented in this section. Basic to the derivation is an uncoiled and rigid walled cochlea in which the cochlear partition is reduced to one single layer separating the cochlea in two parts: the "scala vestibuli" and "scala tympani". The interaction between the membrane and fluid interfaces is computed from the balance of forces and the relationship p = Z.V, in which p is the fluid pressure acting on the impedance Z; V is the volume velocity of the fluid and equivalent to membrane velocity. Also the continuity equation plays a major role in this treatment.

The continuity equation is derived as follows:

The cross sectional areas of scala vestibuli, S_v, and scala tympani, S_t, and the width of the cochlear partition b are all functions of x, the distance from the stapes (Figure I.2). Consider a volume element in the scala vestibuli $S_v dx$; the volume velocity of the fluid at distance x being U_v. The mass inflow into the volume element per unit time is therefore $U_v S_v \rho$; ρ being the fluid density at x. The mass outflow per unit time through surface at x + dx is

$$\frac{\partial}{\partial x}(U_v S_v \rho)\, dx + U_v S_v \rho$$

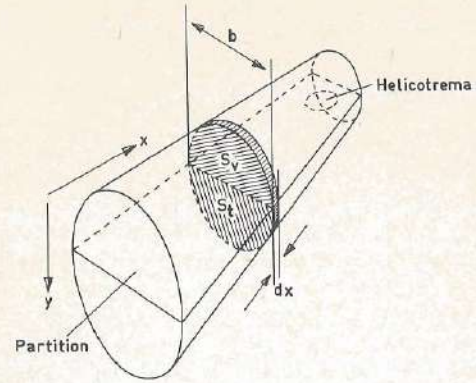

Fig. I.2 Simplified geometry of the cochlea and the volume elements in the scala vestibuli ($S_v dx$) and the scala tympani ($S_t dx$) (after *Dallos*, 1973).

Therefore the net flow through this volume element (is inflow - outflow) is equal to

$$-\frac{\partial}{\partial x}(U_v S_v \rho)dx$$

An additional change of mass in this volume element is due to the up and down motion of the cochlear partition. If the velocity is v then the volume change is equal to vbdx and change of mass is vbdx (per unit time). The total rate of change of mass is per definition

$$\frac{\partial}{\partial t}(\rho S_v dx) = S_v \frac{\partial \rho}{\partial t} dx$$

Conservation of mass implies:

$$S_v \frac{\partial \rho}{\partial t}\, dx + vb\rho dx + \frac{\partial}{\partial x}(U_v \rho S_v)dx = 0$$

This is the continuity equation for volume element $S_v dx$ which somewhat simplified takes the form

$$S_v \frac{\partial \rho}{\partial t} + vb\rho + \rho \frac{\partial (U_v S_v)}{\partial x} = 0$$

This equation applies to the volume element in the scala vestibuli. A similar derivation yields the continuity equation for the scala tympani. Because the cochlear partition moves in the opposite direction the partition velocity has the opposite sign.

Derivation of the balance of forces equation: If the pressure at x is P_v (in the scala vestibuli) acting on the surface S_v at x the force is equal to $P_v S_v$; the force acting on the surface at x + dx is

$$(P_v + \frac{\partial P_v}{\partial x}dx)(S_v + \frac{\partial S_v}{\partial x}dx) = P_vS_v + P_v\frac{\partial S_v}{\partial x} + S_v\frac{\partial P_v}{\partial x}dx$$

(neglecting higher orders in dx) which is equal to

$$P_vS_v + \frac{\partial(P_vS_v)}{\partial x}dx$$

Consequently the net force due to pressure in the scala vestibuli acting on the volume element is the force at x - the force at (x + dx) and is equal to

$$-\frac{\partial(P_vS_v)}{\partial x}dx$$

This force is counterbalanced by inertial and frictional forces in this volume element. The inertial force can be computed according to Newton's second law and results in

$$S_v\frac{\partial(\rho U_v)}{\partial t}dx$$

and with an incompressible fluid reduces to

$$S_v\rho\frac{\partial U_v}{\partial t}dx$$

Frictional forces in a viscous fluid are proportional to the velocity of the moving fluid particles: $(R_vS_v)U_vdx$ in which R_vS_v is the resistance of the scala vestibuli per unit length. The balance of forces requires:

$$S_v\rho\frac{\partial U_v}{\partial t}dx + S_vR_vU_vdx + \frac{\partial(P_vS_v)}{\partial x}dx = 0$$

Assuming that S_v varies slowly with x and after division of common terms the equation reduces to

$$\rho\frac{\partial U_v}{\partial t} + R_vU_v = -\frac{\partial P_v}{\partial x} \quad \circledast$$

Recalling the continuity equation gives

$$S_v\frac{\partial \rho}{\partial t} + vb\rho + \frac{\partial(U_vS_v)}{\partial x}\rho = 0$$

Slow variations of S_v with x result in

$$S_v\frac{\partial \rho}{\partial t} + vb\rho + S_v\rho\frac{\partial U_v}{\partial x} = 0 \quad \circledast$$

A considerable simplification in the calculation can be obtained by assuming incompressibility of the fluid:

$$\frac{\partial \rho}{\partial t} = 0$$

The two equations ⊛ reduce to

$$\left.\begin{aligned}\rho\frac{\partial U_v}{\partial t} + R_vU_v &= \frac{\partial P_v}{\partial x}\\ vb + S_v\frac{\partial U_v}{\partial x} &= 0\end{aligned}\right\}$$

Adopting the notation $\dot{U}_v = \frac{\partial U_v}{\partial t}$ and differentiating the first equation to x and the second to t one arrives at (assuming R_v slowly variable with x):

$$\left.\begin{aligned}\rho\frac{\partial \dot{U}_v}{\partial x} + R_v\frac{\partial U_v}{\partial x} &= -\frac{\partial^2 P_v}{\partial x^2}\\ \dot{v}b + S_v\frac{\partial \dot{U}_v}{\partial x} &= 0\end{aligned}\right\}$$

Substituting the second equation in the first one yields

$$-\frac{\rho b}{S_v}\dot{v} - \frac{R_vb}{S_v}v = -\frac{\partial^2 P_v}{\partial x^2}$$

A similar equation holds for the scala tympani:

$$-\frac{\rho b}{S_t}\dot{v} - \frac{R_tb}{S_t}v = -\frac{\partial^2 P_t}{\partial x^2}$$

Substraction and substitution of $R_v = R_t = R$; $p = P_v - P_t$

and $\frac{1}{S} = \frac{1}{S_v} + \frac{1}{S_t}$ results in $\frac{\rho b}{S}\dot{v} + \frac{bR}{S}v = \frac{\partial^2 p}{\partial x^2}$

A relationship between P and v can be obtained by realising that $P = bvZ$ in which bv is the volume velocity per unit length. This results in

$$\frac{\rho}{SZ}\dot{p} + \frac{R}{SZ}p = \frac{\partial^2 p}{\partial x^2}$$

For a harmonic motion $p = P\,e^{j\omega t}$ one obtains

$$j\omega\frac{\rho}{SZ} + \frac{R}{SZ} = \frac{1}{P}\frac{d^2P}{dx^2}$$

A similar relation holds for the velocity v of the cochlear partition by substituting V for P. The ultimately desired solution of the problem is the displacement Y of the basilar membrane as a function of x. This can be obtained by integrating the solution of the differential equation with respect to time since $Y = \int_0^t v dt$; in the harmonic case this results in

$$Y = \frac{V}{j\omega}$$

I.4.2. *Solution of the wave equation*

The parameters R, S and Z are all functions of x and consequently a general solution is not available. The first task has been to obtain analytical forms for R(x), S(x) and Z(x). Based on measurements of von Békésy, Zwislocki arrived at the following equations

1) $S(x) = S_o e^{-ax}$

2) $R(x) = R_o \omega^{1/2} e^{ax/2}$

3) $Z(x) = R_m(x) + j\left[\omega M(x) - \frac{1}{\omega C(x)}\right]$

a) $C(x) = C_o e^{hx}$ the membrane compliance

b) $R_m(x) = R_{mo} e^{-\beta x} \simeq R_{mo}$

c) $\omega M \ll \frac{1}{\omega C}$

Concerning the membrane impedance it has been observed that the change in compliance is about 100 fold for the total length of the basilar membrane, the change in resistance is only 1.25 fold and the mass influence is negligible with respect to the compliance effect. Therefore in a first approximation

$$Z(x) = R_{mo} + \frac{1}{j\omega C(x)}$$

Substitution into the wave equation and neglecting R_m with respect to $\frac{1}{\omega C}$ results in:

$$-\frac{1}{P}\frac{d^2P}{dx^2} = \frac{\omega^2 \rho C}{S}\left[1 - j\left(\omega R_m C + \frac{R}{\omega \rho}\right)\right]$$

which after lengthy calculations and some simplification results for the magnitude of the pressure wave in:

$$\left|P(x,\omega)\right| = B_1{}^1 \omega^{-1/2} \exp\left[-\frac{(h+a)x}{4} - \frac{Q}{2}\omega^2 R_m C_o e^{(3h+a)x/2} - \frac{Q}{2}\frac{R_o \omega^{1/2}}{\rho} e^{(h+2a)x/2}\right]$$

in which $Q = \frac{2\rho^{1/2} C_o{}^{1/2}}{(h+a) S_o{}^{1/2}}$ is a constant for the cochlea under study. $B_1{}^1$ is a constant times a first order Bessel function.

From this equation one observes that the pressure decreases with increasing frequency and increasing distance from stapes. At a constant frequency then the pressure wave gradually decreases from the stapes toward the helicotrema, becoming negligible at a particular distance. The greater the frequency, the earlier the drop in pressure amplitude. At a constant distance from the stapes the pressure magnitude depends on frequency: the greater the frequency the less the pressure. The pressure at the stapes (x=0) is easily recovered from the formula and for the relative pressure magnitude with respect to the stapes one arrives at

$$\left|\frac{P(x,\omega)}{P(o,\omega)}\right| = \exp\left[-\frac{(h+a)x}{4} - \frac{Q}{2}\omega^2 R_m C_o\left\{e^{(3h+a)x/2} - 1\right\} - \frac{Q}{2}\frac{R_o \omega^{1/2}}{\rho}\left\{e^{(h+2a)x/2} - 1\right\}\right]$$

The phase function of the pressure wave is written as $\angle P = -\omega Q e^{(h+a)x/2} + \frac{1}{4}\pi$, the reference phase at the stapes being $\angle P_o = -\omega Q + \frac{1}{4}\pi$ resulting in the relative phase of the pressure wave

$$\angle P - \angle P_o = -\omega Q\left\{e^{(h+a)x/2} - 1\right\}$$

This represents a phase lag which monotonically increases with respect to increase of both frequency and distance from the stapes.

The quantity of interest is in the displacement of the cochlear partition. This pattern can be obtained from the pressure function by $P = j\omega Z y$ i.e. $y = P/j\omega Z$.

According to *Dallos* (1973a) we may take $|Z| \simeq \frac{1}{\omega C}$ and $\left|\frac{1}{Z}\right| \simeq \omega C$ therefore $|Y| = C|P|$ which results in:

$$\left|\frac{Y(x,\omega)}{Y(o,\omega)}\right| = e^{hx}\left|\frac{P(x,\omega)}{P(o,\omega)}\right|$$

which results in

$$\left|\frac{Y(x,\omega)}{Y(o,\omega)}\right| = \exp\left[-\frac{(-3h+a)x}{4} - \frac{Q}{2}\omega^2 R_m C_o\left\{e^{(3h+a)x/2} + 1\right\} - \frac{Q}{2}\frac{R_o \omega^{1/2}}{\rho} \cdot \left\{e^{(h+2a)x/2} + 1\right\}\right]$$

The phase of the partition displacement is the same as the phase of the pressure wave: $\angle Y = \angle P$

I.4.3. *Phase velocity and travel time*

The phase velocity of the partition displacement is by definition $\frac{dx}{dt}$. Recalling

$$\angle y = \omega t - \omega Q e^{(h+a)x/2} + \tfrac{1}{4}\pi$$

$$\text{Then } \frac{d\angle y}{dt} = -\frac{a+h}{2} Q\omega e^{(h+a)x/2}\frac{dx}{dt} + \omega$$

The points that are one cycle apart have the same phase and therefore

$$\frac{dx}{dt} = \frac{2}{(h+a)Q} e^{-(h+a)x/2}$$

This expression shows that the velocity of the disturbance that travels along the cochlear partition diminishes exponentially as it gets farther and farther away from the stapes. The velocity of propagation is independent of the frequency of stimulation.

The travel time of a particular disturbance from the stapes to a point on the

partition is obtained by integrating the reciprocal of the above expression between $x = 0$ and $x = x_o$:

$$T = \int_o^{x_o} \frac{(h+a)Q}{2} e^{(h+a)x/2} dx = Q(e^{(h+a)x_o/2} - 1) \text{ sec.}$$

I.4.4. *The parameter values in the wave equation for the human cochlea*

$S = 0.0125\, e^{-0.5x}$ $\quad S_o = 0.0125$

$R = R_o \omega^{1/2} e^{ax}/2$ $\quad R_o = 8.15 \text{ gm/cm}^3 \text{ sec}^{1/2}$ $\quad a = 0.5 \text{ cm}^{-1}$

$C = C_o e^{hx}$ $\quad C_o = 4 \cdot 10^{-10} \text{ cm}^4/\text{dyne}$ $\quad h = 1.5 \text{ cm}^{-1}$

$R_m = R_{mo} e^{-0.35x/2} = R_{mo} e^{-0.175x} \simeq R_{mo} = 470$

From these values one calculates $Q = 1.79 \cdot 10^{-4}$.
Since the dimensions of the guinea pig cochlea, as well as the frequency range of hearing are quite different from those in man, adjustments of these parameters will be necessary when applying this theory to the results obtained. These values will be calculated in the appropriate section in Chapter IV.

I.5. *FORMULATION OF THE SUBJECT OF INVESTIGATION*

In order to study the frequency analysing capabilities of the hydromechanical part of the guinea pig cochlea under varying mechanical properties thereof, the cochlear microphonics have been recorded. The changes in mechanical properties are obtained by varying the cochlear temperature. This investigation is performed with respect to three separate points:

1. *Measurement of the amplitude and phase of the cochlear microphonics recorded from the first, second and third cochlear turn with respect to frequency, intensity and cochlear temperature.*
2. *Relating the observed changes in the cochlear microphonic phase data to Zwislocki's theory on cochlear hydrodynamics in order to calculate the compliance of the basilar membrane.*
3. *Separating the effects of changes in mechanical properties and changes in cochlear metabolism due to lowering the temperature with respect to the CM-amplitude data.*

Chapter II

EXPERIMENTAL METHOD

II.1 THE EXPERIMENTAL ANIMAL

The experiments have been performed on guinea pigs. These animals have been chosen because they have a cochlea which is situated entirely free in the bulla tympanica and for that reason have been used most widely for intracochlear recordings. In order to obtain animals of about the same age and presumably with the same inner ear condition the guinea pigs have been sorted out in weight. Sometimes only heavier animals were available and thus the weight composition of the population of the experimental animals shows an oblique distribution to the right. The number of experimental animals used is 29 of which 24 had a body weight between 300 and 600 grams, with an average over the total group of 464 grams. Most animals used were female albinos.

II.2 PREMEDICATION AND ANAESTHESIA

About half an hour preoperative the guinea pig received a premedication of 2,5 micrograms of atropine sulphate per 100 grams body weight i.m., in order to diminish the excitability of the vagal nerve and to reduce the mucous secretion in the respiratory tract. During the experimental procedure this dose was administered every three hours. Simultaneously the animal also received 5 milligrams promethazinehydrochloride (Phenergan®) and 5 milligrams chlorpromazinehydrochloride (Largactil®). Promethazinehydrochloride is administered to prevent shivering during cooling. Chlorpromazinehydrochloride is administered to disrupt temperature regulatory mechanisms. Both drugs also have sedating and analgetic effects. Instead of these last named two chemicals three animals out of the group received 10 milligrams diazepam (Valium®) i.m. to prevent shivering by way of muscle relaxation and as a sedative; another six animals received a neuroleptanalgesia with 0.03 milliliter Thalamonal® (a combination of 2.5 milligram droperidol and 0.05 milligram fentanyl per milliliter) per 100 grams body weight i.m. With exception of the degree of suppression of shivering which was best with the combination of promethazinehydrochloride and chlorpromazinehydrochloride, no difference was observed between these three kinds of premedication. Shortly before the operation a moderate deep anaesthesia was obtained, by administration of urethane intraperitoneally at a dose of 0.1 milligram per 100 grams body weight which was satisfactory for the duration of the experiment.

II.3 SURGICAL PROCEDURE AND ELECTRODE PLACEMENT

To prevent temperature loss during the operation the animal was placed on a small electrically heated sheet. After removal of most of the body hairs, to facilitate cooling later on, tracheotomy is performed to achieve a free air passage. An incision is made in the right mandibular region and the retromandibular band of

blood vessels is ligated. The mandible is fractured in the middle of its horizontal branch and lifted with a retractor under its posterior portion. Pushing down the digastric muscle the bulla tympanica is exposed. Then part of the sterno-cleido-mastoid muscle is severed and the styloid process after cutting it across near its base, is removed together with part of the stylohyoid muscle. The bulla is partially cleaned of periosteum and opened with a dental drill. The cochlea then is fully exposed by removing the bony shell of the bulla with a forceps. Then the surgical procedure is continued under a binocular operating microscope. Part of the mucous membrane is removed from the cochlea. Using a stiff steel-needle ground to a very fine tip, small holes (about 100 microns ϕ) are ground into the cochlear walls of the scalae tympani and vestibuli of the first, second and third cochlear turn (compare Figures I.1 and I.2). Nichrome steel wires (25 microns ϕ) insulated with enamel except at the tip are introduced through the holes in the scalae. Near the tip these active electrodes are provided with a small globule of resinous material to control the depth of penetration into the cochlea and to hinder abundant outflow of perilymph. The electrode pairs are located in the first, second and third cochlear turn at respectively 4, 10 and 14 millimeters from the stapes. They are fastened to the edge of the bulla with orthophosphate cement in a position perpendicular to the cochlear wall. The wound retractor is used as a ground electrode in good contact with the cervical muscles.

II.4 THE MEASUREMENT SETTING

The head of the animal is rigidly clamped in a headholder in a slightly sideways flexed position in order to prevent accumulation of perilymphe and wound secretions in the middle ear and to allow flowing of these liquids outside the animal. The animal body is placed in a double walled U-shaped metal device to allow cooling by introducing cold water therein and to allow rewarming by blowing warm air through it. A modified ear speculum is sewn into the right ear and is attached with a close fitting connection piece to a dynamic headphone (STC-4026-A). Thus a closed sound system between the telephone membrane and the eardrum of about 10 cubic centimeters is obtained. Using a calibrated probe microphone (Brüel & Kjaer ½ inch condensor microphone, consisting of cathode follower type 2615 + cartridge type 4133, in combination with a probe of 1.6 mm diameter and a length of 16 centimeters) the sound pressure level at a point a few millimeters from the eardrum is recorded during the experiments.
In order to facilitate the installation of the animal a slight modification was used comprising a plastic tube with a length of eleven centimeters and a diameter of two millimeters sewn into the right ear and connected to the modified ear speculum. This introduces several peaks in the CM output curve because the pressure is now measured at a certain distance from the eardrum. Because only relative CM amplitudes at the first, second and third turns are measured, this is thought not to influence the final results.
The animal is situated in an electrically shielded sound proof room with an electrical noise level below 5 microvolts p.p. The disturbing noises existed predominantly out of very low frequencies like vibrations in the building. The noise level was

measured with a precision sound level meter (Bruël & Kjaer type 2203 with type 1613 1/3 octave filter set). This was below 12 dB SPL per octave for frequencies above 500 Hz and increased to 44 dB SPL per 1/3 octave at 31.5 Hz. The temperature in the room was adapted to the temperature at which the experiments were carried out in the animal by heating with electrical radiators or cooling by blowing air into the room.

II.5 THE COOLING AND HEATING PROCEDURE

The experiments are carried out in animals in which the body temperature is artificially adjusted between 26 and 39°C. The temperature in the guinea pig is measured rectally using a mercury thermometer. According to *Eggermont* (1972) the temperature on the cochlear surface is equal to the temperature of the bulla tympanica. The temperature in the oropharynx is thought to be nearly the same as the temperature of the bulla tympanica. Because it is easier to measure the temperature in the oropharynx the "cochlear" temperature is determined here using a NTC resistor connected in a bridge circuit. Cooling is achieved by applying ice water in the U shaped device in which the animal is situated. The cold water is removed when the rectal temperature becomes 25°C. Ten minutes later the cochlear temperature becomes stable at about 5°C below the initial temperature at the start. During the measurements in the next 30 minutes the variation in cochlear temperature never exceeds 0.5°C, however, the rectal temperature rises 5°C. The cooling procedure is now repeated until the rectal temperature becomes stable at about 10°C below the initial temperature. In later experiments cooling is achieved in one step of 10°C instead of 2 steps of 5°C each. From *Eggermont* (1972) it is known that temperatures lower than 25°C cause hysteresis in the temperature dependency of several parameters of the cochlear potentials. After the measurements at the lowest temperature ($\cong$ 26°C) are completed the guinea pig is gradually rewarmed in about one hour to its normal body temperature. Artefacts in the CM recordings especially in the phase measurements due to shivering of the animal are prevented by measuring outside the cooling procedure and if necessary by increasing the depth of the narcosis.

II.6 THE STIMULATION AND RECORDING PROCEDURE

As stimuli continuous tones and tone-bursts are presented to the guinea pig ear. The continuous tones are generated by a Rhode and Schwarz wave analyzer type F.T.A. with a coupled tone generator and are presented to the headphone using a continuous variable attenuator (Figure II.1). The tone-bursts consist of sinewaves with a trapezoidal envelope of which the rise and fall time (0.1 - 100 msec.) and the duration of the plateau (0.1 - 200 msec.) can be adjusted independently. The continuous tone stimulus is used in the CM amplitude and phase measurements. In the measurements of the travelling-wave delay tone-bursts are used with a rise and fall time of 5 msec. for stimulus frequencies of 500 Hz and 500 Hz + ½ octave. For higher stimulus frequencies the rise and fall time is 3 msec. In the measurements of the latency of the compound action potential (AP) tone-bursts are used with a rise

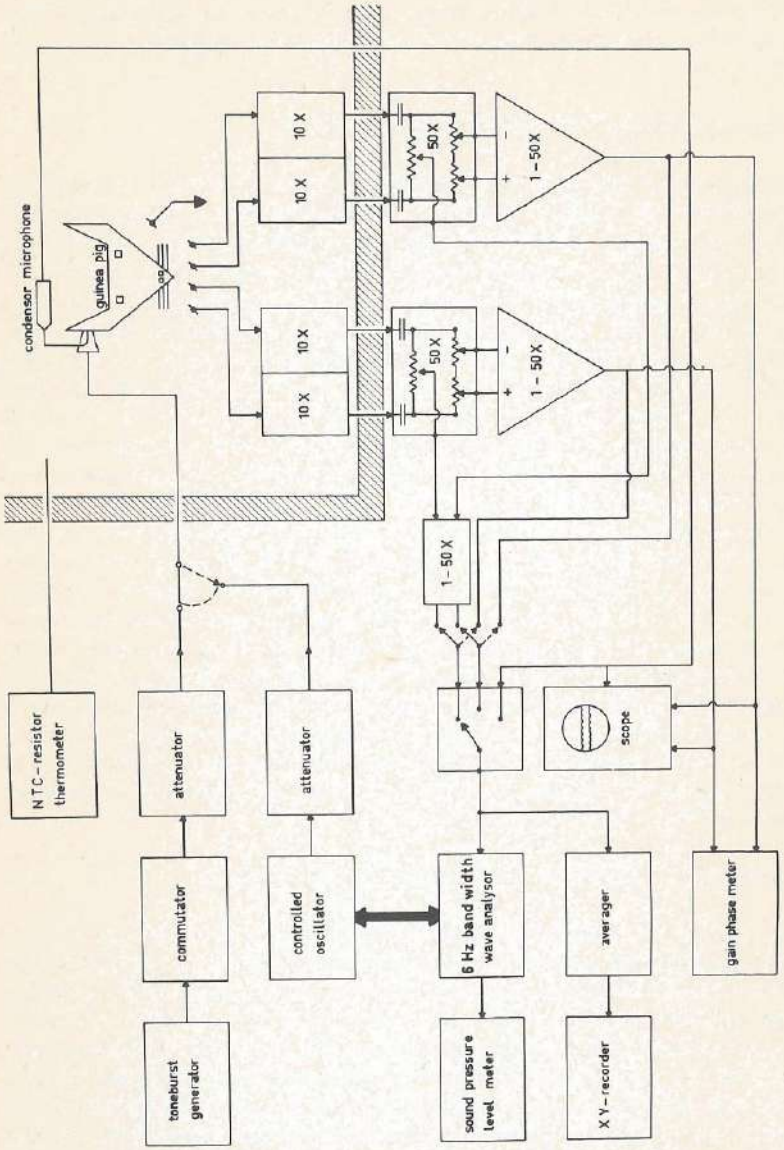

Fig. II.1 Schematic representation of the measurement setting

and fall time of 0.33 msec. In both cases the duration of the plateau of the toneburst is 4 msec. The tone-bursts are generated by a special device so that the phase of the sine wave at the start of the toneburst is always zero (Details in *Spoor*, 1974). The CM and AP are recorded with differential electrodes from the first, second and third cochlear turn. The CM from the scala vestibuli and the scala tympani electrodes is preamplified 10 times in the electrically shielded soundproof room. Outside this room the electrical signal is passed through a differential amplifier. Using a compensation network (*Tasaki et al.*, 1952) the AP and summating potential (SP) can be separated from the CM. The total amplification can be set between 500 and 25000 times. The CM output is measured as μV RMS using the wave analyzer in the narrowband mode (6 Hz bandwidth irrespective of frequency). CM phase measurements are performed without filtering using a gain phase meter (H.P. type 3575A) with a large rejection of harmonic components and insensitive to noise (error caused by odd harmonics in the worst case is about 5 degrees). The phase is determined by measuring time differences between successive zero crossings of the two input signals (from two cochlear turns). For the measurements of the travelling wave delay the CM response to low frequency tone-bursts is recorded using an averager (Data laboratories DL 102 S) and an X-Y recorder (HP 7035 B), the system permitting a time resolution of 0.02 msec. in the calculations. A schematic representation of the experimental set up is shown in Figure II.1.

Cooling and rewarming the guinea pig may cause some hysteresis in the CM generation and a sensitive test for the cochlear functioning during and after the cooling and warming up procedure must be available. Therefore the latency of the AP evoked by a high-frequency tone-burst is measured before and after each series of measurements and during the cooling and warming periods. It is known that the AP-latency is very sensitive to temperature as well as other metabolic disturbances (*Gannon et al.*, 1966; *Eggermont*, 1974) and therefore the requirement is made that the start and end latencies of a complete experiment must be the same. *Coats* (1965) gives an increase for AP-latency with decreasing temperature of 0.06 msec./°C, while also changes of a factor 1.6 for 10°C are reported (*Eggermont*, 1974). This type of control measurement has been performed for each animal and an identical start and end latency was required for using the data of an individual animal in this respect.

Chapter III

EXPERIMENTAL RESULTS

III.1 INTRODUCTION

Differentially recorded CM from the first, second and third cochlear turn is quantified by the amplitude and by the phase lag with respect to the first turn, for stimulus frequencies ranging from 100 Hz to 10 kHz at a level of 70 and 90 dB SPL. By stimulating with short tone-bursts the delay in the CM recorded from the third cochlear turn referred to the first one can be measured. A comparison is made between the results obtained at normal cochlear temperature and at an about 10 degrees lower cochlear temperature. In some animals CM data were also obtained at 5 degrees below normal cochlear temperature.

III.2 THE AMPLITUDE OF THE COCHLEAR MICROPHONICS

The CM amplitude as a function of frequency for an individual guinea pig ear is shown in Figure III.1a at a stimulus level of 70 dB SPL and for an intensity of 90 dB SPL in Figure III.1b at temperatures of 36°C and 26°C. Recordings have been made from the first, second and third cochlear turn. At 70 dB SPL the curve of the CM amplitude recorded from the first turn is relatively flat up to 6500 Hz with the exception of a peak around 1300 Hz. The CM amplitude decreases rather sharply above 2600 Hz in the second turn and above 1000 Hz in the third turn. At 90 dB SPL the CM amplitude is larger but the shape of the curve is basically the same as at 70 dB SPL. At lower temperature the amplitude from the first, second and third turn decreases while the basic shape of the CM frequency response curve does not change.

The mean CM amplitude calculated for nine ears as a function of frequency recorded from the first and the third cochlear turn at a stimulus level of 70 dB SPL is shown in Figure III.1c and at 90 dB SPL in Figure III.1d. The mean CM amplitude curve calculated for the first turn is relatively flat up to 6500 Hz, again with the exception of a peak around 1300 Hz. In the third turn the mean CM value for frequencies above 2 kHz is close to zero. At a stimulus level of 90 dB SPL the mean CM amplitude is larger while the shape of the curve is basically the same as at 70 dB SPL in accordance with the conclusion on the individual data. At lower temperature the amplitude is lower for both turns but the shape of the curves does not change.

The frequency-response curves in Figures III.1c and III.1d for the CM recorded from the first turn show a peak around 1300 Hz not reported in the literature. The peak recorded in our measurements is caused by recording the SPL at a distance from the eardrum i.e. at the end of the plastic tube between the ear and the modified ear speculum (See chapter II). For particular frequencies the SPL at the end of the tube is different from the SPL at the eardrum.

By measuring in three ears successively with the modified ear speculum and with the plastic tube attached to the modified ear speculum an average correction

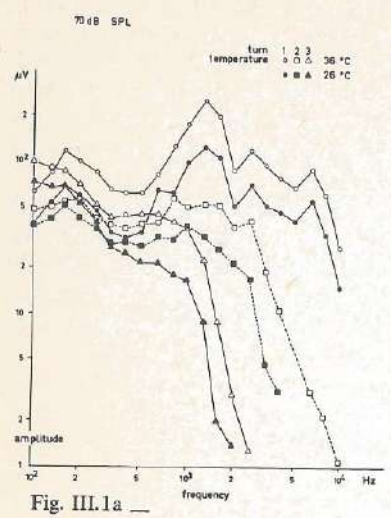

Fig. III.1a

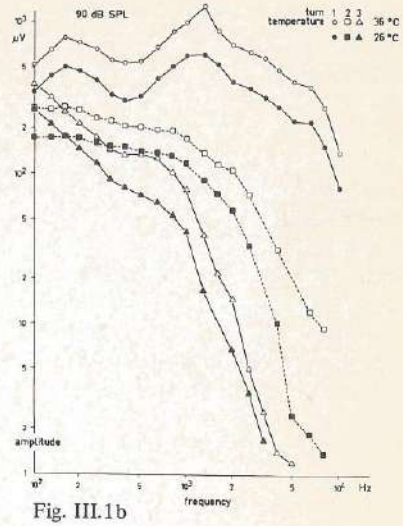

Fig. III.1b

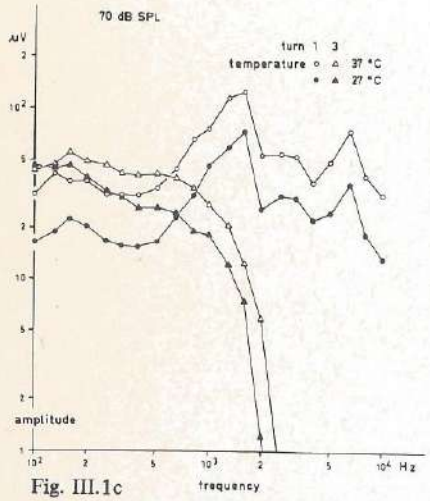

Fig. III.1c

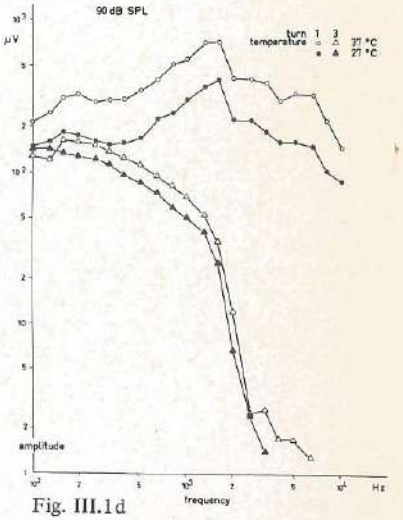

Fig. III.1d

Fig. III.1a-d The amplitude of the CM as a function of frequency for two cochlear temperatures at 70 and 90 dB SPL. At low frequencies (e.g. 100 Hz) the output of the three cochlear turns is nearly the same. For increasing frequency marked differences are observed. The third turn does not produce a clear output for frequencies above 2 kHz while the second turn provides output up to about 5 kHz. In contrast the first turn produces an almost frequency independent CM output. This reflects the frequency selective action of the basilar membrane and attached structures. Due to the differential electrode recording technique this can be measured. (Individual data Figure III.1a-b. Mean data Figure III.1c-d).

factor can be calculated for the data obtained with the plastic ear tube. Correction of the mean data from the Figures III.1c and III.1d with this factor results in disappearance of the peak around 1300 Hz as shown in Figure III.2 for a temperature of 37°C at 70 and 90 dB SPL. The corrected curves are higher between about 300 and 1500 Hz. Outside these frequencies not much difference from the uncorrected curves is observed. In this way a good correspondence is achieved with the data reported in the literature.

Because conclusions in the remaining part of this thesis are only drawn from CM amplitude-ratios, the artificial peak around 1300 Hz in the CM recordings from the first turn will not influence the final results. This is shown in Figures III.3a and III.3b. In the ratio CM_3/CM_1, for an individual ear there is little difference between the results with and without the plastic ear-tube which is especially clear at 70 dB SPL.

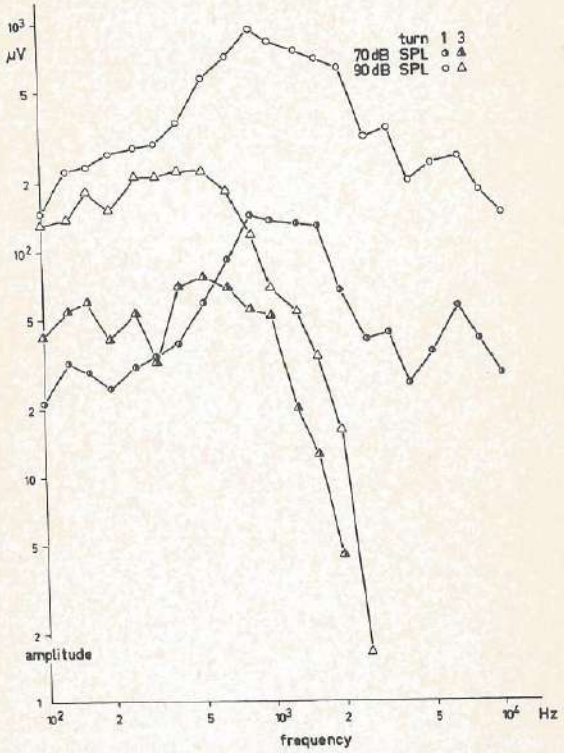

Fig. III.2 The average amplitude of the CM at 37°C as shown in Figure III.1c-d corrected for the plastic tube attached between the ear and the modified ear speculum. A peak in the amplitude of the CM is recorded caused by measurement of the SPL at a distance from the eardrum. Applying a correction factor obtained by measuring at a short distance from the eardrum shows a flatter CM amplitude curve obtained from the first turn in accordance with the data reported in the literature. Most marked is the change between 1 and 2 kHz.

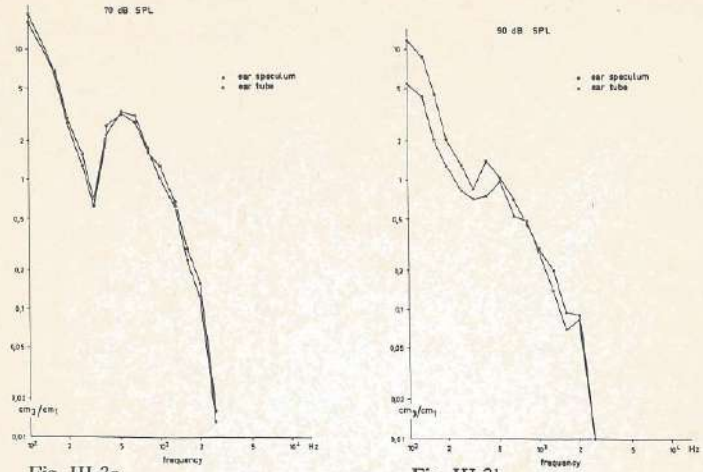

Fig. III.3a Fig. III.3b

Fig. III.3a-b The ratio of the CM amplitude from the third and the first cochlear turn as a function of frequency for an individual ear at normal body temperature. The data are obtained with and without the plastic tube attached between the ear and the modified ear speculum. Little difference is observed between both data. Thus CM amplitude-ratios are not influenced by the artificial peak around 1300 Hz in the CM recordings from the first turn.

III.3 THE AMPLITUDE RATIO OF THE COCHLEAR MICROPHONICS RECORDED FROM THE THIRD AND THE FIRST TURN

For the detection of any shift in the electrical excitation pattern in the cochlea it may be convenient to calculate the ratio of the CM recorded from the third and the first turn (CM_3/CM_1) for different cochlear temperatures. In the Figures III.4a and III.4b the results of such a calculation for an individual ear are shown as a function of frequency at respectively 70 and 90 dB SPL. The curves obtained are steep at the low and high frequency sides but flat in between. Comparing the curves for the two stimulus levels the steep parts have the same slope for both. Measurements at five cochlear temperatures are shown in these Figures. It is observed that the CM_3/CM_1 ratio is higher for lower temperature, however, the ratio tends to increase for the first measurement series during rewarming. This indicates a considerable hysteresis either in the CM generating mechanism or in the cochlear temperature with respect to the oropharynx temperature. The start and end series coincide. Averaged CM_3/CM_1 curves obtained for 9 guina pig ears show an increasing gradient for increasing frequency (Figures III.4c and III.4d). The slope of the steep part is the same for stimulus levels of 70 and 90 dB SPL. Considering the standard deviations, calculated for a few points of the 37°C curve, it is clear that from the mean data no conclusions can be drawn with respect to any significant effect of temperature. In order to study if there is a distinct frequency shift of the CM_3/CM_1 curve for lower temperature this shift is calculated for nine guinea pig ears at 4 different ratios being 0.2, 0.5, 1 and 1.2. In Figure III.5 the frequency shift

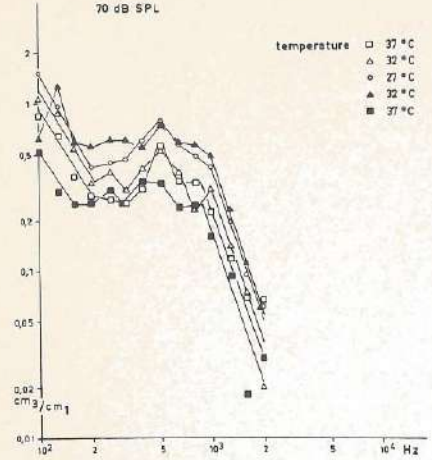

Fig. III.4a

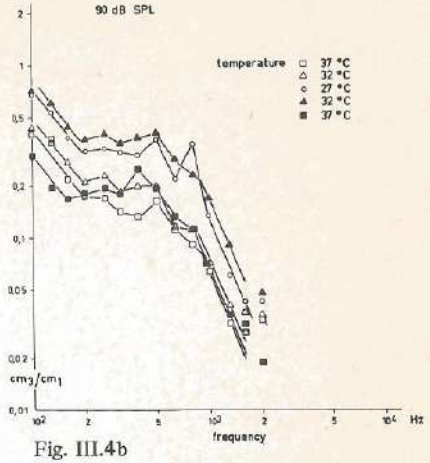

Fig. III.4b

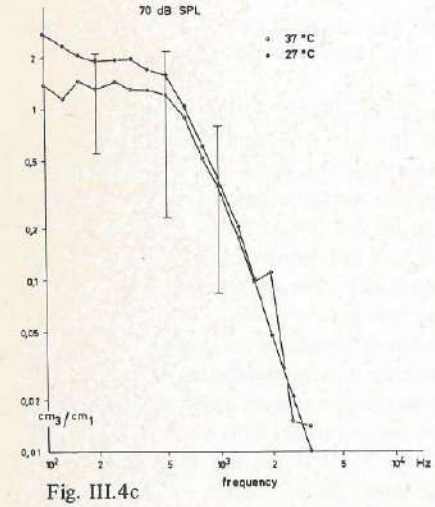

Fig. III.4c

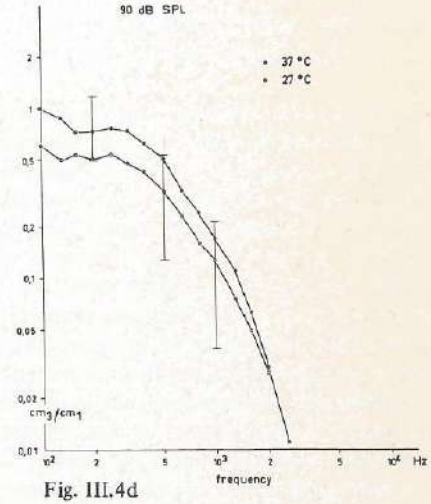

Fig. III.4d

Fig. III.4a-d The ratio of the CM amplitude from the third and the first cochlear turn as a function of frequency for various cochlear temperatures. The ratio is larger for lower temperature. The individual values (Figure III.4a-b) indicate hysteresis. The slope of the curve of the averaged ratios (Figure III.4c-d) increases for increasing frequency. Considering the magnitude of the standard deviations no conclusions can be drawn with respect to any significant effect of temperature.

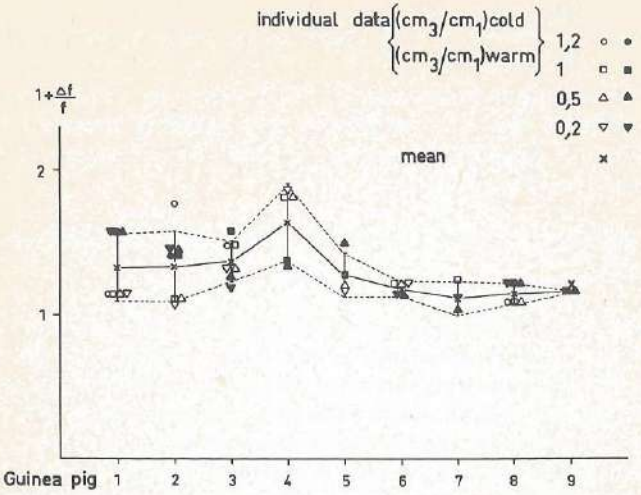

Fig. III.5 The frequency shift calculated for four different ratios of the CM amplitude from the third and the first turn at normal and about a ten degrees lower temperature. As a result of cooling about 10°C an apical frequency shift of about 1/3 of an octave is observed independent of the level. The standard deviations indicate that the frequency shift is significant. (Open symbols 70 dB SPL, closed symbols 90 dB SPL).

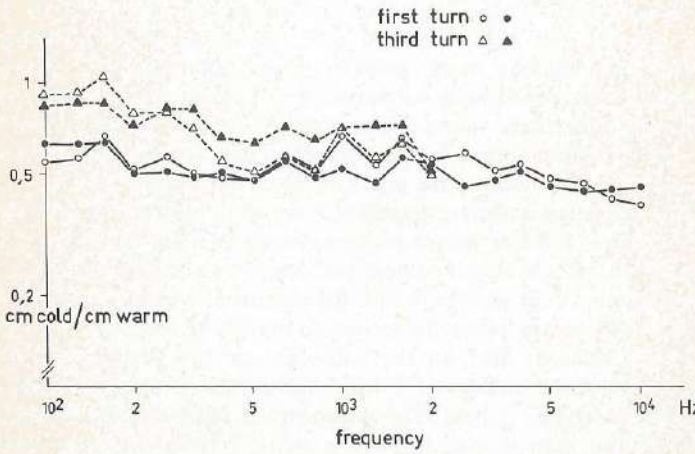

Fig. III.6 The averaged ratio of the CM recorded at low and normal temperature as a function of frequency. For the first turn the ratio is frequency independent and amounts to about 0.5. For the third turn the ratio is frequency dependent and amounts up to about 0.9 at low frequencies pointing to a different effect of temperature in the first and the third turn. (Open symbols 70 dB SPL, closed symbols 90 dB SPL).

calculated from $\frac{(CM_3/CM_1)\ \text{cold}}{(CM_3/CM_1)\ \text{warm}}$ is shown to be independent of the cochlear output level and on the average equals 1.3 ± 0.2 which may be called a significant effect. This indicates that at a ten degrees lower cochlear temperature the frequency at which for example the output of both cochlear turns is the same is shifted by about 1/3 of an octave upwards.

III.4 THE TEMPERATURE COEFFICIENT OF THE COCHLEAR MICROPHONICS

The temperature coefficient of the CM amplitude is calculated for both the first and the third cochlear turn at the levels of 70 and 90 dB SPL. The averaged data for nine guinea pig ears are shown in Figure III.6. It appears that for the first turn the CM-cold/CM-warm ratio (i.e. the temperature coefficient) is almost frequency independent and on the average equals 0.5 ± 0.1. For the third turn at frequencies below 1 kHz this ratio is larger and increases up to 0.9 ± 0.2 for the range of 100 - 200 Hz, however, for stimulus frequencies above 1 kHz the same ratio is found as for the first cochlear turn. One must keep in mind that data for the third turn for frequencies above 2 kHz are probably based upon remote contributions from other turns and these data therefore are not shown in the Figure.

III.5 THE PHASE DIFFERENCE OF THE COCHLEAR MICROPHONICS

Between the differentially recorded CM from the first and the third cochlear turn a phase difference exists. For an individual guinea pig ear this is shown in Figure III.7a at 70 dB SPL and in Figure III.7b at 90 dB SPL for two different temperatures. At low frequencies a nearly constant phase difference of 1/4 π radians is observed, which increases towards 4 π radians at 2 kHz. As cited before with regard to the CM amplitude, data values above 2 kHz must not be considered because they are due to remote contributions. Lowering the temperature by about ten degrees results in an increase of the phase difference at a given frequency of about 1/8 π radians indicating a shorter travelling wave length for the frequencies in this part of the cochlea. For five guinea pig ears averaged data of the phase differences between the first and the third cochlear turn are shown in Figure III.7c at 70 dB SPL and in Figure III.7d at 90 dB SPL for normal and about ten degrees lower temperature. The average phase difference is somewhat larger for the lower frequencies ($\simeq$ ½ π radians) than for the individual ear just considered but also increases up to about 4 π radians at 2 kHz. These data indicate the same general trend as in the individual animal. The increment in phase difference between the first and the third turn due to the lower cochlear temperature is plotted as a function of frequency in Figures III.8a and b. For frequencies below 260 Hz one may hardly speak of a significant change in the phase difference. For frequencies above 260 Hz up to 1600 Hz there is an increase in the phase difference as a result of cooling. The standard deviations of the averaged data are indicated with dashed lines. Because the standard deviation curves are above the line of zero change in

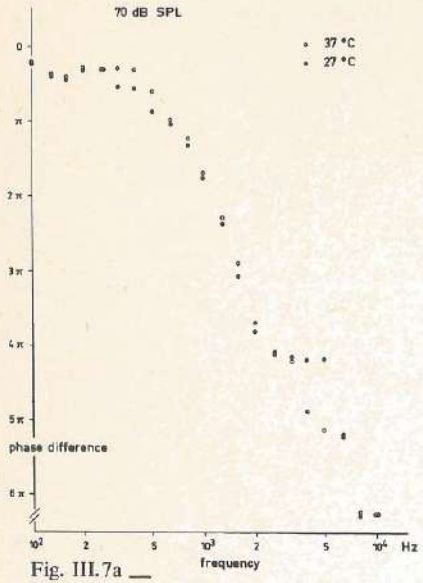

Fig. III.7a —

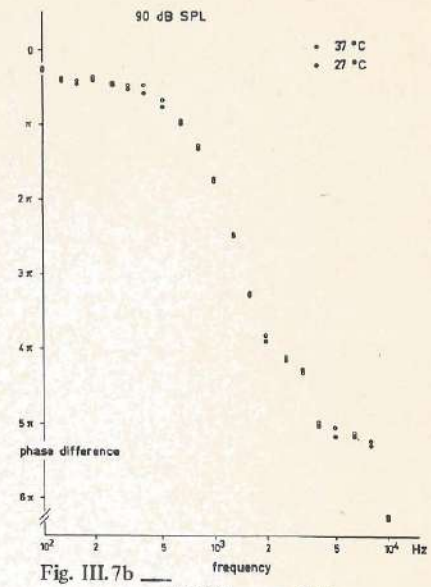

Fig. III.7b —

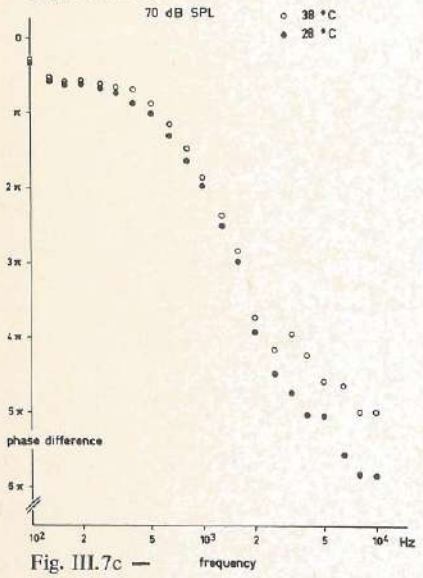

Fig. III.7c —

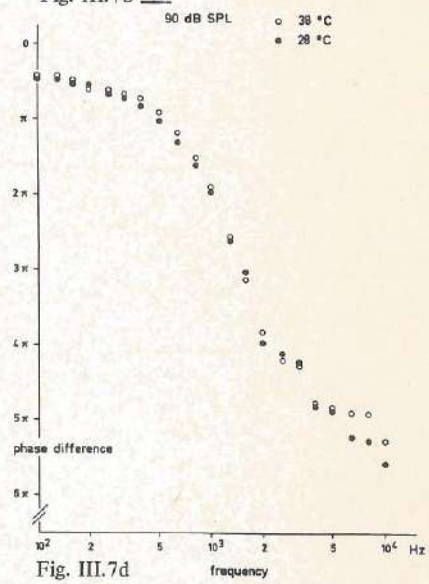

Fig. III.7d

Fig. III.7a-d The phase difference between the CM recorded from the first and the third turn. A frequency dependent phase difference increasing from an almost constant value of ¼ π radians at low frequencies towards 4 π radians at 2 kHz is shown. As a result of about 10°C cooling the phase difference increases by about 1/8 π radians at a given frequency. This is thought to be caused by a shorter travelling wave length for the frequencies in this part of the cochlea. (Individual data Figure III.7a-b. Mean data Figure III.7c-d).

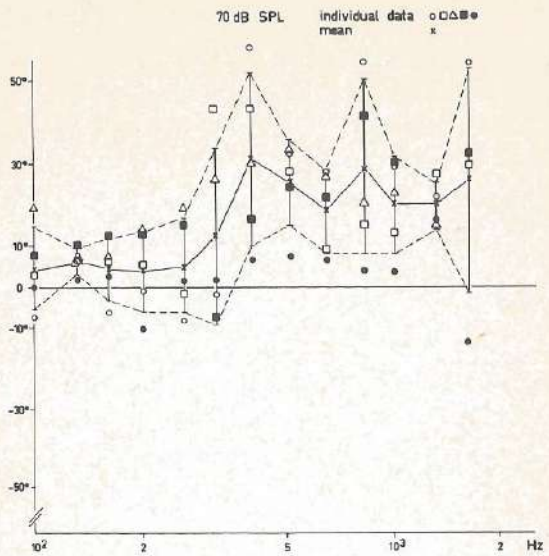

Fig. III.8a

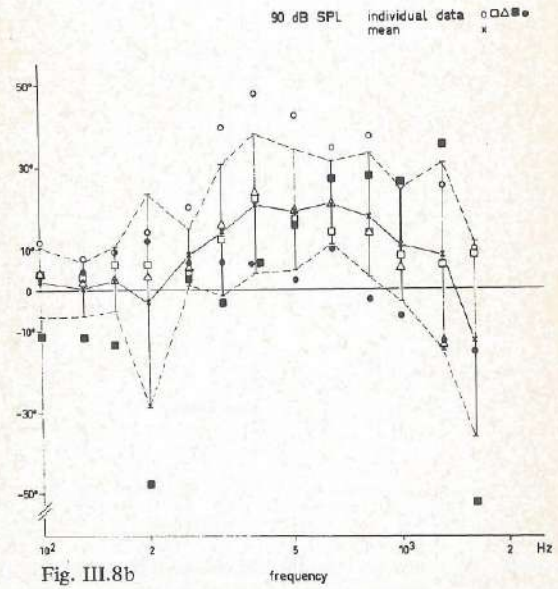

Fig. III.8b

Fig. III.8a-b The increment in phase difference (vertical scale) between the first and the third turn, due to cooling, as a function of frequency. In the mid frequencies an average increase of about 23 degrees phase difference is observed at 70 dB SPL (Figure III.8a) and about 19 degrees at 90 dB SPL (Figure III.8b) as a result of 10°C lower temperature.

phase difference the change can be called significant at 70 dB SPL for frequencies between 400 and 1300 Hz and at 90 dB SPL for frequencies between 400 and 820 Hz. The average increase in the phase difference between the first and the third turn at 70 dB SPL for frequencies between 400 and 1300 Hz amounts to about 23 degrees. At 90 dB SPL this value, between 400 and 820 Hz, amounts to about 19 degrees. These phase changes are in conformity to about 1/8 π radians.

III.6 THE TRAVELLING WAVE DELAY

By stimulating with short tone-bursts the CM recorded from the first and the third cochlear turns shows a characteristic delay in the onset thereof (Figure III.9). The delay amounts from 0.65 to 1.0 msec., depending somewhat on the interelectrode distance. Measurements for tone-bursts of frequencies of 500 Hz, 500 Hz + ½ octave, 500 Hz + ¾ octave and 1000 Hz have been performed for normal and a ten degrees lower temperature. Individual and averaged data for five guinea pig ears are shown in Figures III.10a and b. The standard deviations are indicated with dashed lines. The average travelling-wave delay between the first and the third cochlear turn for normal temperature is 0.8 ± 0.1 msec. For lower temperature one calculates 0.9 ± 0.1 msec. which cannot be called a significant change. However it corresponds with the observed increase in phase difference because $\Delta\varphi = \frac{\Delta T}{T} \cdot 2\pi$ in which $\Delta\varphi$ is the observed phase shift (1/8 π radians) and ΔT is the observed increase in the travelling wave delay. There is a tendency for increasing frequency that the delay becomes smaller, this is found especially at frequencies above 1000 Hz. This does not point to a dispersion of the travelling-wave velocity but is due to remote contributions to the observed CM.

III.7 CONTROL MEASUREMENTS

III.7.1 *Latency*

The latency of the compound action potential in response to an 8000 Hz tone-burst is shown as a function of temperature in Figure III.11a for an individual guinea pig ear that is cooled and rewarmed over a region of about 11°C. It is observed that the AP latency increases with a decrease in temperature and decreases again with an increase in temperature. The start and end latencies coincide but there is some hysteresis (compare Figures III.4a and III.4b) indicating about 1.5°C temperature lag during rewarming. Individual data of the AP latency for the group of nine ears used in the CM amplitude data in the Figures III.1c and III.1d are shown in the Figures III.11b and III.11c. The data confirm the AP latency dependence on temperature as described above for Figure III.11a.

III.7.2 *Temperature*

The relation between the rectal and the oropharynx temperature is shown in

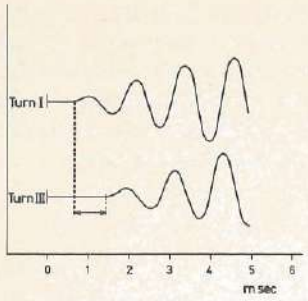

Fig. III.9 The delay in onset of the CM recorded from the first and the third cochlear turn.

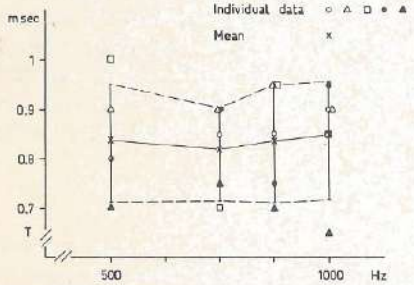

Fig. III.10a

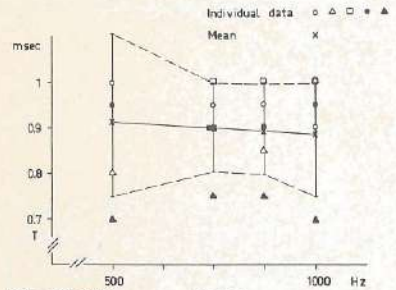

Fig. III.10b

Fig. III.10a-b The travelling wave delay between the first and the third cochlear turn. The average travelling wave delay between the first and the third cochlear turn measured from CM recorded in response to short tone-bursts amounts 0.8 ± 0.1 msec. at normal body temperature (Figure III.10a). The delay increases to 0.9 ± 0.1 msec. at an about 10°C lower temperature (Figure III.10b). Considering the standard deviations the increase is not significant.

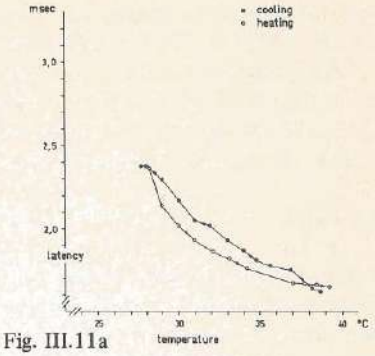

Fig. III.11a

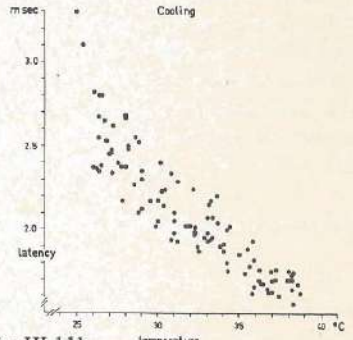

Fig. III.11b

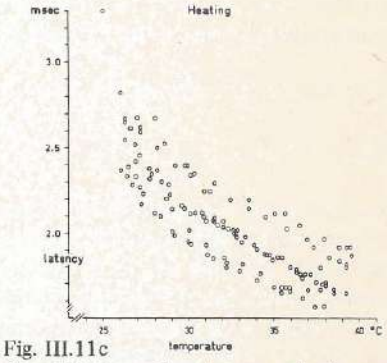

Fig. III.11c

Fig. III.11a-c The latency of the compound action potential as a function of cochlear temperature. The AP latency indicative for cochlear functioning is temperature dependent. Though the start and end series coincide, hysteresis is observed (Figure III.11a). The AP latency of the group of nine ears described in section III.2 is shown in Figure III.11b (Cooling) and III.11c (Rewarming).

Figure III.12 for one individual animal. It is observed that the oropharynx temperature lags behind the rectal temperature during cooling and rewarming procedures. This temperature lag can amount up to about 2°C and may be influenced by the short distance between the rectal thermometer and the cooling and rewarming apparatus on which the animal is situated. Around 33°C both temperature measurements coincide because in this animal measurements were carried out in 5°C steps of cooling and rewarming and thus the temperature has to be stable around 33°C. As a rule the temperature varied no more than 0.5°C during one half hour which was sufficient time to complete the experimental procedure.

III.7.3 *Shunt resistances*

In order to investigate if shunt resistances between the differential electrodes influence the electrical signal measured from the first and the third turn in a different way, the ratio of the amplitude of the first negative deflection of the compound action potential from the third and the first turn is calculated for five ears. Individual and mean data are shown in Figure III.13 as a function of temperature. It is clear that the value $AP_3/AP_1 = 1$ is mostly within one standard deviation from the mean and thus no significant change is observed. It is concluded that shunt resistances influence the electrical signal, measured from the first and the third turn in the same way.

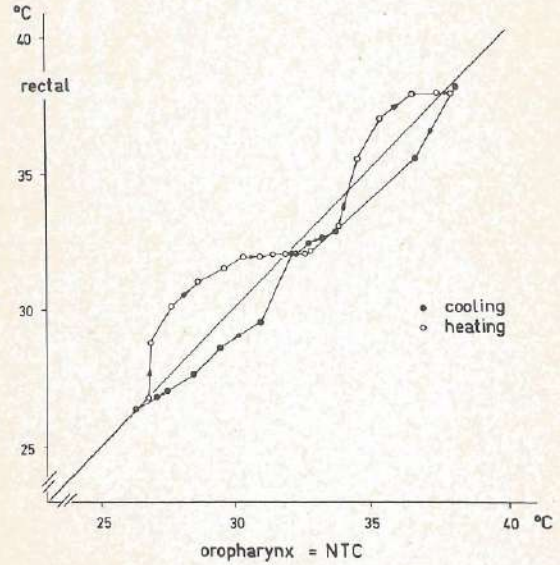

Fig. III.12 The relation between the rectal and the oropharynx temperature. The oropharynx temperature lags behind the rectal temperature. At the points of CM measurement, in this individual animal in 5°C steps, both temperatures coincide.

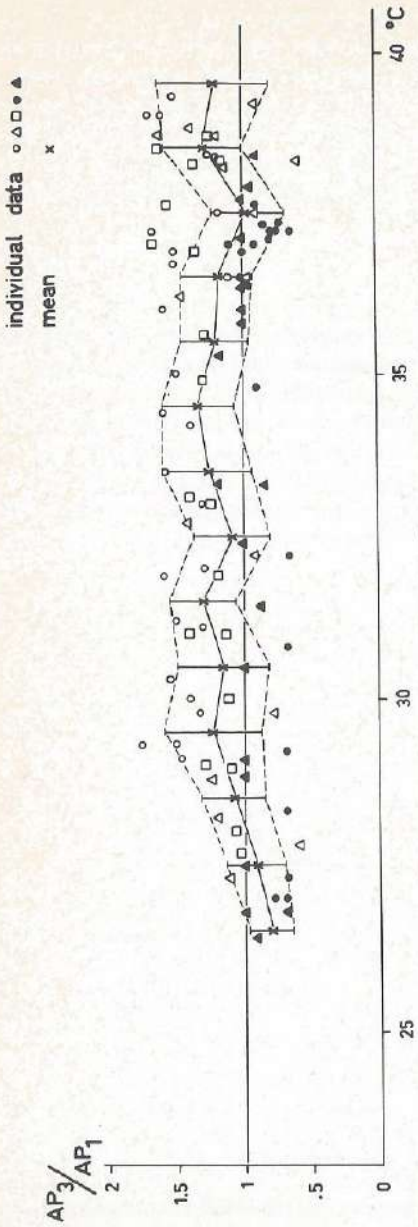

Fig. III.13 The ratio of the amplitude of the first negative deflection of the compound action potential recorded from the first and the third turn. Leak currents may influence the electrical signal recorded from both turns in a different way. The AP amplitude ratio does not change significantly. It is concluded that leak currents have about the same effect on the electrical signal recorded from the first and the third turn.

Chapter IV

DISCUSSION OF THE EXPERIMENTAL RESULTS AND COMPARISON WITH THEORY

IV.1 INTRODUCTION

The CM profile is studied using amplitude as well as phase data. Phase data reflect travelling wave velocities and tuning characteristics of the basilar membrane, while amplitude data offer additional information about the state of the CM generators i.e. metabolic influences become salient.

IV.2 PHASE DIFFERENCES AND TRAVEL TIME IN THE COCHLEA

Dallos (1973b) measured the phase angle between the differential recorded CM and the sound field at the eardrum for the first, second and third turn of the guinea pig cochlea at 50 dB SPL. For the second and third turn a steadily accumulating phase lag of the CM increasing with frequency up to about 6π was observed while for the first turn the phase lag accumulated up to about $\frac{1}{2}\pi$. The accumulating phase difference between the first and the third turn is in accordance with the experimental data reported in Chapter III (Figures III.7a-d), the comparison is shown in Figure IV.1.

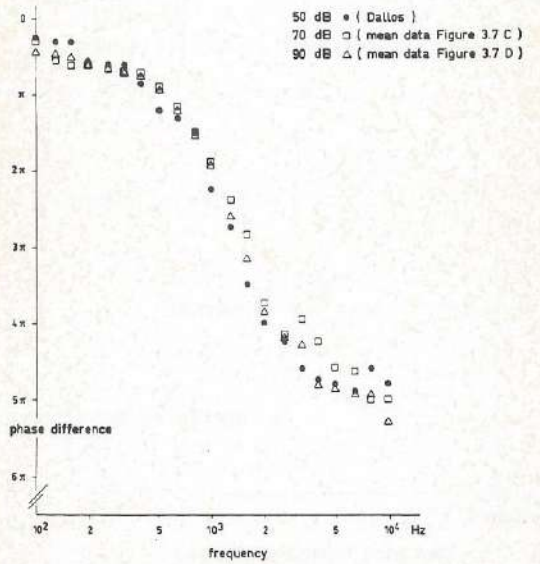

Fig. IV.1 The phase difference between the CM recorded from the first and the third turn. The frequency dependent accumulating phase difference obtained in the experiments at normal body temperature (compare Figure III.7c-d) is observed to be in accordance with the phase difference calculated from the experiments by *Dallos* (1973b).

The travel time of the CM from the first to the third turn, computed from the phase data shown in Figure IV.1 using the formula $T = \varphi/2\pi f$ where φ is the phase difference and f is the frequency, is about 1.1 msec. This does not agree completely with the measured travel time of 0.8 msec. reported in Chapter III. This may be due to a relatively large low frequency phase difference which influences the average value. For example 1000 Hz the observed phase difference is about $1.9\,\pi$ resulting in T = 0.95 msec. which is nearer the measured values.
One may conclude that the results obtained agree in general quite well with those reported by *Dallos* (1973b).

IV.3 THE EFFECT OF COOLING ON THE MECHANICAL ACTION OF THE COCHLEA

According to *von Békésy* (1960) the basilar membrane compliance is predominantly responsible for the shape of the vibratory pattern as illustrated in Figure IV.2 An increase in compliance causes a basal shift while a decrease in compliance causes an apical shift of a particular excitation profile.
Kohllöffel (1972) measured the frequency response curve in the basal turn of the living guinea pig and also at daily intervals post mortem. He also observed a basal shift of the region of best response of about 10% per day during the first three days after death. The phase pattern was measured from the fourth up to the seventh day post mortem. In the curves a tendency to an increase in phase at a fixed place was observed for frequencies of 5 and 9 kHz. It was concluded that after death the parameters of the basilar membrane change.

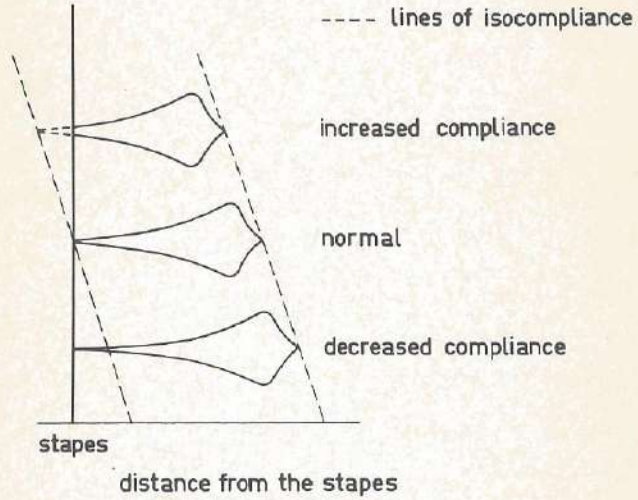

Fig. IV.2 Schematic representation of the effect of a change in compliance of the basilar membrane on the place of the excitation profile. As a result of a decrease in compliance of the basilar membrane the excitation profile shifts towards the apex. (Vertical scale = relative amplitude).

As a result of cooling, our experiments (section III.5) show for each frequency an increase in phase difference between the first and the third cochlear turn. Surprisingly the changes caused by cooling are nearly identical to those produced by the death of the animal in that the basilar membrane compliance apparently increases.

IV.4 COMPARISON WITH THEORY

In order to calculate the compliance of the basilar membrane, the results obtained from the CM-phase and CM-travelling wave delay measurements will be substituted in the formulas derived in Chapter I.
From Chapter I the equation

$$\angle P - \angle P_o = -\omega Q\left\{e^{(h+a)x/2} - 1\right\}$$

is recalled in which

$$Q = \frac{2\rho^{1/2} C_o^{1/2}}{(h+a) S_o^{1/2}}$$

It is assumed that the cross-sectional areas of the cochlear scalae (S) and the cochlear length constants h and a are not changed by cooling 10°C while the change in density is so small that it also may be neglected. Now the only variable in the equation is the basilar membrane compliance (C_o). From both the phase and the travelling wave measurements C_o can be calculated. The cross-section of the scala vestibuli (S_v) and of the scala tympani (S_t) depends on the distance from the stapes (x). According to *Dallos* (1973a) for the human cochlea $S_v + S_t = 0.05\ e^{-0.5x}$. From *Fernandez* (1952) it is known that for the guinea pig the cross-section of the cochlea at the stapes is 0.042 cm^2. Consequently $S_v + S_t = 0.042\ e^{-ax}$ for the guinea pig.
Following the derivation of *Dallos* (op. cit.) assuming for simplicity that $S_v = S_t$ then from the definition

$$S = \frac{S_v\ S_t}{(S_v + S_t)} \text{ one obtains } S = \frac{(S_v + S_t)}{4} = 0.01\ e^{-ax}$$

From the data of *Fernandez* (op. cit.) one derives $a = 2\ cm^{-1}$ as is illustrated in Figure IV.3.
The change in compliance will be about a hundredfold (assuming similarity with the human cochlea) over the whole length of the basilar membrane. In the guinea pig cochlea the length of the basilar membrane is about 2 times shorter than in the human cochlea. In order to maintain the same compliance profile in the guinea pig basilar membrane: $h = 3\ cm^{-1}$.

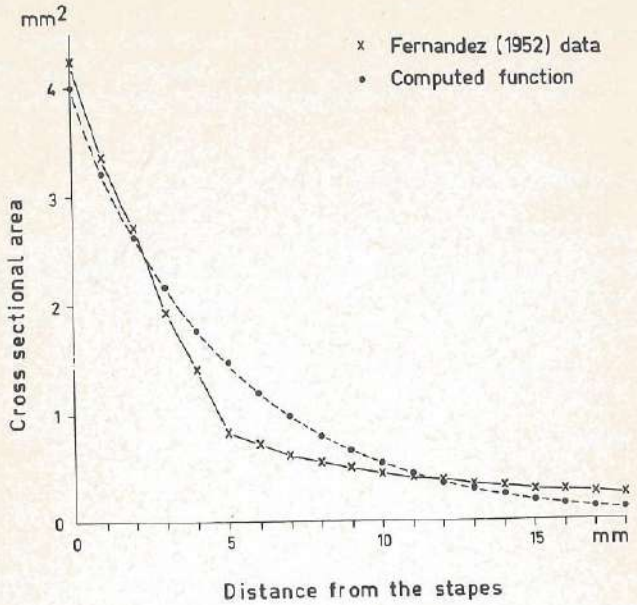

Fig. IV.3 The cross-section of the scala vestibuli and the scala tympani as a function of the distance from the stapes. The formula $S = 0.01e^{-ax}$ approximates the measured data of Fernandez (1952) for a = 2 cm^{-1}.

The parameter values for the guinea pig are:

$S = 0.01\, e^{-2x}$ $\quad S_o = 0.01$ $\quad a = 2\ cm^{-1}$

$R = R_o \omega^{1/2} e^{\frac{ax}{2}}$ $\quad R_o = 8.15$ $\quad h = 3\ cm^{-1}$

$C = C_o e^{hx}$ $\quad C_o = ?$

$R_{mo} = 500 \simeq R_m$

Measurements were made in the first turn at approximately 0.4 cm and in the third turn at approximately 1.4 cm from the stapes. For the phase difference between the third and first cochlear turn, one may derive:

$$\angle p_{III} = \angle p_I = (\angle p_{III} - \angle p_o) - (\angle p_I - \angle p_o)$$
$$= -\omega Q\left\{e^{(h+a)\frac{1.4}{2}} - e^{(h+a)\frac{0.4}{2}}\right\}$$
$$= -2\pi f \frac{2C_o}{5 \cdot 0.1}\left\{33.1 - 2.71\right\}$$
$$= -2\pi f\, 121.6\, C_o^{1/2}$$

From the phase measurements (Figure III.7c-d) C_o is calculated to be $0.59 \pm 0.06\ 10^{-10}$ cm^4/dyne at 38°C which is about a factor 7 smaller than in the human cochlea. This indicates that the resonance frequency at a given distance from the stapes is about $\sqrt{7} = 2.6$ times larger than in man and the travelling wave velocity at a given distance from the stapes will also be about 2.6 times larger than in man *(Zwislocki, 1953)*. At 28°C C_o is calculated to be $0.69 \pm 0.10\ 10^{-10}$ cm^4/dyne (Figure III.7c-d). So the phase data point to an increase in the basilar membrane compliance by about 20% as a result of cooling 10°C.
For the travel time the following equation holds (See Chapter I):

$$T = \int_o^{x_o} \frac{(h+a)Q}{2} e^{(h+a)\frac{x}{2}}\, dx = Q\left\{e^{(h+a)x_o/2} - 1\right\} \text{ sec.}$$

Therefore one obtains for the travelling wave delay between the first and the third turn:

$$T_{III} - T_I = (T_{III} - T_o) - (T_I - T_o)$$
$$= Q\left\{e^{(h+a)\frac{1.4}{2}} - e^{(h+a)\frac{0.4}{2}}\right\}$$
$$= \frac{2\, C_o^{1/2}}{5 \cdot 0.1}\left\{33.1 - 2.71\right\} = 121.6\, C_o^{1/2}$$

$T_{III} - T_I$ is measured to be 0.8 ± 0.1 msec. at 38°C and 0.9 ± 0.1 msec. at 28°C.

One obtaines therefore

$0.8\ 10^{-3} = 121.6\, C_o^{1/2}$ $\quad C_o = 0.43 \pm 0.07\ 10^{-10}$ cm^4/dyne at 38°C

$0.9\ 10^{-3} = 121.6\, C_o^{1/2}$ $\quad C_o = 0.55 \pm 0.07\ 10^{-10}$ cm^4/dyne at 28°C

which is slightly lower than the results from the phase data. So far only information about mechanical data is used and it is concluded that as a result of cooling the basilar membrane compliance increases and the excitation profile of a continuous tone shifts basally.

4.5 AMPLITUDE DATA: PREDICTIONS FROM THEORY

The ratio of the mean CM data in dB obtained at 70 and 90 dB SPL (See Figures III.4c-d) is shown in Figure IV.4 at 38 and 28°C. The CM_3/CM_1 ratio increases as a result of cooling. The increase is somewhat less for higher frequencies. Recalling the formula for the amplitude ratio of the basilar membrane displacement in the first and the third cochlear turn:

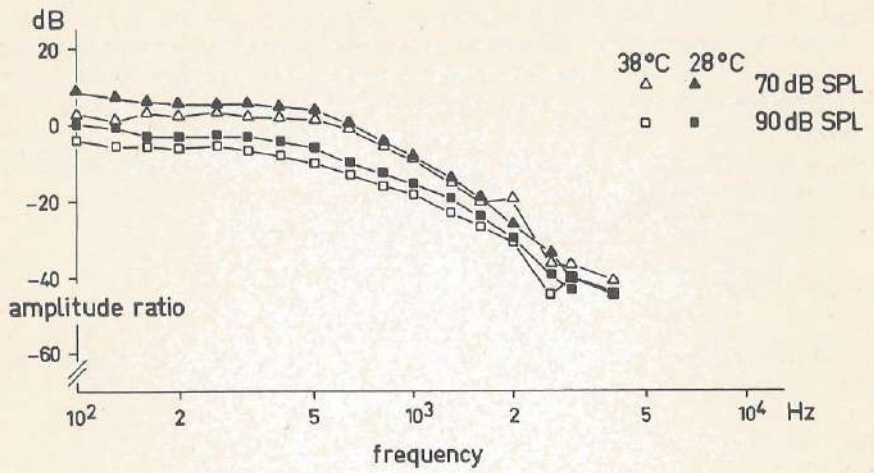

Fig. IV.4 The ratio of the mean CM data obtained from the third and the first turn in dB as a function of frequency for normal and a ten degrees lower temperature (Compare Figure III.4c-d). The ratio decreases towards higher frequencies. Cooling causes an increase of the ratio which is somewhat less for the higher frequencies.

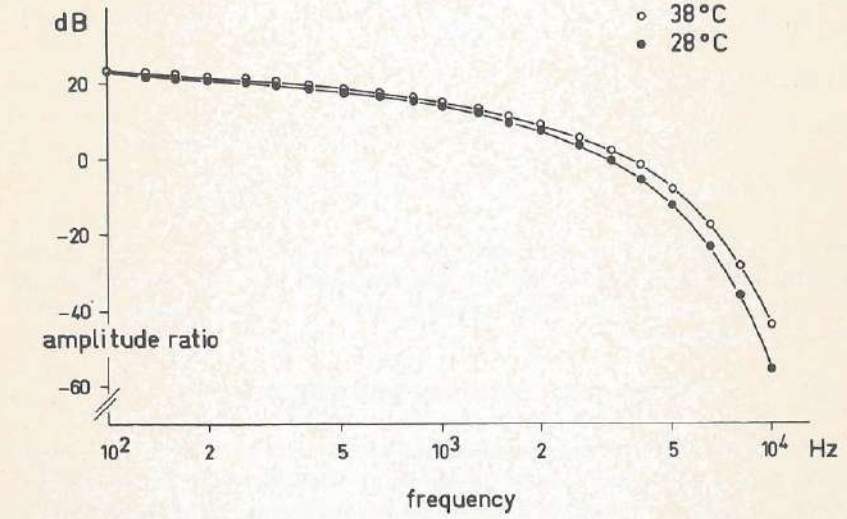

Fig. IV.5 The amplitude ratio of the basilar membrane displacement in the first and the third turn computed on the basis of *Zwislocki*'s (1953) theory on cochlear dynamics. The ratio decreases towards higher frequencies. It is calculated that cooling causes an increase in the compliance of the basilar membrane resulting in a decrease of the computed amplitude ratio of the basilar membrane displacement that is larger for the higher frequencies.

$$\left|\frac{Y_{(x_{III},\omega)}}{Y_{(x_I,\omega)}}\right| = \exp\left[-\frac{(-3h+a)}{4}(x_{III}+x_I) - \frac{Q}{2}\omega^2 R_m C_o \left\{e^{\frac{3h+a}{2}x_{III}} + e^{\frac{3h+a}{2}x_I}\right\} - \frac{Q}{2}\frac{R_o\omega^{1/2}}{\rho}\left(e^{\frac{h+2a}{2}x_{III}} + e^{\frac{h+2a}{2}x_I}\right)\right]$$

Substituting the parameter values results in:

$$\left|\frac{Y_{(x_{III},f)}}{Y_{(x_I,f)}}\right| = \exp\left[3.15 - 8.8\cdot 10^7 C_o^{3/2} f^2 - 5.6\cdot 10^3 C_o^{1/2} f^{1/2}\right]$$

At 38°C $C_o = 0.59\ 10^{-10}$ cm^4/dyne so

$$\left|\frac{Y_{(x_{III},f)}}{Y_{(x_I,f)}}\right| = \exp\left[3.15 - 4\cdot 10^{-8} f^2 - 4.3\cdot 10^{-2} f^{1/2}\right] \text{ or}$$

since $20^{10}\log \exp[\quad] = 8.6[\quad]$ one obtains the ratio in dB:

$$\left|\frac{Y_{(x_{III},f)}}{Y_{(x_I,f)}}\right| = 8.6\left[3.15 - 4\cdot 10^{-8} f^2 - 4.3\cdot 10^{-2} f^{1/2}\right] \text{ dB}$$

At 28°C $C_o = 0.69\ 10^{-10}$ cm^4/dyne. This results in

$$\left|\frac{Y_{(x_{III},f)}}{Y_{(x_I,f)}}\right| = 8.6\left[3.15 - 5\cdot 10^{-8} f^2 - 4.65\cdot 10^{-2} f^{1/2}\right] \text{ dB}$$

The ratio of the displacement of the basilar membrane computed at 38 and 28°C is shown in Figure 4.5. It appears that cooling causes a decrease of the ratio of the basilar membrane displacement which is in accordance with a basal shift of the excitation profile. The decrease is larger for higher frequencies. In comparison the ratio of the mean CM data obtained at 70 and 90 dB SPL is shown in Figures 4.6a-b. The shape of the measured CM profiles is in good accordance with the computed curves. The shift of the CM profile of the measured data as a result of cooling, however, is contradictory to the shift of the computed curves. On the basis of the mechanical factors which determine the CM-amplitude profile a controversial conclusion is drawn with respect to the experimental data. Since the CM-amplitude is also dependent on the potential gradient across the haircells, metabolic factors must also be taken into account.

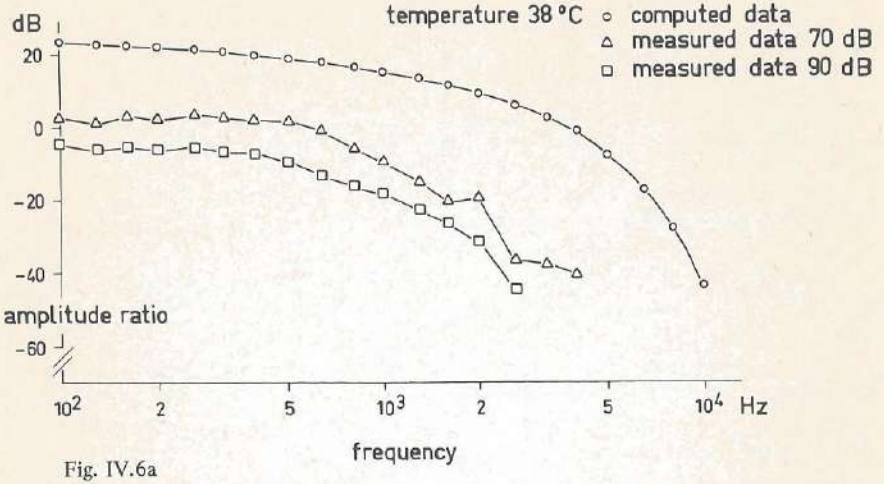

Fig. IV.6a

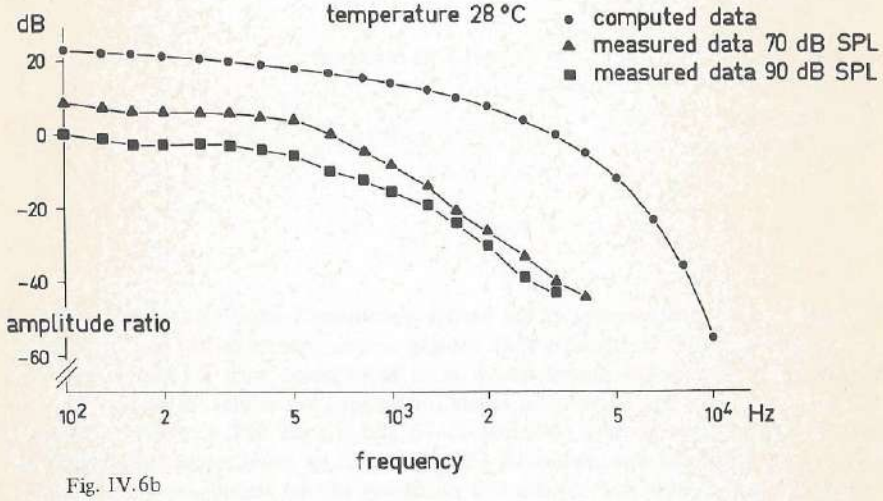

Fig. IV.6b

Fig. IV.6a-b Comparison of the experimental CM ratio and the computed amplitude ratio of the basilar membrane at normal (Figure IV.6a) and a 10 degrees lower temperature (Figure IV.6b). The shape of the curves is quite similar. The shift of the experimentally obtained CM profile is in the opposite direction of the shift of the computed curve.

IV.6 THE INFLUENCE OF COCHLEAR METABOLISM

The energy production in the stria vascularis is thought to be due to primarily oxidative metabolic processes, because in anoxia the endolymphatic potential disappears almost instantaneously (*Konishi et al.*, 1961). Cooling causes a decrease in metabolic rate resulting in a decrease of the CM amplitude. *Butler et al.* (1960) observed in guinea pigs that lowering the cochlear temperature decreased the CM magnitude in an orderly fashion with 6 dB/10°C. This is in accordance with the experimental results in the first turn where a Q_{10} of about 2 is reported (See section III.4). In the third turn, however, a Q_{10} of 1.1 is calculated. Therefore one may conclude that a difference in resistance against lowering of the metabolic rate exists between the first and the third turn. It is known from *Thalmann et al.* (1973) that for the guinea pig inner ear a gradient in energy storage exists in the Organ of Corti. The phosphocreatine concentration decreases by about a factor 5 from the stapes towards the apex, while the glycogen concentration increases by a factor 3. No significant gradient for the adenosinetriphosphate (ATP) or glucose concentration has been reported in the literature. In the stria vascularis and the spiral ligament *Kuypers* (1969) reported a decrease in ATP-ase activity by about a factor 8 from the stapes towards the apex. Since the CM may be due to a stimulus related modulation of a leak current across the reticular lamina (*Davis*, 1965; *Strelioff et al.*, 1972) the CM amplitude will be dependent on the endolymphatic potential as well as on the negative polarization in the hair cells. The endolymphatic potential is maintained by the stria vascularis. The enzymatic activity decreases by cooling and therefore the maintenance of the endolymphatic potential may be disturbed, however, a Q_{10} of only 1.2 was reported by *Butler et al.* (1960) for the endolymphatic potential. It is assumed that the total amount of induced energy determines the potential gradient. The absolute amount of the effect of cooling on the metabolic rate will be largest in the basal turn due to the distribution of ATP-ase. This may explain the difference in the temperature coefficient in the first and the third cochlear turn. The different effect of cooling on amplitude in both turns results in a factor

$$\frac{Q_{10}(x_I)}{Q_{10}(x_{III})} = \frac{2}{1.1} = 1.8$$

by which the first turn amplitude values decrease more than those from the third turn. The apical shift of the CM profile for lower temperature as derived from the amplitude data, is calculated to be a factor 1.3 (See section III.3). From the phase data one calculates a (basal) shift by a factor 0.9. The difference from phase and amplitude measurements, resulting in opposite shifts is about a factor 1.45. This may be explained by the difference in the temperature coefficient in the first and the third cochlear turn.

The contradiction between the shift of the CM profile obtained from the phase and the amplitude is explained by the different effect of cooling on the metabolic rate in the first and the third cochlear turn. It may be concluded that the obtained

CM amplitude data offer incorrect information while the obtained phase data offer correct information with regard to a shift of the mechanical excitation profile as a result of temperature. It seems, therefore, worthwhile to study the shift of the excitation profiles in one turn only. Since the gradient in temperature coefficient from the first to the third cochlear turn is supposed to be a linear function of the distance, the effect thereof will be greatly reduced by studying the CM in a small part of the first turn only. In addition, since the frequency distribution on the cochlear partition is an exponential one, there is no loss in sensitivity by studying the phenomena over a smaller range if the stimulus frequencies are chosen appropriately. The resulting situation provides absolute changes in amplitude which are comparable to a measurement in the first and third cochlear turn but with a greatly reduced influence of different temperature coefficients. This may be accomplished by using the multiple electrode-array technique, as will be described in the next chapter.

Chapter V

MULTIPLE-ELECTRODE ARRAY MEASUREMENTS

V.1 INTRODUCTION

Multiple-electrode array measurements allow analysis of the microstructure of the CM pattern with regard to its longitudinal course. In this method, introduced by *Kohllöffel* (1971) a multiple-electrode array is placed in the basal turn of the scala tympani of the guinea pig, because this part of the cochlea is spacious enough to allow the insertion therein. Recordings have been made from each electrode in reference to a neck electrode. It is known (*Tasaki & Fernandez*, 1952) that the superiority of the differential electrode technique, i.e. recording potentials only from one turn without contamination with electrical signals from other turns, does not hold in the basal turn for frequencies which evoke maximum basilar membrane displacements at more apical positions. In the basal turn CM recorded with one electrode in the neck and the other in the basal turn, either in the scala vestibuli or in the scala tympani is not contamined by the responses from the upper turns (op. cit.). For a frequency range of 200 Hz to 10 kHz *Dallos* (1969) did not find any disadvantage in the technique with one electrode in the basal turn and the other in the neck in respect to the differential electrode technique apart from the fact that differential recorded potentials are a factor 2 larger. The slit in the wall of the scala tympani, necessary to allow installation of the multiple electrode array, seems not to influence the recordings more than other techniques with a few small holes. *Kohllöffel* (1971) could not measure any difference between single electrodes inserted through holes in the scala tympani and the multiple electrode array inserted through a slit therein. The fluid level in the scala tympani has neglible influence on the mechanical response of the basal part of the basilar membrane provided that it is not dried out (*Tasaki et al.*, 1952; *von Békésy*, 1960; *Johnstone & Boyle*, 1967; *Robertson*, 1974). According to *Kohllöffel* (1971) the multiple electrode array has practically no influence on the recorded results and can be regarded as a "passive" sensory device.

V.2 THE EXPERIMENTAL METHOD

V.2.1 *Premedication, anaesthesia and surgical procedure*

The experiments have been performed on ten female albino guinea pigs with an average weight of 676 grams, the smallest weighing 515 grams, the largest weighing 860 grams. Premedication and anaesthesia are obtained in the way as described in Chapter II section II.2. All animals received atropine and urethane. Eight animals also received Thalamonal® and two other animals also received diazepam (Valium®). The cochlea is fully exposed in the way as described in Chapter II section II.3. Using a Portmann-drill with a circular blade, a narrow slit, about 2 mm long and 0.5 mm wide is obtained in the wall of the scala tympani of the basal turn. Using fine wire hooks the bone dust and sometimes tiny blood clots are

removed and the slit in the membranous part of the scala tympani is made as wide as the bony slit. The slit extends from about 3 - 5 mm along the basilar membrane measured from the basal end of the membrane. Then the animal is placed in the electrically shielded sound proof room and mounted in a stereotaxic instrument, with the head rigidly clamped between the upper jaw and hollow earbars that allow sound stimulation.

V.2.2 *The multiple electrode array*

The eleven electrodes composing the probe are made of stainless steel wire of 50 micron Ø. They are assembled side by side at an equal distance of 150 micron and glued with alpha-cyanoacrylate (cyanolit®) in parallel grooves in a rectangular polyvinyl chloride piece (a) with dimensions of 1 x 2.4 x 14 mm, the tips of the electrodes protruding 400 micron. The polyvinyl chloride piece is attached to a trapezium shaped plate of polyvinyl chloride (b) with a length of 75 mm and a base of 25 mm having the same thickness. The steel wire electrodes are glued thereon with alpha-cyanoacrylate. The probe is coated with Acrifix® 92. The electrodes are soldered to eleven wires that are assembled into a bundle of 50 cm length (c) and soldered to eleven golden bars in a connector (d). By this way the probe is connected to a round perspex shaft (e) with eleven embedded messing wires leading to eleven preamplifiers arranged stellated in this shaft (Figure V.1).

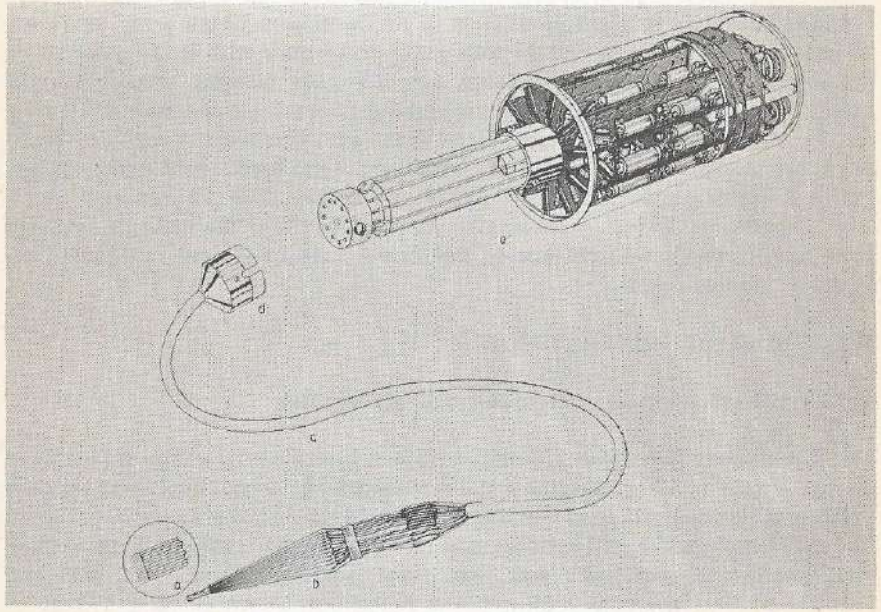

Fig. V.1 Schematic representation of the multiple electrode array. Eleven electrodes are attached to a polyvinyl chloride plate (a) at equal distances. They are connected to a round perspex shaft (e) holding eleven preamplifiers.

V.2.3 *The measurement setting*

With a micro manipulator the multiple electrode array is inserted through the slit into the scala tympani. In this way the basalmost electrode is about 3 mm away from the basal end of the basilar membrane. The tips of the electrodes are thus located in a line at about 500 micron distance parallel to the basilar membrane. Then the animal is turned upside down in order to prevent accumulation of perilymphe and wound secretions in the middle ear and to allow flowing of these liquids outside the animal. A modified ear speculum is attached to the hollow earbar in the animal's right ear and attached to the same dynamic earphone and sound stimulator as in Chapter II sections II.4 and II.6. Also the sound pressure level is recorded in the same way and the animal is situated in the same room.

V.2.4 *The cooling and heating procedure*

The cooling and heating procedure is essentially the same as described in Chapter II section II.5 with the exception that cooling is achieved by attaching a small metal container filled with water and ice to the animal's back. Cooling and rewarming is achieved in one step of 10°C. Rewarming is achieved by blowing warm air directly at the animal.

V.2.5 *The stimulating and recording procedure*

The stimulating procedure is the same as described in Chapter II section II.6. The continuous tone stimulus is used in the CM amplitude and phase measurements. Tone-bursts are used in the AP-latency measurements. The CM is recorded from the scala tympani electrodes alternately from electrodes no 1 to 10 in respect to the basalmost electrode no 0 using the wound retractor which is in good contact with the cervical muscles as a ground and reference electrode. The CM recorded from the eleven scala tympani electrodes is preamplified 10 times, each electrode having its own preamplifier. All preamplifiers are identical and have the same flat frequency response curves from 0.8 Hz - 10 kHz. The preamplifiers no 1 to 10 are successively connected to the main amplifier with an amplification factor ranging from 500 to 25,000 times.

V.3 EXPERIMENTAL RESULTS

The amplitude and phase distribution of the CM is measured along the electrode array for stimulus frequencies ranging from 1 to 14 kHz at a level of 90 dB SPL for temperatures of 35[5] and 28°C. For a typical animal Figure V.2 shows the CM amplitudes in reference to the first electrode (nr. 0) and the CM phase for nine electrodes in the array at 35[5]°C body temperature.
According to *Kohllöffel* (1971) the low frequency recording can be used as a control of the preparation and the uniformity of the recording electrodes because at this frequency all electrodes are situated at about the same distance from the maximum of the CM amplitude envelope located apically in the cochlea. Because

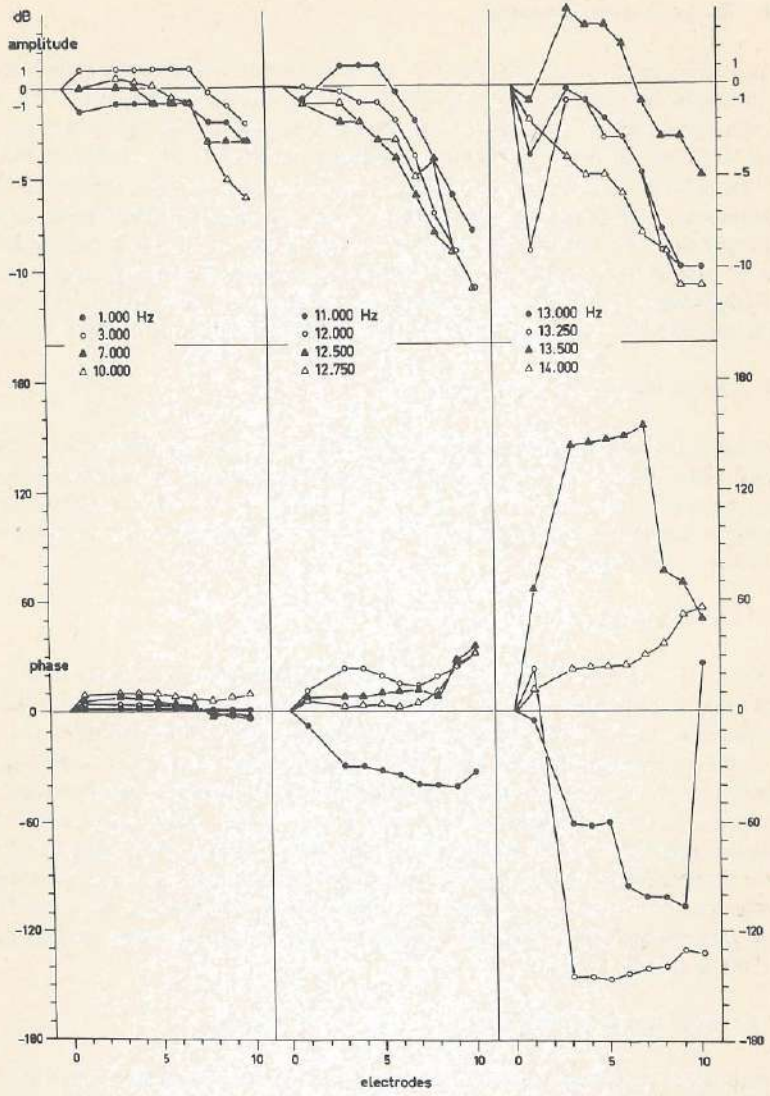

Fig. V.2 The CM amplitude in reference to the first electrode (nr. 0) and the CM phase gradient for nine electrodes in the array at 35^5 °C and 90 dB SPL at various frequencies. The low frequency recordings (e.g. 1000 Hz) can be used as a control. At the electrodes 8, 9 and 10 the CM amplitude declines which is thought to be an artefact because hardly any phase difference is observed between these electrodes. The CM amplitude decreases with increasing frequency. The phase gradient along the array increases with increasing frequency. At very high frequencies phase reversal is observed.

of the long wavelength of the travelling wave in the basilar membrane (about 10 mm) in respect to the width of the electrode array (2 mm) little phase variation is observed (e.g. the 1 kHz recording in Figure V.2). Due to the long wavelength there is almost no phase cancellation in the region of the electrode array and the CM amplitude curve has a pattern in conformity with that of the basal slope of the CM envelope i.e. a very flat slope. At the electrodes 8, 9 and 10 the amplitude declines which, according to *Kohllöffel* (op. cit.) is an artefact due to the strong bending of the basilar membrane in this area. Because the electrodes are arranged in the array in a straight line the apical electrodes are at a larger distance from the basilar membrane that bends away from them. Because almost no phase difference is observed at the apical electrodes the drop in CM amplitude is not caused by phase cancellation. With increasing frequency the maximum of the CM envelope moves towards the stapes and the wavelength of the excitation pattern becomes progressively shorter. This means that the phase change along the basilar membrane increases (per unit of length). Because neighbouring hair cells are driven out of phase there will be mutual cancellation of the hair cell outputs. With increasing frequency the CM amplitude is observed to decrease for all electrodes in the array and progressively towards the apical electrodes (See also Figures V.5a-c). The slope of the CM amplitude curves is observed to be about the same for all frequencies. The phase gradient along the electrode array increases progressively with the stimulus frequency. At 13.5 kHz a large phase variation of 70 degrees is observed between the electrodes 7 and 8. At high frequencies the apical electrodes may lead the basal ones in phase. Phase reversal is observed at 13.000 Hz between electrodes 9 and 10 and at 13.250 Hz between electrodes 1 and 3. These observations are in accordance with the experiments of *Kohllöffel* (op. cit.). The CM amplitude (in dB) in reference to the first electrode (no 0) and the CM phase gradient as a function of frequency are shown in Figures V.3a-b for the electrodes 1, 3, 5, 7, 9 and 10 in the array. The CM amplitude is observed to decrease for the more apical electrodes, especially for the high frequencies, with distinct minima around 13 kHz. The CM phase gradient as a function of frequency shows almost no phase difference up to 10 kHz. Above 10 kHz phase variations occur and at frequencies around 13 kHz phase reversal is observed. The phase reversal is accompanied by an amplitude minimum as a result of phase cancellation. Cooling causes no systematic changes in the phase difference at low frequencies (i.e. below 10 kHz). For higher frequencies the phase difference increases which is in accordance with a basal shift (compare Chapter IV). Around 13 kHz a shift to low frequencies of the phase reversal is observed to be in the order of 250 Hz (Figure V.4). No amplitude shift is observed as a result of cooling. In the Figures V.5a-c the CM amplitude in μV RMS and the CM phase are shown for the electrode array at 35^5 and 28°C. *As a result of cooling the CM amplitude decreases as expected in conformity with the results described in Chapter III.* For high frequencies, i.e. from 12 kHz up to 13.5 kHz the CM amplitude is observed to increase slightly for the more apical electrodes (i.e. 9 and 10). For frequencies up to 12 kHz the phase difference increases for the more apical electrodes when lowering the temperature. Above 12 kHz this results in an increase in phase difference for all electrodes in the array. At 13 kHz the phase reversal shifts from the electrodes 9 and 10 to the electrodes 7 and 8. The corresponding amplitude minimum is situated at the same

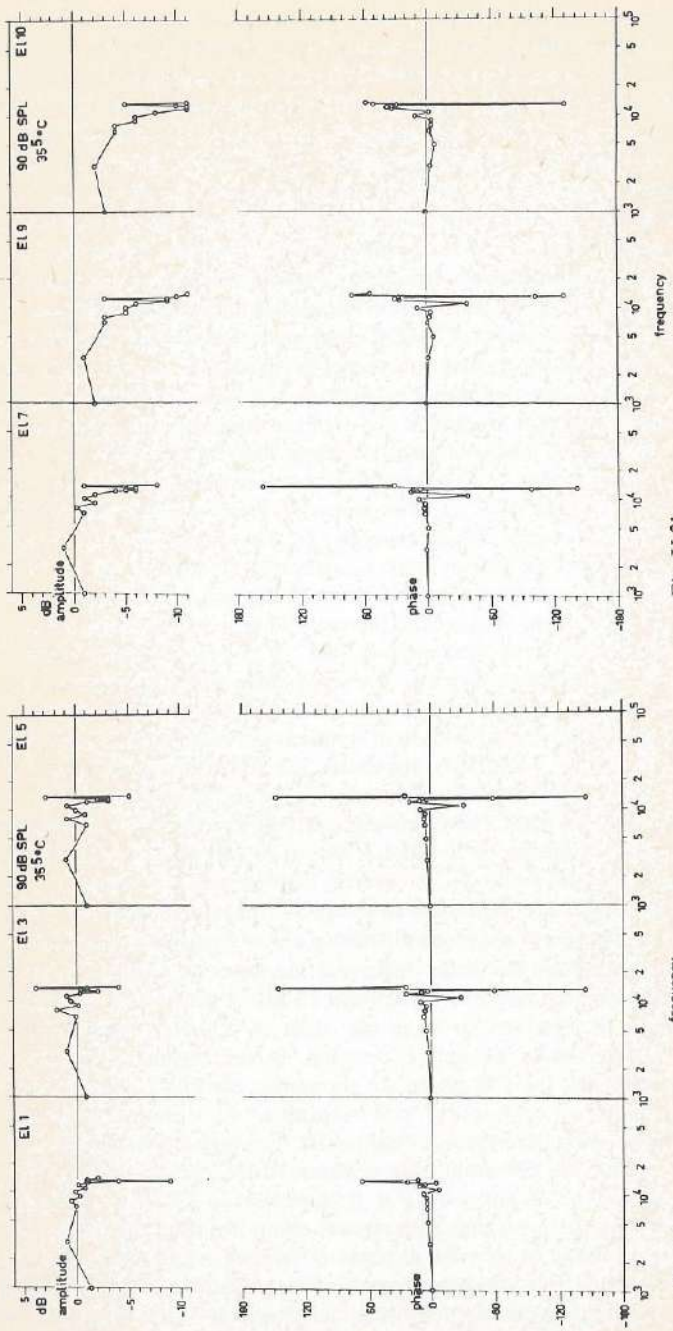

Fig. V.3b

Fig. V.3a

Fig. V.3a-b The CM amplitude (in dB) with reference to electrode nr. 0 and the CM phase gradient as a function of frequency for various electrodes at 35.5 °C. The CM amplitude shows a decrease towards higher frequencies and for more apical electrodes. The amplitude minimum is accompanied by phase reversal.

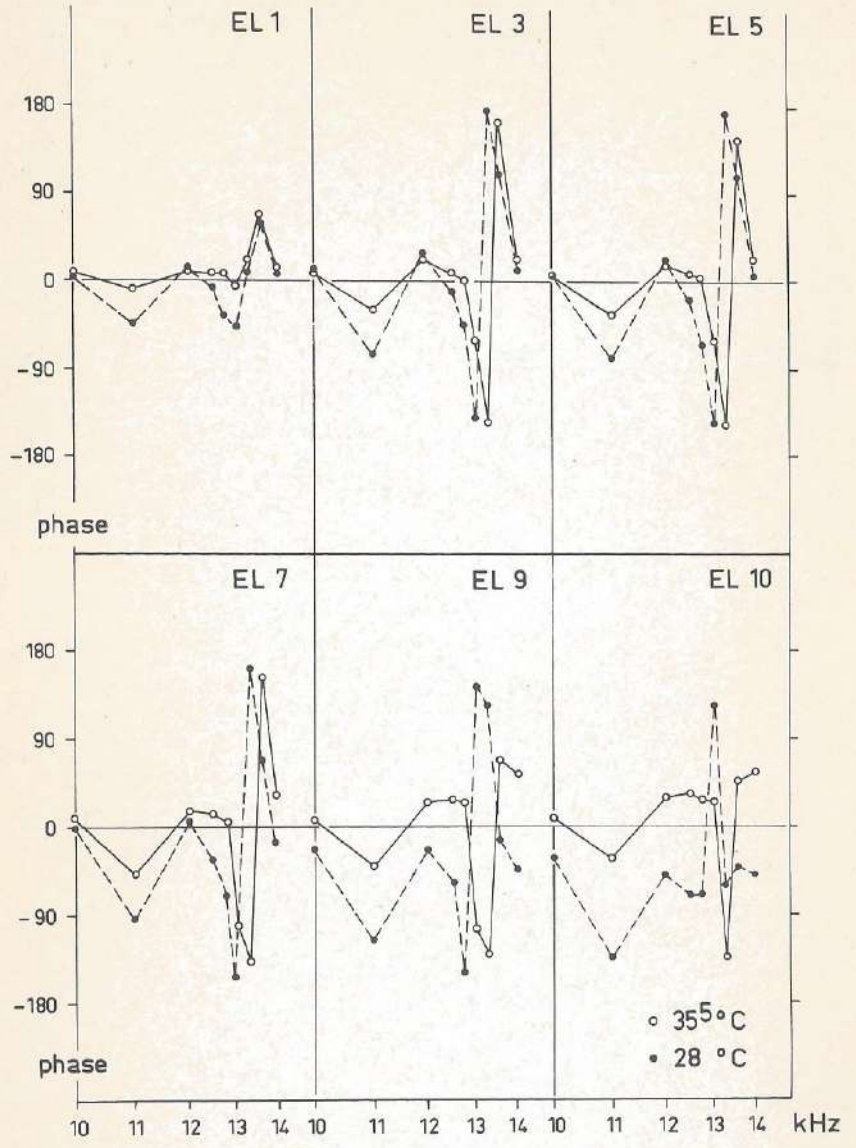

Fig. V.4 The CM phase as a function of frequency at different temperatures. Cooling causes an increase in phase difference in accordance with a basal shift of about 250 Hz.

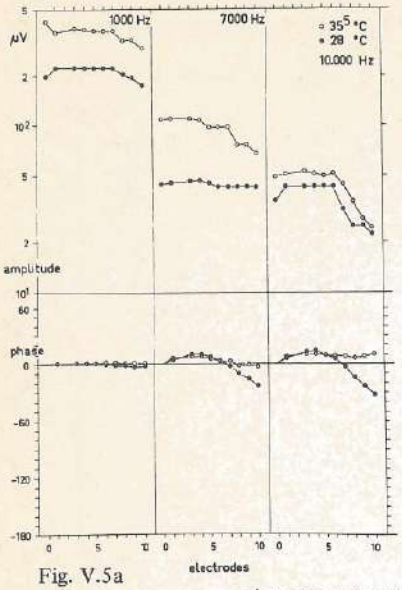

Fig. V.5a

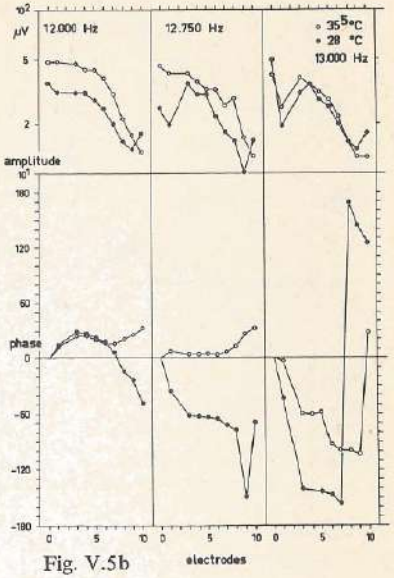

Fig. V.5b

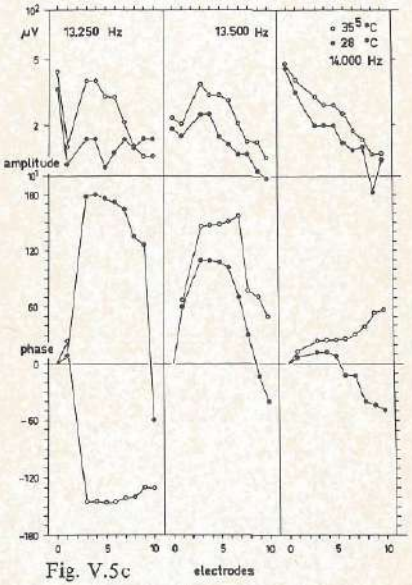

Fig. V.5c

Fig. V.5a-c The CM amplitude in μV RMS and the CM phase gradient along the electrode array at various frequencies and different temperatures at 90 dB SPL. Cooling causes an increase in phase difference and a basal shift of the phase reversal along two electrodes. This is in accordance with a shift of the CM excitation pattern of about 225 micron. The larger decrease of the CM amplitude at 1000 and 7000 Hz stimulus frequency is noted. This can be explained by the position of the electrode array along the basilar membrane.

electrodes. *These results are in accordance with a basal shift of the CM excitation pattern of one to two electrodes (i.e. 150 to 300 micron).* As a result of cooling at 12.750 Hz stimulation, an increase in phase is observed between electrodes 9 and 10 accompanied by an amplitude minimum. Comparing the CM phase gradient at stimulation of 12.750, 13.000 and 13.250 Hz in the Figures V.5b-c no phase reversal is observed at 12.750 Hz at 35^5 °C but at 13.000 and 13.250 Hz a phase reversal is observed between respectively the electrodes 9 and 10 and between 1 and 3. As a result of cooling the phase reversal shifts basally and is already at 12.750 Hz stimulation between electrodes 9 and 10. At 13.000 Hz the phase reversal has shifted two electrodes in the direction of the stapes. At 13.250 Hz the phase reversal has shifted to the most basal electrodes (i.e. between 1 and 3). At 13.500 Hz still part of the phase reversal is visible between electrodes 1 and 3. Out of the series of ten animals, six failed to show any phase difference along the electrode array as a function of frequency at normal temperature. It was concluded that in that situation there was considerable crosstalk between the electrodes. In three additional animals a phase increase occurred as a result of cooling; this being comparable to the results presented above.

V.4 DISCUSSION

As a result of cooling of about 8°C the CM excitation pattern shows a basal shift of about 1 to 2 electrodes corresponding with a distance of 150 to 300 micron. Also as a result of cooling phase reversal as a function of frequency is observed to shift about 250 Hz to a lower frequency. One may conclude that in this particular animal cooling causes a basal shift of the excitation pattern of about 250 Hz corresponding to about 225 micron along the basilar membrane in the basal turn of the cochlea where the electrode array is situated. The relation of the electrode array with regard to the excitation profile is illustrated in Figure V.6.

The measurements in this Chapter concern one individual animal. They are in accordance with the results of *Kohllöffel* (1971) obtained at normal cochlear temperature.

The fact that the multiple electrode array measurements in the first turn (with respect to amplitude as well as phase data) in four animals support the conclusions drawn from the phase data of the differential electrode measurements from the first and third turn, serves as an illustration for the disturbing effects of the difference in temperature coefficient on the amplitude data therefrom.

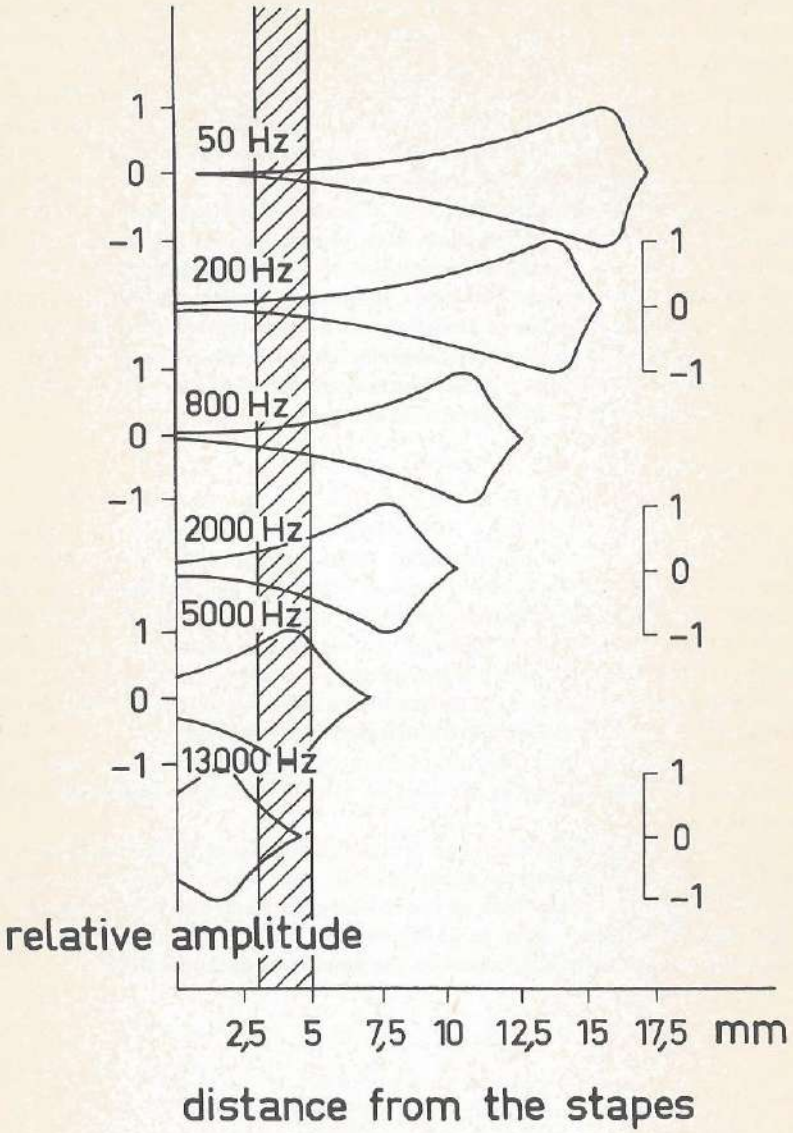

Fig. V.6 Schematic representation of the travelling wave envelope as a function of the distance from the stapes in comparison to the place of the electrode array (modified after von Békésy, 1960). The shaded area indicates the place of the electrode array.

Chapter VI

GENERAL DISCUSSION

This section combines the discussion of chapter IV and chapter V.
The CM-phase data from both the differential electrode measurements and the multiple electrode array measurements indicate a basal shift of the excitation profile for a continuous tone. From substitution of parameter values in the equations derived from Zwislocki's theory one calculates an increase by about 20% in the compliance of the basilar membrane for the first and third cochlear turn.
The influence of metabolism on the CM-amplitude results in changes of different direction for the differential electrode series and the multiple electrode array series, the latter being in accordance with the conclusion from the phase data. Since one should expect that cooling causes an increase in stiffness (i.e.a decrease in compliance) in any physical system these results are quite surprising.
The effects of increased viscosity of the perilymphe will not have any influence on the phase data (according to the theory) but a considerable one for the amplitude pattern. The effect of viscosity is mainly seen in the last term of the equation on the amplitude distribution. As *Dahl & Kleinfeld* (1973) have pointed out the viscosity of the perilymphe increases by a factor 2 for lowering the temperature to 28°C. This will influence the parameter R_o which will increase. This also results in a basal shift of the CM profile maximum due to a smaller effect of the viscosity loading at higher frequencies.
The smallness of the effects produced by cooling of the cochlea may leave the possibility that some counteracting effects are responsible for these results. On the basis of two independent sets of measurements and a calculation based on a generally accepted theory it remains difficult, however, to state other mechanisms besides the increased compliance and increased viscosity responsible for the effects observed.

SUMMARY

The subject of this investigation is the frequency analyzing properties of the guinea pig cochlea in relation to changing mechanical and metabolic properties thereof.

Chapter I

The transducer properties of the peripheral hearing organ are reviewed with respect to basilar membrane movement, sensory transduction in the hair cells and the relation of the cochlear microphonics (CM) to basilar membrane vibration. This is followed by a survey of a mathematical model relating hydromechanical theory to experimental data. The various experimental methods for obtaining direct measurements of basilar membrane displacement and reliable CM data are discussed. For the present investigation the CM is recorded from the first, second and third cochlear turn using the differential electrode technique. The measurements are performed with respect to amplitude and phase of the CM for various continuous tone frequenties, stimulus intensities and cochlear temperatures. Thereafter, the observed data are related to Zwislocki's theory of cochlear dynamics in order to calculate the changes in the compliance of the basilar membrane under varying cochlear temperature. After such calculations a separation of mechanical and metabolic effects on the changing CM stimulation profile in the guinea pig is performed.

Chapter II

The changes in mechanical properties of the guinea pig cochlea are obtained by varying the cochlear temperature. Cooling causes changes in the compliance of the basilar membrane and in the viscosity of the cochlear fluids. As stimuli continuous tones and tone-bursts are presented to the guinea pig ear. The CM and the compound action potential (AP) are recorded by using the differential electrode technique. In this method the CM is recorded from a well defined region along the basilar membrane without intermingled AP or summating potential. The CM amplitude and phase are recorded in response to a continuous tone stimulus. The CM travelling wave delay is measured in response to low frequency tone-bursts. Because temperature change may cause some hysteresis in cochlear metabolism the latency of the AP evoked by high frequency tone-bursts is used as a sensor for cochlear functioning.

Chapter III

The differential recorded CM from the first, second and third cochlear turn is quantified by the amplitude and by the phase lag with respect to the CM of the first turn. The CM amplitude decreases sharply above 2600 Hz in the second turn and above 1000 Hz in the third turn. For normal temperature the results of others were confirmed. A comparison is made between the results obtained at normal cochlear temperature and at about 10°C lower temperature. At lower temperature the amplitude from the first, second and third turn decreases while the basic shape of the CM frequency response curve does not change. The accumulating phase difference between the first and the third turn increases by about $1/8\pi$ radians as a result of 10°C cooling pointing to a basal shift of the CM profile. The travelling wave delay between the first and the third cochlear turn slightly increases as a result of 10°C cooling in accordance with the phase data. An apical shift of 1/3 of an octave is calculated from the frequency shift for various ratios of the CM amplitude from the third and the first turn for normal and a 10°C lower temperature. A different effect of temperature on the CM in the first and the third turn is calculated. In the first turn $Q_{10} = 2$ and in the third turn $Q_{10} = 1.1$.

Chapter IV

The observed changes in the CM phase and CM travelling wave delay are substituted in the formules derived from Zwislocki's theory on cochlear hydrodynamics in order to calculate the compliance of the basilar membrane. The values of the basilar membrane compliance substituted in the formula for the amplitude ratio of the basilar membrane as derived from Zwislocki's theory result in a decrease of the ratio of the basilar membrane displacement in accordance with a basal shift of the CM excitation profile. The ratio of the measured CM amplitude data, however, increases as a result of cooling pointing to an apical shift of the measured CM amplitude profile in contradiction to the results obtained from the phase and travelling wave delay data. On the basis of the mechanical factors which determine the CM amplitude profile a controversial conclusion is drawn with respect to the experimental data.
Since the CM amplitude is also dependent on the potential gradient across the hair cells metabolic factors must be taken into account. From a literature survey on the distribution of the energy storage and relating enzymes (i.e. adenosinetriphosphatase) in the guinea pig cochlea it is concluded that the effect of cooling on the metabolic rate will be largest in the basal turn. This may explain the difference in the temperature coefficient in the first and third cochlear turn. It may be concluded that the CM amplitude data offer incorrect information while the phase data offer correct information with regard to the shift of the mechanical excitation profile as a result of cooling.

Chapter V

Because of the different temperature coefficient of the CM in the first and the third turn the shift of the excitation profile has subsequently been studied in the first turn only, using the multiple electrode-array technique. The amplitude and phase distribution of the CM along the electrode array is measured. Along the electrode array phase differences and phase reversal are observed. The phase reversal is accompanied by an amplitude minimum. As a result of cooling the CM amplitude decreases and the phase difference increases in conformity with the results described in chapter III. From the phase as well as the amplitude measurements a basal shift of the CM excitation pattern is deduced.

Chapter VI

The CM data from both the differential electrode measurements and the multiple electrode measurements indicate a basal shift of the excitation profile for a continuous tone. An increase in the compliance of the basilar membrane is calculated to be about 20%.
Since one should expect that cooling causes an increase in stiffness (i.e. a decrease in compliance) in any physical system, these results are quite surprising. Increase of the viscosity of the perilymphe will not have any influence on the phase data (according to the theory) but some on the amplitude pattern. This increase in viscosity would also result in a basal shift of the CM profile maximum. The smallness of the effects produced by cooling of the cochlea may leave the possibility that some counteracting effects are responsible for the results. On the basis of two independent sets of measurements and a calculation based on a generally accepted theory it remains difficult, however, to state other mechanisms besides the increased compliance responsible for the effects observed.

SAMENVATTING

Dit proefschrift beschrijft een onderzoek naar het frequentie analyserend vermogen van de cochlea van de cavia bij wisselende mechanische en metabole eigenschappen.

Hoofdstuk I

De transducer mechanismen van het perifere gehoororgaan worden beschreven voor wat betreft de beweging van het basilair membraan, de mechanisch-elektrische omzetting in de haarcellen en de relatie tussen de cochleaire microfonie (CM) en de beweging van het basilair membraan. Dit wordt gevolgd door een overzicht van een wiskundig model dat de hydromechanische theorie koppelt aan de experimentele resultaten. De verschillende experimentele methoden voor het verkrijgen van directe meting van de verplaatsing van het basilair membraan en betrouwbare resultaten over de CM worden besproken.
In het onderhavige onderzoek wordt de CM afgeleid uit de eerste, tweede en derde cochleaire winding door middel van twee differente intracochleaire electroden. De amplitude en fase van de CM worden gemeten voor verschillende frequenties van een continue toon, stimulus intensiteiten en cochleaire temperaturen. De resultaten worden gerelateerd aan Zwislocki's theorie betreffende cochleaire hydrodynamica teneinde de compliantieveranderingen van het basilair membraan te berekenen bij wisselende temperatuur. Na deze berekening wordt onderscheid gemaakt tussen mechanische en metabole effecten op het veranderende CM stimulatieprofiel in de cochlea van de cavia.

Hoofdstuk II

De veranderingen in de mechanische eigenschappen van de cochlea van de cavia worden verkregen door de cochleaire temperatuur te wijzigen. Afkoeling veroorzaakt verandering in de compliantie van het basilair membraan en in de viscositeit van de cochleaire vloeistoffen. Als stimuli worden continue tonen en toonstoten gebruikt. De CM en de samengestelde actiepotentiaal (AP) worden afgeleid door middel van twee differente intracochleaire electroden. Bij deze techniek wordt de CM afgeleid uit een scherp omschreven gebied langs het basilair membraan zonder vermenging met de AP of de sommatiepotentiaal. De CM amplitude en fase worden afgeleid na stimulatie met een continue toon. De vertraging van de lopende CM golf wordt afgeleid na stimulatie met laag frequentie toonstoten. Aangezien temperatuurverandering hysterese in het cochleair metabolisme kan veroorzaken wordt de latentie van de AP afgeleid na stimulatie met hoog frequente toonstoten daar dit een gevoelige test is voor het functioneren van de cochlea.

Hoofdstuk III

De met intracochleaire electroden afgeleide CM uit de eerste, tweede en derde cochleaire winding wordt gekwantificeerd aan de hand van de amplitude en het

faseverschil tussen de CM uit de eerste en de derde winding. De CM amplitude neemt sterk af in de tweede winding bij stimulatie met tonen van een hogere frequentie dan 2600 Hz. In de derde winding treedt deze afname reeds boven 1000 Hz op. Bij normale temperatuur werden de resultaten van andere onderzoekers bevestigd. De resultaten tussen metingen bij normale temperatuur en die bij een 10°C lagere temperatuur worden vergeleken. Bij lagere temperatuur daalt de amplitude uit de eerste, tweede en derde winding. De vorm van de karakteristiek van de CM amplitude als functie van de frequentie blijft onveranderd. Het toenemende faseverschil tussen de eerste en de derde winding stijgt met ongeveer $1/8\pi$ radialen als gevolg van 10°C afkoeling. Dit wijst op een verschuiving van het CM profiel in basale richting.
De lopende golf vertraging tussen de eerste en de derde winding toont een geringe toename als gevolg van 10°C afkoeling, in overeenstemming met de fasemetingen. Een apicale verschuiving van het CM stimulatie profiel van 1/3 octaaf wordt berekend uit de frequentie verschuiving voor een aantal verhoudingen van de CM uit de derde en de eerste winding voor normale en een 10°C lagere temperatuur. Een verschillend temperatuureffect op de CM wordt berekend in de eerste en de derde winding. In de eerste winding wordt een temperatuurcoëfficiënt $Q_{10} = 2$ berekend en in de derde winding $Q_{10} = 1.1$.

Hoofdstuk IV

De gemeten veranderingen in de CM fase en de lopende golf vertraging worden gesubstitueerd in de formules afgeleid uit Zwislocki's theorie over de cochleaire hydrodynamica, ten einde de compliantie van het basilair membraan te berekenen. Uit de fasemetingen en de lopende golfvertraging tussen de CM uit de eerste en de derde winding wordt een toename van de compliantie van het basilair membraan berekend ten gevolge van afkoeling. Uit substitutie van deze berekende waarden in de formule voor de bewegingsamplitude verhouding van verschillende punten van het basilair membraan afgeleid uit Zwislocki's theorie volgt een verschuiving van het CM excitatieprofiel naar de stapes toe. Echter de verhouding van de gemeten CM amplitude neemt toe als gevolg van afkoeling hetgeen wijst op een apicale verschuiving van het gemeten CM amplitude profiel. Dit is in tegenspraak met de resultaten uit de metingen van de fase en de lopende golf vertraging. Op basis van de mechanische factoren die het CM amplitude profiel bepalen wordt een tegenstrijdige conclusie getrokken ten aanzien van de experimentele gegevens.
Aangezien de CM amplitude bovendien afhankelijk is van het potentiaal verschil over de haarcellen, moet met metabole factoren rekening worden gehouden. Uit een literatuuroverzicht blijkt dat in de cochlea van de cavia een ongelijke verdeling bestaat van de beschikbare energie en de enzymen (d.w.z. adenosinetrifosfatase) die daarbij betrokken zijn. Geconcludeerd wordt dat het effect van afkoeling op het metabolisme het grootst zal zijn in de basale winding. Dit kan het verschil verklaren tussen de temperatuurcoëfficiënten in de eerste en de derde winding. Men kan concluderen dat de CM amplitude metingen onjuiste informatie geven, terwijl de fasemetingen de juiste informatie leveren ten aanzien van de verschuiving van het mechanische excitatieprofiel ten gevolge van afkoeling.

Hoofdstuk V

Vanwege het verschil in temperatuurcoëfficiënt van de CM tussen de eerste en de derde winding, werd de verschuiving van het excitatieprofiel vervolgens alleen in de eerste winding gemeten met gebruikmaking van de multi-electrode techniek. De amplitude en faseverdeling langs de electrode array worden gemeten. Langs de electrode array blijkt fase verschil en fase omslag te bestaan. De fase omslag valt samen met een amplitude minimum. Ten gevolge van afkoeling daalt de CM amplitude en neemt het fase verschil toe overeenkomstig de resultaten beschreven in hoofdstuk III. Uit zowel de fase als de amplitude metingen wordt een basaalwaartse verschuiving van het excitatieprofiel verkregen.

Hoofdstuk VI

Zowel de gegevens verkregen met de intracochleaire electroden als met de multi-electrode wijzen op een basaalwaartse verschuiving van het CM excitatie profiel voor stimulatie met een continue toon. Een toename van de compliantie van het basilair membraan in de orde van 20% wordt berekend.
Aangezien men voor elk fysisch systeem zou verwachten dat afkoelen een toename in stijfheid (d.w.z. een afname in compliantie) zou veroorzaken, zijn deze resultaten verrassend. Toename van de viscositeit van de perilymfe zal de fasemetingen niet beïnvloeden (volgens de theorie) maar heeft wel enige invloed op de amplitude. Deze toename van de viscositeit zou eveneens een basaalwaartse verschuiving van het maximum van het CM profiel tot gevolg hebben.
De geringe effecten die worden veroorzaakt door afkoeling van de cochlea laten de mogelijkheid open dat tegenstrijdige effecten verantwoordelijk zijn voor de uitkomsten. Op basis van twee onafhankelijke meettechnieken en een berekening gebaseerd op een algemeen aanvaarde theorie blijft het echter moeilijk om andere mechanismen behalve een toegenomen compliantie en een toegenomen viscositeit verantwoordelijk te stellen voor de waargenomen effecten.

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Stellingen behorende bij het proefschrift van H. B. de Brey

1. In tegenstelling tot wat men in analogie met de endolymfatische potentiaal zou verwachten heeft ischaemie geen invloed op de intracellulaire potentiaal in het orgaan van Corti.
   Matschinsky, F. M. & Thalmann, R. in : Biochemical Mechanisms in Hearing and Deafness, 265, 1970. (Ed. M. M. Paparella).

2. Brackmann's verwachting dat het gebruik van electrische prothesen met multichannel electroden spraakdiscriminatie kan produceren op grond van plaatspitch is ongegrond.
   Brackmann, D. E.: Laryngoscope 86, 373, 1976.

3. Veranderingen in de compliantie van het basilair membraam kunnen in vivo alleen door veranderingen in de cochleaire temperatuur teweeg worden gebracht.

4. Electrocochleografie verricht met behulp van een transtympanaal op het promontorium geplaatste electrode is een veilige methode.
   Crowley, D. E. et al.: Ann. Otol. 84, 297, 1975.

5. Het door Shea ingevoerde begrip „fluctuant hearing loss" werkt verwarrend.
   Shea, J. J.: Otolaryng. Clinics of North America 8, 263, 1975.
   Shea, J. J. & Bowers, R. E.: Otolaryng. Clinics of North America 8, 431, 1975.

6. Het te laat ontdekken van congenitale middenoorafwijkingen welke een matige geleidingsslechthorendheid veroorzaken vergroot de kans op atrofie van auditieve kernen in de hersenstam.
   Webster, D. B. & Webster, M.: J.A.S.A. 59, suppl. 1, 007, 1976.

7. Bij operaties van de arteria femoralis en arteria poplitea is de intra arteriële druk- en flowmeting van het aorta en arteria iliaca traject een betrouwbaarder informatie omtrent de centrale arteriële inflow dan het aorta-arteriogram.
   Faris, I.B.: J. Cardiovasc. Surg. 16, 597, 1975.

8. Gezien de huidige diagnostische en microchirurgische mogelijkheden verdient verwijdering van brughoektumoren via de middelste schedelgroeve de voorkeur.
   Bochenek, Z. & Kukwa, A.: Acta Otolaryng. 80, 410, 1975.

9. Na appendectomie is de kans op het ontstaan van een carcinoom belangrijk vergroot. Het profylactisch verwijderen van de appendix dient dan ook te worden afgekeurd.
   McKay, J. R.: Lancet 1, 439, 1967. Ovink, A. H. O. & Verschoof, K. J. H.: Jaarboek Kankeronderzoek en Kankerbestrijding 1974, 135.

10. Decompressie van de nervus facialis later dan 10 tot 20 dagen na het ontstaan van de idiopathische perifere nervus facialis paralyse is obsoleet.
    Mechelse, K. et al. Lancet II, 57, 1971. Adour, K. K. & Swanson, P. J.: Trans. Amer. Acad. Opthal. Otolaryng. 75, 1284, 1971.

11. Het verlangen om medicijnen in te nemen is een opvallend verschilpunt tussen de mens en het dier.
    Sir William Osler : The Principles and Practice of Medicine, 1892.

12. Indien de prijs van diners in de congreskosten is verdisconteerd dient naast het wetenschappelijk programma ook het menu vermeld te worden.