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THE NATURE OF THE NEGATIVE ENDOCOCHLEAR POTENTIALS PRODUCED BY ANOXIA AND ETHACRYNIC ACID IN THE RAT AND GUINEA-PIG

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SUMMARY

1. The alterations in the Na^+ and K^+ concentrations of the cochlear endolymph and in the endocochlear potential were followed simultaneously by means of ion-sensitive and conventional micro-electrodes during simple anoxia, during anoxia after I.V. ethacrynic acid and after I.V. ethacrynic acid alone. The endolymphatic pH changes were measured separately and the effect of perilymphatic ethacrynic acid upon the endocochlear potential was investigated.

2. The over-all Na^+:K^+ permeability ratio for the endolymph system was determined in individual animals for the first time using an indirect method. The normal mean values of 0·27 (rat) and 0·38 (guinea-pig) were increased after ethacrynic acid. Permeability changes occurred during anoxia but were delayed in onset.

3. The negative endocochlear potentials in each situation behaved quantitatively like modified K^+ diffusion potentials largely dependent upon the K^+ and Na^+ gradients between endolymph and perilymph.

INTRODUCTION

The endocochlear potential, a positive potential of 80–90 mV with respect to blood or perilymph found in the endolymph of the mammalian cochlea, consists of two major components. One is a positive potential due to an active electrogenic mechanism in the stria vascularis which is believed to be associated with K^+ transport, although supporting experimental evidence is meagre (Sellick & Bock, 1974). The other is a negative potential of 40 mV which replaces the positive potential in anoxia and after the administration of certain inhibitors. The origin of this negative potential is controversial and two concepts are current.

In the first, the potential is considered to be a pre-existing K^+ diffusion potential dependent upon the chemical gradients between endolymph and perilymph. Other diffusion potentials can be excluded as prime sources since a Na^+ potential would be of the wrong sign and the remaining possibilities would be of insufficient magnitude. A major criticism of this idea is that its quantitative justification (Johnstone, 1965; Kuijpers & Bonting, 1970; Melichar & Syka, 1977) depends on the extrapolation of permeability ratios from single cell studies to the multicellular membranes surrounding the cochlear duct.

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The second hypothesis represents the development of Békésy's (1952) original idea. In it, the potential is thought to be produced by the leakage current associated with the continued presence of the negative intracellular potentials in Corti's organ. These potentials, in turn, will depend mainly on the K^+ gradient between the cell cytoplasm and the fluid around the cell bodies, which is in equilibrium with perilymph. Quantitative support in this case comes from an electrical model based upon a series of experimental impedance measurements (Honrubia, Strelioff & Sitko, 1976).

Further investigation of these two possibilities has proved difficult. Alteration of the perilymphatic composition by perfusion has given conflicting results and can cause effects unrelated to simple changes in the chemical gradients (Honrubia, Johnstone & Butler, 1965; Konishi & Kelsey, 1973). In addition, any alterations induced in the endocochlear potential could be due to changes in the chemical gradients between the perilymph and either the cells or the endolymph. For this reason, alteration of the endolymphatic composition seems a more useful procedure, since it would have little effect upon the gradient between the perilymph and the cells. But control perfusions of the endolymphatic system are often accompanied by a progressive fall in the endocochlear potential (Sellick & Bock, 1974; Sellick & Johnstone, 1975; S. K. Bosher, unpublished observations), so that invalidating damage cannot always be excluded if this method is used.

It was decided, therefore, to study the effects of anoxia and intravenous ethacrynic acid upon the chemical composition of the endolymph and to relate these effects to the negative endocochlear potentials produced. Major disturbances of the chemical gradients between the perilymph and the cells could be avoided because intravenous ethacrynic acid produces no changes in the perilymphatic composition (Bosher, Smith & Warren, 1973) and substantial alterations do not occur before 10 min in anoxia (Melichar & Syka, 1977). Permeability ratios were determined experimentally and the negative endocochlear potentials were found to behave quantitatively as though they were modified K^+ diffusion potentials primarily dependent upon the chemical gradients between endolymph and perilymph. The initial anoxia results have been communicated to the Physiological Society (Bosher, 1977).

METHODS

Animal preparation. Sprague–Dawley-descended albino rats (150–200 g) and Hartley-descended albino guinea-pigs (200–300 g), unselected with regard to sex, were used. The strains were Caesarean-originated and barrier-sustained (Charles River) and the animals' ears had not been subjected to the effects of either infection or antibiotics. Anaesthesia was induced with ethyl chloride in both species. The main anaesthetic agent in the rat was sodium pentobarbitone given intraperitoneally in an initial dose of 60 mg kg^{-1}. Neuroleptanaesthesia (Evans, 1978) was employed in the guinea-pig, 4 mg kg^{-1} droperidol and 1 mg kg^{-1} phenoperidine being administered intramuscularly in the first instance, and 30 mg kg^{-1} sodium pentobarbitone was injected intraperitoneally to continue the narcosis. These doses were supplemented if necessary and repeated as required to produce and maintain surgical anaesthesia; no muscle relaxants were used. The animals' temperature was kept constant (to $\pm 0{\cdot}4$ °C) by means of a heating blanket linked to a rectal thermistor. 35 °C was chosen for the rat, because higher temperatures caused hypernoea, and 37 °C for the guinea-pig.

A small fenestra was made over the stria vascularis of the left middle turn in the rat and the

left basal turn in the guinea-pig for insertion of the microelectrodes (Bosher & Warren, 1968). Perilymph measurements were undertaken through a fenestra made over the scala vestibuli of the middle turn in the rat and through the round window membrane in the guinea-pig. In the perfusion experiments, small openings were made into the scala vestibuli, a quarter turn on either side of the scala media fenestra. The perfusion fluid composition was Na^+ 144 mM, K^+ 6 mM, Ca^{2+} 2 mM, Cl^- 129 mM and HCO_3^- 25 mM, giving an osmolality of 308 m-osmole. It was filtered through a 25 μm Millipore filter, equilibrated with 95 % O_2 5 % CO_2 for at least 30 min, warmed approximately to the animal's temperature, and introduced into the more apical opening by means of a glass pipette (tip diameter, 20 μm) connected to a peristaltic pump. This opening was made much larger than the pipette and free egress of fluid was permitted from the other one; the usual flow rate was 3·5 μl. min^{-1}.

Ethacrynic acid was administered as sodium ethacrynate solution (Edecrin; Merck, Sharp & Dohme), 12·5 mg $ml.^{-1}$, by means of a 60 sec injection into the previously exposed right femoral vein. Anoxia was usually produced by the injection of 2 ml. air through an indwelling catheter in the left femoral vein without disturbing the electrical screening around the preparation. It was often followed after 1–2 min by transient but marked respiratory movements which tended to disrupt the sealing around the electrodes, thus invalidating the subsequent readings in approximately a quarter of the experiments.

Measurement of the endolymphatic ion concentrations and endocochlear potential. Longitudinal variations occur in the endocochlear potential (Bosher & Warren, 1971) and a 1 mV difference would be equivalent to a K^+ concentration change of 5·6 mM at the endolymphatic concentration. A double-barrelled K^+-sensitive electrode was considered essential, therefore, to exclude any artefacts which might arise from the spatial separation of the tips of single electrodes. The micro-electrodes, tip diameter 2 μm, were constructed from 2 mm o.d. borosilicate glass theta-capillary tubing (Clark Electromedical TGC 200). One barrel was siliconized with 5 % tri-*n*-butylchlorosilane in 1-chloronaphthalene, its tip filled with K liquid ion-exchanger (Corning 477317) and the remainder back-filled with 500 mM-KCl as described by Walker (1971). The other barrel was filled with 150 mM-KCl to form a conventional micro-electrode for measuring the endocochlear potential. The butt of the electrode was siliconized with 2 % dimethyldichlorosilane in CCl_4, the filling solutions were covered with a layer of mineral oil and the end of the electrode was filled with silicone rubber sealant, which served to keep the two Ag/AgCl wires as far apart as possible. Representative electrodes were tested during the course of the experiments by determining the effect of earthing one channel upon the readings from the other channel over an 8 hr period. At no time was there any change in the potential from the recording channel, indicating that electrical isolation of the channels was satisfactory.

A major difficulty was discovered in the use of the micro-electrode. During the course of the experiment, the potential from the K^+ channel would suddenly become progressively more positive, apparently indicating a rapid increase in the endolymphatic K^+ concentration which soon reached levels greatly in excess of any possible physiological concentration. The readings from the other channel and the Na^+-sensitive electrode were unaffected and the phenomenon undoubtedly represents some artifact. Occasionally, the vibrations produced by the anoxic respiratory movements caused an abrupt return of the K^+ channel potential to its expected level. Consequently, the artifact could have been due to the impalement of some structure like the tectorial membrane but this is not certain and why it should have such an effect is not clear. In addition, the mechanical stability of the liquid ion-exchanger seemed to be reduced when the electrode was in endolymph and it tended to become displaced. As a result of these problems, successful K^+ recordings were obtained in only 44 % of the experiments.

Single Na^+-sensitive glass micro-electrodes of the recessed-tip type, tip diameter 5 μm, were constructed from NAS 11–18 glass using the method developed by Thomas (1978). Difficulties with Na^+-sensitive electrodes of the reversed-tip type have been reported in previous investigations on endolymph (Sellick & Johnstone, 1972); in particular, the electrodes have not recorded d.c. potential changes accurately and have been non-linear at low Na^+ concentrations. Preliminary studies suggested these problems arose because the resistance of the electrodes increased considerably at low Na^+ levels. An attempt was made to overcome this feature by increasing the length of the cone of Na^+-sensitive glass to 200 μm. Such electrodes recorded all d.c. alterations accurately but their slope deviated from the theoretical value when the Na^+ concentration in the test solutions was below 2 mM. However, a series of experiments was performed in which the endolymph was aspirated and its Na content determined by flame

spectrophotometry (Bosher & Warren, 1968) immediately after the Na^+-sensitive electrode measurement. This revealed the electrode response in endolymph to be linear to the lowest concentration found, 0·24 mM, although why this should be is uncertain. Successful Na^+ recordings were obtained in 65% of experiments, persistently unstable readings of unknown aetiology being present in the remainder.

Recessed-tip pH-micro-electrodes were constructed using H^+-sensitive glass (Corning 0150) as described by Thomas (1978), with tip diameters of 2·5 μm. Microscopic cracks rapidly appeared in the cone of the pH-glass during use and the number of completed experiments was small. This probably arose from some error in the electrode manufacture but the matter was not investigated further as the few successful recordings seemed adequate for the purpose of the study.

Attempts to employ Cl^--sensitive micro-electrodes, constructed after the method of Walker (1971) from chloride liquid ion-exchanger (Corning 477315) were also made. Despite the use of a wide variety of silanes and siliconization procedures, the ion-exchanger was rapidly displaced from the tip of the electrode in most instances. Even if this did not happen, the potential readings were almost always markedly unstable. The electrodes seemed satisfactory if inserted into muscle and the reason for the lack of success in endolymph remains obscure.

Each micro-electrode was connected to a varactor bridge preamplifier (Analog Devices 311J), except the Na^+-sensitive micro-electrode which required a vibrating reed preamplifier (Vibron 62A) because of its greater resistance. A chlorided Ag wire inserted into the left scapular muscles served as a reference electrode and was connected to all preamplifiers in use. The endocochlear potential was subtracted from the potentials recorded by the ion-sensitive electrodes by means of operational amplifiers to give the true ion-dependent potentials. The various potentials were measured by means of a digital voltmeter (Solartron LM 1604), which was switched across all the channels every minute using a Digitimer. When necessary, the scanning rate was increased or continuous readings were made from any one channel. The output of the digital voltmeter was recorded by a printer (Sodeco PE 121), the figures then being transcribed to punched paper tape for subsequent use in a computer.

The ion-sensitive micro-electrodes were calibrated in solutions of constant osmolality (300 m-osmole) maintained at the animal temperature. The response of the Na^+-sensitive micro-electrode was determined between 100 and 10 mM (the additional cation being K^+), the K^+-sensitive micro-electrode between 150 and 75 mM (the additional cation being Na^+), and the pH micro-electrode between pH 6·6 and 7·6 (Tris maleate buffer). Any micro-electrode whose slope differed from the theoretical Nernstian one by a factor greater than 0·05 was discarded. After a successful experiment, the calibration was checked but was not found to have altered in any instance. Repeated measurements of the calibrating solutions gave identical readings and a better estimate of the sensitivity of the methods was required. All potentials were recorded to ± 50 μV. For the Na^+-sensitive micro-electrode this is equivalent to $\pm 0{\cdot}002$ mM at the 1 mM level and $\pm 0{\cdot}02$ mM at the 10 mM level. The corresponding figures for K^+ are $\pm 0{\cdot}28$ mM at 150 mM and for pH $\pm 0{\cdot}001$ u. at pH 7·4.

Expression of the results. The results are routinely expressed in the form: mean, $\pm$S.E. of mean, number of experiments (if this has not been indicated in the adjacent text). The values give ionic concentrations and not activities to enable comparison with previous work to be made more easily. Since the inner ear fluids are essentially simple aqueous solutions of salts, the activity coefficients of the various ions in these fluids are likely to be much the same as in the test solutions. Where the significance of the difference between the findings has been estimated, this has been done by means of Student's two-tailed t test.

RESULTS

Normal endolymphatic and perilymphatic constitution

The normal values from the whole investigation are given in Table 1. The endolymphatic pH results confirm the original report of Misrahy, Hildreth, Clark & Shinabarger (1958) and the other findings are in good accord with the levels now generally accepted.

Endolymphatic changes in anoxia (rat)

Ischaemic anoxia was produced in eleven rats by the I.V. injection of air. Circulatory arrest seemed to be almost instantaneous so the start of the injection was taken as the anoxia-initiation time. The endocochlear potential declined rapidly to become negative usually within 1 min (seven animals) and always within 2 min. A minimum of $-40{\cdot}1$ ($\pm 1{\cdot}3$) mV was reached at 9·0 ($\pm 0{\cdot}6$) min, when the potential began returning towards zero (Fig. 1*B*). At first rapid, the rate of change became progressively slower and, in the one animal followed for 120 min, the final potential was $-6{\cdot}0$ mV.

TABLE 1. Normal endolymph and perilymph composition

	Range	Mean	S.E. of mean	No. of experiments
Rat				
Endocochlear potential (mV)	79·2–97·0	88·1	0·6	54
Endolymph [Na^+] (mM)	0·24–1·42	0·82	0·04	40
[K^+] (mM)	135·8–170·9	150·3	1·8	32
pH	7·430–7·440	7·436	0·001	6
Perilymph [Na^+] (mM)	117–145	131·7	4·1	6
[K^+] (mM)	4·6–9·9	6·8	1·0	6
Guinea-pig				
Endocochlear potential (mV)	75·5–91·3	81·3	1·4	13
Endolymph [Na^+] (mV)	0·36–1·29	0·75	0·09	13
[K^+] (mM)	148·1–170·2	159·4	2·1	13
Perilymph [Na^+] (mM)	141–158	151·7	4·4	6
[K^+] (mM)	3·5–4·0	3·8	0·2	6

Successful Na^+ recordings were obtained in nine animals, but K^+ recordings were satisfactory in only four. The endolymphatic Na^+ concentration began to increase at an approximately constant rate of 0·63 ($\pm 0{\cdot}04$) m-mole min^{-1}. However, after 12–15 min, the Na^+ entry rate increased fairly suddenly by about threefold and became more irregular. The endolymphatic K^+ concentration decreased in a roughly sigmoid fashion (Fig. 1*A*).

When the rates of the concentration alterations are normalized with respect to the appropriate driving forces, a clearer picture emerges (Fig. 1*C*). The initial increases reflect the progressive inhibition of active transport by the anoxia. Here, the discrepancy between the durations of this phase for the two ions is puzzling and the question arises of how long active transport persists after the induction of anoxia. Thalmann and his colleagues (Thalmann, Miyoshi & Thalmann, 1972; Thalmann, Kusakari & Miyoshi, 1973) found that ischaemia produced a decrease in the strial ATP and phosphocreatine concentrations, whose time course parallelled the fall in the endocochlear potential, and that the high-energy phosphates disappeared completely at the time of the minimum peak in the potential. This peak seems to correspond, therefore, with the time of abolition of active strial transport.

The reason for the apparently premature cessation of Na^+ transport in the present experiments is not known. Attention has been restricted, accordingly, to the period

after the negative peak in the endocochlear potential, when it is certain that the alterations are due entirely to the passive forces (Fig. 1*D*). The normalized rates of change in the cation concentrations remained almost constant for 3–6 min after this negative peak and then substantial and initially progressive increases occurred. Although these stable periods may appear relatively short in the context of the whole

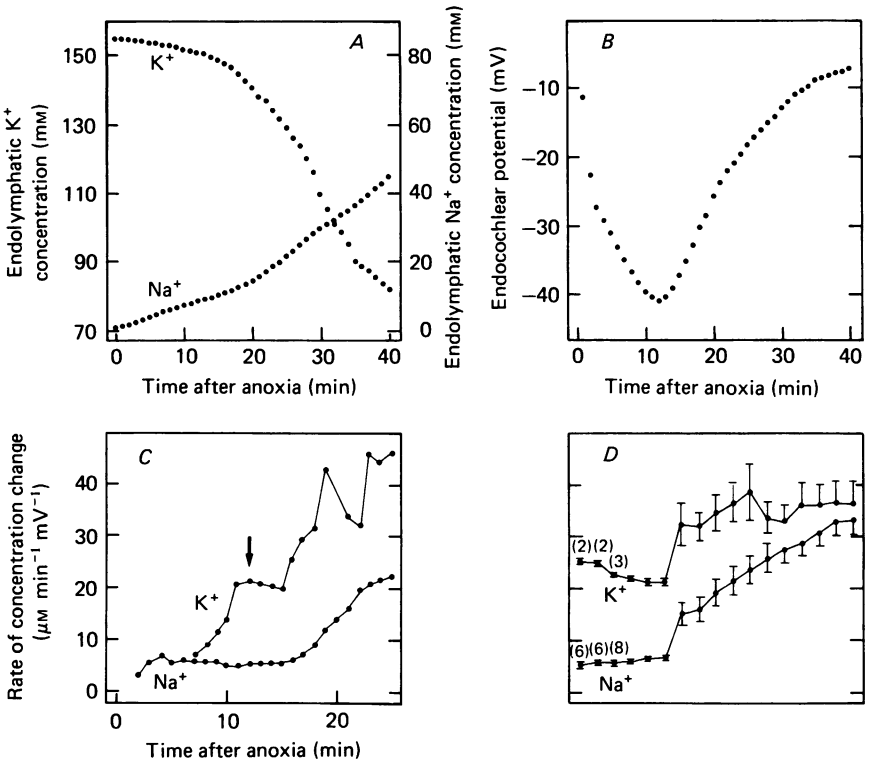

Fig. 1. The changes in the endolymphatic cation concentrations (*A*) and the endocochlear potential (*B*) produced by anoxia in a normal rat (representative experiment). The initial potential was 83 mV. In *C* the rates of change in the cation concentrations for each minute have been normalized with respect to the appropriate electrochemical gradient. The arrow indicates the time of the negative peak in the endocochlear potential. *D* shows the means and S.E. of mean of the normalized rates of change in the K^+ (four experiments) and Na^+ (nine experiments) concentrations for each minute after the minimum endocochlear potential. The ends of the stable periods have been taken as the reference point for the time axis. The different lengths of the stable periods are thus indicated by the reduction in number of the experiments represented by the initial points (shown in parentheses). The general level of the results was consistent in each case so that there was always a significant difference between the K^+ and Na^+ values in an individual experiment.

process, their duration does exclude the possibility that they are due to some transient aberration. During them, the mean rate of increase in the Na^+ concentration was 6 ($\pm 0{\cdot}5$) μmole min^{-1} mV^{-1} and the mean rate of decrease in the K^+ concentration was 22 ($\pm 0{\cdot}2$) μmole min^{-1} mV^{-1}, giving a ratio of 0·27. As discussed later, this figure provides a useful estimate of the overall Na^+:K^+ permeability ratio for the normal endolymphatic system.

Consideration of possible causes of the increases in the cation movement rates suggested that it would be worthwhile to investigate the effect of anoxia upon the endolymphatic pH, about which nothing was known. Attempts to diminish vibratory damage to the pH-micro-electrodes by using the administration of respiratory

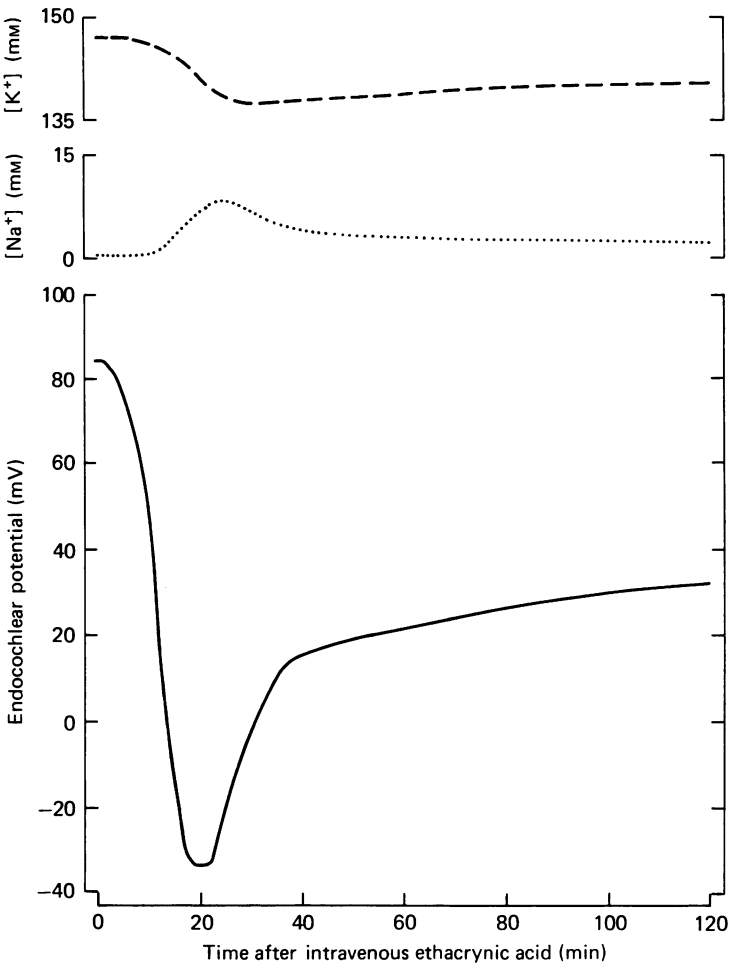

Fig. 2. The effects of intravenous ethacrynic acid (60 mg kg^{-1}) upon the endolymphatic K^+ and Na^+ concentrations and the endocochlear potential in a representative rat experiment. There was a delay of 1 min before the potential commenced to decline. At the same time, the Na^+ concentration began to rise, although this is not evident because of the scale used. The K^+ concentration did not change for 4 min. The minimum endocochlear potential (−35·2 mV) persisted for 2 min.

nitrogen to induce anoxia were not completely successful. Satisfactory results, consequently, were obtained in only two rats but these were in close agreement. The endolymphatic pH remained constant until respiratory movements ceased, after which it decreased in a biphasic manner, a relatively rapid fall being followed at 14 min by a slower rate of change (Fig. 4).

Endolymphatic changes after I.V. *ethacrynic acid (rat)*

A dose of 60 mg kg^{-1} was selected because it is the smallest which produces complete abolition of the positive component of the endocochlear potential in the rat (Bosher *et al.* 1973). Complete results were obtained in seven of the thirty-five experiments undertaken and consideration will be restricted to these; the remaining experiments revealed no additional features.

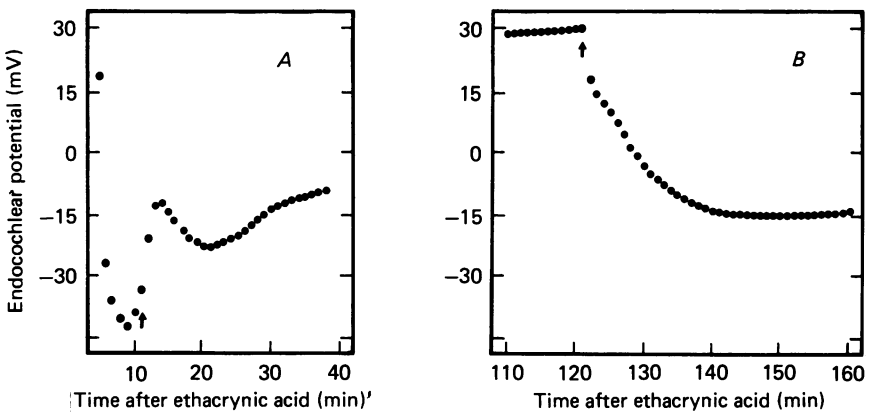

Fig. 3. The effect of anoxia (induced at the times indicated by the arrows) upon the endocochlear potential 2 min (*A*) and 100 min (*B*) after recovery commenced in ethacrynate-treated rats (60 mg kg^{-1} I.V.).

The endocochlear potential began to decrease after a latent period of 2·7 (± 1·1) min, timed from the beginning of the intravenous injection. Both the rate and the extent of its fall were less consistent than in anoxia, the minimum peak of −13·5 to −23·7 mV occurring at 13–31 min. At the same time, the endolymphatic Na^+ concentration increased and, after a variable initial period, the K^+ concentration decreased. These changes were reversed during the subsequent recovery, which was still incomplete after 120 min (Fig. 2).

Endolymphatic changes in anoxia after I.V. *ethacrynic acid (rat)*

The ethacrynic acid experiments were terminated with anoxia produced by the I.V. injection of air at varying times during the initial 120 min of the recovery phase. When anoxia was initiated during the first 10 min of this phase, the endocochlear potential continued to recover for up to 4 min before it began to fall (Fig. 3*A*). With recovery periods of more than 10 min, the potential decreased immediately (Fig. 3*B*). In all instances, the rate of fall was much less than in simple anoxia so that the times taken to reach the minimum peaks in the endocochlear potential, 13–33 min, were longer than the 9 min found in normal animals, even though the magnitudes of the total changes were smaller. Similar effects have been found in the guinea-pig by Kusakari, Ise, Comegys, Thalmann & Thalmann (1978), who were able to relate them to reductions in the rates of decrease in the strial ATP and phosphocreatine concentrations. The values of the anoxic minimums were not

time-dependent and their mean, $-19{\cdot}9$ ($\pm 0{\cdot}7$) mV ($n = 25$), was only about half the normal one.

The pattern of the Na^+ and K^+ alterations was the same as in simple anoxia but the rates of change were slower due to the diminished permeability of the endolymphatic membranes (S. K. Bosher, in preparation). Thus, the early rate of increase in the Na^+ concentration was only about half normal, although the relatively sudden increase in the rate was still approximately threefold, and the rate of decrease in the K^+ concentration was approximately a third normal. When the findings following the cessation of active transport were normalized with respect to the appropriate electro-

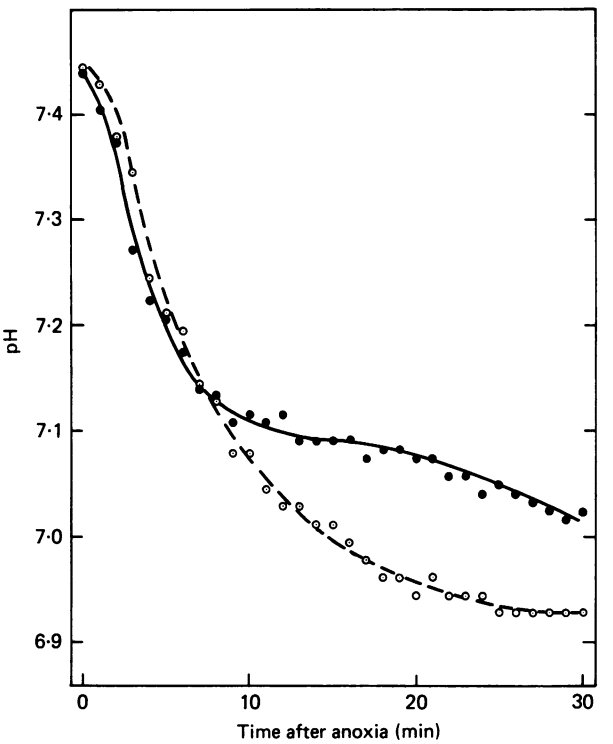

Fig. 4. The endolymphatic pH changes produced by anoxia in a normal rat (filled circles) and in one 59 min after I.V. ethacrynic acid (60 mg kg^{-1}) (open circles). The lines have been drawn by eye through the experimental points.

chemical gradients, short stable periods again became evident. During them, the mean rate of increase in the Na^+ concentration was $3{\cdot}5$ ($\pm 0{\cdot}3$) μmole min^{-1} mV^{-1} ($n = 11$) and the mean rate of decrease in the K^+ concentration was $5{\cdot}4$ ($\pm 0{\cdot}6$) μ-mole min^{-1} mV^{-1} ($n = 9$). These values were considerably smaller than those in the normal animals and they indicated that the Na^+:K^+ permeability ratio had increased.

Because of the technical difficulties, satisfactory pH results were obtained in only two animals in whom anoxic anoxia was produced by nitrogen inhalation 13 and 50 min after recovery commenced. The findings differed little in the two experiments

and there were only minor divergences from the results obtained in the normal animals (Fig. 4).

Correlations of the increase in cation flow during anoxia

A prominent feature of the endolymphatic alterations found during anoxia in both normal and ethacrynate-treated animals is the relatively sudden and pro-

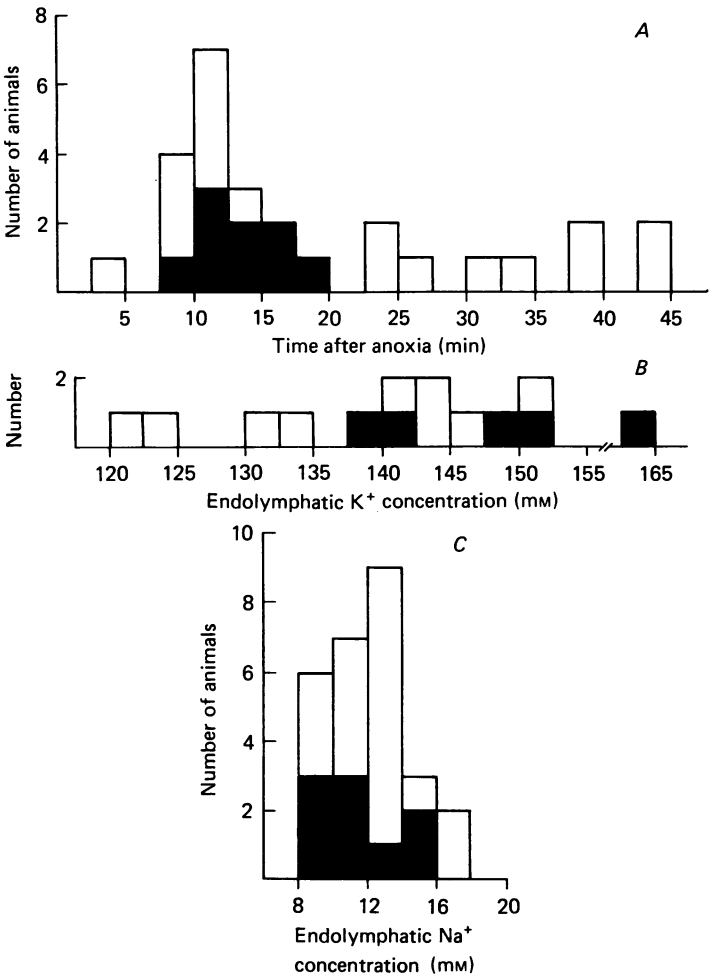

Fig. 5. The relation of the change-over point in the cation flow rates during anoxia to time (*A*), endolymphatic K^+ concentration (*B*) and endolymphatic Na^+ concentration (*C*). The solid columns refer to normal rats and the open ones to ethacrynate-treated animals (60 mg kg^{-1} I.V.).

gressive increase in the normalized rates of change of the cation concentrations. It seems reasonable to interpret this phenomenon as indicating that a significant increase in cation permeability may have occurred. The correlations of the change-over point were investigated, accordingly, in the two situations. The time course of the endolymphatic pH changes was unaltered after ethacrynic acid administra-

tion, so that the time of the change-over point should also be unaltered if the endolymphatic pH level was a determinant factor. The results from sixteen ethacrynate-treated animals revealed the commonest times for the change-over point to lie between 7·5 and 12·5 min but, unlike the normal animals, the overall correlation with time was poor (Fig. 5*A*), which effectively excludes a correlation with the pH alterations.

Again, there was no correlation between the time of the change-over point and the level of the endolymphatic K^+ concentration (Fig. 5*B*) but a good correlation was found with the Na^+ concentration (Fig. 5*C*). The increase in the rates of con-

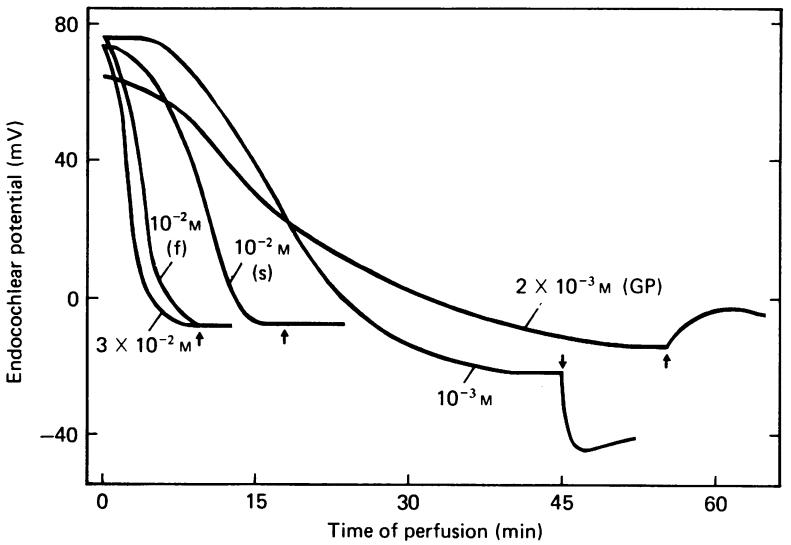

Fig. 6. The effect of scala vestibuli perfusion of ethacrynic acid at the concentrations shown upon the endocochlear potential in the rat. Two flow rates were used for the 10^{-2} M perfusions, the standard 3·5 μl. min $^{-1}$ (s) and 25 μl. min^{-1} (f). The presence of the two scala vestibuli openings reduced the endocochlear potential to 74·5 ($\pm$ 1·5) mV (n = 10). The arrows indicate the times when anoxia was induced. For comparison, the result of similar perfusion in a guinea-pig is also depicted (GP). This confirmed that the absence of an anoxia-sensitive negative component in the rat was not attributable to the experimental conditions.

centration change occurred when the endolymphatic Na^+ concentration rose to 11·64 ($\pm$ 0·78) mM (n = 9) in normal animals and 12·41 ($\pm$ 0·51) mM (n = 16) in ethacrynate-treated ones. The difference between these values is not significant ($0{\cdot}4 > P > 0{\cdot}3$), giving an over-all mean of 12·15 ($\pm$ 0·42) mM.

This finding suggests that the rise in the endolymphatic Na^+ concentration to this relatively modest level might be, at least, one causative factor. However, changes have been reported recently in the endolymphatic Ca^{2+} concentration during anoxia in normal animals, which resemble in a general way those in the endolymphatic Na^+ concentration described here (Bosher & Warren, 1978). The relationship between the two effects has not been investigated yet and the possible role of Ca^{2+} in their aetiology, therefore, has still to be determined.

Endocochlear potential changes during perilymphatic perfusion with ethacrynic acid

In the guinea-pig, a negative endocochlear potential is produced by perilymphatic perfusion with ethacrynic acid which has two components, one being anoxia-sensitive (Kusakari & Thalmann, 1976; Kusakari *et al.* 1978; Sellick & Johnstone, 1974). It was decided to repeat this type of experiment in the rat, primarily as a first step in investigating whether an anoxia-sensitive component was also present after intravenous ethacrynic acid in this species. Anoxia was initiated by the I.V. injection of 2 ml. air when the endocochlear potential had fallen to a constant level for 2 min during the ethacrynic acid perfusion, which was continued.

With a concentration of 10^{-3} M-ethacrynic acid (three experiments), anoxia caused an immediate and rapid decrease in the endocochlear potential (Fig. 6), resembling that seen in normal animals and revealing that only about 76% inhibition of the electrogenic positive component had been achieved. When the ethacrynic acid concentration was increased to 1×10^{-2} M (five experiments), no alteration was produced in the negative endocochlear potential by the anoxia (Fig. 6). The remote possibility that anoxia-sensitive positive and negative components of equal magnitude were present was excluded by increasing the ethacrynic acid concentration to 3×10^{-2} M (two experiments). This would have reduced further any remaining positive component and so would have revealed a negative component, if it were present. In fact, the results were exactly the same as before (Fig. 6). The inhibition of the positive component was thus complete and the absence of a positive shift in the potential, as observed in the guinea-pig, showed that an anoxia-sensitive negative potential was not present in the rat. The differences in the levels of the negative endocochlear potentials found after perfusion with the various concentrations of ethacrynic acid were due to gradations in the drug's effects upon the permeabilities of the endolymphatic membranes (S. K. Bosher, in preparation).

Endolymphatic changes in the guinea-pig

Because of the difference between the effects of perilymphatic ethacrynic acid in the rat and guinea-pig, it was decided to repeat the other experiments in a small number of guinea-pigs. As was expected, no additional qualitative differences were found. However, the quantitative differences were sufficiently large to make detailed extrapolation of the results between the two species undesirable.

In the first group of experiments, anoxia was induced in five animals by the intravenous administration of air. The minimum endocochlear potential of $-32{\cdot}5$ ($\pm 2{\cdot}9$) mV was approximately 7·5 mV less negative than in the rat and the potential subsequently returned towards zero much more slowly (Fig. 7*B*). The secondary rise in the rate of increase in the endolymphatic Na^+ concentration was only half as large and the rate of decrease in the K^+ concentration was considerably reduced (Fig. 7*A*), the latter finding being in good accord with the results of Melichar & Syka (1977). When the rates of change were normalised as before, the mean values during the initial short stable periods (Fig. 7*C*) were 6 μmole min^{-1} mV^{-1} for Na^+ ($n = 3$) and 16 μmole min^{-1} mV^{-1} for K^+ ($n = 2$), which gives a ratio of 0·38 between the two compared with 0·27 in the rat, largely because of the lower value for K^+.

In the second group, 40 mg kg^{-1} ethacrynic acid was injected intravenously, since preliminary experiments revealed this dose to be the smallest which abolished the positive endocochlear potential completely (see also Kusakari *et al.* 1978). Anoxia was produced in the same way as in the first group 60 min after the administration of the ethacrynic acid. Full records were obtained in only two animals but they were in good agreement with each other. The quantitative differences in the changes

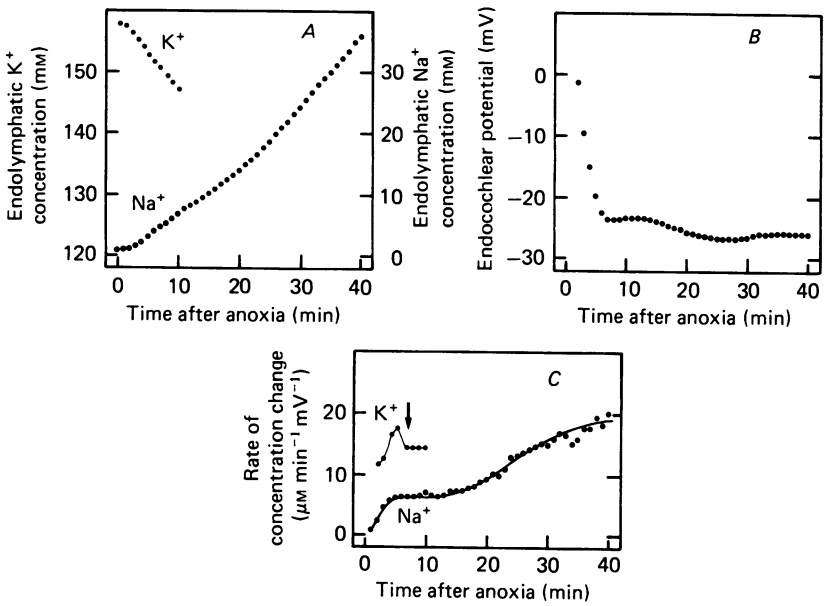

Fig. 7. The changes in the endolymphatic cation concentrations (*A*) and the endocochlear potential (*B*) produced by anoxia in a normal guinea-pig for comparison with Fig. 1 (the increased scale will be noted). This was the only experiment where satisfactory results for both K^+ and Na^+ were obtained in a single animal but no K^+ concentrations are shown after 10 min because the K^+-sensitive electrode developed an artifact at this point. The initial potential was 77·4 mV. In *C* the rates of change in the cation concentrations for each minute have been normalized with respect to the appropriate electrochemical gradient. The arrow indicates the time of the negative peak in the endocochlear potential. The line through the Na^+ results has been drawn by eye.

produced by ethacrynic acid alone in the two species were rather complex and not relevant to the problem of the origin of the negative endocochlear potential (S. K. Bosher, in preparation). The effect of ethacrynic acid upon the alterations during the terminal anoxia was identical to that in the rat and the differences between the two species merely reflected those found in simple anoxia. Thus, the negative peak in the endocochlear potential was only about half the normal one, while the rates of change in the endolymphatic Na^+ and K^+ concentrations were reduced to a half and a third normal respectively, producing an increase in the ratio between their normalized values. The K^+ findings resemble those recently reported by Melichar & Syka (1978).

When both groups of experiments are considered together, the apparent increase in the cation permeabilities of the endolymphatic membranes at the end of the

initial stable periods occurred when the endolymphatic Na^+ concentration reached a mean value of 11·3 mM, which does not differ significantly from that found in the rat.

DISCUSSION

At present, the ionic permeabilities of the cochlear duct membranes can only be estimated by means of the indirect method introduced by Johnstone and his colleagues (Johnstone, 1970; Sellick & Bock, 1974; Sellick & Johnstone, 1975). For any selected minute during anoxia, the rates of change in the endolymphatic con-

TABLE 2. Comparison of the observed and calculated negative endocochlear potentials

Type of experiment	Observed potential	Calculated potential (see text)	Discrepancy (calculated–observed)	No. of experiments
Simple anoxia	−40·6 (±0·6)	−36·5 (±1·5)	+4·1 (±0·9)	4
Ethacrynic acid	−24·7 (±3·9)	−20·0 (±4·4)	+4·7 (±0·9)	7
Anoxia after ethacrynic acid	−18·5 (±1·3)	−13·6 (±3·7)	+4·9 (±3·3)	7

The dose of ethacrynic acid was 60 mg kg^{-1} I.V. and the greater variance of the observed potentials following its administration arose because the magnitudes of the potentials depended on their time of occurrence (S. K. Bosher, in preparation). The magnitudes of the calculated potentials varied with time in a similar fashion as shown by the much smaller variance in the discrepancy column.

centrations, normalized with respect to the relevant electrochemical gradients, are taken as indices of the passive ionic conductances as described by Hodgkin & Katz (1949) in the squid axon. Thus, the permeability ratios can be determined and the relative alteration in the conductances with time can be followed. In addition, Johnstone and his co-workers have argued from the results of ouabain administration that the membrane conductances are unaltered in early anoxia. In the experiments reported here, the normalized rates of change remained constant for a brief period after the abolition of active transport and these values, accordingly, were considered to represent the conditions before anoxia in the few cases where this information was needed.

The method enabled Na^+:K^+ permeability ratios to be determined in individual animals for the first time in those experiments where simultaneous measurements were successful. These ratios, with the measured endolymphatic concentrations and the normal perilymphatic concentrations, were then used to calculate the possible values of the K^+ diffusion potentials by means of the Hodgkin & Katz (1949) equation. The full equation includes the contribution of the Cl^- chemical gradient and this was the reason an attempt was made to follow the alterations in the Cl^- concentrations. As it was unsuccessful, an abbreviated form of the equation,

$$E = \frac{RT}{F} \ln \frac{[K^+_p] + a[Na^+_p]}{[K^+_e] + a[Na^+_e]}$$

was employed, where E, R, T and F had their usual significance, a was the Na^+:K^+ permeability ratio and the subscripts e and p referred to endolymph and perilymph respectively. However, evidence from perfusion studies suggests that the Cl^- con-

tribution is very small and that no significant error was introduced by its exclusion (Kuijpers & Bonting, 1970; Prazma, 1969; Sellick & Johnstone, 1975).

The potentials calculated at the time of the minimum peaks in the endocochlear potential (when the perilymph concentrations are still unaffected) in the three situations which were investigated in the rat are shown in Table 2. The endocochlear potential observed in each situation, it will be seen, was the same as the modified K^+ diffusion potential dependent on the K^+ and Na^+ gradients between endolymph and perilymph, except for a consistent positive discrepancy. The three comparable guinea-pig experiments gave essentially the same result but the average discrepancy was 7·1 ($\pm$ 2·6) mV. When the time course of the potential changes in the anoxic animals was considered, use was made of the perilymphatic concentrations reported previously (Bosher *et al.* 1973; Melichar & Syka, 1977). The close agreement between the calculated and the measured potentials was present for at least 30 min and the positive discrepancy persisted substantially unaltered during this period.

The origin of the small positive discrepancy is uncertain. In the anoxia experiments, all active transport was almost certainly abolished and modification of the K^+ diffusion potential by contributions from ions other than Na^+ seems most likely. But another possibility must be considered after I.V. ethacrynic acid. Perilymphatic perfusion with ethacrynic acid in the guinea-pig has produced evidence of an electrogenic negative potential (Kusakari & Thalmann, 1976; Kusakari *et al.* 1978; Sellick & Johnstone, 1974), which could be the source of the discrepancy. The guinea-pig studies, therefore, were repeated in the rat but a similar potential was not present. Hence, it could not be the source of the positive discrepancy in the latter species, whatever the situation after I.V. ethacrynic acid in the guinea-pig. Further investigation will be needed to decide whether the finding in the rat was due to absence of the underlying mechanism or to its increased susceptibility to ethacrynic acid.

The demonstration that the negative endocochlear potential behaves like a diffusion potential in both rat and guinea-pig immediately raises the question of a possible site for the K^+ selective membrane. Two general possibilities exist. First, the potential could be generated across the endolymphatic poles of some, or all, of the boundary cells. Here, in order to provide the necessary K^+ gradient, the intracellular K^+ concentration would have to differ considerably from usual. This, in turn, would lead to an unusual level for the intracellular potential, although its value would depend on the assumptions made about the intracellular Na^+ concentration and the relative permeabilities of the cell membranes. In addition, such unusual intracellular K^+ concentrations will also be associated with changes in other ionic constituents and, consequently, with alterations in cell function. The notion, therefore, leads to considerable difficulties, especially as it is not easy to explain why variations in the perilymphatic K^+ concentrations would produce rapid endocochlear potential changes (Honrubia *et al.* 1976; Konishi & Kelsey, 1973). The second possible site for the generation of the negative potential is the tight junctions between the cells. While these are known to be cation-selective elsewhere, they do not seem normally to differentiate between the monovalent cations. A special situation would have to be present in the cochlea and it is hard to think what purpose it would serve. Nevertheless, it need not lead to a large loss of K^+, since only the relative and not the absolute permeability is involved.

On the other hand, the alternative hypothesis that the endocochlear potential depends upon the intracellular potentials seems unlikely to be correct because the potential developed by the leakage current would have to mimic, at all times, the diffusion potential which could arise from the chemical gradients between endolymph and perilymph. In particular, the reduction in the negative endocochlear potentials found after intravenous ethacrynic acid poses a severe problem. If the intracellular potentials remain unaltered, the observed reduction could only be explained by a decrease in the membrane resistance of the boundary cells to about half normal. But ethacrynic acid is known to increase membrane resistance (for e.g., see Burg & Green, 1973) and this increase seems to be about threefold in the endolymph system (S. K. Bosher, in preparation). Alternatively, if ethacrynic acid does produce a decrease in the intracellular potentials, it would have to be to 0·2 normal in order to explain the negative endocochlear potential and this again seems most unlikely when the K^+ gradient between the cells and the perilymph is not affected. The evidence, therefore, suggests the negative endocochlear potential to be a modified K^+ diffusion potential. However, this conclusion must be accepted with caution until more is known about its precise origin.

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