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THE EFFECTS OF INTRACOCHLEAR AND SYSTEMIC FUROSEMIDE ON THE PROPERTIES OF SINGLE COCHLEAR NERVE FIBRES IN THE CAT

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SUMMARY

1. Tuning properties and spontaneous discharge rate of single cochlear fibres in the anaesthetized cat were determined during short- and long-term poisoning of the cochlea by locally and systemically applied furosemide.

2. With intra-arterial administration of furosemide, short-term reversible elevation occurred of the low threshold sharply tuned 'tip' segment of the frequency threshold ('tuning') curve (f.t.c.) by up to 40 db, without substantial changes in the threshold of the low frequency 'tail' segment of the f.t.c. These changes could occur in part without changes in the spontaneous activity and entirely without changes in the maximal evoked activity. These effects were observed in all fibres examined, the characteristic frequencies of which ranged from 3·5 to 31 kHz.

3. Intracochlear administration of furosemide in 0·9 mM concentrations produced similar changes, but these were not reversible.

4. The changes correlated with the depression of the amplitude of the gross cochlear action potential. The cochlear microphonic potential, however, was either unchanged, or only slightly reduced.

5. In long-term furosemide poisoning of the cochlea, fibres with anomalous response properties were found alongside fibres having normal tuning. The former exhibited either reduced excitability of the low threshold tip segment, or a tip segment attenuated in both excitability and threshold.

6. It is concluded that the selective effects of furosemide on the tip segment of cochlear fibre f.t.c.s offer further evidence for a physiologically vulnerable 'second filter' in the cochlea. The selective influence of the furosemide on the low threshold tip segment provides support for the hypothesis that the normal f.t.c. is generated by two largely independent processes: one vulnerable, low threshold and sharply tuned, and the other less vulnerable, but high threshold and more broadly tuned.

7. The findings, obtained with an agent known to produce reversible impairment of hearing in man, provide direct physiological evidence in support of the hypothesis that in sensorineural hearing loss of cochlear origin the frequency selectivity of cochlear nerve fibres is impaired.

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INTRODUCTION

This paper, like that preceding it (Evans & Klinke, 1982), is concerned with an investigation of the physiological vulnerability of the cochlear filtering process. In particular, it is concerned with a further test of the hypothesis that the discrepancy between the frequency selectivity of the passive mechanics of the basilar membrane and that of the cochlear nerve fibre responses is due to the action of a physiologically vulnerable 'second filter' process in the cochlea. This concept, and the evidence for it, is detailed elsewhere (see Evans, 1972, 1974*b*, 1975*a*, *b*; Evans & Wilson, 1973; Evans & Klinke, 1982).

From analyses of the suprathreshold properties of cochlear fibres under the conditions of hypoxia (Evans, 1974*a*, *b*; 1975*b*) and KCN administration (Evans & Klinke, 1974, 1982) it has been argued that these effects are not likely to result from non-specific alterations in the sensitivity of the cochlear fibres themselves, in contrast to the conclusion of Robertson & Manley (1974). However, the depressant action of these manoeuvres *is* ultimately non-specific, and the excitability of the nerve fibres themselves can undoubtedly be affected by them. In the present study, therefore, we examine the effects on cochlear tuning of furosemide (frusemide BP), a potent diuretic having known specifically ototoxic properties, but no reported effects on the excitability of nerve fibres or synaptic transmission.

Furosemide is one of the 'loop' diuretics having ototoxic side-effects in clinical experience and in animal investigations (see Brown & Feldman, 1978; Prazma, 1981 for reviews).

In animal experiments, systemic administration produces rapid reduction in endocochlear potential (e.p.), cochlear microphonics (c.m.) and gross cochlear action potential (c.a.p.). The valuable conjunction of systemic effectiveness and immediate, reversible action, offered by this agent compared with others (e.g. ethacrynic acid), led to its choice in the present study. Its systemic action offered the possibility of a further control for possible artifacts in the KCN studies of Evans & Klinke (1982) arising from the method of intracochlear instillation used. The present study therefore utilized both systemic and intracochlear routes of administration.

Preliminary reports of the study have already been published (Evans & Klinke 1974; Klinke & Evans, 1974).

METHODS

Successful experiments were carried out on five cats. For details of the preparation, physiological control, sound stimulation, single fibre recording and computer controlled analysis techniques, see Evans (1979) and the preceding paper (Evans & Klinke, 1982).

Furosemide (Frusemide BP: Lasix, Hoechst Pharmaceuticals, MW 330·8) was administered by two routes. For intracochlear administration, it was instilled into the scala tympani via a needle inserted after complete removal of the round window. It was dissolved in a cerebrospinal fluid (c.s.f.)–artificial perilymph mixture in concentrations of 0·3–0·9 mM, although the final concentration in the organ of Corti is likely to be an order of magnitude lower after diffusion into the cochlear partition (see Evans & Klinke, 1982).

For systemic administration, the subclavian artery on the same side as the cochlear recording site was cannulated so that injections of undiluted furosemide preparation (20 mg in 2 ml.) could be given into the arterial supply of the cochlea. Repeated injections led to a substantial diuresis, amounting in one 3 kg cat to more than 1 l. over a period of 8 hr. In order to compensate for the

loss of fluid and electrolytes, sterile urine was collected by suprapubic cannulation of the bladder and immediately re-infused I.V. This procedure effectively maintained the preparation in a stable physiological condition.

RESULTS

(1) *Effects of furosemide on the gross cochlear potentials*

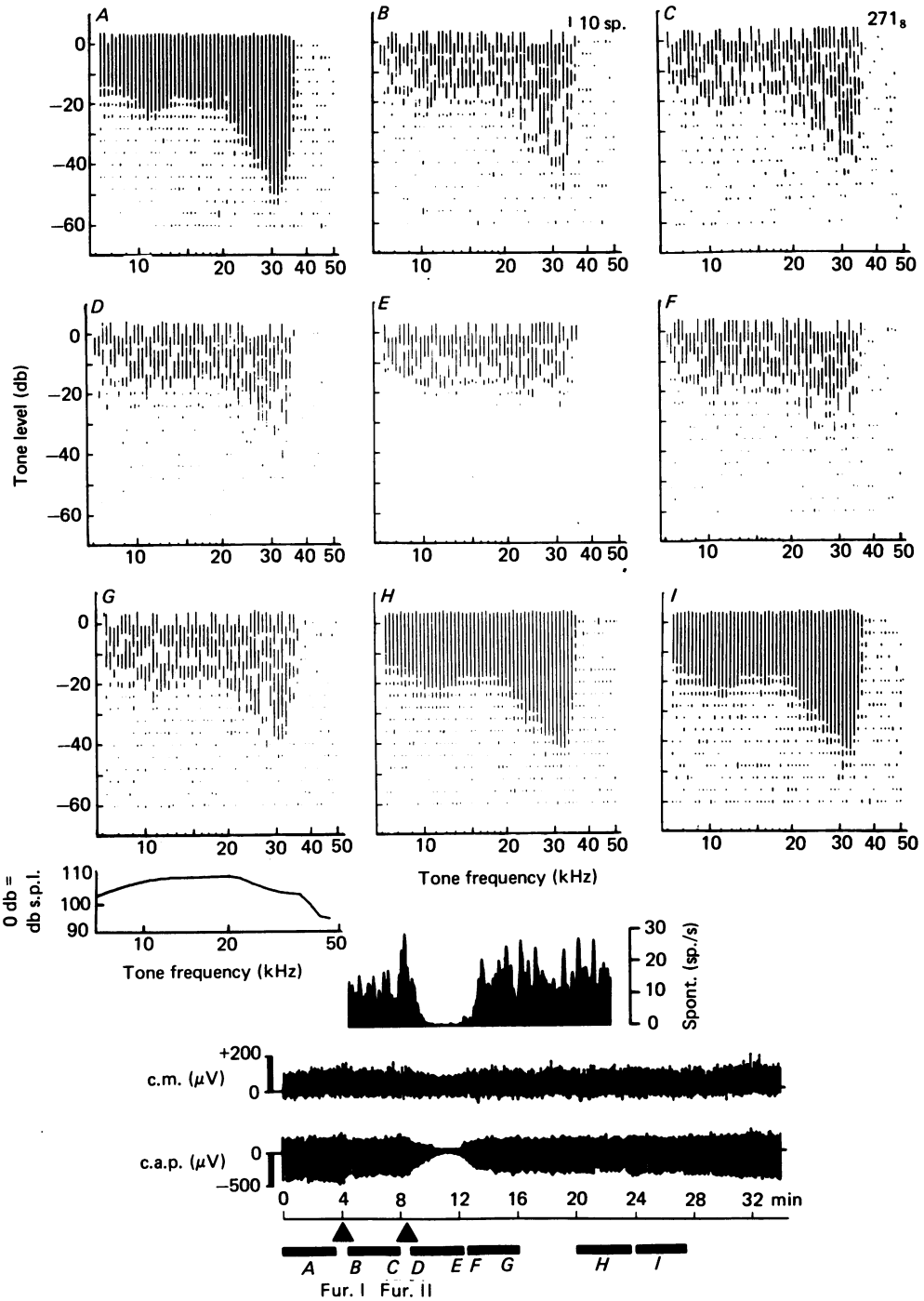
(*a*) *Intra-arterial administration*

Larger doses of furosemide than those reported by Brown & McElwee (1972) were required to produce depression of the gross cochlear action potential c.a.p. A total of at least 19 mg/kg I.A. was necessary in the present experiments to reduce the c.a.p. amplitude by some 30 % for a few seconds (equivalent to a reduction in sound pressure by 20 db). The reversible effects of two separate I.A. injections of furosemide on the c.a.p. and c.m. amplitude in response to a constant level click stimulus 30–40 db above the c.a.p. threshold are illustrated in Fig. 1. The first injection (Fur. I) shown on the Figure, which in fact was the fourth bolus of 20 mg in a 3·2 kg cat, produced a reduction of some 50 % in the c.a.p. for about 10 sec without change in c.m. Subsequent injections of furosemide appeared to be more effective: thus the second injection of the same dose (Fur. II) completely suppressed the c.a.p., and slightly depressed the positive component of the c.m. (c.m.$^+$) for 1 min, whereas the negative component (c.m.$^-$) was unchanged. The equivalent reductions in sound pressure amount to 40 db and 4 db for the c.a.p. and c.m.$^+$ respectively. With up to a total I.A. dose of 30 mg/kg, c.m. and c.a.p. recovered within 10 min. With higher total doses (50 mg/kg), complete recovery was not observed within 2 hr, probably because the re-infusion of urine containing furosemide maintained the blood levels of furosemide (see Methods). Additionally, with these high doses, both c.m.$^+$ and c.m.$^-$ were reduced in amplitude.

(*b*) *Intracochlear administration*

Small effects were observed with concentrations of 0·3 mM, but effects were more pronounced with 0·9 mM concentrations (Fig. 2*A*). The latency of onset of action on the c.a.p. was about 3 min after the furosemide reached the cochlea. This latency is substantially longer than that for instillations with KCN. Fig. 2*B* shows, for comparison, a subsequent KCN instillation in the same cochlea and under similar conditions, where the latency of effect was 1 min or less. The time course of the c.a.p. depression under furosemide was also very substantially longer than that under KCN: maximal depression occurred 26 min after the start of the instillation, and at least 100 min (including 25 min of c.s.f. flushing) were required before recovery to 85 % of control amplitude was obtained.

In the case illustrated in Fig. 2, the c.a.p. was reduced to 32 % and the positive-going component of the c.m. (c.m.$^+$) to 60 %, of control, equivalent to reductions in sound pressure of about 35 and 4 db respectively. The peak-to-peak c.m. amplitude decreased by only about 10 %, which means that the negative going component *increased*. The converse is the case with KCN (Fig. 2*B*). In the case illustrated in Fig. 5, the c.a.p. was eliminated completely about 25 min after the start of furosemide instillation and had not recovered 7 hr later, whereas the c.m. was not completely eliminated, and its peak-to-peak amplitude had recovered to 55 % after 1 hr.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Fur. II

Fig. 1.

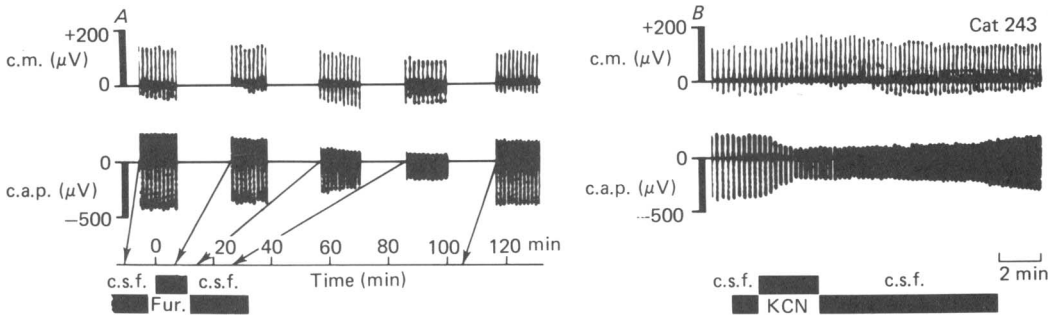


Fig. 2. *A*, records of c.m. and c.a.p. in response to rarefaction clicks before, during, and after instillation of furosemide into scala tympani. c.m. and c.a.p. recorded in response to 50 μsec rarefaction clicks, every 10 sec, presented at about 40 db above visual detection level of the c.a.p. Arrows indicate times at which the sample records were obtained. Fur: period of instillation of 1 mM-furosemide. c.s.f: control instillations of artificial perilymph alone. All at 5 μl./min. *B*, records of c.m. and c.a.p. in response to clicks before, during and after instillation of 1 mM-KCN at 5 μl./min, for comparison with *A*. Conditions as in *A*.

In no case was an increase in the c.m.$^+$ observed under systemic or intracochlear administration of furosemide, contrary to the finding with cyanide (Evans & Klinke, 1982).

(2) *Short-term effects of furosemide on the responses of single cochlear fibres*

(*a*) *Intra-arterial administration*

(i) *Changes in the frequency–threshold curves.* Where a cochlear fibre could be held for an adequate period, the sequence of changes illustrated in Fig. 1 and 3 were observed. The control frequency response analysis (*A*) indicates the typical frequency response area of a fibre with a characteristic frequency (c.f.) of 31 kHz. The analysis

Fig. 1. Reversible effects of two intra-arterial injections of furosemide on the cochlear microphonic (c.m.), the gross cochlear action potential (c.a.p.), the spontaneous activity and the tuning properties of a single cochlear nerve fibre. Fur. I and Fur. II. Two doses, each of 6·25 mg/kg furosemide given into the ipsilateral subclavian artery, following three identical but ineffective doses. *A–I*: frequency response plots of cochlear fibre discharges, obtained at the times indicated by the labelled horizontal bars in relation to the record of cochlear potentials below. Length of vertical bars in each frequency response plot indicates the number of spike discharges evoked by a single 60 msec tone of frequency and stimulus level represented by the centre of the bar. Stimulus level in db of electrical signal to the condenser driver earphone. Inset indicates sound pressure level (in db s.p.l.) at the tympanic membrane, corresponding to 0 db electrical level. Plots *A*, *H* and *I*: full analyses (i.e: 1024 data points addressed); plots *B–G*: half analyses (i.e. 512 points addressed). (See Methods). Spont: record of 'spontaneous' discharge of the fibre plotted on the same time scale as that for the cochlear potentials. The number of spikes (sp.) gated between stimuli were summed over five successive intervals and the result smoothed by a 10-point Hamming window thus occupying fifty interstimulus intervals (i.e: 10 sec real time). Increase in spontaneous activity at time of second injection (Fur. II) is noise induced. c.m. and c.a.p: amplitude of cochlear microphonic and gross cochlear action potential respectively, gated out of the round window record, in response to a 50 μsec rarefaction click every 10 sec (at about 40 db above initial visual detection level of c.a.p.) (see Methods).

was complete, as were those of *H* and *I*, i.e. all 1024 frequency–intensity points had been sampled. In contrast, analyses *B–G*, represent only half analyses, i.e. 512 samples. The outlines of the frequency response areas, the f.t.c.s, are superimposed in Fig. 3 for convenient comparison (after correction to s.p.l. at the tympanic membrane). Following the fourth intra-arterial injection of 6·25 mg/kg furosemide (Fur. I), a small elevation (*ca.* 10 db) of tip threshold occurs (*C*). During the major depression of the c.a.p. following the next injection of an equal dose of furosemide

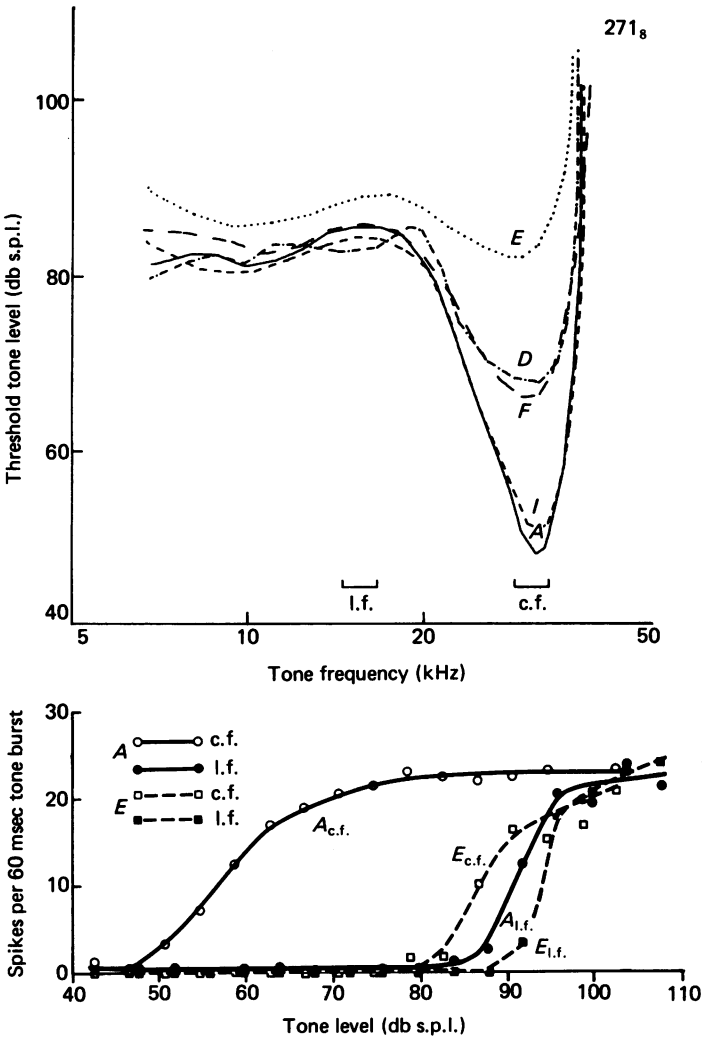


Fig. 3. Frequency–threshold curves and discharge-rate *versus* stimulus-level functions for the cochlear fibre illustrated in Fig. 1, under conditions of intra-arterial furosemide administration. Upper half: *A–I*: f.t.c.s corresponding to frequency response plots *A–I* of Fig. 1, corrected to db s.p.l. at the tympanic membrane. Lower half: Discharge rate *versus* stimulation level functions at two frequency regions, indicated by l.f. and c.f. respectively, for frequency response plots *A* and *E*. Note how the rate *versus* level function at c.f. becomes much steeper under furosemide action ($E_{c.f.}$) compared with control ($A_{c.f.}$), to resemble the tail frequency rate-level functions ($A_{l.f.}$, $E_{l.f.}$).

(Fur. II), the threshold of the sharply tuned tip segment of the f.t.c. was progressively elevated (*D*, *E*) until in (*E*), about 3 min after the furosemide administration, over 30 db of the tip had been lost, leaving a broad high-threshold segment. During recovery of the c.a.p. the f.t.c. tip threshold progressively returned (*F*, *G*, *H*) until at (*I*), 16–20 min after the second furosemide administration, recovery was virtually

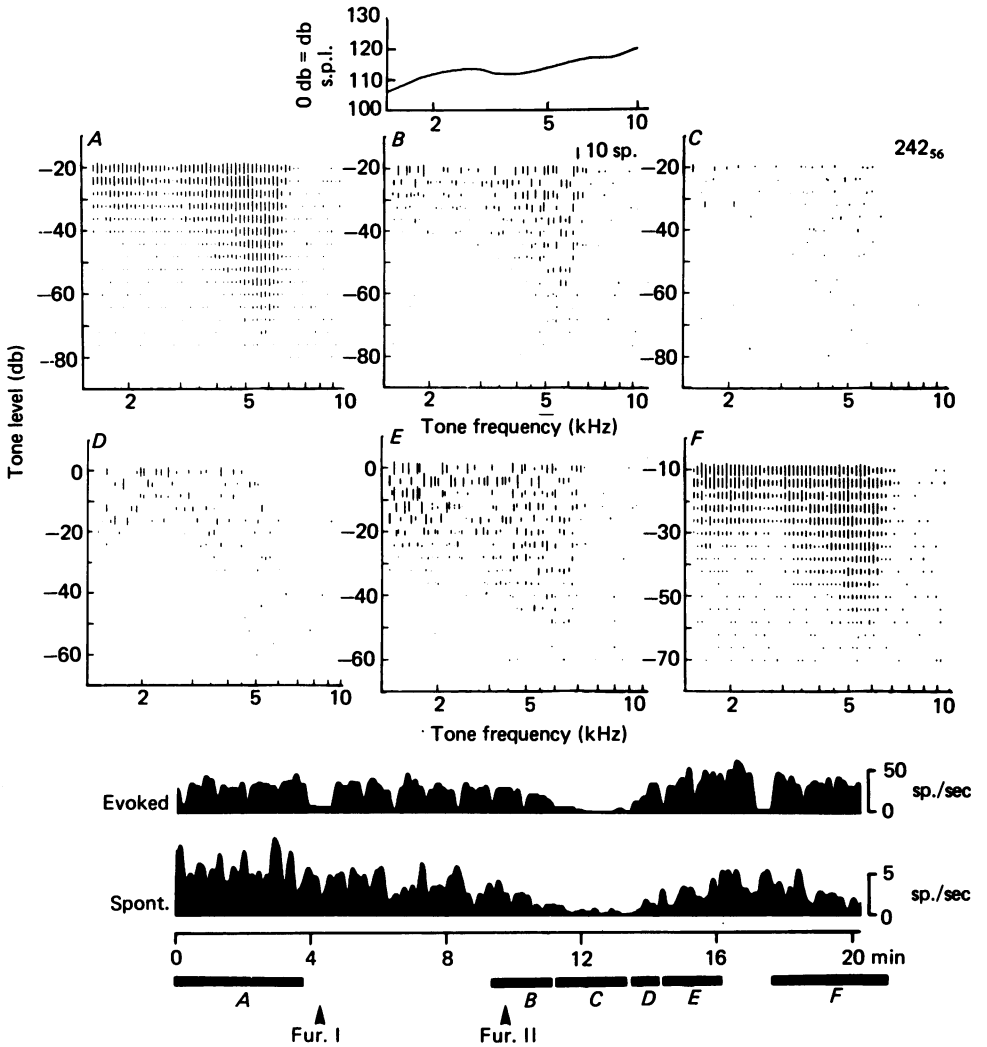
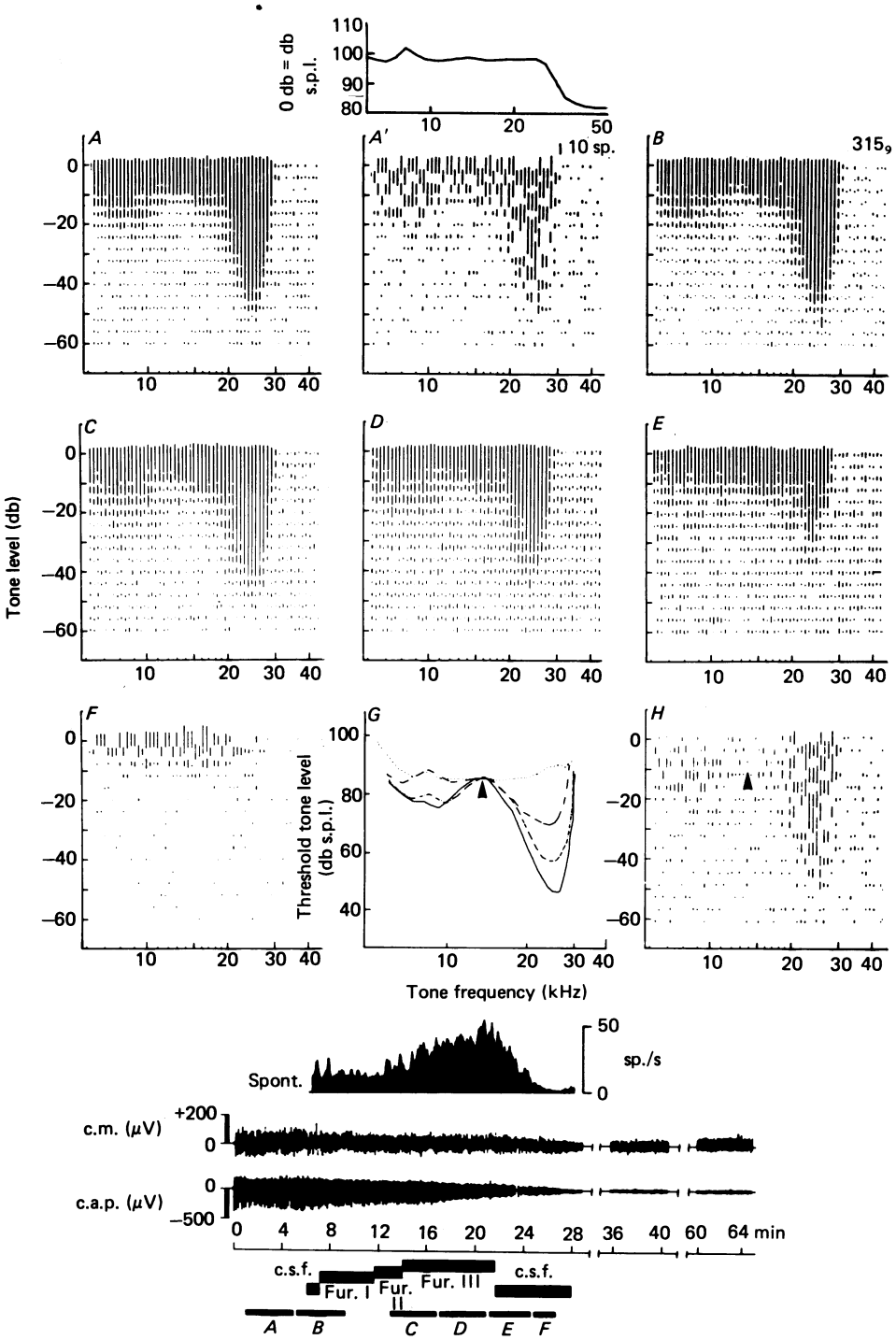


Fig. 4. Substantial effect of furosemide: reversible loss of spontaneous and evoked activity, and of tuning of cochlear fibre from large intra-arterial dose. Fur. I and Fur. II: doses of 4 mg/kg furosemide given into the ipsilateral subclavian artery. *A–F*, frequency response plots obtained before (*A*) and during (*B*, *C*, *D*) the action of the furosemide. Plots *E* and *F* were obtained during recovery. Plots *A* and *F*: full analyses (1024 points addressed); *B*, *C*, *E*: half analyses (512 points addressed); *D*: quarter analysis (256 points addressed). Evoked and Spont. Spike counts gated during and between tone stimuli respectively: summed over five successive intervals and smoothed by a 10-point Hamming window, (ie: 10 sec real time). Note higher stimulus levels in plots *D*, *E* (20 db) and *F* (10 db) relative to *A*, *B* and *C*, as indicated by the ordinates.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A B C D E F

Fig. 5.

complete. The threshold of the low frequency 'tail' segment, on the other hand, remained relatively stable, being elevated less than 5 db even at the height of the action of the furosemide (*E*). The tip segment was sufficiently attenuated that the cochlear fibre f.t.c. became effectively low-pass, and the c.f. became somewhat lower than in the control case.

This sequence of events in whole or in part was observed in five other fibres having c.f.s of 3·5–12 kHz, where doses of more than 19 mg/kg were employed, producing threshold elevations of the tip segment ranging from 10 to 40 db. Fibres having lower c.f.s were not tested.

The most extreme case observed is shown in Fig. 4 in a fibre with a c.f. of 5·2 kHz, where intra-arterial administration of 8 mg/kg furosemide (in two divided doses, Fur. I and Fur. II, 5 min after a previous dose of 8 mg/kg) was sufficient to eliminate briefly the evoked, as well as the spontaneous activity of the fibre. Analyses *A* and *F* are complete; *B*, *C* and *E* half samples; and *D* a one-quarter sample of the frequency–intensity space. *A* is the control analysis. Analyses *B*, *C* and *D* demonstrate the increase in tip threshold and *E* and *F* the partial recovery before contact with the fibre was lost. Analysis *D*, (covering, with analyses *E* and *F*, a range of higher stimulus levels than the earlier analyses) shows that the substantial elevation of tip threshold was accompanied by a 10–20 db elevation of the threshold of the low-frequency tail. This was a greater effect on the tail segment than that observed in other fibres studied.

(ii) *Changes in spontaneous activity in relation to changes in the f.t.c.s*

In two cases, illustrated in Figs. 1 and 4, the fibres' spontaneous activity was briefly eliminated as a result of the action of furosemide.

In the unit of Fig. 4, the changes in evoked and spontaneous activity were such that the time course of the changes in tuning could not be distinguished from that of the spontaneous activity. In the behaviour of the fibre illustrated in Fig. 1, however, the changes in spontaneous activity and tuning followed different time courses. Thus, the threshold of the f.t.c. tip segment was elevated over 10 db after the first injection of furosemide (Fur. I) without significant reduction in spontaneous activity (compare *C* with *B*). Following the second injection (Fur. II), (the mechanical noise of which was responsible for the brief increase in 'spontaneous' activity) the spontaneous discharge rate rapidly decreased (*D*) and virtually disappeared, during which time the remainder of the tip segment of the f.t.c. was lost (*E*). The spontaneous activity returned at the beginning of analysis *F* and rapidly returned to levels at and above the control, although the f.t.c. was only showing slight signs of recovery. No

Fig. 5. Effects of intra-cochlear instillation of furosemide. Instillation commences with artificial perilymph (c.s.f.) as control; then 0·3 mM-furosemide (Fur. I), both at 7 μl./min. The furosemide is then increased in concentration (0·6 mM: Fur. II) and, subsequently, in rate (10 μl./min: Fur. III). Finally artificial perilymph (c.s.f.) is used to flush out the furosemide. *A–F*, frequency response plots of a single cochlear fibre, obtained before (*A*) and during (*B*, *C*, *D*) administration of furosemide. Plots (*E*) and (*F*) obtained during flushing with artificial perilymph (c.s.f.) as effects of furosemide on cochlea were still increasing. Plots *A*, *B*, *C*, *D* and *E* are full analyses; plots *A'* and *F*, half analyses (see text). *A'* represents the first half of analysis *A*. *G:* f.t.c.s. corresponding to plots *A–F*, corrected to db s.p.l. at the tympanic membrane. Plot *H*: data values of plot *F* subtracted from corresponding values of plot *A'*. Spont, c.m. and c.a.p: as in Fig. 1.

further changes in spontaneous activity occurred during the complete recovery of the tip segment of the f.t.c. (*G*, *H*, *I*).

Even during the period of change in spontaneous discharge rate, the level of evoked activity in response to maximal (saturation) level stimuli does not change significantly, as can be seen from the upper row of data points in each of the analyses. This point is made more clearly in the discharge rate-level functions for the fibre, shown in Fig. 3. Here, the rate-level function for the tail frequencies (l.f.) is virtually identical before (*A*) and during (*E*) the furosemide action. It is noteworthy that the rate-level function at the c.f. approaches that for the tail frequencies as the f.t.c. tip is lost. In all cases, the saturated discharge rates are the same.

(*b*) *Intracochlear administration of furosemide*

The changes in tuning properties under intracochlear instillation of furosemide was studied in two fibres in two animals. Each showed the expected elevation of tip threshold, as illustrated for one fibre, in Fig. 5, with concentrations of 0·3–0·9 mM. Analyses *A* and *B* before the furosemide has acted, are the control. The following sequence of events is clearly discerned in analyses *C*–*F*, the f.t.c.s from some of which are superimposed in *G*. First, the spontaneous discharge rate increased, during *C*, with little if any change in the f.t.c. Next, the f.t.c. tip threshold became elevated by 10 db with only a small increase in spontaneous discharge rate (*D*). By analysis *E* the tip threshold was elevated by another 10 db as the spontaneous activity decreased towards the control value. At the beginning of analysis *F* the spontaneous discharge rate virtually disappeared. The f.t.c. had now lost its tip segment completely, leaving behind a high threshold segment with a c.f. substantially lower than that of the control. The completeness of the disappearance of the tip segment is shown by plot *H*, where the discharge values for plot *F* have been subtracted from those for plot *A'* – a half-point plot of analysis *A* corresponding to that of *F* – to demonstrate the component parts of the control f.t.c. missing in analysis *F*. The major loss is the tip segment, but about 10 db of the low frequency 'tail' has also been lost. It is noteworthy that the 'notch' between the low frequency 'tail' and the tip segment (indicated by the arrow in *G*, *H*) is the most stable feature of the f.t.c.

Throughout these changes in f.t.c. and spontaneous discharge rate, the maximum evoked activity of the low frequency 'tail' segment remained virtually constant. Thus, the mean number of spikes evoked by the maximum level of stimulation between 6·5 and 15·7 kHz were 16·4 and 16·7 spikes in the analogous analyses of *A* and *F* respectively.

While the most striking change in the c.f. of this fibre occurred with total loss of the tip segment (c.f. change from 26 to 14 kHz), there was a systematic decrease in c.f. as the threshold of the tip segment was progressively elevated (D, E, F).

As has already been mentioned in section 1(*b*) above, no recovery of the c.a.p. occurred within 7 hr of the instillation; likewise, it was not possible to demonstrate recovery of normal single fibre properties.

(3) *Long-term effects of furosemide on the responses of single cochlear fibres*

In two animals, repeated intra-arterial administration totalling over 30 mg/kg furosemide led to a chronic pathological state of the cochlea. During the period

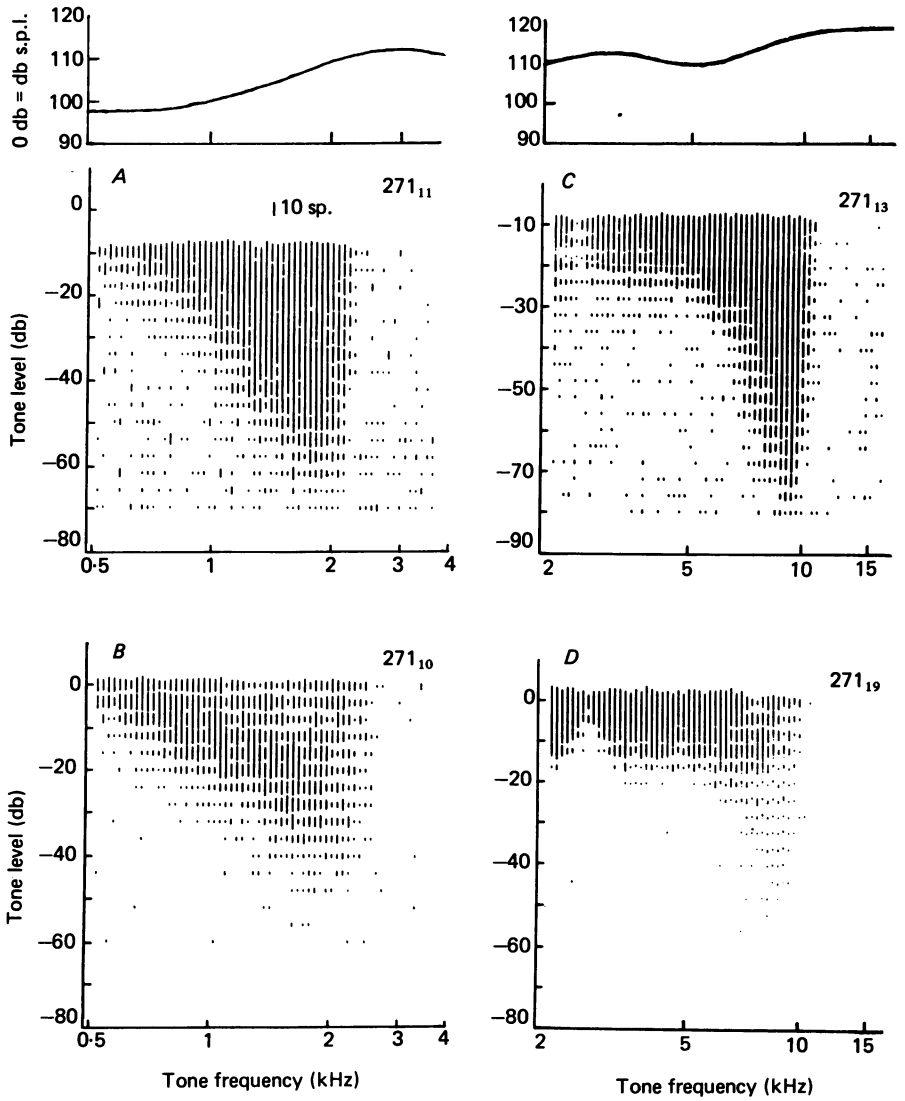

Fig. 6. Examples of anomalous tuning found during long-term intoxication by systemically administered furosemide. *A–D*, frequency response plots of four cochlear fibres obtained 3·8, 3·6, 4·2 and 5·5 hr, respectively, after the first dose of a series of I.V. injections of furosemide, 30 mg/kg in total. *A* and *B*, *C* and *D*, pairs of fibres obtained closely together in the same animal, each pair having similar c.f.s (about 2 and 8 kHz respectively). One member of each pair (*B*, *D*) has anomalous tuning and response characteristics, the other (*A*, *C*) is normal for comparison. Note in *B*, nonmonotonic relation between response and stimulus level for frequencies at the c.f., and reduction in frequency yielding maximum response as level is increased. Note in *D*, lower threshold 'tip' segment having extremely attenuated response, compared with normally responsive 'tail' segment. Sound pressure level corresponding to 0 db: given above respective plots.

3·5–5·5 hr following the first dose, fibres exhibiting anomalous behaviour were encountered alongside normally behaving fibres. Fig. 6 illustrates two of these anomalous fibres of contrasting c.f.s. encountered in the same cochlea, together with examples of virtually adjacent fibres exhibiting normal behaviour.

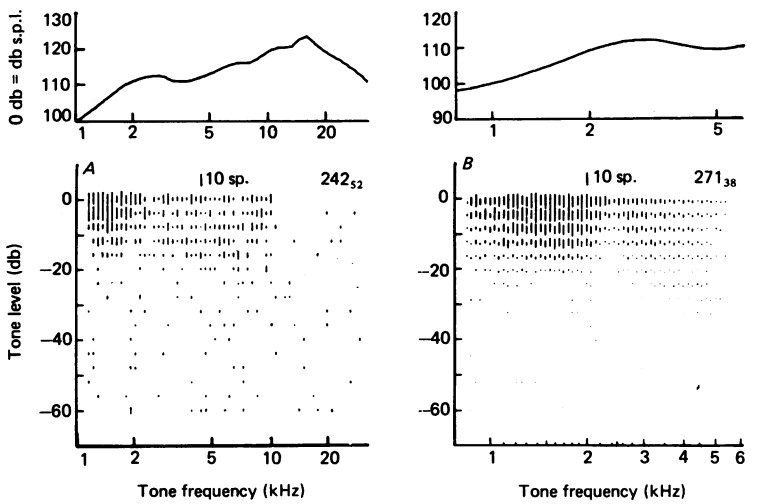

Fig. 7. Further examples of anomalous tuning properties in two fibres from two cats during long-term intoxication with furosemide. *A*, Frequency response plot of fibre obtained 4 hr after first of a total dose of 28 mg/kg furosemide I.V. *B*, obtained 10 hr after first of a total dose of 48 mg/kg I.A. Note in both cases, attenuated responsiveness of higher frequency 'tip' segment, compared with low frequency 'tail' segment. The tip segments in each case have pathologically raised thresholds. Insets indicate db s.p.l. corresponding to 0 db electrical level given in the respective plots.

Fibres *A* and *B* have almost identical c.f.s of about 2 kHz, and were isolated as adjacent fibres within a period of 30 min, fibre *B* before *A*. Fibres *C* and *D* have c.f.s of 9·0 and 8·2 kHz respectively, and were recorded within a period of 1 hr 20 min. Both anomalous fibres (*B* and *D*), in contrast to the normal fibres (*A* and *C*), have greatly attenuated responses in the higher frequency region of their respective response areas. In the case of the lower c.f. fibres (*B*), the response is *reduced* for high level stimulation at the higher frequencies. This means that the response at its c.f. is non-monotically related to stimulus level, and that the maximum response (corresponding to maximum length of the vertical bars) moves rapidly downwards in frequency with increasing stimulus level. In the case of the fibre with higher c.f. (*D*), the attenuated response area corresponds to the lower threshold, high frequency segment of normal f.t.c.s. This barely observable segment corresponds to that *missing* from the f.t.c. under acute furosemide poisoning, as in Figs. 1, 3, 4 and 5.

Fig. 7 *A* and *B* illustrates two further fibres, from two different cats, having c.f.s of about 7 and 4 kHz respectively, and with substantially raised thresholds for what remains of their tip segments, but where, again, the responsiveness of this segment is substantially reduced compared with the low frequency 'tail' area. The frequency response properties of the fibre in Fig. 7 *B* remained unchanged for the period of over 1 hr that the fibre was under observation.

DISCUSSION

(1) *How and where does the furosemide act in the cochlea?*

There is little information available on the mode of action of furosemide in the cochlea. This holds also for other loop diuretics. Their action on the kidney, on the other hand, is known in some detail, and this may help us to understand their effects upon the cochlea.

The main effect of furosemide in the kidney is an inhibition of active transport of Cl^- in the ascending limb of the loop of Henle (Burg, Stoner, Cardinal & Green, 1973). Similar inhibition of Cl^- transport by the loop diuretics occurs in the frog cornea (Candia, 1973; McGahan, Yorio & Bentley, 1977) and in fish intestinal mucosa cells (Zeuthen, Ramos & Ellory, 1978), as at the target site of the crossed olivocochlear bundle in the cat (Desmedt & Robertson, 1975).

A second possibility is that furosemide, and other loop diuretics, produce their effects on the inner ear by interference with energy generation in the hair cells or stria vascularis. Indeed, Kaku, Farmer & Hudson (1973) obtained histochemical evidence for such effects on succinic dehydrogenase and diphosphopyridine nucleotide diaphorase with ethacrynic acid in the outer hair cells of the basal part of the cochlea and in the stria vascularis, in the guinea pig (see also Spector, 1975). Loop diuretics inhibit glycolysis in the kidney (Klahr, Yates & Bourgoigne, 1971). However, these changes were not obtained with I.V. doses of less than 70 mg/kg whereas ototoxic effects on threshold have been reported in the guinea pig with doses as low as 4–10 mg/kg I.P. (Ernstson, 1972; Crifo, 1973), and in the cat, with 19 mg/kg, in the present experiments. Likewise in our experiments, furosemide effects were observed with concentrations of 0·9 mM or less in the instillation fluid (less in the cochlea by dilution, see Evans & Klinke, 1982), whereas in the experiments by Klahr *et al.* (1971), this dose was insufficient to block glycolysis. Also, Bowman, Dolgin & Coulson (1973) found that diuretic doses of ethacrynic acid were insufficient to block glycolysis. As far as oxidative processes are concerned, Brown (1975) argues that because chloramphenicol produces suppression of cochlear respiratory enzyme activity (Koide, Hata & Hando, 1966) without substantial ototoxic effects in guinea pig and cat (e.g. Patterson & Gulick, 1963), it is unlikely that suppression of cochlear respiratory enzyme activity is the primary mechanism whereby ethacrynic acid (and by implication, furosemide) exerts ototoxic effects. Similar conclusions were drawn, by Thalmann, Thalmann, Ise & Paloheimo (1977) and Kusakari, Ise, Comegys, Thalmann & Thalmann (1978), from data with furosemide.

In the absence of further evidence, therefore, it seems more likely that the loop diuretics exert their effects by specifically blocking an hypothetical Cl^- pump in the cochlea. This is consistent with evidence from the present experiments which suggests that furosemide acts differently from the presumably more generalized effects of cyanide. In contrast to the latter, furosemide did not decrease the amplitude of the c.m.$^-$ component (except with very high doses) and did not cause an increase in the c.m.$^+$ component. The changes in endolymph ionic concentrations reported to occur with ethacrynic acid poisoning (Cohn, Gordes & Brusilow, 1971; Brusilow & Gordes, 1973; Bosher, Smith & Warren, 1973*a*, *b*; Bosher, 1979, 1980*a*) are also consistent with the assumption that it exerts its effects upon ionic pumps.

In regard to the anatomical site of action, furosemide has been shown to produce ultrastructural changes in the stria vascularis 4 hr after I.V. injection of 40–50 mg/kg furosemide in the guinea pig (Forge, 1976). Ethacrynic acid produces cytopathological changes in the stria vascularis and in the outer hair cells of the basal part of the cochlea in a number of species, including cat (Quick & Duvall, 1970; Mathog, Thomas & Hudson, 1970; Nakai, 1971; Bosher, Smith & Warren, 1973*a*, *b*, Silverstein & Begin, 1974; Bosher, 1980*b*). The question therefore arises whether the effect of furosemide on the organ of Corti are secondary to primary effects on the stria, mediated via the endocochlear potential (e.p.). In the guinea-pig, doses of furosemide of 50 mg/kg produce substantial changes (to negative values) in the e.p., within about 2 min of I.V. injection (Kusakari *et al.* 1978). In the case of the present experiments, however, this possibility seems less likely in view of the finding that the c.m.$^-$ component is unchanged during furosemide action, and in one case at least (Fig. 2) actually increased, and in view of the fact that changes in cochlear fibre tuning could be demonstrated with I.V. doses as low as 19 mg/kg. Additionally, the *increase* in spontaneous activity observed in a number of cases in the present experiments (e.g. Figs. 1 and 5) is consistent with a direct effect of furosemide on the hair cells themselves. For this to occur secondary to changes in the e.p., the effects of furosemide would have to include an initial *increase* of the e.p. This has not been reported in the literature to occur. On the other hand, the very brief latency of action of systemically administered furosemide, and the difference in recovery rates between systemic and local administration, could be consistent with the effects being secondary to changes in the stria vascularis. Clearly direct demonstration that the e.p. is not primarily involved is required.

However, should there be an action of furosemide on the hair cells, it could be speculated, in view of the cytochemical evidence cited above, that the effects may be primarily exerted on the *outer* hair cells. This would support models of cochlear frequency selectivity based on some form of interaction between the outer and inner hair cell systems, of an electrical or mechanical nature (Davis, 1973; Evans & Wilson, 1973; Manley, 1977; Harrison & Evans, 1977). The finding, by Russell & Sellick (1977, 1978) that the intra-cellular receptor potentials of the inner hair cells (which the majority of cochlear fibres innervate) are as sharply tuned as the responses of cochlear fibres, has eliminated alternative possibilities involving synaptic (Zwislocki & Sokolich, 1974) or electrotonic (Evans, 1974*b*) interaction between the outer and inner hair cell *afferent nerve fibres*.

(2) *Implications for 'second filter' type hypotheses of cochlear tuning*

The reversible changes in the tuning of cochlear fibres under furosemide poisoning of the cochlea provide further evidence for a physiologically vulnerable 'second filter' (Evans, 1972; Evans & Wilson, 1973) between the passive mechanics of the basilar membrane and the cochlear nerve, in the following respects.

The anomalous tuning obtained in long-term furosemide poisoning in some fibres alongside normal tuning in neighbouring fibres (Fig. 7) is consistent with the notion that the 'second filter' is 'private' to individual fibres or groups of fibres. This notion was originally derived from the observation that the tuning (measured for example as the '$Q_{10\ \mathrm{db}}$', Evans, 1972) could show substantial variation from fibre to fibre in

the same cochlear nerve and at the same frequency (Evans, 1972; Evans & Wilson, 1973; Geisler, Rhode & Kennedy, 1974). It is not easily compatible with a distributed, sharply tuned basilar membrane filter unless its tuning depends upon local active processes as has been suggested by some recent evidence (see Discussion in Evans & Klinke, 1982).

Further evidence that the changes in tuning do not derive from changes involving the passive mechanical properties of the basilar membrane is provided by the following observations. Firstly, the time scale of the changes produced by furosemide would appear to be too short for changes to be expected to occur in the mechanics of the basilar membrane itself. Changes in cochlear fibre tuning can be obtained within 50 sec of intraarterial injection of furosemide, as in Fig. 1. Secondly, the profound changes in the gross c.a.p., reflecting deterioration in cochlear fibre tuning and elevation in threshold, can occur with little change in the amplitude of the c.m. (Figs. 1 and 2). This argument depends upon the assumption that the c.m. amplitude correlates with the amplitude of basilar membrane vibration. There is good evidence supporting this assumption (e.g. Dallos, 1973; Wilson, 1974). In view of the possible objections to the use of this argument in the case of cochlear instillations because of the potentially localized action of the instilled agents (see Discussion in Evans & Klinke, 1982), it is important to note that the dissociation between c.a.p. and c.m. amplitudes was observed in the present experiments also with the *systemic* administration of furosemide, where any effects on cochlear mechanics should be generalized, as evidenced by the effects on the cochlear fibres which extended over wide range of c.f.s, from at least 3·5 to 31 kHz. It is likely that had fibres with c.f.s lower than 3·5 kHz been tested, effects of the systemic administration would have been observed. Thus, even if the c.m. potentials in our round window recordings derived from a wider region of the cochlea than the first turn, in the case of the systemic furosemide effects the c.m. should not have been contaminated by potentials from remote cochlear regions unaffected by the furosemide.

It seems unlikely that the furosemide could exert its effect via a change in viscosity by virtue of a local osmotic change. The time scale of effect on cochlear tuning from a single injection appears to be too short for this to happen.

Furosemide is not known to have neurotoxic properties, nor is it known to interfere with synaptic processes. It is therefore unlikely that it exerts its effects via non-specific deterioration in excitability of the cochlear fibres themselves, although this cannot be entirely excluded. Certainly, as with cyanide (Evans & Klinke, 1982), high doses can reduce the spontaneous (Figs. 1, 4 and 5) and evoked discharge activity (Fig. 4). On the other hand, substantial changes in tuning occur without substantial changes in either threshold or maximal (saturated) evoked activity in the low frequency tail of the f.t.c. (e.g. Figs 1, 3, 5, 6), particularly in the region of the 'notch' commonly found between the tail and c.f. segments (e.g. Fig. 5 *G*, *H*, arrow).

One characteristic aspect of the physiological vulnerability of cochlear tuning common to all types of pathology, whether arising from hypoxia (Evans, 1974*a*, *b*; Robertson & Manley, 1974), cyanide (Evans & Klinke, 1974, 1982), noise exposure (Kiang, Liberman & Levine, 1976; Liberman & Kiang, 1978), aminoglycoside poisoning (Kiang, Moxon & Levine, 1970; Kiang *et al.* 1976; Evans & Harrison, 1976, Harrison & Evans, 1977, 1979), phentolamine poisoning (Klinke & Evans, 1977), and

the present furosemide administration, is the vulnerability of the sharply tuned 'tip' segment of the f.t.c., in contrast to the more robust high threshold 'tail' segment. This raises the question whether the two segments of the f.t.c. may be derived from two different mechanisms. Some support for this notion comes from two lines of evidence from our experiments with KCN (Evans & Klinke, 1974, 1982) and furosemide. Firstly, as already noted under conditions of hypoxia (Evans 1974*b*, 1975*a*, *b*; also data of Robertson & Manley, 1974), the c.f. of the f.t.c. shifts towards lower frequencies as the tip segment is lost, so that the c.f. of the remaining segment is substantially different from that of the tip segment (Figs. 3 & 5). Secondly, the excitability of the tip segment can be independently modified with little, if any, effect on the high threshold 'tail' segment. This can be seen in the changes in responsiveness illustrated in Fig. 6*D* and Fig. 7*A*, *B*, and in the discharge-rate *v.* stimulus level functions of Fig. 3.

(4) *Significance for sensorineural hearing loss of cochlear origin*

The hypothesis has been advanced elsewhere (Evans, 1972, 1975*a*, *b*, 1976*a*, *b*, 1978; Evans & Wilson, 1973) that deterioration in auditory frequency selectivity may be associated with sensorineural hearing loss of cochlear origin, as a result of the deterioration in the tuning of cochlear fibres. In as much as reversible changes in cochlear nerve tuning were observed in the present experiments in addition to elevation of threshold, with furosemide, and this agent is known to produce reversible impairment of hearing in man, the present data provide direct physiological evidence in support of this hypothesis.

The consequences of this deterioration in frequency selectivity for the interpretation of clinical measures of hearing loss of cochlear origin and of attempts to compensate, by hearing aids, for the loss of function, are also discussed elsewhere (Evans, 1975*a*, *b*, 1976*a*, *b*, 1978). However, the relevance of the data presented here and in the previous paper (Evans & Klinke 1982) to 'recruitment', a phenomenon characteristic of hearing impairment of cochlear origin, is worthy of comment. Two factors observed in these data could contribute to the abnormally abrupt 'recruitment' of loudness (e.g. Fowler, 1937; Dix, Hallpike & Hood, 1948), and of the amplitude of the gross cochlear action potential (e.g. Eggermont, 1976) found clinically with increase in sound level, in cochlear hearing loss. The first is the deterioration in the frequency selectivity of the cochlear fibres. The broadening of their f.t.c.s would mean that a suprathreshold tone would 'recruit' cochlear fibres of neighbouring characteristic frequency into activity more rapidly with increasing tone level than in the normal case. If loudness on the one hand, and the amplitude of the gross cochlear action potential on the other, are related to the number of fibres active, then one would expect 'recruitment' of both measures to occur in cochlear pathology (see model in Evans, 1975*a*, *b*). The second factor concerns the discharge-rate *v.* stimulus-level functions of the cochlear fibres under the pathological conditions of these experiments. With poisoning of the cochlea by KCN (Fig. 4 of Evans & Klinke, 1982) and furosemide (Fig. 3 of the present paper), the rate *v.* level functions at the characteristic frequency become abnormally steep. In other words, the dynamic range of the cochlear fibres becomes reduced at their characteristic frequency. Data from long-term damage to the guinea pig from poisoning with kanamycin (e.g. Evans &

Harrison, 1976) are in agreement with this: the mean dyanmic range was reduced from about 45 dB in normal fibres to 11 dB (Harrison & Evans, 1979). The net result of these changes can be seen in the 'recruitment' of the gross cochlear action potential amplitude with stimulus level as a result of medium term pathological changes, in Fig. 6 of Evans & Klinke (1982).

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