incorporation of this colloidal dye into some of the gland lobules as a function of haemolymph protein transport (figure 2). Therefore, the above studies give some evidence of haemolymph protein incorporation by the salivary glands[4], as has been established in the case of vitellogenin incorporation by the developing oocytes[18,19]. The present studies suggest that some haemolymph protein fraction is sequestered by the salivary glands, thereby resulting in heterosynthetic saliva. However, it awaits further immunological study.

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Effects of increased potassium in scala tympani on auditory nerve sensitivity

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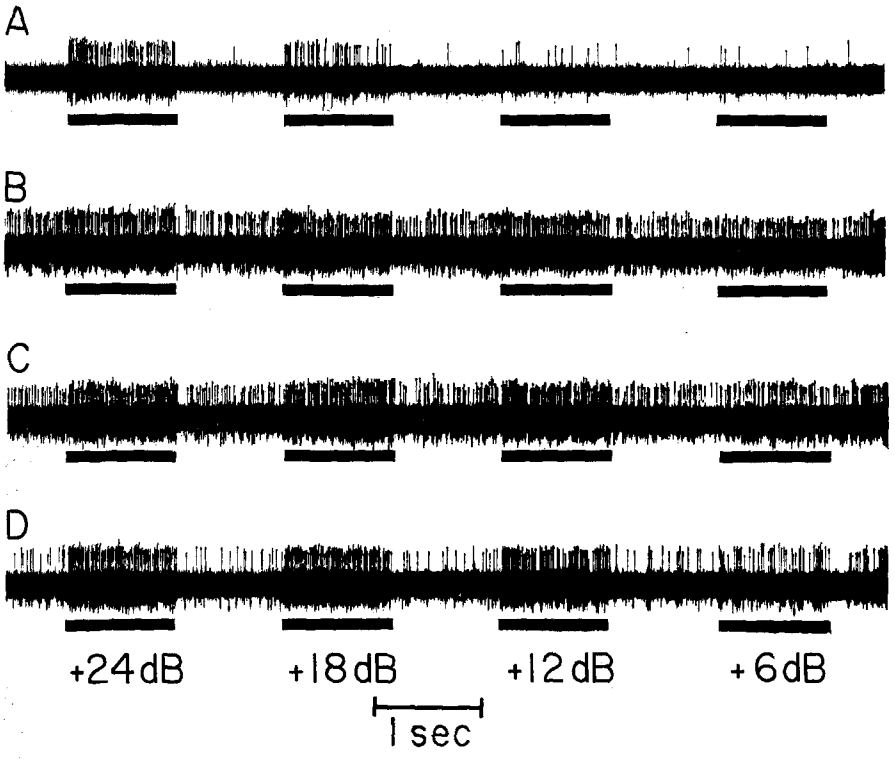
Summary. Raising the K^+ concentration in scala tympani of the guinea-pig cochlea generally caused a substantial increase in the spontaneous firing rate of single auditory nerve fibres. This effect was not accompanied by any observed reduction in the threshold sensitivity of these fibres. These findings cast doubt on current theories of cochlear transduction.

The mechanical event of direct significance to the cochlear receptor mechanism is believed to be deformation of the hairs of the cochlear hair cell. According to the widely held Davis theory of mechano-electrical transduction[3-5] this deformation causes a resistance change in the hair cell membrane, accompanied by a current flow from the positively polarized endolymphatic space into the negatively polarized interior of the hair cell. Thus 2 biological batteries coupled in series provide a store of energy which can be tapped by mechanical events at the hair cell, and the threshold sensitivity of the cochlea derives from the large total polarization provided by these batteries across the hair bearing surface of the hair cell. The batteries give rise to the endocochlear potential (EP) and to the hair cell resting potential, respectively. The current flow will probably be carried mainly by K^+ ions since these are predominant in the endolymph that bathes the hairs and since the effective driving force through the hair cell is greater for K^+ than for the other main ion species in endolymph[4]. Support for the Davis theory has come from reports that agents which depress the EP simultaneously depress the auditory nerve gross action potential and substantially raise the minimum thresholds of primary auditory neurones[7-13]. The degree of dependence of threshold sensitivity in the auditory nerve upon the EP can be estimated from the work of Manley and Robertson[7]. These authors recorded from cochlear ganglion cells and reported that transient hypoxia which induced a reversible drop of 30 mV in the EP simultaneously produced reversible losses of about 60 dB in the minimum thresholds of cochlear neurones.

Because reductions in the EP produce substantial changes in auditory nerve sensitivity, it is to be expected from the Davis theory that reductions in the hair cell membrane potential would have a corresponding effect. We sought to test this by raising the K^+ concentration in scala tympani in order to depolarise the hair cells and to reduce the K^+ gradient between endolymph and perilymph, whilst simultaneously recording from single auditory nerve fibres.

Material and methods. Guinea-pigs were anaesthetised with urethane. The cochlea was exposed ventrolaterally and holes of 50–80 µm diameter were drilled into scala tympani of the basal turn at 2 mm from the round window, and at the helicotrema. A glass micropipette whose tip was broken to fit tightly into the basal hole served to introduce perfusates into the cochlea. This pipette was coupled to a microinfusion pump, which was used to perfuse at rates of 8–14 µl/min over about 1 min. Perfusates were modifications of an artificial perilymph described by Konishi and Kelsey[15]: the K^+ content was raised at the expense of Na^+ to maintain isotonicity. Various K^+ concentrations between 20 and 30 mM were used, most frequently 27.5 mM K^+ and 22.5 mM K^+. Recordings were made from the auditory nerve using glass microelectrodes filled with 4 M NaCl. Units were identified as primary by standard electrophysiological criteria[16].

Once a unit was isolated, elements of its response characteristics were determined manually, and thereafter data were collected with on-line use of a PDP-9 computer. In some units the response to a repeated tone pip at the characteristic frequency (CF) and the discharge in the 'silent' intervals between tone pips were studied. In other units rate-intensity functions for stimulation at the CF were sequentially plotted. For this latter procedure the unit was stimulated by 1-sec tone pips separated by 1-sec silent intervals: the discharge in each successive sec was counted and stored for as long as the unit was held, usually between 5 and 10 min. Starting at a chosen maximum the tone intensity was reduced by 6 dB between pips for each of 10 tone pips, whereupon this cycle was restarted.



Effect on an auditory nerve unit of a 22.5 mM K^+ perfusion of scala tympani. *A* Before perfusion. *B, C* After perfusion, showing increased spontaneous activity but no loss of threshold sensitivity. *D* Some time after the end of perfusion. The spontaneous rate is still higher than in A. Solid bars indicate the periods of sound stimulation at the characteristic frequency (2.8 kHz) at the intensities indicated. (0 db ≃ 18 db SPL).

Results and discussion. Perfusions with unmodified artificial perilymph had no significant and substained effect upon any of 23 units examined. 70 units from 28 animals were tested by perfusion once or more with 20–30 mM K^+ solutions. To most of these perfusions significant effects were seen within 40 sec.

2 units were unaffected by perfusions lasting 3 min: these probably derived from the extreme base of the cochlea, and so were not in the path of bulk fluid flow through the cochlea. 57 perfusions produced a considerable rise in spontaneous activity in the unit under test. The observed increases were typically of the order of 30–80 i/s and were normally substained for several min. In general, units remained responsive to tone stimuli during the period of increased spontaneous activity, and their threshold sensitivity was unimpaired (figure): in some cases a marginal enhancement of sensitivity was observed. Some exceptions to this pattern of intact threshold sensitivity occurred when the rise in spontaneous activity was so great as to apparently saturate the unit. In these cases threshold sensitivity returned as soon as the spontaneous activity began to drop after stopping the perfusion.

After 34 perfusions spontaneous activity declined considerably, in all but 3 cases to below 1 impulse/sec. Such a decline usually took 20–80 sec and was accompanied by total abolition of the responsiveness of the unit. In 14 of the 34 perfusions this decline followed a rise in spontaneous activity. Recovery from a decline was observed in 12 cases. Each time threshold responsiveness returned first: the maximum firing rate was slower to recover. We believe that such a decline in activity probably reflected a failure in action potential transmission due to excessive depolarization of the nerve endings of these units.

A rise in the extracellular K^+ concentration from the normal 5 mM to 20–30 mM should depolarize the hair cell membrane by 30–40 mV, assuming similarities between hair cells and mammalian CNS neurones. Butler[14] perfused scala tympani with solutions similarly high in K^+ concentration and observed a drop in the organ of Corti negative potential consistent with such a large depolarization. Thus whereas Manley and Robertson's result suggest that a 30 mV drop in EP produces a 60 dB loss in auditory nerve threshold sensitivity, present experiments suggest that a comparable reduction in the hair cell membrane potential is consistent with intact threshold sensitivity.

It is possible that the hair cells were not depolarized to the postulated extent. Whereas most workers have estimated the hair cell potential as 60–80 mV[6,14], similar to that of the CNS neurones, Russell and Sellick[17] recently suggested a much lower value, 25–45 mV. If this lower value is correct it may reflect an unexpectedly low K^+ conductance of the hair cell membrane, in which case our perfusates may not have depolarized the hair cells sufficiently. However, if this is the case it seems unlikely that the receptor potential in the hair cell is carried largely by K^+ ions, which reopens the question of the significance of the high K^+ content of endolymph. Our results accordingly seem to be inconsistent with widely held theories concerning the cochlear receptor mechanism.

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