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PRODUCTION OF ENDOLYMPH IN THE SEMICIRCULAR CANAL OF THE FROG *RANA ESCULENTA*

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SUMMARY

1. The mechanisms of secretion of endolymph were studied *in vitro* in the isolated inner ear of the frog. Prior to *in vitro* experiments, the composition of perilymph was evaluated *in vivo* and compared to that of plasma.

2. Composition of perilymph resembled that of an extracellular fluid, although Na and Cl concentrations were higher and K concentration was lower in perilymph than in plasma water. No difference in Ca and Mg concentrations was observed between these two fluids. Osmolality averaged 227 mosmol/kg H_2O in perilymph and 183 mosmol/kg H_2O in plasma.

3. Endolymph in frog inner ear corresponded in chemical pattern to mammalian endolymph. K and Na concentrations in endolymph collected from the ampulla of the posterior vertical semicircular canal averaged 121·1 mM and 2·5 mM, respectively. Osmolality of endolymph was 237 mosmol/kg H_2O. K and Na concentrations were unaltered when inner ears were incubated for 24 h either at 15 °C or at 4 °C.

4. Addition of ouabain (10^{-4} M) to the perilymph-like bathing solution altered greatly Na and K composition of endolymph after incubation for 3 h at 15 °C. The Na and K concentration gradients between endolymph and the bath were abolished after incubation for 24 h.

5. Ligatures of the posterior vertical semicircular canal were performed at different sites to isolate some parts of the canal, i.e. the ampulla and the non-ampullar duct. K concentration in the ampulla after incubation for 24 h remained as high as 20 times that in the bath. This K gradient was abolished in the presence of ouabain (10^{-4} M). High K concentration could be maintained in the non-ampullar part of the semicircular canal only if the latter communicated with the ampulla.

6. It is concluded that endolymph is actively secreted into the ampulla of the semicircular canal. Na^+–K^+-activated ATPase in the ampullar dark cells may energize the ouabain sensitive ionic transports that are involved in the production of endolymph. Endolymph secreted into the ampulla would spread intraluminally to account for the high K and low Na concentrations of the fluid which fills the non-secretory part of the semicircular canal.

INTRODUCTION

In the inner ear, the membranous labyrinth, a tight heterogeneous epithelium (Jahnke, 1975), separates two fluid compartments of differing constitution. The basolateral cellular surfaces are bathed in perilymph, whose chemical composition resembles that of an ordinary extracellular fluid (Bosher & Warren, 1968). The apical pole of the cells faces endolymph, whose characteristic feature is the association of its high K and low Na content with the presence of a positive potential with respect to blood or perilymph (Békésy, 1952; Smith, Lowry & Wu, 1954; Bosher & Warren, 1968). The magnitude of the endolymphatic resting potential varies widely from one part to another part of the inner ear, and from species to species (Schmidt & Fernandez, 1962; Sellick & Johnstone, 1975). Because the administration of ouabain abolishes both this potential and the chemical (Na and K) gradients between endolymph and perilymph (Konishi & Mendelsohn, 1970; Kuijpers & Bonting, 1970; Simon, Hilding & Kashgarian, 1973; Sellick & Johnstone, 1974; Bosher, 1980), some parts of the inner ear epithelium that contain a high Na^+– K^+-activated ATPase activity are thought to produce the endolymphatic resting potential and to maintain the composition of endolymph (Sellick & Johstone, 1975).

High activities of Na^+–K^+-activated ATPase have been identified in the marginal cells of the stria vascularis in the cochlea, in the dark cells in the mammalian vestibular apparatus, and in the dark cells in the inner ear of lower vertebrates (Nakai & Hilding, 1968; Kuijpers & Bonting, 1969; Burnham & Stirling, 1984). The Na^+–K^+-activated ATPase has been located at the basolateral surfaces of these cells (Simon *et al.* 1973; Kerr, Ross & Ernst, 1982; Burnham & Stirling, 1984), and might energize an electrogenic pumping of K^+ from perilymph into endolymph (Sellick & Johnstone, 1975).

Unlike the cochlea where the stria vascularis runs uninterruptedly from its base to its apex, in the mammalian vestibular apparatus and the lower vertebrate inner ear the dark cells are concentrated on a relatively small area around the sensory hair cells (Kimura, 1969; Burnham & Stirling, 1984). This observation supports the hypothesis that endolymph would be secreted only in some restricted areas in the mammalian vestibular apparatus or lower vertebrate inner ear. The role of the other parts of the inner ear epithelium in the formation of endolymph remains questionable.

The object of the experiments described here is to further study the production of endolymph. The frog posterior vertical semicircular canal, which is made of an ampulla and a non-ampullar part, i.e. the semicircular duct, was chosen (Fig. 1*A*). This preparation allowed a comparative evaluation of the role, in the endolymphatic secretion, of the ampulla, that contains both dark cells and sensory hair cells, from the role of the semicircular duct, from which these structures are absent.

METHODS

Adult green frogs (*Rana esculenta*), weighing 25–45 g, unselected with regard to sex, were used. Animals were purchased from Elevage d'Ardenay (France), and kept at 15 °C for a week, at least, before an experiment.

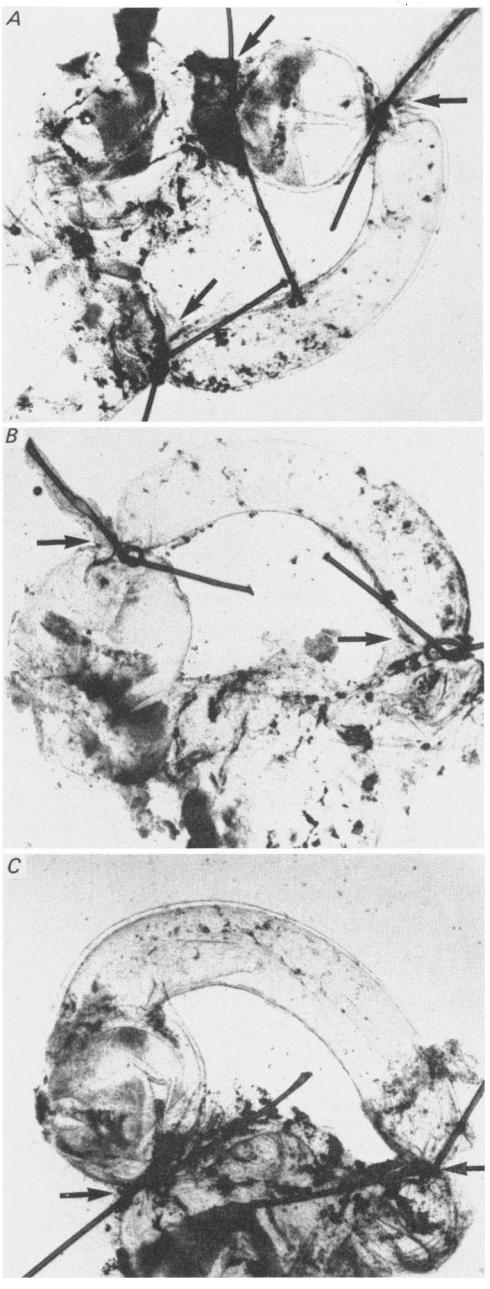

Fig. 1. Ligatured semicircular canals as described in Methods (magnification ×50). *A*, three ligatured semicircular canal to isolate the ampulla and the duct. *B*, two ligatured canal to isolate the duct. *C*, two ligatured canal to isolate the whole canal. Arrows indicate the localizations of the ligatures.

In vivo experiments

Collection of perilymph was performed *in vivo* in frogs anaesthetized with percutaneous urethan (Sigma, St. Louis, U.S.A.) and immobilized with subcutaneous tubocurarine (Abbott, St. Rémy sur Avre, France). The head was fixed with the frog in a supine position. The inner ear was exposed by retracting the opened mouth and removing the mucous membrane and cartilage overlying each labyrinth. The inner ear was bathed with water-saturated mineral oil (Prolabo, Paris, France). A sample of about 2·5 μl of perilymph was taken from each inner ear under stereomicroscopic observation using a bevelled glass micropipette with a 10 μm tip (outside diameter) siliconized and filled with water-saturated mineral oil. The micropipette was fixed to a de Fonbrune micromanipulator (CIT, Alcatel, Paris, France). The specimen was immediately transferred under oil for the determinations of electrolytic concentrations and osmotic pressure (see below). After the last collection of perilymph, a blood sample of about 0·5 ml was obtained from each animal by intracardiac puncture using a heparinized syringe.

In vitro experiments

Collection of endolymph was performed *in vitro* on isolated heads. Under anaesthesia, frogs were decapitated and each isolated half-head was pinned, ventral side up, onto a dissecting tray filled with a solution of artificial frog perilymph (NaCl, 96 mM; KCl, 2·5 mM; $NaHCO_3$, 20 mM; NaH_2PO_4, 0·17 mM; $CaCl_2$, 1·8 mM; $MgCl_2$, 1·2 mM; glucose, 2·8 mM), bubbled with 95 % O_2 and 5 % CO_2 and with a pH adjusted to 7·4. The membranous labyrinth was exposed by removing successively the mucous membrane and cartilage overlying the inner ear, and the membrane lining the perilymph spaces.

In a separate set of experiments, the posterior vertical semicircular canal was ligatured (nylon monofil 10·0, Robert et Carrière-Lederlé, Paris, France) according to two different procedures. In one group (three ligature group), three ligatures were performed in order to isolate the ampulla and the semicircular duct from each other on the one hand, and each of them from the utricule on the other hand (Fig. 1 *A*). In another group (two ligature group), two ligatures were performed either to isolate the duct both from the ampulla, at one extremity, and from the utricle, at the other extremity (Fig. 1 *B*), or to isolate the whole semicircular canal, i.e. the ampulla and the duct, from the utricule (Fig. 1 *C*).

Endolymph was sampled from the ampulla of the posterior vertical semicircular canal, its duct, and when necessary the utricule. No more than one sample of endolymph from each site was collected. Sampling of endolymph was carried out in a manner similar to the *in vivo* sampling technique. Approximately 20–50 nl of endolymph was aspirated using a bevelled glass micropipette with a 8 μm tip (outside diameter) filled with oil and fixed to a de Fonbune micromanipulator (CIT, Alcatel, Paris, France). Only a gentle suction was applied with a 10 ml hand-held syringe in order to prevent a contamination of the specimen with the perilymph-like bathing solution. The specimen was transferred under oil, and the determinations of the solute concentrations and of the osmotic pressure were performed immediately.

Sampling of endolymph from intact and ligatured inner ear preparations was carried out either immediately or after incubation, up to 24 h, into the artifical perilymphatic solution at 15 °C. The effects of temperature and ouabain upon the electrolytic constitution of endolymph were studied by incubating at 4 °C or by adding the drug (10^{-4} M) to the bathing solution, respectively.

Determinations of electrolytic concentrations

Na and K concentrations in endolymph and perilymph were determined by ultramicro-emission spectrometry (Morel & Lucarain, 1967) in aliquots of 1 nl withdrawn with a siliconized volumetric glass micropipette filled with water-saturated mineral oil. Cl concentration in inner ear fluids was determined by micro-electrometric titration (Ramsay, Brown & Croghan, 1955) in aliquots of 1 nl (see above). Concentrations of Ca and Mg in perilymph were determined in aliquots of 2 μl by atomic absorption spectrophotometry (SP9 Pye Unicam, Cambridge, U.K.).

In plasma, Na and K concentrations were determined by flame photometry (Eppendorf, Hamburg, F.R.G.), Cl concentration by electrometric titration (CMTIO, Radiometer, Copenhagen, Denmark), and Ca and Mg concentrations as described above. Arterial blood pH and P_{a,CO_2} were immediately measured at 20 °C (BMS 3Mk2, Radiometer, Copenhagen, Denmark). For that

purpose, blood was collected anaerobically in heparinized capillary tubes. HCO_3 concentration was calculated using the Henderson–Hasselbalch equation with a pK′ of 6·17 and a CO_2 solubility factor of 0·046. The bathing solution was assayed after each experiment for Na, K, Cl concentrations and pH (PHM83 Autolocal pHmeter, Radiometer, Copenhagen, Denmark).

Determination of osmotic pressure

The osmolality of endolymph, perilymph, and plasma was determined immediately by the freezing point depression method in nanolitre samples (Ramsay & Brown, 1955) as previously described (Sterkers, Ferrary & Amiel, 1984*a*).

Statistical analysis

All the results are expressed as mean ±S.E. of mean. Comparisons of multiple groups were made by analysis of variance and subsequent modified *t* test (Snedecor & Cochran, 1967). When appropriate, the unpaired Student's *t* test was used. Differences were considered as significant at a P value $< 0·05$.

TABLE 1. Elemental composition (mM) and osmolality (mosmol/kg H_2O) of plasma and perilymph

	Na	K	Ca	Mg	Cl	Osmolality
Plasma	85·7 ± 1·64 (15)	2·7 ± 0·37 (12)	2·2 ± 0·16 (16)	0·72 ± 0·068 (7)	64·7 ± 1·53 (12)	183 ± 3·6 (10)
Perilymph	119·5 ± 0·79 (27)	1·7 ± 0·073 (27)	2·3 ± 0·23 (15)	0·81 ± 0·023 (7)	102·1 ± 2·55 (10)	227 ± 2·4 (17)
Significance unpaired *t* test	$P < 0·001$	$P < 0·001$	N.s.*	N.s.*	$P < 0·001$	$P < 0·001$

* Not significant.

RESULTS

Plasma and perilymphatic compositions

The ionic concentrations and osmolality of plasma and perilymph are given in Table 1. Blood pH was 7·44 ± 0·012 ($n = 6$) and P_{CO_2} was 3·77 ± 0·14 kPa ($n = 6$). Bicarbonate concentration calculated from blood pH and P_{CO_2} determinations was 25·6 ± 0·65 mM ($n = 6$), a value that fairly filled the anionic gap.

The general feature of the ionic composition of perilymph resembles that of any extracellular fluid, although the perilymphatic composition differed from that of plasma. Na and Cl concentrations were higher and K concentration was lower in perilymph than in plasma. The concentrations of Na salts in perilymph and plasma were in accord with the osmolalities determined by the freezing-point depression method in the two fluids. Similarly, a 30 mosmol difference can be calculated between perilymph and plasma from the Na and K concentrations in frog fluids as reported previously by Schulze (1969, as cited by Rauch & Rauch, 1974). In mammals, no such difference in osmotic pressure was found between perilymph and plasma (Sterkers *et al.* 1984*a*). In frogs, however, there is an extension of the perilymphatic space through an opening to the cranial cavity, limited by the perilymphatic sac whose membranous walls separate perilymph from the fluid of the cranial cavity (Wever, 1973). This perilymphatic sac may be implicated in the secretion of perilymph, a proposal that needs further documentation.

Endolymphatic composition

As shown in Table 2, low Na and high K concentrations were found in frog endolymph collected from the ampulla of the posterior vertical semicircular canal (ampulla), the non-ampullar part of this canal (duct), and the utricule. In the ampulla, the mean value of Na concentration was very low. Most of the Na values

TABLE 2. Elemental composition of endolymph (mM). K and Cl concentrations were not significantly different between the ampulla, the duct and the utricule (K), and between the ampulla and the duct (Cl). Na concentration values were not tested for significance as indicated in Results section

	Na	K	Cl
Semicircular canal			
Ampulla	2·5 ± 0·18 (23)	121·1 ± 1·90 (23)	93·7 ± 2·03 (13)
Duct	5·4 ± 0·90 (4)	120·7 ± 7·37 (4)	94·3 ± 1·66 (4)
Utricule	7·2 ± 0·91 (5)	121·7 ± 2·69 (5)	—

ranged between 1·5 and 2·5 mM, as shown on the histogram of Fig. 2, and consequently all the samples whose Na content was higher than 5 mM were discarded as they were presumed to be contaminated with the bathing solution, i.e. perilymph. For the duct and the utricule, because the number of samples was small, the samples whose Na content were between 5 and 10 mM were included. If the true value of the Na concentration in the endolymph would have been say, 2 mM, a value as high as 10 mM in the sample represents only 7·0% contamination. When the inner ears were incubated for 24 h in artificial perilymph, Na and K concentrations remained fairly constant in the ampulla (Fig. 3) as well as in the duct (at 24 h: 24·0 ± 11·05 mM for Na and 115·7 ± 16·24 mM for K, $n = 4$). Cl concentration in ampullar endolymph increased, however, up to 107·6 ± 5·42 mM ($n = 5$) at 24 h, a value significantly higher than that given in Table 2 ($P < 0·05$).

The value of the osmolality of endolymph in the ampulla averaged 237 ± 4·2 mosmol/kg H_2O ($n = 16$). This is in good agreement with the ionic determinations as the ratio of the osmolality, measured by the freezing-point depression method, to the osmolality calculated as twice the monovalent cationic concentration in endolymph samples (mainly K) was 0·95. This suggests, as already demonstrated for endolymph in mammals (Sterkers *et al.* 1984*a*), that the effective osmotic pressure in endolymph can be accounted for by K and its accompanying anions (mainly Cl). The participation in osmolality of other compounds than KCl in amphibian endolymph would be very low as in mammals (Rauch & Rauch, 1974; Sterkers *et al.* 1984*a*).

Effects of temperature and ouabain upon endolymphatic composition

When the inner ear preparations were incubated at 4 °C for 24 h, no change in Na (6·4 ± 1·04 mM, $n = 3$) and K (114·3 ± 1·61 mM, $n = 3$) concentrations was observed in endolymph collected from the ampulla. Similarly, the composition (Na and K) of endolymph remained fairly unaltered when the inner ears of frogs were incubated for

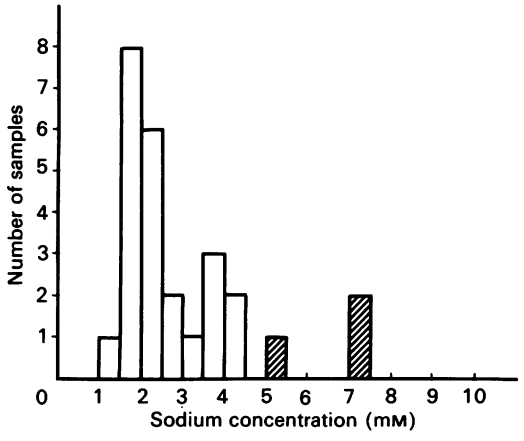

Fig. 2. Histogram of Na concentration values in samples obtained from the ampulla of the semicircular canal. The hatched columns indicate the samples where Na concentration was above 5 mM that were discarded.

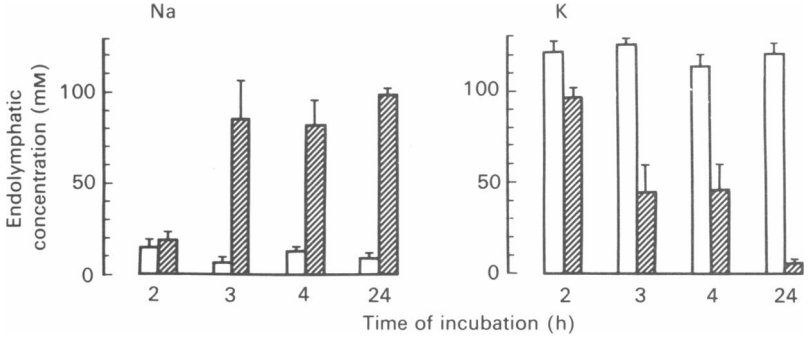

Fig. 3. Na and K concentrations in endolymph obtained from the ampulla of inner ears were incubated from 2 h up to 24 h in the absence (open columns; numbers of samples were 3, 3, 4, 11 at 2, 3, 4, 24 h, respectively) or in the presence of ouabain (hatched columns; numbers of samples were 3, 4, 6, 14 at 2, 3, 4, 24 h, respectively). The averages of Na concentration in absence of ouabain at 2 and 4 h are above 10 mM because some samples in which Na concentration was higher than 10 mM were included.

6 days at 1 °C (Simon *et al.* 1973). These results suggest that the Na–K pump in the inner ear of frogs remains active at low temperatures as it has been demonstrated in brain of frogs (Bowler & Duncan, 1968).

The addition of ouabain (10^{-4} M) to the bathing solution at 15 °C produced progressive changes in Na and K concentrations in endolymph (Fig. 3). At 3 h, Na and K concentration gradients were nearly dissipated between endolymph and bathing solution. After 1 day of incubation in the presence of ouabain, these gradients were abolished. At that time, Cl concentration in ampullar endolymph was $79{\cdot}7 \pm 2{\cdot}05$ mM ($n = 6$), a value lower than that in controls ($P < 0{\cdot}001$).

Effects of three ligatures upon endolymphatic composition

In the three ligatured semicircular canals (Fig. 1*A*), the compositon of endolymph (Na and K) varied according to whether it was sampled from the ampulla or from the duct (Fig. 4). Immediately after the ligatures, changes in Na and K concentrations were observed in the duct but not in the ampulla as compared to the intact inner

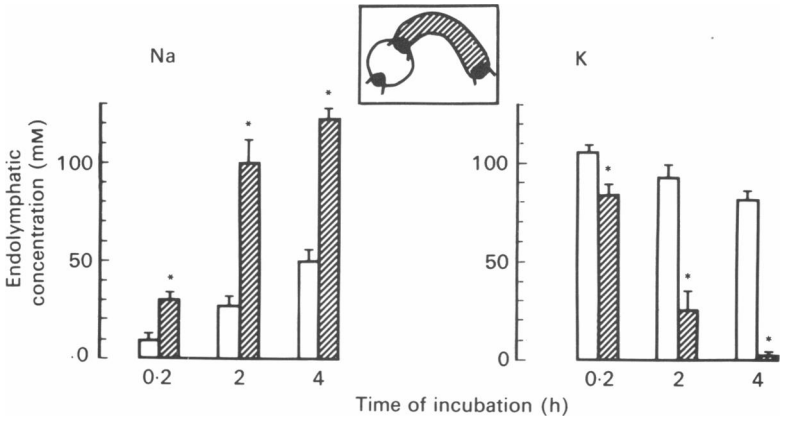

Fig. 4. Na and K concentrations in endolymph as the semicircular canal was ligatured at three different sites as indicated in the upper panel. Open colums refer to endolymph sampled from the ampulla and dashed columns to endolymph obtained from the duct. Sampling of endolymph from the duct was performed at the non-ampullar extremity. Inner ears were incubated for 0·2 h ($n = 5$), 2 h ($n = 3$) and 4 h ($n = 5$). * Signifies significant difference between open and hatched columns ($P < 0{\cdot}02$).

ears. Then, Na and K concentration gradients between the endolymph in the duct and the bathing solution dissipated entirely after a 2 h period of incubation. In contrast, in ampullar endolymph, a slow and progressive increase in Na and decrease of K concentration were observed. When inner ears were incubated for 24 h, K concentration was about 20 times higher in ampullar endolymph than in non-ampullar endolymph and bathing solution ($P < 0{\cdot}001$), and Na concentration was lower in ampullar endolymph than in the two other fluids ($P < 0{\cdot}001$). No difference was found at 24 h in Na and K contents between endolymph in the duct and the bathing solution on the one hand, and between endolymph in the ampulla and the utricule ($86{\cdot}8 \pm 3{\cdot}99$ mM for Na, $47{\cdot}3 \pm 7{\cdot}83$ mM for K, $n = 10$) on the other hand. The similar chemical pattern in ampullar and utricular endolymph suggests that an increase in permeability to Na and K occurred in the tissues that were tied.

The incubation of three ligatured semicircular canals in the presence of ouabain (10^{-4} M) for 24 h abolished the cationic gradients between the bathing solution on the one hand, and, on the other hand, endolymph collected from the ampulla (Fig. 5), from the semicircular duct ($117{\cdot}1 \pm 0{\cdot}76$ mM for Na, $2{\cdot}4 \pm 0{\cdot}070$ mM for K, $n = 6$), and from the utricule ($112{\cdot}3 \pm 3{\cdot}64$ mM for Na, $2{\cdot}5 \pm 0{\cdot}050$ mM for K, $n = 4$).

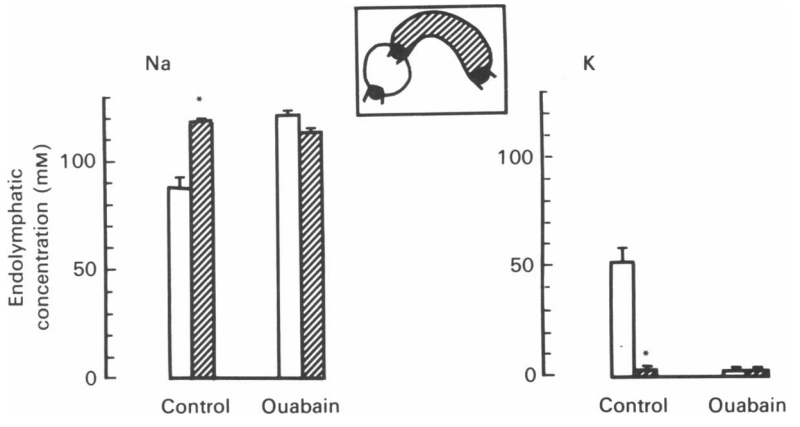

Fig. 5. Na and K concentrations in endolymph of three ligatured semicircular canal, as indicated in the upper panel, after incubation for 24 h in the absence ($n = 18$ for ampullar endolymph and $n = 22$ for non-ampullar endolymph) and in the presence of ouabain ($n = 6$ both for ampullar and non-ampullar endolymph). Open and hatched columns refer to ampullar and non-ampullar endolymph, respectively. * Signifies significant difference between open and hatched columns ($P < 0{\cdot}001$).

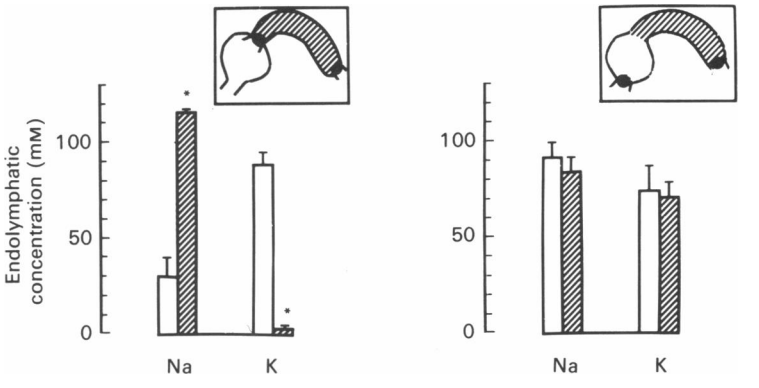

Fig. 6. Na and K concentrations in endolymph of two ligatured semicircular canal as indicated in the upper panels. Open and hatched columns refer to ampullar and non-ampullar endolymph, respectively. Four samples were obtained from the ampulla and six from the duct when the semicircular canal was tied to isolate only the duct. Four samples were taken from the ampulla and three from the duct when the whole semicircular canal was isolated. * Signifies significant difference between open and hatched columns ($P < 0{\cdot}001$).

Effects of two ligatures upon endolymphatic composition

When the semicircular canal was ligatured at two sites, the changes in Na and K contents in endolymph depended upon the location of the ligatures. As shown in Fig. 6, when the non-ampullar part of the semicircular canal was tied at each extremity, Na and K concentrations in endolymph of the semicircular duct equilibrated with those in the bath at 24 h while in ampullar endolymph Na and K concentrations remained low and high, respectively. The values determined in

ampullar endolymph were higher for K ($P < 0.001$) and lower for Na ($P < 0.001$) than those observed when the ampulla was tied at each extremity.

At variance, when the ampulla and the duct together were separated from the utricule by the two ligatures (Fig. 6), the monovalent cationic concentrations remained fairly homogeneous from the ampulla to the non-ampullar part of the semicircular canal. These values were in accord with those found for ampullar endolymph when the ampulla was tied either at each extremity or at only the duct extremity. The observation that the high K and low Na concentrations were maintained in the non-ampullar endolymph, although the duct was tied at one extremity, clearly ruled out that the dissipation of cationic gradients between non-ampullar endolymph and the bath was totally accounted for by changes in Na and K permeabilities that would be produced by the ligatures of the epithelial tissues.

DISCUSSION

The intracellular-like constitution of endolymph in frogs was suggested in the early study of Johnstone, Schmidt & Johnstone (1963) in which the highest K–Na concentration ratio was 30 and, later, that of Henriksson, Gleisner & Johansson (1966) where Na and K concentrations in three samples were about 10 mM and 135 mM, respectively. Wide ranges of Na and K values were then reported from 20 to 70 mM for Na and from 50 to 110 mM for K (Schulze, 1969, as cited by Rossi, Valli & Taglietti, 1973; Simon *et al.* 1973; Rauch & Rauch, 1974; Valli, Zucca & Casella, 1979). In other lower vertebrate species such as the alligator lizard, however, endolymph has a composition similar to endolymph in cats (Peterson, Frishkopf, Lechêne, Oman & Weiss, 1978). In the present study, it is shown that the monovalent cationic constitution of endolymph in frogs is quantitatively similar to that in the vestibular apparatus of mammals (Sellick & Johnstone, 1975). Small differences in ionic constitution (Na, K, Cl) were observed, however, from one part to another one of the inner ear (Sellick & Johnstone, 1975; Sterkers, Saumon, Tran Ba Huy, Ferrary & Amiel, 1984*b*). Heterogeneity of the structures of the inner ear, as regards endolymphatic secretion, may account for these differences. The capacities of the inner ear epithelium to transporting ionic species such as Na and K vary from one place to another place of the inner ear. This could account for the wide range of magnitudes of the resting potential that have been measured in the endolymphatic spaces. In the inner ear of the frog, Schmidt & Fernandez (1962) reported a transmembrane potential of +3 mV when the micro-electrode was introduced into the endolymphatic space. The ionic determinations in the present experiments show that Na and K but not Cl are out of electrochemical equilibrium in endolymph, as already reported by Simon *et al.* (1973). Consequently, K must be transported actively into, and Na out of, endolymph, while Cl distribution may be passive.

This work provides evidence for a secretion, sensitive to ouabain, of endolymph in the ampulla of the posterior vertical semicircular canal. The secretory processes involve presumably an inward K^+ flux into endolymph coupled to an outward Na^+ flux which could be accounted for by the Na^+–K^+-activated ATPase in the dark cells. Actually, the enzyme concentration and the membrane pump density in the dark cells of the frog are high as compared to those in other tissues of the inner ear (Burnham

& Stirling, 1984). These enzymatic parameters in the frog dark cells are of the same order of magnitude as those evaluated in other transporting epithelia such as the mammalian kidney (Katz, Doucet & Morel, 1979; Burnham & Stirling, 1984). The fact that the Cl concentration in endolymph decreased when the Na^+–K^+-activated ATPase was inhibited by ouabain suggests either a coupling of an inward Cl^- flux to the K^+ influx into ampullar endolymph or an increased production of organic acids.

Endolymph does not appear to be secreted actively in the semicircular duct because the alterations of K and Na concentrations in endolymph of the non-ampullar part of the semicircular canal were rapid when the duct did not communicate with the ampulla. The fluid secreted in the ampulla would thus spread towards the semicircular duct, which would account for the chemical homogeneity of endolymph that we observed whether endolymph was sampled from the ampulla or from the duct. Such an intraluminal movement of ions may explain the apparent discrepancy between the rapid binding of [^{3}H]ouabain to the dark cells (less than 10–20 min), as reported by Burnham & Stirling (1984), and the long period required for ouabain to dissipate entirely the chemical gradients between endolymph and perilymph (more than 3 h), as observed in the present work.

The conclusion is that, in the inner ear of the frog, endolymph is secreted in some localized sites, i.e. the ampulla in the semicircular canal, by active ionic transport dependent upon Na^+–K^+-activated ATPase. In other parts of the inner ear, where the dark cells are absent, i.e. the duct in the semicircular canal, the ionic composition of endolymph is not regulated by means of transepithelial active processes but rather dependent on intraluminal spread of the fluid formed in the secretory areas of the inner ear.

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REFERENCES

BÉKÉSY, G. VON (1952). D-C resting potentials inside the cochlear partition. *Journal of the Acoustical Society of America* **24**, 72–76.

BOSHER, S. K. (1980). The effect of inhibition of the strial Na–K-activated ATPase by perilymphatic ouabain in the guinea pig. *Acta oto-laryngologica* **90**, 219–229.

BOSHER, S. K. & WARREN, R. L. (1968). Observations on the electrochemistry of the cochlear endolymph of the rat: a quantitative study of its electrical potential and ionic composition as determined by means of flame photometry. *Proceedings of the Royal Society* B **171**, 227–247.

BOWLER, K. & DUNCAN, C. J. (1968). The temperature characteristics of the ATPases from a frog brain microsomal preparation. *Comparative Biochemistry and Physiology* **24**, 223–227.

BURNHAM, J. A. & STIRLING, C. E. (1984). Quantitative localization of Na–K pump site in frog inner ear dark cells. *Hearing Research* **13**, 261–268.

HENRIKSSON, N. G., GLEISNER, L. & JOHNSTONE, G. (1966). Experimental pressure variations in the membranous labyrinth of the frog. *Acta oto-laryngologica* **61**, 281–291.

JAHNKE, K. (1975). The fine structure of freeze-fractured intercellular junctions in the guinea-pig inner ear. *Acta oto-laryngologica* **336**, suppl., 1–40.

JOHNSTONE, C. G., SCHMIDT, R. S. & JOHNSTONE, B. M. (1963). Sodium and potassium in vertebrate cochlear endolymph as determined by flame microspectrophotometry. *Comparative Biochemistry and Physiology* **9**, 335–341.

KATZ, A. I., DOUCET, A. & MOREL, F. (1979). Na–K-ATPase activity along the rabbit, rat and mouse nephron. *American Journal of Physiology* **237**, F114–120.

KERR, T. P., ROSS, M. D. & ERNST, S. A. (1982). Cellular localization of Na^+, K^+-ATPase in the mammalian cochlear duct: significance for cochlear fluid balance. *American Journal of Otolaryngology* **3**, 332–338.

KIMURA, R. S. (1969). Distribution, structure, and function of dark cells in the vestibular labyrinth. *Annals of Otology, Rhinology and Laryngology* **78**, 542–561.

KONISHI, T. & MENDELSOHN, H. (1970). Effect of ouabain on cochlear potentials and endolymph composition in guinea pigs. *Acta oto-laryngologica* **69**, 192–199.

KUIJPERS, W. & BONTING, S. L. (1969). Studies on (Na^+–K^+) activated ATPase. XXIV. Localization and properties of ATPase in inner ear of the guinea pig. *Biochimica et biophysica acta* **173**, 477–485.

KUIJPERS, W. & BONTING, S. L. (1970). The cochlear potentials. I. The effect of ouabain on the cochlear potentials of the guinea pig. *Pflügers Archiv* **320**, 348–358.

MOREL, F. & LUCARAIN, C. (1967). Un spectrophotomètre à flamme pour le dosage du sodium et du potassium dans des échantillons biologiques de l'ordre du nanolitre. *Journal de physiologie* **59**, 460–461.

NAKAI, Y. & HILDING, D. (1968). Vestibular endolymph-producing epithelium. Electron microscopic study of the development and histochemistry of the dark cells of the crista ampullaris. *Acta oto-laryngologica* **66**, 120–128.

PETERSON, S. K., FRISHKOPF, S. L., LECHÊNE, C., OMAN, C. M. & WEISS, T. F. (1978). Element composition of inner ear lymphs in cats, lizards, and skates determined by electron probe microanalysis of liquid samples. *Journal of Comparative Physiology* **126**, 1–14.

RAMSAY, J. A. & BROWN, R. H. J. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *Journal of Scientific Instruments* **32**, 372–375.

RAMSAY, J. A., BROWN, R. H. J. & CROGHAN, P. C. (1955). Electrometric titration of chloride in small volumes. *Journal of Experimental Biology* **32**, 822–829.

RAUCH, S. & RAUCH, J. (1974). Physico-chemical properties of the inner ear especially ionic transport. In *Handbook of Sensory Physiology*, vol. V/I: *Auditory System*, ed. KEIDEL, W. D. & NEFF, W. D., pp. 647–682. Berlin, Heidelberg, New York: Springer.

ROSSI, M. L., VALLI, P. & TAGLIETTI, V. (1973). Concentrazione in potassio dell'endolinfa della rana. *Bollettino della Società italiana di biologia sperimentale* **49**, 660–665.

SCHMIDT, R. S. & FERNANDEZ, C. (1962). Labyrinthine DC potentials in representative vertebrates. *Journal of Cellular and Comparative Physiology* **59**, 311–322.

SELLICK, P. M. & JOHNSTONE, B. M. (1974). Differential effects of ouabain and ethacrynic acid on the labyrinthine potentials. *Pflügers Archiv* **352**, 339–350.

SELLICK, P. M. & JOHNSTONE, B. M. (1975). Production and role of inner ear fluid. *Progress in Neurobiology* **5**, 337–362.

SIMON, E. J., HILDING, D. A. & KASHGARIAN, M. (1973). Micropuncture study of the mechanism of endolymph production in the frog. *American Journal of Physiology* **225**, 114–118.

SMITH, C. A., LOWRY, O. H. & WU, M. (1954). The electrolytes of the labyrinthine fluids. *Laryngoscope* **64**, 141–153.

SNEDECOR, G. W. & COCHRAN, W. G. (1967). *Statistical Methods*, 6th edn. Ames, IA: Iowa State Univ. Press.

STERKERS, O., FERRARY, E. & AMIEL, C. (1984*a*). Inter- and intracompartmental osmotic gradients within the rat cochlea. *American Journal of Physiology* **247**, F602–606.

STERKERS, O., SAUMON, G., TRAN BA HUY, P., FERRARY, E. & AMIEL, C. (1984*b*). Electrochemical heterogeneity of the cochlear endolymph: effect of acetazolamide. *American Journal of Physiology* **246**, F47–53.

VALLI, P., ZUCCA, G. & CASELLA, C. (1979). Ionic composition of the endolymph and sensory transduction in labyrinthine organs. *Acta oto-laryngologica* **87**, 466–471.

WEVER, E. G. (1973). The ear and hearing in the frog, *Rana pipiens*. *Journal of Morphology* **141**, 461–478.