SODIUM AND POTASSIUM IN THE ENDOLYMPH AND PERILYMPH OF THE STATOCYST AND IN THE EYE OF *OCTOPUS*

By J. E. AMOORE,* KATHLEEN RODGERS and J. Z. YOUNG, F.R.S.

Department of Biochemistry, University of Oxford, Medical Research Unit for Cell Metabolism, University of Oxford, and Department of Anatomy, University College, London

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INTRODUCTION

In the majority of invertebrates the statocyst consists of a single sack; only in the octopod cephalopods is there one sack inside another, so that one can distinguish endolymph and perilymph as in the vertebrate labyrinth. Even in the decapod cephalopods (squids and cuttlefishes) no inner sack is present. The significance of the two sacks has yet to be determined, but it is clearly of interest to discover the composition of the fluids in the two compartments, and in particular whether the endolymph contains high potassium and low sodium concentrations, as in mammals (Smith, Lowry & Wu, 1954; Citron, Exley & Hallpike, 1956; Rauch & Köstlin, 1958). At the same time we have taken for comparison a few samples of fluid from the anterior and posterior chambers of the eye, and from the blood.

The endolymphatic and perilymphatic spaces both contain colourless fluids. The perilymph is crossed by numerous fibrous strands carrying blood vessels to the inner sack. These give it a remarkable resemblance under the microscope to the perilymphatic space of vertebrates. In fixed sections the liquid differs in appearance from blood and contains no cells, but often a coarse precipitated material. The endolymph differs in appearance from both blood and perilymph. In sections it also shows a precipitate but no cells (Young, 1959).

There is no obvious special apparatus for producing the perilymph. The outer wall of the statocyst is formed of a vascular type of cartilage, not lined by an epithelium. Into the endolymphatic space there opens a canal, representing the remains of the embryonic communication with the ectoderm and known as Kölliker's duct. This is ciliated and might serve for either production or absorption of endolymph. There are muscle fibres in the wall of the sack and in life the contents seem to be under a small pressure.

Robertson (1949, 1953) measured the sodium and potassium concentrations in the blood and eye fluids of several cephalopods, but so far as we are aware, these analyses have not previously been performed on the body fluids of *Octopus vulgaris*, nor on the statocyst fluids of any cephalopod.

* Present address: Department of Botany, University of Edinburgh.

METHODS

Collection of samples

A single statocyst contains volumes of 30 μl. of perilymph and 15 μl. of endolymph, or even more in larger specimens. However, in order to obtain uncontaminated specimens, much smaller samples were used, in the range of 0·1–3·5 μl., making 'ultra-micro' methods of analysis necessary. Fluids were collected in capillary tubes of borosilicate glass. These were cleaned in hot chromic acid fluid for 1 hr. and then washed with running tap water and distilled water. Distilled water was drawn through each tube, followed by acetone. When dry the tubes were drawn to tip diameters of 10–50μ and stored in container tubes.

Octopuses were killed by decapitation, without previous anaesthesia. Each statocyst in turn was then immediately exposed by cutting away first skin and then cartilage with a sharp blade under a low-power microscope. When the cartilage had been cut so as almost to open the perilymph its surface was wiped with filter paper and the blade itself was dried. A further cut then opened the perilymph and a little fluid escaped.

A pipette, previously prepared, was then advanced with a simple micro-manipulator into the cavity and fluid was sucked into it with a suction pump, the flow being regulated with a valve under manual control. Considerable suction was necessary to collect the fluid and pipettes with finer tips ($< 10\mu$) frequently became blocked, presumably by the strands in the perilymph. Tips of 50μ were found to be more convenient.

After a small sample of perilymph had been collected the rest of it was removed by suction with a coarse pipette. In this way a dry cavity was obtained. A fresh pipette was then moved towards the turgid endolymphatic sack and with a sharp advance was made to enter it. By immediate suction a small sample could then be obtained directly from the sack itself. However, the fluid rapidly escaped from the sack and collected in the emptied perilymphatic space. In most experiments some of this fluid was also collected (as a separate sample) and was found not to differ from that obtained immediately after puncture. It is therefore thought that there was no serious contamination of the endolymph samples by the remains of perilymph, either on the outside of the sack or in the cavity.

The samples from the first statocyst opened were obtained within 15 min. of death. The second statocyst was then treated in the same way.

Samples of the fluids from the anterior and posterior chambers of the eye were obtained by similar methods, after opening each with scissors and inserting pipettes. These fluids are also colourless and transparent in life. The anterior fluid is in virtual communication with the seawater through the overlapping pseudo-corneal folds (Boycott & Young, 1956).

Blood was obtained from the ventricle or the orbital sinus.

Analytical methods

The methods of handling each sample of fluid and measuring its volume were based on those described by Exley (1956), with the following modifications. The sample (0·1–3·5 μl.) was discharged from the collecting capillary into the centre well of a 4·5 cm. diameter Conway unit. The unit rested on the base of a 500 ml. beaker, which was inverted in a 1500 ml. beaker to prevent draughts. The outer well of the unit, and the space between the beakers, were filled with wet tissue paper. Between samples, the outer beaker was covered with a Petri dish lid to preserve a moist atmosphere. The inside of the smaller beaker was blackened to make the sample more visible. The Conway unit was not silicone-treated; the centre well was wiped clean with moist and then with dry tissue paper between each sample. With minimum delay the sample was drawn into a capillary pipette, the surface (including the tip) was wiped with tissue paper, and the position of the meniscus was marked. The sample was quantitatively transferred into 1·0 ml. of 0·0025 M-$Li_2SO_4.H_2O$ in a 6 ml. stoppered polythene specimen tube (A. Gallenkamp and Co., London, E.C. 2); the capillary was rinsed three times by drawing the lithium sulphate solution up above the mark and blowing it out again. After being washed and dried, the pipette was refilled to the mark with a standard solution containing 0·518 M-NaCl and 0·016 M-KCl (i.e. approximating to seawater with respect to sodium and potassium concentrations). This solution was quantitatively transferred into a second 1·0 ml. of 0·0025 M-Li_2SO_4. Both polythene tubes were stoppered and shaken. The solutions were aspirated in a lithium internal-standard flame photometer (Amoore, Parsons & Werkheiser, 1958). By fitting a no. 16 hypodermic needle to the atomizer the aspiration rate was decreased to 0·5–0·6 ml./min., permitting two readings for sodium and two for potassium on each 1·0 ml. sample.

Calibration curves were prepared from the flame photometer readings obtained when solutions containing known concentrations of sodium and potassium in 0·0025 M-Li_2SO_4 were aspirated. The volume of the octopus fluid sample was calculated from the quantity of sodium found after refilling the pipette with standard saline of known molarity (0·518 M-NaCl) as described above. The concentrations of sodium and potassium in the sample were calculated from the flame photometer readings and the determined volume.

In order to determine that there was no evaporation of the sample during analysis, ten samples of standard saline (0·518 M-NaCl) ranging in volume from 0·3 to 4 μl. were delivered from an ultra-microburette into the Conway unit, drawn up into capillary pipettes and the sodium concentration determined as described above. The mean concentration of sodium found was 519 m.-equiv./l.; S.D. $\pm$ 16. Within these volume limits the accuracy was independent of the sample size. The coefficient of variation for a single flame photometric determination of sodium or potassium was 1·6% for aspirated solutions containing between 0·04 and 2·0 m.-equiv./l., and 5% for solutions containing between 0·012 and 0·04 m.-equiv./l.

RESULTS

The concentrations of sodium and potassium (mean ± S.D.) found in the body fluids of *Octopus vulgaris* are summarized in Table 1. The perilymph and endolymph samples were obtained from the statocysts of seven octopuses. In all cases but one, only a single sample from the appropriate part of each statocyst was analysed; in that one case the figure used in the statistical analysis was the mean of the determinations on the two samples from the same part of the same statocyst. The eye fluids came from three octopuses, each sample being obtained from a different anterior or posterior chamber. The results of the blood analyses are those obtained from a single sample from the ventricle of one octopus, and the mean of three results on samples from the orbital sinus of a second octopus.

Table 1. *Concentrations of sodium and potassium in statocyst, eye and blood of* Octopus vulgaris

Fluid analysed	No. of samples	Volume of samples		Sodium concn. mean ± S.D. (m.-equiv./l.)	Potassium concn. mean ± S.D. (m.-equiv./l.)	Ratio Na^+/K^+
		Range (μl.)	Mean (μl.)			
Perilymph	10	0·12–3·44	1·17	555 ± 32	17 ± 6	33
Endolymph	12	0·10–1·23	0·38	601 ± 46*	20 ± 3	30
Anterior chamber	3	0·31–1·44	0·85	614 ± 68	19 ± 2	32
Posterior chamber	3	0·58–1·69	1·04	603 ± 12	26 ± 11	23
Blood	2	0·23–1·52	0·67	525 ± 3	30 ± 2	17
Seawater†	—	—	—	559	11·9	47·0

* One low value of 182 m.-equiv. Na^+/l. has been omitted from the statistical analysis.
† These values are based on an assumed chlorinity of 22·5 ‰ for the seawater at Naples (Robertson, 1953).

All the *Octopus vulgaris* fluids analysed had approximately the same concentration of sodium as the Naples seawater, but definitely higher concentrations of potassium. The blood in particular contained over twice the potassium concentration of the sea. Robertson (1949, 1953) showed that the bloods of *Eledone*, *Loligo* and *Sepia* contain about twice as high a concentration of potassium as does seawater.

There was no significant difference between the sodium content of blood and perilymph, but endolymph contained significantly more sodium than perilymph or blood ($P < 0·05$). There was no significant difference in potassium concentration between perilymph and endolymph, but both fluids had a lower concentration of potassium than the blood ($P < 0·05$).

The fluid from the posterior chamber of the eye had a potassium concentration near to that of blood, but this was not significantly higher than that in the anterior chamber of the eye. The fluids of the eye both had a similar sodium concentration to that of endolymph.

DISCUSSION

The sodium and potassium concentrations in octopus endolymph are markedly different from those in mammalian utricular and cochlear endolymph. Thus guinea-pig and human endolymph contain about thirty times the concentration of potassium found in the serum, and a correspondingly lower concentration of sodium (1/9 to 1/5 of that in serum), so that the usual high ratio of Na^+/K^+ found in most extracellular body fluids is substantially reversed in mammalian endolymph (Smith *et al.* 1954; Citron *et al.* 1956; Rauch & Köstlin, 1958).

It is not certain at which stage of vertebrate evolution this high concentration of potassium appeared in the endolymph. In elasmobranch fishes both endolymph and perilymph are said to have a high Na^+/K^+ ratio (Kaieda, 1930). The stria vascularis is probably closely involved in either the secretion or regulation of mammalian endolymph (Citron *et al.* 1956; Naftalin & Harrison, 1958). The stria first appears in a rudimentary form in some amphibians (Guggenheim, 1948). It would be interesting to know whether the production of a potassium-rich endolymph coincides phylogenetically with the emergence of the stria vascularis.

It is at present uncertain what connexion (if any) these ionic ratios have with the standing potentials between endolymph and perilymph, and the sensitivity of the hair cells (Davis, 1957). Whatever the significance of the presence of two fluids in *Octopus* may be, it is not connected with large differences in sodium and potassium concentrations. It therefore remains obscure why there should be two fluids in octopods whereas the related decapods, like other invertebrates, have only one sack.

The observations on the fluids of the eye confirm those of Robertson in showing that neither of these fluids is identical with seawater. In *Octopus*, as in the *Sepia* that he examined, there is a potential channel of communication between the anterior chamber and the sea. The pseudocorneal folds of *Octopus* divide the anterior chamber into inner and outer parts (Boycott & Young, 1956). The inner of these (from which the fluid here analysed was obtained) communicates with the outer (and thus with the sea) by a narrow channel. Nevertheless it has a potassium concentration higher than that of seawater, but lower than blood. At the back of this chamber is a specialized epithelium, the anterior chamber organ, connected with a series of strands, the subpedunculate tissue, which run into the optic lobes (Boycott & Young, 1956). This system may well play a part in regulating the composition of the fluid in the anterior chamber.

SUMMARY

1. The concentrations of sodium and potassium were measured in 0·1–3·5 μl. samples of the following body-fluids of *Octopus vulgaris*: perilymph and endolymph from the statocyst, anterior and posterior chamber fluid from the eye and blood.

2. All these fluids had approximately the same sodium concentration as the seawater, but slightly higher concentrations of potassium. The blood had the highest concentration of potassium, 30 m.-equiv./l., or over twice that in the sea. In the endolymph the potassium concentration was 20 m.-equiv./l.

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