

of the micropipette tip did not result in the formation of any blebs around the needle. Moreover, microiontophoretic injection of neither calcium ions nor of 3',5'-cyclic AMP resulted in any change in cell shape. However, when calcium was added to the petri dish, the ability of such cells to form blebs around the point of needle insertion returned, as did the ability to form lobe-like protuberances upon microiontophoretic injection of calcium ions or of 3',5'-cyclic AMP.

In summary, the data suggest there is a system that can be found anywhere beneath the surface of the *Ilyanassa* egg which, when supplied intracellularly with either calcium ions or with 3',5'-cyclic AMP, will generate a lobe-like protuberance of the cell surface so long as exogenous calcium ions are present. One possible explanation for the results may be that microiontophoretic injection of calcium ions or of 3',5'-cyclic AMP quickly initiates changes in the properties of the plasma membrane that lead to an increase in membrane permeability to exogenous calcium ions. The resultant formation of a lobe-like protuberance therefore, although morphologically very similar to normal lobe formation, appears to be an aberrant mimic of the process, for, as noted above, normal polar lobe formation does not appear to be dependent upon exogenous calcium ions.

This work was supported in part by a grant from the National Institutes of Health (HD 07193).

ION HOMEOSTASIS IN CEREBROSPINAL FLUID IN THE DOGFISH, *Squalus acanthias*, AND LITTLE SKATE, *Raja erinacea*.

P. J. Cornell, J. K. Ashby, and Helen F. Cserr. Physiology and Biophysics, Brown University, Providence, R.I.

In higher vertebrates the concentrations of potassium, magnesium and calcium in cerebrospinal fluid (CSF) and in brain interstitial fluid (ISF) are maintained relatively constant, independent of changes in plasma ion concentration. Potassium homeostasis has also been demonstrated for elasmobranch CSF, but only in dogfish (Cserr and Rall, *Comp. Biochem. Physiol.* 21:431-434, 1967). In view of the paucity of information on CSF homeostasis in elasmobranchs and, further, in view of differences in the ultra-structure of the blood-brain barrier between elasmobranchs and higher vertebrates (Brightman et al. *Progr. Neuropathol.* 1:146-161, 1971) we have investigated CSF ion homeostasis in two elasmobranch species, dogfish and little skate. The comparison of CSF electrolytes between dogfish and little skate is also of interest since the ventricular cavities and choroid plexuses are large in dogfish and extremely reduced in the skate.

CSF was obtained from the cerebellar ventricle in dogfish (0.5 to 1.0 ml) and from the third ventricle in skates (5 to 15 microliters). To test for homeostasis, the plasma concentration of one of the three cations was elevated for four hours. Magnesium or calcium was given as an initial i.m. injection (4.8 ml/kg 333 mM MgCl₂ or 6.0 ml/kg 333 mM CaCl₂) followed by 1/4 of the original dose after two hours. For potassium, the initial injection (5 ml/kg 400 mM KCl) was followed by a continuous i.m. infusion (approx. 5 ml/kg-hr). Analyses of plasma before and at hourly intervals after injection confirmed that plasma ion concentration was elevated throughout the experimental period. Extradural fluid (EDF), which bathes the outer surface of the elasmobranch brain, was also sampled and analyzed for electrolytes. Concentrations were converted to mEq/kg H₂O using values for % water content of plasma, CSF and EDF of 94%, 97% and 96%, respectively (Cserr, Fenstermacher and Rall, in press).

Results are summarized in Table 1. CSF concentrations of magnesium and calcium in control dogfish and skates are in the same range as mammalian values (Katzman & Pappius, In: "Brain Electrolytes and Fluid Metabolism," 1973), and further, increasing plasma concentration by 2- to 4-fold resulted in only a small increase in CSF concentration, demonstrating homeostasis of CSF calcium and magnesium in both

Table 1

HOMEOSTASIS OF Mg⁺⁺, Ca⁺⁺ AND K⁺ IN ELASMOBRANCH CSF

Ion	Experiment	mEq/kg H ₂ O					
		Dogfish			Little skate		
		Plasma	CSF	EDF	Plasma	CSF	EDF
Mg ⁺⁺	Control	1.5±0.2(4)	2.3±0.2(4)	1.2±0.1(4)	2.0±0.3(4)	2.7±0.2(4)	1.9±0.4(4)
"	Plasma [Mg ⁺⁺] [†]	6.3±0.3(5)	2.8±0.1(5)	2.3±0.2(5)	6.2±0.3(5)	3.3±0.3(5)	2.7±0.1(5)
Ca ⁺⁺	Control	4.9±0.4(4)	3.5±0.1(4)	3.8±0.2(4)	4.9±0.3(4)	3.8±0.3(4)	3.9±0.4(4)
"	Plasma [Ca ⁺⁺] [†]	10.4±0.5(4)	4.5±0.2(4)	4.5±0.3(4)	8.9±0.3(6)	4.7±0.2(6)	4.7±0.4(6)
K ⁺	Control	4.1±0.1(28)	3.7±0.2(11)	3.4±0.1(6)	4.0±0.3(4)	3.8±0.1(4)	3.4±0.3(4)
"	Plasma [K ⁺] [†]	9.3±1.6(4)	3.8±0.1(4)	4.6±0.5(3)	8.3±0.6(7)	8.2±0.4(7)	5.7±0.1(5)

Values are means ±SEM with number of observations in parentheses. Plasma ion concentration was elevated for four hours. Dogfish potassium values from Cserr and Rall (*Comp. Biochem. Physiol.* 21:431-434, 1967) and from Cserr *et al.* (*Comp. Biochem. Physiol.* 42A:73-78, 1972).

elasmobranch species. Differences between dogfish and skate are apparent from the potassium data. When plasma concentration was elevated, CSF potassium concentration in dogfish remained close to the control value of 3.7 mEq/kg H₂O whereas there was no homeostasis of potassium in skate CSF. Thus, in little skate there was no significant difference between the potassium concentrations of plasma and CSF over a range in plasma concentration from 3.4 to 11.2 mEq/kg H₂O (paired comparison; P > 0.5). EDF concentrations of magnesium, calcium and potassium rose following elevation of plasma concentration, but not to the same extent as plasma. Presumably, failure to reach equilibrium between EDF and plasma is due to the slow rate of exchange between these two compartments (Zubrod & Rall, *J. Pharmacol. Exp. Ther.* 125: 194-197, 1959).

In higher vertebrates, homeostatic transport mechanisms are located both at the blood-brain barrier and the choroid plexus and the electrolyte concentrations of CSF and of ISF are believed to be similar. The ultrastructure of the elasmobranch blood-brain barrier differs from that of higher vertebrates in that tight junctions are located between perivascular glial processes rather than between adjacent endothelial cells. Assuming that CSF and ISF are also similar in elasmobranchs, the results for calcium and magnesium suggest that the elasmobranch "glial" blood-brain barrier is also the site of homeostatic transport systems.

The explanation for the difference between the little skate and dogfish with respect to potassium homeostasis is not clear. Possibly, potassium homeostasis in elasmobranchs depends on the presence of transport mechanisms in the choroid plexus since this secretory epithelium is much more prominent in dogfish, being 2.4% of brain weight in dogfish versus only 0.1% in little skate.

Supported by NIH Grant #NS 11050 and by NSF Grant #GB 28139. H. Cserr is the recipient of RCDA NS 70500.

SAND DOLLAR EMBRYOS AS MONITORS OF ENVIRONMENTAL POLLUTANTS

Richard B. Crawford and Anthony M. Guarino, Trinity College, Hartford, Connecticut and Laboratory of Toxicology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

A large diversity of biological systems is required for adequate testing of the effects of new chemicals on the biosphere. These should include phases of reproductive cycles of a variety of organisms as well as effects on adult forms. Embryos of the sand dollar *Echinarachnius parma* are particularly well suited for such studies, especially as described by Karnofsky and Simmel (Prog. exp. Tumor Res., 3:254-295, 1963) with respect to various drug actions. This preliminary report describes initial studies to demonstrate the usefulness of sand dollar embryos to the monitoring of xenobiotic compounds and for the determination of harmful levels of these compounds. From these studies it is apparent that information regarding embryo toxicity can be obtained reliably and within a short period of time using echinoderms.

Gametes were obtained upon injection of about 1 ml of 0.5 M KCl into the mouth area of the animal. Within 5 minutes of fertilization embryos were placed in the test solutions and incubated at 16°. Observations of the developing embryos were made at times appropriate to detect effects on first cleavage, regularity of early cleavages, cell shapes and sizes during early cleavage, rate of early development, completion of the blastula, hatching of the blastula, and developmental anomalies of the gastrula, prism and pluteus. These observations can all be made within 3 days of fertilization.

All compounds tested were used at concentrations of 10, 1, 0.1, and 0.01 ppm. In the cases of the very insoluble compounds toxaphene, malathion and sevin, solubility was achieved by adding 10 µl of an acetone:Emulphor (8:2) solution to 100 ml of filtered sea water. Results were obtained from 7 compounds as follows:

Pentachlorophenol: at 10 ppm and 1 ppm no cleavage occurred. At 0.1 ppm development was delayed but proceeded to the late blastula whereupon all embryos died. At 0.01 ppm, development was unaffected through the termination of the experiment, at the very late pluteus.

Malathion and Aminotriazole: at all concentrations these compounds had no effect on development through the very late pluteus stage.

Phenol and Sevin: these compounds had no effect on development through gastrulation at all levels tested.

Toxaphene: at 10 ppm development was normal to the prism stage at which it was arrested. The lower concentrations of this compound had no effect through development of the very late pluteus.

DDT: from 0.01 ppm to 10 ppm of this compound, development proceeded normally until the completion of gastrulation. Then all the test embryos died.

It can be seen from testing these few xenobiotic compounds that a variety of effects on early sand dollar development may be observed. Since, by seasonal selection of species, echinoderm gametes may be obtained at any time of the year, extension of these studies may provide ideal model systems for exploring effects of environmental pollutants on embryogenesis.