

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.