EXTENDED CULTURE OF CELLS FROM TISSUES OF THE DOGFISH SHARK (*Squalus acanthias*) AND LITTLE SKATE (*Raja erinacea*)

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Experiments using cell cultures from mammals, insects, and birds have provided essential insights into a variety of basic biological phenomena, but the use of cell cultures from marine organisms has lagged that of other species (Ballitori, N and Villalobos, AR. Tox. and Applied Pharm. 183, 207-220, 2002). This situation is changing for three reasons: (1) successful attempts to culture fastidious cell types from both fish and mammals have led to the understanding that conventional cell culture approaches must be modified to provide conditions designed specifically for the target cell type, (2) marine organism genomic databases are becoming sufficiently large that sequence data can be used to address species-specific questions regarding physiological mediators of cell function, and (3) recombinant DNA technology now can provide sufficient quantities of growth-promoting peptides and other critical molecules to carry out systematic cell culture studies. We are attempting to derive cell lines from tissues of marine organisms that may be useful to ourselves and other investigators in pursuing a variety of research questions related to physiology, toxicology and developmental biology.

Primary cultures of adult and embryonic dogfish shark rectal gland were initiated (Valentich JD and Forrest JN, Jr. Am. J. Physiol. 260 C813-23, 1991). The medium routinely was supplemented with epidermal growth factor and fibroblast growth factor (each 10 ng/ml), insulin (1 ug/ml), fetal bovine serum (5%), forskolin (10 uM) and shark serum (1%). Positive effects on proliferation or survival were seen upon further supplementation of the medium with alpha-tocopherol, retinol and dexamethasone (100 ng/ml each). After two to three months of culture, foci of high cell density appeared. DNA synthesis in the cultures was confirmed by introduction of bromo-deoxyuridine into the medium and immunohistochemical localization of sites of macromolecular incorporation using monoclonal antibody to the nucleotide analogue. Cultures appeared to form dynamic, three-dimensional "domes", evidence of transporting epithelia with tight junctions. Cultures have been maintained for more than eight months; passage of the cells to new culture vessels was best achieved by transferring the cells suspended in a soft collagen gel. Preliminary results indicate that this approach consistently resulted in adherent secondary cultures that appeared healthy, although we have not yet examined proliferative capacity of the secondary cultures, and passaged (secondary) cultures have not yet attained confluency.

Using similar approaches, cell cultures from other adult and embryonic shark tissues

were initiated. Attachment, proliferation and passage was achieved with cells from dogfish shark brain and eye. These cultures are currently in the third passage. Frozen storage of any of the cultured cell types has not yet been attempted. Proliferating cell cultures also were obtained from shark ovary and kidney and from early embryos of the little skate. These cultures may be useful as models of cells from these tissues and species (Dranoff, J, et al., J. Biol. Chem. 275, 30701-30706, 2000; Wellner et al., Bull. MIDBL Bull. 41, 101-102, 2002). In addition these cultures may provide growth factors and attachment molecules in conditioned media or as feeder layers that will promote culture of cells from shark rectal gland and skate liver, another cell type for which we are attempting to develop a culture system.

Cultures showing promise will be studied further to identify conditions optimal for cell proliferation. Cells also will be characterized by immunohistochemical assay of expression of transporter molecules of interest, including the cystic fibrosis transmembrane regulator and related regulatory proteins (Frizzell, R. Physiol Rev. 79, (Suppl): S1-2, 1999), ATP-independent xenobiotic transport proteins (Wang, W et al. Proc Natl Acad Sci USA 98, 9432-9436, 2001) and ABC (ATP-binding cassette) xenobiotic transport proteins (Benyajati S and Renfro L. Am. J.. Physiol. Regul. Integr. Comp. Physiol. 79, R704-12, 2000; Terloux, S. et al. Mol. Pharmacol 59, 1433-1440, 2001). Attention will be given to changes with passage number in gene expression patterns and karyotype, but data on these phenomena are not yet available. These models may be useful for *in vitro* approaches of human medical relevance in the study of membrane transporters and membrane-related toxicology (Forrest JN Jr, et al. J. Exp Zool. 279:530-536, 1997; Kinne-Saffran, E and Kinne, R. Biochim. et Biophy. Acta 1510, 442-451, 2001). In particular, these cell lines may allow homologous system-expression of cloned genes from marine organisms, providing experimental means to understand functional and structural interaction of genes and gene products among species.

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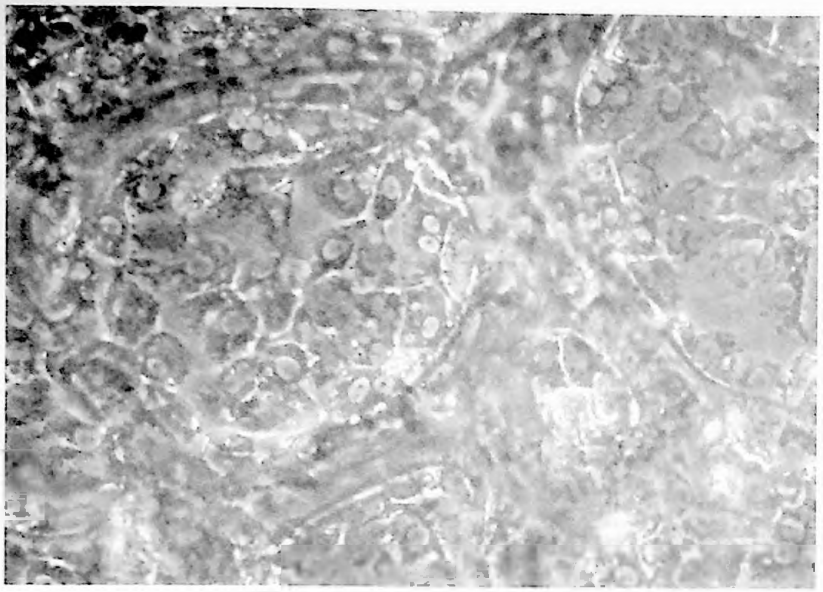

Figure 1. Photomicrograph of a primary culture of shark rectal gland cells. Average Cell diameter is approximately 60 microns. Cells were maintained in a 3% carbon dioxide atmosphere at 20 degrees C. The culture was approximately six months old.

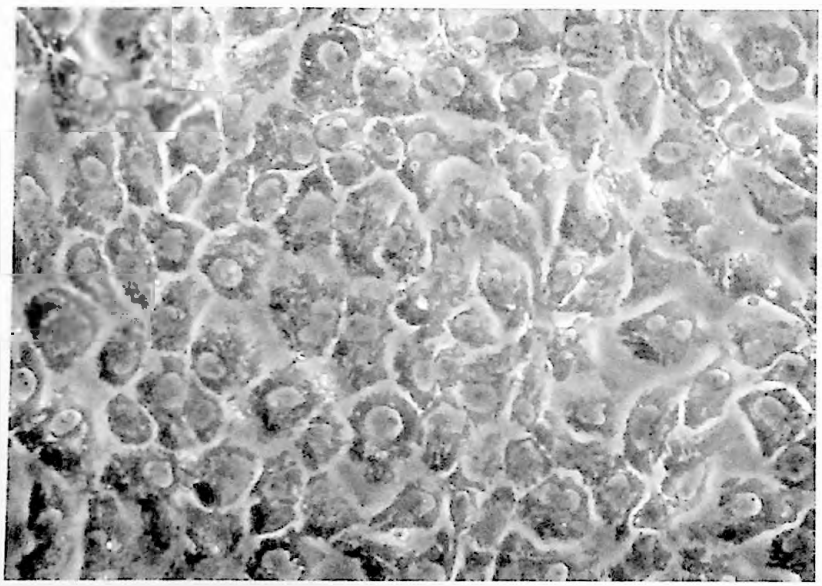

Figure 2. Photomicrograph of primary culture of shark kidney cells. Average cell diameter is approximately 80 microns. Cells were maintained in HEPES-buffered culture medium under ambient air at 20 degrees C. The culture was approximately two months old.