# A LONG-TERM PRIMARY CULTURE MODEL OF HEPATOCYTES ISOLATED FROM THE LITTLE SKATE, *RAJA ERINACEA*

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Isolated mammalian liver parenchymal cells in culture have proven to be an invaluable system for studying a multitude of biochemical, physiological, and toxicological questions (Alpini et al., Hepatology 20:494-514, 1994). Cultured hepatocytes offer a number of advantages over the in vivo or isolated perfused liver models, including elimination of systemic effects, better control of environmental conditions, reduced variability between experiments, simultaneous and repeated sampling in a single experiment, and more efficient use of reagents and time. Short-term cultured hepatocytes (<8 h) closely mimic the functions of cells in the intact liver and provide a useful model for studying acute effects of drugs and xenobiotics. Primary monolayer cultures allow for measurement of long-term effects (up to 2 weeks), although cells tend to dedifferentiate and lose viability over time.

Hepatocytes have been isolated from many different species, including marine and freshwater fish (Baski and Frazier, Aquatic Toxicol. 16:229-256, 1990). We have previously developed a short-term culture system for skate hepatocytes (Smith et al., J. Exp. Zool. 241:291-296, 1987), and have used this system to evaluate mechanisms of hepatic metabolism, detoxification, membrane transport, and cell volume regulation (e.g., Ballatori et al., Toxicol. Appl. Pharmacol. 95:279-291, 1988; Am. J. Physiol. 267:G285-G291, 1994; Mol. Pharmacol. 48:472-476, 1995; Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992). When compared with mammalian hepatocytes, skate hepatocytes are easier to isolate and culture, and exhibit high viability, stability, and more complete maintenance of apical and basolateral membrane polarity (Smith et al., J. Exp. Zool. 241:291-296, 1987; Henson et al., J. Exp. Zool. 271:273-284, 1995).

The objective of the present study was to develop conditions for long-term culture of skate hepatocytes and to begin to characterize this hepatocyte model. The culture medium selected was Dulbecco's Modified Eagle's Medium/Ham's F12 (DMEM/F12; Valentich, J. Tiss. Cult. Meth. 13:149-162, 1991), which was further modified to make it comparable to elasmobranch Ringer. DMEM/F12 containing 15 mM Hepes (Sigma D8900) was supplemented with (in mM), 1.5 $CaCl_2$, 2 $MgCl_2$, 90 NaCl, 350 urea, 8 $NaHCO_3$, 100 trimethylamine N-oxide, 0.1 taurine, and 0.1 betaine. The medium pH was adjusted to 7.5 with NaOH, the solution was sterilized by filtration, and supplemented with 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.1 mM dexamethasone. Skate hepatocytes were isolated by collagenase perfusion as previously described (Smith et al., J. Exp. Zool. 241:291-296, 1987), and were washed three times in culture medium, before plating at a density of near confluency on plastic dishes. Cells were placed in an incubator that circulated room air at 12°C. Sterile conditions were needed to prevent bacterial and fungal contamination of the cultures. Culture medium was changed after the first 18-24 h, and every 2-3 days thereafter. The cells were analyzed over a ten-day period using a variety of morphologic and biochemical assays.

Skate hepatocytes readily attached to plastic culture dishes, and formed a network-type three dimensional structure. Although most of the cells were present as clusters surrounding a bile canaliculus, some cells were also present as single isolated cells and some as couplets. The number of single cells could be increased by lengthening the time of collagenase perfusion during cell isolation. Trypan blue and propidium iodide exclusion was measured on days 0, 1 and 2 of culture, and was found to be >98% for the adherent cells. During the first 24 h a number of cells were found floating (presumably damaged and killed during isolation), but there was minimal loss of cells from day 2-10, as evaluated from both the amount of total protein and of glutathione *S*-transferase enzyme activity on each dish. Because the cells are organized in aggregates around a bile canaliculus, counting the number of cells was sometimes difficult, particularly for the propidium iodide assay, which requires switching between brightfield and fluorescent optics. In contrast to plastic dishes, attachment of the cells to glass cover slips was not as avid, although it was markedly improved by coating the cover slips with poly-L-lysine, type I or type IV collagen. The effects of the coatings was transient, however, as the cells gradually detached from the treated glass over 2-4 days in culture.

Of significance, the cultured skate hepatocytes maintained a relatively normal morphology, and a polarized distribution of apical and basolateral membrane domains as assessed both morphologically and functionally (secretion of fluorescent organic anions into the bile canaliculus). Examination of the microtubular and microfilamentous array by immunofluorescence microscopy revealed maintenance of normal cytoskeletal organization. Because of the formation of a network structure the cells attached firmly to plastic culture dishes and could be lifted as a whole piece from the culture plate for additional processing. The cells secreted organic anions (NBD-taurocholate) into the canalicular lumen, responded to insulin, and exhibited high viability and metabolic activity. Although additional work is needed to characterize this model, these preliminary studies indicate that it should be an excellent system for investigating long-term effects of drugs and xenobiotics. (Supported by ES03828, ES01247, ES06484, DK34989, DK25636, DK48823, EPA R825218, and by the NSF ESI9452682 and DBI9531348).