IN VITRO CULTURE OF RENAL TISSUE OF THE LITTLE SKATE, *LEUCORAJA ERINACEA*

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The kidney of marine elasmobranchs features a nephrogenic zone, where new nephrons are added to the definitive excretory organ in adult sexually mature animals (Hentschel, H., Am. J. Anat. 190:309-333, 1991). We established a renal reduction model with adult little skate, and showed that neonephrogenesis (NNG) can be induced by partial nephrectomy (Elger, M. et al., J. Am. Soc. Nephrol., in press, 2003). For better understanding of regeneration by NNG, we developed an organ culture model. Cells from other organs of skate and dogfish have been successfully cultured previously (rectal gland: Valentich, J. et al., Am. J. Physiol. 271:C1993-C2003, 1996; Aller, S.G. et al., Am. J. Physiol. 271: C442-C449, 1999; liver: Rebbeor, J.F. et al., Am. J. Physiol. 279:G417-G425, 2000; gonad: McClusky, L.M. et al., Bull. MDIBL 35: 96-97,1996). Recent interest has concentrated on derivation of continuous cell lines as model systems (Barnes, D.W. et al., Bull MDIBL 41:45-47, 2002). The apparent lack of information on maintenance of renal tissue of elasmobranch fish in vitro prompted us to establish organ culture for skate kidney, with special emphasis on the nephrogenic zone.

Small tissue samples were dissected from kidney slices of little skate *Leucoraja erinacea* after perfusion with elasmobranch Ringer's solution. The pieces were washed in culture medium according to Valentich 1991 (J. Tissue Cult. Method 13:149-162; Dulbecco's modified Eagle's medium, DMEM, 100mM NaCl, 3.9 mM $CaCl_2$, 2.5 mM $MgCl_2$, 350 mM urea, 72 mM trimethyl amine oxide, 8 mM $NaHCO_3$) supplemented with antibiotics. The tissue was kept at 18°C and in an atmosphere with 1 % CO_2 in Valentich's culture medium supplemented with antibiotic antimycotic solution containing penicillin, streptomycin, and amphotericin B (Sigma, 1ml / 100 ml culture medium), insulin-transferrin-sodium selenite liquid media supplement (ITS+1, Sigma, 1ml / 100ml), and shark serum for 48 hours. Thereafter, culture was continued without antibiotic antimycotic solution and the medium was changed every two days. Cell proliferation was assessed by the incorporation of 5-bromo-2'-deoxy uridine (BrdU) into dividing cells. After one or two weeks in culture, 10 µM BrdU (Roche) was added to the cultures for 2, 4, or 6 hours, and tissue samples were fixed with 4 % paraformaldehyde (PFA) in 0.1 M Soerensen buffer. The buffer was adjusted with NaCl and sucrose to 900 mOsm/l. The samples were embedded in paraffin. Proliferating cells were visualized on deparaffinized sections by indirect immuno-histochemistry with antibody against BrdU. For high resolution light microscopy and transmission electron microscopy tissue pieces were fixed in PFA fixative with the addition of 1% glutaraldehyde. Tissue blocks for microtomy were obtained by embedding in Epon resin.

Histological investigation of tissue sections in the light and electron microscope revealed that the zonation of the renal tissue in mesial tissue, lateral countercurrent bundles, and

nephrogenic zone was retained. Epithelial cells of mature nephrons were well preserved in the late distal tubule and the cilia-bearing neck segment and intermediate segment. Proximal tubule cells and early distal tubule cells of cultured tissue showed minor changes in their morphology as compared to cells *in situ*. However, many proximal tubules (PII) had disintegrated, and their epithelial cells showed all stages of degeneration. In contrast, structure and morphology of glomeruli were well preserved. Podocytes, parietal cells of Bowman's capsule, mesangial cells, and endothelial cells showed the same phenotype as *in situ*. The collecting duct system was virtually intact, and many intrarenal blood vessels were structurally preserved.

The nephrogenic tissue survived well, and proliferation of the developing stages and young tubules was vigorous as shown by the incorporation of BrdU in epithelial cells (Fig. 1).

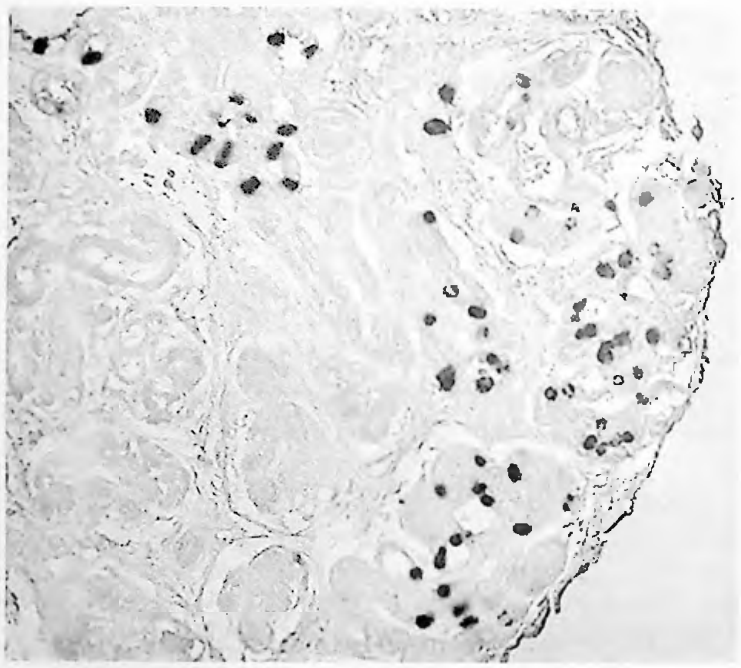

Fig. 1. Skate renal tissue two weeks in culture. Cell proliferation as visualized by immunohistochemical detection of incorporated BrdU is seen in tubules of the nephrogenic zone and in young nephrons. Paraffin section.

In conclusion, organ culture of skate renal tissue provides a promising model for future study of factors governing the controlled development of nephrons during neonephrogenic processes.

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