MICROCYSTIN-INDUCED CYTOSKELETAL REDISTRIBUTION IN CLUSTERS OF ISOLATED POLARIZED SKATE (*RAJA ERINACEA*) HEPATOCYTES

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Microcystins are hepatotoxic heptapeptide compounds produced by aquatic cyanobacteria which have been causally linked to animal and human fatalities. These toxins are generally cellular impermeant, however they affect hepatocytes due to the unique transport systems these cells possess. The molecular mode of action of microcystin is to potently inhibit protein phosphatases 1 and 2A (MacKintosh et al., *FEBS Letters* 264:187-192, 1990). However, it is still unclear which cellular protein targets are most affected by microcystin exposure. A number of previous studies have indicated that the hepatocyte cytoskeleton is disrupted by microcystin treatment and have suggested that hyperphosphorylation of the cytokeratin cytoskeletal networks are a significant event in the toxicologic/carcinogenic effects (Falconer and Yeung, *Chem.-Biol. Interac.* 81:181-196, 1992; Ohta, et al., *Carcinogen.* 13:2443-2447, 1992). In the present study we have examined the effects of microcystin treatment on clusters of polarized hepatocytes isolated from the little skate (*Raja erinacea*). These clusters of cells provide an excellent model system due to their maintenance of structural and functional polarity *in vitro*. To date, the majority of studies on the effects of microcystin on hepatocyte cytoskeleton have been performed using primary cultures of unpolarized mammalian hepatocytes.

Clusters of hepatocytes were isolated from skate liver and maintained in culture according to Smith et al. (*J. Exp. Zool.* 241:291-296, 1988). Hepatocytes were treated for at least 30 minutes with 0.25-10 μM microcystin-LR or -YM in elasmobranch Ringer's at 15°C. Fluorescent localization experiments were carried out as described in Henson et al. (*J. Exp. Zool.* 271:273-284, 1995). Cells were stained for actin filaments either with rhodamine-phalloidin or a mouse monoclonal antibody against a conserved epitope of actin. For cytokeratin localization, cells were stained with a mouse monoclonal antibody raised against trout cytokeratins (clone 2E11) or a mix of the AE1/AE3 antibodies against mammalian cytokeratins (Woodcock-Mitchell et al., *J. Cell Biol.* 95:580-588, 1982). Western blotting of anti-cytokeratin 2E11 against lysates from skate hepatocytes was performed as described previously (Henson et al., 1995). Stained cells were viewed using a Nikon 60X 1.4NA planapo objective lens on a Nikon Optiphot II microscope using either conventional epifluorescence or a Nikon PCM-2000 laser scanning confocal system.

In control skate hepatocytes, actin filaments (Figure 1A) display a cortical distribution with a concentration in the pericanalicular region of the cluster, as has been reported previously by Henson et al. (1995). The bile canalicular lumen is clearly outlined by actin specific labeling (Figure 1A). Cytokeratin labeling of control hepatocytes with either 2E11 or AE1/3 antibodies reveals a limited array of tangled filaments in the deep cytoplasm of the cells (Figure 1C). This cytokeratin assemblage appears to exist in the cytoplasmic interstices between the nucleus and the large lipid droplets present in these cells. Western blotting of isolated skate hepatocytes with the 2E11 antibody results in strong labeling of a band in the vicinity of 50kDa (data not shown), a molecular mass which is consistent with the major mammalian hepatocyte cytokeratins, CK8 (49 kDa) and CK18 (55kDa). In hepatocytes treated with microcystin the actin filament and cytokeratin cytoskeletons are dramatically disrupted. The actin filaments appear to collapse into an array of

foci surrounding the now constricted bile canalicular lumen in the middle of the cluster (Figure 1B). In addition there appears to be a reduction in the basolateral cortical actin labeling (Figure 1B). The previously perinuclear arrangement of the cytokeratin filaments also collapses into the pericanalicular region after exposure to microcystin (Figure 1D). In some images the apical regions of the cells appear to be clearly constricted (arrows in Figure 1D).

The results of our study indicate: (1) skate hepatocytes contain a unique arrangement of cytokeratin filaments which is unlike that reported for mammalian hepatocyte cytokeratins in which the cytoplasm is filled with a distribution of elongated cytokeratin filaments, often clustering at points of cell-cell contact (see Falconer and Yeung, 1992). In addition, the distribution of the cytokeratin staining in the skate hepatocytes is different from that seen in other fish hepatocytes (namely freshwater sucker and trout) labeled with the same 2E11 antibody (J. Holy, unpublished observations). The function of this unusually structured cytokeratin array in the skate cells is not clear, however skate hepatocytes are noteworthy for the presence of large cytoplasmic lipid droplets, and in other cell types intermediate filaments have been linked to lipid sequestration and metabolism processes (reviewed in Marceau and Laoranger, 1995). (2) Microcystin causes an extensive redistribution of the actin and cytokeratin cytoskeletons in the skate hepatocytes. Microcystin also appears to induce a constriction in the bile canalicular structure of the polarized cells. Previous studies have described microcystin-mediated changes in actin and cytokeratin, however they have reported that the cytokeratin array redistributes to the perinuclear region of the cell (Falconer and Yeung, 1992). In our study we clearly see a collapse into the pericanalicular region (Figure 1D). The discrepancy between our results and those of previous studies may be due to the polarized nature of the isolated skate hepatocytes. It is tempting to speculate that microcystin is causing a constriction of the bile canalicular lumen via the hyperphosphorylation of myosin light chain and the resulting interaction of actin and myosin filaments in the circumferential pericanalicular sheath. The constricted apical regions of some cells are particularly suggestive of this mechanism and we have recently localized myosin II in the pericanalicular region of skate hepatocytes using an antibody against human platelet myosin (unpublished observations). In addition myosin light chain phosphorylation levels have been reported to be regulated by the microcystin sensitive protein phosphatase 1. However, a recent study has suggested that myosin light chain phosphorylation levels do not increase in cultured mammalian hepatocytes exposed to microcystin, although these results were reported as data not shown (Toivola et al., *J. Cell Sci.* 110:23-33, 1997). Future studies will focus on determining the exact nature of the effects of microcystin treatment on the organization of the submembranous pericanalicular cytoskeleton in skate hepatocytes.

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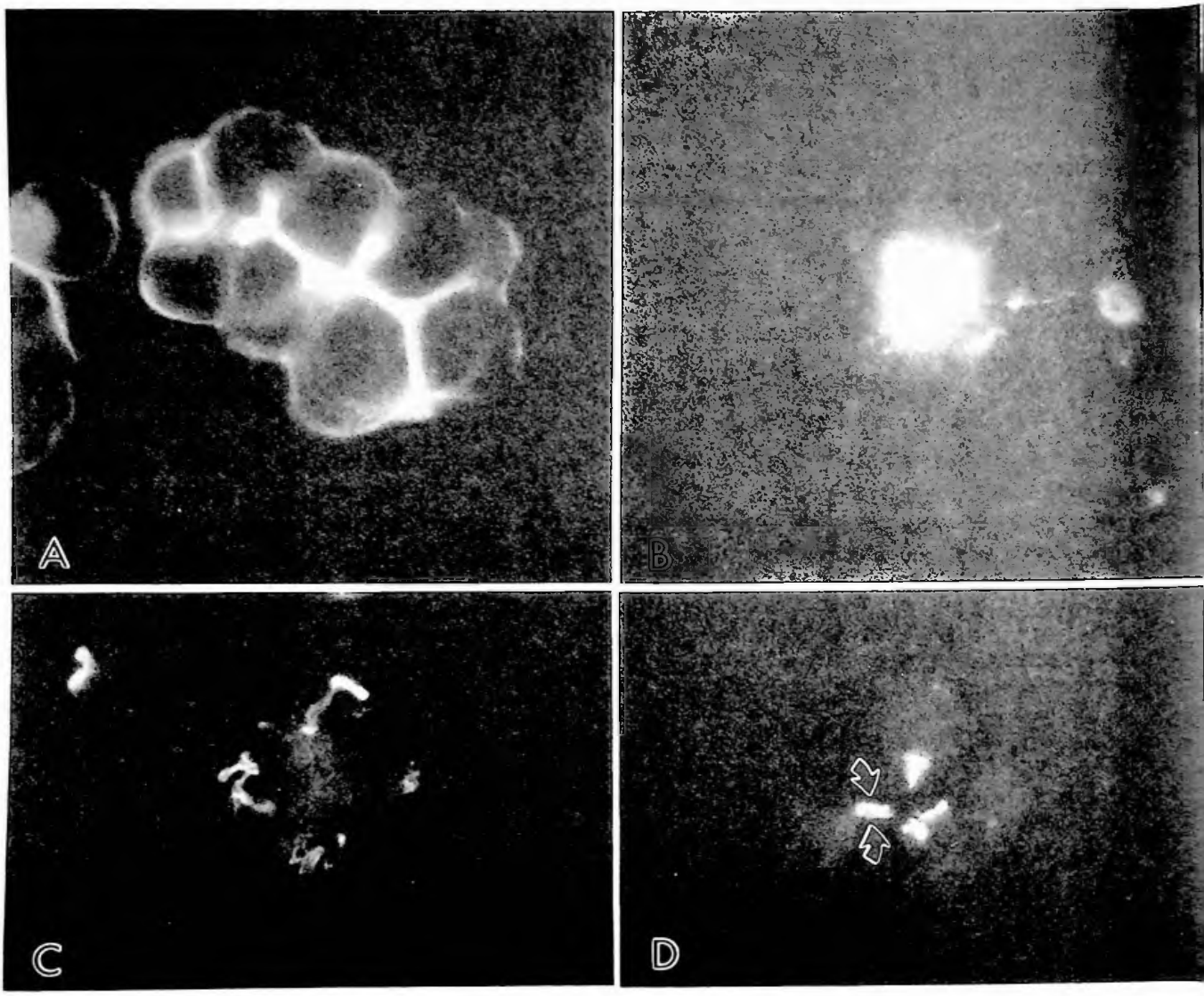
Figure 1: Panels A and B - Filamentous actin labeling in control (A) and microcystin (5 μM) treated (B) clusters of skate hepatocytes. Note that the clear bile canalicular labeling seen in the control cluster has been altered to a central focal distribution in the treated cells. Panels C and D - Cytokeratin (anti-AE1/3) labeling of control (C) and microcystin (5 μM) treated (D) skate hepatocytes. The limited filamentous arrays present in the deep cytoplasm of the control cells collapse into pericanalicular spots within the constricted apical regions (arrows) of treated cells. Magnifications = 800X.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