TABLE 3

Preliminary Assessment of the Effects of DDA, CPR, and BDG on the Clearance of $^{14}$C-PAH by the Winter Flounder*

| Inhibitor | Plasma [Inhibitor] | $C_{PAH}$ | % Inhibition |
|---|---|---|---|
| | μM | | |
| DDA** | 0 | 200.1 | |
| | 1 | 16.4 | 91.8 |
| | 2 | 8.2 | 95.9 |
| | 10 | 2.6 | 98.7 |
| CPR** | 0 | 171.9 | |
| | 1 | 29.7 | 82.7 |
| | 10 | 5.0 | 97.1 |
| BCG** | 0 | 113.4 | |
| | 0.1 | 18.0 | 84.1 |
| | 1.0 | 0.8 | 99.3 |

*Abbreviations: DDA = 2,2-bis@p-chlorophenyl) acetic acid; CPR = chlorophenol red; BCG = bromcresol green.

**DNA results reflect 12 control and 12 experimental clearance periods in 2 fish. CPR and BCG results are from single fish with 6 control and 6 experimental clearance periods in each fish.

## CYTOTOXICITY OF PLASMA AND TISSUE HOMOGENATES OF SHARKS ON MURINE LYMPHOBLASTS IN CULTURE

H. N. Jayaram, M. P. J. S. Anandaraj, J. B. Pritchard*, and A. M. Guarino, National Cancer Institute, National Institutes of Health, Bethesda, Maryland and *National Environmental Health Sciences, Research Triangle Park, North Carolina

There is an increasing interest at the National Cancer Institute and elsewhere in finding antineoplastic substances from the sea (Li et al., Cancer Chemother. Rep. 4(2):97, 1974). Snodgrass et al. (JNCI 56:981, 1976) have demonstrated that significant inhibition of the growth of Lewis Lung Carcinoma is produced in mice by parenteral administration of dogfish serum. In a previous bulletin (MDIBL 15, in press 1976), we have documented the megalocytic (increase in the mean cell volume) and cytotoxic effects of spiny dogfish plasma on a subline of Leukemia 5178Y cells and L1210 cells in vitro. Cytofluorimetric analysis of the DNA distribution of L5178Y/AR cells cultured in the presence of spiny dogfish plasma established that cell cycle progression was arrested at the $G_1$ phase. Preliminary attempts to characterize the factors causative of the megalocytic effect suggested that it was a nondialyzable macromolecule.

In the present report, we have compared the megalocytosis and cytotoxicity produced by plasma from different species of sharks with that of plasma from dogfish. We also have studied the

cytotoxicity of homogenates of various tissues of the spiny dogfish, in order to identify the organ of origin of this megalocytic and cytotoxic principal. The relative megalocytic effect of five species of shark plasma on L5178Y/AR cells is listed in Table 1. Size distribution of cells was measured after 48 hours of culture using a Coulter Channelizer. The plasma of all species

TABLE 1

Megalocytic Effect of Various Shark Plasma

| Plasma | Concentration of shark plasma protein mg/ml of culture | Mean cell volume in cubic microns x 100 (range) | |
|---|---|---|---|
| Control | 0.0 | 12.5 | (10-15) |
| Spiny dogfish (*Squalus acanthias*) | 2.0 | 55 | (50-60) |
| Sandbar shark (*Carcharhinus milberti*) | 3.1 | 42.5 | (35-50) |
| Atlantic sharp-nose shark (*Rhizoprionodon terraenovae*) | 4.0 | 40 | (35-45) |
| Smooth dogfish (*Mustelus canis*) | 2.0 | 40 | (35-45) |
| Scalloped hammerhead shark (*Sphyrna lewini*) | 3.6 | 35 | (30-40) |

Legend: Shark plasma was separated by centrifuging heparinized blood and frozen immediately. Culture flasks in a total volume of 5 ml contained 0.5 ml of centrifuged, sterilized (by filtration) plasma added to 4.5 ml of Dulbecco-Vogt medium containing L-glutamine (3.5 g/L), 5% fetal calf serum and 5 million L5178Y/AR cells. The cells were cultured for 48 hours at 37° in an atmosphere of 95% air and 5% $CO_2$. Electron particle sizing and counting of cells were carried out using a Coulter Channelizer and Counter. All plasma samples were collected from sharks caught off the coast of S. Carolina, except that of dogfish shark, which was from the Gulf of Maine.

produced a prominent megalocytic effect but the plasma of the spiny dogfish was most potent, provoking a five-fold increase in the mean cell volume when compared with cells cultured in the presence of fetal calf serum alone. Of the five species tested, spiny dogfish, sandbar and Atlantic sharp-nose sharks plasma produced total inhibition of cell growth at the concentrations tested whereas smooth dogfish plasma depressed cell growth by 66% and scalloped hammerhead shark plasma depressed cell growth by 30%, respectively.

To identify the origin of the cytotoxic and megalocytic principal, plasma was separated from heparinized blood, bile was collected, tissue homogenates of spiny dogfish were prepared in normal saline (1:4 w/v), centrifuged at 45,000 g for 30 minutes, and examined for megalocytic and cytotoxic properties against L5178Y/AR cells (Table 2). Tissue homogenates of brain, gills, kidney, pancreas, rectal gland, spleen, uterus and ovary were each without effect on inhibition of growth. Of the organs examined, only liver, and to a lesser extent muscle, exhibited cytotoxicity; only plasma produced a significant megalocytic effect. Bile was highly cytotoxic (cytolytic) before but not after exhaustive dialysis against saline; dialysis also decreased the cytotoxicity to liver extracts (by 50%) and of

TABLE 2

Effect of Various Tissue Homogenates of Spiny Dogfish (*Squalus acanthias*) on the Growth of L5178Y/AR Cells in Vitro

| Organ Homogenate | % Inhibition of Growth After 48 Hours |
|---|---|
| Plasma | 100 |
| Liver | 100 |
| Bile | 100* |
| Muscle | 50 |
| Others (see text) | 0 |

Legend: Tissue supernatant was sterilized by filtration and added (0.5 mg protein/ml of medium) to the Dulbecco-Vogt medium supplemented with L-glutamine (3.5 g/L) and 5% fetal calf serum. One million cells/ml L5178Y/AR cells were added and the culture flasks were incubated at 37° for 48 hrs in an atmosphere of 95% air and 5% $CO_2$. After 48 hrs, the cells were counted in the Coulter Counter, Model Fn. The cell concentration had increased to 3-4 millions cells/ml in the control cultures. Trypan blue exclusion counting indicated greater than 90% cells Trypan blue negative in cultures showing no inhibition.

plasma (by 40%). However, the megalocytic principal in plasma was again observed to be retained by cellulose dialysis tubing. Attempts are being made to characterize the megalocytic principla in the spiny dogfish plasma by isoelectrofocusing and ion-exchange chromatography.

THE OPERCULAR EPITHELIUM OF THE KILLIFISH (*Fundulus heteroclitus*) and the Sea Raven (*Hemitripterus americanus*)

Karl J. Karnaky, Jr. and William B. Kinter, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

Studies on intact fish and isolated, perfused gills have provided virtually all of our knowledge of osmoregulatory NaCl transport mechanisms in the teleost gill. The severe limitations of these two preparations, e.g., they are not flat sheets and cannot be studied under the ideal thermodynamic conditions achieved with the short-circuit current technique, have prompted us to examine the epithelium lining the gill chamber as a third preparation to study osmoregulatory electrolyte transport mechanisms in teleosts.

Over twenty-five years ago, Burns and Copeland (Biol. Bull. Mar. Biol. Lab., Woods Hole, 99: 381-385, 1950) showed that the opercular epithelium of the killifish, *Fundulus heteroclitus*, contained chloride cells. Recently, we have examined the histology and ultrastructure of this epithelium in both the killifish and the sea raven, *Hemitripterus americanus* (Karnaky and Kinter, J. Expt. Zool., in press). We observed that the opercular epithelium is indeed a flat sheet containing chloride cells. Significantly, the identity of the chloride cell in this epithelium was definitely established with the electron microscope. Furthermore, we observed that whereas the opercular epithelium of the marine