multiplied by 10 in this figure for clarity. The kinetics of the reaction do not appear to follow simple Michaelis-Menton theory. Others have found complex kinetics in the GSH S-transferase reactions even in purified enzyme preparations (Pabst, Habig, Jakoby, J. Biol. Chem. 249, 7140, 1974).

The characterization of GSH S-transferase activity alkene oxides was carried out in little skate liver since this was the species used previously to characterize GSH S-transferase activity towards 1,2-dichloro-4-nitrobenzene. We also measured GSH S-transferase activities towards styrene oxide in a number of species as shown in Table I.

In most species, specific activiteis of GSH S-transferase in hepatic or renal cytosol fractions towards octene oxide were much lower than towards styrene oxide. Exceptions were the two teleost species studied, the King of Norway (*Hemitripterus americanus*) and the winter flounder (*Pseudopleuronectes americanus*). Specific activities of both epoxide hydrase and GSH S-transferase towards styrene oxide in liver and kidney of the winter flounder were high, especially microsomal epoxide hydrase. This might indicate that the ability to metabolize epoxides to more polar compounds is intrinsicaly important in the flounder. GSH S-transferase activity towards 1,2-dichloro-4-nitrobenzene in flounder hepatic cytosol fraction was an order of magnitude lower than towards either alkene oxide substrate, whereas in the little skate hepatic cytosol fraction, activity towards styrene oxide was about the same as towards 1,2-dichloro-4-nitrobenzene. This could indicate that skate and flounder have different proportions of the GSH S-transferases which show higher specificity for epoxide substrates.

We were also interested to find high spec activities of epoxide hydrase (about double the activi present in rat liver microsomes) in microsomal prepa tions from hepatopancreas of the lobster (*Homa americanus*) and rock crab [*Cancer ittorus*). Microsom prepared from lobster gill filaments also had high epox hydrase activities. The activities. The activities of G S-epoxide transferases towards styrene oxide or octe oxide in cytosol fractions of hepatopancreas or gill fr the crustacean species were variable and low compa with epoxide hydrase specific activities.

There is one interesting difference between several the marine species studied and rat or guinea pig (Jam Fouts, Bend, Biochem. Pharmacol. In Press, 1975) both mammalian speciea hepatic GSH S-transfer activity (both total and specific) greatly exceeds epo hydrase activity (using 1.0 mM styrene oxide as substr in both assays). In several of the marine spec however, epoxide hydrase activity predominated sugg ing that the microsomal hydrase may be relatively m important in the marine animals for epoxide detoxificat than in guinea pig or rat.

In summary, we may say that all marine species have studied are capable of metabolizing epoxides some extent, both by conjugation with GSH or hydration to the dihydrodiol. The existence in ma species of these pathways for further biotransformat of potential carcinogens, mutagens and cytotoxins co be an important protective mechanism towards envir mental contaminants such as polycyclic aroma hydrocarbons, which are known to be released into t ocean.

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Cytotoxicity Of Dogfish Shark Plasma On Murine Lymphoblasts In Culture

H.N. Jayaram, D.A. Cooney, M.P.J.S. Anandaraj, R.L. Dion, V.H. Bono, J.M. Ward, R.L. Reagan and A.M. Guarino, National Institutes of Health, Bethesda, Maryland 20014

Adamson (*Proc. Marine Tech. Soc. Foods - Drugs From The Sea 3033, 1972*) in a preliminary report on the effects of Elasmobrance plasma on L1210 cells *in vitro* noted only a 13% inhibition of the growth of L1210 cells in 24 hrs by dogfish plasma. For some time, we have been examining the ability of the serum and plasma of several species to alter the growth of murine lymphoblasts in culture. In the course of these studies, we observed that the plasma of dogfish shark *Squalus acanthias* is cytotoxic to a subline of Leukemia 5178Y cells rendered resitant to L-asparaginase (L5178Y/AR) and to L1210 cells *in vitro*. The present report characterizes this cytotoxicity.

L5178Y/AR and L1210 cells were cultured in Dulbecco-Vogt medium containing 10% of either Fetal Calf Serum (FCS) or shark plasma except where indicated and incubated for 48 hours at 37° in an atmosphere of 95% air and 5% CO_2. Electronic particle sizing of the cells was carried out using a Coulter channelizer and counter.

Table I

Effect of Fetal Calf and Shark Plasma on the Growth of L5178Y/AR *In Vitro*

Dulbecco-Vogt Medium, Supplemented with Fetal Calf Serum or Shark Plasma		Total Cell Count/ml (x 10^6)	
		Initial Count	Count After 48 hrs
Fetal Calf Serum	5%	1.0	3.8
	10%	1.0	4.0
Shark Plasma	5%	1.0	0.9
	10%	1.0	0.86

Legend: Cells were cultured for 48 hours at 37° in an atmosphere of 95 air and 5% CO_2. Trypan blue exclusion counting indicated grea than 90% cells Trypan blue negative in all cultures.

The frequency distribution of DNA content of individu cells was measured by flow microfluorimetry (*Van Dilla e al., Science 163, 1213, 1969*).

L5178Y/AR cells when cultured in FCS exhibit doubling time of about 16 hrs. When shark plasma wa used instead of FCS, multiplication ceases (Table 1). Th mitotic index in the FCS cultures averaged 3.5 percen whereas no mitotic figures were observed in cultures wit shark plasma. Morphologic examination of cells cultur for 48 hrs in shark plasma revealed a striking enlargeme or magalocytosis of the treated cells. This change wa

documented unequivocally by electron particle sizing of the cultures. The mean cellvolume of lymphoblasts in FCS was 16 ^{3}. When shark plasma was used, the mean cellular volume incresed to 44 ^{3}. Light and electron microscopic studies of the cells illustrated the magalocytic effect of shark plasma and also showed a decrease in the presence of heterochromatin in the nucleus of treated cells (Figure 1).

Because it appeared that shark plasma either failed to support or interrupted cell division, attempts were made to localize the phase of the cell cycle at which this supplement was operating, using cytofluorimetric analysis of the DNAdistribution of L5178Y/AR cells cultured for 24 and 48 hrs. The results of these studies are presentd in Figure 2, and indicate that cell cycle progession in shark plasma is arrested primaril in the G_1 phase. The relative lack of S-phase cells at both time periods suggests tht those cells which have initiated DNA synthesis are capable of completing it. Surprisingly, after 48 hrs, shark plasma augmented the fluorescence of the lymphoblast DNA by 20%, an effect which may be related to the marked increase in the volume of these cells. The frequency distribution curve in Figure 2 has been corrected to account for this effect. Preliminary results on the measurement of synthetic rate of DNA, RNA and protein in L5178Y/AR cells, at early time periods up to 4 hrs indicates that the synthesis of all three macromolecules proceeds at the same rate in both FCS and shark-plsma-treated cells.

Lstly, preliminary attempts were made to characterize the factors present in shark plasma responsible for the magalocytic effect reported here. Exhaustive dialysis did not abolish the cytotoxicity of the plasma. Acrylamide gel electrophoretic analysis of the shark plasma in stacked gels (3.5%, 4.75%, 7% and 12%) revealed three major families of protein bands, one each in the 3.5%, 4.75% and 7% gel. One of the major bands (in the 7% gel region) migrated with the same mobility as mammalian pre-albumin. Attempts are underway to determine which of these proteins is responsible for the effect.

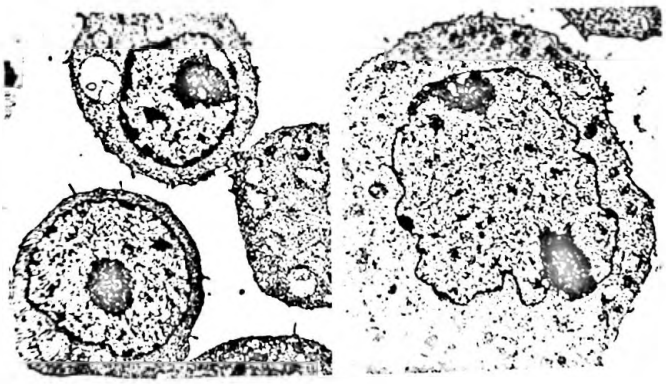

Figure 1. Electron micrographs of L5178Y/AR cells cultured in Fetal Calf Serum (left) and Dogfish Plasma (right). The cells were stained in Uranyl acetate-Lead citrate (x 4000).

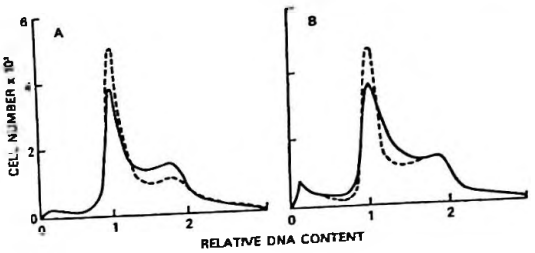

Figure 2. Histograms showing frequency of individual cellular DNA content in L5178Y/AR populations cultured in Dulbecco-Vogt medium supplemented with 10% Fetal Calf serum (—) or 10% shark plasma (——). Curve A, 24 hr culture. Curve B, 48 hr culture.

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Cardiovascular Responses To Hypoxia In *Squalus acanthias*

Barbara Kent, jean Francois Eid, Adrian Bryan-Brown and E. Converse Peirce II. Veterans Administration Hospital, Brons, New York; and Mt. Sinai School of Medicine, New York, N.Y.

Hypoxia and hypercapnia are potent cardio-inhibitory stimuli in the dogfish, *Squalus acanthias*. In past studies the bradycardia produced by CO_2 administration has been shown to be accompanied by a vagally mediated decrease in cardiac output and increse in resistance to blood flow in the gills (*Bull. MDBIBL 9:13, 1969*). In the present investigation the effect of hypoxia on the gill circulation was studied.

Six dogfish of either sex weighing between 1.8 and 5.8 Kg were prepared as described in *Bull MDIBL 8:20, 1968* for measurement of pre-gill ventral aortic pressure (VAP), post gill dorsal aortic pressure (DAP) and cardiac output (Q_B). Cardiac output was measured from an electromagnetic flow probe (Carolina Medical) placed around the ocnus arteriousus. Gill resistance (R_G) was calculated as (VAP-DAP)/Q_B and systemic resistance (R_S) as DAP/Q_B. Heart rate (H.R.) was read from the presure trace. The gills were perfused with fresh 15°C sea water (3L/min) equilibrated in a bubbler (Seal Corp) with 100% oxygen or, during ten minute intervals, with 100% nitrogen. Arterial and venous blood samples (s ml each) were taken after 15 minutes exposure to sea water and 100% O_2, after 10 minutes of sea water and 100% N_2, and again after 10 minutes of sea water equilibrated with 100% O_2. PO_2, oxygen content (cO_2), and pH were measured for each sample s described in *Bull. MDIBL 14:17, 1974* and PCO_2 was derived by a method described in *Bull. MDBIL 7:27, 1967*. Oxygen consumption (VO_2) was calculatedb/100. One fish was given atropine (2mg/Kg) and the above measurements were repeated during $O_2$and N_2 equilibration.

A profound hypoxemia resulted in fish exposed to sea water bubbled with 100% N_2. The PO_2 of arterial blood fell from 483$\pm$47 mmHg with 100% O_2 to 16$\pm$5 mmHg during the hypoxic stimulus as seen in the figure.