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).

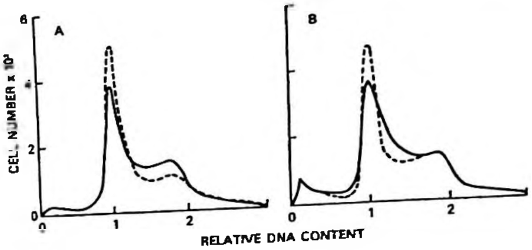

Figure 2. Histograms showing frequency of individual cellular DNA content in L5178Y/AR populations cultured in Dulbecco-Vogt medium supplemented with 10% Fetal Calf serum (—) or 10% shark plasma (-----). Curve A, 24 hr culture. Curve B, 48 hr culture.

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Cardiovascular Responses To Hypoxia In *Squalus acanthias*

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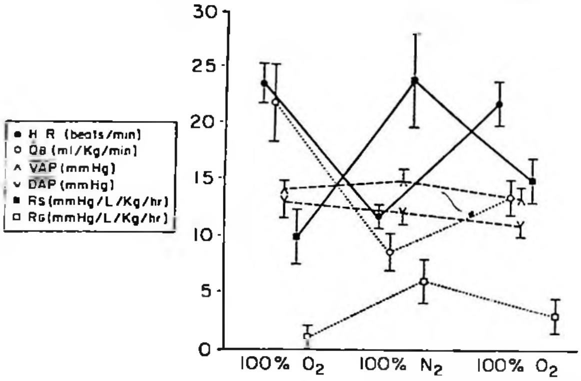
Hypoxia and hypercapnia are potent cardio-inhibitory stimuli in the dogfish, *Squalus acanthias*. In past studies the bradycardia produced by CO_2 administration has been shown to be accompanied by a vagally mediated decrease in cardiac output and increse in resistance to blood flow in the gills (*Bull. 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±47 mmHg with 100% O_2 to 16±5 mmHg during the hypoxic stimulus as seen in the figure.

Oxygencontent and PO$_2$of both arterial and venous blood fell significantly as did the oxygen consumption. There was a statistically significant return of these values when the sea water to the animal was agin oxygenated. pH and PCO$_2$ did not change significantly during the period of hypoxia. With the fall in oxygen tension there was a sharp decrease in heart rate and cardiac output. Ventral aortic pressure, however, rose slightly, dorsalaortic pressure fell, and the gill resistance increased five-fold. There was also an increase in the calculated systemic resistance with hypoxemia. All these hemodynamic variables returned toward pre-hypoxic levels when the fish was returned to sea water equilibrated with 100% O$_2$. In the fish given atropine the changes in response to hypoxemia were abolished.

PaO$_2$(mmHg)	483±47	16±5	357±114
PvO$_2$(mmHg)	25±6	12±3	25±3
CaO$_2$(VOL %)	4.5±0.5	0.5±0.2	4.2±0.7
CvO$_2$(VOL %)	0.7±0.2	0.2±0.1	0.5±0.2
Vo$_2$(cc/Kg/min)	1.10±0.50	0.03±0.01	0.10±0.05
pHa*	7.37±0.10	7.41±0.10	7.45±0.05
PaCO$_2$*(mmHg)	5.1±1.0	4.8±0.6	6.2±1.9
PvCO$_2$*(mmHg)	6.7±1.3	8.9±1.5	9.6±1.1



*NO SIGNIFICANT DIFFERENCE BETWEEN 100% O$_2$ AND 100% N$_2$ VALUES WITH PAIRED T TEST

Vagally mediated bradycardia accompanying anoxia or hypoxemia is commonly observed in many species of lower vertebrates including dogfish. The change in pre-gill and post-gill pressures in response to hypoxemia are similar to those reported by Satchell (*J. Exp. Biol. 39:503, 1962*). The cardiac output values found furing equilibration of inspired sea water with 100% O$_2$ in this study are in agreement with those reported in the literature. The oxygen consumption values during 100% O$_2$ equilibration reported here are higher than those found in dogfish in sea water equilibrated with air (*J. Cell. Physiol. 67:93, 1966*). Since blood is only 85% saturated in free swimming fish (*Resp. Physiol. 1:13, 1966*) with PaO$_2$ of close to 100 mmHg, equilibration with 100%O$_2$ adds a significant amount of oxygen to the arterial blood. The dogfish is an oxygen uptake 'conformer" so an increase in oxygen delivery results in an increased oxygen consumption. The fall in cardiac output and the concomitant rise in gill resistance in response to hypoxia in this study are similar to the response to hypercapnia. In this study, however, there were no changes in pH or PCO$_2$. The pH values for this group of fish is low and the PCO$_2$ is elevated. If the response to hypoxia a hypercapnia are aditive then the excess acid and C might attenuate the observed response to hypoxem The response to hypercapnia, however, might secondary to hypoxia brought about by the effect of h CO$_2$ on the affinity of hemoglobin for oxygen. Since gill reflex response to hypercapnia is present in f equilibrated with 95% O$_2$, it is dobtful that hypoxia is necessary comonent of the response to hypercapnia. T similarity in effect of hypercapnia and hypoxia m indicate a common site of afferent stimulation like t peripheral or central chemoreceptors in mammal systems.

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Relationships Between Cardiac Output And Oxyge Consumption In *S. Acanthias*

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The dogfish *S. acanthias* belongs to a group o animals called oxygen "conformers" because oxyge consumption is dependent on the amount of availab oxygen. Oxygen availability is dependent on man factors. The rate of blood flow or cardiac outpu influences oxygen availability in two ways: a) by the amount of oxygen taken up in the gills; b) by the amoun of oxygen delivered to the tissue. In this study some relationships between cardiac output and oxygen consumption were determined.

Nine dogfish of either sex weighing between 1.5 and 6.0 Kg were prepared for measurement of cardiac outpu (Q$_B$) and oxygen consumption (VO$_2$) as described in report #25 of this bulletin. Fish were volume loaded with 150cc dogfish Ringer's given in ten minutes into the caudal artery and bled by the same amount over a thirty

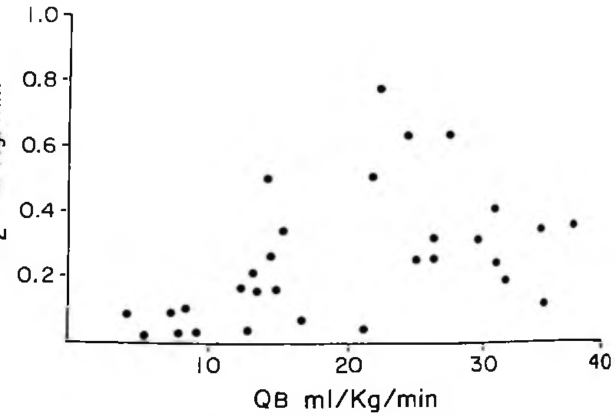

Figure 1. Cardiac output vs. oxygen consumption in 9 fish.