grains. This corresponds to a zone where adjacent cells exhibit very little interdigitation. Nuclei are free of grains. In most areas the grains seem to follow basal and lateral cell borders. This is especially evident in cells that have been pulled apart slightly. Where grains do appear to be over cellular cytoplasm they are probably over cell interdigitations described above. Control binding studies (scintillation counting) were consistent with specific tissue binding of ^{3}H-ouabain, e.g., incubation medium high in potassium markedly reduced binding. Present morphological and autoradiographic observations provide direct evidence that the Na-K-ATPase is located on the basal and lateral plasma membrane in flounder urinary bladder. Thus as in many epithelia this enzyme is located at the cellular pole to which Na is transported.

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EFFECT OF pH ON OXYHEMOGLOBIN DISSOCIATION CURVES IN *Squalus acanthias*

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In earlier studies we reported a gill reflex response in *Squalus acanthias* in which a vagally mediated bradycardia and vasoconstriction of the gill vasculature are induced by an increase in sea water P_{CO_2}. Hypoxia is known to induce a similar response (Satchell, G.H., J. Exper. Biol. 39: 503, 1962). pH influences oxygen affinity of many marine blood pigments. The Bohr effect causes a shift to the left of the relationship between P_{O_2} and percent saturation with increasing pH or decreasing P_{CO_2}. The Root

effect is a decrease in oxygen capacity with fall in pH. It was postulated that the response to hypercapnia in the gill reflex might be secondary to oxygen affinity hypoxia since decrease uptake of O_2 by gill could cause a drop in P_{O_2} in a post-gill chemoreceptor where the pH shift might be less. In order to determine the magnitude of the Bohr and Root effects in dogfish blood, oxygen dissociation curves at several levels of blood pH were studied.

A total of 153 arterial and venous blood samples of 2 ml each were taken from 20 partially restrained dogfish. Sea water flowing through the gills of the fish was equilibrated with air, 100% O_2, 5% CO_2 in air and 5% CO_2 in oxygen. Blood samples were drawn from catheters implanted percutaneously in the ventral and dorsal aortas. The following measurements were made on each sample: hematocrit, P_{O_2} (with Radiometer gas monitor and Clarke type membrane P_{O_2} electrode), oxygen content (Lexington Instrument Oxygen analyzer), and pH (Radiometer pH meter and micro electrode). The P_{O_2} and pH electrodes were kept at 15°C, the same as the temperature of the fish. In four fish blood was collected from the caudal artery, centrifuged and the plasma saved. Oxygen content, P_{O_2}, and osmolality were measured on plasma and sea water samples equilibrated with room air and 100 percent oxygen.

The slope of the linear regression formula between P_{O_2} and oxygen content for plasma was used to determine the solubility of oxygen in the blood. The solubility coefficient of oxygen in dogfish plasma was found to be 0.02ml/ml liquid at 760 mmHg at 15°C. An identical value was found for sea water. In order to compare blood samples of different hematocrits the dissolved oxygen was subtracted and the remaining oxygen content value was corrected to a hematocrit of 20 percent. Percent saturation of hemoglobin was calculated using oxygen content at P_{O_2} greater than 200 mmHg as 100

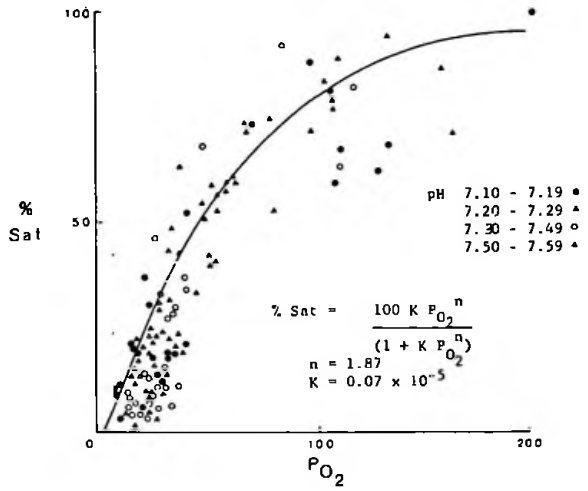

Figure: An oxyhemoglobin dissociation curve for *Squalus acanthias*. Individual points designated by pH levels. Solid line represents best fit of combined data to Hill approximation.

percent. Paired values of P_{O_2} and percent saturation for blood samples were divided into groups according to pH intervals of 0.1 pH unit between pH of 7.10 and pH of 7.60.

Oxygen dissociation curves for each pH interval were generated by fitting the data to the Hill equation: $Y = 100\ K\ (X)^n / 1 + K\ (X)^n$ where Y is percent saturation, X is P_{O_2}, and K and n are constants related to the affinity of hemoglobin for oxygen. K and n were used to calculate the P_{O_2} at which the hemoglobin was 50 percent saturated, the P_{50}, for each pH interval P_{50} was

found to decrease with increasing pH's such that $\log P_{50}/pH = -2.8$. Over the range studied P_{50} shifted from 61 mmHg at pH interval 7.10-7.19 to 44 mmHg at pH 7.50-7.59, indicating a Bohr effect. At P_{O_2} equal to 200 mmHg the percent saturation calculated from K and n increased from 85 percent at the lowest pH interval to 97 percent at the highest. The depressed capacity at low pH is indicative of a Root effect. The difference in the parameters K and n over the pH intervals used was not statistically significant. The combined data shown in the figure yields an n = 1.87 and a $K = 0.07 \times 10^{-4}$. The P_{50} for this data fit to the Hill equation was 50 mmHg.

There are no other reports in the literature of an oxyhemoglobin dissociation curve for blood from *Squalus acanthias*, but in studies on closely related species (Resp. Physiol. 1:13-29, 1966) there was no demonstrable Bohr effect or Root effect and the Hill constant n was close to 1 (Resp. Physiol. 5:326-337, 1968). Average arterial saturation reported in the literature is 84.5 percent. Using 110 mmHg as an average arterial P_{O_2} for *Squalus acanthias* (Robin and Murdaugh, Sharks, Skates and Rays p288) saturation predicted from the dissociation curve found in this study is 82 percent. The curve generated by the data to fit the Hill equation in this study is very similar to a hyperbolic oxygen dissociation curve derived from data from this lab on hemoglobin solutions of dogfish blood equilibrated with gases of varying P_{O_2}. In comparison to human blood at 37°C, the blood of *Squalus acanthias* has considerably less heme-heme interaction (n = 2.4-2.8 for human blood) and a lower affinity for oxygen (P_{50} = 26 mmHg for human blood). Since blood from several fish was used and actual measurement of both oxygen content and P_{O_2} were made, there was considerable scatter in the data. Over the ranges of pH studied here there is evidence of both Bohr and

Root effects. It is concluded that, given a peripheral receptor, oxygen affinity hypoxia may be a factor in triggering the gill reflex response to hypercapnia.

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HEMODYNAMIC RESPONSES TO VOLUME LOADING AND HEMORRHAGE IN *Squalus acanthias*

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Stressing the circulatory system by changing volume is one of the classic methods for studying circulatory control. Hemodynamic responses to large changes in volume were studied in *Squalus acanthias*.

Large female dogfish (4.8±1.2 kg) were placed in a trough in a sea water aquarium and their gills perfused with sea water (15°C) at 1 - 3 L/min. A #16 gauge needle was introduced into the caudal artery for measurement of dorsal aortic pressure and for injection and withdrawal of fluid. Heparin was given (1000 units/fish). Dorsal aortic pressure, sensed by a Statham P23"V" transducer, was recorded on one channel of a two-channel Beckman dynograph recorder. Heart rate was determined from recorder pressure peaks. The opercular opening rate was taken visually with a stopwatch. Blood samples (2 ml) were taken at 15-minute intervals for determination of hematocrit, P_{O_2} (Radiometer gas monitor and Clarke electrode) and pH (Radiometer pH meter and micro electrode). Time intervals for the experiment were as follows: (1) 15 minutes for stabilization and collection of control