

PERIPHERAL VASCULAR CONTROL IN *Squalus acanthias*

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Since the spiny dogfish (*Squalus acanthias*) apparently does not possess a mammalian-type neurogenic control of its peripheral vascular system (Opdyke, et al., *Comp. Biochem. Physiol.* 42A: 611-620, 1972) it is conceivable that arterial blood pressure at any moment is determined solely by the amount of blood in the system. This hypothesis assumes that the pressure-volume relationship (vascular compliance) is fixed and that pressure will vary directly with change in blood volume. Failure to conform to this hypothesis would indicate the presence of a mechanism for controlling system capacity, but not necessarily a baroreceptor control system such as that which operates in mammals.

Experiments have been carried out in which plasma volume has been changed acutely by injection of isotonic and hypertonic saline, hypertonic urea, and hypotonic dextran solutions. Each of these solutes presents the fish with a different problem in maintaining normal plasma osmolality. The dogfish can excrete sodium efficiently via the kidney and rectal gland (Burger, *Physiol. Zoo.* 38:191-198, 1965) but urea only very slowly (Goldstein and Forster, *Bulletin, MDIBL*, 10:20-22, 1970). Sodium distributes chiefly to the extracellular compartment while urea enters both the extracellular and intracellular compartment. Dextran is confined primarily to the vascular compartment, is excreted slowly, and retains water tenaciously.

Unanesthetized dogfish (1.5-2.0 Kgm) were kept in a narrow, shallow tank provided with a continuous flow of fresh, cool seawater. A polyethylene catheter (PE 60) was placed in the dorsal aorta via the caudal artery and connected to a P23AA Statham gage-Electronics-for-Medicine recording system. Dorsal aortic pressure is reported as S(ystolic) and D(iastolic), indicating the highest and lowest pressure recorded during a single typical cardiac cycle. Zero blood pressure reference level was set at the level of the pectoral fin of the submerged fish. The catheter also served for the withdrawal of blood samples and the injection of solutions. Heart and gill movement rates were determined by measurements on the blood pressure records. These did not change significantly during the course of the experiment and are not reported here. Plasma volume was measured by injection of 1-2 mgm Evans Blue Dye (T-1824). Blood samples were taken at 0, 10, 20, and 30 minutes for plasma volume determinations. Plasma osmolality was determined on 1 ml samples once diluted with ion-free water by means of a Precision Systems Osmette A osmometer. Microhematocrits were obtained on all blood samples. Standard statistical methods were employed in the analysis of the data.

Injection of Isotonic Saline (0.55M; 1030 mOsm/Kgm). Table 1 shows the result of injecting 5 ml isotonic saline at one-minute intervals in each of 22 fish until a total of 25 ml had been injected. The total volume injected was calculated to increase the initial plasma volume by approximately 25 percent. Although a slight increase in blood pressure was observed (maximum 116 percent systolic, 106 percent diastolic) the change was not significant. Based on the initial measurement of plasma volume and hematocrits before and after injection it was calculated that plasma volume increased an average of 33 ml. Plasma osmolality was not measured in this series of experiments.

TABLE 1

Injection of 5 ml Increments of Elasmobranch Isotonic Saline
Solution (1030 mOsm/kg) at one minute intervals

N = 22

Blood Pressure Response

% B.P.	Control	5 ml	10 ml	15 ml	20 ml	25 ml
Systolic	100%	108 ± 5.4%*	116 ± 7.4%	112 ± 6.7%	111 ± 6.0%	112 ± 7.1%
Diastolic	100%	103 ± 4.0%	106 ± 5.4%	102 ± 4.6%	101 ± 3.8%	102 ± 4.6%

Avg. Control Plasma Volume: 88 ± 5.0 ml (5.69 ± 0.3% Body Wt.)

Avg. Control Hematocrit: 19.9 ± 1.3%

Avg. Hematocrit After Last Injection: 14.5 ± 1.7%

Calculated Plasma Volume One Minute After Last Injection (Total injection volume, 25 ml):
121.0 ± 6.0 ml

Calculated Net Average Increase in Plasma Volume: 33 ml

*Standard error of means

Injection of Hypertonic Saline - (1.1 M; 2081 mOsm/Kgm). Ten ml of the hypertonic saline solution were injected at 10-minute intervals until a total of 30 ml had been injected. Plasma volume was measured before the first injection and 10 minutes after the third injection. Blood pressure was recorded before, two, four and 10 minutes after each injection. The results are shown in Table 2. The increase in blood pressure between control and 10 minutes after the third injection is not significant. In this respect the response is similar to that observed with isotonic saline. Plasma volume increased significantly ($P < 0.01$), the net increase being 35.5 ml. Plasma osmolality also increased significantly ($P < 0.01$).

Injection of Urea - (3.5 M; 3466 mOsm/Kgm). Injection of the highly hypertonic urea solution did not significantly increase blood pressure either acutely or accumulatively, although a highly significant increase in plasma volume (28 ml) and plasma osmolality (1051 to 1201 mOsm/Kgm) resulted.

Injection of Hypotonic Dextran - (4.5 percent Sol. Clinical Grade Dextran, 7 mOsm/Kgm).

The 10 ml injections of dextran solution increased blood pressure acutely and accumulatively. The difference between control systolic and diastolic pressure and that 10 minutes after the last injection is highly significant ($P < 0.01$). Plasma volume increased significantly by 25.2 ml. Plasma osmolality decreased somewhat but not significantly.

These results do not support the hypothesis that arterial blood pressure in the dogfish is determined solely by the relationship between blood volume and the compliance of the vascular system. The system accommodated, either actively or passively, the increments of isotonic saline and urea without significant pressure increase. The greatest increase in blood pressure occurred as a result of dextran injection which produced the least increase in plasma volume (25.2 ml) and a non-significant decrease in plasma osmolality. Hypertonic saline on the other hand increased blood volume more but blood pressure less. Plasma volume did increase significantly but not significantly greater than in the case of urea injection which only increased blood pressure very slightly. The greatest increase in plasma osmolality was observed after urea injection.

TABLE 2
Blood Pressure, Blood Volume and Plasma Osmolality Response to
Multiple Injections of Hyper- or Hypotonic Solutions

Treatment	Blood Pressure Response mmHg		Blood Volume ml	Plasma Osmolality mOsm/Kg
	Control \pm SEM	10 Min. Post Inj.		
Hypertonic Saline				
1.1 M N = 8				
1st Inj (10 ml)	S 20.1 \pm 2.4 D 13.4 \pm 1.8	25.4 \pm 2.8 16.1 \pm 1.8	Control \pm SEM 96.5 \pm 5.6	Control \pm SEM 1016 \pm 24
2nd Inj (10 ml)	S 25.4 \pm 2.8 D 16.1 \pm 1.8	25.2 \pm 2.9 14.8 \pm 1.6	10 Min. after third Injection \pm SEM 132 \pm 8.2	10 Min. after third Injection \pm SEM 1083 \pm 27
3rd Inj (10 ml)	S 25.2 \pm 2.9 D 14.8 \pm 1.6	26.1 \pm 2.9 14.8 \pm 1.9	= 35.5 ml	= + 67 mOsm/Kg
Urea				
3.5 M N = 12				
1st Inj (10 ml)	S 17.4 \pm 1.0 D 11.3 \pm 1.0	19.5 \pm 1.5 11.7 \pm 0.9	Control \pm SEM 93.6 \pm 6.2	Control \pm SEM 1051 \pm 18
2nd Inj (10 ml)	S 19.5 \pm 1.5 D 11.7 \pm 0.9	20.3 \pm 1.4 12.7 \pm 1.1	10 Min. after third Injection \pm SEM 121.6 \pm 5.4	10 Min. after third Injection \pm SEM 1201 \pm 36
3rd Inj (10 ml)	S 20.3 \pm 1.4 D 12.7 \pm 1.1	21.6 \pm 1.2 14.7 \pm 0.7	= 28 ml	= + 150 mOsm/Kg
Dextran				
4.5% Sol. N = 8				
1st Inj (10 ml)	S 20.1 \pm 1.3 D 14.0 \pm 1.1	23.9 \pm 1.1 17.2 \pm 1.3	Control \pm SEM 96.0 \pm 4.6	Control \pm SEM 1066 \pm 16
2nd Inj (10 ml)	S 23.9 \pm 1.1 D 17.2 \pm 1.3	25.9 \pm 0.6 18.1 \pm 1.3	10 Min. after third Injection \pm SEM 121.2 \pm 5.0	10 Min. after third Injection \pm SEM 1028 \pm 19
3rd Inj (10 ml)	S 25.9 \pm 0.6 D 18.1 \pm 1.3	30.1 \pm 1.9 19.0 \pm 0.8	= 25.2 ml	= - 38 mOsm/Kg

These results suggest that the peripheral vascular system expanded to accommodate the increase in blood volume resulting from urea injection but failed to accommodate a nearly equal increase resulting from dextran injection. In fact considering the greater increase in blood volume resulting from hypertonic saline injection with an intermediate blood pressure response, it seems that possibly the *capacity* of the system decreased in response to dextran injection.

The data can be interpreted tentatively to mean that change in the capacity of the vascular system can be induced. Possibly this is initiated by an unknown stimulus (not pressure) and effected by either an as yet undiscovered neurogenic mechanism or by activation of a neurohumoral system

(such as norepinephrine release from the chromaffin tissue which is characteristic in this species). There is a suggestive relationship that warrants further study of changes in plasma osmolality as a candidate for the role of the unknown stimulus.

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THE MORPHOLOGY OF THE BLOOD CELLS OF THE ELASMOBRANCH *Squalus acanthias*

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The microenvironment of the bone marrow appears to play an essential role in hematopoiesis in higher vertebrates. That the study of the production and release of blood cells in an elasmobranch with neither bones nor bone marrow would aid in the more exact delineation of the role of the marrow microenvironment is obvious. The effects upon the hematopoietic system of elasmobranchs of several antineoplastic agents that in mammals are selectively toxic to hematopoietic tissue are of special interest to us. The spiny dogfish *Squalus acanthias* should be an ideal species for this type of study. It is readily available and blood can be collected easily from it. A perusal of the literature however reveals a paucity of information on the blood of this species. This is a preliminary report of a study designed to identify and describe the blood cells of the spiny dogfish prior to extending the studies of Rall, Ratner, and Roomet (Bull. MDIBL 9: 51-52, 1969) on the toxicity of antineoplastic agents upon the hematopoietic system of this species.

Blood was collected from the caudal vein of dogfish and mixed with heparin. Smears were made and stained with Wright's stain. The remaining blood was centrifuged to concentrate the leukocytes, and the pellets were fixed in glutaraldehyde, processed, and examined in an electron microscope.

Examination of the Wright-stained smears revealed oval-shaped erythrocytes 13 to 17 μ in width and 23 to 27 μ long. These cells averaged approximately 15 by 25 μ . A centrally positioned elongated nucleus with dense granular chromatin, averaging 6 by 10 μ was present in each red blood cell. Many immature erythrocytes are present in the blood. They are recognized by nuclei that are larger and rounder and by cytoplasm that is more bluish but less abundant than mature erythrocytes. Upon ultrastructural examination, the erythrocytes, both mature and immature, reveal mitochondria in the cytoplasm. Thrombocytes in the peripheral blood of spiny dogfish are nucleated and fairly numerous. They are oval-shaped and approximately 8 by 14 μ in the blood smears. The nuclei are large and elongated, approximately 6 by 13 μ , and comprise the major portion of each cell. Some of the nuclei contain a cleft along one side near the center. A scant amount of very faint cytoplasm is present around the nuclei. An occasional thrombocyte contained a single red granule in its cytoplasm. Only one type of granulated leukocyte is found. Based on morphology and staining characteristics these polymorphonuclear cells should probably be called heterophils. They are round in the smears and varied in diameter from 16 to 21 μ . Most of the nuclei are bilobed, some oval, and