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ESTIMATE OF CEREBRAL BLOOD FLOW IN *Squalus acanthias*

Barbara Kent, Ph.D., Marjorie Peirce and E. Converse Peirce II, M.D. Veterans Administration Hospital, Bronx, New York

Arteries directing blood to the brain arise rostral to the confluence of the efferent branchials of the first gill arch. Because there is no convenient injection site it has not been possible to determine brain blood flow with conventional intravascular injection methods. In this study small (15μ) diameter microspheres injected into the cardiac ventricle are used to estimate blood flow to the brain. Seven restrained male dogfish (1.7 ± 0.2 Kg) prepared as described earlier (Bull. MDIBL 26, 1971) were injected with 1 ml (1μCi) amounts of ^{85}Sr tagged 15μ diameter microspheres (3M). After an hour the fish were killed and the gills, brain, and kidneys were dissected and weighed. Analysis proceeded as described in report #29 of this bulletin. An average of 69 percent of the 15μ diameter microspheres were caught in the gills. Of the remaining 0.16 ± 0.02 percent were found in the brain and 0.37 ± 0.10 percent in the kidneys. Although the arterial blood supply to the brain of the dogfish is part of the systemic circulation and is in parallel with it there is no central point for injection of microspheres on the arterial side of the circulation that will include the brain. Microspheres injected into the heart that clear the gill capillaries will be carried in the systemic circulation in proportion to blood flow. Thirty-one percent of microspheres 15μ in diameter will pass through the gills. This is not surprising when one considers the diameter of a dogfish erythrocyte is reported to be 15μ also (Kisch, Exp. M. & Surg., 1951). The minimum estimate of blood flow to the brain based on percent of 15μ microspheres is 0.03 ml/gm/min. This is a small fraction of the blood flow through the first gill arch (2.76 ml/min, Bull MDIBL 26, 1971) and closely approximates average blood flow (0.023 ml/gm/min, Bull MDIBL 29, 1967) in the fish. This work was supported by the Veterans Administration Hospital, Bronx, New York 10468, Project #7240-03.

1973 #27

BLOOD FLOW DISTRIBUTION IN *Squalus acanthias:* A SEQUEL

Barbara Kent, Ph.D., Marjorie Peirce, and E. Converse Peirce II, M.D., Veterans Administration Hospital, Bronx, New York

In a previous report (Bull. MDIBL 26, 1971) a preliminary study of blood flow distribution in *S. acanthias* was described using the tagged microsphere method. The number of tissues studied has been expanded and the results are reported here.

Six male dogfish (1.8 ± 0.4 Kg) swam unrestrained in a 0.3 M^3 tank supplied with fresh running sea water. An injection catheter was threaded from the caudal artery up the dorsal aorta to the level of the fifth gill arch. The fish were given 1000 units of heparin. Blood was sampled from the catheter at time 0 when microspheres were given at 30 minutes and at 90 minutes when the fish were killed.

Determinations of pH (Radiometer micro pH meter), 0_2 content (Lex-0_2-Con), and hematocrit were made. At time 30 minutes 1 ml of 50μ diameter ^{125}I tagged and 1 ml of 50μ diameter ^{46}Sc tagged microspheres (3M Brand Tracer Microspheres) were given into the catheter and were washed in by 1 ml dogfish Ringers. The amount of activity in each dose was approximately 1μCi. An hour after the microspheres were given the fish were killed by section of the spino-medullary junction. The following organs were weighed: gills, heart, esophagus, stomach, duodenum, ileum, liver, pancreas, spleen, gall bladder, kidneys, gonads, rectal gland, and brain. Multiple samples of approximately 1 gm were taken from each organ. Samples of skin were taken from above the pectoral fin, beside the dorsal fin, the abdominal lateral line, and caudal lateral line areas. White muscle underlying the skin samples was taken and red muscle was sampled from the lateral line. Cartilage samples were taken from the skull and from the pelvic girdle. The samples were weighed and analyzed for radioactivity by a Nuclear-Chicago gamma counter. The cpm for each sample for each isotope was calculated per gm of tissue. An average cpm/gm was calculated for each fish for both isotopes as follows:

$$\frac{\text{total cpm injected - cpm in gills}}{\text{body weight - gill weight}} = \frac{\text{Average cpm}}{\text{gm}}$$

The relative accumulation (R.A.) of isotopes for each sample was calculated as follows:

$$\text{R.A.} = \frac{\text{cpm/gm tissue}}{\text{Average cpm/gm}}$$

The percent cardiac output going to each organ was calculated as:

$$\% = \frac{\text{R.A. x 0.023 ml/gm x organ weight (gms)}}{\dfrac{\text{1.4 x average body weight x 1000}}{60}}$$

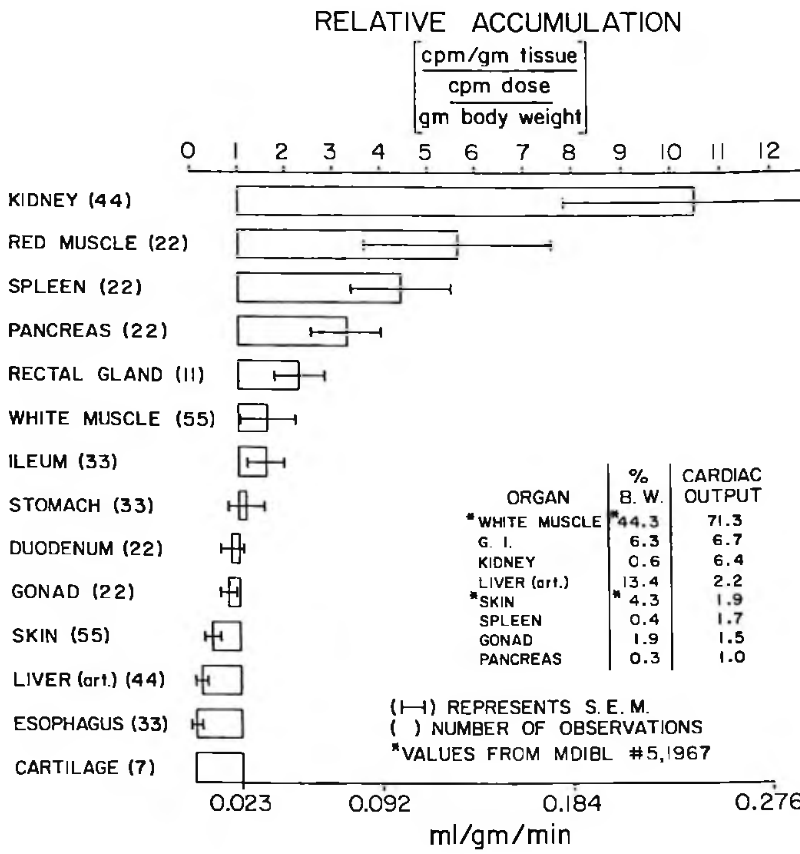
Organ weight for muscle, cartilage and skin were estimated as 43, 14 and four percent of body weight respectively (Bull. MDIBL 5, 1967). Total cardiac output was estimated as 1.4 1/Kg/hr (Bull. MDIBL 29, 1967). A paired T-test was used to compare the distribution of the two isotopic tags.

There was no significant difference in the distribution pattern of the two isotopes. Between one and two percent of the microspheres traversed the systemic circulation and appeared in the gills. The remainder were distributed throughout the body as seen in Figure 1. Kidney tissue on a per gram basis accumulated over 10 times the number of microspheres which would be expected if the dose were evenly distributed throughout the fish. This corresponds to an arterial flow of 0.24 ml/gm/min and a portion of the cardiac output equal to 6.4 percent. White muscle, the largest tissue mass in the body, received 71.3 percent of the cardiac output. Red muscle, spleen, pancreas, and rectal gland are all higher than average flow tissues. Flow in red muscle seems to be related to activity in that the three most active fish had the three highest flow rates per gm values in red muscle. This correlation did not hold for white muscle. Areas of less than average perfusion are the gonads, skin, esophagus, and finally cartilage which is avascular.

In each fish pH values rose over the 90-minute course of the experiment form a mean of 7.44 ± 0.11 to 7.53 ± 0.12 (SD). Oxygen content decreased significantly from 4.2 ± 0.4 to 3.6 ± 0.1 volumes percent with a drop in hematocrit from 15.4 ± 1.8 percent to 13.3 ± 2.1 percent.

The rise in pH over the 90-minute experimental period probably reflects a return toward normal of a pH lowered during the time the animal was taken from the live-car to the tank. The drop in arterial oxygen content is directly related to a drop in hematocrit which resulted from a slight blood loss from the point of insertion of the cannula in the caudal artery and from samples.

In the present study the tissues sampled comprise 86 percent of the body weight. Undoubtedly stomach contents, blood, and other fluid account for a sizeable portion of the remaining body weight. Not included are the heart and organs of the nervous system. The hypobranchial artery which eventually gives rise to the coronary artery leaves the second gill arch collector loop and the internal carotid artery which is the primary arterial blood supply to the brain arises beyond the first gill arch and before the beginning of the dorsal aorta (Vertebrate Dissection, 1970, p. 292). Since only a small percentage of microspheres reaches the gills in this preparation and it has been shown that 93 percent of the 50μ microspheres are cleared by the gills (Bull. MDIBL 26, 1971), there are no detectable microspheres delivered to the heart and brain. Ninety-three percent of the cardiac output is



accounted for in the present study. The remaining seven percent must be distributed to the red muscle, heart, brain, and related sensory organs. An estimate of brain flow is present in report #26 of this bulletin.

Smooth muscle and striated muscle make up 50 percent of the body weight and microsphere accumulation is close to the average in these tissues. Blood flow in muscle then is closely approximated by total body flow measurements. As might be expected those tissues important to the fish for osmoregulation and metabolism are richly perfused while the less active tissues are poorly perfused. This work was supported by the Veterans Administration Hospital, Bronx, New York, Project #7240-03.