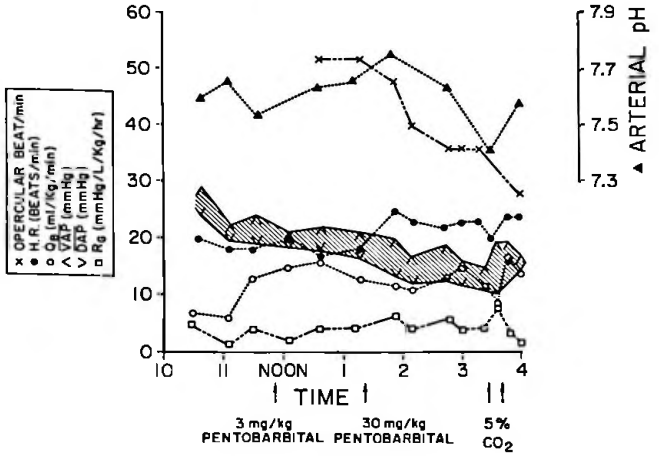

Figure 3. Effects on hemodynamic parameters of 3 mg and 30 mg/Kg pentobarbital followed by hypercapnia. Fish partially restrained and gills artificially irrigated.

on oxygen consumption or cardiac output. Higher doses are lethal unless the gills of the fish are artificially irrigated. Doses lower than 10 mg/Kg have no effect on any of th e parameters measured in this study. It is questionable that doses below 10 mg/Kg are effective at all.

In artificially irrigated fish, large doses of sodium pentobarbital (30 mg/Kg) have a slightly depressant effect on cardiac output and oxygen consumption, but the cardiovascular responsiveness of the animal is not interrupted as demonstrated by the reflex response to hypercapnia. There is, however, a demonstrable uncoupling of the usual cardio-ventilatory interaction. High doses of pentobarbital lead to tachycardia which is probably due to a decrease in vagal inhibition secondary to CNS depression. The concomitant decrease in opercular rate could be explained either as a central, medullary depressant effect, or again as a decrease in vagal inhibition. It has been postulated that vagal afferent, similar to Hering-Breuer stretch receptors in mammalian systems, operate in the fish gill to control opercular rate (Nature 211: 1187-1188, 1966). A direct effect on the vagus could then increase heart rate and inhibit the opercular rate.

The duration of action of the sodium pentobarbital is related to the size of the dose. The range between the dose which effectively changes opercular rate and the lethal dose is narrow. Pentobarbital in doses between 10 and 20 mg/Kg does not show the progressive deleterious effects found with MS-222. That sodium pentobarbital has any anesthetic effect in this range is doubtful, and it has a minimal effect on cardiovascular and metabolic systems. This project supported by Veterans Administration Hospital, Bronx, New York, Project numbers 4901-01 and 4901-02 and NIH General Research Support Grant for Mount Desert Island Biological Lab (#5 507RR05764) and the Atwater Kent Foundation.

A METHOD FOR DETERMINING RELATIVE FUNCTIONAL SURFACE AREA OF THE DOGFISH GILL

Jean-Francois Eid, Michael Levy, Ted Veltfort, E. Converse Peirce II and Barbara Kent, Departments of Surgery, Bronx Veterans Administration Hospital, Bronx, New York, Mt. Sinai School of Medicine, New York, N.Y. and Universitee Libre de Bruxelles, Brussels, Belgium

Although neural and hormonal stimuli cause vasomotion of the gill vasculature the effect on the respiratory exchange area is not clear. A change in resistance in gill vasculature can be estimated from the ratio of pre- and post gill pressure and cardiac output. When cardiac output is maintained constant, a change in driving pressure across the gills reflects a change in resistance. Because of the parallel

lamellar structure of the dogfish gill, two possible mechanisms can bring about a decrease in gill resistance: vasodilatation or recruitment. An increase in gill resistance will result from vasoconstriction or negative recruitment. A resistance change, therefore, may or may not reflect a proportional change in functional surface area. For instance, the increase in gill resistance during a hypoxic stimulus might be related to an increase in functional surface area if the mechanism were narrowing of the intralamellar blood space, analogous to vasoconstriction. On the other hand, the resistance increase might be caused by a decrease in the total number of perfused lamellae, negative recruitment, in which case functional surface area would be less. In order to differentiate between these two mechanisms and to elucidate the action of a variety of stimuli on blood flow in the fish gill, we have developed a method for determining relative functional surface area using thermal wash-out techniques.

Fish are maintained on an extracorporeal perfusion circuit which delivers blood to the gills at a constant rate. The fish temperature is that of the sea water (about 15°C) perfusing the gills and in which the fish is submerged. The blood delivered to the gills is warmed slightly above the fish temperature in a heat exchanger at room temperature (17-20°C). Heat is lost from the blood to the water during the passage of the blood through the gills. The amount of heat lost is assumed to be directly proportional to the functional surface area of the gill. Changes in temperature are recorded by a thermistor in the immediate post-gill circulation. It is assumed that an increase in post-gill temperature reflects a decrease in functional surface area, while a decrease in temperature means more heat was lost in transit across the gills, so functional surface area increased. This assumption is predicated on a constant water space flow, i.e., ventilation is unchanged.

Seven fish (1.5-2.2 Kg) were given 20 mg/Kg sodium pentobarbital and were artificially irrigated with fresh sea water at 2 liters/min. Sea water was equilibrated with appropriate gases (O_2, N_2, CO_2) in a Scholander Corp. bubbler. The fish were maintained on a total body perfusion circuit (Bull. MDIBL 9:45, 1969) at a constant flow rate. Pre- and post-gill pressures were measured alternately using a Statham P23V transducer and Beckman recorder. After removal of the G.I. tract at mid-eosphagus for visualization of the dorsal aorta, a Fenwal GB41M2 mini-probe thermistor was implanted in the immediate post-gill dorsal aorta. The thermistor was coupled with an amplification circuit to another channel of the Beckman recorder. On the linear portion of the thermistor response curve changes in temperature as small as 0.003°C could be read reliably.

Changes in gill resistance were elicited by hypoxia (Bull. MDIBL 15:49-50, 1975), hypercapnia (Bull MDIBL 8:35-38, 1968) and vasoactive drugs (Bull. MDIBL 10:59-63, 1970), while changes in temperature were recorded in the dorsal aorta.

Small pulse changes in temperature (0.003°C) were seen in the dorsal aorta in synchrony with the pump in the perfusion circuit. Much larger changes in temperature (0.10 to 0.20°C) were observed when resistance in the gill changed. Figure 1 shows that as gill resistance increased in response to hypercapnia the temperature of the post-gill blood rose concomitantly. The changes were simultaneous and similar in

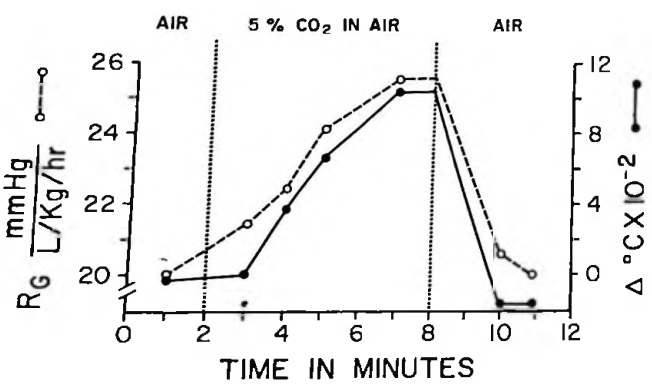

Figure 1. Dorsal aortic blood temperature change and gill resistance (R_G) change recorded during hypercapnia stimulation.

magnitude. Likewise the rise in gill resistance to hypoxia was followed closely by a rise in post-gill blood temperature to 11 x 10^{-2} °C as seen in Figure 2. Table summarizes gill resistance changes to hypoxia in 8 fish and to hypercapnia in 6 fish. In every case an increase in vascular resistance was accompanied by

TABLE 1

Stimulus	#	Δ R_G mmHg/L/Kg/hr	Δ T gm cal/min
Hypoxia	8	+4.1 ± 0.8	+5.4 ± 0.8
Hypercapnia	6	+8.5 ± 1.8	+3.9 ± 0.5
(50 α) Epinephrine	2	+5.2	-3.2
(100 α) Serotonin	2	+10.0	+3.8
(12 mg/Kg) Atropine	1	-1.0	-5.1

+ or - indicates an increase or decrease in resistance and dorsal aortic blood temperature

an increase in recorded temperature in the dorsal aorta, i.e., a decrease in heat loss across the gills. In the two fish given epinephrine, however, the increase in gill resistance was followed by a decrease in dorsal aortic blood temperature. Serotonin mimicked the response to hypoxia and hypercapnia. Gill resistance and post-gill temperature rose concomitantly as seen in Figure 2. In one fish given atropine, a slight

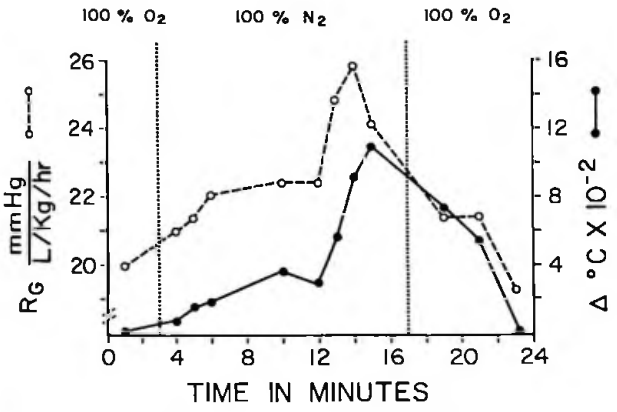

Figure 2. Temperature and resistance changes with hypoxia.

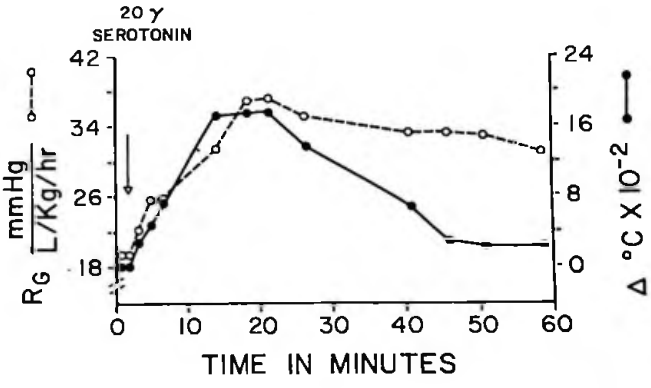

Figure 3. The effect of serotonin on heat loss and resistance to blood flow across the gill.

fall in gill resistance was accompanied by a decrease in dorsal aortic blood temperature.

This study represents the first attempt to quantitate functional surface area in the elasmobranch gill. Several studies using isotopic flux techniques, have been made of relative functional surface area in teleost gills. The rate of ^{22}Na loss was higher in "active" than in "quiet" trout, and addition of norepinephrine increased isotope loss (Comp. Biochem. Physiol. 41A:629-637, 1972). Using ^{14}C urea influx as an indicator of functional surface area, again the catecholamines were found to increase influx in trout (J. Comp. Physiol. 94:267-286, 1974) but serotonin drastically reduced uptake (Fed. Proc. 35(3):528, 1976). Our results, using epinephrine and serotonin in dogfish gill corroborate the teleost studies, i.e., an increase in functional surface area with epinephrine and a decrease with serotonin.

The current debate concerning the existence of non-respiratory blood pathways (shunts) in teleost gills obscures the interpretation of a possible mechanism for changing functional surface area. Dogfish gills

appear to be structurally simpler and the possibility of an anatomical non-respiratory shunt is more rem The elasmobranch is left with two possible mechanisms for adjusting functional surface area, recruitment lamellar narrowing. Although the results of the present study are preliminary, it is clear that hypoxia, hypercapnia and serotonin decrease functional surface area while resistance to blood flow increases. Thi gives evidence in support of the recruitment theory, because a decrease in functional surface area with increasing resistance could occur only if fewer lamellae were being perfused. Epinephrine probably acts decreasing the intralamellar space. In this case the water space may be expanded. This concept is consistent with the findings of an increased gill resistance and increased functional surface area. The eff of atropine, opposite to the effect of hypoxia and hypercapnia, is to be expected. Since vagal pathways carry information which increases gill resistance while decreasing functional surface area, it is not sur prising that atropine brings about the converse.

The thermal wash-out technique provides a sensitive, reproducible, simple method for quantifying chan in functional surface area in the fish gill. The method, in elasmobranchs, provides a way of differenti- ating between recruitment and lamellar narrowing as mechanisms controlling gill blood flow. This project supported by Veterans Administration Hospital, Bronx, New York, Project #4901-01 and 4901-02.

KIDNEY FUNCTION IN SPINY MICE (*Acomys cahirinus*) ACCLIMATED TO WATER RESTRICTION

Howard Haines, Department of Zoology, University of Oklahoma, Norman, OK and Bodil Schmidt-Nielsen, Mount Desert Island Biological Laboratory, Salsbury Cove, ME.

Spiny mice (*Acomys cahirinus*) readily acclimate to conditions of water shortage (Borut et al. Int'l. Congr. Physiol. Paris, 1977). This preliminary study was done to evaluate the role of the kidney in the acclimation process.

Eighteen *Acomys cahirinus* were studied. The animals were maintained several months previous to this study in the animal facilities of the MDIBL. Temperature was 30°C with a 12 hours light, 12 hours dark cycle. One week prior to testing the animals were placed individually in cylindrical wire cages. The ca were equipped with food and water receptacles and could be placed on petri dishes for collection of urine samples. Six control animals received water and food as desired (controls). Six acutely dehydrated animals (ADA) were deprived of water for 24 hours prior to testing. Six chronically dehydrated animals (CDA) were forced to acclimate over a 14-day period to minimal water supply. The acclimation procedure us was described earlier (Haines et al. Am. J. Physiol. 227:958-963, 1974).

Glomerular filtration rate was measured with ^{14}C polyethylene glycol (MW 4000) after the method of Truniger and Schmidt-Nielsen (Am. J. Physiol. 207:971-978, 1964). Urine samples (usually 20-50 µl) were diluted 5-10 times with distilled water. Aliquots of diluted urine were counted by liquid scintillation (Packard, Tricarb 3002) for ^{14}C. Osmolality was measured by vapor pressure osmometer (Wescor), concentrations of Na^+ and K^+ by Instrumentation Laboratory Model 343, and urea by the Conway method (Conway, E.J., and E. O'Malley. Biochem. J. 36:655, 1942). Samples of plasma were counted and analyzed identically to urine samples except that there was no dilution.

The average body weight of controls was 48.5 ± 2.4 g; in acutely dehydrated animals it was 36.0 ± 2.5 and in chronically dehydrated animals it was maintained at 32.6 ± 2.1 g. Acutely and chronically dehydrat animals showed no differences from controls in plasma solute concentrations or osmolality. This observati supports previous findings (Horowitz and Borut, Comp. Biochem. Physiol. 51A:827-831, 1975) and our unpubl data on the capacity of this species to maintain normal plasma concentrations during dehydration.

Urine flow rate (V) in ADA was approximately one-quarter that of the controls. In CDA it was less tha one-tenth that of controls (Table 1). Glomerular filtration rate (GFR) was greatly reduced in the CDA and unchanged in ADA animals (Table 1). Urea clearance (C_u) in ADA was unchanged from controls but was reduc