# ERYTHROCYTES PLAY A SIGNIFICANT ROLE IN $CO_2$ EXCRETION ACROSS THE SHARK GILL

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Despite earlier data to the contrary (Haswell & Randall, Resp. Physiol. 28:17, 1976; Haswell et al., Am. J. Physiol. 238:240, 1980), it is now clear that erythrocyte carbonic anhydrase (CA) is critically involved in the excretion of $CO_2$ across the teleost fish gill (Perry et al., J. Exp. Biol. 101:47, 1982). However, its role in $CO_2$ excretion across the elasmobranch gill is still unclear. Swenson et al. (Bull. MDIBL 22:72, 1982) found that branchial excretion of a bicarbonate load was inhibited by concentrations of benzolamide which were too low to affect erythrocyte CA, and suggested that branchial cell CA, rather than erythrocytic CA, was the site of the dehydration of blood $HCO_3^-$. Swensen and Evans (this volume) have recently found that the isolated perfused head of the Squalus acanthias pup can, at least under some conditions, excrete $CO_2$ when perfused with erythrocyte-free elasmobranch Ringer's solution, and that this excretion is completely inhibited by addition of benzolamide ($10^{-5}$ M) to the perfusate, indicating an important role for branchial cell CA. However, the $CO_2$ excretion rates in these experiments were only approximately 20% of those described for intact adult S. acanthias (Robin et al., J. Cell Physiol. 67:93, 1966), indicating that the preparation was limited in some way. In an effort to examine more directly the role of erythrocytes in elasmobranch $CO_2$ excretion we perfused pup heads with Ringer's solution to which we had added washed S. acanthias erythrocytes.

Pup heads were prepared and perfused as described previously (Evans & Claiborne, J. Exp. Biol. 105:363, 1983) with the exception that irrigation rates were increased to 20 ml·min$^{-1}$; perfusate contained $10^{-7}$ M epinephrine; perfusate was gassed with 0.4% $CO_2$, 8% oxygen, balance $N_2$ to produce $PCO_2$s of 3 torr and a perfusate pH of ca. 7.6; parameters closer to the in vivo condition. Perfusion rate was 700 µl/min and mean fish weight was 40 g. Both the perfusate and irrigate were cooled so that the branchial tissue was maintained at 12 ± 2°C. Erythrocytes were obtained by centrifuging adult blood (from the caudal artery) at 2000 RPM at 5°C for 5-10 minutes, washing with cold elasmobranch Ringer's solution (ERS), and repeating the process 3-5 times. Cells were then resuspended in cold, heparinized ERS to an hematocrit of 3.5 to 8%, 20-50% that found in the intact pup (Kormanik & Evans, this volume). Preliminary experiments determined that higher hematocrits resulted in extraordinary increases in afferent pressures. For the same reason, PVP was not added as a colloidal osmolyte to the perfusate containing erythrocytes. $CO_2$ excretion rates were calculated by A-V differences, monitored from afferent and efferent samples of the perfusate, by use of a Capnicon (Cameron Instruments). Control periods were approximately 30 minutes long, in some experiments the perfusate was then changed to erythrocyte-perfusate (EP). $CO_2$ excretion rates were measured once during the control period and one to four times during the 10 to 60 minutes after the perfusate was changed to EP.

In the present experiments, $CO_2$ excretion rates were negative when the head was perfused with Ringer's solution without erythrocytes (A-V difference = 0.36 mM ± 0.30 S. E., n = 17, a positive value indicating a net increase in perfusate total $CO_2$ as it leaves the branchial epithelium in the dorsal aorta). These data are to be contrasted with our other study (Swensen & Evans, this volume),

where we found measurable A-V differences (-0.38 mM) when ERS perfusate was used. We have no explanation for this discrepancy, except that those experiments utilized heads with closed spiracles, which may have increased the effectiveness of irrigation. However, other experiments with sutured spiracles have also shown no consistent $CO_2$ excretion when the heads are perfused with ERS only (Evans & Robbins, unpublished). When the perfusate was changed (in six heads) to that containing erythrocytes, there was usually a net excretion of $CO_2$ (A-V differences for 13 measurements = $-0.23 \pm 0.09$), resulting in a calculated net $CO_2$ excretion rate of some 1.0 $\mu mol \cdot 100g^{-1} \cdot min^{-1}$ or 0.6 $mmol \cdot kg^{-1} \cdot hr^{-1}$ (assuming that the actual net $CO_2$ extrusion is the difference between the net positive A-V difference under the control (perfusate only) conditions and the net negative A-V difference when the perfusate is changed to EP).

These experiments are too preliminary to withstand rigorous analysis, but they are certainly consistent with the proposition that, in the shark, as in all other vertebrates, the erythrocytes are of major importance for $CO_2$ excretion. Our measured $CO_2$ excretion rates are only 40% of that described previously for the adult (Robin et al., J. Cell. Physiol. 67:93, 1966), but the low hematocrit may account for this discrepancy. Our in vitro excretion rates may also be lowered by perfusion, irrigation, or diffusion limitations of the perfused head preparation. In addition, and the generally positive A-V differences seen under control conditions support this idea, the preparation may be limited by A-V shunts, which do not allow $CO_2$ excretion. Part et al. (Comp. Biochem. Physiol. 79A:29, 1984) have described functional shunts for the trout gill, involving movement of perfusate (or blood) through basal channels in the lamellae, but pre-lamellar arterio-venous anastomoses actually exist in the S. acanthias gill (Olson & Kent, Cell Tiss. Res. 209:49, 1980), which could certainly provide for a significant perfusate flow in filamental channels which never approach the lamellar epithelium for gas exchange. Of course these would only result in a reduction in apparent $CO_2$ excretion if the shunted perfusate re-entered the perfusate stream in the dorsal aorta proximal to the site of sampling in these experiments. This seems unlikely, but is unstudied. Finally, we found that perfusion with even rather low masses of erythrocytes (hematocrits below 10%) resulted, in many cases, in rather substantial hemodynamic effects. Changes in afferent pressures varied from -17 to + 20 torr (around a baseline of approximately 30 torr) when washed cells were used, and final perfusion with erythrocyte-free Ringer's solution never resulted in complete clearance of the gill vessels, indicating that addition of even washed cells (in heparinized ERS) was accompanied by some clotting, and substantial hemodynamic alterations in the perfused head.

Thus, despite some obvious problems, it appears that the perfused pup head may be useful in the investigation of the modes of $CO_2$ transport across the elasmobranch gill epithelium. (Supported by NSF PCM 81-02621 to DHE.)