THE PATTERN OF CARBON DIOXIDE EXCRETION IN THE RAINBOW TROUT *SALMO GAIRDNERI*

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**SUMMARY**

1. Patterns of carbon dioxide excretion were investigated in rainbow trout (*Salmo gairdneri*).

2. The loss of erythrocytic carbonic anhydrase caused by severe anaemia does not affect acid/base regulation or the ability of fish to excrete CO$_2$.

3. Bicarbonate excretion across the saline-perfused gills of trout is significant even though residence time for the saline in the gills is only 1–3 s. CO$_2$ excretion across these saline-perfused gills is blocked by the carbonic anhydrase inhibitor, diamox.

4. The excretion of CO$_2$ in fish is via the movement of plasma bicarbonate into the gill epithelium where branchial carbonic anhydrase catalyses the production of CO$_2$. Fish can adjust pH by regulating bicarbonate movement across the gills.

5. The erythrocytic carbonic anhydrase is not necessary for CO$_2$ excretion in the gills but is involved in facilitating Bohr and Root shifts to augment O$_2$ delivery in the tissues.

**INTRODUCTION**

Plasma bicarbonate is the major form of CO$_2$ in fish blood (Randall & Cameron, 1973) and most of the CO$_2$ excreted across the gills originates from plasma bicarbonate. Carbonic anhydrase is known to be required for normal patterns of CO$_2$ excretion in fish because Hoffert & Fromm (1973) have shown that inhibition of carbonic anhydrase with diamox (acetazolamide) results in a marked rise in $P_a$ CO$_2$ and fall in pH$_a$ in rainbow trout. Haswell & Randall (1976) have shown, in vitro, that intact rainbow trout erythrocytes, although containing carbonic anhydrase, do not catalyse the dehydration of plasma bicarbonate. The gill epithelium in fish contains high levels of carbonic anhydrase (see Table 4) and is a possible site for dehydration of plasma bicarbonate. The following experiments were carried out to determine if plasma bicarbonate is dehydrated within the gill epithelium rather than red blood cells in the rainbow trout.

**METHODS**

All experiments were performed on rainbow trout (*Salmo gairdneri*) weighing 100–300 g. These fish were maintained in large circular tanks provided with oxygenated dechlorinated Vancouver tap water. The water temperature during the course of these experiments was 8–10 °C.
All fish were implanted with chronic indwelling catheters (Smith & Bell, 1964) to facilitate the sampling of dorsal aortic blood. All operative procedures were performed under MS-222 anaesthesia. Fish utilized for gill perfusion studies were immobilized by a blow to the head subsequent to catheterization, the pericardial cavity was exposed by a ventral incision, and a length of P.E. 190 tubing was secured with a ligature in the bulbus arteriosus. All other fish were allowed to recover in individual darkened lucite chambers for at least 24 h before the experiments were begun.

**Blood measurements**

pH determinations were made utilizing a Radiometer PHM-71 acid/base analyser and associated micro pH electrode. Carbon dioxide partial pressures were measured using a mass spectrometer and associated silastic catheters incorporated into thermostatted cuvettes for *in vitro* analysis (M. S. Haswell, in preparation). Carbon dioxide content was determined in a manner similar to that of Cameron (1971). All pH and blood gas determinations were performed at ambient water temperatures (8–10 °C). Gas mixtures utilized for instrument calibration and perfusion experiments were provided by Wöstoff gas mixing pumps.

### Experimental protocol

(a) *Anaemic fish.* After recovery initial blood samples were obtained to establish control levels for pH, $P_{CO_2}$, total CO$_2$ content ($T_{CO_2}$) and haematocrit (Hct.). Severe anaemia was then induced either by intraperitoneal injections of phenylhydrazine (Cameron & Davis, 1970) or by repeated bleeding, the blood lost being replaced by returning the plasma plus Cortland saline to the fish (Wolf, 1963). It was difficult to remove all erythrocytes by either method and the anaemic fish group had haematocrits of less than 4% compared with the control group with haematocrits of 18–25%. Dorsal aortic blood was sampled and pH$_a$, $P_a$, and $T_{CO_2}$ were measured 24 h after anaemia had been established.

Diamox (acetazolamide, Lederle) dissolved in saline was injected (10 mg/kg body weight) into the dorsal aorta of anaemic fish. Six hours later pH$_a$, $P_a$, and $T_{CO_2}$ were measured in blood sampled from the dorsal aorta. Thus pH$_a$, $P_a$, and $T_{CO_2}$ of arterial blood were measured in normal, anaemic, and anaemic diamox-injected fish. The same fish made up the anaemic and the anaemic diamox-injected groups of fish.

(b) *CO$_2$ excretion rates.* The effect of anaemia on CO$_2$ excretion rates was measured by sealing a rainbow trout in a lucite chamber closed except for a water inlet and outlet. The water flow rate through the box and the CO$_2$ content of inflowing and outflowing water were determined (M. S. Haswell, in preparation). Anaemia was then induced by intraperitoneal injections of phenylhydrazine, and 24 h later CO$_2$ excretion rates were determined again.

(c) *Perfused gills.* These experiments were carried out on 14 rainbow trout. A fish was secured ventral side up in a lucite chamber and the gills were perfused via the ventral aorta with heparinized (10 i.u./ml) Cortland saline using a Harvard Apparatus motor-driven syringe pump and a 100 ml glass syringe. The saline was equilibrated with 1% CO$_2$ mixed with air and held at water temperature. The saline passed
Table 1. *Arterial blood pH and $P_{CO_2}$ for rainbow trout subjected to severe anaemia followed by diamox treatment (see text for details)*

($n = 11$, mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>$P_{CO_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control period</td>
<td>7.82 ± 0.08</td>
<td>2.64 ± 0.5</td>
</tr>
<tr>
<td>Anaemic period</td>
<td>7.86 ± 0.08</td>
<td>2.55 ± 0.35</td>
</tr>
<tr>
<td>Diamox + 6 h</td>
<td>7.44 ± 0.16</td>
<td>6.85 ± 1.7</td>
</tr>
</tbody>
</table>

Table 2. *CO$_2$ excretion following phenylhydrazine treatment in rainbow trout (see text for details)* ($n=6$)

<table>
<thead>
<tr>
<th></th>
<th>Before phenylhydrazine treatment</th>
<th>After phenylhydrazine treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ excretion rate (mM/min)</td>
<td>72.1 ± 28.0</td>
<td>70.7 ± 29.0</td>
</tr>
</tbody>
</table>

through the gills, around the body and out through the cut ventricle at a rate of $4.5$ ml/min. The first $100$ ml of perfusate was used to wash out erythrocytes. Measurements were made on the second $100$ ml of perfusate before and after flowing through the gills. The post branchial sample was obtained through an indwelling dorsal aortic catheter. Gill ventilation was maintained at $1000$ ml/min from a constant head reservoir through a rubber tube inserted in the mouth. This rate of water flow should have been adequate to ensure CO$_2$ removal (see Davis & Cameron, 1970). Diamox (10 mg/kg body weight) in saline was injected intraperitoneally into eight of the fourteen trout, the remaining six acting as a control group. Diamox was injected $6$ h before any surgery was initiated.

The Henderson-Hasselbalch equation was used to calculate bicarbonate concentrations. In the perfusion experiments total CO$_2$ content was also calculated utilizing the following equation:

$$T_{CO_2} = (\alpha \cdot P_{CO_2}) + (\alpha \cdot P_{CO_2} \cdot \text{antilog pH-pK}),$$

where $\alpha$ is the solubility coefficient of CO$_2$ in saline at $10$ °C, and pK values are from Albers (1970) for human plasma.

**RESULTS**

(a) *Anaemia*

Anaemia did not result in any change in pH$_a$, $P_{a,CO_2}$ or the CO$_2$ content of arterial blood (Table 1). The addition of diamox, however, caused a marked drop in pH$_a$ and a near tripling of $P_{a,CO_2}$. The injection of diamox into anaemic fish was often lethal whereas injection of the same dose into controls was rarely so. Presumably the difference in effect is due to the buffering power of haemoglobin. All anaemic fish survived the first $6$ h and the values were recorded at this time. There was no further change in CO$_2$ content of the blood $6$ h after diamox injections.

Arterial blood pH was unaffected by haematocrit (Fig. 1) as was $P_{a,CO_2}$ (Fig. 2). CO$_2$ excretion rates were also unaffected by anaemia (Table 2). Anaemia was correlated...
Table 3. Effect of diamox on CO₂ excretion in perfused trout gills (see text for details)

<table>
<thead>
<tr>
<th></th>
<th>Control fish (n=6 mean ± S.D.)</th>
<th>Diamox-injected fish (n=8 mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Perfusate (inflow)</td>
<td>Dorsal sample (outflow)</td>
</tr>
<tr>
<td>pH</td>
<td>7.495 ± 0.008</td>
<td>7.586 ± 0.051</td>
</tr>
<tr>
<td>P₅₀₀₉ (mm)</td>
<td>7.5</td>
<td>5.36 ± 0.69</td>
</tr>
<tr>
<td>HCO₃⁻ (mm)</td>
<td>0.51</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>Total CO₂ (mm)</td>
<td>9.39 ± 0.18</td>
<td>8.40 ± 0.67</td>
</tr>
<tr>
<td>Excretion (%)</td>
<td>11.5%</td>
<td></td>
</tr>
</tbody>
</table>
Carbon dioxide excretion in rainbow trout

with a decrease in blood oxygen capacity, as expected, and marked increase in gill ventilation, as observed by Cameron & Davis (1970).

(b) Gill perfusion

Perfusion of the gills with saline equilibrated with 1% CO$_2$ in air resulted in the removal of 12% of the total CO$_2$ content of the perfusate (Table 3). Only 5% of the total CO$_2$ present in the inflowing perfusate was molecular CO$_2$, the remainder was bicarbonate. Transit time for saline flow through the gills was 1-3 s as judged by the appearance of methylene-blue in the dorsal aortic catheter. The half-time for the uncatalysed reaction velocity for bicarbonate dehydration at 10 °C is 11·7 min (Fig. 3). Treatment of rainbow trout with diamox prior to saline perfusion reduced CO$_2$ excretion to zero in saline-perfused gills (Table 3).

DISCUSSION

In intact rainbow trout, plasma bicarbonate is the major form of CO$_2$ excreted and plasma bicarbonate concentrations are reduced by between 10 and 20% as the blood passes through the gills (Haswell, unpublished observations). Diamox injected into trout results in a marked increase in $P_a$.CO$_2$ and a reduction in pH$_a$ indicating that carbonic anhydrase is important in CO$_2$ excretion (Hoffert & Fromm, 1973). Plasma bicarbonate is excreted as CO$_2$ and the dehydration reaction is catalysed by carbonic anhydrase because, firstly, in the present study, CO$_2$ excretion was reduced to zero in the saline-perfused gill following the application of diamox and, secondly,
Table 4. Ratio of blood v. gill carbonic anhydrase activities

Enzyme activities expressed per gram tissue (wet weight.)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Blood/gill carbonic anhydrase</th>
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<tbody>
<tr>
<td>Lake trout*</td>
<td>0.86</td>
</tr>
<tr>
<td>Perch†</td>
<td>1.32</td>
</tr>
<tr>
<td>Sea bass‡</td>
<td>0.96</td>
</tr>
<tr>
<td>Parrot-fish‡</td>
<td>1.06</td>
</tr>
<tr>
<td>Dogfish§</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Hoffert (1965)
† Maetz (1956)
‡ Smith and Paulson (1975)
§ Hodler et al. (1955).

the transit time for blood flow through the gills is of the order of a second (Randall, 1970), much less than the half time for the dehydration reaction (Fig. 3).

The results of the anaemia experiments indicate that erythrocytes are unnecessary for plasma bicarbonate dehydration, a conclusion consistent with the observations of Haswell & Randall (1976) that fish erythrocytes in vitro do not catalyse plasma bicarbonate dehydration. Normal rates of CO₂ excretion are maintained during anaemia and the saline-perfused gill is able to excrete bicarbonate ions in the absence of erythrocytes.

The ratio of gill epithelial to erythrocytic carbonic anhydrase is about 1:1 (Table 4). Carbonic anhydrase from the gill has a higher substrate affinity than that from erythrocytes (Girard & Istin, 1975; M. S. Haswell, unpublished observations), and we conclude that plasma bicarbonate is dehydrated within the gill epithelium.

What then is the function of erythrocytic carbonic anhydrase in fish? Although fish red cells may be impermeable to plasma bicarbonate (Haswell & Randall, 1976) they are permeable to CO₂ and so bicarbonate will be formed in the erythrocytes as CO₂ enters the blood in the tissues, causing a Bohr shift augmenting oxygen transfer to the tissues. The slow Root ‘on’ shift observed by Berg & Steen (1968) is consistent with the hypothesis that the fish erythrocyte is impermeable to the efflux as well as influx of bicarbonate across the red cell membrane. If this is the case then as blood leaves the tissues and enters the veins, plasma bicarbonate will be formed at the uncatalysed rate following the rise in plasma CO₂ in the tissues. The blood in the veins is a closed system, hence, as plasma bicarbonate levels increase, P₅₀ falls. CO₂ will diffuse from the erythrocytes into the plasma and red cell bicarbonate will be dehydrated. The rate-limiting step will be the uncatalysed hydration reaction velocity in the plasma. Thus some of the bicarbonate formed in the red cells while blood is in the tissue capillaries will be dehydrated, diffuse into the plasma and form bicarbonate before blood reaches the gills. Plasma bicarbonate enters the gill epithelium and is dehydrated to CO₂ before diffusing into water flowing over the gills (Fig. 4). Calculations based on CO₂ excretion rates and changes in plasma HCO₃⁻ before and after the gills indicate that the majority of CO₂ excreted originates as plasma HCO₃⁻.

Trout, like all other aquatic fish, live in a medium relatively poor in oxygen and must continually face the problem of extracting sufficient environmental oxygen to supply tissue needs. Fish cannot utilize changes in ventilation to achieve pH regulation.
as do mammals and birds, without compromising oxygen delivery (Randall & Cameron, 1973). In mammals and birds the dehydration of plasma HCO₃ is never the rate-limiting step in the production of diffusible CO₂. Due to this, regulation of $P_{a,CO_2}$ in mammals and birds is achieved by controlling CO₂ gradients in the lung through changes in ventilation, as environmental oxygen is normally never limiting. In fish, water/blood diffusion distances and ventilation: perfusion ratios are optimized to ensure oxygen transfer. If the dehydration reaction occurring in the blood was not the rate-limiting step, then the control of CO₂ excretion would not be possible. Therefore in fish the production of molecular CO₂, as it occurs in whole blood, is possible only at the uncatalysed rate since red cell CA is unavailable to plasma HCO₃. Due to the long uncatalysed reaction times (especially at lower ambient temperatures) and short residue times for blood in the gill very little CO₂ will be formed from plasma HCO₃⁻ as blood moves through the gill. This is supported by the observation that no excretion of CO₂ occurred in the isolated perfused gills previously treated with diamox. The observed CO₂ excretion in fish is the result of the movement of plasma HCO₃ into the gill epithelium. Unlike molecular CO₂ the movement of bicarbonate across the gill epithelium is likely to be complex; for instance, Randall, Heisler & Drees (1976) have shown that bicarbonate flux can be reversed in dogfish. This observed reversal of HCO₃⁻ flux across the gills modulated
the acidosis caused by elevated CO₂ levels in the blood. When bicarbonate enters the gills from the plasma and forms CO₂ there must be either an equivalent production of H⁺ within the epithelium or the co-transport of H⁺ into the epithelium. This problem has yet to be resolved.

The functional significance of this pattern of CO₂ excretion compared with that seen in mammals and birds is the following. Firstly, the formation of plasma bicarbonate by hydration in the plasma, rather than bicarbonate diffusion from the erythrocytes, results in an elevation in erythrocytic pH and a binding of oxygen to haemoglobin in the veins, lowering P_{VO₂} as blood flows from the tissues to the gills, and augmenting oxygen gradients across the gills. Secondly, the excretion of a significant proportion of total CO₂ as plasma bicarbonate via the gill epithelium allows for the modulation of CO₂ excretion, and therefore blood pH, independent of oxygen-mediated ventilatory adjustments.

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REFERENCES


