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GAS TRANSFER IN A SPONTANEOUSLY VENTILATING, BLOOD-PERFUSED TROUT PREPARATION

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SUMMARY

1. A spontaneously ventilating, blood-perfused trout preparation is described and its suitability for the study of gas exchange in fish assessed.

2. Cardiovascular dynamics closely approximated those found *in vivo*; perfusion flow rate ($\dot{Q}$) = 1·62 $ml^{-1}.100\ g^{-1}$, ventral aortic pressure (VAP) = 58·8 cm H_2O, dorsal aortic pressure (DAP) = 34·8 cm H_2O.

3. Gas exchange characteristics in the branchial and systemic circulations also were similar to those described for resting, intact rainbow trout. All preparations showed consistent oxygen uptake ($\dot{M}_{g,O_2}$, 1·17 $\mu mol.min^{-1}.100\ g^{-1}$) and carbon dioxide excretion rates ($\dot{M}_{g,CO_2}$, 2·05 $\mu mol.min^{-1}.100\ g^{-1}$) across the gills. Across the systemic circulation, oxygen was extracted ($\dot{M}_{s,O_2}$, 1·97 $\mu mol.min^{-1}.100\ g^{-1}$) and carbon dioxide produced by the metabolizing tissue ($\dot{M}_{s,CO_2}$, 1·63 $\mu mol.min^{-1}.100\ g^{-1}$). The respiratory quotient (RE_g) for gas transfer across the gills was 1·85. This high value was a reflection of the fact that much more oxygen than carbon dioxide was added to venous blood in the tonometer. The respiratory quotient for the tissues (RQ_s) was 0·83, a more reasonable value. Breathing rate (f_g) was maintained at 69·4 ventilations.min^{-1}.

4. The mean vascular resistance of blood-perfused gills (R_g) was 14·2 cm $H_2O.ml^{-1}.min.100\ g^{-1}$, a value higher than that usually measured *in vivo*. Mean systemic vascular resistance (R_s) was 19·2 cm $H_2O.ml^{-1}.min.100\ g^{-1}$ which is similar to that measured in intact fish.

5. Cardiovascular responses to hypoxia and adrenergic responses in the branchial and systemic circulations of these preparations also closely approximated those found *in vivo*.

6. This preparation is deemed suitable for studies of the cardiovascular system as well as gas transfer. The results from these experiments are representative of the *in vivo* condition in fish.

INTRODUCTION

Gas exchange in fish has been investigated in some detail (Piiper & Scheid, 1977; Jones & Randall, 1978). These studies however, have been limited in their usefulness

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by the restricted number of parameters measured simultaneously. Due to technical difficulties, ventilation and perfusion of fish gills have not been measured simultaneously while also monitoring gas tension in blood and water as well as the rates of oxygen and carbon dioxide transfer. All these parameters have been measured in concert with a few others, but never all at once. In addition, the degree of control over any of these parameters by investigators has been limited to essentially the inspired water–gas tensions and the volume flow over the gills. These limitations have led researchers to use isolated saline-perfused preparations (see Wood, 1974; Wood & Shelton, 1975; Payan & Matty, 1975; Wood, McMahon & McDonald, 1978). These preparations have been useful in elucidating some aspects of ion exchange and the nature of gill perfusion. However, because of the low oxygen content of the perfusate, oxygen transfer is inadequate and in addition, CO_2 excretion is negligible and hydrogen ion movements are uncontrolled.

A number of investigators have employed some variation of a cardiac bypass/mechanical pump in order to control and manipulate blood flow through the gills of fish (Saunders & Sutterlin, 1971; Opdyke, Holcombe & Wilde, 1979; Hughes, Peyraud, Peyraud-Waitzenegger & Soulier, 1980; Metcalfe & Butler, 1982). In addition, Davie & Daxboeck (see Daxboeck, 1981 for details) have developed a small cardiac pump for controlling blood perfusion in rainbow trout.

This paper describes and characterizes a spontaneously ventilating, blood-perfused rainbow trout (*Salmo gairdneri*) preparation, and its suitability for the study of gas exchange in fish is assessed. Subsequent papers describe the effects of haemodynamic changes on oxygen uptake, and include an estimate of gill epithelium oxygen consumption (Daxboeck, Davie, Perry & Randall, 1982), and the pattern of carbon dioxide transfer across the gills (Perry, Davie, Daxboeck & Randall, 1982).

MATERIALS AND METHODS

Experimental animals

Rainbow trout (*Salmo gairdneri*) weighing between 278–378 g were obtained from the Sun Valley Trout Farm, Mission, B.C. They were held outdoors in large fibreglass tanks supplied with flowing, aerated and dechlorinated Vancouver tap water (5–7 °C), under the ambient light cycle. Fish were fed a daily diet of dried fish pellets, but were not fed during 48 h prior to experimentation.

Blood collection and preparation

Donor fish were anaesthetized with 1:15000 (w/v) aerated MS 222 solution (pH adjusted to 7·0–7·5 with sodium bicarbonate) and then transferred to an operating table (Smith & Bell, 1967). To facilitate blood withdrawal, fish were implanted with chronic indwelling dorsal aortic cannulae (Smith, 1978), and allowed to recover for at least 24 h in darkened Perspex boxes, supplied with flowing, aerated water (7 °C). Generally, 12 fish were cannulated and would supply enough blood for two perfusion experiments. Blood was collected from donor fish immediately prior to each perfusion experiment in the following manner. Approximately 3 ml Cortland saline (Wolf, 1963) containing 2000 U.S.P. units of sodium heparin were injected into the dorsal aorta

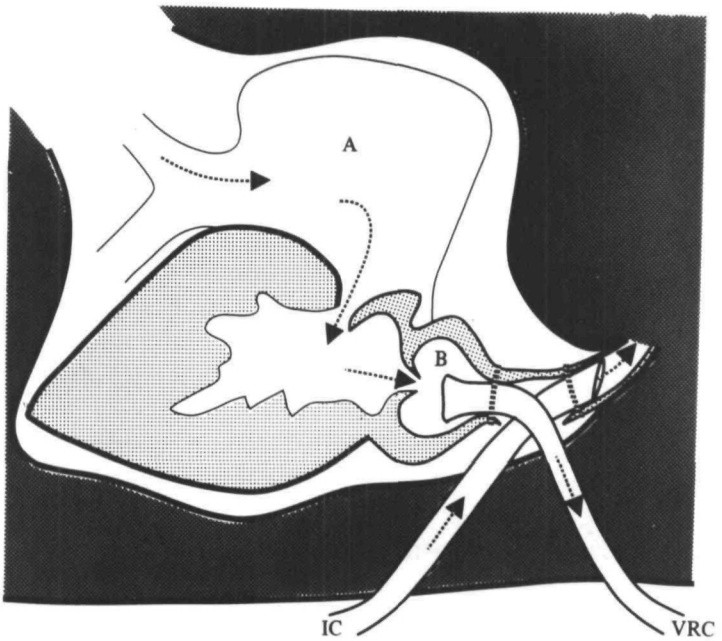

Fig. 1. Schematic sagittal section through the heart of the trout, to illustrate catheter positions used for blood-perfusion experiments, A, Atrium; B, bulbus arteriosus; IC, input catheter into ventral aorta, leading to gills; VRC, venous return catheter, flow aided by ventricular contractions. Arrows within lumens of tubes and heart chambers indicate direction of blood flows.

cannula, and following a 5 min mixing period, blood was withdrawn anaerobically. Typically, 10 ml of blood could be obtained from each fish using this technique. A volume of approximately 100 ml of blood was required for a single perfusion preparation and was prepared by diluting donor blood with saline to a haematocrit of 10–12 %. Blood then was divided into three or four tonometer flasks. These were shaken continuously (Burrell wrist-action shaker) with gas mixtures (0·4 % CO_2:40 % air, remainder N_2) closely resembling trout venous blood gas tensions (Holeton & Randall, 1967). These mixtures were supplied by Wösthoff gas mixing pumps.

Surgical procedures

A fish cannulated the previous day with a patent dorsal aortic cannula was anaesthetized in a 1:15000 MS 222 solution. A second cannula (PE 50; 0·58 × 0·965 mm) was implanted in the buccal cavity following the method described by Saunders (1961) to monitor ventilatory movements. The fish was then transferred to the operating table where the gills were irrigated throughout the operation with an aerated 1:20000 MS 222 solution, either orthograde from a tube in the mouth, or retrograde from tubes in the opercular openings.

The fish was laid supine and the pericardium exposed by cutting the skin above the heart and carefully parting the hypaxial musculature down the midline. Any small vessels which bled into the opening were cauterized with a Birtcher electrosectilis unit

(Birtcher Corp., Los Angeles). Heparin (2000 U.S.P. units in 2 ml saline) was injected into the blood via the dorsal aortic cannula and allowed to circulate for 5 min. As much blood as possible was withdrawn from the dorsal aorta and discarded.

The ventral aortic input catheter consisted of 2·5 cm of silastic rubber tubing (1·45 × 2·30 mm) attached to a curved 13 gauge hypodermic needle shaft. The catheter was connected to a reservoir of Cortland saline (on ice) containing 40 $g.l^{-1}$ PVP (polyvinyl pyrrolidine; av. mol. wt. 40000), a colloid osmotic filler and 1×10^{-5} M noradrenaline (free base, Sigma Chem. Co.). Saline was filtered through Whatman no. 5 paper, then Millipore discs (0·45 μm).

The bulbus was cut immediately caudad of the midpoint and the ventral aortic catheter inserted (Fig. 1) and tied in place. The perfusion flow was started and the fish perfused at a pressure of 50 cm H_2O, to clear all vessels of blood. After exsanguination, the perfusion flow was reduced and the retrograde (venous return, VR) catheter inserted. The venous return catheter was a heat-flared piece of PE 200 tubing (1·4 × 1·9 mm) which was inserted into the bulbus through the same cut as the input catheter and also tied into place (Fig. 1). This catheter was found to offer very little resistance to venous blood flow. Perfusion was resumed while catheters were anchored to the body wall and the incision closed with sutures. The operation took, on average, 45 min. Interruptions to perfusion with either saline or blood lasted less than 1 min.

The fish was transferred to a holding box, which restricted swimming motion, and blood perfusion was started. Water flow over the gills was maintained during recovery from the operation by a tube in the mouth. Once placed into the box and perfused with blood, equilibrium was regained and normal breathing movements resumed within 30 min. Fish were left for 2–3 h to recover from the acute effects of anaesthesia before any experiments commenced.

Occasionally, some bleeding was observed from sutures which closed the incision and anchored catheters to the body wall. Cannulation of the dorsal aorta 24 h prior to experimentation eliminated leakage from around the point of insertion. Some small leaks appeared at other sites and although the blood was heparinized, these usually clotted with time and in no preparation was leakage large. There was no apparent cross reaction between blood from different fish. These fish, however, all came from the same brood stock.

Blood perfusion

Each tonometer, containing approximately 30 ml of blood, had a polyethylene tube (PE 160; 1·14 × 1·57 mm) leading to a set of 3-way taps enabling blood to be drawn into the cardiac pump. These taps allowed switching from any flask to any other without interruption of perfusion. The cardiac pump was accurate to within 1% of gravimetric estimates of the flow rate ($\dot{Q}$), and was not pressure sensitive. This pump allowed independent adjustments of frequency and stroke volume (SV) to be made, without interruptions to the flow.

Pulse pressure was adjusted by changing the size of a gas space at the top of a wide-bore side-arm (Windkessel) in the perfusion line (Fig. 2). Blood was pumped into the ventral aorta via the input catheter and circulated through the entire body. Despite two catheters in the bulbus, ventricular contractions were maintained and pumped

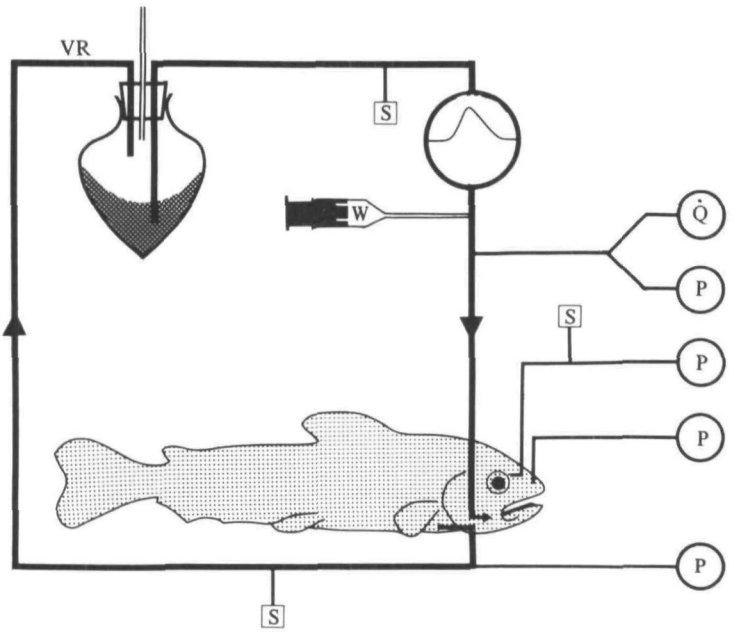

Fig. 2. Schematic representation of the instrumentation used to monitor variables from blood-perfused trout. $\dot{Q}$, Flow record from cardiac pump displayed on chart recorder; P, pressure records, from top to bottom, recorded from: input catheter (ventral aorta, VAP), dorsal aorta (DAP), buccal pressure (for breathing rate), venous return catheter (for intrinsic heart rate), all displayed on chart recorder; S, blood sampling sites; VR, venous return to tonometer flasks containing blood; W, Windkessel to adjust pulse pressure of input. Arrows indicate direction of blood flow.

venous return blood back to the tonometers via a wide-bore silastic rubber tube (1·97 × 3·05 mm).

Perfusion flow was adjusted by altering stroke volume at a cardiac pump frequency of 40 strokes.min^{-1}, necessary to maintain dorsal aortic pressure (DAP) at 40 cm H_2O. This flow rate always was about 16–17 ml.min^{-1}.kg^{-1}, and was taken as the normal value for that preparation.

Blood sampling and analysis

Approximately 0·7 ml of blood were withdrawn simultaneously from each of the three sampling sites (input, DA and VR). Samples were sealed and stored on ice during the analysis period. Generally, input blood was analysed first, followed by dorsal aorta and venous return. Analysis was completed within 15 min of sampling and no measured blood variable was found to change during this period. pH and P_{O_2} measurements were made utilizing an Instrumentation Laboratories Micro 13 pH/blood gas analyser. Total carbon dioxide (C_{CO_2}) and total oxygen (C_{O_2}) contents were determined using the methods of Cameron (1971) and Tucker (1967) respectively, with a Radiometer Copenhagen PHM 71 digital acid-base analyser and associated CO_2 and O_2 electrodes. All pH and P_{O_2} measurements were performed at ambient water temperature, while corresponding C_{CO_2} and C_{O_2} determinations were made at

45 °C, to speed the response of the electrodes used. Partial pressure of carbon dioxi (P_{CO_2}) and bicarbonate concentrations were calculated using the measured pH and C_{CO_2} values, and a reorganization of the Henderson–Hasselbalch equation, as described below:

$$P_{CO_2} = \frac{C_{CO_2}}{[\text{anti-log}\,(\text{pH} - \text{p}K')]\,\alpha CO_2 + \alpha CO_2}. \quad (1)$$

The operational pK' values of carbonic acid were obtained from Severinghaus, Stupfel & Bradley (1956) and the solubility coefficient of CO_2 (αCO_2) was obtained from Albers (1970).

For simplicity, only C_{CO_2} values are reported, while $[HCO_3^-]$ values have been omitted. At physiological pH, HCO_3^- concentration (where

$$[HCO_3^-] = C_{CO_2} - [(\alpha CO_2) \times (P_{CO_2})])$$

comprises approximately 95 % of blood C_{CO_2}, and as such C_{CO_2} is a reasonable approximation of bicarbonate concentration. Following analysis, a small portion of blood was used to determine haematocrit and any remaining blood was returned to the tonometer, or centrifuged and frozen, so that plasma samples could be analysed at a later date. Plasma chloride was determined with a Buchler–Cotlove amperometric titrator and osmolarity was measured using an Osmette freezing point depression osmometer.

Pressure and flow recording

All pressures (input, dorsal aortic, ventricular and buccal) were measured using Statham P23Bd pressure transducers, manometrically calibrated against a static column of water. The water level above the fish was taken as zero. Mean pressures were calculated as diastolic + $\frac{1}{3}$ pulse pressure (Burton, 1972). The pressure drop across the input catheter was measured, with ligatures still in place, after each experiment. This catheter 'resistance' was used to correct measured input pressures for each preparation.

Inflow was measured with an IVM blood flow transducer (electromagnetic pulsed-logic; In Vivo Metric Systems, Los Angeles). Each of the pressure and flow signals was amplified and displayed on a Brush six-channel recorder (Gould Inc., Cleveland, Ohio) (see Fig. 2).

All of the present experiments were conducted at 7 ± 1 °C and temperature did not change during any one experiment. All data are presented as means ± S.E.M. Where appropriate, results were statistically analysed using Students' t test, and 10 or 5 % was taken as the fiducial limit of significance (see Tables).

RESULTS

Blood used for perfusion was taken from donor fish which had recovered from the effects of dorsal aortic cannulation and anaesthesia for at least 14 h (Houston, Madden, Woods & Miles, 1971; Houston & Woods, 1972). Donor fish also did not appear to be agitated during the withdrawal of their blood. This may have avoided increases in the

levels of circulating catecholamines, known to be released into the blood during stress in fish (see Nakano & Tomlinson, 1967).

Surgically prepared fish started to ventilate spontaneously within 20–30 min of the commencement of blood perfusion, although some effects of anaesthesia may have persisted (see Houston *et al.* 1971). Once ventilation became regular, the mouth tube which assisted water flow over the gills was removed. Fish then irrigated their own gills at 69 ± 1 ventilations.min^{-1}. By this time, fish had regained righting and visual tracking reflexes, lost during anaesthesia. Some fish became agitated and attempted to swim during the initial recovery period. Visual disturbances from the surroundings were partly eliminated by masking the holding box with black plastic sheets.

Typically, experiments lasted 4–6 h, which, when combined with 2–3 h for recovery from operative procedures, meant that the measurements were taken from fish which were perfused for no more than 9 h. Over this time period, there appeared to be no appreciable deterioration of blood, as indicated by the lack of cell lysis, or of the fish, as indicated by continuous and stable oxygen uptake and carbon dioxide excretion rates across the gills.

Some preparations were maintained for up to 18 h under resting, normal conditions, without any experimental manipulations. Generally, failure of the preparation was due to mechanical problems associated with the cardiac pump syringe plunger, rather than deterioration of the fish.

Three 0·7 ml blood samples were withdrawn simultaneously from the tonometer, dorsal aorta, and venous return line. No differences were observed in pH or gas tensions of blood from the tonometers or the input catheter. Therefore, blood samples from the tonometer were taken as representative of ventral aortic input values. An advantage of extracorporeal reservoirs is that repeated sampling does not deplete the blood volume in the animal. Consequently, more experiments could be performed on each blood-perfused preparation than on intact fish.

Typical simultaneous recordings of cardiorespiratory variables obtained from a preparation are shown in Fig. 3. Ventilatory interactions on the input pressure trace were observed frequently, especially during a respiratory 'cough' (Hughes & Ardeny, 1977) (see Fig. 3A). This pulse of increased pressure, whether in phase with the cardiac pump cycle or not, usually was transmitted through the gill vasculature to some extent (see also Wood & Shelton, 1980*a*), and was evident in the dorsal aortic pressure trace obtained in the present study. Fig. 3 shows a portion of input trace from a fish in which the pulse pressure had been raised by adjusting the size of the air space in the Windkessel (see Fig. 2).

Bradycardia is associated with exposure of trout to hypoxic water, and is one of the better-described cardiovascular reflexes in fish (see Daxboeck & Holeton, 1978; Smith & Jones, 1978). Blood-perfused fish also showed typical hypoxic bradycardia responses when nitrogen gas was bubbled through the inspired water to lower the oxygen tension (Fig. 3C). Both the 'on' response (bradycardia) and the 'off' response (post-hypoxic tachycardia) were observed. These results indicate that blood-perfused trout have operational neural afferent and efferent reflex pathways by which cardiovascular adjustments may be made.

The variables measured from undisturbed, resting, blood-perfused trout are

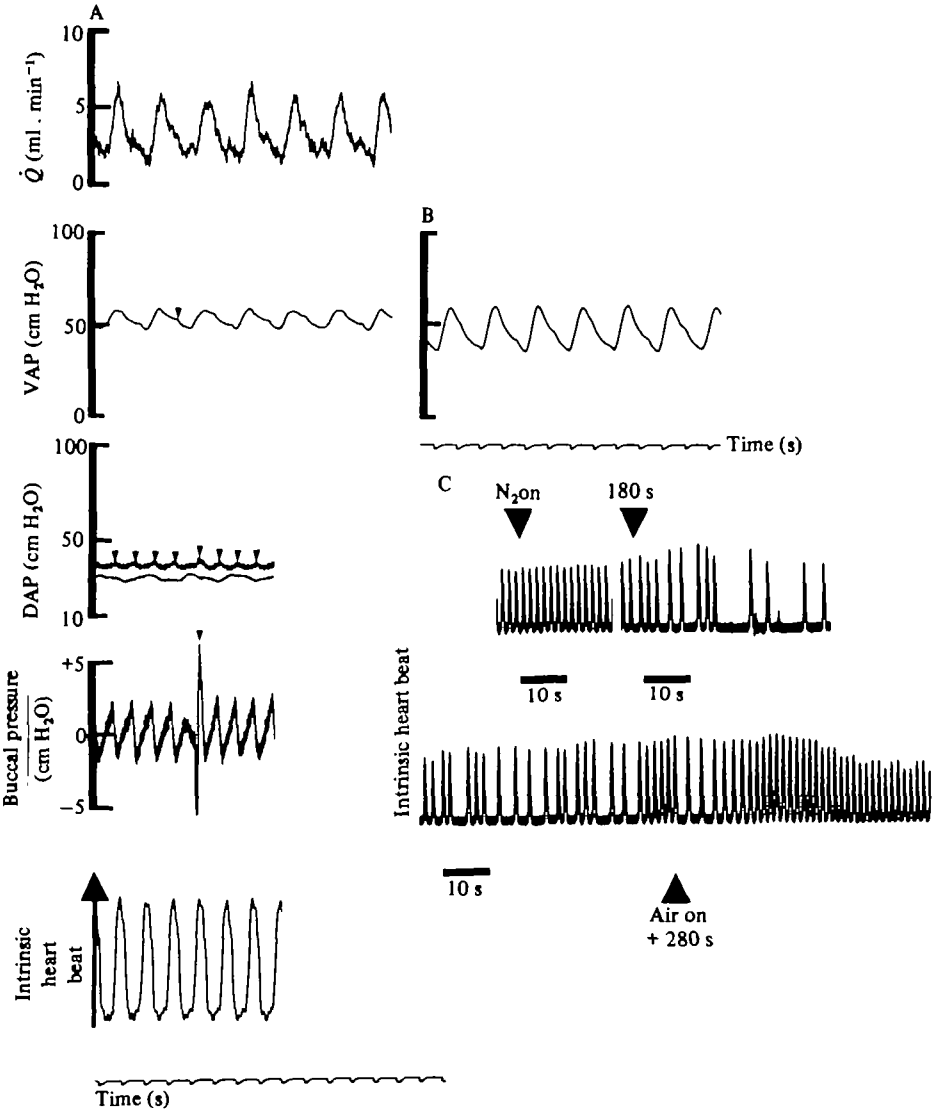

Fig. 3. (A) Simultaneous records of flow and pressure from a spontaneously ventilating, blood-perfused trout preparation. $\dot{Q}$, perfusion flow rate; VAP, ventral aortic (input) pressure; DAP, dorsal aortic pressure. Buccal pressure record of ventilatory movements. Arrow heads show interactions of respiratory movement on VAP, and DAP traces (top trace) – DAP trace below it with no such interaction). Ventricular pressure record of intrinsic heart activity (not calibrated). Arrow indicates contraction. (B) Record of VAP during increased pulse pressure. Note that the heart-like pressure signal is maintained. (C) Record of ventricular contraction during exposure of fish to hypoxic water, showing the 'on' response (bradycardia) after 180 s exposure, and the 'off' response (post-hypoxic tachycardia) after resumption of normoxic water flow. Note that the pressure generated by ventricular contraction increased during hypoxic exposure.

Table 1. *Summary of variables for the normal, resting state of the spontaneously ventilating, blood-perfused rainbow trout at 7 °C.*

(N = 15 fish; 306·2 ± 9·3 g; n = no. of observations.)

	Pressure (cm H_2O)		Cardiac pump		$\dot{Q}$	Hct	Acid/base		P_{O_2}	C_{O_2}	P_{CO_2}	C_{CO_2}	Plasma osmolarity	$[Cl^-]$
	Mean	Pulse	f (bpm)	SV (ml)	(ml.min^{-1}.100 g^{-1})	(%)	$[H^+]$ (nM)	(pH)	(mm Hg)	(mM)	(mm Hg)	(mM)	(m-osmol)	(mM)
						Input								
$\bar{X}$	58·8	10·7	40	0·125	1·619	10·3	17·66	(7·76)	24·9	0·90	3·36	10·28	275·4	111·9
±S.E.M.	2·0	0·2	0	0·003	0·025	0·2	0·40		1·1	0·05	0·15	0·23	0·62	0·70
n	45	45	45	45	45	45	44		44	44	44	45	5 (pooled)	5
						Dorsal aorta								
$\bar{X}$	34·8	2·05	—	—	—	8·80	19·72	(7·72)	103·4	1·58	3·66	9·02	265·7	115·8
±S.E.M.	0·95	0·13	—	—	—	0·2	0·71		2·4	0·08	0·16	0·20	0·30	0·60
n	45	45	—	—	—	42	44		44	44	44	45	5 (pooled)	5
						Venous return								
$\bar{X}$	—	—	—	—	—	9·4	17·55	(7·76)	13·0	0·42	3·65	10·01	273·8	108·2
±S.E.M.	—	—	—	—	—	0·6	0·83		0·5	0·04	0·18	0·20	0·69	1·60
n	—	—	—	—	—	39	42		41	39	41	42	5 (pooled)	5

Breathing rate (f_g) no. min^{-1} = 69·4 ± 0·96 (n = 42).
Intrinsic heart rate, no. min^{-1} = 48·5 ± 0·99 (n = 37).

Table 2. *Summary of the differences in variables across the branchial (input–dorsal aorta) and systemic (dorsal aorta–venous return) circulation in the resting state of spontaneously ventilating, blood-perfused rainbow trout, at 7 °C*

(N = 15 fish, 306·2 ± 9·3 g; n = observations.)

	$[H^+]$ (nM)	$[H^+]$ (%)	P_{O_2} (mm Hg)	P_{O_2} (%)	C_{O_2} (mM)	C_{O_2} (%)	P_{CO_2} (mm Hg)	P_{CO_2} (%)	C_{CO_2} (mM)	C_{CO_2} (%)	ΔP (cm H_2O)
					Δ Input – DA						
$\bar{X}$	+2·06	+13·6	+78·7	+347	+0·69	+79·6	+0·05	+2·32	−1·25	−11·93	23·10
±S.E.M.	0·61	3·4	2·4	20·8	0·05	5·8	0·12	3·36	0·09	0·07	1·75
n	44	44	44	44	44	43	43	44	44	44	45
					ΔDA – Venous return						
$\bar{X}$	−1·36	−6·36	−89·3	−87·4	−1·20	−74·6	+0·07	+4·27	+1·01	+11·66	31·1
±S.E.M.	0·64	3·1	2·2	0·55	0·06	1·67	0·14	4·06	0·06	0·83	0·95
n	41	41	41	41	39	39	41	41	42	42	45

Table 3. *Summary of the vascular resistances to flow, and gas transfer across the branchial (input–dorsal aorta) and systemic (dorsal aorta–venous return) circulation in the resting state of spontaneously ventilating, blood-perfused rainbow trout, at 7 °C*

(N = 15 fish, 306·2 ± 9·3 g; n = observations.)

	R_g (cm H_2O.ml^{-1}.min.100 g^{-1})	$\dot{M}_{g,O_2}$ (μmol.min^{-1}.100 g^{-1})	$\dot{M}_{g,CO_2}$ (μmol.min^{-1}.100 g^{-1})	RE_g ($\dot{M}_{g,CO_2}/\dot{M}_{g,O_2}$)
$\bar{X}$	14·23	1·17	2·05	1·85
±S.E.M.	1·14	0·08	0·15	0·12
n	44	44	44	43
	R_s	$\dot{M}_{s,O_2}$	$\dot{M}_{s,CO_2}$	RQ_s
$\bar{X}$	19·21	1·97	1·63	0·83
±S.E.M.	0·88	0·11	0·09	0·09
n	44	39	44	39

summarized in Tables 1–3. These data are considered to be 'normal'. Under these conditions, all fish showed consistent oxygen uptake ($\dot{M}_{g,O_2}$) and carbon dioxide excretion rates ($\dot{M}_{g,CO_2}$) across the gills. Across the systemic circulation, oxygen was extracted ($\dot{M}_{s,O_2}$), and carbon dioxide produced by the metabolizing tissue ($\dot{M}_{s,CO_2}$). Branchial vascular resistance comprised 43 % of the total vascular resistance to flow in this preparation (Table 3). Haematocrit decreased by 14 %, and plasma osmolarity decreased by 3·5 % as blood flowed from the ventral to dorsal aorta through the gills (Table 1). Data from these preparations are comparable to those available from intact and resting rainbow trout (see Jones & Randall, 1978).

Changes in blood perfusion

The effects of changes in blood perfusion ($\dot{Q}$) on some measured variables are summarized in Table 4. Increases in $\dot{Q}$ caused only a small, non-significant increase in input pressure (VAP) and dorsal aortic pressure (DAP) because of large decreases in gill (R_g) and systemic (R_s) resistance to blood flow. A subsequent decrease in $\dot{Q}$ below the original ('normal') level resulted in a somewhat larger reduction in blood pressure, and an increase in both gill and systemic resistance to blood flow. Haematocrit decreased as blood flowed from ventral to dorsal aorta through the gills, but this decrease showed no change which could be correlated with either changing blood pressure or flow.

Cardiac pump frequency (f) and stroke volume (SV) manipulations

Simulated exercise tachycardia, with no increase in $\dot{Q}$, had no effect upon any of the measured variables, except that ventral and dorsal aortic pulse pressures decreased.

Simulated bradycardia in normoxic water, however, caused a significant decrease in gill resistance (Table 5), despite a slight fall in $\dot{Q}$. This perfusion condition also significantly increased VAP and DAP. The amplitude of the pulse pressure was reduced by the same amount in its passage through the gills during simulated bradycardia or tachycardia, at constant perfusion flow rates. All other measured variables changed very little from normal values.

Table 4. *Summary of the effects of increased and decreased cardiac output on cardiovascular variables from normal, spontaneously ventilating, blood perfused trout*

(N = 7 fish; 292·7 ± 14·3 g.)

	VAP (cm H_2O)		DAP (cm H_2O)		$\dot{Q}$ (ml. min^{-1})	Hct (input) (%)	Hct (DA) (%)	R_g (cm H_2O. ml^{-1}.min. 100 g^{-1})	R_s (cm H_2O. ml^{-1}.min. 100 g^{-1})
	Mean	Pulse	Mean	Pulse					
					Normal $\dot{Q}$				
$\bar{X}$	54·1	11·1	34·4	2·0	4·80	9·6	8·5	12·1	18·5
±S.E.M.	5·1	0·3	1·9	0·2	0·20	0·4	0·5	2·8	2·9
					High $\dot{Q}$				
$\bar{X}$	56·5	15·8	36·3	2·5	7·20	9·6	8·5	8·20*	13·2*
±S.E.M.	4·2	0·7	2·4	0·5	0·40	0·5	0·6	1·40	0·7
					Low $\dot{Q}$				
$\bar{X}$	42·9	7·8	29·4	1·4	2·76	9·34	7·8	14·3	26·9*
±S.E.M.	3·5	0·5	2·0	0·2	0·24	0·5	0·5	2·9	2·0

* Significantly different from normal at 5 %.

Table 5. *Effects of cardiac output ($\dot{Q}$), stroke volume (SV), pulse pressure (PP) and cardiac frequency (f) on gill resistance change (ΔR_g %) in resting, blood-perfused trout*

(o = no change from normal; + = increase from normal; − = decrease from normal.)

Treatment	n	ΔR_g (% change from normal)
(1) SV +; PP +; f o; $\dot{Q}$ +	7	−22·91 ± 9·09
(2) SV +; PP +; f −; $\dot{Q}$ o (bradycardia)	8	−25·22 ± 8·98*
(3) SV −; PP −; f o; $\dot{Q}$ −	7	+35·38 ± 17·96
(4) SV −; PP −; f +; $\dot{Q}$ o (tachycardia)	8	+4·48 ± 18·63
(5) SV o; PP +; f o; $\dot{Q}$ o	8	+15·37 ± 11·16
(6) SV o; PP −; f o; $\dot{Q}$ o	3	+14·99 ± 15·89

Means ± S.E.M.
* Significantly different from normal at 5 %.

Pulse pressure (PP) changes

Increases in input pulse pressure, as occur *in vivo* during exercise, but with no accompanying change in pump frequency or stroke volume, caused no significant changes in any of the variables measured, compared to values found in Table 1. Although ventral aortic pulse pressure was increased above normal, the amplitude of the dorsal aortic pressure did not increase by the same amount, indicative of increased pressure damping within the gill vasculature. Decreased ventral aortic pulse pressure (three fish only) also produced no significant changes in any of the variables measured (see Table 5).

Haematocrit (Hct) changes

Regardless of whether high or low haematocrit blood was perfused through the gills, VAP was increased to the same level above the normal value. Conversely, DAP

Table 6. *Summary of the effects of input haematocrit on cardiovascular parameters from normal spontaneously ventilating, blood-perfused trout*

(N = 6 fish; 320·2 ± 17·2 g and constant $\dot{Q}$ = 5·20 ± 0·04 ml.min^{-1}.)

	VAP (cm H_2O)		DAP (cm H_2O)		Hct (input) (%)	Hct (DA) (%)	R_g (cm H_2O.ml^{-1}.min.100 g^{-1})	R_s (cm H_2O.ml^{-1}.min.100 g^{-1})
	Mean	Pulse	Mean	Pulse				
					Normal Hct			
$\bar{X}$	60·6	10·8	40·2	2·2	11·3	9·3	12·6	22·3
±S.E.M.	1·7	0·5	2·6	0·4	0·5	0·5	1·5	1·8
					Low Hct			
$\bar{X}$	76·2	10·7	28·3	1·4	4·3	3·9	29·6*	15·1
±S.E.M.	7·7	0·7	1·9	0·3	0·4	0·5	4·9	1·4
					High Hct			
$\bar{X}$	76·1	11·8	33·8	1·7	20·2	16·5	26·1*	18·4
±S.E.M.	6·9	0·9	3·1	0·2	1·6	1·2	4·3	1·7

* Significantly different from normal at 5 %.

Table 7. *Effects of* 1 × 10^{-6} M *adrenaline on selected variables in blood-perfused trout preparations*

(N = 4 fish.)

	VAP (cm H_2O)	DAP (cm H_2O)	R_g (cm H_2O.ml^{-1}.min.100 g^{-1})	R_s (cm H_2O.ml^{-1}.min.100 g^{-1})	$\dot{M}_{g,O_2}$ (μmol.min^{-1}.100 g^{-1})
			Normal		
$\bar{X}$	40·4	30·5	6·48	16·38	1·10
±S.E.M.	5·4	5·3	0·82	3·35	0·23
			Adrenaline		
$\bar{X}$	51·3	44·6	4·01*	25·06*	1·30
±S.E.M.	4·7	3·1	1·02	1·97	0·25

* Significantly different from normal at 10 %.

in both instances decreased to similar levels below that found in the normal situation (Table 6). Since the pressure differential across the gills was increased in both cases, with no accompanying changes in $\dot{Q}$, the calculated gill resistance was increased. As a consequence of the decreased DAP, systemic resistance was lower than normal. No other vascular variable was affected by changes in Hct. All data represented in Table 6 are comparable to normal values obtained from similar preparations (see Table 1).

Adrenaline exposure

The levels of circulating catecholamines are thought to increase during exercise in intact trout. The effects of the addition of adrenaline to a final blood concentration of 1 × 10^{-6} M on vascular variables in blood-perfused preparations are summarized in Table 7. Preparations showed a significant decrease in gill vascular resistance to flow of approximately 38 %, associated with a larger rise in DAP than in VAP. Systemic vascular resistance was increased significantly by 56 % above normal in this situation.

The decrease in plasma osmolarity across the gills was twice as large as observed normal blood-perfused preparations.

DISCUSSION

The spontaneously ventilating, blood-perfused trout preparations described in this study behaved in a manner similar to intact rainbow trout. They were capable of maintaining equilibrium, visual tracking, swimming, and showed a typical bradycardia response when exposed to aquatic hypoxia. These prepared fish had blood pressure, flows, and rates of gas transfer at the gills and tissues similar to those reported from *in vivo* studies. This was despite the fact that haematocrit (10–12 %) was lower than normally found in intact trout. Trout have been shown, however, to be quite capable of surviving with haematocrits as low as 3 %, and values of 15 % are not uncommon in hatchery reared trout (see Wood & Shelton, 1980*a*).

Prepared fish may release catecholamines into the blood in response to stress (Nakano & Tomlinson, 1967; Mazeaud & Donaldson, 1977). The effects of any such release will be diluted by the large extracorporeal blood volume. Initial catecholamine levels already were probably low in experimental fish, because of exsanguination and saline perfusion prior to blood perfusion. To elevate blood levels by 15 times over resting levels, as observed in other stressed fish (Mazeaud *et al.* 1977), the experimental fish would have to release enormous quantities of catecholamines into the circulating blood. This did not occur since systemic resistance decreased during the course of an experiment; if catecholamine levels were increasing one would expect the converse, and experimental animals showed a large response to adrenaline infusion.

At the onset of the experiment, blood flow was adjusted to achieve a dorsal aortic pressure of 40 cm H_2O because fish, like most other vertebrates, probably regulate blood pressure by altering $\dot{Q}$ and vascular resistance (Walqvist & Nilsson, 1977, 1980; Smith, 1978; Wood & Shelton, 1980*b*). During the course of the experiment there was no further readjustment of flow, and dorsal aortic pressure decreased to $34{\cdot}8 \pm 1{\cdot}0$ cm H_2O. This is still within the range of values reported for intact trout (Stevens & Randall, 1967*a*; Kiceniuk & Jones, 1977; Wood & Shelton, 1980*a*) and other fish (Stevens, Bennion, Randall & Shelton, 1972; Helgason & Nilsson, 1973; Davie & Forster, 1980). The perfusion rate necessary to maintain this blood pressure is similar to that recorded *in vivo* for resting fish at similar temperatures (Stevens & Randall, 1967*b*; Stevens *et al.* 1972; Jones, Langille, Randall & Shelton, 1974; Kiceniuk & Jones, 1977; Davie & Forster, 1980). Wood & Shelton (1980*a*), however, recorded a much higher cardiac output from trout, due largely to a much higher heart rate than observed in other fish.

Although blood flow is similar in our preparation to that observed in intact fish, gill resistance to flow is higher than previously recorded values, and is associated with an elevated ventral aortic pressure. The mean R_g of blood-perfused gills was 14·2 cm $H_2O.ml^{-1}.min.100\ g^{-1}$ whereas recorded *in vivo* values are generally less than 6 cm $H_2O.ml^{-1}.min.100\ g^{-1}$ (Stevens & Randall, 1967*b*; Kiceniuk & Jones, 1977; Wood & Shelton, 1980*a*). A possible explanation is that blood-perfused fish have a high level of branchial vascular tone. Exposure of these preparations to 1×10^{-6} M

renaline caused a significant decrease in branchial vascular resistance whereas injections of isoprenaline *in vivo* by Shelton & Wood (1980*a*) showed only small effects, indicating a low vascular tone. Another possible contributing factor to the relatively high branchial vascular resistance in these blood-perfused preparations is the small size of the animals. Wood & Shelton (1975) have shown inverse relationships between weight and vascular resistance in isolated saline-perfused preparations.

The results from experiments in which input pressure and flow were changed indicate that increases in stroke volumes of the heart and, to a lesser extent, ventral aortic pulse pressure, cause a decrease in gill vascular resistance. The opposite is observed in the intact animal when stroke volume of the heart increases during either exercise or hypoxia. Thus, in the intact animal, changes in R_g must involve factors other than the action of increases in stroke volume on the gill vasculature.

Gill vascular resistance to blood flow increased in response to both a rise and a fall in haematocrit. In addition, R_s showed the converse response, and fell in response to changes in haematocrit. The decrease in R_s associated with a rise in haematocrit may have resulted from the loss of hypoxic vasoconstriction because of increased oxygen delivery to the tissues. We have no plausible explanation for the increase in R_g with decreasing haematocrit.

In general, systemic vascular resistance (R_s) measured *in vivo* and *in vitro* are in good agreement, better than seen in the comparisons of gill resistance from intact, blood-perfused or saline-perfused gills. Mean systemic vascular resistance to flow in blood-perfused fish was 19·2 cm $H_2O.ml^{-1}.min.100\,g^{-1}$; about 1·35 times the gill resistance. This value of R_s is similar to that measured in intact fish (Stevens & Randall, 1967*b*; Kiceniuk & Jones, 1977) but somewhat higher than that reported by Wood & Shelton (1980*a*).

The breathing rate of the blood-perfused trout is similar to that reported for intact trout at 7 °C (Daxboeck & Holeton, 1978, 1980).

Blood was tonometered with gas containing 0·4 % CO_2; 40 % air, remainder nitrogen. The blood never reached equilibrium with the gas phase of the tonometer because of the continual blood inflow from the fish. Clearly the venous return blood was of higher P_{CO_2} than expected from equilibration of blood with the gas mixture. Despite the fact that equilibration in the tonometer was incomplete, input P_{O_2}, P_{CO_2} and content values were in the physiological range measured *in vivo* (Holeton & Randall, 1967*a*; Cameron & Davis, 1970; Eddy, Lomholt, Weber & Johansen, 1977; Kiceniuk & Jones, 1977) and dorsal aortic blood was about 95 % saturated with oxygen. Considerable excretion of CO_2 across the gills yielded normal dorsal aortic blood CO_2 tensions and contents. Although the rate of CO_2 excretion was comparable to that observed in intact fish (Perry, 1981), the respiratory quotient (RE_g) for gas transfer across the gills was 1·85. This high value was a reflection of the fact that much more oxygen was added to the blood in the tonometer than CO_2. If systemic venous tensions were maintained in the input blood flow then P_{O_2} would have been 13 mm Hg rather than 30 mm Hg in blood flowing to the gills. An increase in venous oxygen content at constant blood flow will reduce oxygen uptake by the blood as it flows through the gills and this undoubtedly accounts for the high respiratory quotient and somewhat reduced oxygen uptake by the blood as it perfuses the gills in this prepara-

tion. Oxygen utilization by the systemic tissues was some 1·75 times that of the g[redacted] oxygen uptake rate, the remainder being added to the blood in the tonometer. The respiratory quotient for the tissues (RQ_s) was 0·83, a more reasonable value, presumably resulting from fat and carbohydrate metabolism.

With the exception of R_g which is higher than observed *in vivo*, and the gill RE, for which we have given an explanation, the blood-perfused, spontaneously ventilating fish displays characteristics which fall well within the range of values reported for intact rainbow trout. Although there is considerable variability in the published data for cardiovascular parameters in fish, comparisons reveal good agreement between our blood-perfused and intact trout. However, there is no reason to expect that experimentally induced variations in cardiac output or venous blood composition should give rise to overall responses similar to those seen in fully integrated, intact fish. In addition, the presence of a second gas exchanger (i.e. the tonometer flasks), and no sensitivity to venous return because of the mechanical heart, while being of experimental advantage, cannot allow authentic replication of *in vivo* values and responses except in a general sense. Nonetheless, this preparation still is suitable for studies of the cardiovascular system as well as gill gas transfer in trout, and is advantageous because blood flow and the composition of venous blood can be controlled and manipulated rather than being measured or estimated.

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