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IN VIVO ANALYSIS OF PARTITIONING OF CARDIAC OUTPUT BETWEEN SYSTEMIC AND CENTRAL VENOUS SINUS CIRCUITS IN RAINBOW TROUT: A NEW APPROACH USING CHRONIC CANNULATION OF THE BRANCHIAL VEIN

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Summary

Freshwater-acclimated rainbow trout were chronically and non-occlusively cannulated in the dorsal aorta (DA), sinus venosus (SV) and branchial vein (BV), the latter returning the blood perfusing the central venous sinus (CVS) of the gill after being shunted away from the systemic circuit. After recovery, blood samples from these three sites were analysed for haematocrit (Hct) and [Hb]. Branchial venous blood was found to have considerably lower Hct and [Hb] (Hct = 3·5 ± 3·1 %; [Hb] = 1·04 ± 0·75 g 100 ml^{-1}) than systemic blood (DA: Hct = 24·3 ± 8·9 %, [Hb] = 6·54 ± 2·90 g 100 ml^{-1}; SV: Hct = 23·1 ± 8·8 %, [Hb] = 6·15 ± 2·55 g 100 ml^{-1}; means ± S.D. $N = 8$), which strongly suggests that plasma skimming occurred at arteriovenous anastomoses and arterioles draining into the CVS. The partitioning of cardiac output, calculated on the basis of the [Hb] data, showed that the systemic flow accounted for 93 ± 4·6 % ($N = 7$) of the total cardiac output with only 7 ± 4·7 % of cardiac output being diverted into the CVS, thus bypassing the systemic vasculature. Previous results using *in vitro* experiments are compared with the present data in an evaluation of the usefulness of the isolated perfused gill and head preparations in the experimental analysis of circulatory physiology in fish gills.

Introduction

Artificially perfused *in vitro* preparations have been increasingly used in recent studies of fish gill physiology. Most commonly, isolated perfused head (IPH) and

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gill arch (IPG) preparations have been employed to study intimate processes of the branchial epithelium, such as respiratory gas exchange (IPH: Perry, Daxboeck & Dobson, 1985*b*; Petterson, 1983; Pärt, Tuurala & Soivio, 1982), ionic transfer (IPH: Avella, Masoni, Bornancin & Mayer-Gostan, 1987; Perry, Booth & McDonald, 1985*a*; IPG: Davis & Shuttleworth, 1985; Farmer & Evans, 1981), control of circulation (IPH: Daxboeck & Davie, 1982; Claiborne & Evans, 1980; IPG: Stagg & Shuttleworth, 1984; Farrell, Daxboeck & Randall, 1979), hormone metabolism (IPG: Nekvasil & Olson, 1986; Olson, Kullman, Narkates & Oparil, 1986) and water permeation (Bennett & Rankin, 1987).

One of the major reasons for the use of *in vitro* perfused preparations has been the technical problems in performing certain measurements under physiological conditions in well-recovered, unanaesthetized fish. It is now well established that the vasculature in the fish gill is made up of two distinct circuits. The arterio-arterial circuit connects the afferent branchial vessels with the efferent ones through the secondary lamellae, and subsequently perfuses the systemic beds. The arteriovenous circuit, however, originates from the efferent side of the arterio-arterial system through short, capillary-sized arteriovenous anastomoses and long, often tortuous arterioles. The blood diverted to the arteriovenous circuit perfuses the central venous sinus (CVS), which is drained by the branchial vein (BV), and finally returns to the heart *via* the major systemic veins. Each circuit is known to be in close contact with a discrete epithelium (see Laurent, 1984, for details of gill anatomy). Such complexity in the architecture of the fish gill requires separate analysis of the different functional units. Several *in vitro* preparations have been developed for this purpose, but doubt has been raised about the reliability of such preparations (Pärt *et al.* 1982; Ellis & Smith, 1983). Apart from the obvious role of respiratory gas exchange in the secondary lamellae, little is known about the localization of other exchange processes in the gill.

Isolation of the head or gill arches from the rest of the body certainly circumvents some of the difficulties encountered during *in vivo* studies. For the results of such *in vitro* experiments to be physiologically relevant, however, the preparations should closely mimic the *in vivo* normal conditions and retain responsiveness as observed in intact animals (Perry *et al.* 1984). A basic requirement is thus the establishment of *in vivo* baseline data as a reference, and the reliability of a particular *in vitro* preparation must be judged on how well *in vivo* baseline values are recovered by the preparation. Unfortunately, *in vivo* baseline data are missing for many aspects of fish gill physiology such as the partitioning of the cardiac output. Paradoxically, it is this difficulty that has largely motivated the use of *in vitro* preparations.

The microcannulation technique of the branchial vein recently developed in our laboratory is capable, together with systemic arterial and venous cannulations, of providing the required basic information on the composition of the inflowing and outflowing blood of the two distinct gill units in well-recovered, unanaesthetized fish. Utilizing this cannulation technique, the present study was aimed at the estimation of blood flow partitioning between the two vascular circuits in the gill,

and at a critical comparison of the resulting data with those obtained in isolated perfused preparations.

Materials and methods

Experimental animals

An essential prerequisite for branchial vein cannulation is free access to the ventral base of the gill during surgery. Accordingly, only fish species with wide gill openings are suitable for successful cannulation. Large specimens are preferable because of the small relative size of the vein. We chose rainbow trout in the mass range 1–2 kg. The animals were obtained from a local trout hatchery near Göttingen, and kept in large, well-aerated glass aquaria (2–3 m^3) at 15 °C with a flow-through of dechlorinated Göttingen tap water of about 100–200 ml min^{-1} $fish^{-1}$. The fish were regularly fed on commercial trout pellets except for the 2 days before surgery.

Design of the cannula for branchial venous cannulation

The cannula was constructed using short lengths of PE 10 (10 cm) and PE 20 tubing (60 cm). One end of the PE 20 catheter was heated over a soldering iron until the lumen became wide enough to hold the short length of PE 10. With a stainless-steel mandril threaded through the PE 10 tubing, it was inserted into the bulged end of the PE 20 tubing and the two tubes were fused over the soldering iron. During this procedure, care was taken not to press the tubes against each other to ensure a wide lumen in the melted portion of the cannula. The mandril was removed and the PE 10 tubing was bent (with application of slight heat) to an angle of 45° about 10 mm from its free end. The PE 10 tubing was then bevelled about 5 mm from the end with a new razor blade. The mandril was reinserted and a slit (about 3 mm long) was cut into the outer wall of the bend (see Fig. 1A). Finally, the PE 10 tubing was again bent perpendicular to, and 10–15 mm from, the first bend to reduce tension after anchorage of the cannula to the body wall (second bend, Fig. 1C). Useful information on fashioning PE tubing has been provided by Heatley & Weeks (1964).

Surgery

The animals were anaesthetized by immersion in 0·01 % MS-222 solution, neutralized with $NaHCO_3$. The fish were positioned ventral side up on a holding rack, and supported by a soft cloth. During surgery, the gills were irrigated with 0·05 % MS-222 solution bubbled with pure oxygen. The same water was used to keep the body surface of the fish wet and cool. The temperature of the irrigation water was thermostatted to 15 °C. The ventral base of the gill arches on one side (usually left) was exposed by widely retracting the operculum. The operculum was held in position by a fine suture threaded through its avascular edge. During the critical stages of branchial vein cannulation, gill irrigation was maintained only on

the contralateral side. The gill filaments of the ipsilateral side were covered with wet cotton pads to avoid any damage during surgery.

Usually the second gill arch was selected, because of the larger space available at its ventral base, and the afferent branchial artery was located. Under microscopic

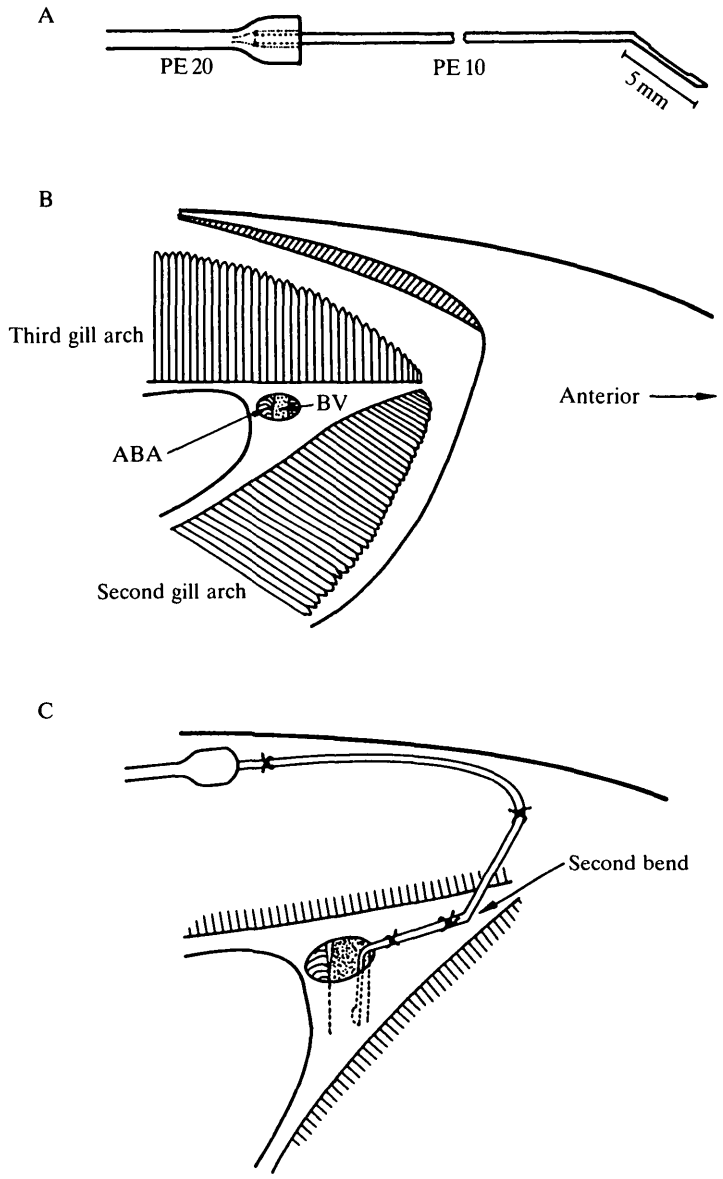

Fig. 1. (A) Cannula construction for branchial vein (BV) cannulation. (B) Diagrammatic view of the surgical site. The BV and afferent branchial artery (ABA) of the second gill arch are exposed. The BV is located anterior and parallel to the ABA. (C) Cannula secured in BV. Note the second bend at the anterior base of the gill arch. Gill filaments are omitted from the figure for clarity.

control the skin over the artery was opened with a short (3 mm) incision at right angles to the axis of the artery (Fig. 1B). Using fine scissors and forceps, the artery was carefully exposed from the posterior side. Then, the tissue anterior to the artery was carefully blunt-dissected. In our experience, it is extremely dangerous to search for the vein directly. Usually the branchial vein (BV) ran parallel to the artery on its anterior side, positioned 1–2 mm below the skin surface. Sometimes it was difficult to distinguish the vein from the surrounding tissue, but it usually looked darker and often carried melanophores on its wall. The entire width of the vein was then exposed. Three loops of fine polyamide threads (5/0) were used to secure the cannula, two on the base of the gill arch and one on the isthmus (Fig. 1C). The cannula was laid on the expected cannulation site and, if necessary, the bend angle was readjusted so that the distal portion of the cannula would be positioned parallel to the vessel wall after cannulation and the side hole would face the central lumen (Fig. 1C). The cannula was filled with heparinized (400 i.u. ml^{-1}) Cortland saline (Wolf, 1963) without glucose. Care was taken to eliminate air bubbles from the cannula.

The cannula was introduced into the vessel after puncturing the anterior side of the vessel wall using a 27-gauge hypodermic needle. The cannula was inserted through the hole at right angles to the wall, carefully advanced and gradually turned till the first bend of the cannula was positioned inside the vein. Patency of the cannula was checked by free flow of blood from the cannula under a slightly negative pressure head. The cannula was then secured by ligatures and the incision closed with fine atraumatic sutures.

The dorsal aorta (DA) was cannulated by the method described by Ultsch, Ott & Heisler (1981). The sinus venosus (SV) was percutaneously cannulated through the common cardinal vein. A polyvinyl tube (o.d. 1·3 mm), with a tapered tip and containing a long needle for puncture, was inserted through the body wall slightly dorsal to the opening of the vein into the sinus venosus and then advanced so that the tip of the cannula was located in the median plane within the sinus. The distance from the puncture site to the centre of the sinus was measured prior to cannulation and the length to be inserted was marked on the cannula to facilitate the procedure. The DA and SV cannulae were filled with heparinized (10 i.u. ml^{-1}) Cortland saline without glucose.

After surgery, the fish was placed in a Plexiglas box with circulating normoxic water kept at 15 °C. The box was covered with black plastic sheets to prevent visual disturbance to the fish. Usually the fish recovered quickly when placed in the box, but if necessary the gills were artificially irrigated to facilitate recovery. After checking for the absence of bleeding, the fish was heparinized (400 i.u. kg^{-1} body mass). A further dose of heparin was administered once a day throughout the experiment.

Blood sampling

Blood sampling began 24 h after surgery. The acid–base status of the DA blood was determined first, and further sampling from all three sites was performed only

when the acid–base status of dorsal aortic blood was in the range of previously reported normal values.

Because of the small bore and the length of the BV cannula, blood was not withdrawn by syringe but by siphoning. The sealed free end of the cannula was cut, and blood was collected by application of slightly negative pressure, lowering the end of the cannula to about 20 cm below the water surface. The first 1 ml was discarded to clear the cannula of saline, and the following 0·3 ml was collected for analysis. Dorsal aortic blood for acid–base analysis was collected anaerobically by the usual procedure in syringes, whereas DA and SV blood for measurement of Hct and [Hb] was collected by siphoning in order not to affect the blood flow partitioning in the gill.

Analytical methods

The blood samples were vigorously agitated to ensure even distribution of red blood cells. The haemoglobin concentration ([Hb]) was determined by the cyanmethaemoglobin method. Haematocrit (Hct) was measured after centrifugation at 11 000 rev. min^{-1} (16 000 *g*) for 3 min. Duplicate or triplicate determinations were performed at each site in every experiment. Plasma pH was determined using a Radiometer BMS3 Mk2 system thermostatted to the animal's body temperature. A sample of blood was centrifuged anaerobically and the plasma was analysed for total CO_2 in a Capnicon III apparatus (Cameron Instruments, Port Aransas, TX, USA). Blood P_{CO_2} values were calculated by application of the Henderson–Hasselbalch equation using appropriate constants (see Heisler, 1984; note: the sign of the last line term of the α-formula is misprinted and should read '+').

Calculation of gill blood flow partitioning

The cardiovascular model we used is shown in Fig. 2. The entire cardiac output flows through the secondary lamellae. Thereafter, the blood has two alternative pathways. One is the systemic circuit, in which the blood is directed to the capillary beds of the whole body through the distributing arteries. The other, termed the central venous sinus (CVS) circuit, originates from the efferent side of the gill vasculature. The blood in this circuit perfuses the CVS and is then collected in the branchial vein which in turn drains into the systemic venous system.

The total cardiac output through the ventral aorta ($\dot{Q}_v$) is thus the sum of blood flow through the systemic ($\dot{Q}_s$) and CVS ($\dot{Q}_{cvs}$) circuit:

$$\dot{Q}_v = \dot{Q}_s + \dot{Q}_{cvs} . \quad (1)$$

Also, if a substance incurs no transfer across the vascular wall in its passage through the gill, then

$$\dot{Q}_v C_v = \dot{Q}_s C_s + \dot{Q}_{cvs} C_{cvs} , \quad (2)$$

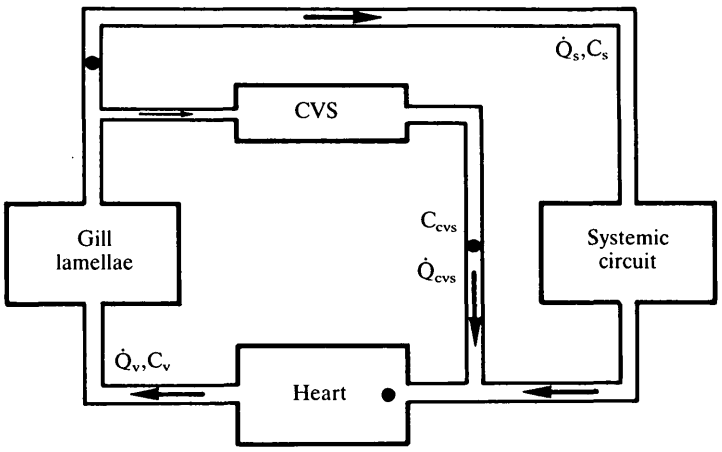

Fig. 2. Schematic presentation of the cardiovascular model of rainbow trout utilized for the present analysis. Solid circles indicate blood sampling sites. $\dot{Q}$, blood flow rate; C, concentration.

where C is the concentration of the substance. The relative blood flow rate for the systemic ($\dot{Q}_s/\dot{Q}_v$) and CVS ($\dot{Q}_{cvs}/\dot{Q}_v$) circuits can then be calculated from:

$$\dot{Q}_s/\dot{Q}_v = (C_v - C_{cvs})/(C_s - C_{cvs}) \quad (3)$$

and

$$\dot{Q}_{cvs}/\dot{Q}_v = (C_s - C_v)/(C_s - C_{cvs}) \, . \quad (4)$$

This analysis was performed using the haemoglobin concentration, since it meets the above criterion and can be measured easily.

Results

The acid–base parameters of our fish determined 24 h after surgery are in fairly good agreement with previously reported values for rainbow trout at 15°C (Wood & Jackson, 1980; Thomas, 1983), indicating that our fish were well recovered and in resting conditions. pH was 7·845 ± 0·161; plasma C_{CO_2} was 8·1 ± 3·79 mmol l^{-1}; and P_{CO_2} was 2·5 ± 0·51 mmHg (0·333 ± 0·068 kPa) ($\bar{x}$ ± S.D., $N = 8$).

The haemoglobin concentration ([Hb]) and haematocrit (Hct) values are shown in Table 1. Blood from the branchial vein (CVS) showed significantly lower values for both [Hb] and Hct than either dorsal aortic (DA) or sinus venosus (SV) blood ($P < 0{\cdot}01$, paired t-test). The dorsal aortic blood had significantly higher [Hb] ($P < 0{\cdot}05$) and Hct ($P < 0{\cdot}01$) than blood from the sinus venosus (paired t-test). The partitioning of blood flow through the systemic and CVS vasculatures was calculated based on the [Hb] as presented in Table 2. Most of the cardiac output was directed to the systemic circuit (87–99 %); on average only 7 % of the total flow was diverted to the CVS circuit.

Table 1. *Haemoglobin concentration ([Hb]) and haematocrit (Hct) in blood from dorsal aorta (DA), sinus venosus (SV) and branchial vein (CVS) of freshwater-acclimated rainbow trout at 15°C*

	[Hb] ($g\,100\,ml^{-1}$)			Hct (%)		
Experiment	DA	SV	CVS	DA	SV	CVS
1	12·8	11·60	0·25	45·3	43·8	0·8
2	3·09	3·05	0·23	17·2	15·3	0·5
3	4·44	4·08	1·22	18·6	17·8	4·2
4	5·57	5·38	0·11	23·0	22·7	0·2
5	7·02	6·28	1·34	23·2	20·4	3·8
6	7·01	6·58	1·98	24·3	22·9	8·2
7	5·31	5·34	2·17	19·0	18·8	7·7
8	7·11	6·89	1·01	23·4	23·0	2·4
$\bar{x}$	6·54	6·15	1·04	24·3	23·1	3·5
S.D.	±2·90	±2·55	±0·75	±8·9	±8·8	±3·1

Table 2. *Fractions of total cardiac output ($\dot{Q}_v$) directed to the systemic vasculature ($\dot{Q}_s/\dot{Q}_v$) and to the central venous sinus ($\dot{Q}_{cvs}/\dot{Q}_v$) circuits in freshwater-acclimated rainbow trout at 15°C*

Experiment	$\dot{Q}_s/\dot{Q}_v$	$\dot{Q}_{cvs}/\dot{Q}_v$
1	0·91	0·10
2	0·99	0·01
3	0·88	0·12
4	0·97	0·03
5	0·87	0·13
6	0·91	0·09
7*		
8	0·96	0·04
$\bar{x}$	0·93	0·07
S.D.	±0·046	±0·047

* For unknown reasons the ratio of $\dot{Q}_s/\dot{Q}_v$ exceeded 1.

Discussion

Critique of the method

Surgical technique

Because of the very small size of the branchial vein even in large fish and the aim of non-occlusive cannulation, the use of PE 10 tubing was indispensable. The small bore of the PE 10 and PE 20 cannulae, however, naturally limited the patency of the cannulae to shorter durations. In our hands, blood could be collected for only 2–3 days after surgery. It will therefore be difficult to apply this technique to long-

term experiments. Some improvement may be possible using Silastic tubing, although this material is much more difficult to handle for both fashioning and cannula placement. Heparin pretreatment of the cannula according to the method of Hagler *et al.* (1975) may help to extend its life-span.

Because of the small bore and the sampling method, the rate of blood flow from the branchial vein cannula is relatively slow. This feature, being advantageous with respect to minimizing the fraction of blood sampled as compared with the flow in the BV, is disadvantageous with respect to the fact that blood is in contact with air-equilibrated polyethylene tubing for a long time. There is therefore some uncertainty in the determination of acid–base parameters in BV blood. Siphoning from the BV cannula took some 5 min to collect 0·3 ml of blood. Based on our estimate of the blood flow partitioning in the gill vasculature, this corresponds to roughly 10 % of the BV blood flow (see below), which is unlikely to affect the flow patterns in the gill.

The BV is probably the venous pathway of the nutrient vasculature of the gill in addition to the CVS (Rowing, 1981). Nutrient vasculature independent of the CVS and draining into the BV, if it occurs, should contaminate the drainage of the CVS and lead to errors in assessing the CVS function with the present technique. The errors caused by this factor cannot be estimated, because neither the vascular connections between the CVS and the nutrient vessels nor their drainage pathways have yet been sufficiently clarified (Cook & Campbell, 1980; Boland & Olson, 1979). Virtually nothing is known about the blood flow distribution within the complex vasculature in the gill filament.

A significant limitation of the present technique is that blood collected from the BV cannula represents, at best, the mixed venous drainage from only one of eight gill arches. Any functional differences among gill arches in terms of CVS function may accordingly be obscured, and this may affect the reliability of extrapolation to the overall function. Blood sampling from more central sites such as the inferior jugular vein, however, is less desirable because of the larger inflow of systemic venous blood.

Model calculations

Our model calculations assume that no net water transfer occurs across the gill epithelium. This assumption is not strictly true, but the possible error caused by net water movement can be assessed by the following considerations. In freshwater fish, the net water gain through the gill epithelium at steady state is approximately the same as the urine flow rate ($\dot{Q}_u$), whereas water gain through the body surface is of minor importance. The effect of the net water flux on the estimate of flow partitioning can be assessed by modifying equations 3 and 4:

$$\dot{Q}_s/\dot{Q}_v = (C_v - rC_{cvs})/(C_s - C_{cvs}) \qquad (3')$$

and

$$\dot{Q}_{cvs}/\dot{Q}_v = (rC_s - C_v)/(C_s - C_{cvs}) , \qquad (4')$$

where $r = (\dot{Q}_u + \dot{Q}_v)/\dot{Q}_v$.

The urine flow rate of freshwater rainbow trout ranges from 0·05 to 0·07 ml kg^{-1} min^{-1} at around 15°C (Hunn, 1982; McDonald, 1983), whereas the cardiac output of rainbow trout is in the range 20–100 ml kg^{-1} min^{-1} (Holeton & Randall, 1967; Kiceniuk & Jones, 1977; Wood & Shelton, 1980; Neumann, Holeton & Heisler, 1983). Net water uptake through the gill epithelium of this magnitude will lead accordingly to an r value of 1·0005–1·004, i.e. the error introduced by neglecting the net water flux is below 0·7 %. Similar considerations apply to marine species, with drinking rate as an indication of net water loss through the gill (neglecting urinary loss and cutaneous gain).

Systemic and central venous sinus Hct and [Hb]

The differences in Hct and [Hb] between systemic and CVS blood are remarkable. Although a low red blood cell concentration in the CVS fluid has been indicated by anatomical (Dunel & Laurent, 1980), as well as physiological studies *in vitro* (Olson, 1984) and *in vivo* (Soivio, Nikinmaa, Nyholm & Westman, 1981), this is the first unequivocal demonstration of the low Hct values of the fluid perfusing the CVS circuit. This is probably due to plasma skimming at capillary-sized arteriovenous anastomoses and/or tortuous arterioles leading to the CVS. Recently, Steffensen, Lomholt & Vogel (1986) have reported microscopic observations of the occurrence of plasma skimming at the junction between the systemic and 'secondary vessel' in glass catfish, the latter connected with the systemic arteries through curled capillary-sized anastomoses and distributed throughout the internal and external surfaces of the fish body. On the basis of morphological similarities, Vogel (1985) considered the CVS circuit in the gill filament to be part of the secondary vessel system. The Hct value of the fluid in the secondary vessel has been estimated by visual observation to be less than 1 % (Steffensen *et al.* 1986).

The differences between ventral and dorsal aortic blood in Hct and [Hb] are rather small and can only be detected by most careful sampling procedures and measurements. Soivio *et al.* (1981) found that Hct and [Hb] values in the dorsal aortic blood were higher than in the ventral aortic blood in chronically cannulated rainbow trout, and ascribed this to plasma skimming occurring in the gill, whereas Jones, Brill & Mense (1986) reported great variability of these parameters among individuals. For unknown reasons, we found a reversed gradient in one of our specimens. This may have been due to incomplete mixing of systemic venous blood in the sinus venosus. The ventral aorta would certainly have been a better site for mixed venous blood sampling, considering the convective blood mixing taking place within the heart. Cannulation of the sinus venosus was chosen for technical reasons. Although *post mortem* dissection revealed that the cannula tip was successfully located in the median plane of the sinus, incomplete mixing of venous blood cannot be ruled out. Greater accuracy may be expected from the use of chromium-labelled red blood cells instead of chemically determined Hb as an indicator.

Table 3. *Comparison of the fraction of cardiac output directed towards systemic tissues ($\dot{Q}_s/\dot{Q}_v$) in isolated perfused gill preparations* (in vitro, *first group) and* in vivo *(second group)*

Species	Perfusate	$\dot{Q}_s/\dot{Q}_v$	References
Ophidon elongatus	Saline	0·33–0·88	Farrell, Daxboeck & Randall, 1979
Platichthys flesus	Saline	0·72	Stagg & Shuttleworth, 1984
Ictalurus punctatus	Blood	0·70	Olson, 1984
Ictalurus melas	Blood	0·51–0·69	Olson, 1984
Salmo gairdneri	Blood	0·55	Olson, 1984
Salmo trutta	Saline	0·51	Smith, 1977
Anguilla anguilla	*In vivo*	0·72*	Hughes, Peyraud, Peyraud-Waitzenegger & Soulier, 1982
	In vivo	0·94*	
Salmo gairdneri	*In vivo*	>0·95	Neumann, Holeton & Heisler, 1983
Salmo gairdneri	*In vivo*	0·93	Present study

* Second value after infusion of catecholamines (see text).

Blood flow partitioning in vivo vs in vitro

Comparison of the data on blood flow partitioning in the gill vasculature of the present study with previous *in vitro* experiments yields considerable differences (Table 3). Only the most careful studies utilizing isolated gill arch techniques have been included, and isolated perfused head preparations were not considered to be comparable. This is because, as a common practice, the dorsal aorta of the head is cannulated and the effluent taken as the 'arterial' flow, and the flow from the sectioned surface of the head as the 'venous' flow (Girard & Payan, 1980; Gardaire *et al.* 1985). Obviously this venous outflow is a mixture of cephalic arterial and true venous outflow, and only a small fraction of the mixture represents drainage from the branchial vein. Accordingly, reliable studies of flow partitioning in the gill cannot be performed using this technique.

Studies with isolated gill preparations generally yield much lower $\dot{Q}_s/\dot{Q}_v$ values (range 33–88 %, Table 3) than those determined by the present technique. These large differences have to be attributed to inadequate separation of the arterial and venous outflows, and/or to unphysiological pressure and flow profiles often applied to these isolated preparations. One important factor may also be the level of catecholamines in the perfusate used to stabilize preparations, which otherwise will deteriorate, or used to test the effects of these drugs on physiological processes occurring in the gills. However, typical concentrations used (10^{-5}–10^{-7} mol l^{-1}) are unphysiologically high. Recently reported plasma catecholamine concentrations of chronically catheterized, recovered rainbow trout using high-pressure liquid chromatography are in the range of 10^{-10}–10^{-9} mol l^{-1} (Boutilier, Iwama & Randall, 1986; Perry & Vermette, 1987; Primmett, Randall, Mazeaud & Boutilier,

1986). Plasma catecholamine levels would not rise to such concentrations even in response to severe stress *in vivo* (see Perry & Vermette, 1987).

There are only a few studies aimed at estimating blood flow partitioning in fish gills *in vivo*. Hughes, Peyraud, Peyraud-Waitzenegger & Soulier (1982) have compared cardiac output in eels directly measured using a Doppler flowmeter with lamellar blood flow determined by the Fick principle. Lamellar blood flow was found to be only 70 % of cardiac output, and they concluded that as much as 30 % of cardiac output bypassed the lamellae and returned directly to the heart. A few teleost species including eels, however, are known to possess afferent, in addition to efferent, arteriovenous anastomoses in the gill (Dunel & Laurent, 1980). In rainbow trout, Neumann *et al.* (1983) showed that there was little, if any, difference between ventral and dorsal aortic blood flow.

The fairly wide variation in Hct and [Hb] values in CVS blood suggests that neural and/or hormonal control mechanisms contribute to the regulation of plasma skimming and flow distribution. In this context, it may be worth mentioning that in our experiments there were many more red blood cells in the fluid from the BV cannula of fish undergoing surgery than in recovered, resting fish. Recently, Bailly & Dunel-Erb (1986) and Dunel-Erb & Bailly (1986) have studied the innervation of a sphincter in the efferent filamental artery in four species of teleosts. They demonstrated dual, cholinergic and adrenergic, innervation of the sphincter, suggesting presynaptic modulation of cholinergic nerve activity by adrenergic fibres. This system could be involved in the regulation of the extent of red blood cell entry into the CVS as well as of the distribution of cardiac output between the systemic and CVS circuits.

The ubiquity of the CVS in fish gills may reflect its important role in the proper function of the gills in the regulation of homeostasis. Several hypotheses have been suggested for the function of the CVS, but none of them has received convincing experimental verification (Randall, 1985). The present *in vivo* preparation provides a new approach to gain more precise and detailed information for further insight into the complex overall function of fish gills.

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