# Oxygen exchange and vascular resistance in the totally perfused rainbow trout

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WOOD, CHRIS M., B. R. McMAHON, AND D. G. McDONALD. *Oxygen exchange and vascular resistance in the totally perfused rainbow trout*. Am. J. Physiol. 234(5): R201-R208, 1978 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 3(3): R201-R208, 1978.—A whole trout preparation (*Salmo gairdneri*) externally ventilated with water and internally perfused with artificial medium via a cardiac pump is described for the study of $O_2$ exchange and vascular resistance. As cardiac output ($\dot{Q}$) was raised, ventral and dorsal aortic pressures increased while branchial (Rg) and systemic (Rs) vascular resistances fell, reflecting considerable passive distensibility. Arterial oxygenation was negative at low $\dot{Q}$'s due to significant internal $O_2$ demand by the gill tissue, but increased to zero or positive values at intermediate $\dot{Q}$'s, and eventually declined at high $\dot{Q}$'s because of transit time limitation. $O_2$ uptake from the ventilatory flow rose with increasing $\dot{Q}$. Epinephrine ($10^{-5}$ M) decreased Rg, increased Rs, and enhanced arterial oxygenation. Artificial elevation of dorsal aortic pressure decreased Rg but did not affect arterial oxygenation. A 10-fold elevation of ventilatory flow increased arterial oxygenation but did not alter Rg or Rs. Endogenous metabolism of branchial tissue accounted for 11.7% of resting $O_2$ uptake in vivo, and comprised an internal component taking $O_2$ from perfusion flow and an external component drawing $O_2$ from ventilatory flow.

fish gill; ventilation; passive distensibility; branchial vascular resistance; epinephrine; gill metabolism

STEEN AND KRUYSSE (24) described respiratory and nonrespiratory pathways of blood flow through the teleost gill, and proposed that fish can vary their functional respiratory surface area or permeability by adjusting the pattern of branchial blood flow. Perfusion of the respiratory pathway was thought to be associated with adrenergic vasodilation, and perfusion of the nonrespiratory pathway with cholinergic vasoconstriction (13, 18). More recent work indicates that the circulation in the gill is far more complex than originally described, and the principal "nonrespiratory shunt" of Steen and Kruysse (24) is now considered a venous channel returning oxygenated blood to the heart (6, 16, 26–28). Current theories envisage that autonomic and humoral stimuli act both to vary the number of secondary lamellae perfused (16, 29) and to redistribute blood flow between efferent venous and efferent arterial pathways (7, 19, 22) rather than simply to shunt perfusion between respiratory and nonrespiratory vessels. Nevertheless, the concept of a respiratory surface adjustable in size or permeability to satisfy varying metabolic and osmoregulatory demands remains a basic principle (e.g., 11).

Isolated-perfused gill preparations have been employed to study various aspects of this concept, including vascular resistance (7, 15, 19, 22, 29, 30), urea influx as an index of branchial surface area (1, 8), heat exchange as an index of branchial vascular geometry (23), and various ionic exchanges (15, 20, 21). However, the fundamental parameter on which the concept hinges, respiratory gas exchange, has received no such experimental attention. Indeed, the only information indicating that autonomic or humoral agents can affect respiratory gas exchange remains the original work of Steen and Kruysse (24), who demonstrated that the intravenous administration of epinephrine increased arterial $P_{O_2}$ and $O_2$ content in restrained eels.

The present work describes a totally perfused whole trout preparation. The objectives have been to examine the effects of variations in internal and external perfusion flow rates, adrenergic stimulation, and blood pressures on $O_2$ exchange and vascular resistance in the branchial and systemic vascular beds.

## SYMBOLS

| | |
|---|---|
| $\dot{V}g$ | external ventilatory flow |
| $\dot{V}o_2$ | $O_2$ uptake from the ventilatory flow |
| $\dot{Q}$ | output of cardiac pump or heart in vivo |
| HR | stroke rate of cardiac pump or heart in vivo |
| SV | stroke volume of cardiac pump or heart in vivo |
| $Pi_{O_2}$ | inhalant $O_2$ tension |
| $Pe_{O_2}$ | exhalant $O_2$ tension |
| $Pa_{O_2}$ | dorsal aortic $O_2$ tension |
| $Pv_{O_2}$ | ventral aortic $O_2$ tension |
| $Ps_{O_2}$ | $O_2$ tension of perfusate returning from systemic circulation |
| $\alpha w_{O_2}$ | solubility of $O_2$ in water |
| $\alpha b_{O_2}$ | solubility of $O_2$ in perfusion medium or blood in vivo |
| Pa | dorsal aortic blood pressure |
| Pv | ventral aortic blood pressure |
| Pt | transmural blood pressure |
| Rg | gill vascular resistance |
| Rs | systemic vascular resistance |

## MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*, 110–260 g) were

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obtained from commercial suppliers and held at 7.5 ± 1.5°C for at least 2 wk prior to experimentation. All experiments were performed at 7.5 ± 0.5°C.

*Preparation of Animals*

A trout was anesthetized with 1:15,000 MS-222 on an operating table. The dorsal aorta was cannulated at the junction of the efferent branchial arteries with PE-60 tipped with a no. 21 Huber point needle (9); 500 IU of sodium heparin were infused and allowed to circulate for 5 min. The ventral aorta was sectioned at its point of curvature and cannulated anteriorly with PE-190 tipped with a short length of soft rubber tubing. The cut posterior end of the ventral aorta was similarly cannulated. Perfusion medium was then infused anteriorly through the aorta at a constant pressure of 40 $cmH_2O$ until the venous effluent appeared free of blood (10–15 min). A flared tube for external ventilation of the gills was sewn onto the roof of the mouth (29), entering anteriorly through a hole punched in the lower jaw; the mouth was then sewn tightly shut, resulting in opercular abduction.

*Perfusion System (Fig. 1)*

The head was passed through a plastic collar which encircled the animal and formed a pressure seal just posterior to the cleithrum. The whole fish was placed inside a rectangular Plexiglas tube with which the collar made a watertight seal, effectively dividing the tube into a posterior body compartment and an anterior head compartment. The tube fitted inside a larger chamber to form a seal against the anterior surface of the latter. External water flow ($\dot{V}g$) entered through this anterior surface and was directed over the gills by the flared ventilation tube. The $\dot{V}g$ leaving the opercular openings was directed anteriorly by the plastic collar and exited through anterioventral holes joining the tube to the outer chamber. A slight positive-pressure gradient (0.5 $cmH_2O$) prevented backflow of water from the outer to the inner chamber. Thus the anterior compartment of the tube formed a mixing chamber from which mixed expired water samples could be drawn. $\dot{V}g$ was monitored with a Gilmont flowmeter on the inflow, periodically checked by timed collection from the constant-level overflow of the outer chamber. Dye pulses were regularly administered through the ventilation tube to assure a relatively even distribution of $\dot{V}g$ over the branchial basket, lack of leakage through the mouth and jaw, and good mixing in the anterior chamber.

A Harvard 1405 cardiac pump of fixed phase (35% systole, 65% diastole) but variable SV and HR was employed for internal perfusion ($\dot{Q}$). In vivo measurements of $\dot{Q}$ in fish indicate that systole normally accounts for 20–40% of the cardiac cycle (Wood and Shelton, unpublished results; 10). The advantages of constant flow perfusion have previously been documented (29, 30, 32). The perfusion medium has been described previously (29) and was Millipore-filtered (0.22 $\mu$m) before use. The medium carried $O_2$ only by physical solution with $\alpha b_{O_2}$ = 5.126 $\mu$l $O_2$/Torr per ml $H_2O$, as calculated from physical tables. Direct measurements of $\alpha b_{O_2}$ obtained by injecting samples of known $Po_2$ into a "Lex-$O_2$-Con" analyzer (Lexington Instruments) gave a comparable figure. The perfusion reservoir rested on a top-loading balance for exact determination of $\dot{Q}$ (30) and was gassed with an air-$N_2$ mixture by a Wosthoff pump. The output of the cardiac pump passed through a temperature-equilibration coil in the outer chamber and then into the ventral aorta of the preparation. The exiting venous perfusate was collected outside the chamber at a height equal to the water level in the outer chamber.

*Measurements*

$Po_2$ was determined with a Radiometer microelectrode maintained at the experimental temperature. Inspired ($Pi_{O_2}$ = 140–145 Torr) and mixed expired ($Pe_{O_2}$) water samples were drawn from the ventilation tube

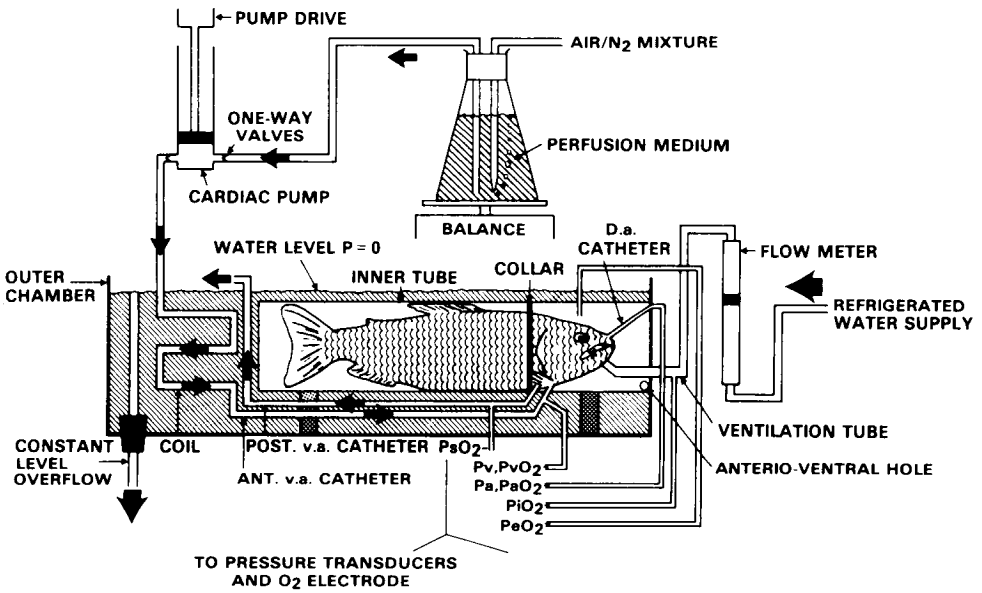


FIG. 1. Semidiagrammatic representation of apparatus used in perfused trout preparation. *Small arrows* indicate direction of flow of internal perfusion medium, *large arrows* direction of external ventilatory flow. Da = dorsal aortic; va = ventral aortic. See text for details.

and from the mixing chamber, respectively. $Pv_{O_2}$ samples were taken from a T junction in the perfusion system immediately proximal to the point of anterior ventral aorta cannulation. $Pa_{O_2}$ samples were taken from the dorsal aortic catheter, and $Ps_{O_2}$ samples from the posterior ventral aortic catheter. Pv and Pa were measured from the respective sampling catheters with Hewlett Packard 267 BC pressure transducers. The water level in the outer chamber was considered zero pressure. Integration of the pulsatile pressure wave form produced by the cardiac pump indicated that area mean pressure (2) could be closely estimated by (1 systolic + 2 diastolic)/3. Pv was corrected for the resistance of the perfusion system distal to the point of pressure measurement (32). Perfusate samples were allowed to fill the $Po_2$ electrode under their own pressure. The height of the electrode was adjusted for each measurement so that actual blood pressure in the vessel sampled did not fall more than 5 $cmH_2O$ during the sampling period, as judged from independent pressure measurements in pilot experiments.

### *Experimental Treatments*

In all four treatment groups, $Pv_{O_2}$ was set to 30–40 Torr, the normal $Pv_{O_2}$ of intact trout (4), by manipulating the air-$N_2$ mixture of the Wosthoff pump relative to $\dot{Q}$. Initially, the preparation was perfused at $\dot{Q}$ = 1.0 ml/100 g per min (HR = 50/min) and $\dot{V}g$ = 25 ml/100 g per min, the normal $\dot{V}g$ of intact trout at this temperature (4) for a 30-min stabilization period. During this time, a spontaneous decrease in Pa to a base-line level always occurred. This was due to a loss of endogenous $\alpha$-adrenergic tone in the systemic vasculature, as has been previously documented (32). Pv remained generally stable over this period. The experimental condition was then applied, $\dot{Q}$ being kept at 1 ml/100 g per min for an initial paired comparison of the effects of the treatment. Measurements were then taken on each preparation at $\dot{Q}$'s = 0.50, 1.0, 1.5, 2.0, 2.5, and 3.0 ml/100 g per min, $\dot{Q}$ being varied by manipulation of SV only (HR = 50/min). These $\dot{Q}$'s represent the normal range measured in intact trout at a comparable temperature, while 50/min is the normal in vivo HR (25). At each $\dot{Q}$, a stabilization period of 20 min was allowed before measurements were performed. $Po_2$'s were determined in the order $Pi_{O_2}$, $Pe_{O_2}$, $Pa_{O_2}$, $Pv_{O_2}$, and $Ps_{O_2}$. Because each experiment lasted 6–8 h, the order of application of $\dot{Q}$'s was randomized to negate any effects of time-dependent changes in the preparation.

*Control group* ($N$ = 9). Standard perfusion medium and $\dot{V}g$ = 25 ml/100 g per min were employed throughout. $Ps_{O_2}$'s were measured only in this group.

*Epinephrine group* ($N$ = 9). The perfusion medium was modified by addition of $10^{-5}$ M $l$-epinephrine bitartrate (Sigma). $\dot{V}g$ = 25 ml/100 g per min.

*High Pa group* ($N$ = 7). A steel tourniquet clamp was applied to the whole body posterior to the plastic collar and tightened to increase Rs. Rs was varied at each $\dot{Q}$ so as to maintain Pa in the range of 30–40 $cmH_2O$, the normal Pa of intact trout (4). At the lowest $\dot{Q}$ (0.50 ml/100 g per min), it was not always possible to attain the desired elevation of Pa with this method. Consequently, the mean Pa at this $\dot{Q}$ was actually only 26.0 $cmH_2O$ (Fig. 2*D*). $\dot{V}g$ = 25 ml/100 per g min. Standard perfusion medium was used.

*High $\dot{V}g$ group* ($N$ = 7). $\dot{V}g$ was elevated 10-fold to 250 ml/100 g per min. At this $\dot{V}g$, differences between $Pi_{O_2}$ and $Pe_{O_2}$ were not measurable, so only $Pa_{O_2}$ and $Pv_{O_2}$ were determined. Standard perfusion medium was used.

The significance ($P \leq 0.05$) of experimental treatments was assessed by comparison with values at the same $\dot{Q}$ in the control group (unpaired two-tailed Student $t$-test) and by comparison within preparations of the initial effects of the treatment when applied at 1.0 ml/100 g per min (paired two-tailed Student $t$-test).

### *$\dot{V}o_2$ In Vivo*

Resting $\dot{V}o_2$'s were determined by flow-through respirometry on four trout in darkened metabolism boxes at 7.5 ± 0.5°C. These fish were in fact being used in a renal function study, and the measurements were taken 5 days after recovery from MS-222 anesthesia and urinary bladder catheterization.

## RESULTS

### *Blood Pressure and Vascular Resistance*

Blood pressure-flow relationships accompanying the experimental variations in $\dot{Q}$ are shown in Fig. 2 for the four treatment groups, and the initial effects of experimental treatments within preparations at $\dot{Q}$ = 1.0 ml/100 g per min are summarized in Table 1. In all groups, both Pv and Pa increased as $\dot{Q}$ was raised from 0.5 to 3.0 ml/100 g per min. However elevation of $\dot{V}g$ from 25 to 250 ml/100 g per min had no effect on pressure-flow

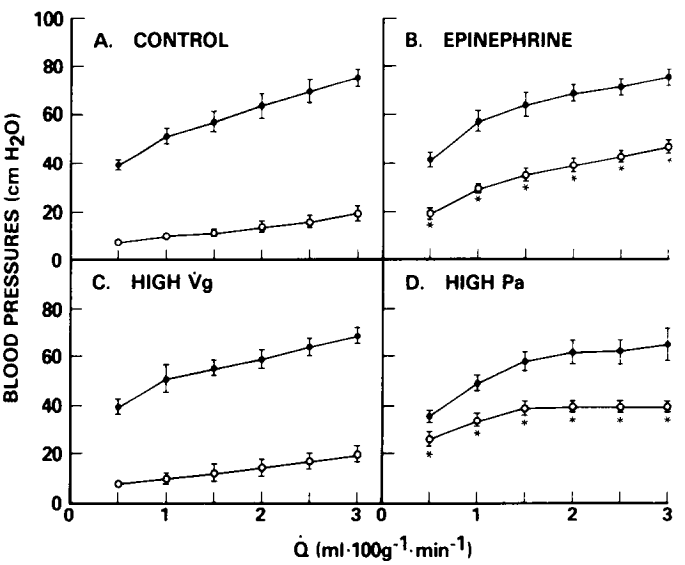


FIG. 2. Effect of variations in $\dot{Q}$ on ventral aortic (Pv = ●) and dorsal aortic (Pa = ○) blood pressures in perfused trout preparation under *A*, control ($n$ = 9); *B*, epinephrine ($n$ = 9); *C*, high $\dot{V}g$ ($n$ = 7); and *D*, high Pa ($n$ = 7) treatments. Means ± 1 SE are given. *Mean significantly different ($P \leq 0.05$) from comparable value at same $\dot{Q}$ in control group.

TABLE 1. *Pv, Pa, $Pa_{O_2} - Pv_{O_2}$, and $\dot{V}O_2$ before and after different experimental treatments in perfused trout preparation*

| | Before | | | | After | | | |
|---|---|---|---|---|---|---|---|---|
| | Pv | Pa | $Pa_{O_2} - Pv_{O_2}$ | $\dot{V}O_2$ | Pv | Pa | $Pa_{O_2} - Pv_{O_2}$ | $\dot{V}O_2$ |
| Epinephrine, $10^{-5}$ M ($n$ = 9) | 54.7 | 14.4 | −21.8 | 2.63 | 60.4 | 39.0* | −8.3* | 2.56 |
| High Pa ($n$ = 7) | 49.8 | 11.2 | 23.2 | 3.87 | 50.9 | 36.1* | −24.2 | 4.20 |
| High $\dot{V}g$ ($n$ = 7) | 52.5 | 9.8 | −19.1 | | 52.4 | 10.3 | −7.4* | |

Values are means. See SYMBOLS for explanation of abbreviations. Pv and Pa are measured in $cmH_2O$, $Pa_{O_2} - Pv_{O_2}$ in Torr, and $\dot{V}O_2$ in $\mu l$ $O_2$/100 g per min. Before values taken 30 min after start of experiment at $\dot{Q}$ = 1.0 ml/100 g per min. After values taken 30 min after application of experimental treatment at this same $\dot{Q}$ (60–90 min after start of experiment). *Significantly different ($P \leq 0.05$) from before values by paired two-tailed Student $t$-test.

relations within the preparation (Fig. 2, *A* and *C*; Table 1).

Epinephrine ($10^{-5}$ M) in the perfusate significantly elevated Pa at all values of $\dot{Q}$, whereas Pv was not significantly changed (Fig. 2, *A* and *B*; Table 1). Consequently the Pv − Pa differential was markedly reduced at all $\dot{Q}$'s relative to the control group (Fig. 2, *A* and *B*). The response of the systemic vasculature, and thus Pa, to epinephrine tended to decline with time. This can be seen by comparing the Pa value of the epinephrine group in the initial comparison data (39.0 ± 1.5 (9) $cmH_2O$ (mean ± SE (*N*)); Table 1) with that at the same $\dot{Q}$ in the regular experiments (28.9 ± 3.9 (9) $cmH_2O$; Fig. 2*B*). The former value was obtained 30 min after the initial application of epinephrine, whereas the latter represents a mean value for later times (1–8 h) due to the randomized nature of $\dot{Q}$ variation (see MATERIALS AND METHODS). This phenomenon can be prevented by oxygenation of the perfusate (32), but oxygenation could not be employed in the present experiments because it would have interfered with the $O_2$ exchange determinations. No marked changes in branchial adrenergic reactivity (i.e., Pv − Pa) were seen over the same period.

Artificial manipulation of Pa to the 30–40 $cmH_2O$ range effected a twofold (high $\dot{Q}$) to threefold (low $\dot{Q}$) elevation of Pa relative to the control group, although Pv was not significantly altered at any $\dot{Q}$ (Fig. 2, *A* and *D*; Table 1). The actual values of Pa attained at each $\dot{Q}$ did not differ significantly from those in the epinephrine treatment (Fig. 2, *B* and *D*). As with the epinephrine treatment, the Pv − Pa differential was markedly reduced at all $\dot{Q}$'s relative to the controls (Fig. 2, *A* and *D*).

Vascular resistances were calculated as Rg = (Pv − Pa)/$\dot{Q}$ and Rs = Pa/$\dot{Q}$. Both Rg and Rs fell markedly in all treatment groups as $\dot{Q}$ increased, the largest changes occurring at the lowest $\dot{Q}$'s (Fig. 3, *A* and *B*). Rg was unaffected by the high $\dot{V}g$ treatment but was significantly lowered at all $\dot{Q}$'s by both epinephrine and high Pa (Fig. 3*A*). The latter treatment was in fact slightly more effective, resulting in significantly lower values of Rg than the epinephrine treatment at $\dot{Q}$ = 0.50 and 1.0 ml/100 g per min. Conversely, Rs was also unaffected by high $\dot{V}g$ but was significantly elevated by epinephrine and of course by the high Pa treatment (Fig. 3*B*). There were no significant differences in Rs between the latter two groups.

### *Oxygen Exchange*

$O_2$ exchange by the internal perfusate during its passage through the gills has been expressed as $Pa_{O_2} - Pv_{O_2}$ to compensate for slight differences in $Pv_{O_2}$ (30–40 Torr) in different experiments (Figs. 4 and 5). Since $O_2$ was carried entirely by physical solution in the perfusion medium, $(Pa_{O_2} - Pv_{O_2}) \cdot \alpha b_{O_2}$ will equal the $O_2$ concentration difference between arterial and venous samples. In the control group, $Pa_{O_2} - Pv_{O_2}$ increased from a negative value at $\dot{Q}$ = 0.5 ml/100 g per min (net $O_2$ loss) to an apparent asymptotic maximum at $\dot{Q}$ = 3.0 ml/100 g per min; the latter value was not significantly different from zero (no net $O_2$ flux) (Fig. 4).

The relative forms of the $Pa_{O_2} - Pv_{O_2}$ versus $\dot{Q}$ curves were very similar in the other three groups, though the absolute values differed (Fig. 5). The asymptotic nature of these relationships is at least partly an artifact of averaging. In all four groups, the majority of preparations showed a maximum value of $Pa_{O_2} - Pv_{O_2}$ at some intermediate value of $\dot{Q}$, followed by a decline at the highest values, as illustrated by the example in Fig. 4. The actual $\dot{Q}$ at which $Pa_{O_2} - Pv_{O_2}$ reached a peak varied from preparation to preparation, whereas in

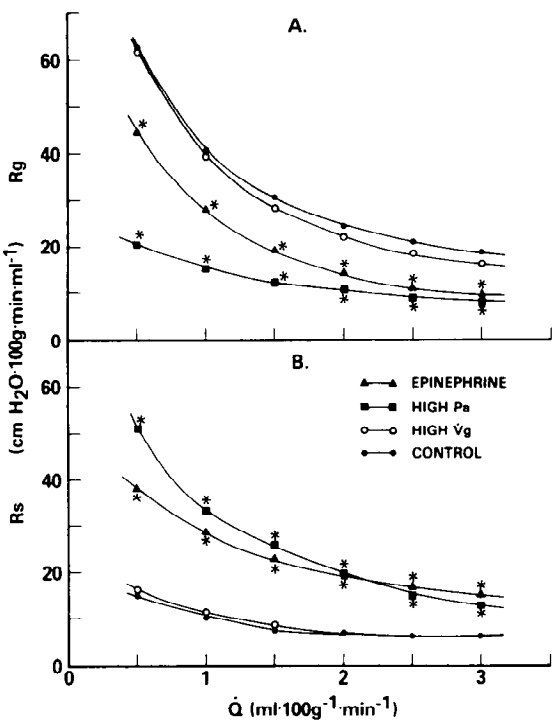


FIG. 3. Effects of control ($n$ = 9), epinephrine ($n$ = 9), high $\dot{V}g$ ($n$ = 7), and high Pa ($n$ = 7) experimental treatments on the relationship between *A*, branchial vascular resistance (Rg) and $\dot{Q}$, and *B*, systemic vascular resistance (Rs) and $\dot{Q}$ in the perfused trout preparation. Means are given (standard errors omitted for clarity). *Mean significantly different ($P \leq 0.05$) from comparable value at same $\dot{Q}$ in control group.

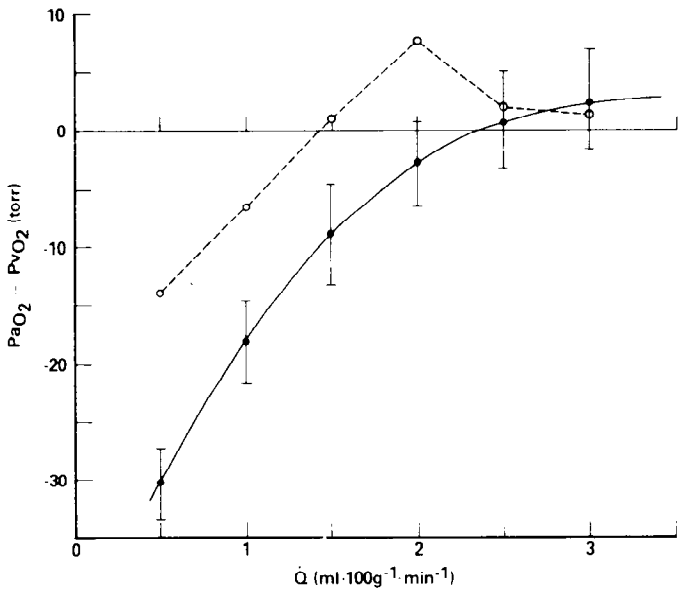


FIG. 4. Effect of variations in $\dot{Q}$ on $Pa_{O_2} - Pv_{O_2}$ in perfused trout preparation under control conditions ($n$ = 9). Means ± 1 SE are given. *Dotted line* illustrates results from a single preparation (weight = 189 g); note that highest value of $Pa_{O_2} - Pv_{O_2}$ is attained at an intermediate $\dot{Q}$, and that $Pa_{O_2} - Pv_{O_2}$ declines at higher $\dot{Q}$'s. This effect is obscured by averaging in mean results.

some animals $Pa_{O_2} - Pv_{O_2}$ was still increasing at the highest $\dot{Q}$ tested. Averaging effectively obscured this optimum $\dot{Q}$ effect.

Epinephrine ($10^{-5}$ M) significantly elevated $Pa_{O_2} - Pv_{O_2}$ by about 15 Torr relative to the control group over the whole range of $\dot{Q}$ tested (Fig. 5). This effect was also seen in the initial comparison data (Table 1). Thus at $\dot{Q} \geq 2.0$ ml/100 g per min, there occurred a significant net uptake of $O_2$ by the perfusate in the presence of epinephrine. The high $\dot{V}g$ treatment increased $Pa_{O_2} - Pv_{O_2}$ in a similar manner to epinephrine (Fig. 5; Table 1), though this rise was significant (relative to the control group) only at $\dot{Q} \leq 1.5$ ml/100 g per min. Nevertheless, net $O_2$ uptake by the internal perfusate was significantly greater than zero at $\dot{Q} \geq 2.5$ ml/100 g per min in the presence of high $\dot{V}g$. The high Pa condition had no significant effect on the $Pa_{O_2} - Pv_{O_2}$ versus $\dot{Q}$ relationship (Fig. 5; Table 1).

$\dot{V}o_2$ from the external ventilatory flow was calculated by the Fick principle as $\dot{V}o_2 = (Pi_{O_2} - Pe_{O_2}) \cdot \alpha w_{O_2} \cdot \dot{V}g$. However $Pi_{O_2} - Pe_{O_2}$ differences were generally in the order of 0.5–5.0 Torr, which approaches the precision (±0.5 Torr) of the analytical system. Consequently, measurement error was substantial and the variability in the data was large. Nevertheless $\dot{V}o_2$ tended to rise with increasing $\dot{Q}$ in all three treatments (Fig. 6), though this trend was significant ($P < 0.01$) only in the control group (as assessed by the significance of the correlation coefficient). No significant differences in $\dot{V}o_2$ from control values occurred in either the high Pa or the epinephrine groups, though the mean values in the latter were regularly lower than in the other two (Fig. 6). No effect of epinephrine or high Pa could be seen in the initial comparison data (Table 1). Overall, $\dot{V}o_2$ averaged about 5 μl $O_2$/100 g per min (Fig. 6); this figure may be compared with a value of 51.2 ± 4.5 (4) μl $O_2$/100 g per min measured in resting trout in vivo.

$Ps_{O_2}$, the $O_2$ tension of the perfusate returning from the systemic circulation, was extremely low (6.5 ± 1.9 (9) Torr) and did not vary significantly with $\dot{Q}$, despite an increase in $Pa_{O_2}$ from 7.8 ± 2.8 Torr at 0.5 ml/100 g per min to 37.5 ± 4.0 Torr at 3.0 ml/100 g per min. Thus $O_2$ uptake by the systemic tissues was limited by the rate of $O_2$ delivery, and the observed increases in Pa – Ps from 1.2 ± 2.1 Torr at the lowest $\dot{Q}$ to 31.6 ± 2.6 Torr at the highest $\dot{Q}$ simply reflected the increasing oxygenation of the arterial perfusate. In two animals, $Ps_{O_2}$'s between 0.5 and 2.0 Torr were consistently recorded, indicating the remarkably low tensions from which trout cells can take up $O_2$.

## DISCUSSION

### *Blood Pressure and Vascular Resistance*

In the present preparation, the branchial and systemic circulations were perfused in situ under conditions closely duplicating the in vivo situation with respect to the serial arrangement of the vascular beds, the normal range of $\dot{Q}$, the pulsatile wave form and HR of $\dot{Q}$, the $Po_2$ of the branchial perfusate, and the external $\dot{V}g$. The results largely confirm the findings of earlier work (29, 32) in which the branchial and systemic vascular beds were isolated and perfused separately under less physiological conditions.

In the branchial results, the principal point of interest is the marked passive distensibility of the system. For example, in the control group, note the 66% drop in Rg as $\dot{Q}$ was raised from 0.5 to 3.0 ml/100 g per min (Fig. 3*A*); this was associated with a doubling of Pt (1/2 (Pv + Pa)) from 23.3 to 47.2 $cmH_2O$ (Fig. 2*A*). Similarly, in the epinephrine group, a doubling of Pt (30.4 to 61.1 $cmH_2O$) over the same $\dot{Q}$ range caused an 80% decrease in Rg (Figs. 2*B* and 3*A*). This passive distensibility can also be seen in the results for the high Pa group (Figs.

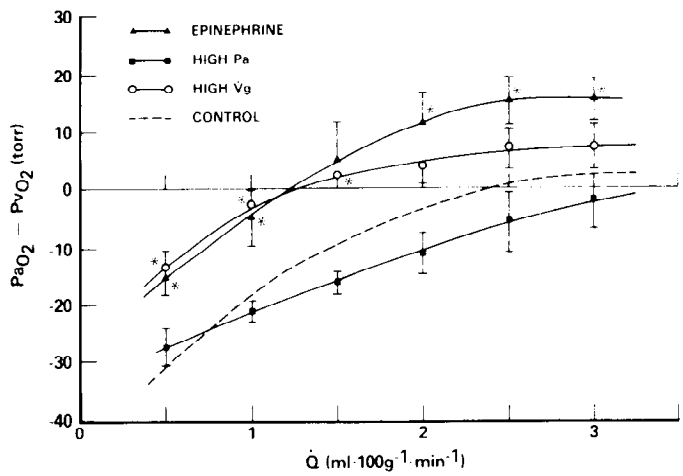


FIG. 5. Effects of epinephrine ($n$ = 9), high $\dot{V}g$ ($n$ = 7), and high Pa ($n$ = 7) experimental treatments on relationship between $Pa_{O_2} - Pv_{O_2}$ and $\dot{Q}$ in perfused trout preparation. Position of control relationship from Fig. 4 is indicated by *dotted line*. Means ± 1 SE are given. *Mean significantly different ($P \leq 0.05$) from comparable value at same $\dot{Q}$ in control group.

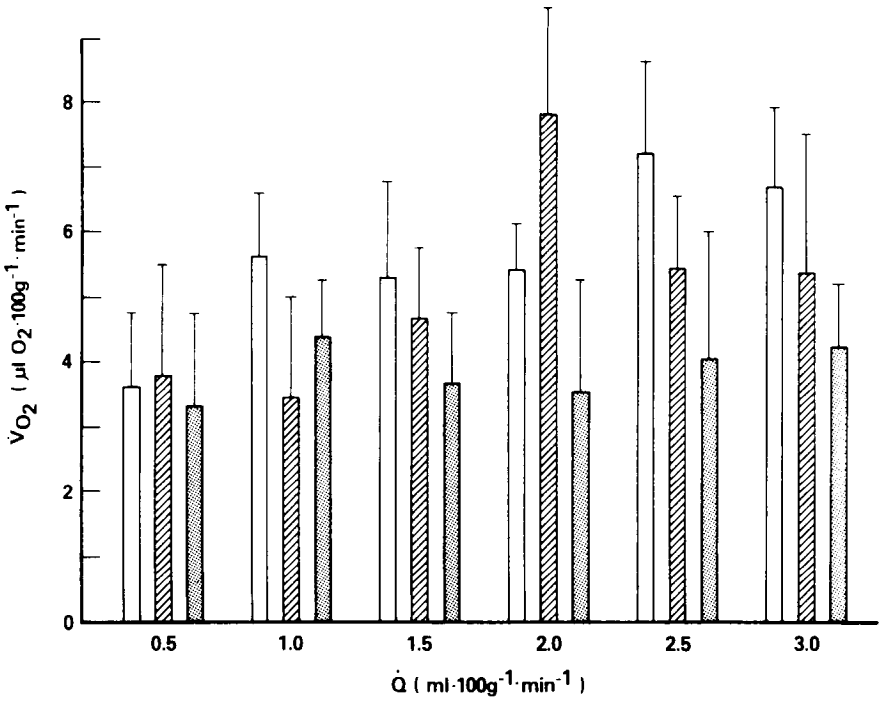


FIG. 6. Effect of variations in $\dot{Q}$ on $O_2$ uptake ($\dot{V}_{O_2}$) from external ventilatory flow by gills in perfused trout preparation under control (*white*; $n$ = 9), high Pa (*crosshatched*; $n$ = 7), and epinephrine (*stippled*; $n$ = 9) experimental treatments. Means are given; bars = +1 SE.

2*D* and 3*A*) in which Rg was significantly lower than in the controls at all values of $\dot{Q}$. Earlier, by applying a simple hydraulic model to the branchial circulation, we indicated that for a given Pt, the contribution of Pa would be more effective than that of Pv in reducing Rg (29). This is directly confirmed by the present work. An illustration is given in Table 2. Note that while Pt's were identical in the two groups, the Rg in the high Pa treatment was significantly lower. This difference reflected the greater contribution of Pa and reduced contribution of Pv to Pt in the latter relative to the control group. Many changes in Rg observed in vivo are probably explicable in terms of passive distensibility associated with changes in Pv and Pa (9, 11, 29, 32).

Epinephrine ($10^{-5}$ M) significantly reduced Rg at all $\dot{Q}$'s relative to the control group (Fig. 3*A*). This effect is associated with the stimulation of dilatory $\beta_1$-adrenoreceptors in the branchial vessels; a much smaller $\alpha$-adrenoreceptor constriction is masked (19, 29, 30). However, Pa's were significantly elevated by epinephrine (Fig. 2*B*), a phenomenon caused by systemic constriction. This effect is due to stimulation of constrictory $\alpha$-adrenorecptors in the systemic vessels; a smaller $\beta_2$-adrenoreceptor dilation is probably hidden (31). As Rg was reduced to a comparable or greater extent by the high Pa treatment (Fig. 3*A*), one possible interpretation is that the branchial dilation in the epinephrine group simply reflected physical distension by elevated Pa rather than a true decrease in vasomoter tone caused by epinephrine. There are several reasons for believing this interpretation to be untrue. First, epinephrine ($10^{-5}$ M) causes a 50% decrease in Rg even in the presence of a constant high Pa = 40 $cmH_2O$ (29). Second, the situation is complicated by the presence of efferent venous pathways as well as an efferent arterial (i.e., dorsal aortic) pathway from the gill (see introduction). Epinephrine decreases venous outflow and increases dorsal aortic outflow (7, 19, 22). The high Pa treatment

TABLE 2. *Example situation illustrating that, for a given Pt, contribution of Pa is more effective than that of Pv in reducing Rg*

| | Control ($n$ = 9) | High Pa ($n$ = 7) |
|---|---|---|
| Pt, $cmH_2O$ | 47.3 ± 2.8 | 48.4 ± 2.6 |
| Rg, $cmH_2O \cdot 100\ g \cdot min/ml$ | 18.5 ± 1.4 | 12.4 ± 2.7* |
| Pv, $cmH_2O$ | 75.0 ± 4.2 | 57.9 ± 3.7* |
| Pa $cmH_2O$ | 19.5 ± 2.3 | 39.0 ± 1.7* |
| $\dot{Q}$, ml/100 g per min | 3.0 | 1.5 |

Values are means ± 1 SE. *Significantly different ($P \leq 0.05$) from control values by unpaired two-tailed Student $t$-test.

on the other hand will increase the effective resistance at the dorsal aortic outflow (i.e., Rs), but should not affect that at the venous outflow. Calculation of Rg by (Pv − Pa)/$\dot{Q}$ will therefore tend to underestimate the vascular resistance of the gill-dorsal aortic route (arterial outflow) to a greater extent in the high Pa treatment than in the epinephrine condition.

The presence of both arterial and venous outflow routes from the gill also poses a problem for the calculation of Rs by Pa/$\dot{Q}$, because the actual $\dot{Q}$ perfusing the systemic circulation will be reduced by the amount of the venous outflow from the gill. Girard and Payan (7) determined this loss to be 10–20% of $\dot{Q}$, but used a preparation in which the normal pressure relations of the outflow channels were markedly abnormal. Smith (22) employed more realistic outflow pressures, and found a 20–60% loss of $\dot{Q}$ (estimated from Figs.). In light of this uncertainty, we have elected to use simply an uncorrected $\dot{Q}$ in the calculation of Rs, recognizing that this will tend to underestimate the real Rs to some extent. Nevertheless, the levels of base-line Rs computed in the control group (Fig. 3*B*) agree closely with those determined at comparable $\dot{Q}$'s in the isolated, directly perfused trunk (32).

The data provide evidence of passive distensibility in

the systemic vascular bed, Rs falling by about 60% in the control group as $\dot{Q}$ was raised from 0.5 to 3.0 ml/100 g per min (Fig. 3*B*). However, due to the lack of endogenous $\alpha$-adrenergic tone in the preparation (32), the Pt's (Pa/2 = 3.8–9.8 $cmH_2O$) were far below the physiological range (15–25 $cmH_2O$). Extrapolation of the Pa and Rs curves in Figs. 2*A* and 3*B* to the normal Pt range indicates that there should be little passive distensibility in Rs under in vivo conditions, in agreement with earlier work (32). In the epinephrine group, the Rs data are complicated by the time-dependent decrease in systemic adrenergic reactivity (see RESULTS), and cannot be used to assess distensibility over the physiological range of Pt's.

*Oxygen Exchange*

Both the forms and relative positions of the $Pa_{O_2} - Pv_{O_2}$ versus $\dot{Q}$ curves (Figs. 4 and 5) were surprising. One might expect that $Pa_{O_2} - Pv_{O_2}$ would be greatest at the lowest $\dot{Q}$ (i.e., highest $\dot{V}g/\dot{Q}$ ratio) and then fall as perfusate transit time decreased (i.e., diffusion limitation – cf. Fig. 4 of Jones, Randall, and Jarman (12)). On the contrary, under all treatments, $Pa_{O_2} - Pv_{O_2}$ was lowest and negative at the smallest $\dot{Q}$ and increased to a maximum value at higher $\dot{Q}$'s (Figs. 4 and 5). This behavior can only be explained by a significant uptake of $O_2$ from the internal perfusate by the branchial tissue. At low $\dot{Q}$'s, this $O_2$ demand drove $Pa_{O_2}$, and thus $Pa_{O_2} - Pv_{O_2}$, down to a negative value. As $\dot{Q}$ increased, the influence of this demand per unit perfusate flow decreased, so $Pa_{O_2} - Pv_{O_2}$ rose. Furthermore, this demand must have taken place at sites distal to the oxygenation sites or else $Pa_{O_2}$ would have been reelevated in the oxygenation sites (presumably the secondary lamellae). The influence of transit time limitation was seen in the decline of $Pa_{O_2} - Pv_{O_2}$ at the highest values of $\dot{Q}$, an effect which caused the asymptotic form of the averaged curves (Figs. 4 and 5).

Current anatomic evidence (6, 26–28) indicates that all the perfusate must flow through the secondary lamellae before passing to the various efferent pathways. Therefore it seems reasonable to assume that the oxygenation sites and $O_2$ demand sites are perfused in series. On this basis, an estimate was made of the magnitude of this $O_2$ demand from the internal perfusate by the gills. For this calculation in the control group at $\dot{Q}$ = 0.5 ml/100 g per min, it has been assumed that perfusate entered the gills at the set $Pv_{O_2}$ = 35 Torr, was oxygenated to 100 Torr in the lamellae, and was then deoxygenated to $Pa_{O_2}$ = 4.6 Torr (i.e., observed $Pa_{O_2} - Pv_{O_2}$ = 30.4 Torr; Fig. 4) by the $O_2$ demand sites. Thus the internal $O_2$ demand is given by (100 – 4.6 Torr)$\cdot\dot{Q}\cdot\alpha b_{O_2}$ = 2.44 $\mu$l $O_2$/100 g per min. The major assumption in this estimate is the "oxygenated" $Pa_{O_2}$ = 100 Torr. This value was chosen on the grounds that $Pa_{O_2}$ in vivo at approximately this temperature and $\dot{V}g$ level is about 100 Torr (4). In vivo, $\alpha b_{O_2}$ is much higher and the $\dot{V}g/\dot{Q}$ ratio is much lower (4), so that estimate should be conservative.

Even if the extreme case was assumed, i.e., that "oxygenated" $Pa_{O_2}$ reached equilibrium with $Pi_{O_2}$, the measured $\dot{V}o_2$ still exceeded the amount of $O_2$ transferred from the external water to the internal $O_2$ demand sites. Consequently, there must also have occurred a significant external $O_2$ demand, i.e., $O_2$ taken up from the external $\dot{V}g$ by the gills but not transferred to the internal perfusate. This external $O_2$ demand was estimated in the control group at $\dot{Q}$ = 0.5 ml/100 g per min as equal to the measured $\dot{V}o_2$ (3.63 $\mu$l $O_2$/100 g per min; Fig. 6) minus the uptake transferred to the internal perfusate ((100 – 35 Torr)$\cdot\dot{Q}\cdot\alpha b_{O_2}$). The resulting figure for external $O_2$ demand (1.97 $\mu$l $O_2$/100 g per min) was comparable to that for internal $O_2$ demand (2.44 $\mu$l $O_2$/100 g per min).

The combined internal and external $O_2$ demand probably increased as $\dot{Q}$ was raised from 0.5 to 3.0 ml/100 g per min, for the measured rise in $\dot{V}o_2$ (3.07 $\mu$l $O_2$/100 g per min; Fig. 6) was larger than that accounted for by the observed increase in $(Pa_{O_2} - Pv_{O_2})\cdot\alpha b_{O_2}\cdot\dot{Q}$ = 1.16 $\mu$l $O_2$/100 g per min (Fig. 4). Such a rise in endogenous $O_2$ demand probably reflected the perfusion of greater numbers of both secondary lamellae and $O_2$ demand sites as $\dot{Q}$ was raised, the increasing Pt surpassing the critical closing pressures of individual lamellae (29). The internal $O_2$ demand sites could include regions of Rg control such as arteriovenous anastomoses and smooth muscle on the efferent lamellar and efferent filamental arterioles, whereas the external $O_2$ demand sites are probably largely ion-transporting cells located in the filamental and lamellar epithelia (16, 26–28). Eddy (5), on the basis of measured NaCl fluxes and gill potentials, has calculated a very similar value to the present external $O_2$ demand estimate for the cost of branchial ion regulation in the freshwater goldfish, about 1.65 $\mu$l $O_2$/100 g per min (i.e., 1.68–2.24 J/100 g per h).

Overall, the combined internal and external $O_2$ demands of the branchial tissue accounted for essentially all of the $\dot{V}o_2$ in the control and high Pa groups, and averaged 5.98 $\mu$l $O_2$/100 g per min, or about 11.7% of the normal $\dot{V}o_2$ of resting intact trout at 7.5°C (51.2 $\mu$l $O_2$/100 g per min). This endogenous branchial metabolism has previously been neglected but may be of considerable importance. For example, it is of sufficient magnitude to account for the production of all the $CO_2$ involved in $HCO_3^-/Cl^-$ exchange (3) and all the $NH_3$ involved in "basal" ammonia excretion (20), assuming normal $\dot{V}CO_2/\dot{V}O_2$ and $\dot{V}NH_3/\dot{V}O_2$ ratios. Furthermore, it will cause a definite overestimation of $\dot{Q}$ by the Fick technique commonly employed for determining this parameter in fish in vivo, expecially when combined with the 13% of resting $\dot{V}o_2$ which is consumed cutaneously (14).

The increase in $Pa_{O_2} - Pv_{O_2}$ caused by epinephrine in the present preparation (Fig. 5; Table 1) constitutes the first experimental confirmation that epinephrine enhances arterial oxygenation (cf. 24). The concentration of epinephrine ($10^{-5}$ M) employed is probably about the maximum that ever occurs in vivo (17). The effect may be attributed to the lamellar recruitment thought to be mediated by epinephrine (16, 29). One might expect a rise in $\dot{V}o_2$ to accompany the action of epinephrine on $Pa_{O_2} - Pv_{O_2}$; on the contrary, measured $\dot{V}o_2$'s were lower (though not significantly) than in the control group (Fig. 6). Therefore epinephrine may have enhanced $Pa_{O_2} - Pv_{O_2}$ by depressing endogenous $O_2$ de-

mand rather than by elevating branchial surface area. On the other hand, no change in $\dot{V}O_2$ was detected in the initial comparison data (Table 1). In view of the uncertainties involved in the $\dot{V}O_2$ measurements (see RESULTS), this matter clearly deserves further experimental attention.

The high Pa treatment was employed because our previous analysis of the branchial circulation indicated that the effects of epinephrine and high Pa on gas exchange might be similar, both acting to increase lamellar recruitment (29). The lack of influence of high Pa on $Pa_{O_2} - Pv_{O_2}$ in the present study (Fig. 5) casts doubt on this prediction. As discussed above, the actual mechanisms of branchial vasodilation stimulated by epinephrine and high Pa may differ.

The high $\dot{V}g$ treatment was administered to test whether inefficiencies in ventilation (e.g., unstirred layers, ventilatory dead space) contributed to the low $O_2$ transfers recorded in the present preparation. The small but significant enhancement of $Pa_{O_2} - Pv_{O_2}$ which accompanied a 10-fold elevation of $\dot{V}g$ (Fig. 5; Table 1) indicated that ventilatory limitation was indeed a factor, though not a large one. Clearly the principal reason for the low values of $Pa_{O_2} - Pv_{O_2}$ was the high internal $O_2$ demand of the branchial tissue superimposed on the low $\alpha b_{O_2}$ of the perfusate. $\alpha b_{O_2}$ of trout blood is actually 10–15 times larger than that of the perfusion medium employed (4), so the relative influence of this internal $O_2$ demand on arterial oxygenation will be much smaller in vivo. Future perfusion studies must employ a medium with a much higher $\alpha b_{O_2}$ and/or artificially high values of $Pi_{O_2}$ to overcome this problem.

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REFERENCES

1. BERGMAN, H. L., R. R. OLSON, AND P. O. FROMM. The effects of vasoactive agents on the functional surface area of isolated-perfused gills of rainbow trout. *J. Comp. Physiol.* 94: 267–286, 1974.
2. BURTON, A. C. *Physiology and Biophysics of the Circulation* (2nd ed.). Chicago, Ill.: Year Book, 1972, p. 87–88.
3. CAMERON, J. N. Branchial ion uptake in arctic grayling: resting values and effects of acid-base disturbance. *J. Exptl. Biol.* 64: 711–725, 1976.
4. CAMERON, J. N., AND J. C. DAVIS. Gas exchange in rainbow trout (*Salmo gairdneri*) with varying blood oxygen capacity. *J. Fisheries Res. Board Can.* 27: 1069–1085, 1970.
5. EDDY, F. B. The effect of calcium on gill potentials and on sodium and chloride fluxes in the goldfish, *Carassius auratus*. *J. Comp. Physiol.* 96: 131–142, 1975.
6. GANNON, B. J., G. CAMPBELL, AND D. J. RANDALL. Scanning electron microscopy of vascular casts for the study of vessel connections in a complex vascular bed—the trout gill. *Ann. Proc. Electron Microscopy Soc. Am., 31st. New Orleans, La.* 1973.
7. GIRARD, J.-P., AND P. PAYAN. Effect of epinephrine on vascular space of gills and head of rainbow trout. *Am. J. Physiol.* 230: 1555–1560, 1976.
8. HAYWOOD, G. P., J. ISAIA, AND J. MAETZ. Epinephrine effects on branchial water and urea flux in rainbow trout. *Am. J. Physiol.* 232: R110–R115, 1977 or *Am. J. Physiol.: Regulatory Integrative Comp. Physiol.* 1: R110–R115, 1977.
9. HOLETON, G. F., AND D. J. RANDALL. Changes in blood pressure in the rainbow trout during hypoxia. *J. Exptl. Biol.* 46: 297–305, 1967.
10. JONES, D. R., B. L. LANGILLE, D. J. RANDALL, AND G. SHELTON. Blood flow in the ventral and dorsal aortas of the cod, *Gadus morhua*. *Am. J. Physiol.* 226: 90–95, 1974.
11. JONES, D. R., AND D. J. RANDALL. Respiration and circulation during exercise in fish. In: *Fish Physiology*, edited by W. S. Hoar and D. J. Randall. New York: Academic, 1978, vol. VIII.
12. JONES, D. R., D. J. RANDALL, AND G. M. JARMAN. A graphical analysis of oxygen transfer in fish. *Respiration Physiol.* 10: 285–298, 1970.
13. KEYS, A., AND J. B. BATEMAN. Branchial responses to adrenaline and pitressin in the eel. *Biol. Bull.* 63: 327–336, 1932.
14. KIRSCH, R., AND G. NONNOTTE. Cutaneous respiration in three freshwater teleosts. *Respiration Physiol.* 29: 339–354, 1977.
15. KIRSCHNER, L. B. Ventral aortic pressure and sodium fluxes in perfused eel gills. *Am. J. Physiol.* 217: 596–604, 1969.
16. MORGAN, M., AND P. W. A. TOVELL. The structure of the gill of the trout, *Salmo gairdneri* (Richardson). *Z. Zellforsch.* 142: 147–162, 1973.
17. NAKANO, T., AND N. TOMLINSON. Catecholamine and carbohydrate metabolism in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. *J. Fisheries Res. Board Can.* 24: 1701–1715, 1967.
18. ÖSTLUND, E., AND R. FÄNGE. Vasodilation by adrenaline and noradrenaline, and the effects of some other substances on perfused fish gills. *Comp. Biochem. Physiol.* 5: 307–309, 1962.
19. PAYAN, P., AND J.-P. GIRARD. Adrenergic receptors regulating patterns of blood flow through the gills of trout. *Am. J. Physiol.* 232: H18–H23, 1977 or *Am. J. Physiol.: Heart Circ. Physiol.* 1: H18–H23, 1977.
20. PAYAN, P., AND A. J. MATTY. The characteristics of ammonia excretion by a perfused isolated head of trout (*Salmo gairdneri*): effect of temperature and $CO_2$-free Ringer. *J. Comp. Physiol.* 96: 167–184, 1975.
21. PAYAN, P., A. J. MATTY, AND J. MAETZ. A study of the sodium pump in the perfused head preparation of the trout *Salmo gairdneri* in freshwater. *J. Comp. Physiol.* 104: 33–48, 1975.
22. SMITH, D. G. Sites of cholinergic vasoconstriction in trout gills. *Am. J. Physiol.* 233: R222–R229, 1977 or *Am. J. Physiol.: Regulatory Integrative Comp. Physiol.* 2: R222–R229, 1977.
23. SORENSON, P. R., AND P. O. FROMM. Heat transfer characteristics of isolated-perfused gills of rainbow trout. *J. Comp. Physiol.* 112: 345–357, 1976.
24. STEEN, J. B., AND A. KRUYSSE. The respiratory function of teleostean gills. *Comp. Biochem. Physiol.* 12: 127–142, 1964.
25. STEVENS, E. D. *Cardiovascular Dynamics during Swimming in Fish, Particularly Rainbow Trout (Salmo gairdneri)*. (PhD thesis). Vancouver, B. C.: University of British Columbia, 1968.
26. VOGEL, W., V. VOGEL, AND H. KREMERS. New aspects of the intrafilamental vascular system of a euryhaline teleost, *Tilapia mossambica*. *Z. Zellforsch.* 144: 573–583, 1973.
27. VOGEL, W., V. VOGEL, AND M. PFAUTSCH. Arterio-venous anastomoses in rainbow trout gill filaments. A scanning and transmission electron microscopic study. *Cell Tissue Res.* 167: 373–385, 1976.
28. VOGEL, W., V. VOGEL, AND W. SCHLOTE. Ultrastructural study of arterio-venous anastomoses in gill filaments of *Tilapia mossambica*. *Cell Tissue Res.* 155: 491–512, 1974.
29. WOOD, C. M. A critical examination of the physical and adrenergic factors effecting blood flow through the gills of the rainbow trout. *J. Exptl. Biol.* 60: 241–265, 1974.
30. WOOD, C. M. A pharmacological analysis of the adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* 53: 1569–1577, 1975.
31. WOOD, C. M. Pharmacological properties of the adrenergic receptors regulating systemic vascular resistance in the rainbow trout. *J. Comp. Physiol.* B107: 211–228, 1976.
32. WOOD, C. M., AND G. SHELTON. Physical and adrenergic factors affecting systemic vascular resistance in the rainbow trout: a comparison with branchial vascular resistance. *J. Exptl. Biol.* 63: 505–523, 1975.