A STUDY OF THE CARDIOVASCULAR SYSTEM
OF THE RAINBOW TROUT (*Salmo gairdneri*)
AT REST AND DURING SWIMMING EXERCISE

by

Charles Daxboeck
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We accept this thesis as conforming
to the required standard

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June 1981

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Department of  

Zoology

The University of British Columbia  
2075 Wesbrook Place  
Vancouver, Canada  
V6T 1W5

Date  
4 Sept. 1981
ABSTRACT

The effects of steady-state, aerobic swimming exercise upon blood volume and flow distribution in the rainbow trout (*Salmo gairdneri*) were examined. Isotopic Rubidium-86, and radiolabelled microspheres were injected into trout forced to swim against a current at 80% of their critical velocity ($U_{\text{crit}}$) in a Brett-type water tunnel respirometer. The results gathered from experiments using these radioactive tracers within the circulatory system of the trout indicated that blood flow during exercise was redistributed to favour working muscles, at the expense of diminished blood flow to those organs and tissues in the systemic circulation which could tolerate periods of transient hypoxia. Active hyperaemia in the skeletal muscle and vasoconstriction of the coeliacomesenteric artery, via adrenergic receptor mechanisms are proposed as the main sites of the control for blood volume and flow redistribution in the systemic circulation in trout during exercise.

The gills of these fish must be able to maintain adequate gas transfer in order to keep pace with the increased metabolic demands of the working muscles during exercise. An isolated, saline-perfused trout head preparation and a spontaneously ventilating, blood-perfused whole trout preparation were developed in order to study how increases in the pulsatility of input and increases in the cardiac output through these gills; cardiovascular alterations known to occur during exercise *in vivo* in these fish, affect fluid flow distribution through, and within the branchial vasculature, and gas exchange across the gills. Data from these preparations indicated that pulsatility of flow increased venolymphatic fluid drainage from within gill tissues, as well increasing the fluid flux/reflux across the branchial microvasculature. However, these changes in fluid distribution associated with increased pulse pressure did not significantly change the rate of gas transfer across the gills. Although gill vascular resistances to flow were
very sensitive to alterations in pulse pressure and flow rate, only perfusion flow rate through the gills could cause significant changes in the rate of mass transfer of gases across the gills. The gills of trout therefore were found to be perfusion and not diffusion limited for gas transfer, under conditions which simulated those found at rest and during exercise, \textit{in vivo}.

It also was shown that, given oxygen uptake and cardiac output data from the literature, combined with those for blood flow redistribution during exercise from the present study, the working muscles, which were operational during steady-state, aerobic swimming exercise in rainbow trout, could account for nearly all the measured increase in the total oxygen uptake at this level of exercise. The circulatory system of the rainbow trout, both branchial and systemic, was shown to be quite efficient in its ability to take up and distribute oxygen to the tissues during prolonged, aerobic swimming exercise. The numerous cardiorespiratory adjustments noted during exercise account for this animal's ability to maintain swimming activity in the face of increased oxygen demands put upon the circulation by enforced activity.
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GENERAL INTRODUCTION
GENERAL INTRODUCTION

Increased skeletal muscle activity, or exercise, is the most commonly encountered form of stress in most vertebrates. Exercise generally is associated with increases in cardiac output, ventilation volume and oxygen consumption. The cardiorespiratory system of mammals adjusts to maintain an adequate supply of oxygen to, and for the removal of waste products from the metabolizing tissues during steady-state exercise conditions. Blood flow to these skeletal muscles can increase 10 to 15-fold during maximal exercise, while cardiac output increases by only five-fold (Folkow and Neil, 1971). There must be concomitant blood flow redistribution elsewhere in the body. The splanchnic and renal circulation in man at rest receives as much as 25% of the total cardiac output (Rowell et al., 1964; Rowell, 1974). During mild exercise, the mesenteric blood flow decreases (Eklund, 1967; Scher et al., 1972) and therefore, this splanchnic circulatory blood "pool" is considered to be the major site of control for blood redistribution and arterial blood pressure regulation during exercise. Mean arterial blood pressure is increased during exercise, even in the face of an almost 50% decrease in the total peripheral resistance to blood flow once a steady-state condition is attained. Increases in the heart rate during this exercise state are proportional to the decrease in peripheral resistance (Bevergard and Shephard, 1967).

Numerous studies have been conducted to evaluate the effects of swimming exercise on the cardiorespiratory and metabolic responses of fishes to this naturally encountered form of stress (see Fish Physiology, Vol. 7. W.S. Hoar and D.J. Randall, eds. Academic Press, New York, for review). In fishes, some of the possible limiting factors of maximum oxygen consumption during exercise are: the rate and efficiency of oxygen uptake across the gills, the rate of oxygen delivery to the tissues by the circulating blood,
or the rate and efficiency of oxygen extraction by the working muscles.

Exercise can be categorized into burst activity, and prolonged exercise at maximal or submaximal energy expenditure. Regardless of the type of exercise performed, it can be divided further into initiation, steady-state and recovery phases. Although some of the energy budget during prolonged, steady-state exercise may be provided by anaerobiosis, its contribution has been found to be minimal in salmonids (Driedzic and Kiceniuk, 1976). There also may be a larger relative anaerobic phase at the onset of exercise, but once a steady-state condition is established, nearly all metabolism is aerobic.

The first section of this study examines the effects of prolonged aerobic exercise on the circulatory system as a whole, investigating changes in blood volume and flow in various tissues from rest, and their possible means of regulation.

No studies to date however, have investigated the effects of different perfusion regimes on vascular fluid distribution in the intact gills and head of teleost fish in detail, although the effects of pulse pressure, mean pressure and perfusion flow on gill vascular resistance in isolated holobranchs have been studied (Farrell et al., 1979). Similarly, the dynamics of fluid flow through the recurrent or venolymphatic circulation in teleost gills have not been examined to any extent. The anatomy of the recurrent blood vessel system however, has been described in some detail (Gannon et al., 1973; Laurent and Dunel, 1976; Vogel et al., 1976; Farrell, 1979). These descriptions indicate that the venolymphatic sinus compartment of the gills could contain as much as 12% of the total blood volume of the fish. Low haematocrits have been reported for this recurrent blood space (Hughes and Wright, 1970; Booth, 1978). Plasma skimming at the anastomoses between efferent filament vessels and the recurrent circulation could
account for the observed low haematocrit. How this venolymphatic/extra-vascular space behaves under different physiological conditions found \textit{in vivo} also has not been investigated, although previous studies have indicated that pulsatile perfusion of isolated gill arches decreases vascular resistance to flow. This was thought to be due, in part, to increases in venolymphatic fluid clearance (Farrell et al., 1979). In the second section, changes in the gill circulation are discussed in detail. In particular, I examine the effects of changes in pressure and flow on gill vascular resistance and fluid distribution during exercise.

Gas exchange in fish gills has been examined in some detail (see Randall, 1979). There also have been extensive studies of oxygen consumption of intact fish (see Brett, 1972), and measurements of oxygen uptake and gas transfer across the gills are relatively common (see Fisher et al., 1969; Cameron and Davis, 1970; Davis and Cameron, 1971). From these studies, fish gills are assumed to be primarily diffusion limited for oxygen uptake. Because of this assumption, it has been suggested that cardiorespiratory adjustments during exercise are for the purpose of decreasing the diffusion barrier for gases, such that oxygen uptake, as well as carbon dioxide excretion, are maintained at levels sufficient to supply the aerobic metabolic requirements of the fish during swimming activity. Cardiac output and ventilation volume increase during exercise in trout. Oxygen uptake is augmented by presumed decreases in the diffusion barrier and, to a lesser degree, greater perfusion of an increased respiratory surface area, brought about by lamellar recruitment (Jones and Randall, 1978).

Vertebrate respiratory vessels generally are subjected to blood pressures which are much lower than in any other part of the systemic circulation. The capillary beds of the respiratory tissues of fish gills, the secondary lamellae, however, are exceptional in that they have blood
pressures which are appreciably higher than systemic pressures (Johansen, 1972). These vessels also have high pulse pressures (Farrell, 1979); higher than in any other blood vessel of similar size. There are two distinct barriers to fluid movement across fish gills; the first or basal (blood-facing) barrier, and the much more permeable water-facing barrier (Isaia et al., 1978). The structure of the blood/water barrier of fish gills has been described in detail by Hughes and Morgan (1973). Briefly, an outer epithelial cell layer separates an interstitial fluid space from the external environment. Beneath this interstitium lies a basement membrane overlying the supportive pillar cells of the blood spaces. This epithelium/basement membrane/pillar cell layer complex is referred to as the functional respiratory epithelium.

Exchange of fluid across small vessels (capillaries and alveolar blood vessels) is governed by a "Starling equilibrium" (1896), in which filtration rate is proportional to the hydrostatic pressure of the blood and the permeability of the vessel wall (Landis and Pannenheimer, 1963). Filtration across the wall also has a pulsatile component, but its effects are restricted to a boundary layer near the blood/vessel wall interface (Kenyon, 1979). Given that fish lamellar blood pressures are high, compared to other vertebrate respiratory tissue (Johanssen, 1972), and the thickness of the blood/water barrier is small, fluid movements across the endothelium of fish gills could be expected to be different from fluid movements across other low pressure capillary walls.

In Section III, I examine the effects of the changes in pressure and flow on gas transfer across the gills of blood- and saline-perfused trout. Results from these simulated-exercise, cardiovascular dynamics experiments lead to discussions of their effects on the diffusing capacity of fish gills during exercise, in vivo.
In the final section I have attempted to bring the results of Section I, II and III together, to examine the role of the circulation in gas transport during exercise.

The object of my study therefore is to examine various components of the cardiovascular system of the rainbow trout (*Salmo gairdneri*), at rest and during steady-state, submaximal, aerobic swimming exercise. The adjustments and possible underlying mechanisms responsible for the maintenance of adequate oxygen transfer across the respiratory organ, the gills, are investigated.
MATERIALS AND METHODS
MATERIALS AND METHODS

THE FISH

All experiments were carried out on rainbow trout (*Salmo gairdneri*) of both sexes, weighing between 200 and 600 grams. Fish were obtained from the Sun Valley Trout Farm, Mission, B.C. After transport to U.B.C., they were held outdoors in large fibreglass tanks supplied with running, aerated and dechlorinated Vancouver tap water (5 - 11°C, depending on season; see Table 1), and kept on ambient light cycle. Fish were fed a daily diet of dried fish pellets (Moore-Clarke Co.) *ad libitum*, but were not fed 24 hours before or during experimentation. All experiments were conducted at the temperature to which the fish were acclimated.

SWIMMING EXERCISE: THE APPARATUS AND METHOD OF PERFORMANCE ASSESSMENT

Exercise performance was assessed for trout during sustained, aerobic swimming against a water current in a Brett (1964) respirometer/swim tunnel. The fish used for these experiments had been pre-conditioned to swim by continuous exposure to a mean water velocity of 20 cm·sec\(^{-1}\) (zero cm·sec\(^{-1}\) in centre; 35-40 cm·sec\(^{-1}\), at edge) in a large, circular holding tank equipped with a pump. The time period was never less than 2 weeks. Selected fish were introduced into the water tunnel through the access port and allowed to accommodate to this confinement for at least 18 h at a water velocity of approximately 8.7 cm·sec\(^{-1}\), the lowest speed on the pump, before being tested.

The respirometer/water tunnel incorporated a cylindrical Plexiglass chamber (126.5 cm\(^2\) cross-sectional area) through which a variable-speed water pump could maintain the speed at a desired value (± 3%; Kiceniuk, 1975). The water in the present experiments was renewed at a rate of 1 L·min\(^{-1}\). The temperature was controlled (± 0.5°C) by a heat exchanger system. The water tunnel used in the present experiments was identical to the one used by Kiceniuk (1975), who gives a detailed description of the
Table 1. Analysis of Vancouver tap water.

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<th>April 1980</th>
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<tr>
<td>pH</td>
<td>6.5</td>
<td>6.4</td>
</tr>
<tr>
<td>Alkalinity (mg•L⁻¹ CaCO₃)</td>
<td>6.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Conductivity</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>EDTA Hardness (mg•L⁻¹ CaCO₃)</td>
<td>-</td>
<td>5.4</td>
</tr>
<tr>
<td>PO₄ as P (µg•L⁻¹)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Fe (µg•L⁻¹)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Na⁺ (µg•ml⁻¹)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total Organic Carbon (µg•L⁻¹)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cl⁻ (variable between 0.015 - 0.03 µg•L⁻¹)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
apparatus. Partial pressure of oxygen of the water flowing into the respirometer was always greater than or equal to 155 mm Hg.

The critical velocity swimming test is a measure of exercise performance. In the present experiments, where \( U_{\text{crit}} \) was directly determined, the formula described by Brett (1964) was used as follows:

\[
U_{\text{crit}} = u_1 + \left( t_1 / t_2 \times u_2 \right),
\]

where \( u_1 \) is the highest velocity maintained for the prescribed 30 min period (cm·sec\(^{-1}\)); \( u_2 \) is the velocity increment (which was approx. 0.5·fork length·sec\(^{-1}\)·fl·sec\(^{-1}\)); \( t_1 \) is the time (min) that each fish swam at the "fatigue" velocity; and \( t_2 \) is the prescribed period of swimming (30 min). In these experiments, fatigue was defined as the point at which a fish was unable to remove itself from the electrified downstream grid of the water tunnel after repeated efforts and electrical stimulation. I chose to follow the procedure described by Smit et al. (1971), whereby once a fish had fallen against the grid, the water velocity was reduced by 0.25 fl·sec\(^{-1}\). If the fish then continued to swim, even after the "fatigue" velocity was regained, the first failure was ignored. However, the subsequent failure terminated that experiment and that time to "fatigue" was recorded. This was the time used in the calculation of \( U_{\text{crit}} \). All swimming speed experiments were performed over the same time period of the day, to overcome possible diel differences in exercise performance.

Several corrections to the obtained \( U_{\text{crit}} \) value were made, or at least considered, before \( U_{\text{crit}} \) was reported. The solid-blocking effect (Pope and Harper, 1966) resulting from the decrease in the effective cross-sectional area of the tunnel due to the presence of the fish, obviously varied with the cross-sectional area of each fish. If the cross-section of the fish at the level of the pectoral fin approximated an ellipse, a correction factor, \( k \), was derived from

\[
k = \frac{\pi r^2}{\pi r^2 - \Pi \tau}
\]

where \( r \) is the inside
tunnel radius (6.35 cm) and a and b are the semi-axes of the fish at the widest point, for each fish. The corrected \( U_{\text{crit}} \) value was estimated by \\
\[ k \times U_{\text{crit}} \]

A "condition factor", described by Fulton (1911, in Ricker, 1975) was considered in the present experiments, where \( C.F. = \text{Mass (grams)} \times \text{fork length}^{-3} \text{(cm)} \). However, this factor never accounted for more than a 1% increase in the uncorrected value obtained for \( U_{\text{crit}} \), and therefore was considered relatively unimportant to the overall estimate of exercise performance. Also, data presented by Brett (1964, 1965a) indicate that there is little difference between the levels of swimming activity attained by either male or female salmonids, and thus no sexual distinction was made for \( U_{\text{crit}} \) values in the present study.

In the radiolabelled experiments, \( U_{\text{crit}} \) was not determined directly. However, fish were exercised in a manner similar to that described above (i.e., 0.5\( \text{fl} \cdot \text{sec}^{-1} \) velocity increments at 30 min time intervals), to an estimated 80% \( U_{\text{crit}} \). The \( U_{\text{crit}} \) value was determined from Figure 9, Kiceniuk (1975), but was corrected in order to include the added drag due to the dorsal aortic cannula in the water stream. Therefore, the estimated \( U_{\text{crit}} \), from Fig. 9, was reduced by 20% to account for added resistance (D.R. Jones, personal communication), and then 80% of that value taken as the estimated 80% \( U_{\text{crit}} \) used for that fish.

THE EXPERIMENTS. I. CARDIAC OUTPUT AND BLOOD VOLUME DISTRIBUTION

Preparation

Pre-trained fish initially were anaesthetized in aerated water containing 1:10,000 MS-222 (w/w methane tricaine sulfonate, adjusted to pH 7.0 - 7.5 with NaHCO₃), and transferred to an operating table (Smith and Bell, 1964, 1967). There, gills were irrigated continuously with aerated 1:15,000 MS-222 at 4°C. Fish were laid supine and a dorsal aortic cannula (75 cm of
PE 50 tubing (0.58 mm I.D., 0.965 mm O.D.) was inserted through the roof of the buccal cavity between gill arches 1 and 2. This cannula was pre-filled with Cortland saline (Wolf, 1963) and plugged with a pin. The surgical procedure followed has been described in detail by Smith (1978). This cannula served as the site of injection for radio-labelled substances into the circulation, as a blood sampling port, a place from which to measure dorsal aortic blood pressure (DAP) and a site for the injection of saturated KCl solution, used to quickly arrest circulation at the end of the experiments.

**Experimental Protocol**

Following surgery, a fish was placed inside the tunnel respirometer and allowed to recover for at least 18 h (Houston and Woods, 1972; Houston et al., 1971) at 8.7 cm·sec⁻¹ water flow. Water temperature during these experiments was maintained at 4 - 5°C. Once recovery was complete, DAP was recorded for at least one hour before starting the testing. DAP was recorded from the cannula connected to a Statham P23 BB pressure transducer, which was manometrically calibrated against a static column of water. The output was displayed on a Gilson MP5 pen recorder. The pulsatile pressure traces allowed heart rates to be derived. A pre-exercise blood sample was withdrawn and haematocrit (Hct) determined.

A total of 6 fish (415.1 ± 40.3 g) were forced to swim against a water current, in 0.5·fl·sec⁻¹·30 min⁻¹ increments up to an estimated 80% $U_{crit}$. After 25 min swimming at the highest velocity, 0.05 mCi $^{86}$RbCl (NEN) (0.30 ml Cortland saline vehicle + 0.5 ml "cold" saline wash) were introduced into the circulation via the cannula. The small injection volume did not appear to affect the pressure trace in any way. Five minutes were allowed for tracer equilibration within the body, and then the fish were sacrificed by a 0.20 ml injection of KCl.
Six other fish (384.4 ± 40.5 g) were prepared as above, but were allowed to recover in darkened Perspex holding boxes, supplied with running aerated water at the same temperature as swimming fish. After recovery (18 h minimum), DAP was recorded and heart rates determined for several hours, and Hct taken as described before. These "resting" fish then were injected with $^{86}$Rb and sacrificed in the same manner as described above.

Five more fish (316.8 ± 36.6 g) were sacrificed by over-anaesthesia (1:10,000 MS-222, w/w) and fresh excision of selected tissues was made, to determine the relative percentage of the total body weight accounted for by each sample. Care was taken to blot excess blood and other fluids from all tissues. Gut contents were removed before weighing, as was the blood contained within the heart.

**LIQUID SCINTILLATION COUNTING (LSC)**

**Tissue selection and Preparation**

Once each fish had been sacrificed, 200 mg samples of the tissues described in Table 2 were carefully excised and weighed into NEN low potassium, borosilicate glass scintillation vials (22 ml capacity). Care was taken not to cross-contaminate any sample with blood or fluid from another. To each vial was added 1.5 - 2.0 ml of Protosol tissue solubilizer (0.5 M quaternary ammonium hydroxide solution), and the capped contents were allowed to digest at 55°C for 18 - 24 h in a temperature-controlled shaking water bath.

Once digested, coloured solutions were partially decolourized by the dropwise addition of 0.1 - 0.5 ml 30% $\text{H}_2\text{O}_2$ at room temperature, then rewarmed and shaken for an additional 30 min at 55°C. 50$\mu$l glacial acetic acid was added to these vials per 0.5 ml Protosol. Once cooled again, 10 ml Econofluor R (NEN - a premixed scintillation solution with characteristics similar to those of toluene base cocktails) were added. Once mixed and
Table 2. List of tissues sampled from rainbow trout, to determine cardiac output distribution during rest and exercise, using Rb-86.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosaic muscle</td>
<td>Left and right side epaxial mass under dorsal fin</td>
</tr>
<tr>
<td></td>
<td>Left and right side from the caudal peduncle, with all red muscle carefully removed, from under the region of the adipose fin</td>
</tr>
<tr>
<td></td>
<td>Left and right cheek muscle (cephalic portion of adductor mandibularis)</td>
</tr>
<tr>
<td>Red muscle</td>
<td>Left and right side lateral line muscle, with lateral line organ removed from;</td>
</tr>
<tr>
<td></td>
<td>a) Just caudad of the opercula</td>
</tr>
<tr>
<td></td>
<td>b) The region under the dorsal fin</td>
</tr>
<tr>
<td></td>
<td>c) The caudal peduncle</td>
</tr>
<tr>
<td>Organs</td>
<td>Spleen, stomach (fundus and pyloric region sampled, with contents removed), intestine (mid-region and highly vascularized absorptive region, with contents removed), kidney (cephalic region - &quot;head kidney&quot; - and from region under dorsal fin), ventricle (blood removed from lumen), gonad (whether mature or immature), brain, gills (left and right second arch).</td>
</tr>
</tbody>
</table>

Duplicate 200 mg samples of each tissue in the above list were taken and count rate determined.
shaken, vials were kept in dark before counting, and were allowed to remain in the counter for at least 60 min before counts were begun. Light emissions due to β-interactions with the fluor were monitored using a ISOCAP (Model 300, Nuclear Chicago) Liquid Scintillation counter. Each sample was counted for 10 min in the $^{32}\text{p}$ window setting of the counter. Data were converted to counts per minute per gram tissue (cpm.g$^{-1}$). Since the %age body weight of each tissue had been determined previously, and the weight of each tissue sample was known (usually 200 mg), the total cpm's in the fish could be estimated. This value was compared to the known injection cpm's for each fish, since a standard for each injection was counted with each fish. All values are expressed in the tables as means ± S.E.M. with the number of observations given.

Precautions and Corrections for Liquid Scintillation Counting (LSC)

Rubidium-86 ($T_{1/2} = 18.7$ days) is a β-emitter (91% 1.77 MeV; 9% 0.69 MeV), as well as being a weak γ-emitter (9% 1.08 MeV). Phosphorus-32 ($T_{1/2} = 14.3$ days) is a pure β-emitter (1.71 MeV). $^{86}\text{Rb}$ emission counts were monitored in the $^{32}\text{p}$ window of the ISOCAP since this counter had no direct provision for the measurement of isotopic Rb activity.

Inherent in LSC are many interfering processes. One of these is chemiluminescence resulting from chemical reactions between additives or specimen and the components of the fluor, thus causing more counts than would be due solely to the emitter/fluor reaction. The decay of chemiluminescence is temperature dependent and proceeds faster at higher temperatures. Therefore samples were kept at least at room temperature for a 3 hour period before counting, to allow this interference to decay. Additionally, chemiluminescence can be diminished or completely abolished by acidification of the Protosol with glacial acetic acid before the addition of fluor, as was done in the present case.
Phosphorescence is another interfering process which could give counts above those directly attributable to the emitter interaction itself. Many proteinaceous substances, e.g. liver, when digested and diluted with a toluene base solvent and exposed to sunlight, or fluorescent light, emit light and thus give high count rates, even in the absence of radioactivity (Lloyd et al., 1962). This problem was eliminated by keeping samples in the dark and/or acidification of the samples before counting.

Quenching interferences are those processes in which maximum photon yield is not achieved for a given radioactive source. In the present study, colour quenching was the most prevalent source of interference, especially from highly pigmented tissues, even after partial decolourization. This was corrected by the channels-ratio method, since there was no internal standard in the counter. However, counting efficiency ($\varepsilon = \text{cpm}/\text{dpm}$) was never determined, and therefore only cpm's are reported in this study. Since different tissues had different colour quenching characteristics, individual colour quench curves were constructed for liver, kidney, spleen, red muscle, and white muscle and similar, light coloured tissues. This was accomplished by adding a constant known cpm's source to uncoloured, and varying amounts of tissue digests, and plotting the channels-ratios obtained against the un-quenched counts (100%). The ISOCAP printout presents simultaneous data for the count rate from the sample as well as the channels-ratio associated with that sample, and therefore each sample could be corrected for quenching to obtain the actual cpm's.

Several vials containing only the appropriate volumes of Protosol and Econofluor, as used in the experiments, were included in each series to determine the background count rate inherent in the system from a variety of sources. Although these sources will not be discussed, suffice to say that, after quench correction, background counts were subtracted from the cpm's obtained.
Although counting "efficiency" was increased, and corrected for by the methods outlined above, no correction was made for any possible interference due to Cerenkov radiation (Jelley, 1956), from the highly energetic β-emitter, $^{86}$Rb.

**γ-Counting and Radiolabelled Microspheres**

To determine total blood volume and the blood volumes of various tissues in resting and exercising rainbow trout, 6 fish (371.0 ± 36.7 g) were maintained and cannulated as previously described. Chromium-51 ($T_{1/2} = 27.8$ days), supplied as sodium chromate (NEN - 1 mCi·ml$^{-1}$ total activity) was used to label red blood cells by the method described by Conte et al., (1963). Briefly, 5 - 6 ml blood were withdrawn via ventricular puncture from donor fish of the same stock, and transferred from the syringe into a 50 ml conical tonometer flask kept on ice. 10 ml Cortland saline with 1000 IU sodium heparin was added, and the flasks gently agitated. 50 μCi total $^{51}$Cr were added to the blood cell suspension and incubated 1.5 - 2 h on ice with gentle agitation every 5 min. After incubation, blood was transferred to round bottom test tubes and centrifuged (2,500 - 3,000 G's) for 2 min. The supernatant was carefully aspirated and discarded, except for 3, 50 μl aliquots which were placed in γ-counting tubes for subsequent analysis. The low centrifugal force used did not pack the cells too tightly, and they were readily resuspended in 10 - 15 ml cold saline, for washing. Again, the cell suspension was centrifuged and the supernatant treated as before. This washing procedure was repeated 2 - 3 times. After the final wash, the supernatant and the top layer of cells (white cells and other debris) was discarded. The $^{51}$Cr-labelled red cells were transferred to an equal volume of cold saline and resuspended. Several 10 or 25 μl aliquots of this final blood suspension were removed, to be analysed for total $^{51}$Cr count rate, as well as Hct. The Hct determination also allowed an assessment of the
degree of blood lysis due to the incubation and mixing procedure to be made. Little damage was evident prior to injection of this blood into recipient fish. No radioactivity was found in the supernatant following the second wash.

One ml of blood was removed from the recipient fish and Hct determined. This volume was replaced by 1 ml of $^{51}\text{Cr}$-labelled blood (Hct = 52.3 ± 10.1%), prepared as described above, so as not to change total blood volume. From preliminary experiments, it was found that the activity in the blood, determined from sequential 50 μl dorsal aortic samples, became constant 1 - 1.5 h after the injection of labelled blood. The labelled blood always was higher in Hct than normal. Dorsal aortic pressure tended to be elevated above normal for approximately 1 hour after injection of labelled blood, after which the pressure declined to normal levels. Labelled red cells therefore were allowed to circulate for a minimum of 2 hours. After the equilibration period, a small sample of dorsal aortic blood was taken and Hct determined, and compared with the pre-injection value. As well, 0.5 ml mixed radioactive/cold DA blood was taken and counted, to determine the blood volume of each fish. This could be calculated since the total injected count rate (corrected for residual counts remaining in the syringe and cannula) was known, and the count rate in 0.5 ml mixed blood also was known. The decrease in the total count rate was due to the unlabelled blood volume diluting and mixing with labelled cells. This ratio (cpm·ml$^{-1}$/total cpm injected) gave total blood volume (ml), and since total body weight was known, the volume was expressed as percent body weight, for comparison with other published data.

Regional distribution of cardiac output at rest and during swimming exercise was determined in 7 fish, using $^{141}\text{Ce}$- and $^{95}\text{Nb}$-labelled microspheres, having a density of 1.3 g·cm$^{-3}$ (blood = 1.05 g·cm$^{-3}$). The
supplier of these spheres (NENTRAC – New England Nuclear, Lachine, P.Q.) provided size specifications with each batch (141 Ce-spheres, 26.1 ± 1.1 μm (±S.D.); 95 Nb-spheres, 25.6 ± 1.3 μm (±S.D.) diam.). Hales et al. (1979) and Jones et al. (1979) have examined similar microspheres using haemocytometry, as well as S.E.M. to check manufacturer's stated sphere concentrations, size ranges etc., and have found no aggregation, irregularly shaped spheres nor any other deviations from specifications. Each batch of spheres had 1 mCi total activity, supplied in 10 ml isotonic saline, to which 0.01% Tween 80 (a surfactant for bead dispersal) had been added. Supplier's data also indicated no appreciable leaching of activity from the spheres into saline.

Subsamples were taken from the supplied stock vials and diluted with Cortland saline to give approximately 2 x 10^5 ± 5 x 10^4 spheres in 10 ml total saline volume, for both 141 Ce- and 95 Nb-labelled spheres. Before injection, the diluted sample vials, containing a magnetic stirring bar, were agitated on a vortex mixer and then ultrasonified for several minutes to evenly disperse the spheres. The vials then were placed on a magnetic stirrer and mixed continuously. An 0.25 ml sample was withdrawn into a 1 ml disposable syringe and attached to the sidearm of a plastic 3-way tap, fixed to a mechanical vibrator, to prevent spheres from settling out of suspension (see Fig. 1). Another syringe contained saline for rinsing residual spheres from cannulae (0.5 ml). 0.25 ml sphere suspension was injected prior to, and another 0.25 ml spheres were injected after 25 min of swimming at the estimated 80% Ucrit for each fish. Since 1.0 ml 51 Cr-labelled red cells already had been injected at rest, to determine blood volumes during exercise, 2 x 0.25 ml sphere volumes, followed by 0.5 ml wash each, for a total 3.0 ml fluid were injected into each fish due to experimental protocol, an equivalent 3.0 ml blood initially were removed from each fish. Hct was determined and the rest of this blood was discarded, in order to keep the total blood volume unchanged. Following 30 min exercise at 80% Ucrit, fish were
sacrificed by injection of 0.2 ml saturated KCl solution into the circulation.

Usually, 5, 10 or 25 μl aliquots of each sphere suspension were prepared at the time of injection, as a check for maximum possible injected activity (after appropriate multiplication to correct for volume differences). In order to determine the total number of injected counts, the entire assemble (see Fig. 1) was cut up and placed into counting tubes. Residual counts in the assembly as well as those remaining in the DA cannulae, were added up and subtracted from the measure of the total possible injected activity, thus giving a value for the total counts injected.

**Tissue Preparation and Gamma (γ)-Counting**

Tissues sampled were essentially the same as those listed in Table 2. The exception was that, where possible, whole organ activity was counted, rather than only 200 mg samples, as in the previous case. As well, skin samples taken from over the lateral line were included, these having had all adhering muscle carefully scraped away. Tissues were excised and weighed immediately following sacrificing of the fish. The wet weights of all tissues could be expressed a %age of total body weight. All samples were dried in an oven overnight (70 - 80°C), on foil. Once dried, all samples were placed in counting tubes (polystyrene) and count rates determined using a Nuclear Chicago multichannel pulse-height γ-counter. ⁵¹Cr-blood, ¹⁴¹Ce- and ⁹⁵Nb-microsphere radiations were counted simultaneously with window settings spanning at least 85% of the total photopeaks. These were determined separately for each isotope (see Figs. 2, 3 & 4). The energy spectra were identical to those found by Heymann et al. (1977) for the same isotopes. There was considerable spillover from the high energy window (⁹⁵Nb) into the other windows. As well, ⁵¹Cr had a spillover component into the ¹⁴¹Ce window. However, no interfering counts from the other two labels were
Figure 1. Schematic representation of the microsphere injection assembly. Numbers with associated arrows indicate the sequence of events in the injection of any dose of radio-labelled microspheres.

1, withdrawal of 1 ml dorsal aortic blood and hold
2, slow and steady injection of 0.25 ml spheres into DA
3, flush of cannula with the previous 1 ml blood
4, wash cannula of blood and any remaining spheres with 0.5 ml non-radioactive Cortland saline.
200.25 ml spheres 0.5 ml 'cold' saline wash to fish in swim tube

Mechanical vibrator

DA cannula 0.25 ml spheres 0.5 ml 'cold' saline wash 1 ml blood wash

to fish in swim tube
Table 3. Assessment of cross-interference in energy spectra from the three isotopes counted simultaneously in tissue samples from resting and swimming rainbow trout.

<table>
<thead>
<tr>
<th></th>
<th>Chromium-51 counts found in Cerium-141 window</th>
<th>Niobium-95 counts found in Chromium-51 window</th>
<th>Niobium-95 counts found in Cerium-141 window</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium-51</td>
<td>$20.34%\quad ^{51}\text{Cr}$</td>
<td>$26.46%\quad ^{95}\text{Nb}$</td>
<td>$22.39%\quad ^{95}\text{Nb}$</td>
</tr>
</tbody>
</table>
Figure 2. Energy spectrum derived from a subsample of Chromium-51 stock solution used to label red blood cells. The energy of radiation was scanned from 0 through to 1000 KeV for this emitting source.
Figure 3. Energy spectrum derived from a subsample of Cerium-141-labelled microspheres stock suspension. The energy of radiation was scanned from 0 through to 1000 KeV for this source.
Figure 4. Energy spectrum derived from a subsample of Niobium-95-labelled microspheres stock suspension. The energy of radiation was scanned from 0 through to 1000 KeV for this source.
Figure 5. The decrease in known activity (100%) with increasing height of water added to source, above bottom of the gamma-counting tube.
found in the $^{95}$Nb window (see Table 3).

Samples were counted for 10 min or until $1 \times 10^5$ counts had been accumulated, and then converted to cpm's. Sequential spillover corrections were made (using Table 3), as well as the correction for background count rate in each window for each sample. Counting geometry did not appear to be a significant determining factor in the obtained count rate, since independent experiments indicated that identical counts were obtained when a standard count rate was:

a) applied to and wrapped in foil and counted in the bottom of a dry counting tube,

b) wrapped in foil and placed in distilled water up to a critical height above the bottom of the counting tube (see Fig.5),

c) placed on wet tissue with or without foil wrapping, either dry or in water in the tube, or

d) placed on dried tissue with or without foil wrapping, either dry or in water in the tube.

The % of body weight represented by each tissue was calculated, and the total counts in each tissue or organ were calculated from the total counts measured in that sample. These values were added up and compared to the estimated total injection counts, and then expressed as % recovery. The % total counts found in each tissue was taken as the percentage of the total cardiac output (actual value unknown) flowing to that tissue during rest and exercise. Where $^{51}$Cr measurements were sufficient in any tissue to be unobscured by the spillover from other windows, tissue blood volume during exercise also could be determined. Additionally, after samples from 2 fish were removed and prepared as described, the remaining tissue was ashed completely in a muffle oven (1200 - 1500°C), and the residue counted. Thus, the total counts in the whole body obtained in this way were compared
with that value which was assumed to have been injected initially (after corrections). Despite extensive smoking of the ashing tissue, and the probability that this smoke, as well as heat convection may have carried away some radiolabelled particles, on average 78% of the estimated total injected counts were recovered.

Since the number of counts per sphere was determined, and the half-life \( T_{1/2} \) of activity was known, the number of spheres also was known for each tissue sample. Only samples with at least 384 spheres are included in the results (Buckberg et al., 1971; Heymann et al., 1977). Count rates from samples taken from left and right sides of fish were compared, to test for evenness of microsphere mixing. No apparent left/right preference was noted for any tissue so compared. Gill tissue and heart samples were taken from each fish to assess the degree of sphere entrapment in the post-branchial arterial system. Any counts in the gills or heart were an indication of capillary bypass. However, these counts never accounted for more than 0.5% of the total activity in the tissues and thus it was concluded that arterial capillary entrapment of the injected spheres was very effective.

In a subsidiary experiment, 4 additional fish (534.5 ± 34.4 g) were exercised at 80% \( U_{\text{crit}} \) and injected with one dose of labelled spheres, as before. These fish then were exercised further to complete exhaustion, and injected with a second volume of spheres. Tissue samples were taken, prepared and counted as described previously.

Validity of Tracer Data

Elemental rubidium and its isotope \( ^{86}\text{Rb}^+ \) have biological properties which are very similar to those of potassium (Love et al., 1954). When \( ^{86}\text{RbCl} \) of known activity is administered intravenously as a single injection, the amount of the indicator in an organ remains essentially unchanged for an appreciable time interval after the first circulation (Sapirstein, 1956,
Since $^{86}\text{Rb}^+$ equilibrates and mixes with the blood, and is distributed throughout the body in the same proportions as the blood flow itself, the subsequent fraction of the total injected counts found in any excised tissue sample will be the same as that fraction of the total blood flow (or cardiac output) directed to that tissue (Sapirstein, 1967), under rest or exercise conditions.

Data, obtained from the $^{86}\text{Rb}$ study, require close examination as to their validity for the description of cardiac output distribution at rest and during exercise in the rainbow trout. Even though all tissue activities were corrected to account for most systematic errors, other errors cannot be assessed. Consider the fact that a small volume of saturated KCl solution was injected into the dorsal aortic cannula in order to quickly arrest the heart, and thus the circulation of blood, at the termination of an experiment. After tissue uptake of $^{86}\text{Rb}^+$, re-equilibration of $K^+$ and $^{86}\text{Rb}^+$ pools may occur, leaching $\text{Rb}^+$ from some cells and ultimately leading to an underestimate of the actual $^{86}\text{Rb}^+$ retention (Cameron, 1975).

Furthermore, although the circulation time of rainbow trout is relatively slow, compared to that of mammals; in the order of one to two minutes (Davis, 1970; Itazawa, 1970), a considerable amount of $^{86}\text{Rb}^+$ redistribution may occur following the initial injection and subsequent sacrificing of the fish. Also, the amount of counts contained in the blood volume within each tissue sample should have been taken into account.

Differences in resistances of various tissue cell membranes to the transport of $^{86}\text{Rb}^+$, or in the extraction ratios for $^{86}\text{Rb}^+/K^+$ could exist in fish, as have been found in mammalian cell membranes (Sheehan and Renkin, 1972). Data for fish are not available, but if such a situation were the case, it certainly would lead to errors in the interpretation of distribution results, since analyses rely on the assumption that all tissues have similar
membrane properties, with respect to $^{86}$Rb uptake. The fact that the extraction ratio for $^{86}$Rb$^+$ of isolated, perfused mammalian skeletal muscles appears to be inversely related to the rate of blood flow through that tissue (Friedman, 1968; Sheehan and Renkin, 1972) also raises many questions as to the interpretation of any $^{86}$Rb-based data. It also is apparent that the relative contributions of these perturbing factors to any data analyses are not readily quantifiable.

The potential problems encountered with the use and evaluation of $^{86}$RbCl data for the study of blood flow patterns in the circulation were eliminated with the use of radio-labelled microspheres (see Rudolph and Heymann, 1967). The advantages of this method are obvious. An appropriate size range of spheres was injected into the circulatory stream, with a known activity, and these spheres subsequently became lodged in the capillary beds of tissues. Because the spheres did not exchange their radioactivity with the tissues or plasma, there was no problem of redistribution of counts following tissue entrapment. The total injected activity must remain distributed in the fish, and given that these particles became uniformly distributed in the circulation if injected at a slow, constant rate, the tissue activities reflect the proportion of the cardiac output directed to any particular sample examined, at rest or during exercise. Few studies using microspheres for the examination of blood flow patterns in fish have been conducted (Cameron, 1974a,b, 1975), and a stringent evaluation of haemodynamic consequences of the injection of non-deformable particles into the circulatory system of fish is not available. This technique, however, has been used extensively in mammalian circulatory investigations (Rukasan and Blahitka, 1974; Warren and Ledingham, 1974; McDevitt and Nies, 1976; Foster and Frydman, 1978a,b). Data from numerous studies confirm uniformity of distribution and non-disruptive haemodynamic effects upon the circulation
despite the injection of these particles. Nor was there any indication of serious impairment of tissue function from partial capillary bed blockage by the spheres. Once entrapped, these spheres did not change position over a long period of time (Hales and Cliff, 1977).

Recent studies by Mendall and Hollenberg (1971) and Foster and Frydman (1978a) have compared the $^{86}$Rb tracer method with the radiolabelled microsphere technique for the evaluation of blood flow distribution in mammals. They have come to the conclusion the $^{86}$Rb measurements have very limited quantitative validity, at least in their experiments. It therefore seems rather fortuitous that my $^{86}$Rb$^+$ and microsphere data, describing apparent cardiac redistributions during steady-state swimming exercise in the rainbow trout are equable.

It has been demonstrated that the characteristics of blood, especially haematocrit, change in their passage through the gill vasculature (see Section III). As well, the red cells differ from different sections of the mammalian spleen (Groom et al., 1971). The question arises as to whether the dorsal aortic blood samples were truly representative or valid for the reference of subsequent determinations of tissue blood volumes, using $^{51}$Cr-labelled red blood cells. If indeed haematocrits vary widely throughout each individual capillary network of each tissue, how much confidence can be placed on volume measurements from these dorsal aortic blood reference samples? As I was unable to arrive at any logical alternative reference point, if indeed one exists for intact animals, I have accepted data obtained as being representative of the in vivo condition for each tissue, with the above reservations noted.

II. ISOLATED SALINE-PERFUSED TROUT HEADS

For studies designed to measure fluid distribution in gills during perfusion by pulsatile and non-pulsatile flow, an isolated saline-perfused
trout head preparation was used. Trout for this series of experiments (271.1 ± 13.8 g) were obtained and maintained as described previously. Experiments were conducted at holding temperatures (7 - 9°C) and varied only ± 0.5°C during the course of any experiment.

Fish were prepared for and maintained during surgery as described previously (see Preparation I). Isolated heads were prepared in a manner similar to Wood (1974a), with some modifications (Fig. 6). Briefly, a catheter (15 cm, PE 160 - 1.14 mm I.D. x 1.57 mm O.D., Clay Adams) was inserted into the dorsal aorta, in a retrograde direction, to a point approximately 5 mm posterior of the dorsal edge of the opercular opening. This catheter was connected to a wide bore tube (6 mm I.D.) and was maintained at the same level as the back-pressure reservoir (40 cm H₂O). A side-arm of PE 50 (0.58 mm x 0.965 mm) tubing was attached to this catheter near the point of entry into the fish, to facilitate sampling of dorsal aortic perfusate outflow. In addition to the ventral aortic catheter used for the input of perfusate to the gills, a second cannula (PE 160) was tied tightly into the ventricle, and securely anchored by sutures to the lower jaw. The mid-ventral incision was closed carefully with sutures. A side-arm, similar to that in the dorsal aortic catheter, allowed sampling of saline from the anterior venous flow which was going back into the heart. In all other respects, the preparation was as described by Wood (1974a). A flared tube (9 mm I.D.), used to direct water flow over the gills, was inserted and sealed into the mouth with plasticine. The jaws were sutured tightly closed around this tube, using size-2 silk. Ventilatory flow (1 L·min⁻¹) thus was maintained over the gills at all times. The Latex rubber dental dam surrounding the body of the fish was sealed with Poly-Grip (Block Drug Co., Toronto). This compound was very effective in preventing water leakage around the sutures used to secure the dam to the skin.
Figure 6. Schematic representation of the experimental apparatus used in isolated, saline-perfused trout head preparations. Gills were perfused via the catheter in the ventral aorta. Dorsal aortic effluent and anterior venous effluent were collected from DA and AV catheters respectively.

\[ Q_{DA}, \text{dorsal aortic outflow} \]
\[ Q_{AV}, \text{anterior venous outflow} \]
\[ P_i, \text{input perfusion pressure} \]
\[ \text{DAP, dorsal aortic pressure} \]
\[ W, \text{adjustable Windkessel} \]

gas mixture = 0.4% CO₂; 4.1% O₂; 95.5% N₂
Perfusate and Perfusion

Perfusate used throughout these experiments was double-filtered (0.45 μm) Cortland Salmonid saline (Wolf, 1963), to which 40 g·L⁻¹ PVP (polyvinylpyrrolidone; Matheson, Coleman and Bell, Norwood, Ohio; av. mol. wt. = 40,000), a colloid osmotic filler, had been added. This concentration was chosen since it approximated the plasma protein concentration found in fish (see Holmes and Donaldson, 1970). Saline reservoirs, kept at the experimental temperature, were vigorously bubbled with gas mixtures set to physiological venous gas tensions, delivered via Wösthoff gas mixing pumps. This mixture (0.4% CO₂; 4.1% O₂; 95.5% N₂) gave PCO₂ and PO₂ values similar to those reported by Holeton and Randall (1967a) for ventral aortic blood of S. gairdneri.

The three input regimes used to perfuse the isolated trout head preparations were:

1) constant pulsatile flow delivered by a Harvard Blood Pump Harvard Instrument Co., Inc., Mass.), as modified by Davie and Daxboeck (1981) (see Appendix B). The pressure and flow profiles produced by this pump were found to be coincident and superimposable (see Fig. 12), and simulated in vivo heart action.

2) constant pressure perfusion, from an adjustable pressure head reservoir, and

3) constant non-pulsatile flow, delivered via a Harvard Infusion Pump (Model 976; Harvard Apparatus Co., Inc., Mass.).

Experimental Protocol

After the gills had been cleared of blood (see Farrell et al., 1979), the fish were placed in the apparatus (see Fig. 6) and perfused at constant pulsatile flow (17.0 ± 1.0 ml·min⁻¹·kg⁻¹; Kiceniuk and Jones, 1977),
at a ventral aortic pressure of 10 cm H$_2$O (set by variable Windkessel), for 10 - 15 min. The pulse rate used was set at 40 \text{ min}^{-1}. Input pressure was monitored continuously by a Statham P23Db pressure transducer (Hewlett Packard). This transducer was calibrated against a static column of water. Pressure was recorded on a Gilson chart recorder (Gilson Medical Electronics Inc.). After input pressures had stabilized, inflow, dorsal aortic outflow and anterior venous outflow were collected for five min, and determined gravimetrically on a Mettler P1200 top-loading balance. Perfusion then was changed to either constant pressure (regime 2), or constant non-pulsatile flow (regime 3), and the same variables measured. During constant pressure perfusion, the input pressure was adjusted to match the mean input pressure (diastolic + 1/3 pulse; Burton, 1972) produced by pulsatile perfusion (regime 1). The flow rate selected for constant non-pulsatile flow was as near as possible to 17.0 ml\text{ min}^{-1}\text{ kg}^{-1} as could be obtained from the range available on the Harvard infusion pump. Fish were selected for suitable weights, to be compatible with flows obtainable from the infusion pump. Since flows and pressures were not significantly different for each of the regimes, pulsatility of flow and pressure was the experimental variable.

The order of perfusion regimes was randomized during different experiments. Changes from one perfusion regime to another were accomplished with minimal disturbance to the preparation.

In some experiments, samples drawn from the ventral and dorsal aortic catheters and the anterior venous catheter were analysed for pH, and $P_{O_2}$, using an I.L. Micro 13 blood gas analyser (Instrumentation Laboratories, Mass.). Total carbon dioxide ($C_{CO_2}$) in these samples as determined us the method of Cameron (1971). Partial pressures of CO$_2$ ($P_{CO_2}$) were calculated using a reorganization of the Henderson-Hasselbach equation as
follows:

\[ P_{CO_2} = \frac{C_{CO_2} \text{(mM)}}{\text{antilog (pH - pK')}} \times \alpha_{CO_2} + \alpha_{CO_2} \]

The operational pK' values of carbonic acid were obtained from Severinghaus et al. (1956) and the solubility coefficient of CO₂ (\(\alpha_{CO_2}\)) was obtained from Albers (1970).

Data are presented as means, plus or minus 1 standard error. Results were analysed by a one-way analysis of variance, combined with Duncan's Multiple Range test, and 5% considered the fiducial limit of significance.

**FLUID EXCHANGE IN GILL TISSUES**

For experiments designed to examine fluid exchange between perfusate and respiratory tissues during flow and pressure conditions mimicking those found *in vivo*, a similar trout head preparation as just described above, was employed, with the following modification. Ventilatory water could be switched from the flow-through reservoir supply (1 L·min⁻¹), to the recirculation of 4 L of water at the same flow rate, with no interruption in flow. This flow was maintained by an in-line impeller-type water pump (see Fig. 6). Perfusate inflow (\(Q_{VA}\)), dorsal aortic outflow (\(Q_{DA}\)), and anterior venous outflow (\(Q_{AV}\)) were determined as above, during perfusion with constant pulsatile flow, and constant pressure only.

**In Vivo Marker Tests**

A number of vital dyes were used in an attempt to mark the extracellular fluid space of trout gills (see Fig.7 for a description of the anatomy). These dyes were injected via a dorsal aortic cannula, to approximate a plasma concentration of 0.1%. Alternately, dyes were added to the external medium at a concentration of 0.1% and allowed to be irrigated by the fish.
for 1 hour. Histological examination under light microscopy of fixed gill tissues (Serra's fixative), which had been cleared and embedded in parafin (section at 10 μm), revealed that none of the dyes tested was an adequate extravascular marker, regardless of administration method.

Ethanol (EtOH) then was considered as a marker because it is rapidly taken up by tissues and easily monitored. However, equilibration of ethanol throughout all body tissues in goldfish can take between 3 and 60 hours (E. Shoubridge, personal communication). The in vivo uptake of ethanol across the gills of five rainbow trout was measured from dorsal aortic blood samples, removed via chronic cannulae, at 45s, 60s, 90s, 120s, 5 min, and 15 min intervals following the addition of ethanol to the water to make a concentration of 3 mM (95% EtOH used). After 60s, plasma ethanol concentration was approximately 45% of water concentration. After 15 min, blood ethanol concentration was 58.4 ± 5.2% of water concentration. These data show that measurable amounts of ethanol were taken up across the gills of intact fish in less time than one circulation (1 - 2 min) of blood through the body (Davis, 1970; Itazawa, 1970).

**Ethanol Washout Curves**

Gill tissues were loaded with ethanol by introducing 10 ml 95% EtOH into the recirculating ventilatory water of the saline-perfused head preparation. After 15 min, ventilatory water was changed from recirculating ethanol solution, to water from the fresh water reservoir. Samples (0.3 ml each) of "inspiratory" water, dorsal aortic outflow, and anterior venous outflow were taken at time zero (15 min after EtOH introduction), 30s, 60s, 90s, 120s, 5 min, 10 min, and 15 min after the return of flow-through water. These samples were frozen and stored for subsequent determination of ethanol concentrations. Input perfusate (ventral aortic) samples also were taken, and served as blanks.
Figure 7. Schematic cross-section through the secondary lamellae and filament of a teleost gill, to show vascular and extra-vascular spaces which could have contained the vital dyes tested (redrawn from Randall et al., 1981).
SECTION THROUGH A SECONDARY LAMELLA

epithelia

blood channel with RBC

flanged pillar cell

extracellular or lymph space

chloride and mucous cells

body of filament pocket valve

epithelium around central veno-lymphatic sinus
Ethanol was assayed according to the method of Bernt and Gutmann (1974), on duplicate 50 μl samples. The formation of acetaldehyde from ethanol was followed by measuring the increase in absorption at 340 nm on a spectrophotometer (PYE Unicam SP3-200 U.V.), due to the formation of BNADH (see 1).

\[(1) \text{Ethanol} + \text{BNAD} \rightleftharpoons \text{Acetaldehyde} + \text{BNADH} + \text{H}^+ \quad (\text{see footnote 1})\]

Chemicals used in the above assay were obtained from Sigma Chemical Company.

Washout curves were plotted on semilog graph paper and standard curve-peeling techniques were applied (Shippley and Clark, 1972). All EtOH concentrations were normalized, the initial concentration (after 15 min EtOH introduction) being set to equal 1.0, to facilitate compartmental analysis. Data are presented as mean ± standard error of the mean. The fiducial limit of significance was set at 5% for all statistical tests.

III. SPONTANEOUSLY VENTILATING, BLOOD-PERFUSED TROUT PREPARATION

A. CHARACTERIZATION OF THE PREPARATION

All fish used were the same as those described previously. Experiments were carried out at 7°C.

Blood Collection and Preparation

Donor fish were anaesthetized with 1:15,000 w/w aerated MS 222 solution (pH adjusted to 7.0 - 7.5 with Sodium bicarbonate) and then transferred to an operating table (Smith and Bell, 1964), as in previous cases. To facilitate blood withdrawal, fish were implanted with chronic indwelling dorsal aortic cannulae (Smith, 1978), and allowed to recover for at least 24 hr in darkened Perspex boxes, supplied with running aerated

Footnote 1: ADH = alcohol dehydrogenase

NAD = nicotineamide adenine dinucleotide

NADH = reduced NAD
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 in the following manner. Approximately 3 ml Cortland saline (as described previously) containing 2000 U.S.P. units of sodium heparin were injected into the dorsal aortic 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 final blood volume of approximately 100 ml usually was required for a single perfusion preparation and was prepared by diluting donor blood with saline to a desired 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% CO₂ in air; see Haswell et al., 1979) closely resembling trout venous blood gas tensions. These mixtures were supplied by Wösthoff gas mixing pumps.

**Surgical Procedures**

A fish cannulated the previous day and which had a patent dorsal aortic cannula was anaesthetized in a 1:15,000 MS 222 solution. A second cannula (PE 50) was implanted in the buccal cavity as described by Saunders (1961) to monitor ventilatory movements. The fish was transferred to the operating table where the gills were irrigated throughout the operation with aerated 1:20,000 MS 222 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 closed with a Britcher electrosectilis unit (Birtcher Corp., Los Angeles). The pericardium was opened and two 3-0 silk threads were placed around the bulbous arteriosus. Herparin (2000 U.S.P. units in 2 ml
saline) then was injected into the blood via the dorsal aortic cannula and allowed to circulate for 5 min. As much blood as possible then was withdrawn from the dorsal aorta and discarded.

The ventral aortic input catheter consisted of 2.5 cm of silastic rubber tubing (1.45 mm x 2.30 mm; Sargent Welch Scientific) attached to a curved 13 gauge hypodermic needle shaft. The catheter was connected to a reservoir of Cortland saline (on ice) to which 40 g*L⁻¹ P.V.P. and 1 x 10⁻⁶ M noradrenaline (free base, Sigma Chem. Co.) had been added. As before, saline was filtered through Whatman No. 5 paper and Millipore discs (0.45 μm).

The bulbus was cut just caudad of the midpoint and the ventral aortic catheter inserted (Fig. 8) and tied in place with one of the threads, using a 2+2+1 knot (Tera and Auber, 1976). The perfusion flow was started and the fish was perfused at a pressure of 50 cm H₂O, to clear all vessels of blood. After exsanguination, the perfusion flow was reduced and the retrograde (venous return) catheter inserted. The venous return catheter was a heat-flared piece of PE 200 tubing (1.4 mm x 1.9 mm) which was inserted into the bulbus through the same cut as the input catheter and tied into place with a similar knot as before (Fig. 8). This catheter was found to offer very little resistance to "venous" blood return and "normal" heart function, under the present circumstances. Perfusion was resumed while catheters were anchored to the body wall and the incision closed with sutures.

The fish was transferred to a van Dam-type box (see Davis and Cameron, 1971), and blood perfusion was started. Water flow over the gills was maintained during recovery from the operation by a tube in the mouth. The operation took, on average, 45 min. Interruptions to perfusion with either saline or blood were less than one minute. During saline perfusion on the operating table, fish often exhibit ventilatory movements, a
Figure 8. Schematic sagittal section through the heart of the trout, to illustrate catheter positions used for blood-perfusion experiments. Top of the page is dorsal, right is cephalid.

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 lumena of tubes and heart chambers indicate direction of blood flows.
particularly coughing. Once placed into the box and perfused with blood, equilibrium was regained and regular ventilation resumed within 30 min. Fish were left 2 - 3 h to recover from the acute effects of anaesthesia before any experiments commenced.

**Blood Perfusion**

Blood was held in three or four tonometer flasks. Each tonometer, containing approximately 30 ml of blood, had a polyethylene tube (PE 160) leading to a set of 3-way taps to enable blood to be drawn into a cardiac pump. These taps allowed switching from any flask to any other without interruption of perfusion. The cardiac pump used, as described in the previous methods (II), was accurate to within 1% of gravimetric estimates of the flow rate (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 the gas space at the top of a wide-bore side-arm (Windkessel) in the perfusion line (Fig. 9). Blood was pumped into the ventral aorta through the input catheter and circulated through the entire body. Despite two catheters in the bulbus, ventricular contractions were maintained and pumped venous return blood back to the tonometers via the wide-bore silastic rubber tube (1.97 mm x 3.05 mm).

Perfusion flow was adjusted by altering stroke volume at a cardiac pump frequency of 40 strokes·min⁻¹, to that necessary to maintain dorsal aortic pressure at 40 cm H₂O. This flow rate was always about 16 - 17 ml·min⁻¹·kg⁻¹.

**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
Figure 9. Schematic representation of the instrumentation used to monitor variables from blood-perfused trout.

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)

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.

Note: See Appendix C for photographs of setup in situ.
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 deter-
mined 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 polarographic 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 up the response time of the
electrodes used. Partial pressure of carbon dioxide ($P_{CO_2}$) and bicarbonate
congestions were calculated using the measured pH and $C_{CO_2}$ values, and
the reorganization of the Henderson-Hasselbach equation as described
previously on page 40. For simplicity, only $C_{CO_2}$ values are reported in
the results, 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 approxi-
mation of bicarbonate concentration. Following analysis, a small portion
of blood was used to determine haematocrit (Hct) 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**

Pressures (input, dorsal aortic, ventricular and buccal) all were
measured using Statham P23Db pressure transducers, calibrated against a
static column of water. The water level above the fish was taken as zero.
Mean pressures were calculated as in the previous section. The pressure drop across the input catheter was measured, with ligatures still in place, after each experiment. Catheter "resistance" was used to correct measured input pressures for each preparation.

Inflow was measured with an IVM blood flow transducer and Biotronix 610, electromagnetic pulsed-logic amplifiers (In Vivo Metric Systems, Los Angeles Biotronix Lab., Maryland, U.S.A.). Each of the pressure and flow signals was amplified and displayed on a Brush six-channel recorder (Gould Inc., Cleveland, Ohio) (See Fig. 9).

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 ± standard error from the mean. Where appropriate, results were statistically analysed using Student's t-test, and 10% or 5% was taken as the fiducial limit of significance.

B. EFFECTS OF HAEMODYNAMIC CHANGES ON RESISTANCES AND OXYGEN UPTAKE

Fish used for this series of experiments were maintained and surgically prepared as described above. The cardiac pump allowed the way in which blood was delivered to the gills to be altered. A set of normal blood samples was taken and analysed before any haemodynamic manipulations. Then, changes were made with no interruption in perfusion flow, and a 5 min equilibration and stabilization period was allowed before another set of samples was taken for analysis. The sampling method and analyses were the same as described in the above section.

The alterations performed were as follows:
1) increasing cardiac output (Q) by 1.5 times normal, by decreasing stroke volume (SV), while the delivery frequency was kept normal (f = 40 bpm)*;
2) decreasing cardiac output by 0.5 times normal, by decreasing stroke volume, while the pump frequency was kept normal*;
3) increasing stroke volume and decreasing the pump frequency, so that cardiac output remained close to normal \( (f = 20 \text{ bpm}; SV = 2 \times SV_n) \), thus simulating hypoxic bradycardia in normoxic water;

4) decreasing stroke volume and increasing pump frequency so that cardiac output remained close to normal \( (f = 60 \text{ bpm}; SV = 0.5 \times SV_n) \);

5) increasing input pulse pressure to approximately 2 times normal pulse pressure recorded from the ventral aorta, independent of any changes in cardiac output or pump frequency \( (40 \text{ bpm}) \);

6) increasing input haematocrit approximately 2 times that of "normal" blood \( (2 \times \text{Hct}_n) \), while keeping all other conditions normal, by adding red blood cells (rbc's) to a tonometer;

7) decreasing input haemotocrit to approximately 4%, while keeping all other conditions normal, by removing rbc's and adding plasma to a tonometer;

8) adding \( 1 \times 10^{-6} \text{ M} \) final conc. of adrenaline (Sigma; free base) to a tonometer, while cardiac output and method of delivery were kept normal.

The above sequence of haemodynamic alteration was alternated among the preparations, and no differences due to sequencing was noted in any measured blood variable. Aerated water flow to the preparation was maintained throughout these manipulations, supplied from a flow-through reservoir \( (7^\circ \text{C}) \).

**Direct Measurement of Oxygen Uptake and Ventilatory Flow Across the Gills: A Comparison of Cardiac Output by Direct and Fick Methods**

Fish used for these experiments \( (N = 4; 340.1 \pm 13.2 \text{ g}) \) were maintained and surgically prepared as above, with the following exception. In order to measure ventilatory water flow \( (\dot{V}_g) \) directly, a thin Latex surgeon's glove \( \#8 \text{ 1/2} \) was sutured (3-0 silk) around the mouth of a fish in order to avoid interfering with movements of the branchial apparatus.

*Pulse pressures were not adjusted to a constant normal level.*
When a fish was confined within the holding apparatus, this dam effectively separated inspired and expired water, and thus ventilatory flow could be measured volumetrically from the overflow in the caudal compartment (Fig. 10). During recovery from anaesthesia, the water level in the cephalic compartment was raised approximately 2 cm above that in the caudal compartment, thereby maintaining a positive water flow (approx. 750 ml·min⁻¹) over the gills. When dye was added to the water in the cephalic compartment there was no indication of leakage around the sutures between the dam and skin. Once regular spontaneous ventilatory movements were established, water levels in the two compartments were kept equal for the duration of experimentation. In all other respects, the apparatus was identical to that already described by Davis and Cameron (1971).

Inspired water (for $P_{I_02}$ measurements) was taken one cm in front of the mouth of the fish. Expired water samples (for $P_{E_02}$ measurements) were drawn from one cm inside the gap at the back of the box confining the fish. Other expired water sampling sites have been found to be unsatisfactory (see Davis and Watters, 1970; Davis and Cameron, 1971). Once out of the operculum, expired water could emerge only from this gap, as the tightly fitting cover over the fish allowed no exchange with water from the rest of the apparatus. At the same time as water samples were taken, blood samples also were withdrawn and analysed as before. $\dot{V}_g$ and the respiratory frequency ($f_g$) were determined at this time as well.

Inflowing water passed through a gas exchange column, and air was bubbled through the water ($P_{I_02} = 151.4 \pm 0.8$ mm Hg). All data are analysed and presented as described in the previous section.
Figure 10. Diagrammatic representation of the modified van Dam apparatus used in the present experiments. The Latex dam separated inspiratory water from expired water, such that the overflow in the caudal compartment of the holding chamber was that volume of water which the fish ventilated over its gills ($V_g$). A constant supply of aerated water at a rate far in excess of the ventilation volume was maintained.
overflow = V_g

water level

holding box

CAUDAL COMPARTMENT

CEPHALIC COMPARTMENT

Latex dam

partition

P_{buccal} DAP

overflow = V_g

5 cm
SECTION I

BLOOD VOLUME AND FLOW DISTRIBUTION DURING EXERCISE
INTRODUCTION: SECTION I

In the general introduction I pointed out that in mammals during exercise, blood flow to the working skeletal muscles can increase two to three times above the increase measured in the total cardiac output. That there must be redistribution of blood flow elsewhere in the body is obvious, and the splanchnic circulation was noted as the most probable site of blood flow and pressure control during exercise. Previously, Stevens and Randall (1967a) had recorded blood pressure and flow changes in the sub-intestinal vein of rainbow trout during exercise, and at that time suggested that these changes also were associated with the shunting of blood away from one tissue towards another. Stevens (1968a) nonetheless found no significant changes in the blood volume distribution to different organs between resting and exercising trout, except for a volume reduction in the spleen. In contrast, no studies have been conducted, to date, which investigate the possibility of blood flow redistribution during exercise in fish, as is known to occur in mammals.

The experiments in this section therefore are designed to examine not only the distribution of tissue blood volume, but also the distribution of cardiac output (blood flow) to various organs in the systemic circulation of rainbow trout at rest, and during aerobic, steady-state exercise at 80% of their estimated critical swimming speed. These experiments were carried out using various radiotracer techniques, to assess the circulatory pattern of trout forced to swim against a water current in a Brett-type tunnel respirometer.

From the data obtained, possible underlying mechanisms for the control of systemic circulation at rest and during swimming exercise in rainbow trout, Salmo gairdneri, are discussed.
RESULTS I

Blood Volumes and Flow Distribution

Table 4 lists representative percentages of total body weight for various tissues sampled from fish used in the \(^{86}\)Rb-injection, critical swimming velocity experiments. Whether values were determined directly (given as means \(\pm\) S.E.M.), or taken from the literature, those listed in this Table were used in the present study for the calculations of total counts per minute (cpm) in each whole tissue and within the whole body, at rest and during exercise. Normal resting trout were injected with 1.0 ml \(^{51}\)Cr-labelled blood cell suspension which had an haematocrit of 52.3 \(\pm\) 10.1%, and a total activity of 70,094 \(\pm\) 8,723 cpm. The injection of \(^{51}\)Cr-labelled red blood cells allowed the blood volume distribution in the various tissues to be assessed, at rest. These data indicated a resting blood volume of 6.08 \(\pm\) 1.31% of total body weight, as determined by the degree of dilution of radioactivity in the dorsal aortic blood samples after 2 hours of equilibration. Also, the injection of 1 ml of high haematocrit, labelled blood had minimal effects on the measured post-equilibration dorsal aortic Hct (Table 4).

A total of 87.3% of the body weight of the fish was accounted for from measurements made and presented in Table 4. From this, the percentage of total blood volume accounted for by the tissues listed was 2.43% of body weight. This volume however, did not take into account the blood volume contained within the heart cavities, ventral aorta, arteries, the Cuverian sinus and the veins. Another 12.7% of the body weight also was still unaccounted for. If this 12.7% of the remaining tissues were as poorly vascularized as the mosaic muscle, then only 2.54% body weight value for the total blood volume is indicated. However, this value only represents approximately 40% of the total blood volume, contained in the capillary network, arterioles and small veins of the sampled tissues. When this was
taken into account, then the corrected total blood volume from the fish used here, using information from Table 4, was 6.35% body weight, or 6.35 ml·100g⁻¹. This value compared well with independent values obtained from dorsal aortic blood samples and $^{51}$Cr activity in the blood (6.08 ml·100g⁻¹).

The data in Table 4 also indicated that red muscle at rest contained an average 2.6 times the blood volume per gram tissue of mosaic muscle. Also indicated was the fact that the spleen contained the most blood per gram tissue of any other organ, and approximated 0.7% of the calculated total blood volume. This organ only accounted for 0.23% of the body weight.

There was a measurable decrease in the apparent total blood volume during exercise, which was accompanied by a concomitant haemoconcentration, as indicated by an increase in the dorsal aortic haematocrit of 32.9 ± 9.0% above that found at rest. Fish which were forced to swim at 80% of their critical velocity were injected not only with $^{51}$Cr-labelled red cells so that tissue blood volumes could be determined at 80% $U_{crit}$, but also were injected with microspheres, for the determination of blood flow distributions. However, because of cross-channel counts interference (see Table 3), only those counts above this interference could be included in Table 5. These data indicated that blood volumes in the liver, spleen, kidney and stomach were lower during exercise than they were at rest. Nonetheless, only splenic and red muscle blood volumes appeared to change significantly during exercise at 80% $U_{crit}$, while other tissue blood volumes remained unchanged from resting values.

The percent of cardiac output distributed to various tissues in the resting rainbow trout, as determined by the percentage of the total injected counts found in each tissue sampled, is summarized for both tracer methods in Table 6. These values were derived using data in Table 4. Therefore, data in Table 6 represent the absolute percentage of cardiac output or flow
Table 4. Blood volume distribution to various tissues from normal, resting rainbow trout (N = 6; 371.0 ± 36.7 g).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Blood Volume $\text{ml} \cdot \text{g}^{-1}$</th>
<th>Percent Body Weight</th>
<th>Percent Total Blood Volume $\text{ml} \cdot 100 \text{g}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosaic muscle</td>
<td>0.0084 ± 0.002</td>
<td>66$^b$</td>
<td>0.512</td>
</tr>
<tr>
<td>Lateral red muscle</td>
<td>0.0211 ± 0.040</td>
<td>2.5$^c$</td>
<td>0.053</td>
</tr>
<tr>
<td>Gills (2nd arch)</td>
<td>0.1594 ± 0.040</td>
<td>3.9$^b$</td>
<td>0.622</td>
</tr>
<tr>
<td>Liver</td>
<td>0.2255 ± 0.060</td>
<td>1.156</td>
<td>0.261</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.0883 ± 0.820</td>
<td>0.227</td>
<td>0.700</td>
</tr>
<tr>
<td>Ventricle (emptied)</td>
<td>0.0647 ± 0.014</td>
<td>0.136</td>
<td>0.009</td>
</tr>
<tr>
<td>Pseudobranch</td>
<td>0.2566 ± 0.072</td>
<td>0.12</td>
<td>0.031</td>
</tr>
<tr>
<td>Stomach and esophagus</td>
<td>0.0204 ± 0.006</td>
<td>1.275</td>
<td>0.026</td>
</tr>
<tr>
<td>Intestine and caeca</td>
<td>0.0329 ± 0.008</td>
<td>1.645</td>
<td>0.054</td>
</tr>
<tr>
<td>Gonads</td>
<td>0.0238 ± 0.007</td>
<td>4.218</td>
<td>0.100</td>
</tr>
<tr>
<td>Skin</td>
<td>0.0164 ± 0.007</td>
<td>4.0$^b$</td>
<td>0.066</td>
</tr>
<tr>
<td>Eyes</td>
<td>0.0296 ± 0.006</td>
<td>1.12</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Pre-injection Hct        | -                                           | 16.25 ± 1.92% (at time = 0) |
Sample Hct               | -                                           | 17.72 ± 2.62% (at time = +2 hours) |
Blood volume             | -                                           | 6.08 ± 1.31% body weight |

$^a$All volumes determined from dorsal aortic (arterial) blood sample reference cpm's.

$^b$Stevens (1968a).

$^c$Kiceniuk and Jones (1977).
Table 5. Blood volume<sup>a</sup> distribution to various tissues from rainbow trout swimming at 80% $U_{\text{crit}}$ ($N = 7; 445.0 \pm 27.7$ g).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Resting blood volume (ml·g⁻¹)</th>
<th>Exercise blood volume (ml·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White muscle</td>
<td>0.0084 ± 0.002</td>
<td>0.0085 ± 0.001</td>
</tr>
<tr>
<td>Lateral red muscle</td>
<td>0.0211 ± 0.003 (n = 36)</td>
<td>0.1250</td>
</tr>
<tr>
<td>Liver</td>
<td>0.2255 ± 0.820</td>
<td>0.0654 ± 0.022</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.0883 ± 0.820</td>
<td>1.546 ± 0.253</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.3265 ± 0.101</td>
<td>0.1174 ± 0.026</td>
</tr>
<tr>
<td>Stomach and esophagus</td>
<td>0.0204 ± 0.006</td>
<td>0.0122 ± 0.008</td>
</tr>
<tr>
<td>Intestine and caeca</td>
<td>0.0329 ± 0.008</td>
<td>0.0340 ± 0.004</td>
</tr>
<tr>
<td>Skin</td>
<td>0.0164 ± 0.007</td>
<td>0.0183 ± 0.007</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>20.5 ± 1.66%</td>
<td>26.4 ± 1.36% (at $t = 25$ min at 80% $U_{\text{crit}}$)</td>
</tr>
<tr>
<td>Total blood volume</td>
<td>6.08 ± 1.31%</td>
<td>3.72 ± 0.40% body weight (at $t = 25$ min at 80% $U_{\text{crit}}$)</td>
</tr>
</tbody>
</table>

<sup>a</sup>All volumes determined from dorsal aortic (arterial) blood sample reference cpm's.
Table 6. Percentage of cardiac output to various tissues from rainbow trout at rest, expressed as percentage of total injected cpm's.

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Mosaic Muscle</th>
<th>Red Muscle</th>
<th>Spleen</th>
<th>Liver</th>
<th>Stomach</th>
<th>Intestine</th>
<th>Gonad</th>
<th>Kidney</th>
<th>Cheek</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>24</td>
<td>36</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

**USING $^{86}$Rubidium**

<p>| | | | | | | | | | | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>384.4</td>
<td>36.4</td>
<td>6.51</td>
<td>0.74</td>
<td>8.73</td>
<td>1.86</td>
<td>5.82</td>
<td>1.99</td>
<td>9.26</td>
<td>0.16</td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>40.5</td>
<td>8.0</td>
<td>0.53</td>
<td>0.32</td>
<td>2.88</td>
<td>0.47</td>
<td>1.34</td>
<td>0.67</td>
<td>3.46</td>
<td>0.05</td>
</tr>
</tbody>
</table>

7.68 (mostly immature) ±1.75

**USING RADIOLABELLED MICROSPHERES**

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<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>28</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>X</td>
<td>429.4</td>
<td>49.0</td>
<td>11.2</td>
<td>1.06</td>
<td>4.5</td>
<td>7.40</td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>26.4</td>
<td>7.6</td>
<td>2.6</td>
<td>0.40</td>
<td>1.9</td>
<td>3.10</td>
</tr>
</tbody>
</table>

89.3 (mostly mature) 7.9

Skin

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</tbody>
</table>
directed to the whole tissue listed at rest. Had the actual cardiac output been known, then the absolute blood flow to each tissue or organ (in ml·min⁻¹) could have been calculated simply by multiplication of the cardiac output by the proportion of absolute cardiac output to that tissue. Nonetheless, the two data sets are comparable, and indicate that red and mosaic muscle masses, which constitute approximately 70% of the total body weight of the fish also received the largest proportion of the blood flow (43 to 60% of the cardiac output) at rest. At rest, mosaic muscle received only 4.4 to 5.6 times more of the total blood flow than did the red muscle, yet mosaic muscle accounted for 26.4 times more of the total body mass. These data indicated that, at rest, red muscle received almost 5 times the amount of blood flow on a per gram basis than does mosaic muscle. The remaining blood flow was more or less evenly divided between flow through the coeliacomesenteric artery, to supply the viscera, and flow via the dorsal aorta to supply the kidney, skin and gonads.

During swimming exercise at 80% $U_{crit}$, there was an apparent redistribution of blood flow, as indicated by data presented in Table 7. At this level of exercise, red and mosaic muscle accounted for 56 to 82% of the total cardiac output. Proportional blood flow to red muscle now was increased over that directed to mosaic muscle, which actually showed a decrease in the proportion of total cardiac output flowing to this tissue mass, compared to the resting condition. However, it must be remembered that the absolute cardiac output at this level of swimming could have been increased by 3-fold over that found at rest in trout (Kiceniuk and Jones, 1977), and thus the absolute blood flow to red as well as mosaic muscle was increased above that at rest. Nonetheless, the data indicated that the red muscle, under these circumstances, received approximately 43 times the amount of blood flow per gram tissue over mosaic muscle flow. Proportionate
blood flow to the spleen, liver and gut was consistently lower during exercise than flow measured at rest. It is interesting to note that, whether using $^{86}$Rb or microsphere techniques to assess blood flow distribution during exercise, the percentage change from rest in those organs supplied by the celiacomesenteric artery was the same for each organ (Table 7).

The data for blood flow changes in kidney circulation indicate an increased perfusion of this organ during exercise, while the skin circulation apparently was decreased. Other tissues showed some degree of variability in flow (Table 7), with no consistent trends being of note.

Initially, dorsal aortic pressure (DAP) was recorded from resting and exercising fish, and these data are presented in Table 8. However, these data were collected only during the $^{86}$Rb studies, but not for microsphere experiments. Data from Table 8 indicated that during swimming exercise at 80% $U_{crit}$, heart rate was not changed from resting levels. However, dorsal aortic pressure was elevated some 41% above the resting level. If cardiac output was increased during exercise, then in the present case, these fish obviously accomplished this by increasing their stroke volume rather than the heart rate. As a consequence, the dorsal aortic pressure was elevated during exercise.

**Exhaustion**

Complete exhaustion after strenuous swimming exercise resulted in further changes in the cardiac output distribution to various tissues from the pattern established at 80% $U_{crit}$. The data in Table 9 indicate a decrease in the proportional cardiac output distribution to most tissues sampled, compared to that found during steady-state swimming at 80% $U_{crit}$. The only exception was the apparent proportional increase in mosaic muscle blood flow, at the expense of less flow to red muscle. Nonetheless, red and mosaic muscle vasculatures still constituted the major proportion of the
Table 7. Percentage cardiac output to various tissues from Rainbow Trout swimming at 80% $U_{crit}$, expressed as % of total injected cpm's.

<table>
<thead>
<tr>
<th></th>
<th>wt g(6)</th>
<th>white muscle g(6)</th>
<th>red muscle g(36)</th>
<th>spleen (12)</th>
<th>liver (12)</th>
<th>stomach (12)</th>
<th>intestine (12)</th>
<th>gonad (12)</th>
<th>kidney (12)</th>
<th>cheek (24)</th>
<th>brain (12)</th>
<th>% cpm injected recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>415.1</td>
<td>21.0</td>
<td>35.4</td>
<td>0.37</td>
<td>5.49</td>
<td>1.32</td>
<td>4.55</td>
<td>1.13</td>
<td>11.93</td>
<td>0.21</td>
<td>0.017</td>
<td>87.4</td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>40.3</td>
<td>2.7</td>
<td>2.5</td>
<td>0.15</td>
<td>1.30</td>
<td>0.37</td>
<td>1.15</td>
<td>0.28</td>
<td>1.77</td>
<td>0.04</td>
<td>0.007</td>
<td>4.6</td>
</tr>
</tbody>
</table>

**USING $^{86}$Rb - BLOOD**

**USING RADIO-LABELLED SPHERES**

<table>
<thead>
<tr>
<th>(n)</th>
<th>7</th>
<th>14</th>
<th>14</th>
<th>7</th>
<th>7</th>
<th>7</th>
<th>7</th>
<th>14</th>
<th>12</th>
<th>14</th>
<th>skin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>429.4</td>
<td>32.2</td>
<td>49.5</td>
<td>0.09</td>
<td>0.77</td>
<td>3.40</td>
<td>8.32</td>
<td>2.62</td>
<td>2.70</td>
<td>86.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>26.4</td>
<td>8.5</td>
<td>8.2</td>
<td>0.02</td>
<td>0.66</td>
<td>2.60</td>
<td>5.60</td>
<td>0.80</td>
<td>0.50</td>
<td>9.4</td>
<td></td>
<td>(mostly mature)</td>
</tr>
</tbody>
</table>

$\Delta$% of rest %

<table>
<thead>
<tr>
<th></th>
<th>$\Delta$% of rest %</th>
<th>$\Delta$% of rest %</th>
<th>$\Delta$% of rest %</th>
<th>$\Delta$% of rest %</th>
<th>$\Delta$% of rest %</th>
<th>$\Delta$% of rest %</th>
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<th>$\Delta$% of rest %</th>
<th>$\Delta$% of rest %</th>
<th>$\Delta$% of rest %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-43.8</td>
<td>+412.8</td>
<td>-83.5</td>
<td>-78.4</td>
<td>-83.0</td>
<td>-6.48</td>
<td>-12.6</td>
<td>-56.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>9.5</td>
<td>55.7</td>
<td>7.6</td>
<td>12.5</td>
<td>14.4</td>
<td>37.6</td>
<td>23.8</td>
<td>9.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8. Summary of dorsal aortic pressure (DAP) and heart rate changes during 80% $U_{crit}$ swimming exercise in rainbow trout

<table>
<thead>
<tr>
<th></th>
<th>RESTING</th>
<th>EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAP (cm H$_2$O)</td>
<td>$29.8 \pm 0.63$</td>
<td>$42.1 \pm 1.46$</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>$37.4 \pm 0.52$</td>
<td>$37.9 \pm 1.46$</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>$384.4 \pm 40.5$</td>
<td>$415.1 \pm 40.3$</td>
</tr>
</tbody>
</table>

$n = 59$ observations of 6 fish

$n = 18$ observations of 6 fish
Table 9. Percentage cardiac output to various tissues from Rainbow Trout swimming at 80% $U_{\text{crit}}$ and at point of exhaustion, expressed as %age of total injected cpm's.

<table>
<thead>
<tr>
<th>wt(g)</th>
<th>white muscle</th>
<th>red muscle</th>
<th>spleen</th>
<th>liver</th>
<th>guts</th>
<th>gonad</th>
<th>kidney</th>
<th>skin</th>
<th>% cpm injected recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>(4)</td>
<td>(24)</td>
<td>(16)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(21)</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>534.5</td>
<td>20.8</td>
<td>60.7</td>
<td>0.97</td>
<td>2.35</td>
<td>4.12</td>
<td>3.37</td>
<td>2.82</td>
<td>3.18</td>
</tr>
<tr>
<td>$\pm$ S.E.M.</td>
<td>34.4</td>
<td>7.2</td>
<td>10.1</td>
<td>0.95</td>
<td>2.00</td>
<td>3.14</td>
<td>2.82</td>
<td>0.88</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**EXHAUSTION**

| $\bar{x}$ | 534.5 | 31.8 | 44.6 | 0.08 | 0.28 | 0.13 | 2.39 | 1.90 | 90.5 |
| $\pm$ S.E.M. | 34.4 | 15.7 | 13.2 | 0.05 | 0.10 | 0.07 | 1.00 | 0.66 | 5.1 |
total blood flow. Interestingly, at exhaustion, only 81.2% of the cardiac output distribution could be accounted for in the capillary networks of the tissues sampled. This decreased from a value of 98.3% found when tissues were sampled from fish swimming at 80% of their critical velocity.

**DISCUSSION**

It is known that exercise causes a diuresis in trout (Wood and Randall, 1973) and the measured increase in dorsal aortic pressure during exercise in my experiments could explain the observed increases in the kidney ultrafiltration rate. Also, data from the $^{86}$Rb investigation indicate a 29% increase in the blood flow to the kidney during exercise, over that at rest. Increased kidney function therefore can account for the measured decrease in the total blood volume as well as the haemoconcentration during exercise in the present study. During exercise, an increase in the water flux across the gill tissues into the blood (Wood and Randall, 1973) imposes an increased water load on the fish, but this excess water is eliminated by increased urine production by the kidneys, as well as possible water loss to the tissues (oedema) from the blood. Microsphere data indicate a 13% decrease in the proportion of cardiac output distributed to the kidneys under the same exercise conditions. If there is an exercise diuresis, then kidney vascular dilation could lead to the noted decrease in the entrapment of the injected microspheres, compared to resting values.

My data also indicate that the lateral red muscle has a very substantial increase in the percent cardiac output during steady-state exercise. The proportion of cardiac output to mosaic muscle is decreased below that found at rest, but as pointed out previously, the absolute flow to this tissue mass is in fact increased as well, since cardiac output at this level of exercise in rainbow trout can increase by at least 3-fold above that found at rest (Kiceniuk and Jones, 1977). Working muscles therefore
are preferentially perfused over other tissues, as is found to occur in mammals during exercise.

Most teleost fishes have two types of skeletal muscle fibres. It is generally accepted that the highly vascularized red muscle of fishes, especially salmonids, is used primarily for sustained swimming, as during migratory periods (Bilinski, 1974). Mosaic muscle contains a small percentage of red fibres in rainbow trout (Webb, 1975) and is poorly vascularized, but comprises the largest bulk of the body mass. It is thought to act as a powerful but temporary propulsive force for burst swimming activity. The contractility of red muscles, used for prolonged swimming exercise, is maintained by energy supplied by aerobic metabolism. White muscle on the other hand, is geared to have its metabolic requirements met primarily from anaerobic processes (Driedzic and Hochachka, 1978). Certainly the difference in muscle capillaries between red and white fibres is indicative of their modes of metabolism. The capillary densities of fish red muscle are some 2 to 10 times those of white muscle (Cameron and Cech, 1970; Mosse, 1978), depending upon species. As such, blood volume per unit weight in resting red muscle of rainbow trout is 2 to 3 times that of mosaic muscle. Also it has been shown that anywhere from 5% (Krogh, 1919) to 50% (Wright and Sonnenschein, 1965) of the muscle capillaries of fish are patent at rest. During exercise, a range of from 2 to 20 times increase in the potential blood volume therefore could be expected, given that all capillaries are perfused. In the present study, a 6-fold increase in red muscle blood volume over that measured at rest, is indicated during exercise in the rainbow trout. Mosaic muscle blood volume however did not show any change at the same level of exercise.

White muscle, however, has been shown to be active during prolonged swimming. In brook trout (Salvelinus fontinalis) for example the thres-
hold for the recruitment of white muscle fibres, based on E.M.G. recordings, was approximately 1.8 forklenght·sec⁻¹ (Johnston and Moon, 1980a). This swimming speed, in the present experiments, is almost equivalent to the 80% $U_{\text{crit}}$ exercise level for rainbow trout. Therefore, both muscle fibre types could have been functional during exercise in the rainbow trout during my experiments. Davison and Goldspink (1977) and Walker and Emerson (1978) found that with exercise for 3 to 4 weeks, rainbow trout not only showed hypertrophy of red muscle fibres, both in terms of fibre diameters and lengths, but white fibres also hypertrophied, to an even greater extent than red fibres. Such muscle fibre hypertrophy indicates that white muscle may be utilized during exercise training.

Johnston and Moon (1980b) considered that the aerobic capacity of white muscle was sufficient to supply the energy requirements of this tissue during sustained swimming, since they were unable to detect any increase in blood lactate (an indication of anaerobic metabolism) during exercise. Driedzic and Kiceniuk (1976) also failed to note any increase in blood lactate in rainbow trout swimming at velocities as high as 93% $U_{\text{crit}}$. They concluded, however, that the gills were able to oxidize any lactate present in the blood, due to white muscle anaerobiosis, so that it would not be expressed in the dorsal aortic blood. Additionally, Hulbert and Moon (1978) have shown that eel (*Anguilla anguilla*) red muscle can perform a metabolic recycling function, in addition to contraction (*in vitro*), so that a gluconeogenic process, which converts white muscle lactate to glucose is in existence in this tissue. There is no reason however, to assume that white muscle capillaries supply red muscle fibres *in vivo* in the rainbow trout. The lack of any measured increase in blood lactate during exercise is more likely to indicate primarily an aerobic metabolic route for the energy of the muscles. The erratic burst-glide swimming behaviour exhibited by trout as they near 100% $U_{\text{crit}}$ is
indicative of increased white muscle activity. The possibilities of metabolic acidosis being expressed in the blood as the aerobic capacity of white muscle is exceeded are therefore high at this level of exercise. Muscle cells of some fish (i.e. *Pleuronectes platessa*) however appear to possess the ability to regulate the release or retention of their lactate load to blood during exercise, and increased blood flow to these muscles does not affect lactate release (Wardle, 1978). The release appears to be activated by a catecholamine (as yet unidentified) circulated in the blood stream only following the termination of stress. Whether such a mechanism is operational in trout in order to minimize the effects of metabolic acidosis in the general circulation during prolonged exercise, is not known.

If an organ can be effectively isolated from the general circulation by constriction of its afferent and/or efferent blood pathway during exercise, then even though there may have been no measurable volume change, there will be dramatic changes in blood flow through that organ. Data presented in Tables 6 and 8 of this thesis indicate that such is indeed the case for exercising rainbow trout. Splenic blood volume and flow, as well as the flow through the other organs supplied by the coeliacomesenteric artery are reduced during exercise. That the coeliacomesenteric artery is the main site of control for flow in the spleen, liver, stomach and intestines is based on observations from both Rubidium and microsphere studies, that the percentage decrease in cardiac output distribution to these organs during exercise is of the same magnitude. The situation whereby this circulation is curtailed during exercise in the rainbow trout, in favour of increased blood flow to the working muscles is very similar to the splanchnic blood "pool" in man (Rowell et al., 1964). This circulation in fish probably functions in a similar capacity during exercise. The ultimate result of the overall blood shunting is to increase the effective circulating blood volume,
to decrease the oxygen consumption of tissues not directly related to the maintenance of swimming, and to augment oxygen delivery to the working muscles, through increases in haematocrit and haemoglobin concentrations during exercise (see Stevens, 1968b).

There still appears to be considerable controversy over the mode of systemic vasomotor tone maintenance in resting fishes. It has, however, been repeatedly demonstrated that at least it is under general $\alpha$-adrenergic control, although it is not known whether it is neuronally or hormonally mediated. Walhqvist and Nilsson (1977) state that adrenergic tonus in *Gadus* is maintained by circulating catecholamines rather than adrenergic nerves. Santer (1977) has shown that the chromaffin system in fish, which in function is similar to the adrenal glands of higher vertebrates, is much better developed than is the sympathetic nervous system. This led him to conclude that circulating (hormonal) adrenergic control is of greater importance for the maintenance of systemic vascular tone. To this end, Vanhoutte (1978) has noted that most blood vessels contain vesicles which store or sequester dopamine and convert this to noradrenaline, or actively take up noradrenaline directly from the blood. Although the levels of circulating catecholamines in salmonids are reported to increase during stress (Nakano and Tomlinson, 1967), these catecholamines have a long half-life in the blood, and so it seemed unlikely that these could maintain fine enough control of vascular tonus in these fish. Thus Wood and Shelton (1975) conclude that vasomotor tone is maintained by autonomic innervation, in *S. gairdneri*. The persistence of Mayer waves (measured as cyclical variations in the mean dorsal aortic blood pressure) in rainbow trout suggests that tone is reflexly regulated through a negative feedback control for blood pressure maintenance, via the sympathetic nervous system (Wood, 1974b). Therefore, it seems likely that systemic vascular resistance (tone) is maintained in resting fish through $\alpha$-adrenergic constrictor responses (Randall and Stevens, 1969; Helgason and
Nilsson, 1973; Wood, 1976; Wahlqvist, 1980; Wood and Shelton, 1980a). As well, there may be $\beta_2$ dilatory adrenoceptors in fishes, as in the systemic vasculature of mammals. However, these dilatory responses are thought to be seen only against a background of primarily $\alpha$-adrenergic constrictor responses (Wood, 1976, 1977). Whether $\beta$-adrenergic vasodilation is operative in trout during exercise still is not resolved.

In elasmobranch fishes, in the absence of any significant sympathetic nervous system control, the chromaffin tissues release substantial amounts of adrenaline, and more importantly noradrenaline into the circulatory system. These catecholaminergic species are aspirated directly into the general circulation with each beat of the heart. Vascular tone in these fish obviously is controlled by circulating catecholamines at all times (Satchell, 1971; Capra and Satchell, 1977). The circulatory system of elasmobranchs also is thought to be devoid of any direct neural means by which they can control blood flow distribution to the musculature, even during exercise, except possibly by autoregulation of the microvasculature (Opdyke et al., 1979).

The effects of exercise on blood pressure and heart rate in fishes have been well documented (see Jones and Randall, 1978 for review). Generally, mean dorsal aortic pressures are found to increase with activity, as do ventral aortic mean and pulse pressure, venous (subintestinal) pressures, and cardiac output. Since the dorsal aorta and vessels supplying blood to the body musculature in trout appear to be also devoid of any adrenergic innervation (Gannon, 1972), direct nervous vasodilatory action was minimal. The measured increase in regional blood flow to these tissues must reflect, to some degree, the passive dilation of capacitance vessels, brought about by the increased pressure head reaching these vessels during exercise. It has been suggested that intercapillary anastomoses in mammalian skeletal
muscles provide alternative pathways around transient obstructions in the capillary network, due to contracted muscles, (Honig et al., 1977) in addition to increasing oxygen supply to these muscles through increases in blood volume and flow.

Autoregulation of microcirculation is thought to involve such things as metabolic vasodilator substances which lead to active hyperaemia, and myogenic tonus activity within these vessels (Jones and Berne, 1963; Green and Rapela, 1964; Johnson, 1964; Stainsby, 1964; Strandell and Shephard, 1967; Vanhoutte and Janssens, 1978). At lower venous $P_{O_2}$, the sensitivity of mammalian hind limb resistance vessels (the capillaries) to metabolic and haemodynamic disturbances increases (Granger et al., 1976), and these vessels are more sensitive than any other vessel within the circulatory system (Mellander, 1978). This can be of obvious importance to the observed flow changes in trout muscle during exercise, since its vasculature appears to lack any neuronal pathway for vasodilation. Resistance vessels also suggested to have the ability to escape from the constrictor action of catecholamines when tissue oxygenation is lower than normal (Mellander, 1978). Therefore, the vasoconstrictor effects of any increase in the level of circulating catecholamines, should this in fact occur during exercise in trout, could be overridden in the muscle vasculature by such a mechanism. This would indicate that substrate changes in the metabolically active muscle tissues are responsible for vasodilation in these tissues during exercise. In this manner, red and mosaic muscle blood flow could be increased, as was measured in my experiments, within a background of general catecholaminergic vasoconstriction elsewhere in the body during exercise. Smith (1978) has shown that $\alpha$-adrenergic constriction of systemic vessels at the onset of swimming exercise in rainbow trout (5 min at 2 fl·sec$^{-1}$, from a standing start) controls the measured changes in blood pressure. Smith however, may
have been measuring the "start-up" responses, initiated by nervous stimulation, while in my experiments, the observed flow and volume changes, using the gradual increase in exercise level, may have been maintained through circulating catecholamines in this steady-state condition.

Although the details of the mechanisms responsible for the maintenance of blood volume and flow redistribution during exercise in fish are not fully understood, it appears that α-adrenoceptor stimulation of the coeliacomesenteric artery, and local hyperaemia in muscle vasculature are the main sites for this control.

Gonadal blood flow in immature trout is decreased to a greater and less variable degree than is the case for mature trout under the same swimming exercise conditions (see Table 8). The high variability in data from mature fish may reflect a migratory strategy of maintaining adequate blood supply to the gonads for the ultimate purpose of spawning at the end of a long period of migration.

Although the data are tentative for the experiments where trout were exercised to complete exhaustion, they do indicate that exhaustion may be associated with periodic cessations of the circulation (i.e. heart stoppage). Since microspheres are slightly more dense than red blood cells, they have a stronger tendency to settle out of suspension if not kept in continuous motion. If the circulation were to be temporarily stopped during the infusion period at exhaustion, as in the present study, then an uneven distribution of spheres in the circulation would be expected. If some spheres settled out into large arteries and/or veins, and were not entrapped in the microcirculation of the tissues at the time when the animal was sacrificed, then a decreased percent of the total injected counts which are recovered in the sample tissues can be expected. Only 81% of the cardiac output is accounted for in the tissues samples from fish which were exhausted, as
opposed to 98% in the tissues at $80\% U_{\text{crit}}$. Because short burst activity is quite prominent before exhaustion, it is not surprising that white (mosaic) muscle blood flow is increased over that found at $80\% U_{\text{crit}}$. The failure of the oxygen transport system to maintain adequate supply for aerobic metabolism may be indicated by the decreased relative flow to the red muscle, compared to that found at $80\% U_{\text{crit}}$. If indeed massive circulatory catecholamine release is stress related (Mazeaud et al., 1977), and the systemic vasculature is under $\alpha$-constrictor vasomotor control, then tissue flow to all other capillary networks, as measured by microsphere entrapment, will be drastically reduced at exhaustion, over the flows found at $80\% U_{\text{crit}}$. However, this area of research needs further investigation before a precise description of the cardiovascular events which participate in the inability of the trout to maintain any further swimming activity can be given.

The previous discussion suggests that systemic vascular motor control of *Salmo gairdneri*, although generally understood, is rather difficult to describe precisely. Figure 11 is presented as a general summary of some of the control mechanisms which have been found to exist in the rainbow trout. The plasticity and variability of the relative extent of neuronal versus humoral (hormonal) responses in the vasculature are not readily quantifiable for the resting condition, and appear to be even less so during steady-state, aerobic exercise in these fish.
Figure 11: Summary of the possible sites of direct neural, and circulating catecholamine action in the circulatory system of *Salmo gairdneri*. The vasculature can be divided into branchial and cardiac, and general systemic regions. Direct innervation is from cranial nerves IX and X to the branchial vasculature, stomach, spleen, heart, hepatic and coeliacomesenteric arteries.

$Ch^+ = \text{cholinergic constriction}$

$Ch^- = \text{cholinergic dilation/inhibitory cardiac (chronotropic)}$

$\alpha^+ = \text{adrenergic construction}$

$\beta^+ = \text{\(\beta\)-adrenergic stimulation (inotropic cardiac)}$

$\beta^- = \beta_1 \text{ or } \beta_2 \text{-adrenoceptor dilation}$

$\beta_1 = \text{neuronal } \beta\text{-adrenoceptors}$

$\beta_2 = \text{humoral } \beta\text{-adrenoceptors}$

(Adrenoceptor terminology after Alquist, 1948)
Autonomic nerves - terminal release of catecholamines to stimulate $\alpha$ and $\beta$ receptors

Chromaffin tissue - kidney, cardiac, venous - circulating catecholamines to stimulate $\alpha$ and $\beta$ - receptors

Branchial vascular receptors $\text{Ch}^+, \alpha^+, \beta^-$

Cardiac receptors, $\text{Ch}^-, \beta^+$

Systemic vascular receptors - esp. coeliacomesenteric and splenic $\alpha^+$, muscle $\beta^-$
SECTION II

VASCULAR RESISTANCE TO FLOW IN SALINE- AND BLOOD-PERFUSED PREPARATIONS OF RAINBOW TROUT
INTRODUCTION: SECTION II

In the previous section, I have shown that in intact trout, systemic blood flow during steady-state swimming exercise is redistributed to favour working muscles. Blood pressure and cardiac output increase during exercise (see Jones and Randall, 1978) associated with systemic blood flow redistribution. The gills maintain the necessary gas transfer to meet the increased aerobic metabolic requirements of the muscles. The increased blood pressure and flow associated with swimming exercise could have profound effects upon the resistance to flow through the gills, and upon the pattern of fluid distribution within the gills, given that these tissues, at rest, already function under elevated hydrostatic (blood) pressure. One object of this study therefore, is to examine how changes in input pressure and flow to the gills could alter their function during exercise in the rainbow trout.

In order to examine gill function in fishes, a variety of in vitro perfusion techniques have been employed. Most animals seek to maintain constant arterial pressure by changing blood flow or vascular resistance (Mellander and Johansson, 1968). Most gill preparations therefore have been perfused using either constant input pressure (Keys, 1931; Rankin and Maetz, 1971; Stray-Pederson and Steen, 1975; Payan and Matty, 1975) or constant input flow (Keys and Bateman, 1932; Kirschner, 1969; Shuttleworth, 1972; Bergman et al., 1974; Wood, 1974a; Smith, 1977; Clairborne and Evans, 1980). However, Bergman et al. (1974) have stated that perfusion of rainbow trout gills with constant pulsatile flow was qualitatively superior to perfusion at constant pressure. Furthermore, few preparations have used physiological dorsal aortic pressure, even though the effects of alterations in dorsal aortic pressure on gill vascular resistance to flow can be profound (Wood, 1974a).

In the following section, an isolated, saline-perfused rainbow trout head preparation is described. This preparation is used since it
allows the measurement of fluid distribution in the gills to be made during perfusion with three different input regimes. These data allow the effects of absolute and pulsatile pressure on recurrent flows and gill resistances to be assessed. Haemodynamic alterations mimicking those known to occur during exercise in vivo, and their effects on in vitro fluid distribution and gill vascular resistance to flow are discussed with respect to their possible roles in gill function at rest, and during exercise in the intact trout.

A spontaneously ventilating, blood-perfused whole trout preparation also is described in detail. This preparation overcomes many of the problems inherent in the in vitro, saline-perfused studies. This blood-perfused preparation is characterized and, in this section, its suitability for the study of the effects of haemodynamic changes on branchial and systemic vascular resistances to blood flow in fish, is assessed.
RESULTS II.

Effect of Pulsatility of Input on Resistance to flow and Fluid Flow Distribution In the Gills of Saline-Perfused Trout Heads

A description of the surgical procedures used for the preparation of the saline-perfused trout heads has been given in the Materials and Methods section on pages 34 to 35. A total of 24 fish were used, but only results from preparations which gave continuous and stable flow from both dorsal and anterior venous catheters throughout all experiments are presented. Two-thirds of these preparations failed to satisfy these criteria, leaving eight complete sets of data. Where time permitted, repeat measurements were made with pulsatile and/or constant pressure perfusion. Initial measurements were not significantly different from those taken later, for any of the perfusion regimes tested, despite the noticeable swelling (tissue oedema) in the preparations at the end of the 1.5 to 2 hours of experimentation.

Saline was bubbled with a gas mixture which simulated gas tensions in venous blood of intact and resting rainbow trout. Haswell et al. (1978) have reported that low oxygen tension in the saline prolongs preparation viability, and is qualitatively better for the perfusion of isolated fish head preparations.

Table 10 summarizes the values for flows and the estimates of other parameters obtained from the saline-perfused trout head preparations. These data indicate that only anterior venous outflow \( Q_{AV} \) was increased significantly during pulsatile perfusion, over values found with either constant pressure or constant flow perfusion. Other variables and parameters showed no significant differences among different regimes. Gill vascular resistance to flow \( R_g \) was calculated from the pressure difference between the dorsal and ventral aortae, divided by the rate of input perfusion \( Q_{VA} \). Ventral aortic pressure was taken as the measured input pressure minus the pressure drop across the catheter alone, at the same flow rate (see Wood and Shelton,
Table 10. Summary of dorsal aortic and anterior venous outflow from the isolated trout head (*Salmo gairdneri*) preparation during perfusion by three different input regimes. N represents number of observations.

<table>
<thead>
<tr>
<th>Perfusion Regime</th>
<th>Constant pulsatile flow (1)</th>
<th>Constant Flow (2)</th>
<th>Constant non-pulsatile Flow (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. = 16</td>
<td></td>
<td>N = 12</td>
<td>N = 8</td>
</tr>
</tbody>
</table>

Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Perfusate</th>
<th>Flow Rate</th>
<th>Resistance</th>
<th>Saline Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q_{DA}</td>
<td>(ml min^{-1})</td>
<td>1.54 ± 0.13</td>
<td>1.43 ± 0.22</td>
<td>1.34 ± 0.18</td>
</tr>
<tr>
<td>Q_{AV}</td>
<td>(ml min^{-1})</td>
<td>1.39 ± 0.09</td>
<td>1.09 ± 0.11</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>R_{g}</td>
<td>(Cm H_{2}O ml^{-1} min^{-1} 100g^{-1})</td>
<td>14.41 ± 1.36</td>
<td>18.11 ± 2.85*</td>
<td>23.53 ± 5.22</td>
</tr>
<tr>
<td>R_{AV}</td>
<td>(Cm H_{2}O ml^{-1} min^{-1} 100g^{-1})</td>
<td>11.67 ± 0.98</td>
<td>14.97 ± 1.83</td>
<td>17.83 ± 2.60</td>
</tr>
<tr>
<td>Q_{VA}</td>
<td>(ml min^{-1})</td>
<td>4.79 ± 0.19</td>
<td>4.41 ± 0.29</td>
<td>4.62 ± 0.13</td>
</tr>
<tr>
<td>% Saline loss</td>
<td></td>
<td>38.71 ± 3.08</td>
<td>42.26 ± 3.75</td>
<td>48.86 ± 4.33</td>
</tr>
</tbody>
</table>

Underlined value is the only one which was significantly different due to changes in the perfusion regime used. *note: R_{g} (Wood, 1974a) at same dorsal aortic pressure, and flow rate was 18.8 Cm H_{2}O ml^{-1} min^{-1} 100g^{-1}.*
Resistance of the anterior venous circulation was calculated from the pressure difference between the dorsal aorta and the anterior venous catheter (set to zero pressure), divided by $Q_{AV}$. Resistance values were expressed in units of cm H$_2$O•ml$^{-1}$•min$^{-1}$•100g$^{-1}$. These values were not different among the perfusion regimes. Percent of perfusate lost from each preparation was determined and was found to remain constant at approximately 43% of the inflow. Percent loss was not significantly different among the perfusion regimes tested. A dye (patent Blue; α-Zurine, Sigma) was introduced into the saline to check how evenly the gills were perfused, and to assess the degree of vascular leakage. Visual inspection of the gills during dye infusion showed even colouration during all perfusion regimes. The tips of gill filaments were the last areas to colour with dye. Occasionally, small leaks were observed from the gills, often where mechanical damage was evident. However, most of the saline loss seen during dye infusion was from the hypobranchial area, which was cut during catheterization of the heart.

**Studies of Gill Blood Flow in a Spontaneously Ventilating, Blood-Perfused Trout Preparation: Characterization of the preparation.**

Description of the surgical procedures used during the preparation of blood-perfused trout have been given in the Materials and Methods section, pages 45 to 49. Blood used for perfusion was taken from donor fish which had recovered from the effects of cannulation of their dorsal aortae for at least 14 hours. This was done in order that the blood which was used to perfuse the recipient fish was free of anaesthetic. Donor fish also did not appear to be agitated during the withdrawal of their blood. This avoided possible increases in the levels of circulating catecholamines, known to be released into the blood during stress in fish (see Nakano and Tomlinson, 1967).

Surgically prepared fish started to ventilate spontaneously within 20 - 30 minutes of the commencement of blood perfusion. Once ventilation
became regular, the mouth tube which assisted water flow over the gills was removed and the 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 eliminated, in part, by masking the holding box with black plastic sheets.

Typically, experiments lasted 4 - 6 hours, which, when combined with 2 - 3 hours for recovery from operative procedures, meant that measurements were taken from fish which were perfused for no more than 9 hours. 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 rates, as well as carbon dioxide excretion rates across the gills.

Some preparations were maintained for up to 18 hours 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. 12. Ventilatory interaction
Figure 12. Records of pressures and flow from spontaneously ventilating blood-perfused trout. A. Simultaneous records of flow and pressure from a preparation which displayed cardiorespiratory interactions. Arrow heads show interactions of respiratory movements on the pressure and flow traces. \( Q \), perfusion flow rate; VAP, ventral aortic (input) pressure; DAP, dorsal aortic pressure; buccal pressure record of ventilatory movements; ventricular pressure record of intrinsic heart activity. 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) and after 180 seconds exposure, and the 'off' response (post-hypoxic tachycardia) after resumption of normoxic water flow. Note that the pressure developed by the ventricle increased during hypoxic exposure. This pressure was not calibrated.
on the input pressure trace were observed frequently, especially during a respiratory "cough" (Hughes and Ardeny, 1977) (see Fig. 12A). 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 and Shelton, 1980a), and was evident in the dorsal aortic pressure trace obtained in the present study. Figure 12B shows a portion of input trace from a fish in which the pulse pressure has been raised by adjusting the size of the air space in the Windkessel (see Fig. 9).

Bradycardia is associated with exposure of trout to hypoxic water, and is one of the better described cardiovascular reflexes in fish (see Daxboeck and Holeton, 1978; Smith and Jones, 1978). Blood-perfused trout preparations also showed typical hypoxic bradycardia responses when nitrogen gas was bubbled through the inspired water to lower the oxygen tension (Fig. 12C). Both the "on" response (bradycardia) and the "off" response (post-hypoxic tachycardia) were observed. These results indicated that blood-perfused trout had operational afferent and efferent reflex pathways available, by which cardiovascular adjustments may be made.

Variables which were measured routinely from undisturbed and resting, blood-perfused trout preparations are summarized in Tables 11 and 12. These data were considered to be "normal" for this preparation, as defined by the criteria set out earlier in the Materials and Methods Section of this thesis. Under these conditions, all fish maintained consistent oxygen uptake ($M_{\text{g}O_{2}}$) and carbon dioxide excretion rates ($M_{\text{g}CO_{2}}$) across the gills. Across the systemic circulation, oxygen was extracted ($M_{\text{s}O_{2}}$), and carbon dioxide produced by the metabolizing tissues ($M_{\text{s}CO_{2}}$). Further treatment of the data concerning gas transfer in these preparations is presented in Section III in detail, and will not be discussed in this section.
Table 11. 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).

<table>
<thead>
<tr>
<th>Pressure (cm H₂O)</th>
<th>Cardiac pump</th>
<th>Q</th>
<th>Hct Acid/Base</th>
<th>P₀₂</th>
<th>CO₂</th>
<th>P₀ Cô₂</th>
<th>C₀ Cô₂</th>
<th>Plasma Osmolarity</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (cm H₂O)</td>
<td>mean* pulse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INPUT (VENOUS) BLOOD</td>
<td>58.8 ± 2.0</td>
<td>40</td>
<td>0.125</td>
<td>1.169</td>
<td>10.3</td>
<td>17.66 (7.76)</td>
<td>24.9</td>
<td>0.90</td>
<td>3.36</td>
</tr>
<tr>
<td>DORSAL AORTIC BLOOD</td>
<td>34.8 ± 0.95</td>
<td>2.05</td>
<td>0</td>
<td>0.003</td>
<td>0.025</td>
<td>0.2</td>
<td>0.40</td>
<td>1.1</td>
<td>0.05</td>
</tr>
<tr>
<td>VENOUS RETURN BLOOD</td>
<td>69.4 ± 0.96</td>
<td>48.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
<td>0.83</td>
<td>0.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Mean pressures have been calculated as (1 systolic + 2 diastolic)/3 (Burton, 1972).
Table 12. Summary of the differences in variables across the gill (Δinput - dorsal aorta) and the systemic (Δdorsal aorta - venous return) circulations in the resting state of spontaneously ventilating, blood-perfused rainbow trout at 7°C (N = 15 fish; 306.2 ± 9.3 g).

### ΔINPUT - DORSAL AORTA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>S.E.M.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>H+</td>
<td>+2.06</td>
<td>0.61</td>
<td>44</td>
</tr>
<tr>
<td>P0</td>
<td>+13.6</td>
<td>3.4</td>
<td>44</td>
</tr>
<tr>
<td>P0</td>
<td>+78.7</td>
<td>2.4</td>
<td>44</td>
</tr>
<tr>
<td>CO2</td>
<td>+347</td>
<td>20.8</td>
<td>44</td>
</tr>
<tr>
<td>H2O</td>
<td>+0.69</td>
<td>0.05</td>
<td>44</td>
</tr>
<tr>
<td>PO2</td>
<td>+2.32</td>
<td>0.12</td>
<td>44</td>
</tr>
<tr>
<td>PCO2</td>
<td>-1.25</td>
<td>3.36</td>
<td>44</td>
</tr>
<tr>
<td>CO2</td>
<td>-11.93</td>
<td>0.09</td>
<td>44</td>
</tr>
<tr>
<td>Sw</td>
<td>23.1</td>
<td>77</td>
<td>44</td>
</tr>
<tr>
<td>G</td>
<td>14.23</td>
<td>1.14</td>
<td>44</td>
</tr>
<tr>
<td>Pr</td>
<td>1.17</td>
<td>0.08</td>
<td>44</td>
</tr>
<tr>
<td>S</td>
<td>2.05</td>
<td>0.15</td>
<td>44</td>
</tr>
<tr>
<td>RQ</td>
<td>1.85</td>
<td>0.12</td>
<td>44</td>
</tr>
</tbody>
</table>

### ΔDORSAL AORTA - VENOUS RETURN

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>S.E.M.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔP</td>
<td>-1.36</td>
<td>0.64</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>-6.36</td>
<td>3.1</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>-89.3</td>
<td>2.2</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>-87.4</td>
<td>-0.55</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>-1.20</td>
<td>0.06</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>-74.6</td>
<td>1.67</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>+0.07</td>
<td>0.14</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>+4.27</td>
<td>0.06</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>+1.01</td>
<td>0.83</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>+11.66</td>
<td>0.95</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>31.1</td>
<td>0.88</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>19.21</td>
<td>0.11</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>1.97</td>
<td>0.09</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>1.63</td>
<td>0.09</td>
<td>41</td>
</tr>
<tr>
<td>S</td>
<td>0.83</td>
<td>0.09</td>
<td>41</td>
</tr>
<tr>
<td>RQ</td>
<td>0.83</td>
<td>0.09</td>
<td>41</td>
</tr>
</tbody>
</table>

n = Number of observations
The data in Table 11 also indicate that blood was diluted in its passage through the gills of blood-perfused trout, as haematocrit fell by 24%, and plasma osmolarity decreased by 3.5%. These changes could be caused by a number of factors, including cell volume changes associated with CO₂ excretion, water uptake across the gill epithelium, or simply as an artifact of the sampling procedure.

Branchial vascular resistance (R₀) comprised 43% of the total vascular resistance to flow in these preparations (Table 12). Data presented in Tables 11 and 12, taken from spontaneously ventilating, blood-perfused whole trout preparations, are comparable to those available from intact and resting rainbow trout (see Jones and Randall, 1978). The previously described preparation therefore was used to assess the effects of specific changes in cardiovascular parameters which are known to occur during exercise in vivo, on gill and systemic vascular resistances, but under normoxic, resting conditions.

**Cardiac Output (Q) Changes**

Cardiac output increases in intact trout during swimming. Table 13 summarizes the effects of changes in the perfusion flow rate (Q) on the measured vascular variables in spontaneously ventilating, blood-perfused trout at rest. Input pressure (VAP), and dorsal aortic pressure (DAP) increased above normal values by small, but non-significant amounts, with increasing Q. As a result, with increased flow, both the calculated gill resistance (R₀), and the systemic resistance (Rₛ) to flow were decreased from values calculated under normal conditions. Conversely, these resistances increased as the rate of perfusion was decreased below normal, and both VAP and DAP were decreased from normal values and decreasing Q.

**Cardiac Pump Frequency (f) and Stroke Volume (SV) Manipulations**

Simulated exercise tachycardia, but with no increase in Q, had no
Table 13. Summary of the effects of increased and decreased cardiac output on cardiovascular variables from normal, spontaneously ventilating, blood-perfused trout (N = 7; 292.7 ± 14.3 g).

<table>
<thead>
<tr>
<th></th>
<th>VAP (cm H₂O)</th>
<th>DAP (cm H₂O)</th>
<th>Q  (ml·min⁻¹)</th>
<th>Hct (input) %</th>
<th>Hct (DA) %</th>
<th>R₉ mg·cm⁻¹·min⁻¹·100g⁻¹</th>
<th>Rₛ cm H₂O·ml⁻¹·min⁻¹·100g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW Q</td>
<td>X 42.9 7.8</td>
<td>29.4 1.4</td>
<td>2.76</td>
<td>9.34</td>
<td>7.8</td>
<td>14.3</td>
<td>26.9</td>
</tr>
<tr>
<td></td>
<td>± S.E.M. 3.5</td>
<td>0.5</td>
<td>0.24</td>
<td>0.5</td>
<td>0.5</td>
<td>2.9</td>
<td>2.0</td>
</tr>
<tr>
<td>NORMAL Q</td>
<td>X 54.1 11.1</td>
<td>34.4 2.0</td>
<td>4.80</td>
<td>9.6</td>
<td>8.5</td>
<td>12.1</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>± S.E.M. 5.1</td>
<td>0.3</td>
<td>0.20</td>
<td>0.4</td>
<td>0.5</td>
<td>2.8</td>
<td>2.9</td>
</tr>
<tr>
<td>HIGH Q</td>
<td>X 56.5 15.8</td>
<td>36.3 2.5</td>
<td>7.20</td>
<td>9.6</td>
<td>8.5</td>
<td>8.20</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>± S.E.M. 4.2</td>
<td>0.7</td>
<td>0.40</td>
<td>0.5</td>
<td>0.6</td>
<td>1.40</td>
<td>0.7</td>
</tr>
</tbody>
</table>
effect upon any of the measured variables, except that the ventral and
dorsal aortic pulse pressures were decreased. However, unlike most intact
tROUT which have been exercised (see Jones and Randall, 1978), my fish did
not show an increase in heart rate (Table 8).

Simulated bradycardia in normoxic water however, significantly
decreased gill resistance (Table 14), despite a slight decrease in 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 and tachycardia, at constant perfusion
flow rates. All other variables measured changed very little from normal
values.

**Pulse Pressure (PP) Changes**

Increased input pulse pressure, as occurs 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 12. Although ventral aortic pulse pressure was increased
above normal, the amplitude of the dorsal aortic pulse pressure did not
increase by the same amount, indicative of pressure damping within the
gill vasculature. Decreases in ventral aortic pulse pressure (3 fish only)
also produced no significant changes in any of the variables measured,
although an increase in the gill resistance was observed (Table 14).

**Haematocrit (Hct) Changes**

Regardless of whether high or low haematocrit blood was perfused
through the gills of blood-perfused trout, VAP was increased to the same
level above the normal value. Conversely, DAP in both instances fell to
similar levels below that found in the normal situation (Table 15). Since
the pressure differential across the gills was increased in both cases, with
no accompanying changes in Q, the calculated gill resistance was increased.
Table 14. Effects of cardiac output (Q), stroke volume (SV), pulse pressure (PP) and cardiac frequency (f) on gill resistance change (ΔRg%), in resting, blood-perfused trout (0 = no change from normal; + = increase from normal; - = decrease from normal). Means ±S.E.M.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>N</th>
<th>ΔRg% (from normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) SV +; PP +; f 0; Q +</td>
<td>7</td>
<td>-22.91 ± 9.09</td>
</tr>
<tr>
<td>(2) SV +; PP +; f -; Q 0</td>
<td>8</td>
<td>-25.22 ± 8.98</td>
</tr>
<tr>
<td>(bradycardia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) SV -; PP -; f 0; Q -</td>
<td>7</td>
<td>-35.38 ± 17.96</td>
</tr>
<tr>
<td>(4) SV -; PP -; f +; Q 0</td>
<td>8</td>
<td>+ 4.48 ± 18.63</td>
</tr>
<tr>
<td>(tachycardia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5) SV 0; PP +; f 0; Q 0</td>
<td>8</td>
<td>+15.37 ± 11.16</td>
</tr>
<tr>
<td>(6) SV 0; PP -; f 0; Q 0</td>
<td>3</td>
<td>+14.99 ± 15.89</td>
</tr>
</tbody>
</table>
Table 15. Summary of the effects of variable input haematocrit on cardiovascular parameters from normal spontaneously ventilating, blood-perfused trout (N = 6 fish; 320.2 ± 17.2 g and constant Q = 5.20 ± 0.04 ml.min⁻¹).

<table>
<thead>
<tr>
<th></th>
<th>VAP (cm H₂O)</th>
<th>DAP (cm H₂O)</th>
<th>Hct (input)</th>
<th>Hct (DA)</th>
<th>Rg (cm H₂O.ml⁻¹.min⁻¹.100g⁻¹)</th>
<th>Rs (cm H₂O.ml⁻¹.min⁻¹.100g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL Hct</td>
<td>X 60.6 10.8</td>
<td>40.2 2.2</td>
<td>11.3</td>
<td>9.3</td>
<td>12.6</td>
<td>22.3</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>1.7 0.5</td>
<td>2.6 0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>1.5</td>
<td>1.8</td>
</tr>
<tr>
<td>HIGH Hct</td>
<td>X 76.2 10.7</td>
<td>28.3 1.4</td>
<td>4.3</td>
<td>3.9</td>
<td>29.6</td>
<td>15.1</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>7.7 0.7</td>
<td>1.9 0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>4.9</td>
<td>1.4</td>
</tr>
<tr>
<td>LOW Hct</td>
<td>X 76.1 11.8</td>
<td>33.8 1.7</td>
<td>20.2</td>
<td>16.5</td>
<td>26.1</td>
<td>18.4</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>6.9 0.9</td>
<td>3.1 0.2</td>
<td>1.6</td>
<td>1.2</td>
<td>4.3</td>
<td>1.7</td>
</tr>
</tbody>
</table>
As a consequence of the decreased DAP, systemic resistance was affected, being lower than normal levels. No other vascular variables were affected by changes in Hct. All data presented in Table 15 are comparable to normal values obtained from similar preparations (see Table 11).

**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 $1 \times 10^{-6}$ M on vascular variables in blood-perfused preparations are summarized in Table 16. This concentration may have been slightly higher than *in vivo*. Preparations showed significant ($P<0.10$) decreases 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 ($P<0.10$) by 56% above normal in this situation.

The fall in plasma osmolarity across the gills was twice as large as observed in normal blood-perfused preparations. This was equivalent to and approximate 2-fold increase in the calculated net water influx across the gills during adrenaline exposure. This result was consistent with changes observed in isolated, saline-perfused trout heads exposed to an equivalent level of adrenaline (Isaia, Maetz and Haywood, 1978).
Table 16. Effects of $1 \times 10^{-6}$ M adrenaline on selected variables in blood-perfused trout preparations ($N = 4$ fish).

<table>
<thead>
<tr>
<th></th>
<th>VAP (cm H$_2$O)</th>
<th>DAP (cm H$_2$O)</th>
<th>$R_g$ (cm H$_2$O ml$^{-1}$ min$^{-1}$ 100g$^{-1}$)</th>
<th>$R_s$ (cm H$_2$O ml$^{-1}$ min$^{-1}$ 100g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NORMAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{X}$</td>
<td>40.4</td>
<td>30.5</td>
<td>6.48</td>
<td>16.38</td>
</tr>
<tr>
<td>$\pm$ S.E.M.</td>
<td>5.4</td>
<td>5.3</td>
<td>0.82</td>
<td>3.35</td>
</tr>
<tr>
<td><strong>ADRENALINE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{X}$</td>
<td>51.3</td>
<td>44.6</td>
<td>4.01</td>
<td>25.06</td>
</tr>
<tr>
<td>$\pm$ S.E.M.</td>
<td>4.7</td>
<td>3.1</td>
<td>1.02</td>
<td>1.97</td>
</tr>
</tbody>
</table>
DISCUSSION II: The Effects of Haemodynamic Alterations on Flow Distribution and Branchial Vascular Resistance to Flow in Saline-Perfused Trout Head Preparations

Saline-perfused head preparations were perfused at flow rates similar to those used for blood-perfused trout, with a dorsal aortic pressure maintained at 40 cm•H₂O. These values were chosen to mimic those found in intact, unrestrained rainbow trout at rest (Stevens and Randall, 1967a; Kiceniuk and Jones, 1977).

Anterior venous flow (Qₐᵥ) from saline-perfused trout heads was greater during pulsatile perfusion than during either of the non-pulsatile perfusion regimes. None of the other variables were altered significantly by perfusion with different regimes. The anterior venous circulation is comprised of head vessels, as well as gill venolymphatic vessels. Pulsatility in high pressure gill arteries may have been transmitted to the surrounding low pressure venolymphatic system. This mechanical interaction therefore may be important in generating the observed increase in Qₐᵥ during pulsatile perfusion of the gills. Pulsatile pressures in venolymphatic vessels found in vivo in the lingcod, Ophiodon elongatus (Farrell, 1979) support the notion of transmission of pulse pressure from arteries to venolymphatic vessels. This is a close proximity of vessels to facilitate mechanical interactions, since the venolymphatic parallel the filamental arteries, and often surround the lamellar arteries in doughnut-like structures (personal observations from polymer casts of gills of Amia calva; see also Daxboeck et al., 1981).

These regular pressure pulsations in the extensively valved venolymphatic vessels (Gannon, unpublished observations) will augment lymphatic space drainage. Thus, the pulsatility, rather than the absolute pressure, causes the increases in Qₐᵥ measured in the saline-perfused trout head, by increasing venolymphatic flow, primarily from the gills.
Exercise in fish is a natural condition with which dramatic increases in blood pulse pressure are associated (Kiceniuk and Jones, 1977). During exercise, rainbow trout show increases in ventral, and to a lesser extent, dorsal aortic pressures (Stevens and Randall, 1967a; Kiceniuk and Jones, 1977) as well. The question is: What role could increased venolymphatic flow play in gill function, when the respiratory gas exchange demands placed on the gills are higher than normal? The nutritive function of these vessels becoming more important as more of the gill tissue becomes involved in gas transfer, because of lamellar recruitment (Farrell et al., 1979), is one possible explanation. The other contribution to venolymphatic flow is lymph itself. It is possible that pulsatile flow and/or pressure increases lymph formation and its clearance from interstitial spaces, into the lymphatic vessels, thereby reducing gill tissue oedema during exercise in vivo. Other consequences of these flow changes brought about by increased pulsatility on gas transfer across the gills of rainbow trout at rest, and during swimming exercise will be explored in Section III of this thesis.

The vascular resistance to flow through trout gills, in vivo, is lower than is found from in vitro measurements in the present preparations, regardless of whether the gills are perfused with pulsatile or non-pulsatile flow. $R_g$ values from isolated, saline-perfused preparations are higher than those measured in vivo principally as a result of elevated trans-gill pressure differentials, rather than low flow rates. Wood (1974a) has shown that elevation of dorsal aortic pressure from 0 to 40 cm H₂O in isolated trout gill preparations can reduce $R_g$ by as much as 50%, at flow rates of approximately 17 ml·min⁻¹·kg⁻¹. Saline perfusion "haemodynamics" therefore, do not mimic exactly the in vivo situation. However, data obtained from these isolated preparations still are useful since they describe qualitatively, possible blood and lymphatic flow distribution patterns, and some underlying
mechanisms responsible for these, during periods of increased input pressure pulsatility to the gills, as during exercise, \textit{in vivo}. In addition, if the purpose of perfused gill preparations is to mimic the \textit{in vivo} condition, then from the present results, pulsatile constant flow is considered the most appropriate method, and a physiological dorsal aortic pressure must be maintained in order to obtain representative data.

The Spontaneously Ventilating, Blood-Perfused Whole Trout Preparation:

\begin{itemize}
  \item[a)] \textbf{How representative is it of \textit{In vivo} conditions?}
\end{itemize}

Spontaneously ventilating, blood-perfused trout show cardiovascular dynamics which are essentially identical to those of intact fish. Similarly gas exchange at the gills and tissues is very similar to data available from \textit{in vivo} measurements, despite the fact that the haematocrit of blood used for perfusion in these preparations is lower than normally found in intact fish. Blood was diluted with saline so that fewer fish need to be sacrificed for each preparation. Trout have been shown to be quite capable of surviving with haematocrits as low as 3%, and values of 15% are not uncommon for normal trout \textit{in vivo} (See Wood and Shelton, 1980a). An Hct of 10 - 12%, as is used in my preparations, can be considered as a reasonable compromise between economy and simulation of \textit{in vivo} conditions.

Blood-perfused fish behave in a manner similar to intact animals. They are capable of maintaining equilibrium in the water, of visual tracking, of swimming motions, and show a typical bradycardia response when exposed to aquatic hypoxia. Occasionally, when bleeding is observed from sutures which close the incision and anchor catheters to the body wall, these small leaks tend to clot with time, while larger leaks usually are indicative of a dislodged catheter. Cannulation of the dorsal aorta 24 hours before experimentation eliminates leakage from around this point of insertion. In no successful preparation is leakage so large as to require addition of more
blood to the tonometers.

The levels of the anaesthetic, MS-222, remaining in the body of fish perfused with blood clearly were too low to maintain anaesthesia, although some unmeasured effects of anaesthesia on the fish still may have persisted after only 2 - 3 hours of recovery from surgery in my experiments (Houston et al., 1971). Had any anaesthetic been present in these tissues, and subsequently reappeared in the blood, then it could still be eliminated rapidly from the blood, across the gills into the water (see Daxboeck and Holeton, 1980), and its effects on the preparation would have been insignificant.

There is a large number of publications which report some of the variables in Tables 12 and 13, while few workers have measured a significant number of these variables simultaneously. For comparative purposes, only those sets of data in which as many of the above mentioned variables have been measured simultaneously, were chosen for the following discussion of individual variables from blood-perfused trout.

**Flow Rate - \( Q \)**

Mean perfusion rate was 16.2 ml·min\(^{-1} \cdot \text{kg}^{-1}\) in the blood-perfused trout preparations. Cardiac output (\( Q \)), measured directly in other fish species, range between 6 and 21 ml·min\(^{-1} \cdot \text{kg}^{-1}\) (Ophiodon elongatus 6.0, Stevens et al., 1972; Gadus morhua 20.8 ml·min\(^{-1}\), Jones et al., 1974). From these limited data for direct measurements of cardiac outputs from other resting, intact fish, it is apparent that \( Q \) for the blood-perfused fish approximate in vivo values.

Cardiac output also has been measured directly in resting rainbow trout by Wood and Shelton (1980a). These authors report a value of 36.7 ml·min\(^{-1} \cdot \text{kg}^{-1}\), whereas cardiac output of resting rainbow trout, estimated by the Fick principle are 21.6 ml·min\(^{-1} \cdot \text{kg}^{-1}\) (Stevens and Randall, 1967b) and 17.6 ml·min\(^{-1} \cdot \text{kg}^{-1}\) (Kiceniuk and Jones, 1977). Thus, mean resting cardiac
output reported by Wood and Shelton (1980a) is at least twice that of estimates using the Fick principle, despite the fact that this method for the calculation of cardiac output is known to give overestimates of the actual flow (Wood et al., 1978; see also Section III).

Stroke volumes calculated from all available in vivo data for trout are similar (approximately 0.46 ml·kg⁻¹). Consequently, the above differences in Q from rainbow trout appear to arise from heart rate differences. The 5°C temperature difference between the studies of Wood and Shelton (1980a), and those of Kiceniuk and Jones (1977) and Stevens and Randall (1976b) is insufficient to account for the high heart rates recorded by Wood and Shelton.

**Ventral Aortic (input) Pressure - VAP**

Mean VAP in normal blood-perfused fish was 58.8 cm H₂O. Other species in which resting ventral aortic pressures have been measured (Gadus morhua 58 cm H₂O, Helgason and Nilsson, 1973; Ophiodon elongatus 59.2 cm H₂O, Stevens et al., 1972) give values comparable to those measured in blood-perfused trout. However, this value appears to be slightly high for measurements from intact rainbow trout (47.1 cm H₂O, Stevens and Randall, 1967b; 52.7 cm H₂O, Kiceniuk and Jones, 1977; 42.5 cm H₂O, Wood and Shelton, 1980a). This possibly is indicative of input flow, or gill vascular resistance to flow being slightly higher in blood-perfused trout, than found in intact ones. However, ventral aortic pressures in blood-perfused fish still fall within a range of values obtained from in vivo studies.

Venous pressure was not measured in blood-perfused fish, but was assumed to be 4 cm H₂O (Wood and Shelton, 1980a) for the purpose of systemic vascular resistance calculations.
Dorsal Aortic Pressure - DAP

Q initially was adjusted to achieve a DAP of 40 cm H$_2$O, because fish, like most other vertebrates, probably regulate pressure by altering Q and vascular resistance (Walqvist and Nilsson, 1977; 1980; Smith, 1979; Wood and Shelton, 1980b). As DAP records from trout are relatively common, and the dorsal aorta lies between the two major vascular resistance sites, I chose to set other cardiovascular parameters around this point. Once Q was set, DAP fell during the life of a preparation, to give a mean DAP of 34.8 ± 1.0 cm H$_2$O. However, DAP's measured in resting intact trout range from 34 to 42 cm H$_2$O (Stevens and Randall, 1967a,b; Kiceniuk and Jones, 1977; Wood and Shelton, 1980a; Table 9, Section I) and other species in which DAP's have been measured in vivo have given similar values (see Stevens et al., 1972; Helgason and Nilsson, 1973; Davie and Forster, 1979). Mean DAP in blood-perfused fish compares well with published in vivo data.
Branchial Vascular Resistance to Flow - $R_g$

The pressure drop across the gills divided by the flow rate gives a numerical value for the vascular resistance of the gill vessels to fluid flow through them. Mean $R_g$ of blood-perfused fish gills is 14.2 cm H$_2$O·ml$^{-1}$·min·100g$^{-1}$. In vivo $R_g$ values however, are typically around 6 cm H$_2$O·ml$^{-1}$·min·100g$^{-1}$ (Stevens and Randall, 1967b, 3.4; Kiceniuk and Jones, 1977, 6.02; and Wood and Shelton, 1980a, 3.4 cm H$_2$O·ml$^{-1}$·min·100g$^{-1}$). The differences arise from higher gill resistance values found in blood perfused fish, or overestimates of $Q$ in the other in vivo studies. When blood-perfusion preparations were exposed to 1 x $10^{-6}$ M adrenaline, there was a significant decrease in branchial vascular resistance, and a significant rise in systemic vascular resistance to flow (see Table 18). In vivo measurements may have been taken from fish in which there was little vascular tone. Pharmacological experiments designed to elicit falls in $R_g$ in intact fish (eg. injection of isoprenaline, a specific β-adrenergic agonist, Wood and Shelton, 1980a) showed only small effects, indicating low vascular tone in the gills of these animals. Unfortunately these authors did not measure both $Q$ and VAP in the same animals, which make their values more difficult to compare to the ones from the present study.

In vivo measurements of $R_g$ generally have been taken from fish of greater weight than those used for blood perfusion in the present experiments. Wood and Shelton (1975) and Payan and Matty (1975) have shown inverse relationships between weight and vascular resistance in isolated saline-perfused preparations. The larger size of fish used for in vivo measurements may partly explain why these calculated $R_g$ values are lower than those from blood-perfused fish. Over the range of $Q$, VAP and DAP of normal blood-perfused preparations, $R_g$ was significantly correlated only with VAP. Regression of $R_g$ against VAP showed a highly significant linear relationship ($p < 0.001$) as follows:
Systemic Vascular Resistance to Flow - $R_s$

Mean systemic vascular resistance to flow in blood-perfused fish is $19.2 \text{ cm H}_2\text{O} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$. This value is similar to those measured by Stevens and Randall (1967b) of 16.6, and Kiceniuk and Jones (1977) of $22.8 \text{ cm H}_2\text{O} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$. The value of $R_s$ from Wood and Shelton (1980a) of $7.1 \text{ cm H}_2\text{O} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ however, is lower than is found in the blood-perfused preparations. The latter value is low principally as a result of smaller pressure differentials across the body, rather than their higher measured cardiac output. Suffice to say the $R_s$ calculated for blood-perfused preparations is similar to those measured in vivo. In general systemic vascular resistances measured in vivo, and in vitro from either blood-perfused or saline-perfused preparations are in good agreement; better than seen in the comparisons of the gill resistance values from these types of preparations.

Stress

Blood-perfused fish undoubtedly are subject to a great deal of stress. Acute stress evokes a number of responses (see Mazeaud et al., 1977), many of which are mediated through the release of catecholamines into the blood. However the large total blood volume, taken from resting fish which had recovered from the stress of the cannulation procedure for at least 14 hours ensured that low levels of circulating catecholamines would be present in this blood. The frequent switching from one tonometer to another meant that, to elevate blood catecholamine levels by 15 times over resting levels, as seen during stress in some fish (Nakano and Tomlinson, 1967; Mazeaud et al., 1977), a large amount of catecholamines would have had to be released into the blood by the experimental animal in the present study. Discarding the blood of operated animals, and saline-perfusion prior to the commencement of blood-
perfusion meant that initial concentrations of endogenous circulating catecholamines were small in experimental animals.

If the levels of catecholamines in the blood however, were elevated above a "normal" concentration then one could expect a number of responses in the cardiovascular system of the blood-perfused trout preparation. Catecholamines are known to cause systemic vasoconstriction in trout. Systemic resistance to flow however, decreased rather than increased during the life of any individual preparation. Heart rate and ventilatory responses to elevated levels of circulating catecholamines also were not apparent. Preparations were able to respond to physiological doses of administered adrenaline in a normal manner (i.e. branchial vascular resistance decrease; systemic vascular resistance to flow increases), indicating that blood levels of catecholamines were not elevated unduly. It was concluded that stress responses in blood-perfused preparations were diminished because of the experimental protocol employed, and probably did not affect the results significantly.

The cardiovascular system of blood-perfused trout therefore exhibit pressures, flows and vascular resistances intermediate between those in vivo and isolated, saline-perfused studies. Large differences between data sets usually are explicable in terms of the particular experimental techniques. Where techniques were the same, comparisons reveal good agreement between data which I have presented, and those of other studies. The spontaneously ventilating, blood-perfused trout preparation is deemed suitable for the examination of the effects of cardiovascular haemodynamics on a variety of cardiorespiratory problems, as well as for the study of gas transfer under a number of different physiological conditions. These investigations are made less complicated than in intact fish, since cardiac output (Q), and venous blood gas tensions, and its chemical makeup
are experimental parameters which can be controlled and manipulated, rather than being measured or estimated variables.

b) **Haemodynamic Alterations in Blood-Perfused Trout Preparations: Effects on vascular resistance to flow**

The six treatments involving manipulations of perfusion frequency/stroke volume/pulse pressure, and flow rate (Q), and their effects on gill resistance (Rg) to flow in blood-perfused preparations have been presented in Table 16. As had been noted during exercise in intact trout from my experiments, increased blood flow (i.e. cardiac output), should it in fact be increased above resting values, can be achieved by increasing only the stroke volume while the heart rate remains at a normal level (see Table 8). This was accompanied by an increase in pulse pressure. Simulation of this *in vivo* condition in the resting, blood-perfused trout preparation resulted in a significant decrease in Rg. Either the stroke volume increase, the pulse pressure increase, the increase in Q, or any combination of these factors could have caused the observed fall in Rg. When pulse pressure alone was increased however, while other parameters were kept constant, Rg increased only slightly. Thus, increased pulse pressure alone cannot explain the decrease in Rg. However, pulse pressure changes associated with stroke volume increases and cardiac pump frequency decreases, while flow rate was kept constant, were capable of causing much larger decreased in Rg. Thus, the decrease in gill vascular resistance to flow can be accounted for by large increases in stoke volume, or decreases in heart rate, but it appears more likely that this decrease in Rg was a result of increased stroke volume, and the attendant increase in pulse pressure.

Data from blood-perfusion trout preparations therefore indicate that the principle physiological effect of increased pulse pressure is to lower ventral aortic mean pressure below that found under normal perfusion conditions.
As long as a constant perfusion flow rate is maintained at normal levels, the gill vascular resistance to flow is decreased. And, although increased ventral aortic pulse pressure in itself has no effect on blood-perfused gills resistance to flow, decreased ventral aortic pulse results in large increases in $R_g$. The most probable cause of this resistance change when input pulse to the gills is decreased below normal, is the collapse of some vessels whose critical closing pressures ($15-25$ cm H$_2$O; Wood, 1974a: Farrell et al., 1979) are not exceeded by peak systolic pressure. Decreases in stroke volume and/or pulse pressure, or a decrease in the perfusion flow rate also result in an increase in $R_g$. These conditions tend also to decrease ventral aortic peak pressure, and in the absence of any concomitant changes in the stroke volume or flow rate from normal, a decrease in pulse pressure alone can result in an increase in $R_g$. Pulse pressure therefore may be important in maintaining normal gill vascular resistance to flow, by assisting extravascular fluid clearance from gill tissues, as pointed out in the saline-perfused trout heads, but appears to be of little importance by itself, in actually reducing $R_g$.

Conclusions which can be drawn from these experiments are tentative but do indicate that increases in stroke volume, which are known to accompany increases in cardiac output during exercise in vivo, can decrease gill vascular resistance to flow, while decreases in pulse pressure increase $R_g$, at least in blood-perfused preparations at rest. Decreased cardiac frequency (bradycardia), and increased flow rate also play a role in lowering gill resistance, although frequency changes in themselves are of little consequence. In situations where cardiac output increases above resting levels, as during exercise in intact trout, it may actually be advantageous to achieve this by stroke volume rather than just by frequency increases. If frequency alone were to be used to increase $Q$ during exercise, then gill resistance could be expected to rise, and the competence of the respiratory tissues could be
threatened by excessively high ventral aortic pressures, leading to gill tissue oedema.

As I have pointed out in Section I, systemic vasomotor tone in trout is under general $\alpha$-adrenergic vasoconstrictor control. Stress is known to increase the levels of circulating catecholamines in fish (Mazeaud et al., 1977), and perhaps this also occurs during exercise in vivo. The principle effects of increased levels of adrenaline in the blood of blood-perfused trout were upon vascular resistance and a rise in systemic vascular resistance to flow. The concentration of adrenaline used in these experiments was chosen to illustrate the differences in sensitivity of branchial and systemic vascular beds to this catecholamine, which is found in vivo in trout (Wood and Shelton, 1975; Wood, 1976). Blood-perfused fish showed a percentage fall in $R_g$ which was identical to that observed in perfused trout heads exposed to the same level of adrenaline (Wood, 1974a). The rise in systemic vascular resistance in the present preparation was only one-half that seen in isolated saline-perfused trout trunks (Wood and Shelton, 1975a). Blood-perfused fish probably had significant systemic vascular tone, and consequently some $\alpha$-constriction may have been masked by $\beta_2$-adrenoceptor vasodilation of the systemic circulation. The effects of adrenaline on vascular resistance in blood-perfused trout therefore, are precisely those which would have been predicted from previous studies.

It appears that the gills of isolated, saline-perfused trout are more sensitive to alterations in stroke volume and pulse pressure than to any other haemodynamic variable. The effects of these changes on fluid flow distribution within the branchial vasculature, as brought about by conditions which mimic those found in the cardiovascular system in vivo, and their effects on vascular resistances have been discussed. The relevance
of these findings to the function of the gills for gas exchange will be examined in the following section.
SECTION III
GAS TRANSFER ACROSS THE GILLS OF SALINE- AND BLOOD-PERFUSED PREPARATIONS OF RAINBOW TROUT
INTRODUCTION: SECTION III

In the previous section, I have provided data which show that haemodynamic alterations which mimic those found to occur during exercise in vivo, can have profound effects upon vascular resistances and fluid flow distributions within the branchial circulation of trout. That these changes also may have effects upon gas transfer across the gills is investigated in the following section. Studies of the amount of oxygen taken up by the blood itself in fish at rest, or during exercise however, are relatively few in number (Stevens and Randall, 1967b; Eddy et al., 1977; Kiceniuk and Jones, 1977). Studies involving intact fish are limited by the difficulty in obtaining appropriate simultaneous blood samples, and the low blood volumes of fish (Holmes and Donaldson, 1970). Low blood volumes necessarily limit the number of blood samples which can be withdrawn from a single animal. Moreover, cardiac outputs usually have been calculated from oxygen consumption by the whole fish, by the application of the Fick principle. Where cardiac output has been measured directly, blood gas data necessary for calculations of oxygen uptake and carbon dioxide excretion rates by the blood are lacking.

These technical difficulties have led investigators to use isolated, saline-perfused preparations in order to study gill function in fish (Wood, 1974a; Wood and Shelton, 1975; Payan and Matty, 1975; Wood et al., 1978). However, it has been shown that these saline-perfused preparations can be limited in their usefulness for studies of gas transfer in fish gills (see Wood et al., 1978). The spontaneously ventilating, blood-perfused whole trout preparation (see Section II) overcomes the problem of inadequate rates of gas transfer across the gills, a perturbing consequence of saline-perfused preparations, and enables the simultaneous measurements of all the variables necessary for the determination of blood, as well as ventilatory oxygen uptake to be made. The effects of haemodynamic alterations, which simulate
those found to occur during exercise in trout, *in vivo*, upon oxygen uptake across the gills of resting, blood-perfusing rainbow trout also are assessed.

Because cardiac output is set precisely by an external perfusion pump, the blood-perfused trout preparation also presents a unique opportunity to test the applicability of the Fick principle in predicting the cardiac output, given the measured oxygen uptake rate across the gills, and by the blood itself, as it passes through the gills. The consequences of the findings from these experiments are presented in some detail, as the Fick principle is used widely in cardiorespiratory physiology for estimating cardiac output and flows in various organs, with a minimum of surgical intervention. Precautions for its use, and recommendations for the interpretation of numerical values obtained with the use of the Fick principle are discussed. From the data collected from the above experiments, the contention that fish gills are diffusion limited for gas transfer is examined in detail.

During exercise *in vivo*, mean and pulse pressures in the gills of fish increase. In this section, the isolated, saline-perfused trout head preparation is used in order also to investigate the effects of the pulsatility of constant perfusion flow rate on the nature of fluid exchange across the branchial vasculature of fish, using an ethanol loading/tissue washout technique. In addition, this saline-perfused preparation is used to examine the effects of perfusion regime alteration (i.e. pulsatility of flow) upon gas transfer across the gills of these trout heads in normoxia. The data from these studies lead to discussions of the possible effects of fluid exchange and flow distribution patterns within gill tissues upon gas transfer efficiency in trout, *in vivo*, at rest and during swimming exercise.
RESULTS III: The Effects of Haemodynamic Alterations on Gas Exchange in the Blood-Perfused Trout Preparation

The effects of changes in cardiac output on the resting, blood-perfused trout preparation are summarized in Table 17. Description of the surgical procedures used for the preparation of these fish have been given in the Materials and Methods pages 45 to 49. The data indicated a significant positive correlation between the rate of perfusion and both oxygen uptake (\( \dot{M}_{O_2} \)), and carbon dioxide excretion (\( \dot{M}_{CO_2} \)) across the gills (Fig. 13). That is, \( \dot{M}_{O_2} \) as well as \( \dot{M}_{CO_2} \) increased as \( Q \) is raised. A linear relationship between cardiac output and oxygen uptake is represented by equation 3,

\[
(3) \quad \dot{M}_{O_2} (\mu M \cdot min^{-1} \cdot 100g^{-1}) = 0.63 \times Q (ml \cdot min^{-1} \cdot 100g^{-1}) - 0.16 \\
(p<0.01; n = 21).
\]

A comparable relationship also can be derived for carbon dioxide excretion, and is represented by equation 4,

\[
(4) \quad \dot{M}_{CO_2} (\mu M \cdot min^{-1} \cdot 100g^{-1}) = 1.043 \times Q (ml \cdot min^{-1} \cdot 100g^{-1}) - 0.13 \\
(p<0.01; n = 21).
\]

\( P_{aO_2} \) remained constant (106.0 ± 2.0 mm Hg; \( n = 21 \)) over the range of \( Q \) used in these experiments, indicating that blood oxygen saturation was maintained (Fig. 13). \( P_{aCO_2} \) also remained unchanged (3.43 ± 0.12 mm Hg; \( n = 21 \)) during changes in the perfusion flow rate through the gills of this preparation.

The rate of blood delivery to the systemic circulation also appeared to limit the oxygen consumption of the systemic tissues, since a constant 87% decrease in \( P_{aO_2} \), and a constant 76% of the oxygen content (\( C_{aO_2} \)) were extracted by these tissues, regardless of the rate of blood flow through them. Of course increased blood flow during exercise, in vivo, involves greater \( O_2 \) utilization by tissues, and \( O_2 \) extraction increases.

As the rate of perfusion was decreased, a longer equilibration time was afforded the blood in the tonometer, and this was reflected by increases
Table 17. Summary of the effects of increased and decreased cardiac output on blood gases from normal spontaneously ventilating, blood-perfused trout (N = 7 fish; 292.7 ± 14.3 g).

<table>
<thead>
<tr>
<th></th>
<th>( \dot{Q} )</th>
<th>Hct</th>
<th>( P_{inO_2} )</th>
<th>( C_{inO_2} )</th>
<th>Hct</th>
<th>( P_{aO_2} )</th>
<th>( C_{aO_2} )</th>
<th>RQ (_g)</th>
<th>RQ (_s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/min(^{-1})</td>
<td>%</td>
<td>mm Hg</td>
<td>mM</td>
<td>%</td>
<td>mm Hg</td>
<td>mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NORMA ( L) Q</strong></td>
<td>4.80</td>
<td>9.6</td>
<td>28.7</td>
<td>0.761</td>
<td>8.5</td>
<td>103.6</td>
<td>1.34</td>
<td>1.65</td>
<td>1.06</td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>0.20</td>
<td>0.4</td>
<td>1.9</td>
<td>0.052</td>
<td>0.5</td>
<td>4.7</td>
<td>0.09</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>HIGH Q</strong></td>
<td>7.20</td>
<td>9.6</td>
<td>21.6</td>
<td>0.740</td>
<td>8.5</td>
<td>109.5</td>
<td>1.42</td>
<td>1.34</td>
<td>0.82</td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>0.04</td>
<td>0.5</td>
<td>1.6</td>
<td>0.60</td>
<td>0.6</td>
<td>3.1</td>
<td>0.13</td>
<td>0.33</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>LOW Q</strong></td>
<td>2.76</td>
<td>9.34</td>
<td>41.3</td>
<td>1.06</td>
<td>7.8</td>
<td>104.9</td>
<td>1.46</td>
<td>2.82</td>
<td>1.23</td>
</tr>
<tr>
<td>±S.E.M.</td>
<td>0.24</td>
<td>0.5</td>
<td>3.0</td>
<td>0.14</td>
<td>0.5</td>
<td>2.4</td>
<td>0.15</td>
<td>0.87</td>
<td>0.19</td>
</tr>
</tbody>
</table>
FIGURE 13. The effects of changes in cardiac output on oxygen uptake and $P_aO_2$ (A), and carbon dioxide excretion and $P_aCO_2$ (B) across the gills of spontaneously ventilating, blood-perfused trout.
in both input $P_{O_2}$ and $C_{O_2}$ (Table 17), although all other input blood variables remained unchanged.

The effects of changing haematocrit on the gas transfer across the gills of the blood-perfused preparations are shown in Table 18. Oxygen uptake increased as Hct was raised (Fig. 14), while $R_{Og}$ was decreased. Input blood oxygen content ($C_{iO_2}$) was negatively correlated with Hct, while input $P_{O_2}$ increased with decreasing Hct. Nonetheless, $P_{aO_2}$ and $\Delta P_{O_2}$ across the gill circulation (venous - arterial) were not affected significantly by changes in Hct.

The effects of changes in the perfusion rate, stroke volume and pulse pressure on oxygen uptake are summarized in Table 19. These data indicate that, as long as $Q$ was maintained at a constant normal rate, all other changes had non-significant effects upon the measured value of $M_{O_2}$. Pressure and vascular resistance changes associated with these manipulations have been presented elsewhere (see Table 14).

Oxygen uptake across the gills of blood-perfused trout ($n = 4$) was $1.10 \pm 0.23 \mu M \cdot min^{-1} \cdot 100 g^{-1}$, and was not changed by the addition of $1 \times 10^{-6}$ M adrenaline to the blood ($1.30 \pm 0.25 \mu M \cdot min^{-1} \cdot 100 g^{-1}$). The effects of adrenaline exposure at this concentration on the pressure and resistance to flow in the gills of these blood-perfused preparations also have been presented elsewhere (see Table 16).

The Fick Equation, and the Direct Measurement of Ventilation Volume - $V_g$

Table 20 summarizes the data obtained from blood-perfused trout in normoxia, using the modified van Dam apparatus (see Materials and Methods, pages 54 to 57). These data, when compared with data obtained from normal preparations without the van Dam modification (see Tables 11 and 12), reveal that all variables are essentially identical. However, a most interesting finding from the present experiments was that the amount of oxygen removed
Table 18. Summary of the effects of variable input haematocrit on blood gases for normal, spontaneously ventilating, blood-perfused trout (N = 6 fish; 320.2 ± 17.2 g and constant $\dot{Q} = 5.20 ± 0.04$ ml·min$^{-1}$).

<table>
<thead>
<tr>
<th>Hct</th>
<th>$P_{inO_2}$</th>
<th>$C_{inO_2}$</th>
<th>$P_{aO_2}$</th>
<th>$C_{aO_2}$</th>
<th>$MgO_2$</th>
<th>RQ$_g$</th>
<th>RQ$_s$</th>
<th>Hct (input)</th>
<th>Hct (DA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>X 20.3</td>
<td>0.95</td>
<td>95.3</td>
<td>1.77</td>
<td>1.34</td>
<td>2.0</td>
<td>0.67</td>
<td>11.3</td>
<td>9.3</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>2.5</td>
<td>0.20</td>
<td>6.6</td>
<td>0.20</td>
<td>0.19</td>
<td>0.1</td>
<td>0.11</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>LOW</td>
<td>X 39.4</td>
<td>0.59</td>
<td>108.7</td>
<td>0.87</td>
<td>0.46</td>
<td>2.9</td>
<td>0.85</td>
<td>4.3</td>
<td>3.9</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>4.2</td>
<td>0.03</td>
<td>5.8</td>
<td>0.12</td>
<td>0.18</td>
<td>0.4</td>
<td>0.44</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>HIGH</td>
<td>X 16.3</td>
<td>1.37</td>
<td>91.6</td>
<td>2.88</td>
<td>2.45</td>
<td>1.7</td>
<td>0.87</td>
<td>20.2</td>
<td>16.5</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>2.6</td>
<td>0.26</td>
<td>13.2</td>
<td>0.40</td>
<td>0.40</td>
<td>0.3</td>
<td>0.16</td>
<td>1.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Figure 14. The effect of variable input haematocrit on oxygen uptake across the gills of spontaneously ventilating, blood-perfused trout.
O₂ UPTAKE, μM·min⁻¹·100 g⁻¹

y = 0.13x - 0.14
r = 0.87

HAEMATOCRIT, %
Table 19. Effects of cardiac output (Q), stroke volume (SV), pulse pressure (PP) and cardiac frequency (f) on oxygen uptake ($\dot{M}gO_2$) in resting, blood-perfused trout (0 = no change from normal; + = increase from normal; - = decrease from normal). Means ± S.E.M.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>$\dot{M}gO_2$ μM•min$^{-1}$•100g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) SV +; PP +; f 0; Q +</td>
<td>7</td>
<td>1.41* ±0.28</td>
</tr>
<tr>
<td>(2) SV +; PP +; f -; Q 0</td>
<td>8</td>
<td>1.07 ±0.31</td>
</tr>
<tr>
<td>(3) SV -; PP -; f 0; Q -</td>
<td>7</td>
<td>0.38* ±0.06</td>
</tr>
<tr>
<td>(4) SV -; PP -; f +; Q 0</td>
<td>8</td>
<td>0.99 ±0.26</td>
</tr>
<tr>
<td>(5) SV 0; PP +; f 0; Q 0</td>
<td>8</td>
<td>0.73 ±0.18</td>
</tr>
<tr>
<td>(6) SV 0; PP -; f 0; Q 0</td>
<td>3</td>
<td>0.84 ±0.15</td>
</tr>
<tr>
<td>Normal</td>
<td>44</td>
<td>1.17 ±0.08</td>
</tr>
</tbody>
</table>

* Significantly different from normal (5%).
Table 20. Summary of the cardiorespiratory variables from normal spontaneously ventilating, blood-perfused trout in a modified van Dam apparatus (n = 10 observations on 4 fish; 340.1 ± 11.2 g. $P_{CO_2} = 151.4 ± 0.8$ mm Hg).

<table>
<thead>
<tr>
<th>VAP</th>
<th>DAP</th>
<th>$\dot{Q}_{\text{actual}}$</th>
<th>$\dot{Q}_{\text{Fick}}$</th>
<th>$V_g$</th>
<th>$\dot{V}_g$</th>
<th>$K_{O_2}$</th>
<th>$\dot{V}_{O_2}$</th>
<th>$C_{A02}$</th>
<th>$C_{VRO2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm H$_2$O</td>
<td>cm H$_2$O</td>
<td>ml min$^{-1}$ kg$^{-1}$</td>
<td>ml min$^{-1}$ kg$^{-1}$</td>
<td>ml min$^{-1}$</td>
<td>ml min$^{-1}$</td>
<td>$\mu$M min$^{-1}$ 100g$^{-1}$</td>
<td>$\mu$M min$^{-1}$ 100g$^{-1}$</td>
<td>mM</td>
<td>mM</td>
</tr>
<tr>
<td>X</td>
<td>52.6</td>
<td>35.4</td>
<td>16.81</td>
<td>25.12</td>
<td>57.4</td>
<td>9.47</td>
<td>1.083</td>
<td>1.653</td>
<td>1.26</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>1.1</td>
<td>2.4</td>
<td>0.48</td>
<td>2.18</td>
<td>6.42</td>
<td>0.82</td>
<td>0.103</td>
<td>0.229</td>
<td>0.07</td>
</tr>
<tr>
<td>X</td>
<td>$\Delta CO_2 (V-M)$</td>
<td>$R_g$</td>
<td>$R_f$</td>
<td>$\Delta CO_2 (v-a)$</td>
<td>$RQ_g$</td>
<td>$\Delta CO_2 (a-V)$</td>
<td>$RO_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>µM min$^{-1}$ 100g$^{-1}$</td>
<td>cm H$_2$O ml$^{-1}$ min$^{-1}$ 100g$^{-1}$</td>
<td>cm H$_2$O ml$^{-1}$ min$^{-1}$ 100g$^{-1}$</td>
<td>µM</td>
<td>cm</td>
<td>mm</td>
<td>µM min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>0.726</td>
<td>8.80</td>
<td>17.58</td>
<td>-14.4%</td>
<td>2.55</td>
<td>+12.7%</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± S.E.M.</td>
<td>0.184</td>
<td>0.99</td>
<td>1.33</td>
<td>1.33</td>
<td>1.30</td>
<td>0.30</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
from the water which was ventilated across the gills (\( \dot{V}_{g02} \)) was consistently in excess (by 0.726 ± 0.184 µM·min\(^{-1}·100g^{-1} \)) of that amount of \( O_2 \) uptake which was measured in the blood across the gills (\( M_{g02} \)). A very important consequence of the "extra" oxygen uptake, which was not expressed in the blood, was found when the calculated cardiac output, using the Fick equation (5).

\[
(5) \dot{Q} (\text{ml·min}^{-1}·\text{kg}^{-1}) = \frac{\dot{V}_{g02} \text{ (µM·min}^{-1}·\text{kg}^{-1} \text{)}}{C_{a02} - C_{v02} \text{ (µM)}}
\]

was compared with the actual cardiac output (controlled by the cardiac pump of the apparatus used in the experiments). A consistent overestimate of 49.2 ± 11.4% of the actual \( Q \) was indicated, when using the Fick equation.

The Saline-Perfused Trout Head Preparation: Factors affecting gas transfer across the gills

The effects of variable input flow characteristics on the gas transfer across the gills of isolated, saline-perfused trout heads (see Materials and Methods, pp.34-9 are presented in Table 21. In general, changes across the gills (\( VA - DA \) differences) were smaller than changes across the head circulation (\( DA - AV \) differences). None of the changes of any of the variables was significant for either circulation.

Changes in \( P_{02} \) across the gills were comparable to those changes observed by Wood et al. (1978) under similar experimental conditions. Consistent \( CO_2 \) excretion, as measured by changes in total \( CO_2 \), across the lamellar circuit was observed during all perfusion regimes in the present experiments. The small increase in \( pH \), except during perfusion with constant pressure, is compatible with \( CO_2 \) loss across the gills. During perfusion at constant pressure, a small change in \( C_{CO2} \) across the gill circuit was accompanied by a decrease in \( pH \). Perfusate \( pH \) and \( P_{O2} \) decreased, while \( P_{CO2} \) (calculated) increased as it perfused the head vascular bed. This was
Table 21. Summary of gas exchange data in the isolated, saline-perfused head of *S. gairdneri* (pH determinations included for reference).

<table>
<thead>
<tr>
<th>Perfusion Regime</th>
<th>Variable</th>
<th>Constant Q (pulsatile) (1) MEAN ± SE</th>
<th>Constant Pressure (mean of pulse) (2) MEAN ± SE</th>
<th>Constant Q (non-pulsatile) (3) MEAN ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VA</td>
<td>DA</td>
<td>VA</td>
<td>DA</td>
</tr>
<tr>
<td><strong>GILL CIRCULATION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P&lt;sub&gt;02&lt;/sub&gt; (mm Hg)</td>
<td>45.1 ±2.6</td>
<td>42.5 ±2.6</td>
<td>48.7 ±2.6</td>
<td>51.4 ±2.9</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(13)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>pH</td>
<td>7.73 ±0.27</td>
<td>7.82 ±0.02</td>
<td>7.85 ±0.04</td>
<td>7.82 ±0.02</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(13)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td><strong>HEAD CIRCULATION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P&lt;sub&gt;02&lt;/sub&gt; (mm Hg)</td>
<td>42.5 ±2.5</td>
<td>29.9 ±2.5</td>
<td>51.4 ±2.9</td>
<td>30.0 ±3.7</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(13)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>pH</td>
<td>7.82 ±0.02</td>
<td>7.61 ±0.03</td>
<td>7.82 ±0.02</td>
<td>7.77 ±0.04</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(13)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

*numbers associated with data are observations*
an expected situation, given an actively metabolizing preparation. However, only the effects of haemodynamic alterations upon oxygen uptake will be discussed subsequently.

**Analysis of Water Flux in Gill Tissues**

**Influx and equilibration of ethanol (EtOH)**

Figures 15 and 16 depict typical EtOH concentrations in the recirculated ventilatory water, dorsal aortic, and anterior venous outflows during loading of the gills of an isolated, saline-perfused trout head preparation (see Materials and Methods, pp. 34-44). During this period of EtOH loading, the preparation was perfused with constant pulsatile flow. The higher EtOH concentrations in the water for the first 4 - 5 minutes probably reflects mixing of the added EtOH with the 4 L recirculating water volume. Dorsal aortic EtOH concentrations follow closely the water EtOH concentration. The rate of fall of EtOH in the water was the same as that found in the dorsal aortic outflow. After 15 min, dorsal aortic and anterior venous EtOH concentrations both were approximately 1/20 of that found in the water (see Table 22). This Table also indicates that the concentration of EtOH in the dorsal aortic outflow was not statistically different (Student's t-test) for the two perfusion regimes used in these experiments.

Figure 17 illustrates EtOH concentration in the dorsal aortic effluent during washout, while the head was perfused at constant pulsatile flow (17.0 ml·min⁻¹·kg⁻¹), and constant pressure (flow = 16.2 ml·min⁻¹·kg⁻¹). Flows and pressures from preparations used in these experiments were not significantly different from those reported in Table 10 (Student's t-test). This figure illustrates that the EtOH was washed from the gills more completely and more rapidly during pulsatile perfusion than during non-pulsatile perfusion. Addition of EtOH to the water, and ultimately to the perfusate by transepithelial diffusion, did not in any way alter the vascular resist-
Figure 15. Typical ethanol concentrations in the water, dorsal aortic perfusate and anterior venous perfusate during loading of gill tissues. The decline in dorsal aortic EtOH concentration after 5 min follows the fall in water EtOH concentration. After 15 min of loading, dorsal aortic and anterior venous EtOH concentrations are equal. Influx refers to net movement of EtOH across the tills, from water to perfusate in the vasculature.

- Water EtOH concentration
- Dorsal aortic EtOH concentration
- Anterior venous EtOH concentration
Figure 16. Representation of the data presented in Fig. 15, normalized to the final concentrations in perfusate after 15 min. This figure indicates that dorsal aortic loading proceeds at a faster rate than does the anterior venous outflow, but they reach equilibration at the same time span chosen for loading. Rest of legend as in Fig. 15.
Table 22. Ethanol concentrations in water, dorsal aortic perfusate and anterior venous perfusate. Preparations were exposed to ethanol for 15 minutes to load gill tissues during perfusion by either constant pulsatile flow or constant non-pulsatile pressure perfusion regimes. (n = 5).

<table>
<thead>
<tr>
<th></th>
<th>Water (mM)</th>
<th>Dorsal aortic perfusate (mM)</th>
<th>Anterior venous perfusate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsatile Perfusion</td>
<td>3.57 ± 0.74</td>
<td>0.15 ± 0.05</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Non-pulsatile perfusion</td>
<td>3.33 ± 1.04</td>
<td>0.19 ± 0.08</td>
<td>0.15 ± 0.08</td>
</tr>
</tbody>
</table>
Figure 17. Dorsal aortic ethanol (EtOH) concentrations during washout from gill tissues. EtOH is washed from gills more rapidly and more completely during pulsatile perfusion, compared to non-pulsatile perfusion, of the same mean pressure.

- Pulsatile constant flow
- Non-pulsatile constant pressure
ance of the gills, or the pressure profile of the perfusion pulse (see Fig. 18).

Ethanol concentrations in the anterior venous perfusate during washout are shown in Fig. 19. The points on these curves for washout during the two regimes are not significantly different, although the concentration during pulsatile perfusion appears to be falling more rapidly toward the end of the 15 min period.

Compartmental Analysis of EtOH Washout Curves

Semilog plots of EtOH washout curves from dorsal aortic samples revealed two distinct compartments. By curve peeling, straight lines were fitted to plots of the natural logs (ln) of the ethanol concentrations, versus time. The normalized equation for the EtOH washout curve during pulsatile perfusion is (1).

\[
(1) \quad \text{Amount of EtOH at time} = t = 0.872e^{-1.28t} + 0.127e^{-0.28t}
\]

The \( r^2 \) values (coefficients of the determination) for both lines generated from the data were 0.98. The equation for the curve of EtOH washout during non-pulsatile flow is (2).

\[
(2) \quad 0.606e^{-0.203t} + 0.394e^{-0.0352t}, \text{having } r^2 \text{ values of 0.96 and 0.99, for the fast and slow components respectively.}
\]

Since EtOH concentrations were measured only in the second, or downstream (fluid vascular space) compartment, a complete analysis would not normally have been possible. However, these arguments apply only to the loading of tissues. As gill tissues first were equilibrated with EtOH and then its subsequent washout measured, it was possible to completely analyse the system for all rate constants and volumes of distribution.

The model used for the analysis is presented in Fig. 20. Implicit in this model are the assumptions that; during washout, part of the EtOH in the tissues is washed out into the external medium, and, there is
Figure 18. Record of input pressure during pulsatile constant flow perfusion. Note that the systole phase lasts for one third and diastole phase two thirds of each pump cycle. Introduction of ethanol (EtOH, at arrow) to the water has no effect on input pressure.
Figure 19. Anterior venous ethanol concentrations during washout from gill tissues. These were normalized to the final perfusate concentration after 15 min loading with EtOH. Pulsatile perfusion had no significant effect on the washout of EtOH from the anterior venous circulation.

- Pulsatile constant flow
- ▲ Non-pulsatile constant pressure
exchange of EtOH between gill tissues and the perfusate within the gill vessels. Although anatomical identification of the two compartments is not possible, it is postulated that the fast compartment is the vascular space, and that the slow compartment is comprised of cells, and perhaps the extracellular/extravascular volume.

Figure 20 illustrates a number of differences in the curves presented in Fig. 17. Firstly, the overall washout rate (rate constant $k_{oa}$) during pulsatile perfusion is some 12 times greater than that for non-pulsatile perfusion. The relative volumes of the fast and slow compartments during pulsatile perfusion show that 7/8 of the EtOH in the gills is in the fast or vascular compartment. During non-pulsatile perfusion however, only 2/3 of the EtOH is in this fast compartment. Probably the most important feature of this analysis is that exchange between the two compartments (rate constants $k_{ba}$ and $k_{ab}$) is four times greater during pulsatile perfusion as compared to non-pulsatile perfusion.

Ethanol concentrations in ventilatory water during washout of EtOH from the gills show that there was less than 50% of the initial ethanol in the water after 30 seconds, and none was detectable after 90 sec.
Figure 20. Two-compartment models illustrating the results of the analysis of the dorsal aortic ethanol washout curves during:

(A) Pulsatile constant flow perfusion, and
(B) non-pulsatile constant pressure perfusion.

During pulsatile perfusion EtOH is lost more rapidly from the gills ($k_{oa}$), and exchanges between the fast and slow compartments ($k_{ab}$; $k_{ba}$) are greater. Also during pulsatile perfusion, the fast (vascular) compartment is relatively larger than the slow compartment, when compared to the compartment sizes during non-pulsatile perfusion.
A

To water

\[ b \]
\[ 0.37 \text{ ml} \]

\[ k_{ba} \] 0.27

\[ k_{ab} \] 0.41

\[ a \]
\[ 3.12 \text{ ml} \]

\[ k_{oa} \] 0.88

Slow compartments

B

To water

\[ b \]
\[ 0.89 \text{ ml} \]

\[ k_{ba} \] 0.06

\[ k_{ab} \] 0.10

\[ a \]
\[ 1.37 \text{ ml} \]

\[ k_{oa} \] 0.07

Fast compartments
DISCUSSION III: Gas Exchange in the Spontaneously Ventilating, Blood-Perfused Trout Preparation: Comparisons with *in vivo* data.

**Blood Gas Tensions and Contents**

**Input Blood**

Blood in the tonometers was exposed to gas containing 0.4% CO₂ in 40% air (P\(_{CO_2}\) = 3.0 mm Hg; P\(_{O_2}\) = 60 mm Hg, P\(_{N_2}\) = 697 mm Hg). Measured mean P\(_{O_2}\) of the input blood was 24.9 mm Hg, principally because venous return blood had a low P\(_{O_2}\). Ventral aortic P\(_{O_2}\) in resting rainbow trout, *in vivo* is around 30 mm Hg (Cameron and Davis, 1970; Eddy *et al.*, 1977; Kiceniuk and Jones, 1977), although lower values have been measured (19.0 mm Hg, Stevens and Randall, 1967a). Therefore, input P\(_{O_2}\) values from the blood-perfused preparations were in the physiological range measured *in vivo*.

Mean input oxygen content was 0.9 mM L\(^{-1}\) for the blood-perfused fish. Kiceniuk and Jones (1977) measure oxygen contents of ventral aortic blood *in vivo*, from resting rainbow trout of about 3.2 mM L\(^{-1}\). Given that the blood-perfused preparations are perfused with blood of lower Hct (10.3% compared to 24.2% for the fish used by Kiceniuk and Jones), and that my P\(_{O_2}\) is lower by 25%, C\(_{O_2}\) of the input blood in my experiments is close to that predicted from available data (see Holeton and Randall, 1967a).

*In vivo* venous P\(_{CO_2}\) values range between 1 and 5 mm Hg in resting rainbow trout (Holeton and Randall, 1967a; Stevens and Randall, 1967b; Eddy *et al.*, 1977; Wood *et al.*, in press). The calculated value for input P\(_{CO_2}\) (3.36 ± 0.15 mm Hg) in blood-perfused fish more closely resembled that of the gas mixture. CO₂ content generally was higher in the input blood than has been reported in the literature (Eddy *et al.*, 1977). However, recent values (Wood *et al.*, in press; Perry and Heming, in press) are very close to those reported here.
Dorsal Aortic (arterial) Blood

Mean dorsal aortic blood $P_{O_2}$ in blood-perfused fish was 103 mm Hg and $C_aO_2$ was 1.58 mM L$^{-1}$. For a mean Hct of 8.8%, this indicated over 98% oxygen saturation (from $C_aO_2 = 0.311 \times \text{Hct} + 0.7$ (Vol. %), Holeton and Randall, 1967b). $P_aO_2$ was lower than some in vivo values (133.2 mm Hg, Cameron and Davis, 1970; 117 mm Hg, Eddy et al., 1977; 137 mm Hg for trout in a swim tube, Kiceniuk and Jones, 1977), yet higher than those of Stevens and Randall (1967b) of 85 mm Hg. However, each of the above mentioned $P_aO_2$ values indicates better than 95% saturation of the blood in their particular cases as well.

$C_aCO_2$ measurements and calculated $P_aCO_2$ values from blood-perfused fish were similar to in vivo values, although variability of the in vivo data precludes any detailed comparison.

Oxygen Uptake Across the Gills - $\dot{M}_gO_2$

There are no direct in vivo measurements of $\dot{M}_gO_2$ available in the literature. However, Stevens and Randall (1967b) and Kiceniuk and Jones (1977) measured $N_{O_2}$, the oxygen consumption by the whole fish in a respirometer, for rainbow trout, and found values of 2.68 and 2.5 $\mu$M O$_2$ min$^{-1} \cdot 100$g$^{-1}$, respectively. Mean oxygen uptake by the blood during its passage through the gills in blood-perfused fish was 1.17 $\mu$M min$^{-1} \cdot 100$g$^{-1}$. After correction for temperature differences between in vivo experiments and mine, the in vivo values for $\dot{M}_gO_2$ still were some 60% higher.

Blood-perfused preparations may have shown different $\dot{M}_gO_2$ values than those from in vivo experiments for the following reasons. Tonometering blood with the particular mixture of gases used tended to increase input $P_{O_2}$ from 13 mm Hg (measured mean value for venous return blood) to 25 mm Hg, which increased input $C_O$ two-fold. Had no attempt been made to raise input $P_{O_2}$ to what were considered physiological levels (in vivo values from the
literature), then blood entering the gills would have had a greater capacity for oxygen uptake, by virtue of an increased diffusion gradient for oxygen across the gills. As dorsal aortic blood always was greater than 98% oxygen saturated in these preparations, \( M_{O_2} \) may have been limited by high input \( C_{O_2} \). Oxygen consumption by the skin (Kirsch and Nonnotte, 1977) and the gill tissues (see Section III) in \( N_{O_2} \) (or \( V_{g_2} \)) determinations also could have contributed to the noted differences between \( M_{g_2} \) values obtained from blood-perfused and \textit{in vivo} experiments.

**Oxygen Uptake across the Systemic Circulation** - \( \dot{M}_{s_2} \)

Oxygen extraction by the systemic tissues was some 1.75 times the measured value for the oxygen uptake rate across the gills. This finding was a consequence of raising input \( P_{O_2} \) by tonometry, rather than allowing input blood to reach its own \( P_{O_2} \) level from venous return blood alone. Had this approach been used, then one could presume that \( M_{g_2} \) would increase by an appropriate factor to accommodate the measured \( M_{s_2} \), since these values were much closer to the estimates from \textit{in vivo} experiments. Systemic tissues nonetheless extracted a very large proportion of the delivered blood oxygen content.

**Carbon Dioxide Excretion Across the Gills, and Production by Systemic Tissues**

Mean carbon dioxide excretion across the gills of blood-perfused trout was 2.05\( \mu \text{M} \cdot \text{min}^{-1} \cdot \text{100g}^{-1} \). As venous \( C_{CO_2} \) and arterial \( C_{CO_2} \) have yet to be measured simultaneously with cardiac output (Q) \textit{in vivo}, no values for comparison of \( M_{g_2} \) are available from the literature. Eddy \textit{et al.} (1977) have described a 27% fall in \( C_{CO_2} \) across the gills of two fish, where both venous and arterial \( C_{CO_2} \) were measured. Although mean \( C_{CO_2} \) change across the gills of blood-perfused fish was 12%, some individual fish did show upwards of a 28% decrease in \( C_{CO_2} \) under normal conditions. Blood-perfused fish were able to excrete sufficient net \( CO_2 \) to keep pace with its production
The mean ratio of CO₂ excretion over O₂ uptake across the gills (RQ₉) of blood-perfused fish was 1.85. This high value resulted from low \( M_{O_2} \) measurements, which in turn were the result of the elevation of input \( P_{O_2} \) and \( C_{O_2} \). Mean ratio of CO₂ production by the systemic tissues, over the O₂ utilization by these tissues (RQ₅) was 0.83. Typically, this represents fat catabolism (Robinson, 1974). However, the metabolic substrates used by the present preparation are unknown.

**Blood Chemistry**

Plasma osmolarity and Cl⁻ concentrations were similar to those reported by Holmes and Donaldson (1970), and to measurements made by Perry and Heming (in press). Table 16 indicates some dilution of the blood as it passes through the gills of blood-perfused fish. Hct fell by 14%. There are a number of possible explanations, including dilution during sampling. Water uptake rates, calculated from the plasma osmolarity difference between input and dorsal aortic blood of my preparations, were in the order of 0.06 ml·min⁻¹·100g⁻¹. Net water influx across trout gills, measured in other preparations, is approximately 0.17 ml·min⁻¹·100g⁻¹ (Isaia et al., 1978). Gills of blood-perfused trout behave as they do in vivo, with respect to transepithelial water flux.

**Ventilation Rate**

The mean ventilation rate of blood-perfused trout was 69.4 breaths·min⁻¹. The wealth of data available from intact fish indicates that at the temperature of the present preparations (7°C), this rate is normal (Holeton and Randall, 1967a; Davis and Cameron, 1971; Daxboeck and Holeton, 1978, 1980). Interactions between respiratory movements and blood pressure were evident in the traces from blood-perfused preparations (Fig. 12), and were similar to those described by Wood and Shelton (1980a) from trout in vivo. These
interactions can cause moderate increases in blood flow through the gills, as proposed by Johansen et al. (1966), and have been discussed in detail by Hughes (1973).

**Haemodynamic Alterations and Their Effects on Gas Exchange**

The gills of fish, *in vivo*, are presumed to be diffusion limited for gas transfer. As such, haemodynamic changes which could possibly decrease the gill diffusion barrier to gases should thereby increase oxygen uptake measured across these gills. Data collected from spontaneously ventilating, blood-perfused trout indicate that gill resistance to flow, and not the mass transfer of oxygen (\(\dot{M}_{\text{O}_2}\)) is the variable most sensitive to haemodynamic alterations, other than cardiac output. The data from the blood-perfused study show that the gills of rainbow trout, at rest are PERFUSION LIMITED for gas transfer under these conditions. For gills to be termed perfusion limited, oxygen uptake by the blood (\(\dot{M}_{\text{O}_2}\)) must increase as the rate of perfusion through the respiratory capillary network increases. Such is the case in these preparations (see Fig. 13), and carbon dioxide excretion (\(\dot{M}_{\text{CO}_2}\)) shows a similar relationship (see Fig. 13). The residence time of blood in the gills over the range of flow rates tested also is sufficient for blood oxygen saturation, since \(P_a\text{O}_2\) remains constant at a level equivalent to 98% saturation. As well, \(P_a\text{CO}_2\) is unaffected by changes in perfusion flow; and a constant gradient between blood and water is maintained over a wide range of \(Q\).

If the carrying capacity of the blood is decreased, by decreasing Hct, then a corresponding decrease in the oxygen uptake rate across the gills is noted in the blood-perfused trout preparation, given the perfusion rate remains constant. Dorsal aortic \(P_0\text{O}_2\), as well as the change in \(P_0\text{O}_2\) between input and arterial blood across the gills however are not significantly different from other Hct conditions, even with perfusion with an Hct as low
as 4%. The maintained level of $P_{aO_2}$ is due, in part, to an increased contribution by dissolved $O_2$ in a larger volume of plasma, augmented by the presence of the red blood cells. Unlike severely anaemic trout, which increase cardiac output in response to this stress in vivo (Wood et al. 1979; Wood and Shelton, 1980a), in order to maintain relatively constant oxygen uptake rates, a constant cardiac output in the preparations which I used eliminates any possible masking effects due to changes in $\dot{Q}$. Although there are gill vascular resistance changes to flow with either higher than or lower than "normal" Hct blood perfusion of whole trout preparations, these pressure changes by themselves have no effect on gas transfer, as previously discussed. Therefore, the results with variable haematocrit perfusion reflect only the contribution of the number of erythrocytes upon $O_2$ transfer across the gills.

The gas exchange efficiency of trout gills is said to be increased during exercise, in vivo (Kiceniuk and Jones, 1977), due to attendant cardiorespiratory adjustments. From the data collected during manipulations of cardiac frequency/stroke volume/pulse pressure in the blood-perfused trout preparations at rest, in normoxia, while $\dot{Q}$ is kept constant, negligible changes in oxygen uptake, and carbon dioxide excretion rates across the gills are noted. Neither was oxygen uptake across the gills from the water ($\dot{V}_{gO_2}$), or by the blood ($M_{gO_2}$) changed from normal when bradycardia was simulated. As dorsal aortic blood always is greater than 98% $O_2$ saturated, increases in $M_{gO_2}$ only could have arisen from increases in the amount of oxygen dissolved in the plasma, whether the gills are diffusion or perfusion limited. As plasma, or saline oxygen carrying capacity is small, compared to that of the red cells, no significant changes in $M_{gO_2}$ can be expected under these perfusion conditions: a situation analogous to the saline-perfused preparations. What these data do indicate however, is that the
proposed changes in gill diffusion capacity for gases, associated with changes in gill blood flow and pressure (Farrell et al., 1979, 1980; data presented for isolated head preparations in this thesis) are relatively unimportant to gas transfer. These data again indicate that the gills of rainbow trout at rest, under the experimental conditions, are perfusion limited for oxygen uptake, as well as carbon dioxide excretion, independent of mean and pulse pressure.

One may still argue that gas transfer at the gills is diffusion rather than perfusion limited. Because of the rather low "normal" haematocrit of the blood used in the blood-perfusion experiments, and the attendant increases in input $P_{O_2}$ and $P_{CO_2}$ (reasons for this have been discussed previously), a higher percent $O_2$ saturation for the input is measured, higher than may have been measured had the blood not been tonometered with the chosen gas mixture used. As a consequence, a lower change in the $P_{O_2}$ between input and dorsal aortic blood is measured, than potentially could have been achieved during haemodynamic alterations, had Hct been "more representative" of that found in vivo, and $P_{O_2}$ and $P_{CO_2}$ been allowed to fall to whatever level they could have attained.

Also, one could suggest that, since a diffusion limited exchange system is dependent upon the ability of that system to somehow increase the diffusional area as well, all the secondary lamellae in the blood-perfused preparations are being perfused already, and thus any possibility of demonstrating changes in diffusion capacity by this avenue already is saturated. Steen and Kruysse (1964) and Payan, Girard, Peyraud and Waitzenegger (unpublished observations reported by Girard and Payan, 1980) note an increase in branchial $O_2$ permeability in trout exposed to adrenaline. These data indicate possible decreases in diffusion barriers as a result of the adrenaline exposure. Although ventilation rates (Peyraud-Waitzenegger, 1979), and intrinsic heart rates (Gannon, 1971) might be expected to rise during
adrenaline exposure, neither of these events occur in the blood-perfused preparations. Since the major effect of adrenaline on trout hearts is inotropic, increases in the magnitude of ventricular pressures are observed during the administration of adrenaline in my preparations, but no changes in $P_{aO_2}$ or $C_{aO_2}$ are measured. Neither are $M_{gO_2}$ nor $M_{gCO_2}$ significantly altered during adrenaline exposure in these preparations. An increase in $M_{gO_2}$ however, may not have been expressed in the present adrenaline exposure experiments because, unlike in vivo, the cardiac pump which replaces the actual heart can not make the appropriate responses to this catecholamine. Increases in oxygen uptake in vivo during periods of increased levels of circulating catecholamines, as presumed during exercise in trout, therefore may not be due to the direct effect of changes in the diffusion barrier at the gills, but as a result of the changes which adrenaline promote in causing an increase in the rate of blood flow through the gills. The results with the addition of adrenaline to the blood in my experiments never give occasion to presume that the blood-perfused preparation demonstrates any significant diffusion limitations. If stress had induced an above normal amount of mucous to be produced to cover the gills of blood-perfused fish, a situation often noted with the saline-perfused preparations, it has been demonstrated that, within limits, this mucous layer is primarily a resistance barrier for ionic exchange rather than for gas diffusion (Ultsch and Gros, 1979).

If the haematocrit were to increase to such an extent that the blood was so viscous that the red blood cells would actually interfere with each other, causing diffusive gas exchange problems, notwithstanding the pressure and flow effects brought about by the increased viscosity, then $O_2$ uptake measurements under these conditions could lead to the conclusion that the gills are still diffusion limited. During exercise, Hct does rise, but obviously not to a level which is detrimental to the effectiveness
of gas transfer under the physiological conditions which promote this situation. Also, if cardiac output were to increase to the point where blood residence time in the gills was so abbreviated that the diffusive process of haemoglobin oxygenation were the limiting step, the gills are then diffusion limited. However, the vascular effects of such a situation would surely be completely disruptive, and so outside the scope of reasonable physiological function, that such a condition is untenable.

Applicability of the Fick Principle

The blood-perfused preparation affords the opportunity to test the applicability of the Fick equation for calculating cardiac output, as the actual $\dot{Q}$ already is known. When such a comparison is made, any measurement of oxygen uptake from the water across the gills ($\dot{V}_{gO_2}$) consistently is in excess of the amount of oxygen which is actually picked up by the blood ($\dot{M}_{gO_2}$). This discrepancy is due to the fact that gill tissues are metabolically active and therefore consume a significant amount of oxygen from the water and blood. Data from experiments conducted by Johansen and Pettersson (1981), using isolated perfused gill arches, have indicated that approximately 40% of the metabolic oxygen demand of gill tissues is met by direct uptake and utilization from the water. The difference between $\dot{V}_{gO_2}$ and $\dot{M}_{gO_2}$ in the blood-perfused preparations ($0.726 \pm 0.184 \mu M O_2 \text{ min}^{-1} \cdot 100 \text{ g body weight}^{-1}$) represents the measured $O_2$ consumption of the gills. Since gill tissues constitute 3.9% of the total body weight in trout (Stevens, 1968a), a value of $18.6 \mu M O_2 \text{ min}^{-1} \cdot 100 \text{ grams of gill tissue}^{-1}$ is derived for normoxic rainbow trout gills. This value compares well with the available data for other fish gills (Amazonian fish - measurements corrected to $30^\circ C$: Hoplias malabaricus, 10.0; Osteoglossum bicirrhosum, 12.8; Hoplerythrinus unitaeniatus, 13.3; and Arapaima gigas, 21.3 $\mu M O_2 \text{ min}^{-1} \cdot 100g \text{ tissue}^{-1}$; P.W. Hochachka, unpublished data). One can envisage that under normal conditions, gill
tissues will account for a certain proportion of \( V_{gO_2} \). Then and only then can
the remainder be expressed in the blood as \( M_{gO_2} \). During exercise, \( O_2 \) consump-
tion by gill tissues becomes proportionately less because consumption by the
whole fish may increase by an order of magnitude over that at rest. Thus,
gill oxygen consumption becomes almost insignificant compared with the
measured oxygen uptake during exercise \( \text{in vivo} \).

It is \( V_{gO_2} \) values which are used in the Fick equation to calculate
the cardiac output, despite these values being different from the amount of
oxygen actually entering the blood. This difference can be accounted for by
gill metabolism, as well as having some of the difference accounted for by
oxygen consumption by the skin, in some methods of measurement (Kirsch and
Nonnotte, 1977). Because \( V_{gO_2} \) is found to be an overestimate of the actual
oxygen uptake by the blood, the mean cardiac output (\( \dot{Q} \)) value derived from
Fick is a \( 49.2 \pm 11.4\% \) (\( n = 9 \)) overestimate of the actual \( \dot{Q} \) set by the exter-
nal cardiac pump. The median \( M_{O_2} \) for gill tissues is 27\% of the resting \( V_{gO_2} \).
This observation is in contrast with the findings made by Hughes \textit{et al.} (1980),
who have made similar measurements from eels. These authors found that the
actual \( \dot{Q} \) exceeded those obtained from Fick measurements by 39\%, and attributed
the difference to the fact that nearly 30\% of the cardiac output in eels is
not involved in gas exchange at the gills. Such a situation is unlike that
found in trout, where nearly all blood must pass through the secondary lamel-
lae to be oxygenated. Johansen and Pettersson (1981) recommended that esti-
mates of cardiac output from Fick also be corrected, to account for the amount
of blood \( O_2 \) content removed by the gill tissues between venous and arterial
blood. Such a correction obviously is not necessary, since inherent in the
use of the Fick equation is the use of the actual arterial/venous oxygen
content difference. This measured value already has had whatever \( O_2 \) content
needed by metabolizing gill tissues removed. Needless to say, from the evi-
dence presented, it is quite apparent that considerable care must by exercised
when applying the Fick equation to aquatic animals.

The arguments which I have presented clearly demonstrate that the gills of blood-perfused trout, under the conditions of my experiments, and probably also in vivo, are perfusion limited for gas transfer. Implicit in this contention is the fact that haemoglobin oxygen binding is not the rate limiting step in oxygen uptake, but that mass transfer of oxygen, as well as carbon dioxide excretion are primarily dependent upon the rate of perfusion of the gills, in fish.

The data from saline-perfused preparations, while showing that pulsatility of input flow increases venolymphatic clearance of gill and head spaces in the trout, however, do not provide any clear evidence for or against these gills being diffusion limited for gas transfer. The low oxygen uptake rate by saline-perfused gills, regardless of the type of perfusion regime used, is largely the result of low oxygen carrying capacity of the saline, and the high metabolic rate of the gill tissues. The gas exchange data obtained from saline-perfused gills in my experiments indicate that, under all perfusion regimes, net CO₂ excretion is maintained to a certain degree, while net oxygen uptake is not always accomplished. P⁰₂ and C⁰₂ decrease across the gills as perfusate pH rises. Similarly, in the general head circulation, what oxygen is presented to the tissues via the dorsal aortic saline flow is used by the metabolizing tissues. This is indicated by consistent decreases in P⁰₂ and pH, with attendant increases in the P⁰₂ of the venous return perfusate in all regimes used.

The venolymphatic vessels in the gills of either saline-perfused or blood-perfused preparations could act as fluid "shunts", allowing some blood to pass through the gills twice, without going through a systemic vascular bed. The purpose of this could be to increase the arterial oxygen tension, or to lower the arterial carbon dioxide tension in the perfusing fluid, so that the net result would be more truly "arterial" blood being
delivered to the systemic circulation. Neither of these explanations seem plausible, as dorsal aortic blood is close to fully saturated with oxygen after a single pass through the gills, as illustrated in the blood-perfused preparation. Additionally, the CO$_2$ produced as saline passes through the anterior venous circuit in the isolated head is greater than that lost across the gills. Therefore, no benefit to enhanced gas transfer is apparent, if such shunting of blood were to occur in vivo. Increased venolymphatic clearance from gill tissues could however, decrease the diffusion distance through which gases must pass to be picked up by the blood. This would be of some significance during exercise, when input pulse is increased.

In this section, I have shown that haemodynamic alterations which mimic those recorded during exercise in trout, in vivo, can change the pattern of flow through the branchial circulation, and can affect vascular resistance to flow. Increases in cardiac output and ventral aortic pulse pressure affect not only branchial vascular resistances, but also result in measureable increases in oxygen uptake rates in blood-perfused preparations. And, although I have shown gills to be primarily perfusion limited for gas transfer, a small diffusion limitation still could exist in these tissues, given the complex nature of the intra- and extravascular fluid spaces within these respiratory tissues. Water flux across the gills is known to increase during swimming exercise in trout (Wood and Randall, 1973), and an increase in this flux may indicate that the concomitant increase in O$_2$ flux can in some way be affected by the nature of the fluid exchange across these tissues during exercise.

The nature of this fluid exchange within gill tissues during simulated exercise conditions was investigated with the saline-perfused trout head preparation, and an ethanol loading/washout technique. The choice of ethanol as a marker of fluid spaces in gill tissues was made for the following reasons. Data from independent in vivo studies have shown that sufficient
ethanol crosses into the vascular space through the epithelia from the water within one pass of the perfusate through the gills, to yield measureable EtOH concentrations. As the saline perfusate is presumed to pass through the gills only once, it is essential that sufficient ethanol be taken into the vascular space during this single pass, and equilibrate throughout the gill and head tissues over the time period chosen for loading in these experiments. Ethanol concentrations are measured easily, to a high degree of accuracy, even at very low concentrations. The enzymatic assay used to determine these concentrations obviates the use of radioactive tracers, with their attendant problems. Stray-Pedersen and Steen (1975) have derived values for the permeability constant of eel gills to water, ethanol and a variety of other substances. Steen and Stray-Pedersen (1975) also have shown that these gills have permeabilities to water and ethanol which are almost identical, and these permeabilities change in the same manner in response to perturbations. For these reasons, ethanol was a marker of extra- and intravascular space in the gill tissue of the saline-perfused trout head preparation, and was judged superior to either dyes, or radiotracer methods.

The permeability constant of ethanol across trout gills from the present study is calculated in a manner similar to that described by Stray-Pedersen and Steen (1975). Values for the permeability constant, $k$, during pulsatile perfusion and non-pulsatile perfusion are $4.12 \times 10^{-8}$ cm$^2$·sec$^{-1}$ and $5.30 \times 10^{-8}$ cm$^2$·sec$^{-1}$ respectively. The oil/water partition coefficient of ethanol is 0.022. The thickness of the respiratory barrier is taken to equal 6 µm. Therefore the diffusion coefficient for ethanol ($D_{EtOH}$) can be calculated. During pulsatile perfusion, $D_{EtOH} = 1.12 \times 10^{-9}$ cm$^2$·sec$^{-1}$, and for non-pulsatile perfusion, $D_{EtOH} = 1.44 \times 10^{-9}$ cm$^2$·sec$^{-1}$. These values are in close agreement with those calculated for the eel gill ($7.3 \times 10^{-9}$ cm$^2$·sec$^{-1}$) by Stray-Pedersen and Steen (1975). It must be pointed out that these
authors used very low perfusion rates and pressures, which may account for
the difference between their value for $D_{\text{EtOH}}$ and the ones presented for the
saline-perfused head, notwithstanding any species differences. As ethanol
permeability across trout gills in my experiments is similar to that found
for eel gills, ethanol movements probably mimic water movements in these
tissues as well.

After loading the present preparation with ethanol for 15 min,
dorsal aortic and anterior venous effluent ethanol concentrations are not
different. This time period therefore is sufficient to allow for complete
equilibration of gill and head tissues with ethanol. As ethanol enters
cells and all other fluid spaces (Goodman and Gilman, 1975), subsequent
washout of this marker is from all the possible compartments in the perfused
head preparation.

Compartmental analysis of Washout Curves

Gill vascular (blood) volume in S. gairdneri has been found to be
approximately $0.16 \text{ ml} \cdot \text{g}^{-1}$ of gill tissue (see Table 4). Gill tissues con­
stitute 3.9% of the total body weight of trout (Stevens, 1968a). Since the
fish used in this study had an average weight of 289 g, the calculated gill
vascular volume is 1.8 ml. During non-pulsatile perfusion, when it is pro­
posed that little exchange (reflux) of fluid between the fast and slow
compartments occurs (see later discussion), the fast compartment should
approximate the vascular volume. The volume, during non-pulsatile perfusion
was calculated to be 1.37 ml, which is a reasonable estimate, considering
that in the absence of pulsatile pressure, fewer than 100% of the gill
lamellae would be perfused (see Farrell et al., 1979). The volume of the
fast compartment, as measured by EtOH washout analysis during pulsatile
perfusion could be expected to be larger than independent estimates of vascu­
lar volume because of the extensive reflux of fluid between vascular and
extravascular fluid spaces as pressure rises and falls with each passing pump cycle. The fast compartment during pulsatile perfusion was calculated to be 3.12 ml, and this volume is almost twice that of independent estimates.

**Total Amount of Ethanol Washed from the Gill Tissues**

Using the measured dorsal aortic outflow rate of 1.54 ml min⁻¹, and the equations for the ethanol concentration in the dorsal aortic effluent (1 and 2), the total amount of ethanol washed from the gills via this arterial route could be calculated. During pulsatile perfusion there are $5.54 \times 10^{-7}$ moles of EtOH, and for non-pulsatile there are $7.52 \times 10^{-6}$ moles of EtOH washed from the tissue fluid spaces into the vascular space. Given that the same gills were used from the tissue fluid spaces into the vascular space. Given that the same gills were used for both perfusion regimes, and that the input flow rates were not different, then the 14-fold difference in the above ethanol values must have resulted either from differences in the concentration of ethanol in the gill vessels at time = 0, or in different volumes of distribution and/or concentration within the gill tissues. Since concentration in the dorsal aorta (fast compartment) was not different with perfusion by either pulsatile or non-pulsatile flow, it is difficult to imagine how vascular or extravascular volumes could have changed by more than a 2-fold increase, as suggested from the previous calculation of vascular volumes. It therefore appears that the ethanol concentration in the tissues (slow compartment) is different for the two perfusion regimes.

The proposed model of the ethanol concentration gradients across the respiratory barrier in the gills is illustrated in Fig. 21. This model includes the two barriers to water movement, in which the basal, or blood-facing barrier is eight times less permeable than the apical or water-facing barrier (Isaia et al., 1978). In the model, the amount of ethanol in the respiratory barrier, washed into the perfusate, therefore is one-
Figure 21. Hypothetical ethanol concentration profile across the respiratory barrier of the gills. As the basal (blood-facing) barrier is eight times less permeable than the apical (water-facing) barrier, ethanol in the tissues to the left of the heavy vertical dashed line is assumed to be lost to the water, while that on the right is assumed to be removed by the perfusate in the blood vessels. If exchange between fast (vascular) and slow (tissue) compartments is rapid, and extends across the basal barrier, then the solid line better represents the ethanol concentration. When exchange is slow and extends only to the basal barrier, then the dotted line better represents the ethanol concentration profile. From the figure, the amount of ethanol in the tissues during non-pulsatile perfusion is some 11 times greater than during pulsatile perfusion. See the text for further explanation.
eighth of the total ethanol in the barrier. Within the space cleared to the blood (perfusate) side, the amount of ethanol is some eleven times greater during non-pulsatile perfusion, when there is apparently less exchange between blood and tissues, as can be estimated by the summation of the areas under the curves in Fig. 21. This high ethanol concentration in the slow compartment, combined with the relative increase in volume during non-pulsatile perfusion (see Fig. 18), can be shown to account for the difference in the calculated amounts of ethanol washed from the preparation when perfused by different regimes.

I have shown that pulsatile perfusion increases clearance of ethanol from saline-perfused trout gills, probably because of increased exchange between tissue and perfusate. What is not clear is the mechanism by which pulsatile perfusion could increase ethanol, and most probably body fluid (i.e. lymph and water) clearance. Increased clearance of these fluids from gill tissue interstitia, especially during exercise, when mean and pulse pressures within gills are increased, would reduce oedema, and possibly the diffusion barrier to gas transfer.

In a description of water fluxes through the walls of blood vessels which are subjected to high mean and pulse pressures (arteries, it appears that the stress of the applied pressure is shared by the tissue water and the tissue matrix or skeleton (Harrison and Massaro, 1976; Kenyon, 1979). Kenyon (1979) analysed the water movements in artery walls during application of pressure pulses in the lumen. His analysis allows predictions to be made about the pressure gradients across the artery. From this, and with the applications of diffusion equations, fluxes of water through arterial tissues can be estimated. Kenyon also calculates the boundary layer in the vessel wall, $\delta$, through which water must move, and across which pressure gradients can be established during application of pulsed pressure. The boundary layer thickness is calculated by using $\delta = \sqrt{D/\beta}$, where $D =$ the
diffusion coefficient \( (\text{cm}^2 \cdot \text{sec}^{-1}) \), and \( f \) = the frequency of pulses (Hz).

From my previous calculations, the diffusion coefficient for ethanol (or water) across the whole trout gill is approximately \( 1 \times 10^{-9} \text{ cm}^2 \cdot \text{sec}^{-1} \cdot D_{H_2O} \)

in these respiratory vessels will be approximately \( 8 \times 10^{-8} \text{ cm}^2 \cdot \text{sec}^{-1} \), if one assumes that \( 7/8 \) of the barrier to water movement is located near the blood, and \( f = 30 \text{ bpm} \) (0.5 Hz) at rest, \textit{in vivo} (Kiceniuk and Jones, 1977). The boundary layer thickness in the lamellar vessels of the fish gill then is equal to approximately 4 \( \mu \text{m} \) at rest. During exercise, when \( f = 0.9 \text{ Hz} \) (Kiceniuk and Jones, 1977), the thickness of the boundary layer is approximately 3 \( \mu \text{m} \). These estimates indicate that pressure gradients and trans-epithelial fluid flows, caused by pulse pressures, could act over a significant proportion of the total blood/water respiratory barrier \textit{in vivo}.

\textit{In vivo} blood pressure, applied to an artery wall, is composed of a pulsatile \( P'_A \), and a non-pulsatile or mean component, \( \overline{P}_A \), such that the total pressure applied, \( P = \overline{P}_A + P'_A \cdot \sin \omega t \). From Darcy's Law of filtration through porous membranes, the filtration rate caused by the mean pressure along, \( \overline{W} \) is equal to \( (k/u) \cdot (\overline{P}_A/h) \), where \( \overline{P}_A/h = \) the pressure gradient across the artery wall, and \( k/u = \) the hydraulic resistance of the vessel wall (see Vargas \textit{et al}., 1979).

For the pulsed portion of the pressure, the filtration rate, \( |W'| = \)

\[
\frac{(k/u) \cdot P'_A \cdot R}{2 \cdot h \cdot \delta},
\]

where \( P'_A \cdot R \) is the pulsatile pressure gradient across \( 2 \cdot h \cdot \delta \) the boundary layer.

By rearrangement of these two components of the filtration rate across the wall, it can be seen that

\[
|W'| = \frac{\overline{W} \cdot R \cdot P'_A \cdot h}{2 \cdot h \cdot \overline{P}_A \cdot \delta}
\]

or,
In resting trout gills, there are estimates of each of these parameters:

\[ R = \text{the radius of the lamellar vascular "vessels" of } 8.36 \times 10^{-4} \text{ cm} \]  
(Farrell, 1979)

\[ f = 30 \text{ beats per minute or } 0.5 \text{ Hz} \]  
(Kiceniuk and Jones, 1977)

\[ P_A' = \text{mean pulse pressure in the lamellae} \]
\[ = 1/6 P_{VA}' = 5/6 P_{DA}' \]
\[ = 10.8 \text{ cm } H_2O \]  
(calculated from Kiceniuk and Jones, 1977)

\[ \delta = \text{boundary layer through which fluid moves with each heart beat} \]
\[ = \sqrt{\frac{D}{f}}, \text{ as defined above} \]
\[ = 4 \times 10^{-4} \text{ cm, and} \]

\[ \overline{P}_A = 43.8 \text{ cm } H_2O \]  
(Kiceniuk and Jones, 1977).

Therefore, \( |W'| = \overline{W} \times 0.25 \). That is, there is 1/4 of the net water flux across the gill endothelium moving across this layer and back during each beat of the heart.

Consider now the effects of changing heart rate and mean and pulse pressures as during exercise. After 50 minutes of swimming exercise at 80% \( U_{\text{crit}} \), \( \overline{P}_A = 55.8 \text{ cm } H_2O \)

\[ f = 52 \text{ bpm or } 0.86 \text{ Hz} \]

\[ P_A' = 16.97 \text{ cm } H_2O \]

\[ R = 9.197 \times 10^{-4} \text{ cm, and} \]
\[ \delta = 3 \times 10^{-4} \text{ cm} \]  
(from Kiceniuk and Jones, 1977).

In this instance, \( |W'| = \overline{W} \times 0.46 \). Now, almost half of the net fluid flux across the gill endothelium is moving across this layer and back with each heart beat. These data provide a mechanism for the observed increase in the clearance of ethanol from gill tissues during pulsatile perfusion.
The "sloshing" of perfusate back and forth across the lamellar endothelium may play an important role in the exchange of solutes within gill tissues.

Diffusion is the principal process whereby transcapillary exchange of solutes takes place. However, Lundgren and Mellander (1967) have shown that increased net capillary filtration rate also increased the rate of tracer (Iodine, Rubidium, Potassium) transfer across capillaries. All measurements of pressures in capillaries have been shown to be pulsatile (Intaglietta et al., 1970; Cokelet, 1980), and are not static. As most measurements of capillary permeability and filtration rates have been made during non-pulsatile perfusion or pressure application, perhaps an important para-cellular pathway for solute exchange has been ignored.

It is well known that resting trout do not perfuse all lamellae at all times (Booth, 1978), *in vivo*. Non-perfused lamellae would have reduced, if not zero, pulse pressure, and the exchange of solutes within the gill tissues of non-perfused lamellae may be much reduced. This would ultimately result in the conservation of water and ions, depending on the medium, by decreasing exchange efficiency, and still may play an as yet unresolved role in determining the diffusion barrier to gas transfer in gills, even though gills are primarily perfusion limited.
SECTION IV
OXYGEN TRANSPORT DURING EXERCISE
IN THE RAINBOW TROUT
INTRODUCTION: SECTION IV

Exercise in fish can be categorized as sustained, prolonged or burst swimming (Hoar and Randall, 1978). Further discussion, however, will concentrate on those cardiovascular changes in the rainbow trout, *Salmo gairdneri*, associated with prolonged swimming exercise at 80% of their critical velocity (80% *U* _crit_). This level of steady-state exercise is characterized by aerobic metabolism.

When rainbow trout are forced to swim at levels which approach their critical velocity, oxygen uptake increases by a factor of 6 to 12 times the value found at rest. The arterial-venous oxygen content difference increases by 2 to 3 times, and the cardiac output increases by 3 times the resting value (Kiceniuk and Jones, 1977). Ventilation volume and rate also increase, while oxygen utilization from the water flow across the gills drops from an average 80% at rest, to as low as 10 - 30% during exercise (Shelton, 1970). The oxygen transport system of the exercising trout however, still has the capability of increasing its delivery up to 12 times that of the standard metabolic rate (Brett, 1964, 1965, 1972), through cardiorespiratory alterations.

At the onset of exercise, there is a large increase in the mean and pulse input blood pressure to the gills. These pressures peak between 10 to 15 minutes after the onset of exercise and then decline to levels which still are several mm Hg above the resting value (Stevens and Randall, 1967a). Similar but smaller changes are observed in the dorsal aorta. Although ventral aortic pressure is elevated during exercise, and the gill vascular bed is elastic and distensible (Farrell et al., 1979), there is little change in gill resistance to flow. There is, however, a large reduction in systemic vascular resistance to flow, associated with a rise in blood flow to the working muscles during exercise. Haemoglobin content, plasma protein
levels (Stevens, 1968a) and haematocrit all increase during exercise, possibly associated with a release of erythrocytes from the spleen, and a reduction in plasma volume due to an increase in urine formation (Wood and Randall, 1973). An increase in haemoglobin level would augment the oxygen carrying capacity of the blood during exercise. These and other changes in the cardiorespiratory systems of fish during exercise have been presented previously (see Table 23), and are reviewed by Jones and Randall (1978). There are surprisingly few qualitative differences between the adjustments made by man and fish in order to cope with this stress.

The increase in cardiac output is achieved by an increase in both heart rate and stroke volume, mediated in part by a release of vagal cholinergic tone, and perhaps an increase in circulating catecholamines and sympathetic tone, associated with a rise in venous pressure and flow returning to the heart. Because of the single-circuit design of the fish cardiovascular system, all this cardiac output must probably pass from the heart directly through the gills; the site of gas exchange. The gills therefore must be able to adjust in such a manner so as to ensure adequate blood oxygenation, in order to meet the increased metabolic demands of the fish during exercise. At rest, only about 60% of the gill secondary lamellae are perfused in a rainbow trout (Booth, 1978). It is known if lamellar recruitment occurs during exercise. However, it seems probable that all lamellae will be perfused continuously during exercise, as a rise in ventral aortic pressure is known to cause lamellar recruitment in vitro (Farrell et al., 1979), and increases in blood catecholamines cause lamellar recruitment in vivo (Booth, 1979). It is not known if circulating catecholamine levels increase and remain elevated above normal during prolonged exercise. However, it is known that they do so if fish are stressed (Nakano and Tomlinson, 1967; Mazeaud et al., 1977; Wahlqvist and Nilsson, 1977). Increases in ventral aortic pressure, changes in blood catecholamines, or activity in adrenergic
Table 23. Summary of the cardiorespiratory changes associated with submaximal, aerobic exercise in man and fish.

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<th></th>
<th>IN MAN</th>
<th>IN FISH</th>
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<tr>
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<td>(from numerous sources)</td>
<td>(from numerous sources)</td>
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<td>Heart Rate</td>
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<td>Cardiac Output</td>
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<td>Arterial Pressure</td>
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<td>Diastolic</td>
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<td>Ventral Aortic</td>
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<tr>
<td>Systolic</td>
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<tr>
<td>Blood Pressure</td>
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<td>Diastolic</td>
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<tr>
<td>Dorsal Aortic</td>
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<td>Systolic</td>
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<td>Blood Pressure</td>
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<td>Diastolic</td>
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<tr>
<td>Arterial pH</td>
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<td>Haematocrit</td>
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<td>Haemoglobin</td>
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<td>Oxygen Concentration</td>
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<tr>
<td>Consumption</td>
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<td>Ventilation Rate</td>
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<td>Ventilation Volume</td>
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<td>A-V O₂ Differences</td>
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<tr>
<td>Blood Gas Tensions</td>
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<td>(P_a O₂)</td>
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<tr>
<td>(P_v O₂)</td>
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<tr>
<td>Circulating Cathecholamines</td>
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<td>Total Peripheral Resistance to Blood Flow</td>
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<td>Blood Flow Distribution</td>
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<td>to Working Muscles</td>
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<td>To Splanchnic Region</td>
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<td>To Gas Exchange Organ</td>
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<td>To Heart and Brain</td>
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</table>
fibres innervating the gill vasculature (Gannon, 1972) therefore may cause lamellar recruitment in exercising trout. The rise in ventral aortic pressure probably results in a rise in lamellar blood pressure as well, and this may be enhanced by dilation of the afferent lamellar arterioles due to possible effects of increased adrenergic activity or circulating catecholamines in dilating the smooth muscle sphincter around the afferent lamellar arterioles (Farrell, 1981). An increase in lamellar pressure also causes a more even distribution of blood in the secondary lamellae, and an increased perfusion of the exposed area of the lamellae (Farrell et al., 1980). In addition, there may be some thinning of the blood/water barrier as a result of expansion of the lamellar blood sheet, associated with a rise in lamellar pressure. There is no direct evidence for these changes in exercising fish, but if they do occur, then the diffusion capacity of the gills for oxygen should increase during exercise. The carbon monoxide diffusing capacity of the catfish gill (*Ictalurus nebulosus*) on the other hand, has been measured, and has been shown to increase in hypoxia, compared with that measured in normoxia (Fisher et al., 1969). The mass transfer of carbon monoxide ($M_{CO}$) across the gills of *I. nebulosus* also is found to be proportional to the partial pressure of carbon monoxide in the water. Based on these, and other observations, it was concluded that oxygen uptake, like $M_{CO}$, in fish gills is diffusion limited (Hughes, 1972, 1980). The thicker epithelium of the gill, compared to that of lung, and the boundary layer of unstirred water next to the respiratory surface of fish impose additional diffusion limitations (Hills and Hughes, 1970). Thus the diffusion distance between the medium and blood is even larger than simply the thickness of the epithelium in fish gills. Indeed it is obvious from the literature that blood in fishes never achieves equilibration with the partial pressure of oxygen in the water (Randall, 1970). There also is a large oxygen content difference between air (209.5 ml·L$^{-1}$·STP) and water (6.34 ml·L$^{-1}$ fresh water
at 20°C). Therefore, fish must ventilate at least 30 times more water
minute volume to obtain an equivalent amount of oxygen as air breathing
animals. But, it has been shown that during aquatic hypoxia, when the heart
rate declines, but the cardiac output remains constant and ventilation
increases, gas transfer across the gills of trout is "facilitated" (Randall
et al., 1967; Holeton and Randall, 1967a,b). Therefore, although there
are differences in heart rate and cardiac output between exercise and
hypoxic exposure in trout, both stroke volume and ventral aortic pressures
increase, and this may lead to a rise in the gill oxygen diffusing capacity,
shown in the hypoxic catfish, and supposed in exercising rainbow trout.

Dorsal aortic blood in normoxic fish however, is always greater
than 95% oxygen saturated, despite the fact that arterial oxygen partial
pressure is never equal to that of the surrounding water. Additionally,
during swimming exercise, when the cardiac output and ventilation are
increased, the measured oxygen uptake is augmented, not only by presumed
decreases in the diffusion barrier to gases across the gills, but also, to
a lesser extent, an increased perfusion of a greater respiratory surface
area, brought about by possible lamellar recruitment. It is suggested that
cardiovascular adjustments during this imposed stress are for the purpose
of decreasing the diffusion barrier, such that oxygen uptake can be maintained
at sufficient levels to meet the metabolic requirements of the fish.

Blood transit times through the secondary lamellae are between 1 and
3 seconds in fish (Hughes et al., 1981; Randall, 1981). From lamellar volumes,
derived from plastic casts of the gills, and my 51Cr-labelled red cell blood
volume measurements, and using the cardiac output values from Kiceniuk and
Jones (1977), corrected for a median 27% of resting oxygen uptake being
utilized by the gill tissues and not entering the blood (see Discussion,
Section III), then the calculated blood transit time in resting trout is
3 seconds, if all lamellae are perfused. If however, only 2/3 of these
lamellae are perfused; a more probable situation (for *in vivo* data, see Booth, 1978; *in vitro* data, see Farrell *et al.*, 1979), then this time is reduced to 2 seconds. It will be reduced even further, to one second during exercise, when it is assumed that all the secondary lamellae are being perfused. Since it appears that the stroke volume of the heart probably never exceeds gill lamellar blood volume, even during maximal exercise, a complete cardiac cycle will occur within the time for blood to flow through the lamellae, even though blood velocity changes during each heart beat. As long as all of the cardiac output passes through these secondary lamellae, and the cardiac output and volume of perfused lamellae are known, transit times can be accurately determined. However, the fate of this cardiac output distribution once out of the gills still is a matter for speculation, since not all cardiac output goes directly to the dorsal aorta. For example, the general head circulation, the hypobranchial muscles, and the heart must also be supplied from efferent branchial arteries.

Arterial oxygen tensions nonetheless remain within the same range over a wide range of prolonged swimmings and the blood remains more than 95% oxygen saturated. Even at the most rapid gill transit times, the diffusion capacity of the gills therefore must be adequate to achieve haemoglobin oxygen saturation. That arterial blood never equilibrates with inflowing water $P_{O_2}$ has been pointed out previously. The reasons for this still are not clear, but must in part be due to a large diffusion barrier, consisting of the unstirred water layer, the gill epithelium, the pillar cell and the blood space. This epithelium is much thicker than that in the lung presumably because the cell layer is much more active; being involved in ion and water movements as well as gas transfer. 27% of the oxygen leaving the water at rest in trout is utilized by the gill epithelium and only 73% enters the blood to be distributed to the systemic circulation. The utilization of
oxygen by the gill tissues also will increase the apparent diffusion barrier between water and blood. Why some fish have a $P_aO_2$ of 120 mm Hg, while others have 95 to 100 is probably a reflection of the size of the diffusion barrier and the rate of oxygen consumption by the gill tissues. Since hypercapnia results in a marked increase in $P_aO_2$ in the dogfish (Randall et al., 1976), this could indicate a reduction in gill metabolism due to the prevailing acidic conditions associated with hypercapnia.

RESULTS AND DISCUSSION IV

In the spontaneously ventilating, blood-perfused trout preparations, an increase in blood flow through the gills was associated with an increase in oxygen uptake rate, but not with any change in arterial oxygen tension. These gills therefore appear to be perfusion limited for oxygen uptake. The diffusion capacity of the gills for oxygen is probably reduced in resting fish to ameliorate water and ion transfer, as discussed in Section III. During exercise, any possible increase in the diffusing capacity associated with a rise in cardiac output, and a reduction in venous oxygen content permits a marked rise in oxygen uptake across the gills. An increase in diffusing capacity therefore may be only of significance at high levels of exercise, because manipulations of input pressure in resting, blood-perfused trout neither increases in mean nor pulse pressures had any effect on oxygen uptake rate or $P_aO_2$.

The input blood to the systemic circulation therefore remains nearly oxygen saturated over a wide range of prolonged swimming speeds, and generally shows small increases in mean and pulse pressure during exercise. There is however, a presumed marked increase in blood flow to red muscle during exercise, a smaller increase in kidney blood flow, and flow to the "white" or mosaic muscle actually increases during swimming in the rainbow trout. This white muscle mass constitutes 66% of the total body weight (B.W.)
(Stevens, 1968a), consisting of 64.5% B.W. white fibres and 1.5% B.W. red fibres (Webb, 1975). Therefore, the trout has 2.5% B.W. red fibres in the lateral red muscle and 1.5% B.W. embedded in the white muscle. If one assumes the increase in blood flow to be the same to all red muscle fibres during exercise, then more than all of the flow increase measured within the mosaic muscle is directed towards the red fibres, and flow to the white fibres may be severely curtailed at this level of activity ($80\% U_{\text{crit}}$).

That the factors leading to blood redistribution during steady-state exercise are quite complex and as yet, unresolved, has been pointed out earlier (Section I). In most other studies, the rise in dorsal aortic pressure is small (Jones and Randall, 1978). The elasticity of the systemic circuit would have to be very high to respond to such small changes in input pressure in order to allow systemic resistance to fall in proportion to the increase in flow. However, I observed larger changes in dorsal aortic pressure than either Kiceniuk and Jones (1977) or Stevens and Randall (1967a), on the same species. There were differences in the exercise regime and the extent of cannulation, and these may explain some of the observed differences. Although the larger increase in systemic input pressure observed in my experiments certainly will play some role in increasing systemic blood flow, it probably does not account for all the increase, nor can it account for the observed redistributions among other tissues.

The decrease in liver, stomach and spleen blood flow can be accounted for by constriction of the coeliacomesenteric artery (Holmgren and Nilsson, 1974; Johansson, 1979). An adrenergically innervated muscle sphincter at the base of this artery has been shown to exist (Gannon, 1972). Constriction of the sphincter therefore would account for the flow changes observed during exercise. Contraction of the muscular spleen itself, which also is under adrenergic control (Nilsson and Grove, 1974; Holmgren and Nilsson, 1975)
would account for the noted reduction in splenic volume. This splenic contraction therefore is a small contributing cause to the observed haemocontraction during exercise, by adding red blood cells to the general circulation.

It has been discussed previously that it is difficult to ascribe causes to the increase in muscle blood flow during exercise. The trunk vasculature of the trout shows powerful α-adrenergic constrictor responses to infused catecholamines (Wood et al., 1978). The muscle vasculature lacks any direct adrenergic nerve innervation. Therefore, if these receptors are located within muscle blood vessels, they must respond to circulating catecholamines. These are known to increase in stressed fish, and may increase in exercising fish, thereby causing systemic vasoconstriction. What is observed in intact fish however, is a very significant vasodilation associated with increased blood flow to red muscle fibres. Davie (1981) has investigated the responses of a perfused, isolated eel tail preparation to catecholamines and reported the existence of both α- and β₂-adrenoceptors. Davie suggested that the β₂-receptors, which caused vasodilation, might be located in red muscle and the others could be associated with white fibre blood vessels. Adrenergic nerves and/or circulating catecholamines therefore may be involved in coeliacomesenteric and splenic constriction, possible vasoconstriction in white muscle vasculature, and vasodilation in red muscle. However, it is unlikely that this is a complete description of the control mechanisms involved, since infusion of catecholamines do not mimic the steady-state systemic response seen in intact trout during exercise. Active hyperaemia probably is the major cause of the marked reduction in vascular resistance to flow in red muscle. Both muscle contraction itself, and movements of the tail also may augment muscle blood flow (see Randall, 1968, for details of mechanisms) during swimming exercise in trout.
Exercise at 80% $U_{\text{crit}}$ in trout is largely aerobic and is associated with a large increase in oxygen uptake, most of it presumably being utilized by the working muscles. The oxygen is delivered to these working muscles (primarily the red muscle fibres) by the increased blood flow. The calculations presented in Tables 24, 25, and 26, based on the flow distributions reported previously in this thesis, indicate that the increase in flow is adequate to supply the increased oxygen requirements of the muscles. 93% of the increase in oxygen taken up by the fish at 80% $U_{\text{crit}}$ is delivered to the muscle, and red muscle oxygen utilization increases by a factor of 12 at this level of swimming activity. The calculations, assuming resting rates of oxygen extraction from non-muscle tissues during exercise, indicate 96% oxygen utilization from blood flowing through red muscle. This would mean that red muscle $P_{O_2}$ must be reduced to only 1 or 2 mm Hg during exercise. The resulting calculated mixed venous oxygen content during exercise shows very close agreement with recorded values at the same swimming speed, on the same species (Table 25).

I have demonstrated that the circulatory system of the rainbow trout, *Salmo gairdneri*, is efficient in its ability to distribute the necessary oxygen to the tissues during prolonged, aerobic swimming exercise. The numerous cardiorespiratory adjustments, involving both systemic blood flow redistribution, and changes within the branchial vasculature during exercise account for this animal's ability to maintain swimming activity in the face of increased oxygen demands by the working muscle.
Table 24. Calculation of muscle and systemic blood flow (ml·min⁻¹·kg⁻¹) during rest and exercise in rainbow trout.

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>EXERCISE (80% (U_{crit}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red + White muscle</strong></td>
<td>5.49</td>
<td>10.15</td>
</tr>
<tr>
<td><strong>White muscle</strong></td>
<td>4.81</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Red muscle</strong></td>
<td>0.68</td>
<td>9.73</td>
</tr>
<tr>
<td><em>(in white muscle)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Red Muscle</strong></td>
<td>1.14</td>
<td>16.21</td>
</tr>
<tr>
<td><em>(lateral)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rest of Body</strong></td>
<td>6.22</td>
<td>11.79</td>
</tr>
<tr>
<td><strong>Total blood flow</strong></td>
<td>12.85</td>
<td>38.15</td>
</tr>
<tr>
<td><em>(cardiac output)</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[a\] Assuming that white plus red muscle (66% of body weight - Stevens (1968a) consists of 64.5% B.W. white muscle and 1.5% B.W. red muscle (Webb, 1975), and that red muscle flow in this tissue is the same as calculated for lateral red muscle.

\[b\] Data from Kiceniuk and Jones (1977), corrected for 27% of resting oxygen uptake by gills when calculating cardiac output using the Fick principle.
Table 25. Oxygen uptake and distribution to the systemic circulation of resting and exercising rainbow trout.

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>EXERCISE (80% $U_{\text{crit}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total $O_2$ Uptake$^a$</td>
<td>0.409</td>
<td>2.969</td>
</tr>
<tr>
<td>(ml·min$^{-1}·$kg$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial $O_2$ content$^b$</td>
<td>10.4</td>
<td>10.2</td>
</tr>
<tr>
<td>(vol %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous $O_2$ content$^b$</td>
<td>7.1</td>
<td>2.9</td>
</tr>
<tr>
<td>(vol %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue $O_2$ extraction (%)</td>
<td>31.7</td>
<td>71.6</td>
</tr>
<tr>
<td>$O_2$ uptake by rest of body during exercise, assuming 31.7% $O_2$ extraction from blood</td>
<td>$= 0.381$ ml·min$^{-1}·$kg$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$O_2$ uptake by muscles only</td>
<td></td>
<td>$= 2.588$ ml·min$^{-1}·$kg$^{-1}$</td>
</tr>
<tr>
<td>Total $O_2$ delivered by blood to muscles during exercise</td>
<td>$= 2.689$ ml·min$^{-1}·$kg$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Oxygen extraction from blood (%)</td>
<td></td>
<td>$= 96.2$</td>
</tr>
<tr>
<td>Mean venous oxygen content during exercise (calculated)</td>
<td>$= 2.42$</td>
<td></td>
</tr>
<tr>
<td>Mean value (vol. %)$^b$</td>
<td></td>
<td>$= 2.9 \pm 1.47$</td>
</tr>
</tbody>
</table>

$^a$Data from Kiceniuk and Jones (1977) corrected for 27% $O_2$ uptake by gills in calculating $O_2$ uptake by blood,

$^b$Data from Kiceniuk and Jones (1977).
Table 26. Increase in oxygen uptake by the whole fish and by red muscle at rest and during exercise at 80% $U_{\text{crit}}$.

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_2$ uptake</td>
<td>0.409</td>
<td>2.969</td>
</tr>
<tr>
<td>(ml·min$^{-1}$·kg$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$O_2$ uptake by muscle</td>
<td>0.219$^a$</td>
<td>2.588</td>
</tr>
<tr>
<td>(ml·min$^{-1}$·kg$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in $O_2$ uptake</td>
<td>$= x 12$</td>
<td></td>
</tr>
<tr>
<td>by muscle (factor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in $O_2$ uptake</td>
<td>$= 2.369$</td>
<td></td>
</tr>
<tr>
<td>by muscle (ml²·min$^{-1}$·kg$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in $O_2$ uptake$^b$</td>
<td>$= 2.56$</td>
<td></td>
</tr>
<tr>
<td>by whole fish at 80% $U_{\text{crit}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ml·min$^{-1}$·kg$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$%O_2$ increase going to working muscle at 80% $U_{\text{crit}}$</td>
<td>$= 93%$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Assumes an extraction rate of 31.7% i.e. the same as that for whole animal at rest.

$^b$Data derived from Kiceniuk and Jones (1977).
LITERATURE CITED
LITERATURE CITED


Krogh, A. 1919. The number and distribution of capillaries in muscle with calculations of the oxygen pressure head necessary for supplying the tissue. J. Physiol. 52: 409 - 415.


Peyraud-Waitzenegger, M. 1979. Simultaneous modifications of ventilation and arterial Po2 by catecholamines in the eel, Anguilla anguilla L. Participation of α and β effects. J. Comp. Physiol. 129: 343 - 354.


APPENDIX A

EFFECTS OF CORONARY ARTERY ABLATION ON EXERCISE PERFORMANCE IN RAINBOW TROUT
INTRODUCTION: APPENDIX A

Fish hearts commonly are referred to as being venous hearts, since they only pump venous blood around a "simple", single circulation; the vascular beds being arranged in series with each other. The spongy, trabeculated inner myocardium is supplied by lacunar venous blood, while the compact cortical myocardium is richly supplied with coronary vessels (Ostadal et al., 1970). An estimated 39% of the ventricular mass is comprised of this compact cortical layer in Salmo gairdneri (Santer and Walker, 1980). The coronary arterial blood supply to the cortical layer is derived from the second efferent hypobranchial arteries and the dorsal aorta, and is comprised of two branches (Watson and Cobb, 1979). One branch supplies the pericardium and the whole adventitia of the bulbus, while the other runs along the ventral surface of the ventral aorta (the prominent coronary artery seen in trout). Both run together to supply arterial blood to the ventricle.

The heart is an aerobic tissue in most vertebrates, although those species which appear less active than trout, and have higher tolerances to hypoxia, can maintain myocardial contractility longer in vitro, at low levels of oxygen (Gesser, 1977). On the basis of the weight of the ventricle, and assuming this tissue to have an \( O_2 \) demand similar to that of stimulated red muscle in vitro, or that of the human heart, Cameron (1975) estimated that the heart's oxygen consumption has an upper limit of 4% of the total oxygen uptake of the fish at rest. The question however still remains as to the functional advantage of having the bulk of the contractile tissue of trout devoid of an arterial coronary blood supply, while the cortical layer is so richly vascularized. If the patency of an arterial blood supply is necessary for the maintenance of myocardial contractility in an active fish like the rainbow trout, then given the heart must work harder during exercise, ablation of this coronary artery may affect the level of exercise performed by the fish. The heart may be unable to increase in work capacity during
periods of increased demand, as during exercise, because of a decrease in the 
supply of arterial oxygen to the myocardium. The following experiments were 
performed in an attempt to test the previous hypothesis on exercising rainbow 
trout.

MATERIALS AND METHODS
A total of 26 rainbow trout (316 ± 15.6 g) were used for these 
experiments. The critical swimming velocity of fish in a tunnel respirometer 
was determined as described previously (see page 6). Once \( U_{\text{crit}} \) had been 
determined for each fish, 10 of these fish (292.0 ± 9.0 g) then were selected 
at random and surgically prepared as follows. Each fish was anaesthetized 
and placed supine on an operating table (as described in Materials and Methods 
pp. 9 to 10). A small mid-ventral incision was made through the skin and 
underlying pectoral musculature (Fig. 22A) to expose the pericardium containing 
the heart (Fig. 22B). Care was taken not to disrupt the pectoral girdle 
symphasis. The pericardium was opened carefully to expose the coronary 
artery (Fig. 22C) seen on the ventral surface of the ventral aorta (at arrow). 
This vessel was ablated by electrocautery (Birtcher Electroostasis Unit). 
Thus the main arterial supply to the ventricle was disrupted. The muscula-
ture was sutured closed, and the skin then was closed separately (Fig. 22D 
and 22E). The operated fish were allowed to recover and heal for 5 - 7 days 
before the \( U_{\text{crit}} \) procedure was repeated. Immediately after fish had regained 
equilibrium following anaesthesia, they started to use their pectoral fins in 
a normal manner, indicative of little impairment of muscle function due to 
surgical procedures. Also, six of the previous 26 fish (342.0 ± 21.0 g) were 
sham-operated in a manner similar to that just described, except that the 
coronary circulation was left intact. Recovery and subsequent \( U_{\text{crit}} \) re-evalu-
uation were the same as those of operated fish.

Intact, operated and sham-operated hearts were examined visually
Figure 22. Photographs of the sequence of events during the operation where the coronary artery was ablated.

A. Small incision made in skin and epaxial pectoral musculature, retracted to expose pericardium

B. Pericardium carefully opened to expose heart with coronary artery clearly visible on ventral surface of ventricle and bulbus, running to ventricular mass

C. Coronary arterial supply to ventricle is cauterized and no evidence of any large patent vessel is visible

D. Pectoral musculature sutured closed over the heart

E. Skin over heart is closed separately from musculature

After 5 - 7 days recovery, fish show little sign of tissue necrosis due to the operational procedure and healing is complete.
immediately after exercise. Fish were sacrificed following fatigue, after being removed from the downstream electrified grid of the respirometer, by a sharp blow to the head. In post-mortem autopsy, hearts were excised and placed in 20 ml of 2% glutaraldehyde in 0.14 M Sorensen's phosphate buffer for at least 24 h, so as to fix the tissue. Whole hearts were subjected to progressive alcohol dehydration, through xylene, and embedded in paraffin. These tissues then were sectioned (at 8 μm) on a steel blade microtome and sections were mounted on warmed glass slides. These were stained with haemotoxylin and eosin, and all sections were viewed and selected sections photographed using a Zeiss Photomicroscope.

RESULTS

Following 5 - 7 days recovery from surgical procedures for coronary ablation, U_{crit} values were not different (p<0.05) from values obtained from fish which were sham-operated, or completely intact (Table 27). Bigger fish attained greater absolute speeds (cm·sec^{-1}), but all fish tested attained the same relative U_{crit} (fl·sec^{-1}). The data comparing intact and operated fish's swimming performance are graphically presented in Figs. 23 and 24. The log U_{crit} (cm·sec^{-1}) versus cumulative percentage of fish "fatigued" (on probit scale), both from original data (Fig. 23A), or from data transformation where critical velocities were grouped into 10 cm·sec^{-1} intervals, indicate no differences (p<0.01) between the two lines generated. In addition, a significant linear relationship between fork length and U_{crit} (fl·sec^{-1}) was found (p < 0.05), even over the very limited range of fork lengths for the fish used in this study. This relationship (Fig. 24) indicated that the smaller fish had higher U_{crit} values than did larger fish, when expressed in fork lengths·sec^{-1} (see also Brett and Glass, 1973).

Post-mortem examination of the pericardial cavity of the rainbow trout indicated that the cauterized coronary artery did remain closed during
Table 27. Effects of coronary artery ablation on swimming exercise performance in rainbow trout.

<table>
<thead>
<tr>
<th></th>
<th>Fork length (fl - cm)</th>
<th>Weight (g)</th>
<th>$U_{crit}$ cm·sec$^{-1}$</th>
<th>$U_{crit}$ fl·sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NORMAL (N = 26)</td>
<td>SHAM OPERATED (N = 6)</td>
<td>CORONARY ABLATION (N = 10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X 29.2</td>
<td>316.4</td>
<td>73.8</td>
</tr>
<tr>
<td>± S.E.M.</td>
<td></td>
<td>± 0.44</td>
<td>15.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Figure 23. Logarithmic-probit plot of critical swimming velocity versus percent fish fatigued at that velocity. Upper frame represents the original data, and indicates that the operated fish □ and the intact fish ● lie along the same straight line. In the lower frame, the original data were grouped into 10 cm·sec⁻¹ intervals, with the attendant n numbers included. The two lines are not different. Rest of frame as in upper one.
Figure 24. Linear representation of the relationship between critical swimming velocity ($U_{\text{crit}}$) and fork length for intact $\bullet$, and operated fish $\square$. The equation of the line is only for data from intact fish ($N = 26$) while the operated fish data ($N = 10$) is presented to show that they too lie within the range described for intact fish.
\[ y = -0.056x + 4.16 \]
\[ r = 0.4004 \]
exercise, whereas after exercise in intact fish, this vessel was visibly dilated, and patent. Histological examination of cross-sections of the ventral aorta/bulbus indicated that the coronary vessel was the dominant and appeared to be the only vessel visible in all sections in "intact" fish (Fig. 25-1 and -2). Once the main arterial supply was cauterized however, numerous smaller vessels around the outer layer of the ventral aorta appeared to open up, with red blood cells inside their lumens (Fig. 25-3, -4, and -5). These could be traced along their course to the myocardium. No attempt was made to quantify the total cross-sectional area contributed by these "new" vessels, compared to that of the patent coronary artery. Nor was any attempt made to ascertain the time course of "new" vessel patency, following coronary artery ablation.

DISCUSSION

Swimming exercise in trout is associated with a 3 to 6-fold increase in the cardiac output, over that found at rest (Kiceniuk and Jones, 1977). Although the heart must work harder during exercise, the ablation of the coronary artery has no effect on exercise performance in these fish, compared to intact trout. The inner myocardial mass acquires the necessary metabolic oxygen for contractility from the venous blood pool within the ventricular lumen. It also has been shown that this inner myocardium of the trout (Salvelinus fontinalis) has the ability to aerobically metabolize lactate, produced as a byproduct of anaerobic metabolism (Lanctin et al., 1980) in the myotomal mass of these fish. The venous $P_{O_2}$ is much lower during exercise than at rest in trout (Kiceniuk and Jones, 1977) and therefore, the ventricle must increase oxygen extraction efficiency during exercise (Randall, 1970).

Oxygen to mammalian hearts is supplied primarily via an extensive coronary circulation. If man has a small coronary arteriolar blockage, he runs the risk of myocardial infarction, due to local ischaemia. Exercise with
Figure 25. A(1). Cross-section through the bulbus arteriosus of the rainbow trout, to show the prominent coronary artery (CA) on the ventral surface. In the normal trout, no patent vessels are visible in the adventitia of the bulbus (2), other than the coronary artery. B(1,2,3). The bulbus, 5 - 7 days recovery from having the main coronary artery supply completely ablated using electrocautery. In this case, the main coronary supply is no longer patent, but small vessels in the adventitia (at arrows) are open, and these can be traced to the ventricular myocardium. Sections cut at 8 \( \mu \)m, stained with H and E.
an arterial blood supply to the heart which is interrupted, therefore could lead to some rather serious consequences. Given the differences between myocardial oxygen delivery in fish and man, but that in all other respects, cardiorespiratory adjustments to exercise are qualitatively similar, one also might have expected some change in the level of exercise performance in trout with an ablated coronary artery. Since this is not the case when the main arterial oxygen supply to the trout heart is interrupted, the heart may have adopted an alternate metabolic pathway, anaerobiosis, to supply the energy requirements for maintained contractility. This seems unlikely since Gesser (1977) has shown that trout hearts, in vitro, are especially intolerant of low oxygen levels, and rapidly diminish in their strength of contraction when exposed to such conditions. It is noted however, that when the coronary artery is ablated in trout, small arterioles in the adventitia of the ventral aorta/bulbus appear to become patent, following 5 to 7 days of recovery from surgery. These therefore maintain arterial blood supply to the ventricular cortex, in place of the main blood pathway. While the precise reason for the arterial vascular arrangement of the trout heart is not clear, it does seem that the maintenance of coronary arterial blood flow is important to these fish at all times.

What is not known from this study is whether these trout would have been able to attain the same level of exercise performance during the intermediated period, just following coronary artery ablation, assuming the surgery itself would not have been a perturbing factor in these tests. The fish in the present study were allowed to recover for several days before being tested. The small patent vessels noted after this time in my experiments may have taken some time to open up following ablation of the main coronary arterial blood supply. In this case, the heart may have used the alternate metabolic pathway, alluded to earlier, to sustain contractility. However, anaerobic capacity of the myocardium of trout is very limited,
and fish may not have been able to exercise at all in these early stages of recovery. Alternatively, these small vessels immediately became patent, so that arterial blood flow to the heart was continuous, even when the coronary artery is ablated. However, the end result is that the trout heart does have the capacity to have its vascular blood supply adjust over a period of several days in order that arterial blood oxygen delivery is maintained, and exercise performance is not affected by coronary artery ablation.
APPENDIX B

A MODIFIED PERFUSION PUMP
MODIFICATION OF A PISTON-TYPE PERFUSION PUMP FOR DELIVERY OF LOW FLOW RATES

Harvard Blood Pumps (model 1405; Harvard Apparatus Co., Inc., Mass. U.S.A.) are insensitive to back pressure, and produce flow in a fixed phase of systole:diastole of 1:2. However, these pumps and their associated valves were found to not operate well at low flow rates, when stroke volumes also were small. Therefore, a replacement piston system, with newly designed valves was constructed, to allow the delivery of low minute flow, with stroke volumes in the range of 0.05 - 0.3 ml per stroke.

The existing connecting shaft was attached to the plunger of a disposable 1 cc syringe (Tuberculin; B & D Co., Mississauga, Canada) by a U-shaped steel connector (Fig. 26). The barrel of the syringe was cut off at the 1.0 cc mark and clamped into a machined Perspex block (Fig. 26). This block, with the syringe barrel, then was attached to the existing valve mounting bracket on the pump chassis by the machine bolts provided. The syringe barrel was connected to a redesigned remote pumping head by a length PE 240 tubing (1.67 x 2.42 mm) (Clay Adams, Intramedic). Figure 27A is a diagram of this pumping head, which is similar in concept to that available from Harvard Apparatus Co.

Newly designed valves, which attach to input and output Luer-Lok needles on the redesigned remote pumping head, are shown in Fig. 27B. The checking mechanism of these unidirectional valves was provided by a piece of Latex rubber dental dam stretched lightly over a collar cut from a 1.5 ml Eppendorf centrifuge tube (Brinkmann Co.). The collar and dam (valve assembly) was press-fitted over the tip of a 5 cc disposable Luer-Lok syringe (B & D Co.), and then this assembly was mounted inside a housing constructed from the tip of two 10 cc disposable Luer-Lok syringes (B & D Co.).

When assembled and attached to a Harvard Blood Pump, the new remote pumping head and valve unit allowed only unidirectional flow, regardless of its orientation, and flow rates were independent of outflow pressure.
at stroke volumes as small as 0.05 ml. Also, there was no discernable fluid backflow during the suction (diastolic) phase of the pumping cycle. All other features of the Harvard Blood Pump (i.e. systole:diastole ratio, pressure profile, frequency and stroke volume adjustments) were unaffected by these modifications. Valves were therefore inexpensive and easily constructed.

Figure 26. Modified clamping block and syringe barrel assembly.

A. Side view

B. Top view
clamping block

for 10-32 bolts

1 cc syringe

HARVARD pump shaft

U-connector

5 cm

2 cm

for chassis guide-pin

1 cm
Figure 27. A. Side (a) and frontal (b) views of the redesigned remote pumping head. This was manufactured from machined Perspex.

B. The checking mechanism (a) and the unidirectional valves (b) in exploded view, which were attached to inflow and outflow of the remote pumping head.

All joints and inserts were sealed with Araldite® epoxy cement.
RESEARCH INTERESTS:
Comparative physiology of lower vertebrates: controls and regulations of cardiorespiratory adjustments affecting gas transfer during exercise in fish. Aspects of gas exchange in bimodally breathing fishes.

PUBLICATIONS:


PUBLICATIONS: (Continued)


Davie, P.S. and C. Daxboeck. Effects of pulse pressure on fluid exchange between blood and tissue in trout gills. (MS submitted to Can. J. Zool.)


Daxboeck, C., D.J. Randall and D.R. Jones. Cardiac output distribution during rest and steady-state swimming exercise in the rainbow trout: A comparison of Rubidium-86 and radiolabelled microsphere tracer techniques. (MS submitted to Am. J. Physiol.)


PRESENTATIONS:


