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PHYSICAL AND ADRENERGIC FACTORS AFFECTING SYSTEMIC VASCULAR RESISTANCE IN THE RAINBOW TROUT: A COMPARISON WITH BRANCHIAL VASCULAR RESISTANCE

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SUMMARY

The passive distensibility and adrenergic reactivity of the systemic vascular resistance (*Rs*) in *Salmo gairdneri* have been studied using perfused trunk preparations, and the data compared with previous results on the branchial resistance (*Rg*). At normal levels of efferent blood pressure, *Rs* is relatively more distensible than *Rg* in response to afferent pressure increases, but this difference may not be important *in vivo*. α-adrenergic constrictory receptors predominate in *Rs*, in contrast to β-adrenergic dilatory receptors in *Rg*; a significant α-adrenergic tone in *Rs* is lost during perfusion. *Rs* is far less sensitive than *Rg* to circulatory catecholamine levels. It is suggested that the sympathetic nervous system, rather than plasma catecholamines, provides the effective adrenergic control of *Rs in vivo*.

INTRODUCTION

Since the classic demonstration by Keys & Bateman (1932) of the effects of adrenaline on the branchial and systemic vasculature of the eel (dilatory and constrictory respectively), a large number of studies have been made on the gills in various teleosts. However, the vascular resistance of the trunk is much larger than that of the gills *in vivo* (Stevens, 1968*a*) and may be expected to be dominant in overall haemodynamic control. With the exception of investigations on the circulation of the swimbladder (Fange, 1953; Stray-Pedersen, 1970; Nilsson, 1972) there has been surprisingly little work on adrenergic function in the systemic vasculature of teleosts. Reite (1969) has confirmed the original observations of Keys & Bateman (1932), while Holmgren & Nilsson (1974) have characterized the adrenergic receptors of isolated coeliac artery strips from the cod and the trout. Although many workers have invoked changes in systemic vascular resistance to explain the blood pressure responses to injected sympathomimetic agents (Mott, 1951; Randall & Stevens, 1967; Stevens *et al.* 1972; St Helgason & Nilsson, 1973), there exist no quantitative data on the adrenergic reactivity of the trunk circulation as a whole.

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Present opinion emphasizes the importance of circulatory catecholamines in regulating vasomotor tone in fish (Randall & Stevens, 1967; Burnstock, 1969; Campbell, 1970), and the results of a perfusion study of the trout gill have not conflicted with this concept (Wood, 1974*a*). However, it is difficult to reconcile this viewpoint with the observations that, in trout, blood adrenaline and noradrenaline levels rise during exercise (Nakano & Tomlinson, 1967) while systemic vascular resistance falls (Stevens, 1968*a*). An explanation of this paradox, at least in part, could be the influence of passive distensibility (Green *et al.* 1944) on vascular resistance; for swimming activity is accompanied by a significant rise in dorsal aortic blood pressure (Stevens & Randall, 1967), and this may cause passive changes in vascular resistance unrelated to vasomotor tone. However, it may mean that factors other than blood catecholamine levels (e.g. autonomic nervous system, local effects of metabolites) are important in controlling the calibre of the trunk resistance vessels.

The aims of the present study were therefore quantitatively to assess the influence of circulatory catecholamines and blood pressure on the tone and resistance of the trunk vasculature as a whole in *Salmo gairdneri*, and to characterize the adrenergic receptors therein by the basic method of agonist potency comparison (Ahlquist, 1948). A perfused trunk preparation was developed for this purpose. The investigation paralleled previous work on the gill circulation (Wood, 1974*a*) and thus the methods employed were designed to facilitate comparison between the two studies.

MATERIALS AND METHODS

Rainbow trout (100–600 g) were acquired, maintained, and acclimated to 14·5 ± 1·5 °C as described previously (Wood, 1974*a*). Experiments were performed in a constant-temperature room at 5 ± 1 °C on 14 preparations studied with the 'vertical tube' technique, and 46 pump perfused preparations. Additional data have been taken from a further 160 pump perfused trunks used in studies of the adrenergic and cholinergic pharmacology of the systemic vasculature (Wood, in preparation). The latter were set up under identical conditions, and the data used here (on spontaneous changes in vascular resistance) were taken before the administration of any drugs.

I. *Preparation of animals*

A trout was anaesthetized in 1 : 15000 MS-222 (Sandoz) on an operating table at the acclimation temperature. The heart was exposed and 500 i.u. of sodium heparin (Sigma)/100 g was injected into the ventricle and allowed to circulate for 5 min. (A few fish were prepared without anaesthesia and killed by decapitation; in these trout 5000 i.u. of heparin was administered intraperitoneally 30 min before the experiment.) The animal was then completely transected at the level of the anterior end of the heart, the atrium and ventricle removed, and the dorsal aorta cannulated with PP 190 or 200 tubing (Portex). The normal preparation thus consisted of the whole body posterior to the heart (the viscera were contained by the transverse septum), and averaged 82·5% of total body weight. In a few experiments it was necessary to keep the spinal cord intact, so these fish were not transected. Instead, the dorsal aorta was approached ventrally at a point slightly posterior to the heart (necessitating rupture of the transverse septum), exposed by carefully scraping away the kidney

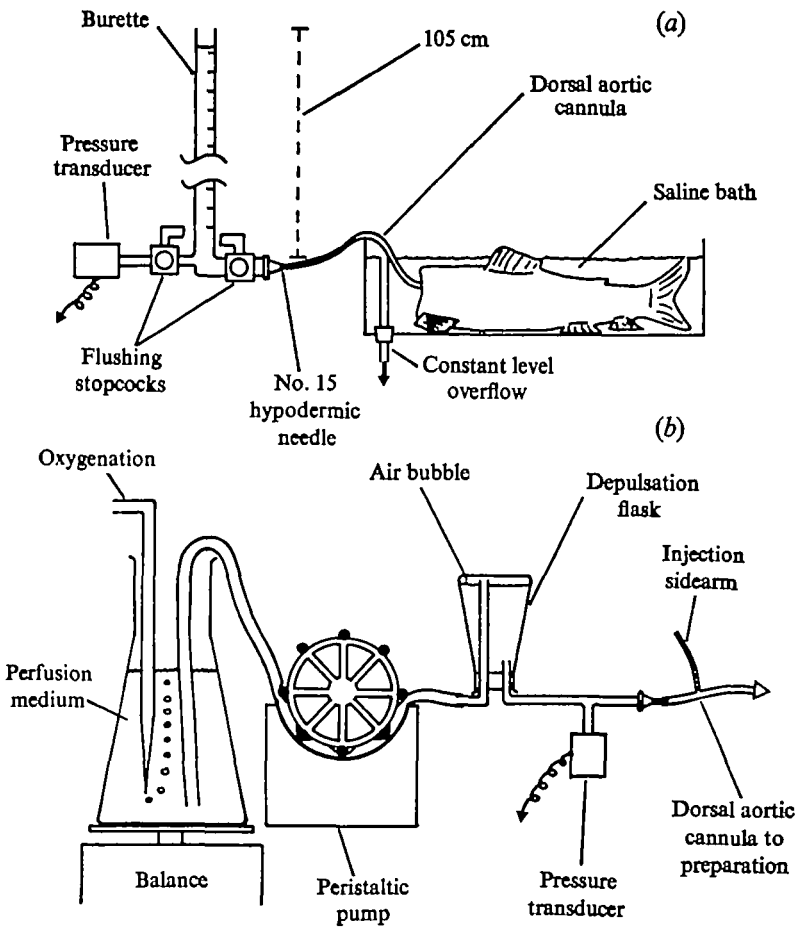

Fig. 1. Schematic diagrams of the apparatus used in (*a*) vertical tube perfusion and (*b*) pump perfusion of the trunk preparation.

and cannulated as above. The trunk was then perfused through the dorsal aorta at a head of 30 cm until blood-free saline dripped from the cut end of the sinus venosus (5–10 min). The perfusion medium was identical to that used in the gill study (Wood, 1974*a*). The preparation was then immersed in a constant-level bath of Cortland saline (Wolf, 1963), and perfusion immediately recommenced by one of the following techniques. All pressures were measured relative to the bath surface as zero and expressed in units of cm of saline (S.G. at 5 °C = 1·013).

II. *Vertical tube perfusion* (Fig. 1*a*)

This system, which permitted the construction of complete pressure differential/flow (*Pd*/*Q*) profiles in the trunk, was similar to that used in the gill study, but modified to permit accurate measurement of absolute critical closing pressures (*CCPd*) down to 1 cm. A glass burette extending 105 cm vertically above the bath surface was connected, at its lower extremity, both to the preparation and to a Sanborn 267 BC pressure transducer through separate stopcocks. The output of the transducer was

monitored via a Sanborn 350–1100 C carrier pre-amplifier on a Smiths RE 5rr Servoscribe chart recorder. As the perfusate flowed from the burette into the preparation, the fall of the head in the vertical tube was recorded by the pressure transducer. The slope of the trace multiplied by the known volume per unit height of the tube (about 0·5 ml/cm) gave the perfusion flow (Q) for any particular head in the burette. The efferent pressure (*Pe* in the gill study) was always 0 cm in the present arrangement, so the pressure applying at the dorsal aortic inflow represented the differential pressure (*Pd*). *Pd* and the vascular resistance of the trunk (*Rs*) were calculated by correcting for the resistance of the delivery system as described previously (Wood, 1974*a*). It should be made clear that *Rs* does not, in this preparation, represent the whole systemic vascular resistance since the circulation through the head is not included. We anticipate that the effect of including the resistance of the head circulation would be to produce small changes in *Rs* values but not to affect the more general relationships described below. Full *Pd*/Q profiles were determined by allowing the fluid level in the vertical tube to fall from 105 cm to the absolute *CCPd* (where $Q = 0$). At low Q, the procedure could be accelerated by opening one of the stopcocks; time for construction of a full *Pd*/Q profile was about 10 min. The perfusion medium was vigorously bubbled with oxygen before use.

III. *Pump perfusion* (Fig. 1*b*)

A Quickfit peristaltic pump with pressure independent delivery was used to provide perfusion at constant flow (Q). The reservoir of perfusion medium rested on a Mettler top-loading balance (for measurement of Q), and was bubbled with oxygen throughout the experiment. In the majority of runs, Q was set at 0·50 ml/100 g total body weight/min (range: 0·45–0·55); higher flows (up to 1·50 ml/100 g/min) were used in about 35% of the preparations. The pump output was somewhat pulsatile, so an inverted 20 ml Erlenmeyer flask containing a small air bubble (2–3 ml) was inserted as a depulsator between the pump and the trunk, providing a pulse pressure of 3–5 cm. This arrangement damped rapid pressure changes but had no effect on the mean pressure associated with any stable resistance in the preparation. The response time (time to proceed to $1-(1/e)$ of an applied pressure change) was approximately 2 s; this factor had little effect on the slow changes in pressure, caused by alterations in systemic resistance, measured in the study. Perfusion pressure was monitored via a T-joint between the depulsator and the trunk with the same instrumentation as used in the vertical tube system. An injection sidearm immediately proximal to the cannulation site was used for the administration of drug doses in subsequent pharmacological studies (Wood, in preparation). At the end of an experiment, the pressure drop across the perfusion catheter distal to the point of measurement was determined at the experimental Q. Resistance (*Rs*) changes in the trunk were calculated from changes in the corrected perfusion pressure (*Pd*) at constant Q.

IV. *The preparation*

As long as the perfusion medium was oxygenated, the trunk displayed relatively stable adrenergic responsiveness for at least 12 h with both perfusion techniques. Oedema was minimal but positively correlated with the duration of perfusion, although the relationship was significant only in the case of pump perfusion (Table 1). Millipor

Table 1. *Percentage weight gain (oedema), perfusion duration, and their correlation (r) in trunks perfused by two methods. Means* ± 1 S.E.

	Vertical tube perfusion $N = 14$	Pump perfusion $N = 25$
Weight gain (%)	2·35 ± 0·40	1·74 ± 0·63
Perfusion duration (h)	4·26 ± 0·27	5·42 ± 0·36
r	+0·373	+0·684
p	n.s.	<0·001

Only data from those preparations to which blocking drugs (yohimbine, atropine, phenoxybenzamine) were not administered have been used.

filtration of the medium (Rankin & Maetz, 1971) had no effect on the pattern of spontaneous vascular resistance change, the responsiveness, or the weight gain of the preparation, and so was not routinely employed. Spontaneous swimming movements appeared in about 25% of the preparations, almost invariably during periods when perfusate concentrations of adrenaline or noradrenaline were high (> 10^{-6}M). The mechanism is unknown. However, the phenomenon was usually associated with an irregular fall of up to 25% in *Rs* (which had previously been elevated by the catecholamines in the perfusate).

V. *Drugs*

The sympathomimetic agents L-adrenaline bitartrate (AD), L-noradrenaline bitartrate (NAD), L-isoprenaline bitartrate (ISO), D,L-isoprenaline hydrochloride (D,L-ISO), and L-phenylephrine hydrochloride (PHE) (all Sigma), the adrenergic blocking agents yohimbine hydrochloride (Sigma) and phenoxybenzamine hydrochloride (Smith, Kline, and French), and the cholinergic antagonist atropine sulphate (Sigma) were used. The five sympathomimetic agents and phenoxybenzamine were added directly to the perfusion medium. Yohimbine and atropine were injected into the ventricle of the intact animal prior to cannulation.

RESULTS

I. *Vertical tube perfusion*

The typical *Pd*/*Q* profile of the trunk differed greatly from that obtained from the gills perfused under virtually identical conditions. [The efferent pressure (*Pe*) of course differed in the two studies, being set to approximate the normal dorsal aortic pressure (40 cm) for the gill, and the venous pressure (0 cm) for the trunk.] Comparison of Fig. 2 with the branchial *Pd*/*Q* relationships of Fig. 2*b*, *c* of Wood (1974*a*) reveals superficial similarity in the form of the curves; however the *Q* axis is an order of magnitude greater in the trunk profiles. Thus systemic vascular resistance (*Rs*), even when highest at the start of perfusion, was generally much lower than gill resistance (*Rg*) under the conditions of these experiments.

In contrast to the gill profiles, where *Rg* increased with time, successive trunk profiles were displaced towards the *Q* axis (Fig. 2). The differential pressures required to maintain particular values of *Q* decreased with time by approximately the same relative amounts over the whole flow range. This relationship also extended to zero flow, where *CCPd* also fell by the same relative amounts as the higher differential

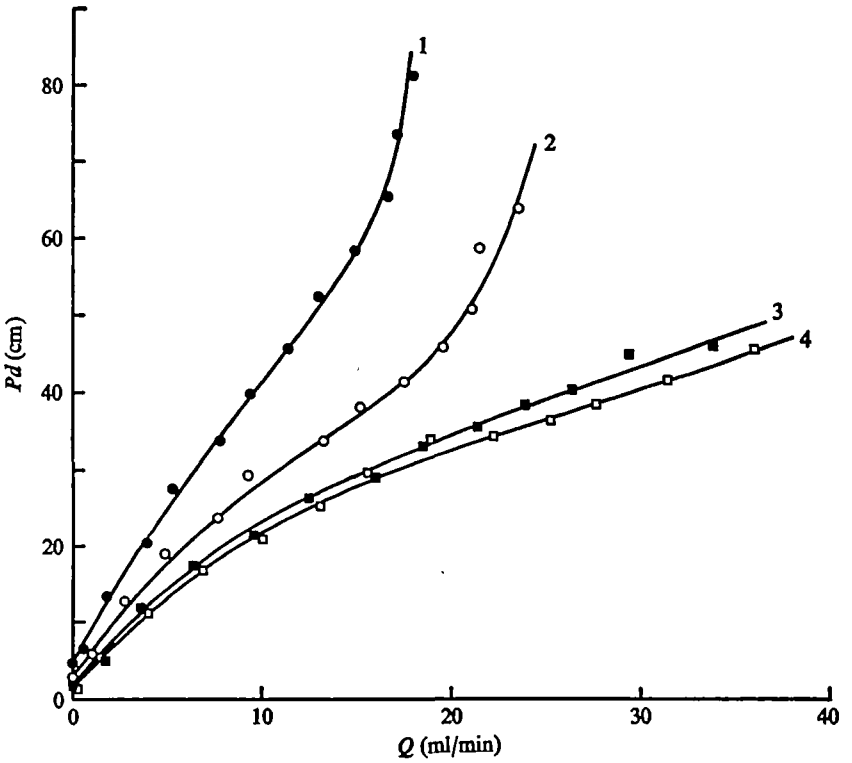

Fig. 2. Typical successive *Pd*/*Q* profiles in a perfused trunk preparation. *Pe* = 0 cm. 1, 2, 3, and 4 denote profiles taken at 0–10, 10–20, 20–30, and 30–40 min respectively after the start of perfusion. All ensuing profiles were identical to 4. Weight = 188·2 g.

pressures. The actual rate of *Rs* decline varied, but in all preparations a stable profile, termed the 'baseline' profile, was attained after 10–70 minutes. Initial profiles often displayed a marked curvilinearity where the slope (and *Rs*) was greater at high pressures than at intermediate pressures (e.g. curve 1 of Fig. 2). The baseline profiles never displayed this pattern; instead all exhibited a simple pronounced convexity to the *Pd* axis (e.g. curve 4 of Fig. 2).

Mean *Pd*/*Q* profiles for the initial and baseline states of *Rs* were calculated by averaging the individual curves at the same *Q*/100 g (Fig. 3). Data will be presented subsequently (Fig. 6) to show the desirability of expressing *Q* on a body weight basis. *Q* values considerably higher than the limit of the graph (11 ml/100 g/min) were shown by some trunks, but any extension of the mean curve beyond the greatest *Q*/100 g recorded in all fish would have been biased in favour of those with lower *Rs* due to the limited height of the vertical tube.

The mean initial profile was virtually linear, while the mean baseline curve retained a slight convexity (a property common to all preparations) (Fig. 3). The initial *CCPd* declined from 4·3 ± 0·4 (mean ± 1 S.E.) to 2·9 ± 0·3 cm. Both profiles indicated considerable distensibility; for example between *Pd* = 10 and 40 cm, the initial and baseline *Rs* declined by 37% and 40% respectively. Inclusion in Fig. 3 of the mean *Pd*/*Q* profile of the gills from Fig. 3 of Wood (1974*a*) (*Pe* = 40 cm; values determined when *Rg* was minimal) emphasizes the great difference between branchial

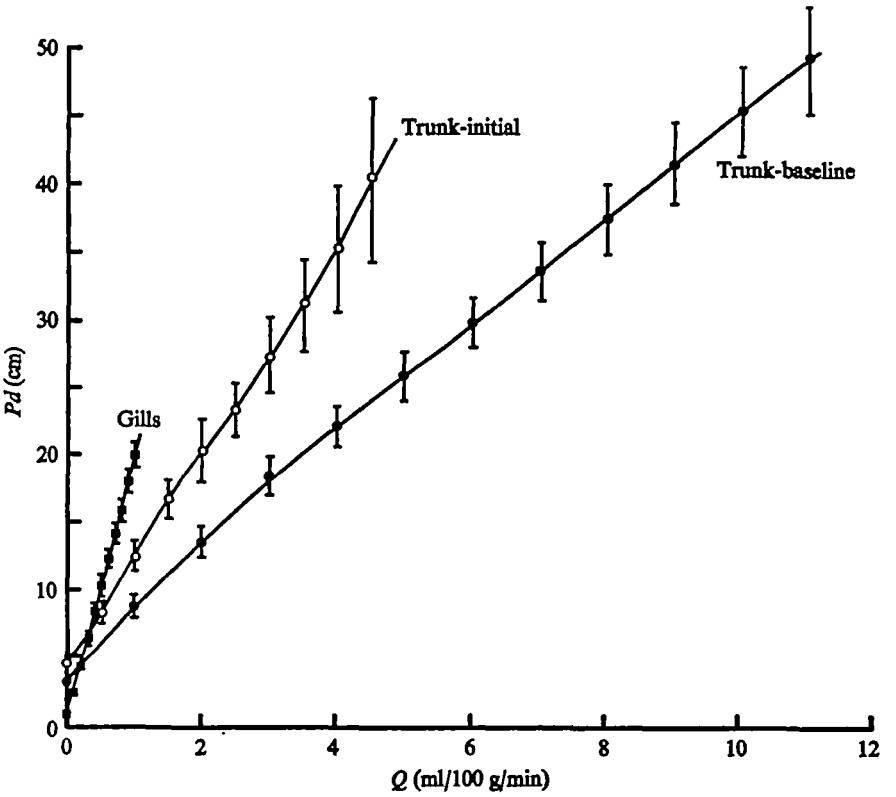

Fig. 3. The average initial (0–10 min) and baseline *Pd*/*Q* relationships in 14 trunk preparations perfused by the vertical tube technique. *Pe* = 0 cm. Mean weight = 190·4 ± 11·3 g; mean coefficient of condition = 1·071 ± 0·031. The average *Pd*/*Q* relationship in the gills at *Pe* = 40 cm has been included for comparison (data from Fig. 3 of Wood, 1974*a*). Means ± 1 S.E.

and systemic resistances in these experiments. It is also apparent that for comparisons made at the normal *in vivo Pe*, the trunk vascular bed was considerably more distensible on a relative basis. Thus a rise in *Pd* from 5 to 20 cm reduced *Rg* by only 8% but *Rs* by 60–80%.

As in the gill work, it was desirable to derive a single expression which would quantitatively describe a change in vascular tone over the whole *Pd*/*Q* relationship. Sympathomimetic agents were added to the perfusion medium to create different levels of vasomotor tone. AD at 10^{-7} M or greater caused a dose-dependent vasoconstriction manifested as an upward shift of the *Pd*/*Q* profile and the *CCPd* (Fig. 4). There was often a curvilinearity similar to that of the initial profiles (cf. curve 1 of Fig. 2). Analysis of profiles under different levels of AD indicated that distensibility decreased as adrenergic tone increased. D,L-ISO by itself had no effect on baseline *Rs*, but, at concentrations of $3{\cdot}2 \times 10^{-5}$ M and above, the drug dilated preparations that had previously been constricted by AD (Fig. 4). This relaxation was of variable magnitude but never resulted in a *Pd*/*Q* relationship lower than that associated with baseline *Rs*. [Subsequent pharmacological work has shown that baseline *Rs* reflects a condition where vasomotor tone (i.e. that tone attributable to neural or hormonal mechanisms) is absent (Wood, in preparation).]

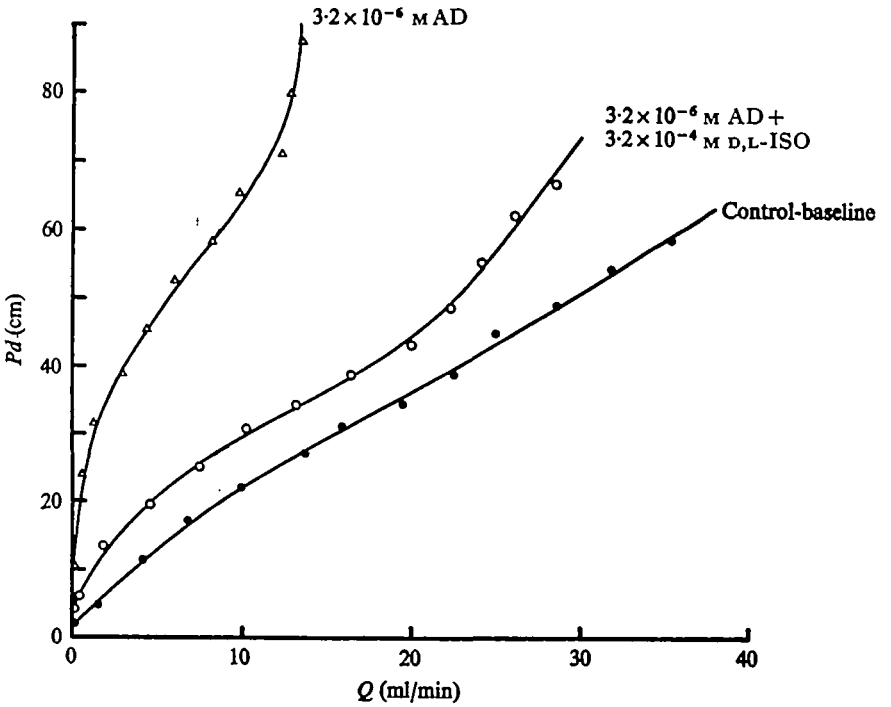

Fig. 4. Typical constrictory effect of L-adrenaline ($3{\cdot}2 \times 10^{-6}$ M) and dilatory effect of D,L-isoprenaline ($3{\cdot}2 \times 10^{-4}$ M) on this constriction in a trunk preparation perfused by the vertical tube technique. Weight = 294·2 g.

Comparisons of *Rs* exp/*Rs* con (exp = experimental, con = control) at the same *Pd* (i.e. *Q* con/*Q* exp) with the analogous ratio at the same *Q* (i.e. *Pd* exp/*Pd* con) were made for a variety of large and small constrictions and dilations caused by AD and D,L-ISO respectively. Because of the lack of vasomotor tone at baseline *Rs*, it was necessary to use experiments in which the constrictory effect of AD had been reversed, by D,L-ISO, for the dilation calculations. In every case (e.g. Fig. 5), comparison of *Rs* values at the same *Q* produced far greater consistency over the whole range of overlap of control and experimental curves than did comparison at the same *Pd*. Variation in *Pd* exp/*Pd* con was generally less than 1·5 fold (maximum = 2 fold), while that in *Q* con/*Q* exp commonly reached 5 fold. Thus, as in the gill, tone changes of the trunk (expressed as *Rs* exp/*Rs* con) determined at only one point on a *Pd*/*Q* profile can be extrapolated to the remainder of the relationship with some reliability when *Rs* has been measured at the same *Q*. The analogous index at $Q = 0$ is the ratio of the *CCPd* values, and this agrees well with *Rs* exp/*Rs* con at the same *Q* over the rest of the profile (Fig. 5). In light of the demonstrated reliability of measuring vasomotor tone changes in the trunk by *Rs* exp/*Rs* con at the same *Q*, subsequent experiments were preformed under constant *Q* perfusion.

II. *Pump perfusion*

As in the vertical tube studies, *Rs* (and thus perfusion pressure) spontaneously decreased with time at a variable rate, a constant baseline level being attained after

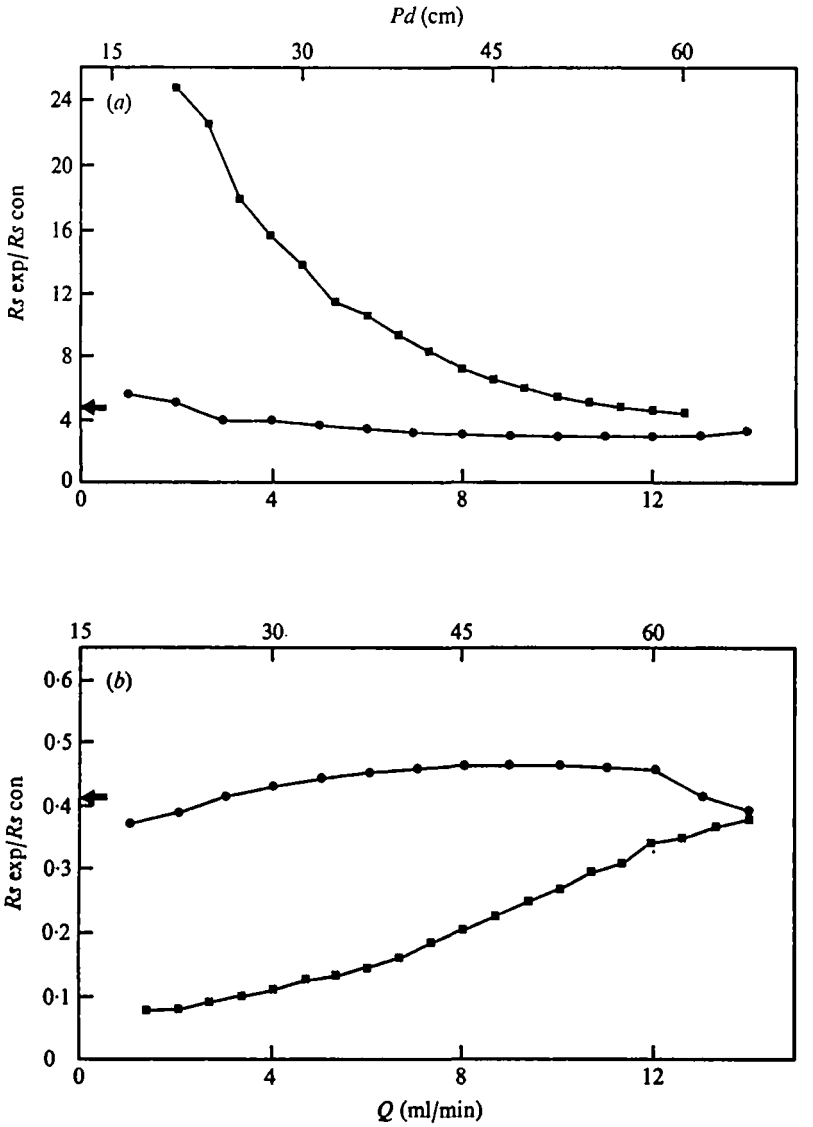

Fig. 5. A comparison of two indices of change in systemic vasomotor tone for (*a*) vasoconstriction and (*b*) vasodilation, based on the data of Fig. 4. For (*a*) constriction: baseline profile = control, $3{\cdot}2 \times 10^{-6}$ M L-adrenaline = experimental. For (*b*) dilation: $3{\cdot}2 \times 10^{-6}$ M L-adrenaline = control, $3{\cdot}2 \times 10^{-6}$ M L-adrenaline + $3{\cdot}2 \times 10^{-6}$ M D,L-isoprenaline = experimental. The two indices are the ratios of vascular resistances in the experimental and control states (*Rs* exp/*Rs* con) at constant *Pd* (■) and at constant *Q* (●). Note the much greater consistency of the latter over the whole range of overlap of control and experimental profiles. Even at $Q = 0$, the analogous index (*CCPd* exp/*CCPd* con; indicated by arrows) agrees with *Rs* exp/*Rs* con at the same Q ($Q > 0$).

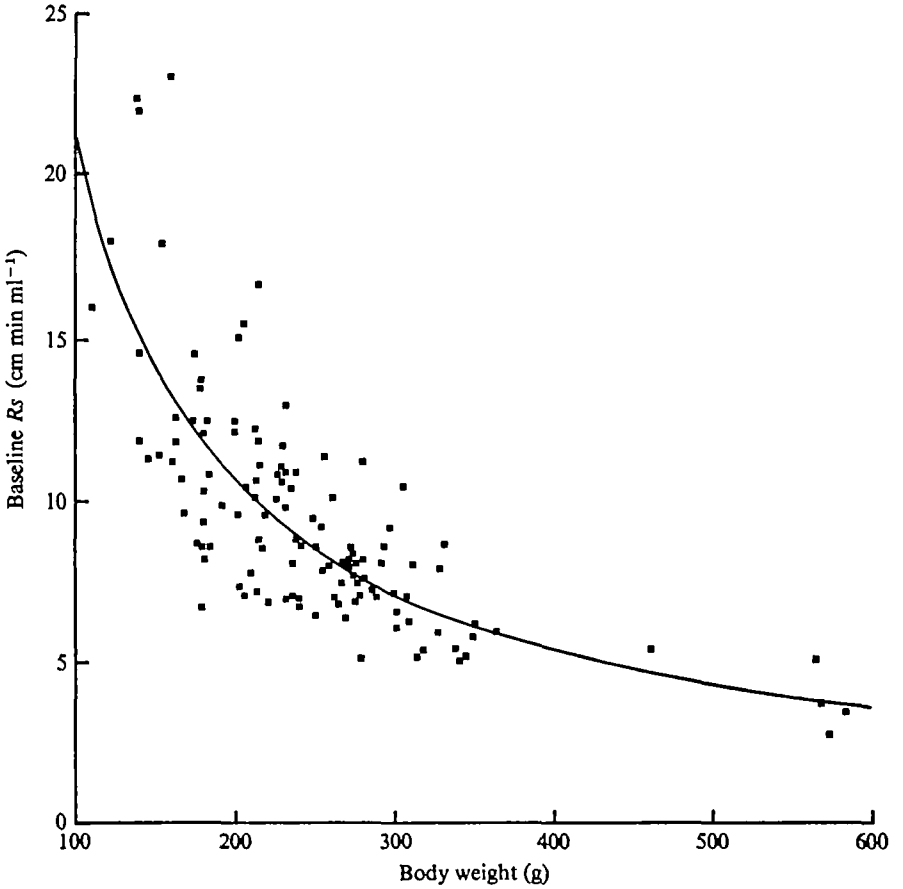

Fig. 6. The relationship between baseline vascular resistance (*Rs*) and total body weight in 119 trunks pump perfused at 0·50 ml/100 g/min (range: 0·45–0·55). Mean coefficient of condition = 1·058 ± 0·006. The line fitted to the data is:

$$Y = \frac{21{\cdot}68 \text{ cm min } 100 \text{ g ml}^{-1}}{X},$$

where $Y = Rs$, X = body weight.

5–80 min. A plot of individual baseline *Rs* values versus total body weight for all preparations perfused at 0·50 ml/100 g/min indicated a simple inverse relationship between the two parameters (Fig. 6). The mean baseline *Rs* on a body weight basis was 21·68 cm min 100 g ml^{-1}. The line fitted to the data is therefore,

$$Y = \frac{21{\cdot}68}{X} \text{ cm min } 100 \text{ g ml}^{-1},$$

where $Y = Rs$, and X = body weight.

There occurred no significant deviation from this curve over the range of weights encountered in the study (Fig. 6). A similar inverse relationship,

$$Y = \frac{40{\cdot}40}{X} \text{ cm min } 100 \text{ g ml}^{-1},$$

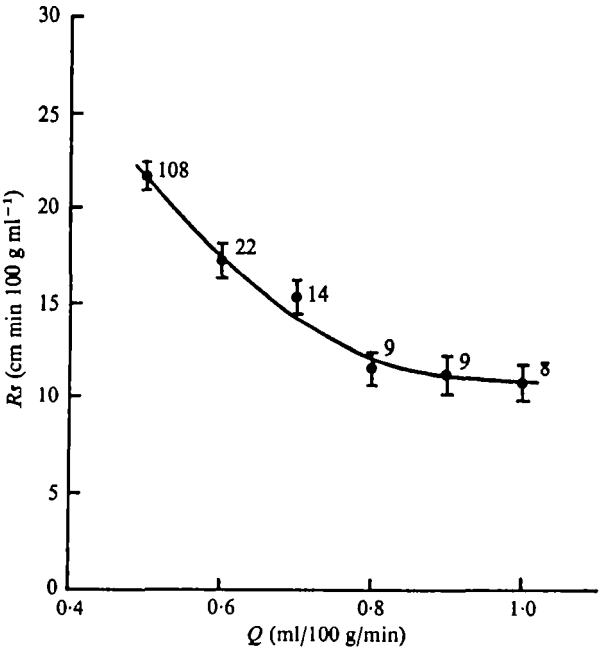

Fig. 7. The effect of increasing perfusion flow (Q) on the baseline vascular resistance (Rs) of the pump perfused trunk. Rs and Q are expressed on a body weight basis. Data have been taken from different groups of preparations at each flow. Mean ± 1 S.E. (N).

fitted the initial Rs data (though there was considerably greater scatter here). Thus expression of Rs on a body weight basis would appear to be a valid method for data comparison.

The baseline Rs (21·68 cm min 100 g ml^{-1}) with pump perfusion at 0·50 ml/100 g/min was considerably greater than that (12·56 cm min 100 g ml^{-1}) which could be estimated from the mean vertical tube Pd/Q profile at this Q (Fig. 3). It is possible that this disagreement reflects a basic difference in the nature of profiles determined with changing Pd and Q (vertical tube) and stable Pd and Q (pump) due to the influence of slowly adapting 'viscous' elements in the vasculature. More probably the difference stems from a lack of accuracy in vertical tube Pd/Q profiles at this low Q. Although particular care was taken to determine the $CCPd$ accurately, the actual Pd/Q relationship was not usually recorded close to the $CCPd$ because of the low flows encountered; consequently the vertical tube Rs value of 12·56 cm min 100 g ml^{-1} was derived from a straight line interpolation between the mean $CCPd$ and the mean Pd at 1·0 ml/100 g/min. Other pump perfusion experiments with different trunks indicated that the Pd/Q relationship is not linear in this range and therefore the interpolation was probably inaccurate. Thus as Q was raised by small increments from 0·5 to 1·0 ml/100 g/min, Pd did not rise significantly; consequently Rs fell markedly (Fig. 7). By 1·0 ml/100 g/min, the baseline of Rs of pump perfusion [10·97 ± 0·65 (8) cm min 100 g ml^{-1}; Fig. 7] was not significantly different from that of the mean vertical tube perfusion profile [9·04 ± 0·71 (14) cm min 100 g ml^{-1}; Fig. 3].

It was suspected that the large spontaneous decline in Rs after the start of perfusion

Table 2. *Initial vascular resistance (Ri), baseline vascular resistance (Rb), and initial resistance as a percentage of baseline resistance in pump perfused trunks subjected to different pre-treatments. Means* ± 1 S.E. (*N*)

	*Ri** (cm min 100 g ml^{-1})	*Rb** (cm min 100 g ml^{-1})	*Ri*/*Rb* (%)
Control	40·40 ± 1·46 (99)	21·68 ± 0·45 (119)	179·02 ± 6·37 (156)
No MS-222	41·29 ± 3·20 (8)	24·60 ± 2·29 (8)	175·15 ± 16·36 (8)
	P = n.s.	*P* = n.s.	*P* = n.s.
Spinal intact	34·02 ± 4·15 (4)	19·80 ± 0·53 (4)	174·51 ± 23·60 (4)
	P = n.s.	*P* = n.s.	*P* = n.s.
Atropine (100 nM/100 g)	40·15 ± 6·60 (5)	24·87 ± 2·73 (5)	159·83 ± 19·56 (5)
	P = n.s.	*P* = n.s.	*P* = n.s.
Yohimbine (100 nM/100 g)	17·45 ± 1·78 (5)	18·85 ± 0·76 (5)	93·15 ± 9·83 (5)
	P < 0·01	*P* = n.s.	*P* < 0·01

P = probability with respect to corresponding control value.

* Absolute resistance values have only been taken from those preparations perfused at 0·50 ml/100 g/min.

might reflect the effect of MS-222, or spinal section, in either elevating the initial *Rs* or depressing the final value. However, neither the pattern of *Rs* decrease, nor the absolute size of initial *Rs*, baseline *Rs*, or their ratio, were significantly different from controls in trunks prepared either without anaesthesia or without spinal section (Table 2). To test the possibility that the phenomenon represented a spontaneous fall in latent vasomotor tone, separate groups of fish were pre-treated with specific pharmacological blocking agents. The muscarinic cholinergic antagonist, atropine (100 nM/100 g), and the α-adrenergic antagonist, yohimbine (100 nM/100 g), were injected into the ventricle of the intact anaesthetized animal 10 min before cannulation. [These doses were chosen on the basis of their known effectiveness and specificity against injected agonists from experiments on intact, unanaesthetized trout (Wood & Shelton, unpublished results)]. Atropine was ineffective, but yohimbine completely abolished the phenomenon so that initial *Rs* was equal to baseline *Rs*; the latter in turn was equal to control baseline *Rs* (Table 2).

Concentration ('dose')/response curves for AD, NAD, PHE, and ISO in the perfusate were determined by a process of cumulative addition (Ariens, 1964; Waud, 1968). To avoid possible interactions, only one agonist was tested in each preparation; full dose/response relationships from threshold to maximum were recorded in a trunk starting from baseline *Rs*. It was desirable that the data obtained with this technique on pump perfused trunks be comparable to the results on the gills determined with a single addition procedure and vertical tube perfusion (Wood, 1974*a*). Apart from any difference between the results of pump and vertical tube perfusions, cumulative addition may produce a different dose/response curve to single addition due to desensitization (Waud, 1968). However, comparison of the individual values for tone change in the trunk associated with different AD levels from the vertical tube perfusions (single addition technique) with the mean AD concentration/response curve from pump perfusion (cumulative addition technique) indicated that these differences in

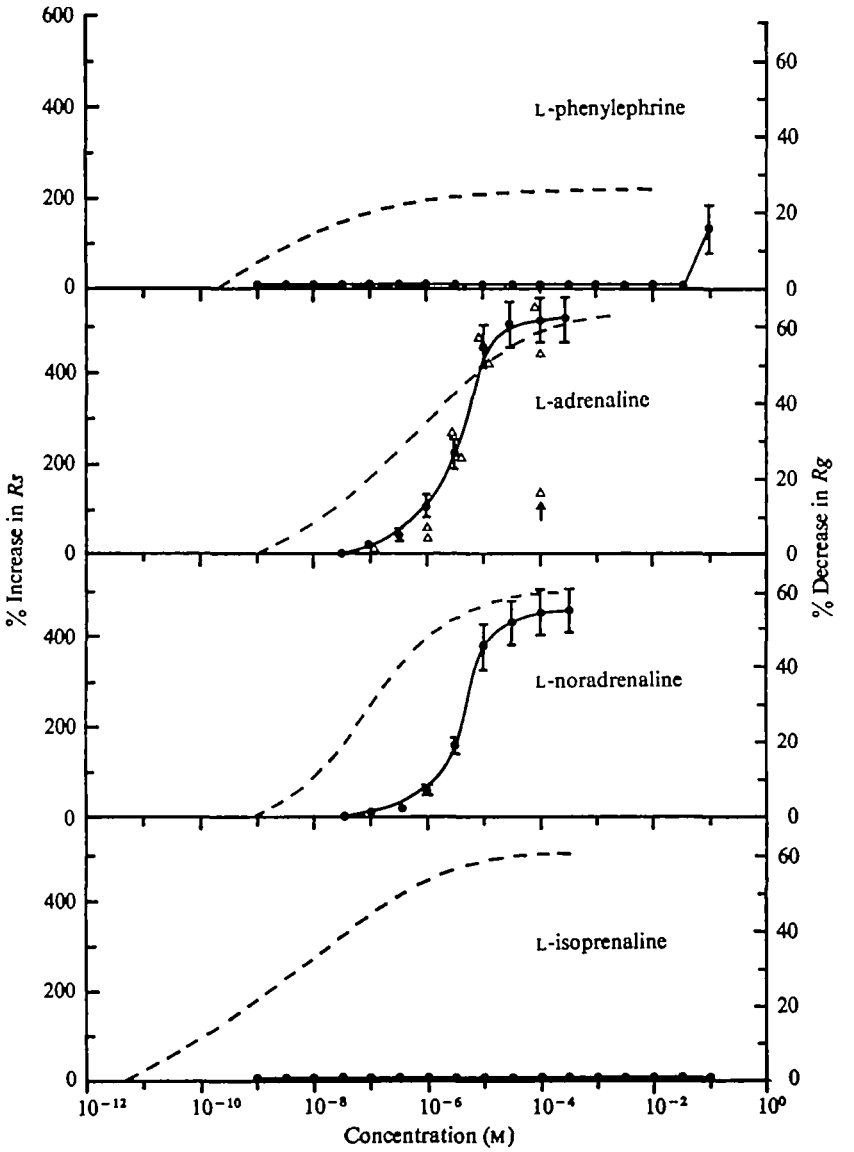

Fig. 8. Concentration/response (constriction) relationships for four adrenergic agonists in the systemic vasculature determined in pump perfused preparations by a cumulative addition procedure. Concentration is plotted on a logarithmic scale. Each point (●) represents the mean ± 1 S.E. of 6 preparations each for L-adrenaline and L-noradrenaline and 4 preparations each for L-phenylephrine and L-isoprenaline. Individual points for L-adrenaline determined by a single addition procedure in preparations perfused by the vertical tube technique are shown by △. A single aberrant point is indicated by the arrow. See text for experimental details. The analogous concentration/response (dilation) relationships in the gills [data from Figure 7 of ---ood (1974*a*)] are shown by dotted lines, the scale having been chosen to equalize the maximum effects of L-adrenaline and L-noradrenaline in the two vascular beds.

methodology were not a problem (Fig. 8). With the exception of one anomalous point from the vertical tube study (cause unknown, labelled in Fig. 8), there was good agreement between the results of the two techniques.

AD and NAD produced typical sigmoidal log concentration/response curves for constriction with similar thresholds (10^{-7} M), slopes, and maximal effects (500% increase in *Rs* at the same *Q*) (Fig. 8). Expressed on this basis, there were no significant differences between the mean responses to AD and NAD at any dose level. The analogous curves from the branchial studies (Wood, 1974*a*) have been plotted for comparison in Fig. 8 with a scale chosen to equalize the maximum responses. Both AD and NAD were obviously far less potent in causing constriction of the systemic vasculature than in causing dilation of the branchial vessels. ISO, which was the most active agonist in the gill, was completely without constrictory effect in the trunk (Fig. 8). PHE was similarly inactive except at the extremely high level of 10^{-1} M where it caused a small excitation (Fig. 8). Pre-treatment of the preparation by perfusing for one hour with the α-adrenergic antagonist, phenoxybenzamine (10^{-5} M) abolished or severely reduced responses to AD and NAD, but the action of PHE at 10^{-1} M persisted. None of the agents caused relaxation from baseline *Rs*.

Since the efficacies (maximal effects) of AD and NAD were equal, and complete concentration/response curves were obtained in each preparation, it was possible to construct mean dose/response curves in terms of percent maximum effect for both agents. This treatment should provide a sounder framework for potency comparison (Ariens, 1964). On this basis, the responses to NAD were slightly but significantly lower ($P < 0.05$) than those to AD at several points on the curves; the AD : NAD potency ratio (Furchgott, 1967) was 1·26 : 1.

DISCUSSION

Relative to the gill preparation (Wood, 1974*a*), the perfused whole trunk of *S. gairdneri* was easy to prepare and extremely durable. The minimal oedema, the long term retention of adrenergic responsiveness, and the spontaneous swimming activity (at high levels of AD and NAD), indicated the suitability of the perfusion conditions. That such movement commonly reduced *Rs* agrees with the observations of Satchell (1965) on the perfused shark trunk. In the trout, the phenomenon could be due to a pumping action of the dorsal aortic ligament (Priede, 1975) or to a muscle pump mechanism facilitated by valves on myotomal segmental vessels (Satchell, 1965; Sutterlin, 1969). Therefore a purely mechanical effect, or a vasodilation mediated by local metabolic factors as commonly seen in exercising mammalian muscle (Scher, 1965), may contribute to the observed fall in *Rs* during exercise in the trout (Stevens, 1968*a*).

The finding that changes in *Rs* at constant *Q* reliably measure variations in vasomotor tone over the whole *Pd*/*Q* profile agrees with mammalian work (Green *et al.* 1944; Kuida, 1965). The situation is parallel to that in the gills (Wood, 1974*a*), and facilitates comparison between the two studies. The pump perfusion *Q* of 0·50 ml/100 g/min employed in most experiments was close to the estimated *in vivo* value at 5 °C. (0·76 ml/100 g/min; Stevens, 1968*b*), but the *Pd* associated with this *Q* was much lower than *in vivo* (i.e. dorsal aortic pressure) due to the very low baseline *Rs* of the

preparation (see below). Nevertheless, the demonstrated consistency, over the whole Pd/Q relationship, of the index of tone change used makes the actual Q chosen unimportant for the purposes of the present study.

The constrictory potency order AD > NAD > ISO provides the classic indication of α-adrenergic receptors in the systemic vasculature (Ahlquist, 1948; Furchgott, 1967). This finding is supported by the effectiveness of the α-adrenergic antagonist, phenoxybenzamine, in blocking the actions of AD and NAD. However, the lack of response to PHE (except at a concentration of 10^{-1} M) was surprising. On mammalian vascular tissue, PHE has excellent α-receptor specifity and is about $\frac{1}{4}$–$\frac{1}{3}$ as potent as AD (Furchgott, 1967). In the trout, even the response at 10^{-1} M was apparently non-specific as it was resistant to phenoxybenzamine. The ineffectiveness of this drug in the trunk may indicate a basic difference in the nature of the α-adrenergic receptor of this lower vertebrate. However, the potency ratio (AD:NAD) of 1·26 : 1 in the trunk is extremely close to that reported for the α-receptors of mammalian arteries (Furchgott, 1967; Sheys & Green, 1972) as well as to values for the *in vitro* coeliac arteries of the cod and the trout (Holmgren & Nilsson, 1974). The whole question of the adrenergic pharmacology of the systemic vasculature will be examined in much greater detail elsewhere (Wood, in preparation). In the present study, it is sufficient to note that α-adrenergic constrictory receptors are dominant in the trunk in contrast to the β-adrenergic dilatory receptors of the gills (Wood, 1974*a*). β-adrenergic dilatory receptors may be present in the systemic vessels, but the action of D,L-ISO in relaxing preparations previously constricted by AD (vertical tube perfusions; Fig. 4) is complex and does not simply comprise β-receptor mediated dilation (Wood, in preparation).

The systemic vasculature is far less sensitive than the branchial vasculature to the naturally occurring catecholamines AD and NAD (Fig. 8). The measured natural range of variation of catecholamine levels in trout blood (Nakano & Tomlinson, 1967) covers most of the gill dose/response curves, but reaches only the very lower end of the trunk curves. Consequently, the highest circulatory levels of AD and NAD (2×10^{-6} M; recorded during exercise) would be sufficient to account for the branchial vasodilation accompanying swimming activity in the trout (Wood, 1974*a*) but would create a systemic constriction little greater than that already present in the resting animal as estimated by initial *Rs* (see below). Thus plasma catecholamine levels may be a major factor regulating *Rg*, but they are probably of only minor importance in the adjustment of *Rs in vivo*. The much greater local concentrations of catecholamines associated with sympathetic nerve activity (Ross, 1971) are more likely to cover the range of the systemic dose/response curves and therefore be effective in controlling *Rs*. Extensive adrenergic (presumably sympathetic) innervation of the systemic vessels has been revealed histochemically in teleosts (Burnstock, 1969; Campbell, 1970), and the recent demonstration of Mayer vasomotor waves in *S. gairdneri* (Wood, 1974*b*) has furnished physiological evidence for a nervous control of *Rs in vivo*.

The initial decrease in *Rs* at the start of perfusion was not eliminated either by omitting anaesthesia or leaving the spinal cord intact. Its abolition by yohimbine, a very selective α-adrenergic antagonist (Boyd *et al.* 1962), but not by atropine, indicated that a decrease in α-adrenergic tone was responsible, with no muscarinic cholinergic involvement. In accord with the preceding argument that circulating catecholamines are relatively unimportant in regulating *Rs*, this α-adrenergic tone

appeared to be of neural rather than hormonal origin for several reasons. Firstly, the trunk was flushed completely free of blood before setting up, yet the tone commonly persisted for 30–40 min. From the fact that initial *Rs* was about 80% greater than baseline *Rs* (Table 2), the original level of circulating catecholamines, if responsible for the initial tone, must have been at least 10^{-6} M (from Fig. 8). Yet Nakano & Tomlinson (1967) found plasma concentrations of only 10^{-8} M in resting *S. gairdneri* killed under similar conditions to the unanaesthetized fish of the present study. Finally, yohimbine at the same dose similarly blocked the apparent sympathetic control of *Rs* implicated in Mayer waves in the trout (Wood, 1974*b*).

If the α-adrenergic tone was of neural origin, then why did it decline? In mammals, spinal section drastically reduces sympathetic vasomotor activity by removing the influence of the vasomotor centre in the medulla. However, some autogenous sympathetic activity persists (Heymans & Neil, 1958). In the trout, no vasomotor tone remains at baseline *Rs* (Wood, in preparation), and the *Rs* fall occurs whether or not the spinal cord is cut (Table 2). The latter finding does not completely exclude the involvement of the removal of a higher vasomotor centre influence, for it may have been functionally abolished by non-perfusion of the head region in these experiments. Nevertheless, it seems more probable that some deficiency in the experimental conditions resulted in a progressive and eventually complete loss (at baseline *Rs*) of function by the sympathetic system which normally maintains a significant α-adrenergic tone in *Rs*.

This loss of α-adrenergic tone probably explains why, under approximately normal afferent and efferent pressure levels, baseline *Rs* was so much lower than *Rg*. *In vivo*, *Rs* is somewhat greater than *Rg*, and probably much higher than even the initial *Rs* measured here, because 5–10 min of perfusion on the operating table had already taken place before recording commenced. The convexity of the *Pd*/*Q* profile to the *Pd* axis at baseline *Rs* is typical of a vascular bed lacking vasomotor tone and simply reflects the marked passive distensibility of the system (Kuida, 1965). At levels of tone above baseline (e.g. initial profiles, or constriction caused by AD), the curvilinearity of the *Pd*/*Q* relationship may, as in the gills (Wood, 1974*a*), indicate a capacity for autoregulation in the trunk vasculature in which compensatory changes in resistance tend to limit *Q* at high *Pd*. The observation that the *CCPd* increased in approximate proportion to the increase in *Rs* at constant *Q* under vasomotor tone agrees with the contention of Burton (1951) that the *CCPd* is an index of vasomotor tone associated with active vessel closure.

Perhaps the major reason why the relative distensibility of the trunk was so much greater than that of the gills over the same *Pd* range was that the trunk, as *in vivo*, was operating at a much lower transmural (distending) pressure ($Pt = Pd/2 + Pe$). *Pe* was set to 0 cm in the trunk and 40 cm in the gills. With the present preparation, it was unfortunately not possible to examine the effect on *Rs* of altering *Pe* (i.e. venous pressure). In many mammalian vascular beds and in the trout gill, increases in *Pe* may produce significant passive decreases in resistance (see Wood, 1974*a*). Venous pressure can be quite variable in the trout (0–15 cm; Wood & Shelton, unpublished results); its influence on *Rs* may not be insignificant.

An increase in *Pd* over the physiological range (from 30 to 40 cm) would decrease resistance by about 7% at baseline *Rs* and by only 0·5% at initial *Rs* (from Fig. 3). The resting level of α-adrenergic tone in the trunk is thought to be even higher than

Table 3. *An estimate of the absolute range of vascular resistance (R) and flow (Q) over which the branchial and systemic vascular beds can vary due to alterations in adrenergic activity alone*

	Gills		Trunk	
	Rg (cm min 100 g ml^{-1})	*Q* (ml 100 g^{-1} min^{-1})	*Rs* (cm min 100 g ml^{-1})	*Q* (ml 100 g^{-1} min^{-1})
Maximum *R*	20·27*	0·74*	74·07†	0·54†
Minimum *R*	7·98†	1·88†	4·66*	8·58*

* Zero adrenergic activity. † Maximum adrenergic activity.
See text for details of calculation.

that measured at initial *Rs* in the present study, and distensibility appears to decrease as adrenergic tone increases. Therefore, despite the higher relative distensibility in the trunk than the gills, it is doubtful that a significant capacity for passive dilation resides in the systemic vasculature under normal levels of *Pd* and tone, at least for blood pressure increases on the afferent side. Some distensibility may become available if tone falls markedly, and perhaps if venous pressure (*Pe*) rises.

Some estimate can now be made of the absolute levels over which the branchial and systemic beds can operate due to alterations in adrenergic factors alone (Table 3). These calculations are based on the maximum changes observed in the dose/response studies (Fig. 8; 60% decrease in *Rg* and 500% increase in *Rs* at the same *Q*) as applied to the mean *Pd*/*Q* profiles in the gills and trunk (Fig. 3). For the analysis, it has been assumed that ventral aortic pressure = 55 cm, dorsal aortic pressure, = 40 cm, and venous pressure = 0 cm (therefore branchial *Pd* = 15 cm, systemic *Pd* = 40 cm). Table 3 presents the estimated values of *Rg* and *Rs* and the associated values of *Q* through the two vascular beds under levels of zero and maximum adrenergic stimulation.

Maximum vascular resistance occurs in the trunk with maximum adrenergic activity and in the gills with no adrenergic activity (Table 3). Under these conditions, *Rs* is much greater than *Rg*, but because the pressure gradient across the trunk vascular bed is about three times larger than that across the gills, the minimum branchial and systemic flows are remarkably similar; these figures are close to the estimated resting cardiac output (0·76 ml/100 g/min) of *S. gairdneri* at a comparable temperature (Stevens, 1968*b*). Minimum resistance occurs in the trunk with no adrenergic activity (baseline *Rs*) and in the gills with maximum adrenergic activity. In this case, *Rs* is somewhat lower than *Rg*, and the maximum possible systemic flow much greater than the branchial value. However, adrenergic dilation of the gills is approximately large enough to account for the decrease in *Rg* (68%) during swimming activity in the trout calculated by Stevens (1968*a*), especially when the effects of passive distensibility are considered (Wood, 1974*a*). The potential for decreasing *Rs* by relaxation of adrenergic tone is obviously great; and more than adequate to account for the calculated decrease (71%) in *Rs* during exercise (Stevens, 1968*a*). It is possible that, despite the increase in circulatory catecholamines accompanying

exercise, systemic adrenergic tone actually declines due to a fall in sympathetic nervous activity. This effect, in combination with mechanical and local metabolic factors, may contribute to the observed systemic dilation.

The calculations in Table 3 are of course based on a grossly oversimplified representation of the fish circulation. Changes in *Rg* and *Rs* will clearly affect blood pressures in the dorsal aorta, ventral aorta, and venous system in an interactive fashion since blood flow is the same through the serially connected systems in the intact animal. It is known, for example, that during swimming activity, the rise in cardiac output is accompanied by elevated pressures throughout the circulation despite the fall in *Rg* and *Rs* (Stevens & Randall, 1967). Further work on the intact trout will be needed to establish the exact roles of adrenergic alterations of *Rg* and *Rs* in the haemodynamic changes occurring under various conditions.

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