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A CRITICAL EXAMINATION OF THE PHYSICAL AND ADRENERGIC FACTORS AFFECTING BLOOD FLOW THROUGH THE GILLS OF THE RAINBOW TROUT

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INTRODUCTION

Branchial resistance decreases as cardiac output and oxygen consumption increase during exercise in trout (Stevens & Randall, 1967*a*, *b*; Stevens, 1968*a*, *b*) so blood flow rises to a much greater extent than blood pressure. Similar changes also probably occur during real or CO-induced anaemia (Cameron & Davis, 1970; Holeton, 1971) but not during hypoxia (Holeton & Randall, 1967*a*, *b*). A large number of studies on excised and/or perfused teleostean gill preparations have demonstrated that catecholamines cause branchial vasodilation (Krawkow, 1913; Keys & Bateman, 1932; Östlund & Fänge, 1962; Maetz & Rankin, 1969; Kirschner, 1969; Reite, 1969; Rankin & Maetz, 1971; Belaud, Peyraud-Waitzenegger & Peyraud, 1971; Randall, Baumgarten & Maylusz, 1972), accompanied by the opening of respiratory shunts in the secondary lamellae (Steen & Kruysse, 1964; Richards & Fromm, 1969). Investigations on intact animals have implied that exogenous adrenaline has a similar inhibitory effect on the gills *in vivo* (Mott, 1951; Randall & Stevens, 1967; Reite, 1969; Maetz & Rankin, 1969; Johansen, 1972). It is therefore thought that the observed rise in circulatory catecholamine levels during swimming activity (Nakano & Tomlinson, 1967) mediates the branchial adaptations to exercise in *Salmo gairdneri* (Randall, 1970; Johansen, 1972).

Yet, for reasons long recognized in mammalian physiology, the aforementioned work is subject to severe limitations. Calculations of branchial resistance shifts based on blood pressure and flow changes measured *in vivo*, such as those performed by Stevens (1968*a*, *b*) and Johansen (1972) do not necessarily indicate alterations in vascular tone. Resistance can change quite significantly in response to purely physical influences when blood pressures and flows vary, without any alterations in tone of local, humoral, or autonomic origin (Whittaker & Winton, 1933; Green *et al.* 1944; Burton, 1951; Kuida, 1965). These factors are the distensibility of resistance vessels and the anomalous viscosity characteristics of blood, the former being considered predominant (Kuida, 1965). The peculiar situation of the gills as a resistance between two arterial pressures which are to a certain extent independently controlled may make passive changes in blood-vessel radius or number of particular importance in their function. Because of this potential distensibility, the aforementioned studies on isolated/perfused preparations must be considered quantitatively applicable only to

the precise afferent and efferent pressure conditions pertaining in each investigation. A further difficulty is that in no case have dose (or concentration)/response relationships been determined for catecholamines. Only through the construction of such relationships can classical pharmacological analyses, such as potency comparisons (Furchgott, 1967), be used to characterize the system.

The first objective of the present study was therefore the development of a whole-gill preparation of the rainbow trout in which complete pressure-differential/flow (Pd/Q) relationships (e.g. Green *et al.* 1944; Nichol *et al.* 1951) could be described with a Newtonian perfusate. The next step was an examination of the effect of the absolute level of afferent and efferent pressures on the Pd/Q profile in order to evaluate possible passive changes in resistance with shifts in ventral and dorsal aortic pressures. A further aim was the assessment of the influence of changes in vasomotor tone on the complete Pd/Q relationship, and, if possible, the derivation of a quantitative expression of tone change which would be applicable to the whole profile, and therefore to the *in vivo* situation where Pd, Q, and gill resistance (Rg) alter simultaneously. We hoped that it would be possible to use such a measure in constructing concentration/response curves for the effects of a number of sympathomimetic substances on Rg. The adrenergic receptors in teleost gills have been variously characterized as α-dilatory (Östlund & Fänge, 1962; Johansen, 1972), α- and β-dilatory (Randall *et al.* 1972), α-constrictory and β-dilatory (Reite, 1969; Belaud *et al.* 1971), and β-dilatory (Rankin & Maetz, 1971; Belaud *et al.* 1971). In originally defining α- and β-receptor sites, Ahlquist (1948) compared the potencies of a series of sympathomimetic amines with known differences in 'α-' and 'β-' stimulating activities. Such potency comparison remains the only method by which adrenoreceptors can be strictly differentiated (Arnold & McAuliff, 1971; Arnold, 1972). However, previous studies on the teleost gill have not used this technique; by applying it to the perfused trout gill, we hoped to clarify the situation. Finally, a simple mathematical model describing Rg/branchial dimensions relationships (with particular reference to tunas) has recently been presented by Muir & Brown (1971). By using values in the literature and approximations from other species, these workers were able to apply the same equations to the rainbow trout with some success. In the present investigation, all necessary variables have been determined directly in *S. gairdneri*, and this information has been used to critically evaluate the model and some of its implications.

MATERIALS AND METHODS

Rainbow trout (*S. gairdneri*; 130–440 g) were obtained from commercial suppliers in Norfolk and maintained in an outdoor tank under seasonal conditions. In an attempt to standardize the thermal history of animals, all fish were kept at $14{\cdot}5 \pm 1{\cdot}5$ °C for at least 1 week prior to experimentation. Gill-perfusion experiments were performed on 95 preparations in a constant-temperature room at 5 ± 1 °C.

I. *Gill-perfusion studies*

(i) *The perfusion medium*

The medium consisted of Cortland salmonid saline (Wolf, 1963) containing 10 i.u./ml of heparin (Evans Medical) and 4% polyvinylpyrrolidone (P.V.P.) (averag

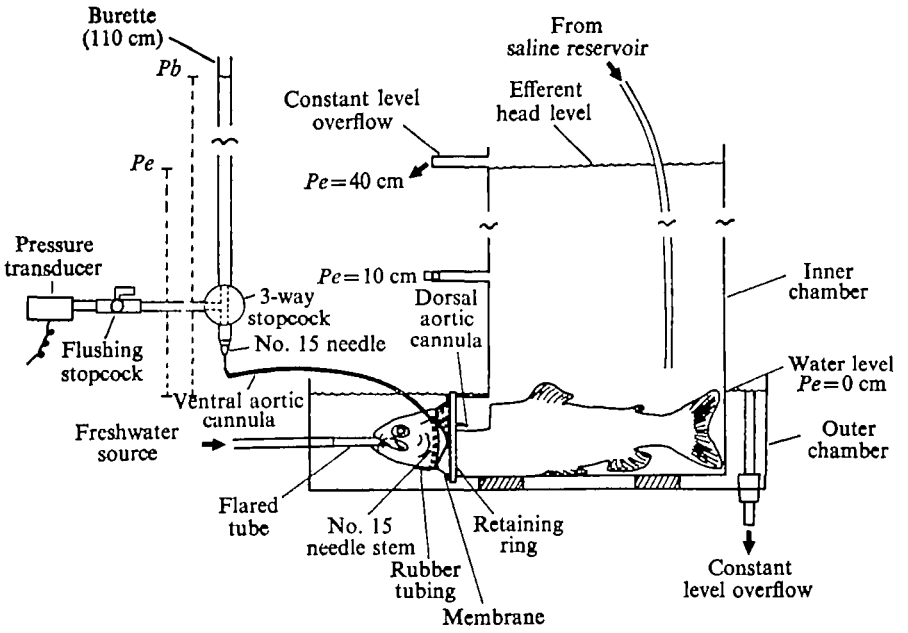

Fig. 1. The apparatus used for determination of branchial pressure/flow profiles in the rainbow trout at different levels of efferent pressure (*Pe*).

molecular weight = 40000; Sigma) as a colloid substitute for plasma proteins. Before use the solution was adjusted to pH 7·4–7·5 and in some experiments was passed through a 0·22 μm Millipore filter (Rankin & Maetz, 1971).

(ii) *Preparation of animals*

A trout was anaesthetized with 1:15000 MS-222 (Sandoz) on an operating table (Smith & Bell, 1964) at the acclimation temperature. A flared plastic tube was sewn onto the roof of the mouth so as to terminate immediately anterior to the first gill slits, and the jaws were sewn tightly around the stem of the tube; this served for external perfusion with fresh water (Fig. 1). The trout's head was inserted through a hole in a Portland latex rubber dam (S.S. White Dental Mfg.), and this membrane was sewn tightly to the skin around the circumference of the body at a level just posterior to the opercular openings (Fig. 1). Removal of the pectoral fins was necessary to ensure a good seal. 500 i.u. of sodium heparin (Sigma)/100 g was then injected into the ventricle and allowed to circulate for 5 min. The ventral aorta was cannulated as far anteriorly as possible with 1 cm of soft rubber tubing attached to the stem (5 cm) of a no. 15 needle (Fig. 1). The needle was bent to the curvature of the animal's body and ran dorsoventrally along the margin of the cleithrum, just anterior to the rubber membrane. In turn, the needle was joined to 15 cm of PP200 (Portex). The cannula was filled with perfusion medium and attached to a perfusion bottle at a head of 30 cm. The ventricle was ruptured to permit flushing of blood from the animal and a few minutes later the ventral incision was stitched up. A section of the fish's dorsal musculature, extending 4 cm immediately posterior to the membrane and vertically to the depth of spinal cord, was removed and a short length (2–3 cm) of tightly fitting tubing (PP200 or greater) was inserted into the dorsal aorta and advanced anteriorly to the

approximate level of the last efferent branchial artery. This cannula served to prevent collapse of the dorsal aorta under the efferent pressure which was later applied.

(iii) *The perfusion system* (Fig. 1)

The trout was placed in a Perspex chamber from which the head protruded; the membrane was secured across a hole in the bottom front of the container by a Perspex retaining ring and brass screws. The chamber could be filled with saline to apply a constant 'efferent' or dorsal aortic pressure (*Pe*; in most cases, 40 cm of saline) to the whole cut end of the head including the dorsal aorta. This container was then placed inside a second Perspex chamber which was attached to an aerated freshwater source and equipped with a constant-level overflow. The external mouth-perfusion tube was connected directly to the freshwater source and the overflow was adjusted so that the water surface was approximately 1 cm above the animal's head. All pressures were measured relative to this water surface as zero and expressed in units of cm of saline (S.G. at 5 °C = 1·013). Branchial water flow (100 ± 20 ml/100 g/min) could be checked by timed collection from the overflow drain. The membrane served as a barrier between the freshwater irrigating the gills externally and the internal medium. At high external pressures (*Pe*), a slight leakage across the membrane from the efferent head to the freshwater compartment was unavoidable; constant supply from a saline reservoir at a rate slightly greater than the leak (and overflow through the vents provided in the chamber) maintained the *Pe*. In a few early experiments, the fish was held in a chamber similar to the above but without provision for a positive efferent pressure.

The perfusion system utilized the principle of the classic 'vertical tube' technique of Nichol *et al.* (1951). The ventral aortic cannula for internal perfusion was attached via a no. 15 needle to a glass burette which extended to a height of 110 cm above the water surface. This vertical tube was attached through a three-way stop-cock and sidearm to a Sanborn 267 BC pressure transducer whose signal was amplified by a Sanborn 350–1100C carrier pre-amplifier and displayed on either a Servoscribe or a Bausch and Lomb V.O.M. 5 chart recorder. When the stop-cock was in the position shown in Fig. 1, the perfusate flowed from the burette into the preparation so that the fluid level in the burette gradually fell. The pressure trace was thus a record of the descent of the meniscus with time, of which the slope at any given point multiplied by the volume per unit height of the tube (about 0·5 ml/cm) gave the perfusion flow rate (Q) for that particular head in the burette. However, in such a system, the actual pressure applying at the ventral aorta (*Pg*) for any calculated Q is lower than the applied head in the vertical tube (*Pb*) due to the resistance of the delivery system (*Rd*). Therefore at the end of an experiment the perfusion cannula was dissected free from the ventral aorta, care being taken that all ligatures remained intact, and *Rd* was measured alone. The value obtained was invariably independent of *Pb*, indicating that all effective resistance resided in the cannula tubings, rather than in the burette itself. The true *Pg* and the resistance of the gill preparation (*Rg*) could therefore be calculated for any *Pb*:

$$\left[\frac{Pb-Pe}{Q}\right]-Rd = Rg,$$

$$[Rg.Q]+Pe = Pg.$$

In determining full Pg/Q profiles the fluid level in the burette was allowed to fall from 105 cm to a height close to or at which flow ceased, a procedure generally lasting about 15 min. Pg and Q were calculated at points corresponding to 5 cm intervals on the vertical column. At low Q, the procedure could be accelerated by opening a stopcock on the sidearm (Fig. 1), thereby quickly shifting to the next desired Pb. This valve was also used for changing solutions and flushing the burette during the drug studies.

(iv) *The preparation*

A persistent increase in Rg with time during perfusion was encountered; this difficulty was never completely overcome, but was greatly reduced by the following factors, in order of importance: low temperature (5 °C); the addition of 4% P.V.P. to prevent oedema; and external irrigation with aerated freshwater (to stop constriction caused by local hypoxia). There was no evidence that low temperature altered adrenergic reactivity or increased the absolute value of Rg (beyond that expected by the viscosity effect) in the preparation (cf. Kirschner, 1969). Reduction of particulate contamination of the perfusion medium by Millipore filtration (0·22 μm) and by thoroughly pre-washing the system with Millipore-filtered water (Rankin & Maetz, 1971) had a slight but definite effect in slowing the rate of Rg rise. However, the small benefit afforded by this tedious procedure made its use worthwhile only for those runs in which an absolute minimum rate of Rg rise was desirable. The use of an efferent column of saline in contact with the cut posterior surface of the head at high Pe caused noticeable oedema. However, the rate of Rg rise was no greater than in preparations at $Pe = 0$, indicating that this oedema was not transmitted to the branchial circulation.

(v) *Drugs*

The following sympathomimetic agents were added to the internal perfusion medium in various concentrations: L-adrenaline bitartrate, L-noradrenaline bitartrate, L-isoprenaline bitartrate, and L-phenylephrine hydrochloride (all Sigma). To avoid interactions, only one agent was tested in each preparation. The effect of each drug concentration was tested in at least 4 animals, 51 being used in total.

II. *Viscosity measurements*

The relative viscosities of trout blood, plasma, and the perfusion medium at 5 °C were determined by comparing their flow rates with that of water in a wide-bore Ostwald U-tube (capillary diameter = 1·44 mm). Corrections were applied for differences in specific gravities of the various fluids, densities being determined gravimetrically. Relative viscosities were converted to absolute values by multiplying by the tabulated viscosity of water at 5 °C (1·519 cp). Blood was drawn into syringes wetted with a 5000 i.u./ml sodium heparin solution from the ventricle and/or the haemal arch of anaesthetized trout. Dilutions due to heparinization were less than 5%. Because of the large volumes needed for each measurement (10 ml), viscosities were assayed on pooled blood samples from two to three animals. Each sample was then centrifuged for plasma separation and haematocrit determination; the viscosity of the plasma alone was then measured.

III. *Branchial structure studies*

The weighted-averages technique of Muir & Hughes (1969), with minor modifications, was utilized for branchial area measurements. The second arch was divided into sections of 11 filaments; secondary lamellar areas were recorded at a minimum of three sites along the length of the central filament of each section. It was found that measurements with the microscope were most easily and accurately performed on very fresh material. Accordingly, the second branchial arch of one side was divided and sampled for determinations of lamellar area while the animal was maintained alive but anaesthetized on an operating table. The fish was later killed and the arches of the other side were used for assessment of filament length and lamellar spacing. Calculations were performed as described by Muir & Hughes (1969). Measurements of pillar-cell channel and marginal channel lengths were taken from the tracings of the individual lamellae used for branchial area determinations. A second series of similarly anaesthetized trout was employed for measurement of the diameters of the marginal channels, and of the channels between the pillar cells. Single lamellae were dissected free and examined in Cortland saline on a glass slide.

RESULTS

I. *Pressure/flow relationships*

The results of early experiments at Pe = 0 cm clearly indicated great differences from the *in vivo* situation (Fig. 2*a*). Pd/Q profiles were linear or slightly convex to the pressure axis, intersecting the ordinate at a pressure always greater than 12 cm, and sometimes as much as 30 cm. This positive pressure at which flow ceases corresponds to the 'critical closing pressure' (*CCPd*) of Burton (1951). Yet in living trout, the Pd measured across the branchial vascular bed was only about 12 cm in some studies (Stevens & Randall, 1967*a*; Holeton, 1971). Only at the highest pressure applied (40–60 cm) did Q (0·50–1·25 ml/100 g/min) approximate the estimated cardiac output (0·76 ml/100 g/min) of resting *S. gairdneri* at a comparable temperature (Stevens, 1968*b*). These profiles did show, however, that the branchial vasculature was quite distensible; in the first curve of Fig. 2*a*, for example, Rg was halved as Pd increased from 35 to 55 cm, a range over which the Pd/Q relationship was approximately linear.

In light of this distensibility it seemed likely that more normal Pd/Q relationships would be obtained when Pe was set at the normal dorsal aortic pressure. Figs. 2*b* and 2*c* illustrate typical results at Pe = 40 cm, which approximates dorsal aortic pressures measured in intact rainbow trout by many workers. It is important to note that in these plots Pd equals the difference between Pb and Pe, while in Fig. 2*a* Pd equalled Pg as Pe was 0 cm. Critical closure now occurred only at Pd = 4 cm or less in fresh preparations, and Q (0·5–1·0 ml/100 g/min) at the *in vivo* Pd (10–20 cm) was similar to the *in vivo* cardiac output (0·76 ml/100 g/min). Except at points close to the *CCPd*, where it abruptly increased to infinity, Rg was relatively stable over the whole profile indicating little further distensibility due to Pg elevations. Successive Pd/Q curves were displaced upwards and towards the Pd axis as Rg increased with time. Millipore filtration (0·22 μm) retarded this trend (Fig. 2*c*). *CCPd* (where Q = 0) also rose, but to a lesser relative extent than did Pd required to maintain equal finite Q over th

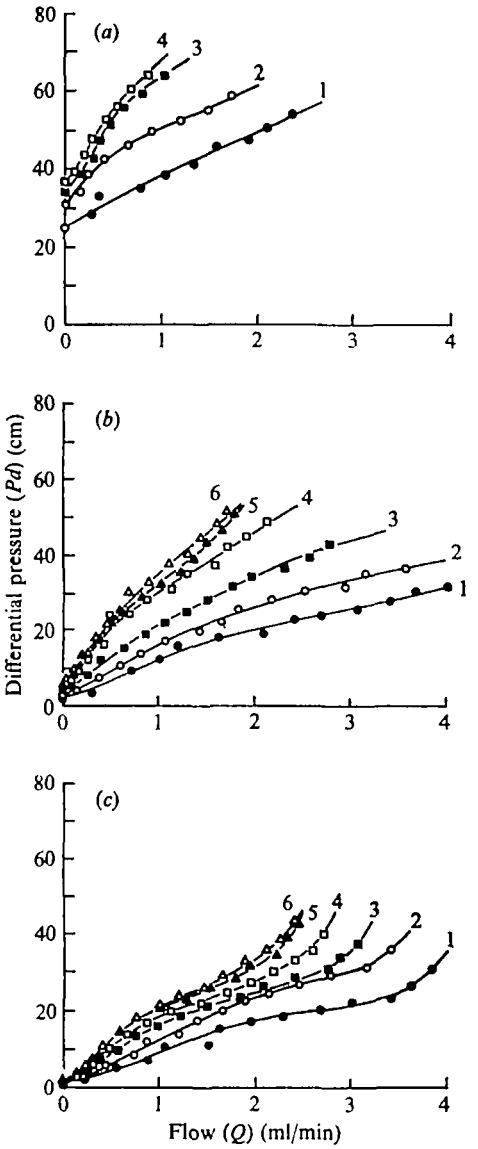

Fig. 2. Successive *Pd*/*Q* profiles in the gills of three trout under different conditions. 1, 2, 3, 4, 5, and 6 denote profiles taken at 10–25, 30–45, 50–65, 70–85, 90–105, and 110–125 min respectively after the start of perfusion. (*a*) *Pe* = 0 cm, 223·4 g. (*b*) *Pe* = 40 cm, 230·0 g. (*c*) *Pe* = 40 cm, Millipore filtration (0·22 μm), 363·0 g.

remainder of the curve. The rate of upward shift of the profiles diminished with time (Fig. 2). Despite the overall constancy in *Rg* (within curves) most profiles showed slight but definite deviations from linearity manifested either as a slight convexity to the pressure axis (Fig. 2*b*) or a sigmoidal form (Fig. 2*c*). These characteristic shapes of the individual curves were maintained in successive profiles as *Rg* increased with time (Figs. 2*b* and 2*c*) or decreased due to the administration of adrenergic drugs (Fig. 5).

Before computing a mean *Pd*/*Q* relationship based on the 54 complete profiles (*Pe* = 40 cm) determined during the period 10–25 min after the start of perfusion

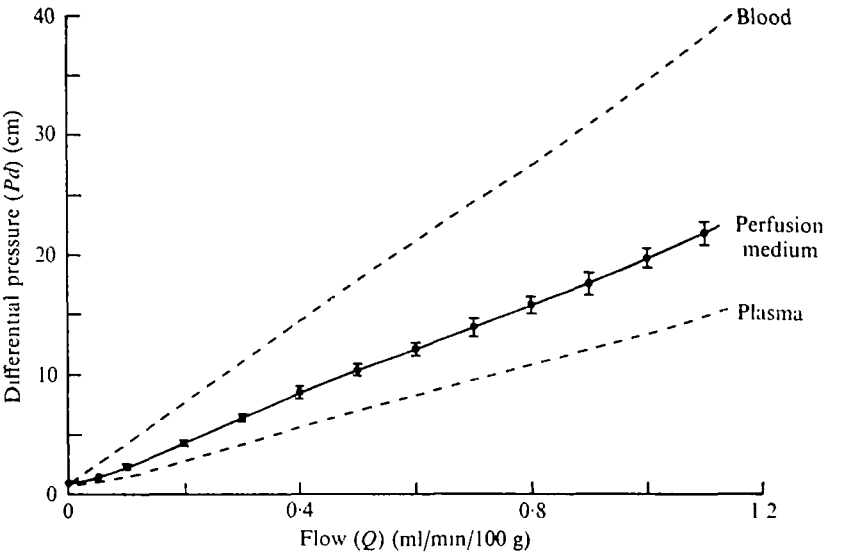

Fig. 3. The average Pd/Q relationship at Pe = 40 cm with the perfusion medium (absolute viscosity = 3·42 cp) from 54 trout (means ± 1 S.E.) determined 10–25 min after the start of perfusion at 5 °C. Mean weight = 272·3 ± 9·2 g; mean coefficient of condition = 1·056 ± 0·014. The dashed lines represent the same curve calculated for absolute viscosities equal to those of whole blood (5·97 cp) and plasma (2·36 cp) from Table 1.

(when *Rg* was minimal), it was necessary to test for the possible size-dependence of *Rg*. Plots of *Rg* against body weight at low, intermediate, and high Q (0·50, 1·75, and 3·00 ml/min) revealed highly significant ($P < 0.001$) negative correlations under all three conditions ($r = -0.530$, -0.647, -0.701; $n = 54$). Sufficient reciprocity was evident between the two parameters to indicate the benefit of expression of Q on a body-weight basis.

Fig. 3 presents the mean Pd/Q relationships (Pe = 40 cm, $n = 54$) determined by averaging profiles at the same Q/100 g. Constant Q was preferable to constant *Pd* as a basis for compilation because in our experimental technique, Q was essentially the independent (measured directly) and *Pd* the dependent variable (estimated by calculation). The mean *CCPd* was less than 1 cm, and *Rg* was virtually constant over most of the relationship at 20 cm/ml/100 g/min, a value close to that which can be deduced from *in vivo* measurements in resting trout (Stevens, 1968*b*). Averaging eliminated the convexities and sigmoids seen in individual profiles, and the relationship was strictly linear except near the *CCPd*. This mean curve has only been calculated up to the highest Q/100 g which was observed in every preparation (1·1 ml/100 g/min). Much greater values of Q/100 g were recorded in some animals, but any extension of the average profile beyond this point would have been biased in favour of the fish with lower *Rg* due to the limited *Pb* capacity (i.e. height) of the vertical tube.

Changing *Pg* and *Pe* revealed more fully the passive distensibility characteristics of the branchial vasculature. Pd/Q profiles were recorded in six preparations at a series of *Pe* values (0, 10, 20, 30 and 40 cm) using approximately the same range of Q in each case (by manipulation of *Pb*). Millipore filtration and rapid profile determinations (10 min) were used to minimize the spontaneous *Rg* rise. To negate its relative effects

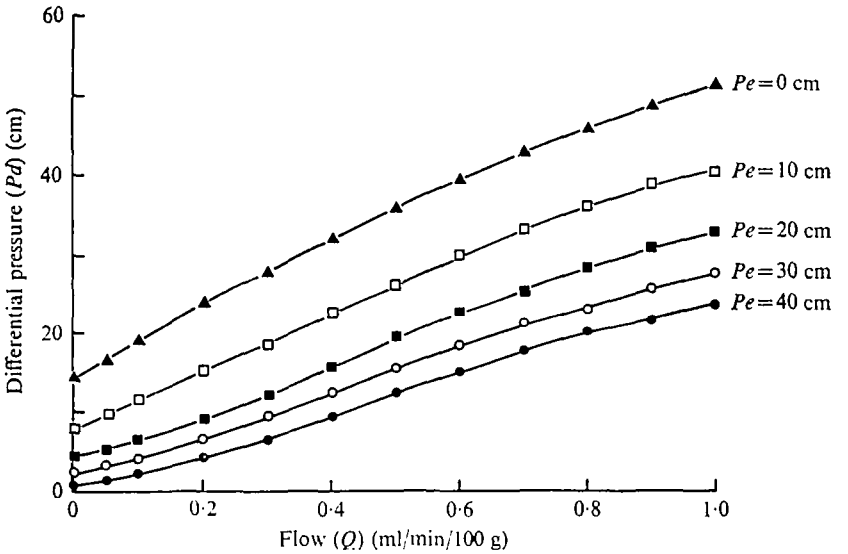

Fig. 4. The effect of five different levels of dorsal aortic pressure (*Pe*) on the mean *Pd*/*Q* profile in the trout gill. Millipore filtration (0·22 μm). $n = 6$; mean weight = 372·7 ± 18·3; mean coefficient of condition = 1·062 ± 0·012. In three preparations *Pe* was sequentially increased with time, and sequentially decreased in the other three.

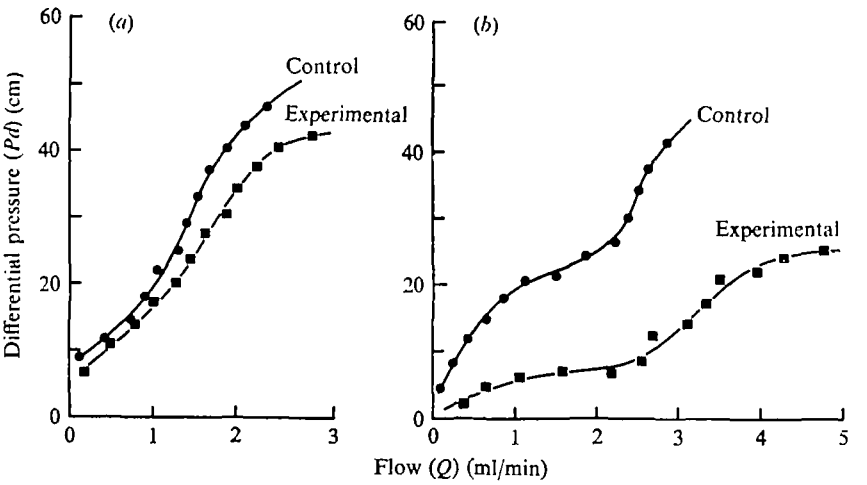

Fig. 5. Typical full *Pd*/*Q* profile comparisons for (*a*) small vasodilation caused by $5{\cdot}5 \times 10^{-3}$ M L-phenylephrine; wt = 287·3 g, and (*b*) large vasodilation caused by $5{\cdot}5 \times 10^{-5}$ M L-adrenaline; wt = 220·5 g. Millipore filtration (0·22 μm). The comparisons were made 1–2 h after the start of perfusion. The experimental curve was recorded 10–20 min after addition of the drug.

Pe was applied in increasing magnitude in three trout and decreasing magnitude with time in the other three preparations; the results were averaged as in Fig. 3. Increasing *Pe* while holding *Pd* constant obviously had a profound effect in decreasing *Rg* through simple physical distension of the vessels (Fig. 4). The vertical shift in the *Pd*/*Q* profile was greatest between *Pe* = 0–10 cm, and least between *Pe* = 30–40 cm. Nevertheless, the displacement in the latter case was still large enough to indicate that

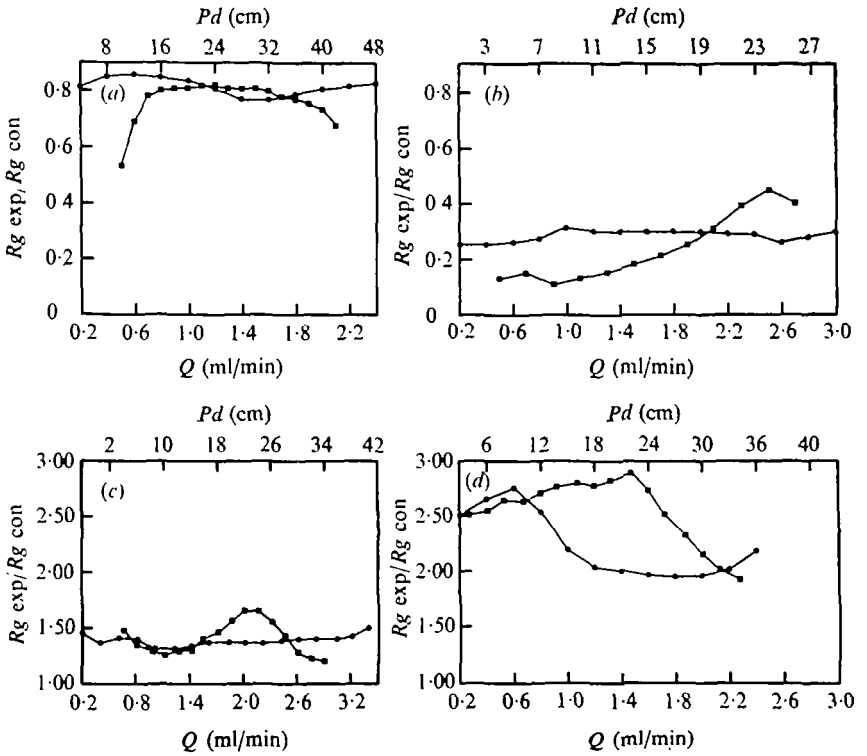

Fig. 6. A comparison of two indices of change in branchial vascular tone under conditions of (*a*) low vasodilation, data from Fig. 5*a*; (*b*) high vasodilation, data from Fig. 5*b*; (*c*) low vasoconstriction, data from curves 1 and 2 in Fig. 2*c*; (*d*) high vasoconstriction, data from curves 1 and 6 in Fig. 2*c*. The two indices are the ratios of vascular resistances in the experimental and control states (Rg_{exp}/Rg_{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.

changes in blood pressure levels of a size which occur *in vivo* will significantly alter *Rg* in the absence of changes invascular tone. The results were qualitatively similar in all six trout, though of course the five profiles showed greater vertical separation in the three fish in which *Pe* was reduced with time.

II. *The expression of change in vascular tone*

In order to derive a single expression which would quantitatively describe a change in vascular tone over the whole *Pd*/*Q* relationship, it was first necessary to record complete profiles at different levels of vasomotor tone. For active vasodilation sympathomimetic agents were added to the perfusate. Again to minimize the effect of *Rg* increase with time Millipore filtration was employed, profiles were determined rapidly, and the comparisons were performed at 1–2 h after the start of perfusion (by which time the rate of *Rg* rise was considerably reduced). Fig. 5 displays typical results for small ($5{\cdot}5 \times 10^{-8}$ M L-phenylephrine), and large ($5{\cdot}5 \times 10^{-5}$ M L-adrenaline) vasodilations, both determined 10–20 min after the administration of the drug. Such runs were not carried out for active vasoconstriction. However, for reasons to be presented subsequently, we believe that the persistent time-dependent increase in *Rg* was a

least partially reflective of an elevation of tone, and that the contribution of active vasoconstriction to this phenomenon was maximal when Millipore filtration was used. Thus the profiles at 30–45 min and 110–125 min in Fig. 2*c*, in comparison to the initial curve at 10–25 min, have been considered as examples of vasoconstriction.

The most reasonable quantifications of tone change would appear to be *Rg* experimental/*Rg* control either at the same *Pd* (i.e. Q_{con}/Q_{exp}) or at the same *Q* (i.e. Pd_{exp}/Pd_{con}). In Fig. 6, these comparisons have been made for all four situations (high and low vasodilation and vasoconstriction) from Figs. 5 and 2*c*. For dilation, these results clearly showed that comparison of *Rg* values at the same *Q* yields much greater consistency over the whole range of overlap of control and experimental curves than does comparison at the same *Pd* values. Variation in Pd_{exp}/Pd_{con} was negligible but up to three fold in Q_{con}/Q_{exp}. The shapes and relative positions of the curves in Fig. 5 can be seen to account for this difference. For small vasoconstrictions, the same differences were evident, but were not clear-cut for the larger ones, probably because factors in addition to vasoconstriction contributed to the change. Nevertheless, Pd_{exp}/Pd_{con} was generally more consistent than Q_{con}/Q_{exp} even in this situation. Measurements of vascular tone change (as Rg_{exp}/Rg_{con}) at one point on a *Pd*/*Q* profile can thus be extrapolated to the rest of the relationship with reasonable accuracy when *Rg* has been assessed at the same *Q*.

III. *The effects of sympathomimetic agents on gill resistance*

As long as suitable correction was applied for the spontaneous *Rg* rise, the size of the change in vasomotor tone (expressed as Rg_{exp}/Rg_{con} at the same *Q*) caused by adrenergic agents was independent of the absolute level of *Rg*. Thus each preparation could be used to test a number of different drug concentrations. Maximum effects were invariably seen 5–20 min after administration. When making *Rg* comparisons at the same *Q*, full *Pd*/*Q* profile determinations during the experimental period were unnecessary, although a fairly comprehensive control curve was required to ensure that Q_{exp} could be matched with Q_{con}. The following protocol was therefore devised: at 10–25 min an extensive *Pd*/*Q* profile was recorded for this control curve; the drug was then added to the perfusion medium, and separate, restricted *Pd*/*Q* relations determined at 25–30, 30–35, 35–40, and 40–45 min; a 15 min period of drug washout at high *Pb* then followed. The cycle was then repeated up to three times. The average Rg_{exp}/Rg_{con} for the 5 min period in which the agent's effect was greatest was computed, the Rg_{con} value having first been corrected for spontaneous *Rg* increase. To obtain such correction, the regime was carried out in 10 preparations for four successive cycles without the addition of drugs. Corrections factors, by which Rg_{con} was multiplied in the drug studies, were 1·4090, 1·2262, 1·0783, and 1·0044 in cycles 1, 2, 3 and 4 respectively. These values reflected the decreasing rate of *Rg* rise with time which by the fourth cycle (160+minutes) had become negligible. The calculated Rg_{exp}/Rg_{con} ratios were finally expressed as percentage decreases in *Rg*.

The true catecholamines L-isoprenaline (ISO), L-adrenaline (AD), and L-noradrenaline (NAD) all caused branchial dilation, producing typical sigmoidal log concentration/response curves with similar slopes (on a log/linear basis) and nearly identical maximal effects (60% decrease in *Rg*) (Fig. 7). The slope of the NAD curve appeared slightly steeper than those of the other two, but the difference was not

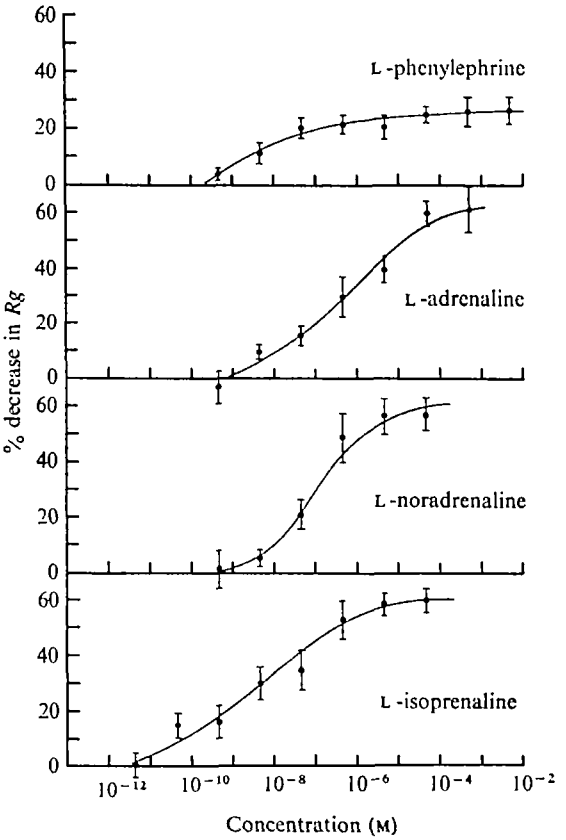

Fig. 7. Concentration/response relationships for four adrenergic agonists in the rainbow trout gill. See text for experimental details. Concentration is plotted on a logarithmic scale. Each point represents the mean ± 1 S.E. of 4–9 determinations, each from a different preparation.

significant. L-phenylephrine (PHE) also effected vasodilation but with a very different relationship, having a similar threshold (10^{-9} M) to AD and NAD, but quickly rising to a much lower maximum effect (25 % decrease in *Rg*) at 10^{-6} M without evidence of sigmoidal form. The potencies of the three catecholamines, calculated as the ratios of concentrations producing equal effects corresponding to 50 % of the maximum response (Furchgott, 1967), were ISO = 1000: NAD = 33: AD = 10. The anomalous PHE curve could not be similarly analysed, but on account of its much lower efficacy PHE may be considered the least potent of the four agonists.

IV. *Viscosities*

The viscosities and specific gravities of whole blood, plasma, and the perfusion medium are given in Table 1. Viscosity values for blood were largely reflective of erythrocyte concentrations, a low haematocrit in one sample (no. 4) being associated with a reduced viscosity. Whole-blood figures were 2–3 times greater than the corresponding plasma viscosities, while that of the perfusion medium was intermediate.

Table 1. *Viscosities and specific gravities of trout blood, trout plasma, and the perfusion medium at 5 °C*

Sample	1 *$n = 3$	2 $n = 3$	3 $n = 3$	4 $n = 2$	Mean
Hematocrit (%)	36·2	36·1	35·2	25·0	33·1
Blood specific gravity	1·040	1·040	1·041	1·038	1·040
Relative blood viscosity	4·25	4·24	4·04	3·18	3·93
Absolute blood viscosity (cp)	6·46	6·44	6·14	4·83	5·97
Plasma specific gravity	1·027	1·028	1·021	1·023	1·025
Relative plasma viscosity	1·57	1·60	1·50	1·55	1·56
Absolute plasma viscosity (cp)	2·38	2·43	2·28	2·35	2·36

*n = number of animals contributing to the pooled sample.
Perfusion medium: specific gravity, 1·013; relative viscosity, 2·25; absolute viscosity, 3·42.

Table 2. *Branchial dimensions of the rainbow trout*

Fish	1	2	3	4	Mean
Weight (g)	333·5	397·6	307·8	322·1	340·3
Coefficient of condition $\frac{\text{weight} \times 100}{(\text{fork length})^3}$	1·098	1·213	1·096	1·040	1·112
Total number of filaments					
Anterior hemibranchs	798	810	844	814	816·5
Posterior hemibranchs	824	852	872	876	856·0
Total filament length (mm)					
Anterior hemibranchs	6268·40	6611·14	6405·22	6302·38	6396·79
Posterior hemibranchs	6192·00	6168·58	6329·08	6292·92	6245·65
Weighted average lamellar spacing (no./mm), both sides of filament					
Anterior hemibranchs	42·24	39·41	38·12	42·54	40·58
Posterior hemibranchs	37·87	37·36	37·37	37·34	37·49
Total number of secondary lamellae					
Anterior hemibranchs	264777	260545	244167	268103	259398
Posterior hemibranchs	234491	230458	236518	234978	234111
Total number of secondary lamellae/fish	499268	491003	480685	503081	493509
Weighted average secondary lamellar area (mm^2)					
Anterior hemibranchs	0·1544	0·2054	0·1815	0·1654	0·1767
Posterior hemibranchs	0·1539	0·2077	0·1836	0·1591	0·1761
Total secondary lamellar area (mm^2)					
Anterior hemibranchs	40881·60	53515·95	44316·31	44344·28	45764·54
Posterior hemibranchs	36088·17	47866·16	43424·65	37384·94	41190·98
Total secondary lamellar area/fish (mm^2)	76969·77	101382·11	87740·96	81729·22	86955·52
Total secondary lamellar area/g body weight (mm^2/g)	230·79	254·99	285·06	253·74	256·15

Table 3. *The distribution of filaments, filament lengths, and secondary lamellar areas over the four gill arches of one side of the rainbow trout*

Fish ...	1	2	3	4	Mean	
			Total number of filaments/arch			
Arch 1	186	195	207	195	193·75	
Arch 2	214	217	222	215	217·00	
Arch 3	213	224	228	222	221·75	
Arch 4	198	195	201	213	201·75	
			Total filament length/arch (mm)			
Arch 1	1582·10	1697·60	1656·55	1633·20	1642·36	
Arch 2	1891·27	1768·64	1674·57	1700·60	1758·77	
Arch 3	1619·63	1672·76	1668·20	1650·80	1652·85	
Arch 4	1137·20	1250·86	1367·83	1313·05	1267·24	
			Total secondary lamellar area (mm^2)			
Arch 1	9771·18	13463·02	11414·15	10553·95	11300·58	*
	25·39%	26·56%	26·02%	25·83%	25·95%	(27·64%)
Arch 2	11643·85	14019·71	11537·20	11024·35	12056·28	
	30·26%	27·66%	26·30%	26·98%	27·80%	(29·43%)
Arch 3	10014·45	13276·02	11495·09	10740·32	11381·47	
	26·02%	26·19%	26·20%	26·28%	26·17%	(25·76%)
Arch 4	7055·43	9932·29	9424·03	8545·98	8739·43	
	18·33%	19·59%	21·48%	20·91%	20·08%	(17·17%)

* Data from Hughes (in Paling, 1968) for 175 g *Salmo trutta*.

V. *Branchial structure*

Within the accuracy of measurement no differences could be found in representative lamellar areas between anterior and posterior hemibranchs at the same level in the arch. Filaments from either hemibranch were therefore selected randomly from the middle of the 11 filament section demarcated on the anterior surface. However, as small but consistent differences were noted in filament length, total filament number, and lamellar spacing, the two surfaces were treated separately in the analysis (Table 2). In all fish the anterior hemibranch bore slightly fewer but longer filaments with more closely packed lamellae. Consequently, the total number of secondary lamallae and the total lamellar area of the anterior hemibranchs exceeded those of the posterior surfaces. Over the limited weight range examined, variation in most parameters between animals was slight, the most noticeable being in weighted average lamellar area, and therefore in total secondary lamellar area.

The data have been broken down to the level of the individual gill arches in Table 3. Arches 2 and 3 consistently carried more filaments than 1 and 4. Total filament length was greatest in arch 2, somewhat lower and about equal in 1 and 3, and markedly smaller in 4. Thus arch 2 accounted for the greatest proportion of gill area, 1 and 3 for slightly less, and 4 for only about one fifth of the total. The only comparable information on branchial area distribution in salmonids, that of Hughes (reported in Paling, 1968) for a single 175 g *S. trutta*, is in agreement with the present work (Table 3).

The internal structure of a lamella was seen as a complex anastomosis of blood channels separated and indeed formed by individual pillar cells. A conspicuous marginal channel, with connexions to the pillar-cell channels, bordered the free edg

Table 4. *The weighted mean lengths of the secondary lamellar blood channels*

Fish ...	1	2	3	4	Mean
Marginal channel length (mm)	0·7678	0·8402	0·8339	0·7962	0·8095
Pillar-cell channel length (mm)	0·4668	0·4565	0·4687	0·4472	0·4598

Table 5. *The diameters of the secondary lamellar blood channels*

Fish ...	5	6	7	8	Mean
Weight (g)	193·6	294·3	274·9	185·7	237·1
Coefficient of condition $\frac{\text{weight} \times 100}{(\text{fork length})^3}$	1·064	0·924	1·039	1·069	1·024
Marginal channel diameter (mm)					
Mean	$6{\cdot}04 \times 10^{-3}$	$8{\cdot}28 \times 10^{-3}$	$6{\cdot}40 \times 10^{-3}$	$6{\cdot}46 \times 10^{-3}$	$6{\cdot}80 \times 10^{-3}$
±	±	±	±	±	
1 S.E.	$0{\cdot}37 \times 10^{-3}$	$0{\cdot}51 \times 10^{-3}$	$0{\cdot}36 \times 10^{-3}$	$0{\cdot}42 \times 10^{-3}$	
N	17	26	26	29	
Pillar-cell channel diameter (mm)					
Mean	$3{\cdot}50 \times 10^{-3}$	$4{\cdot}07 \times 10^{-3}$	$3{\cdot}96 \times 10^{-3}$	$3{\cdot}91 \times 10^{-3}$	$3{\cdot}86 \times 10^{-3}$
±	±	±	±	±	
1 S.E.	$0{\cdot}17 \times 10^{-3}$	$0{\cdot}22 \times 10^{-3}$	$0{\cdot}18 \times 10^{-3}$	$0{\cdot}17 \pm 10^{-3}$	
N	20	32	30	41	

of the lamella. Because of the multiple interconnexions and lack of linearity of the pillar-cell vessels, it was not possible to assess accurately the number of channels per lamella. However, it appeared that individual lamellae contained the equivalent of 15–30 such channels each, the blood space underlying about 50% of the lamellar surface area. When gentle pressure was applied to a freshly excised preparation, individual erythrocytes could be seen moving through the pillar-cell channels, undergoing 'squeeze' in the process. A significant component of flow was obviously also carried by the marginal channels, a steady stream of red blood cells passing along the periphery. As noted by Muir & Brown (1971), pillar-cell channels ran the length of a lamella. However, lamellae were not rectangular, but decreased in length towards their outer border, so the outer channels were shorter than those nearest the junction with the filament. Average pillar-cell channel length has therefore been calculated as the mean, rather than the basal, length of the lamellar body; the short length provided by the raised leading edge of the structure has been omitted from the average. The marginal channel length has been taken as simply that of the free border of the lamella. The data have been subjected to a weighted averages analysis analogous to that used for lamellar area (see Muir & Hughes, 1969). The weighted mean lengths of the pillar-cell channels were slightly greater than half the marginal channel values; variability between fish was again small (Table 4).

Because of the need to deal with extremely fresh material, it was necessary to use a different set of animals for determinations of channel diameters. Both marginal channel and pillar-cell channel widths were extremely variable even within individual lamellae, as reported by Muir & Brown (1971). Thus it did not appear worthwhile to analyse trends over the gill arch or between hemibranchs. Nevertheless, the means,

derived from multiple measurements on five to eight lamellae per fish, were surprisingly uniform (Table 5). The average diameters of the pillar-cell channels were approximately one half the marginal channel values.

DISCUSSION

Previous studies on the perfused teleostean gill have been plagued by problems of deterioration manifested either as increasing vascular resistance, increasing permeability, or both (Keys, 1931; Bateman & Keys, 1932; Schiffman, 1961; Östlund & Fänge, 1962; Kirschner, 1969; Rankin & Maetz, 1971; Randall *et al.* 1972). In the present investigation, the former was a significant problem. A slight leakage of colloid from the branchial epithelium could be seen after several hours' perfusion in a few preparations, but this was not of particular importance in a haemodynamic study. The approach adopted has been to reduce the rate of 'deterioration' by choice of experimental conditions, and then to quantify it as a predictable phenomenon for which correction factors could be derived and applied. As the *Rg* rise was not accompanied by a change in sensitivity or responsiveness to catecholamines (after correction), the approach seemed valid for the present purposes.

Oedema and particulate blockage did not seem to be important in the spontaneous *Rg* increase. Certainly the trunk of *S. gairdneri* has been perfused for many hours under identical conditions with negligible measured fluid uptake and rise in vascular resistance (Wood, unpublished results). It is of interest that *CCPd* as well as *Rg* at the same Q increased with time. Burton and co-workers (Burton, 1951; Nichol *et al.* 1951) found that the *CCPd* was principally an index of vascular tone and unaffected by even extensive oedema. This suggests that a progressive increase in tone may have accounted for a good proportion of the *Rg* elevation in our preparation. Such active vasoconstriction could have been neural or local (e.g. myogenic, metabolic) in origin. The time-dependent *Rg* rise has therefore been tentatively attributed to a real tone increase complicated by minor problems of oedema and particulate blockage.

Although the mean Pd/Q relationship at Pe = 40 cm was approximately linear, individual profiles displayed either convexity to the pressure axis or a sigmoidal form over parts of their range, these shapes remaining evident even when vasomotor tone changed. The use of a Newtonian perfusion fluid eliminated the involvement of the anomalous viscosity of blood (Burton, 1965) in this curvilinearity. Convexity has been observed in a wide variety of mammalian tissues (Kuida, 1965) and probably reflects a slight passive distensibility. Sigmoidal shapes have been recorded more rarely in mammalian work (Green *et al.* 1944; Girling, 1952*a*; Burton, 1965; Lutz *et al.* 1969), sometimes being seen as autoregulation in which vascular resistance varied to buffer flow from pressure changes over the normal range. In some of our preparations the 'distribution' of the sigmoid was such as to satisfy this purpose, while in others exactly the opposite occurred – i.e. $\Delta Q/\Delta Pd$ was greatest in the physiological range (10–20 cm). Nevertheless, this pattern is important for it shows that the branchial vessels do not always behave as simple distensible tubes, but can actively alter their tone in response to pressure changes. Again, the control may be local or central in origin.

Blood viscosity determinations were made in a tube of sufficiently wide bore (d = 1·44 mm) to avoid the Fahraeus–Lindqvist effect (Burton, 1965). Cameron and

Davis (1970) have reported somewhat lower relative viscosity values for the blood of *Salmo gairdneri*, but used a capillary small enough (d = 0·55 mm) to introduce Fahraeus–Lindqvist error and worked at a higher temperature (Hock, 1964). The *Pd*/*Q* profiles plotted for viscosities equal to those of plasma and whole blood represent extremes between which the physiological blood curve must lie (Fig. 3). Due to the Fahraeus–Lindqvist phenomenon and other complications, the effective blood viscosity *in vivo* will be much lower than the viscometer figure, the actual value being determined by the proportion of the total resistance offered by vessels of different size (Whittaker & Winton, 1933; Burton, 1965; Jay, Rowlands & Skibo, 1972). As arterioles of 55 μm would reduce viscosity by about 50%, and lamellar channels of 4 μm would render it approximately equal to the plasma value, the *in vivo Pd*/*Q* relationship may in fact be quite close to that provided by the perfusion medium.

The results of experiments in which *Pg* and *Pe* were varied have revealed that both ventral and dorsal aortic blood pressures can have a profound influence on *Rg*, presumably through passive distension. It will subsequently be shown that the relative influence of *Pe* was apparently greater than that of *Pg* on *Rg*. These factors may be of great importance for branchial function *in vivo*. For example, Stevens (Stevens & Randall, 1967*a*, *b*; Stevens, 1968*a*, *b*) measured ventral and dorsal aortic blood pressures and estimated cardiac output in *S. gairdneri* during moderate exercise. He calculated that *Rg* decreased 68% while *Pd* changed from 11·3 to 24·4 cm and *Pe* from 35·8 to 42·5 cm. From Fig. 4 it can be estimated that *Rg* would decrease by 17% through passive factors alone resulting from the pressure changes. This indicates that both passive distensibility and an active decrease in tone are involved in the branchial vasodilation accompanying exercise. Similarly Holeton (1971) found that *Rg* fell by at least 44% (probably more) during CO-induced anaemia; from his data, we calculate that distensibility alone could have accounted for a 35% decrease. On the other hand, moderate hypoxia (P_{O_2} = 60 mm Hg) elicited a 14% elevation of *Rg* (Holeton & Randall, 1967*a*, *b*) while we estimate a passive decline of 20% due to hypertension. Thus in the latter case the real change in vasomotor tone must have been much greater than simple resistance-change calculations would indicate.

Mammalian studies on the effects of venous pressure manipulation appear somewhat analogous to the present work. Similar results have been commonly found – i.e. a decrease in resistance and/or the *CCPd* when the efferent (venous) pressure was raised (Girling, 1952*b*; Burton & Rosenburg, 1956; Rosenburg, 1956; Folkow & Löfving, 1956; Levy, 1958; Read, Kuida & Johnson, 1958; Hinshaw *et al.* 1963). An increase in resistance of uncertain genesis ('veno-vasomotor reflex') has been observed in other investigations (Yamada & Burton, 1954; Burton & Rosenburg, 1956; Johnson, 1959; Lutz *et al.* 1969). There is no evidence of such a reactive constriction in the present data. By waterfall theory (see Permutt & Riley, 1963), Fig. 4 might be thought to indicate the presence of a waterfall set at 10–20 cm in the trout gill; there was a high *CCPd* (14·3 cm) at *Pe* = 0 and for a constant *Pg* (e.g. 50 cm), *Q* was approximately stable between *Pe* = 0 and 20 cm. However, this phenomenon is more easily explained by a high distensibility at low *Pe* which would maintain *Q* in the face of a reduced *Pd*. As waterfall theory has received little experimental verification when relating to innate vascular tone (Caro *et al.* 1969), it seems unnecessary to apply the concept to the teleost gill at present.

The superiority of Rg_{exp}/Rg_{con} determined at the same Q over that determined at the same Pd as an index of vascular tone is in agreement with mammalian work (Green *et al.* 1944; Kuida, 1965). However, all previous quantitative studies on the perfused teleostean gill [with the exception of those of Reite (1969) and Kirschner (1969)] have measured Rg change at the same or varying Pd which would make the results valid only under those particular pressure conditions. This restriction is compounded by the fact that in none of the investigations, as far as we can determine, has the resistance of the cannula, etc. (Rd) been taken into account. In our experience the pressure drop across Rd can equal or exceed that across Rg in some circumstances and is of course a variable dependent upon Q.

The high vasodilatory potency of ISO (Fig. 7) indicates the presence of β-adrenoreceptors in the trout gill, a conclusion confirmed by blocking studies (Wood, unpublished results). However, the potency order ISO > NAD > AD > PHE is not characteristic of the traditional mammalian β-receptor where AD activity greatly exceeds that of NAD (Ahlquist, 1948; Furchgott, 1967). Three other studies have also suggested NAD > AD in the teleost gill (Östlund & Fänge, 1962; Rankin & Maetz, 1971; Belaud *et al.* 1971). As pointed out by Keys & Bateman (1932) and Rankin & Maetz (1971), the coronary circulation of higher vertebrates arose from the branchial vessels of fish; NAD > AD has also been demonstrated in the β-dilatory receptors of this tissue (Zuberbuhler & Bohr, 1965; Bohr, 1967). Although still a matter of some dispute because of disagreement between agonist and antagonist data, there is now good experimental support for a dual β-receptor hypothesis (Arnold & McAuliff, 1971; Arnold, 1972). The potency order ISO > NAD > AD and ratio 1000:33:10 indicate that the β-receptors mediating branchial vasodilation in the trout, in common with those relaxing mammalian coronaries, are of the β_1 variety, as opposed to the 'traditional' β_2 type serving bronchodilation and vasodepression in higher vertebrates.

PHE is a potent α-adrenoreceptor stimulant with little or no β-activity in mammals (Goodman & Gilman, 1970). The anomalous PHE concentration/response curve (Fig. 7) showing a low threshold but reduced efficacy could be interpreted in several ways: α-dilation; β-dilation by a partial agonist; α- or β-dilation antagonized by simultaneous α-constriction; release of endogenous catecholamines; or a non-specific effect. We favour one of the two latter hypotheses because α-dilation has never been proven in any preparation, PHE was inactive on other vascular α-receptors in the trout (Wood, unpublished results), and the PHE dilation was erratic and poorly sustained even at very high doses. Randall *et al.* (1972) suggested that α-dilatory receptors were present in the trout gill because NAD relaxed the branchial vessels; this result is better explained by the β_1-receptor theory.

The threshold concentrations (10^{-9}–10^{-8} M) for the two naturally occurring catecholamines (AD and NAD) were similar to the resting *in vivo* plasma levels of *S. gairdneri* (Nakano & Tomlinson, 1967). During activity, blood levels increased to a total of approximately 2×10^{-6} M which, from Fig. 7, would have caused about a 45% fall in Rg at the same Q. By extrapolation from Fig. 4, the absolute decrease in Rg during exercise due to elevated plasma catecholamines and passive distensibility would have been about 60%, a figure remarkably similar to the actual value of 68% calculated by Stevens (1968*a*). Thus the data in no way conflict with the hypothesis that circulatory AD and NAD may control branchial vasodilation *in vivo*.

Muir & Brown (1971) have modelled the relationship between vascular resistance and branchial dimensions in the teleost gill (through an extension of the Hagen–Poiseuille equation) assuming that laminar flow occurs, that all cardiac output passes through the pillar-cell channels, and that *Rg* resides wholly in these channels:

(1) $Pd = \frac{128\mu Ql}{n\pi d^4}$;

(2) $Ag = 2ndl$;

(3) $Pd = \frac{256\mu Ql^2}{\pi Agd^3}$;

where

Pd = differential pressure across gills (dynes/cm^2);

μ = viscosity (p);

Q = flow (cardiac output) (ml/sec);

l = pillar-cell channel length (cm);

d = pillar-cell channel diameter (cm);

n = number of channels;

Ag = surface area overlying the channels (cm^2).

All the above variables have been quantified in *S. gairdneri* permitting an appraisal of the model and some of its implications. There were slight differences in fish size between groups on which different measurements were made, but it is unlikely that variation of this magnitude was a large source of error in evaluation of the equations. All calculations have been performed for a 270 g animal.

The following values have been initially assumed; a normal *in vivo* Pd of 15 cm at Pe = 40 cm; μ = 3·42 cp (Table 1); Q = 2·009 ml/min (Fig. 3); l = 0·4598 mm (Table 4); and $d = 3{\cdot}86 \times 10^{-3}$ mm (Table 5). From these data, equation (3) predicts an Ag of 232·80 cm^2 which is 33·66% of the measured total secondary lamellar area of 691·60 cm^2 (from Table 2); this seems a quite reasonable figure for the respiratory surface area of a resting trout. Equation (2) in turn gives n = 6,558,383 for Ag = 232·80 cm^2; dividing this n by the measured number of secondary lamellae/fish (493,509; Table 2) yields 13·29 pillar-cell channels/lamella, a figure not far from our visual estimate of 15–30/lamella. Agreement between the two values would be improved if some lamellae were unperfused as may be indicated by the fact that the calculated Ag was smaller than the measured Ag.

Thus at least superficially the model would appear to have good predictive value. Yet it must be noted that the trout gill shows considerable passive distensibility (Fig. 4). In the above calculations d has been taken from excised preparations where the distending pressure was negligible, whereas *in vivo* a distending (i.e. transmural) pressure of about 47·5 cm would apply. It therefore became necessary to estimate the possible influence of passive changes in d and n on the model predictions.

From the data of Fig. 4, the dimension factor (d^4n) in the model was evaluated at different average transmural pressures [$Pt = \frac{1}{2}(Pg+Pe)$] by application of equation (1) with the previously assigned values for μ, Q, and l. Calculations were performed at Q = 0·2, 0·4, 0·6, 0·8 and 1·0 ml/100 g/min at each level of Pe (0, 10, 20, 30,

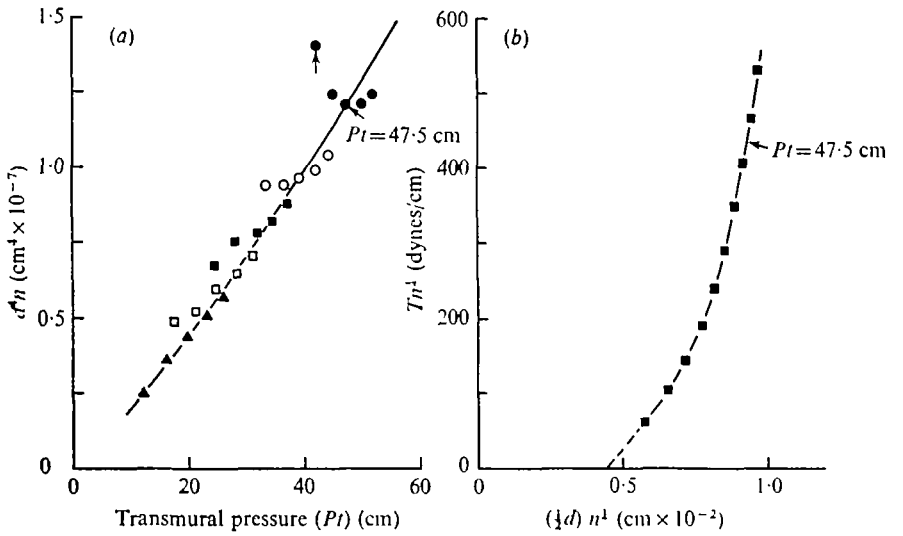

Fig. 8. (*a*) The relationship between d^4n (the gill dimension factor in the Muir-Brown (1971) model) and average transmural pressure at different levels of *Pe*: 40 cm (●), 30 cm (○), 20 cm (■), 10 cm (□), 0 cm (▲). The labelled point has been given less weight in the fitted line. (*b*) A plot of wall tension (T) × $n^{\frac{1}{2}}$ against radius $\frac{1}{2}d \times n^{\frac{1}{2}}$ for an average pillar-cell channel derived by Laplace's Law from the curve of Fig. 8*a* assuming that all distensibility reflects changes in *d*. See text for explanation of extrapolation (dashed line).

and 40 cm). Despite the fact that *Pt* values were in some cases the average of widely separated values and in others of closely similar values, there existed a well defined positive relationship between d^4n and *Pt* (Fig. 8*a*). The single divergent point (labelled) has been given less weight in the fitted line as it was obtained close to the *CCPd* (0·2 ml/100 g/min).

Fig. 8*a* indicates that increases in *Pt* caused by elevating *Pe* had a greater dilatory effect than did comparable increases caused by raising *Pg*. For each *Pe* level, the points from separate profiles produced by augmenting *Pg* formed lines with slopes less than that of the common curve. As *Pe* was raised, the slopes of these individual relationships changed from a value nearly equal to that of the common line (at *Pe* = 0 cm) to essentially zero (at *Pe* = 40 cm), indicating an upper limit to distensibility for *Pt* increases from the ventral aortic side. No such limit was evident for the effect of *Pt* increase caused by *Pe* elevation, although this was not taken above 40 cm. The phenomenon may indicate that the resistance vessels on the dorsal aortic side of the resistance midpoint (by which point one half of the total *Pd* occurs) are more compliant than those on the ventral aortic side (Folkow & Löfving, 1956; Levy, 1958). Certainly it shows that alterations in pressures in the outflow produce greater effects than do similar changes in the inflow at physiological levels.

Two extreme situations may be analysed: firstly, that all distensibility occurs in *d*, *n* remaining constant, and secondly, that all changes in d^4n reflect variation in *n*, *d* being indistensible. In the first case, use may be made of Laplace's Law (Burton, 1951) which states that wall tension (T) in a blood vessel is equal to the product of the distending pressure (*Pt*) and the radius. Thus by incorporating $n^{\frac{1}{2}}$:

$$Tn^{\frac{1}{2}} = Pt\tfrac{1}{2}dn^{\frac{1}{2}}$$

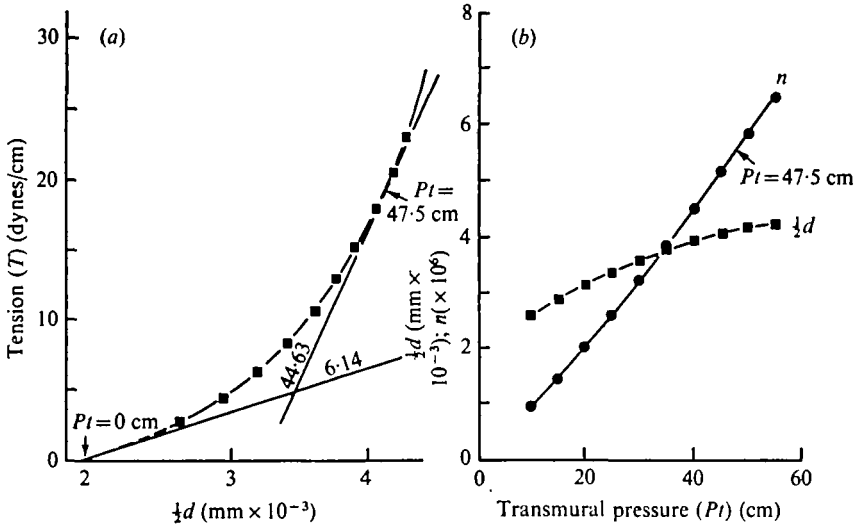

Fig. 9. (*a*) The relationship between wall tension (*T*) and radius ½*d* of an average pillar-cell channel if all distensibility is caused by changes in *d*. The figures on the tangents at transmural pressures (*Pt*) = 0 cm and 47·5 cm represent elastances calculated from the slopes (see text). (*b*) The changes in pillar-cell channel radius ½*d* or number (*n*) with transmural pressure required to explain Fig. 8*a* when all distensibility resides in either factor alone.

the data of Fig. 8*a* provides the plot of Fig. 8*b*. Extension of the curve to $Tn^{\frac{1}{2}} = 0$ yields an estimate of *n*, because at this point (*x*), *Pt* = 0 while *n* and *d* are finite; the microscopically measured $d = 3{\cdot}86 \times 10^{-3}$ mm (determined at *Pt* = 0) may be substituted in the equation:

$$\tfrac{1}{2}dn^{\frac{1}{2}} = x.$$

The extrapolation is difficult for the slope of the curve constantly decreases towards the origin. We have therefore been conservative, using the final slope from the measured range of the curve; this procedure yields a maximum value of *x*(0·0044 cm) and therefore of *n* (270,134). Yet this latter figure is extremely low, smalle reven than that recorded for the total number of lamellae/fish (493,509). At the physiological *Pt* = 47·5 cm, Fig. 8*b* gives $\frac{1}{2}dn^{\frac{1}{2}} = 0{\cdot}00936$ cm, or $d = 8{\cdot}21 \times 10^{-3}$ mm. Thus if *n* remains constant, *d* must more than double between *Pt* = 0 cm and *Pt* = 47·5 cm. From equation (2), at *Pt* = 47·5 cm, *Ag* is only 20·39 cm², or 2·95 % of the measured area. Using equation (3) and the mean *Pd*/*Q* relationship provides a similar result, *Ag* = 24·19 cm² or 3·50 % of the recorded *Ag*. From this approach, the assumptions appear unrealistic.

Roach & Burton (1957, 1959) have shown that elastances [the product of Young's modulus (*Y*) and the wall thickness (*W*)] may be computed from the slopes of plots such as Fig. 9*a*, which was constructed by replotting the curve of Fig. 8*b* on real tension and radius co-ordinates:

$$YW = \frac{\Delta T}{\Delta d/d_0}.$$

Elastances of 6·14 and 44·63 dynes/cm/100 % elongation at *Pt* = 0 and 47·5 cm respectively were obtained. Comparable increases (seven fold) in elastance between *Pt* = 0 and the physiological *Pt* have been observed in mammalian vessels containing collagen

but no elastin (Roach & Burton, 1957), as in the pillar cells of teleosts (Hughes & Weibel, 1972). The effective W is difficult to assess because one pillar cell forms the walls of two or more channels, but a figure of about 1×10^{-3} mm would appear reasonable, producing Young's moduli of $6{\cdot}14 \times 10^4$ and $4{\cdot}46 \times 10^5$ dynes/cm^2/100% elongation at Pt = 0 and 47·5 cm respectively. These values are much lower than expected for a collagen-bearing tissue (Roach & Burton, 1959), which may again indicate the inadequacy of the assumption that n is fixed while d varies with Pt.

If the other extreme is true, the changes in n (with d fixed at the measured value of $3{\cdot}86 \times 10^{-3}$ mm) necessary to explain Fig. 8*a* are much greater than those in d for the previous assumption (Fig. 9*b*). Nevertheless, at Pt = 47·5 cm, n is 5,522,771 (Fig. 9*b*) yielding by equation (2) an Ag of 196·04 cm^2 or 28·34% of the measured branchial area, which compares favourably with the previously calculated value of 33·66% from the mean Pd/Q profile. It might be argued in such a situation one would expect to see most of the pillar-cell channels closed under the microscope (at Pt = 0 cm). That this does not occur may simply reflect the fact that recruitment is at the level of the individual lamella, rather than the pillar-cell channel. Critical closure also occurs without apparent occlusion of these channels, indicating that closing phenomena must take place in the lamellar arterioles or filamental arteries. The collagen content of the pillar cells may in fact prevent the collapse of the vessels of which they form the walls.

The equations may also be applied to the changes in branchial vasomotor tone caused by catecholamines. At a maximum dilation of 60% decrease in Rg at the same Q (Fig. 7), Q increases from 2·009 ml/min to 5·076 ml/min (from Fig. 3). The model predicts that at a constant Pt = 47·5 cm, the decrease in Rg could be effected by either a 26·09% increase in d or 152·68% rise in n. These in turn would cause 26·09% and 152·68% elevations of Ag respectively. One of the prime purposes of the adrenaline vasodilation is thought to be an increase in the transfer factor for respiratory gases (Randall *et al.* 1972). This function would be best served by the larger increase in respiratory area mediated by recruitment of n. Again assuming d to be $3{\cdot}86 \times 10^{-3}$mm, n would increase from 6,558,383 to 16,572,028 and Ag from 232·80 cm^2 to 588·23 cm^2. This expansion would provide a maximum utilization of gill area (85·06%), but the figure is, in fact, probably too high as inadequate room would remain for the pillar cells. Nevertheless, rather small increases in d (accompanied by large rises in n) would be sufficient to remove this objection. The infra-red photographic observations of Davis (1972) on live trout and description of Hughes (1972) of perfused trout gills both support the concept that catecholamines increase the number of lamellae perfused and thus increase n.

Despite the general agreement between theory and results, some of the original assumptions of the model may be criticized. It is, of course, impossible that all Rg resides in the pillar-cell channels, and even the contention that the major proportion occurs there conflicts with the traditional role of the arterioles in this context (Burton, 1965), though many exceptions exist (Kuida, 1965). The model suggests the unusual situation whereby vessel closure occurs outside the sites in which most of the effective resistance resides. A more serious and experimentally substantiated objection is that not all flow necessarily passes through the pillar-cell channels. Vascular shunts through the body and around the tip of the gill filaments have been demonstrated in the trout

Steen & Kruysse, 1964; Richards and Fromm, 1969), although the functional significance of the former has been discounted by Hughes (1972). We observed erythrocytes passing through the apical but not the central filamental pathways in excised filaments. The magnitude of this source of error cannot be assessed. The marginal lamellar channels also obviously carried significant flow *in vitro* and some estimates of their contribution can be made.

Assuming the marginal channels to be indistensible ($d = 6{\cdot}80 \times 10^{-3}$ mm, $l =$ 0·8093 mm), equations (2) and (3) predict that 1, 198, 816 channels or 2·43 times the actual number present (493,509, 1 channel/lamella) would be needed to carry the total Q. However, a relatively small expansion (24·85 %) of d to $8{\cdot}49 \times 10^{-3}$ mm would enable the marginal channels to conduct the total Q.

From equation (1) the total resistance offered by the two parallel circuits (undistended) can be computed on the following basis: pillar-cell channel $n = 11{,}103{,}952$ (i.e. $22{\cdot}5 \times 493{,}509$); marginal channel $n = 493{,}509$. The calculation shows that the marginal channel resistance (49·01 cm/ml/100 g/min) is 4·11 times greater than the pillar-cell channel figure, (11·91 cm/ml/100 g/min) producing a total Rg of 9·58 cm/ml/100 g/min. Thus if all the vessels are rigid, the influence of the marginal channels may be relatively slight, but if even slight distension occurs, their potential contribution to branchial haemodynamics could be great.

In summary, the model of Muir & Brown (1971), although containing many possible sources of error, adequately explains the present data and suggests that both passive and active changes in Rg are best explained by changes in the number of secondary lamallae perfused.

SUMMARY

1. Perfusion of the whole gill of *Salmo gairdneri* with a Newtonian medium under different afferent and efferent pressures has revealed considerable passive distensibility in the branchial vasculature. A capacity for autoregulation may exist.

2. Changes in dorsal aortic pressure are relatively more effective than changes in ventral aortic pressure in altering branchial vascular resistance.

3. Measurements of changes in vascular tone in the gills determined as the ratio of resistances in the experimental and the control states at one point on the pressure differential/flow profile can be extrapolated to the rest of the profile when the comparisons have been made at the same flow.

4. True catecholamines cause a maximum 60 % decrease in vascular resistance (at the same flow) by stimulation of β_1-adrenergic receptors in the gills.

5. Branchial dimensions and perfusate viscosities have been measured and, together with the pressure differential/flow data, have been used to evaluate the Muir-Brown model of vascular resistance in the teleost gill. The model, with some limitations, fits the present data and suggests that both active and passive resistance changes are best explained by alterations in the number of secondary lamellae perfused.

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