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HAEMODYNAMIC EFFECTS OF ADRENALINE ON THE ISOLATED, PERFUSED HEAD OF THE DOGFISH 'PUP' (*SQUALUS ACANTHIAS*)

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SUMMARY

1. The isolated, perfused head of the dogfish 'pup' (*Squalus acanthias*) maintained pressure: flow relationships near to those described for the *in vivo* adult for at least 3 h when perfused at a constant rate. The addition of 3 % polyvinylpyrrolidone reversibly increased branchial resistance, and the postbranchial outflow (arterial + venous) equalled the inflow.

2. 10^{-5} M adrenaline reversibly reduced gill resistance (in some cases after a transient increase in resistance) and stimulated perfusate outflow from the dorsal aorta, at the expense of flow from the cephalic and branchial venous system. Phentolamine did not alter the effect of adrenaline on pressure and flow pattern; addition of propranolol inhibited both adrenaline effects and resulted in a slight increase in afferent pressure, indicating that α-adrenergic receptors are present, but that the dominant haemodynamic effects are mediated via β-adrenergic receptors.

3. The isolated, perfused 'pup' head may provide a vehicle for investigation of transport phenomena in the elasmobranch branchial epithelium.

INTRODUCTION

Isolated, perfused, head preparations of teleost fishes have often been used to investigate haemodynamics (Payan & Girard, 1977; Colin, Kirsch & Leray, 1979; Pettersen & Nilsson, 1979; Claiborne & Evans, 1980; Nilsson & Pettersson, 1981; Oduleye, Claiborne & Evans, 1982) and osmoregulation (Girard, 1976; Girard & Payan, 1977*a*,*b*; Payan, 1978; Claiborne & Evans, 1981; Oduleye & Evans, 1982). Haemodynamics and osmoregulation in elasmobranchs have not been studied using such preparations, but some haemodynamic studies have been performed on anaesthetized, perfused, intact *Squalus acanthias* (Kent & Peirce, 1978; Opdyke,

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Holcombe & Wilde, 1979; Kent, Levy & Opdyke, 1980) and the perfused gills of *Scyliorhinus canicula* (Davies & Rankin, 1973).

We have initiated studies on the isolated, perfused head of prenatal *S. acanthias* 'pups' to define a preparation which may allow us to examine more carefully the transport parameters of the elasmobranch branchial epithelium. We have first examined some aspects of the system's haemodynamics and responsiveness to adrenaline to test its viability.

MATERIALS AND METHODS

Near-term dogfish 'pups' were removed from females (captured in Frenchman's Bay, Maine) and maintained in running sea water as described previously (Evans, 1982). They weighed approximately 50–70 g (including yolk sac) and similar individuals have been maintained in sea water for more than 40 days (Gilbert, 1958; D. H. Evans & K. More, unpublished). In recent years we have found that these 'pups' display the major hallmarks of elasmobranch osmoregulation (Evans, Oikari, Kormanik & Mansberger, 1982). Fifteen to 30 min before dissection, an individual was injected intraperitoneally with 1000 i.u. of heparin carried in dogfish Ringer's solution (Forster, Goldstein & Rosen, 1972). Fish were anaesthetized with 0·01 % MS 222 and the head prepared in basically the same manner as described previously for a marine teleost (Claiborne & Evans, 1980). Changes from the original technique included suturing small diameter (3 mm o.d.) plastic tubing into the mouth for irrigation, use of a small length of flared PE 50 tubing attached to PE 90 tubing for the perfusion cannula, and securing the plastic ring inside the body cavity *via* surgical staples. PE 50 tubing was used to cannulate the dorsal aorta. A cut toy balloon was used to secure the head in a plastic cylinder drilled with two holes to allow drainage of the irrigate into a beaker for recycling.

The irrigation and perfusion circuits were as previously described (Claiborne & Evans, 1980). The temperature of the perfusate (dogfish Ringer's solution) and the irrigate (sea water) was maintained in the range of 15–20 °C by immersing beakers containing the solutions in baths of sea water. Afferent perfusion pressures were monitored *via* a Statham (P23AC) pressure transducer connected through a custom built d.c. preamplifier to a strip-chart recorder (Linear). The recorder was calibrated by raising the Ringer's-filled cannula tip up a meter stick and noting the deflection in the tracing. In addition, the pressure produced by the resistance of the tubing between the perfusion pump (Gilson Minipuls 2) and the tip of the cannula was measured at different flow rates and was subtracted from any pressures measured during the experiments.

Irrigation and perfusion flow rates were set at 175 ml min^{-1}, and 0·75 ml min^{-1}, respectively. Post-branchial perfusate was collected from the cannula in the dorsal aorta (DA) and from the cut muscle mass in tared plastic vials for comparison of inflow and outflow rates and outflow partitioning. The drainage from the cut muscle mass has been termed 'venous' by Payan & Girard (1977), but is actually a combination of branchial and cephalic venous flows. For simplicity this flow will be termed BCV (branchial-cephalic-venous).

Drugs used in this study were dissolved in dogfish Ringer's before use and were

L-epinephrine hydrochloride (Parke-Davis), propranolol hydrochloride (Ayerst), and phentolamine mesylate (Ciba).

Results are expressed as mean ±S.D. (N) and differences were tested using Student's t-test for paired data and two-tailed analysis.

RESULTS

Long term viability and afferent pressure vs *perfusion flow*

It was found that, immediately after completion of the cannulation and mounting of the head, the afferent pressure steadily increased until a relatively stable reading was reached after some 10–30 min, depending on the individual. After that, maintenance of relatively stable afferent pressures (±2 Torr: 1 Torr ~ 133·3 Pa) was possible for periods of at least 3 h, with one experiment lasting for 4·5 h. However, most experiments were performed in the period of 1–2 h after preparation. At a perfusion flow rate of 0·75 ml min^{-1} (approximately 1·5 ml $100g^{-1}$ min^{-1}) the afferent pressure for 27 perfused heads was 16·8 ± 4·9 Torr.

Effect of colloid

The addition of 3 % polyvinylpyrrolidone (PVP, average M_r of 40 000 Da) resulted in a significant increase (5·4 ± 2·4 Torr, $N = 7$) in afferent pressure, which could be reversed when the head was again perfused with PVP-free Ringer's (Fig. 1A).

Inflow vs *outflow*

In 22 fish the control outflow (DA + BCV) was compared with the known perfusion inflow at periods from 1–2 h after initiation of perfusion. The outflow, at a perfusion inflow of 0·75 ml min^{-1}, was 0·79 ± 0·07 ml min^{-1}; thus it is clear that no significant loss of perfusate across ruptured branchial vessels into either extracellular or irrigation fluids was taking place.

Effect of adrenaline

Addition of 10^{-5} M adrenaline to the perfusate resulted in a significant decline in afferent pressure which stabilized some 3–5 min after the first response was seen (Fig. 1A). The apparent lag period after the addition of the drug is due to the significant dead space in the perfusion line. In four heads the afferent pressure fell by 5·1 ± 1·6 Torr, or approximately 25 % below the control. During the same time period, after the addition of adrenaline, DA/BCV increased to 188 ± 4 % (4) of the control. In other words, the addition of adrenaline produced a preferential flow of blood into the dorsal aorta, at the expense of the perfusate flow into venous drainage from the gills and the head. In some instances, the initiation of the adrenaline response was characterized by a slight, and very rapid (less than 1 min), increase in afferent pressure, followed by the usual decline in pressure (Fig. 1B). The decline in afferent pressure produced by adrenaline was not transitory (reversed in 6 min) as described by Davies & Rankin (1973), but was reversible when adrenaline-free Ringer's solution was re-introduced (Fig. 1A).

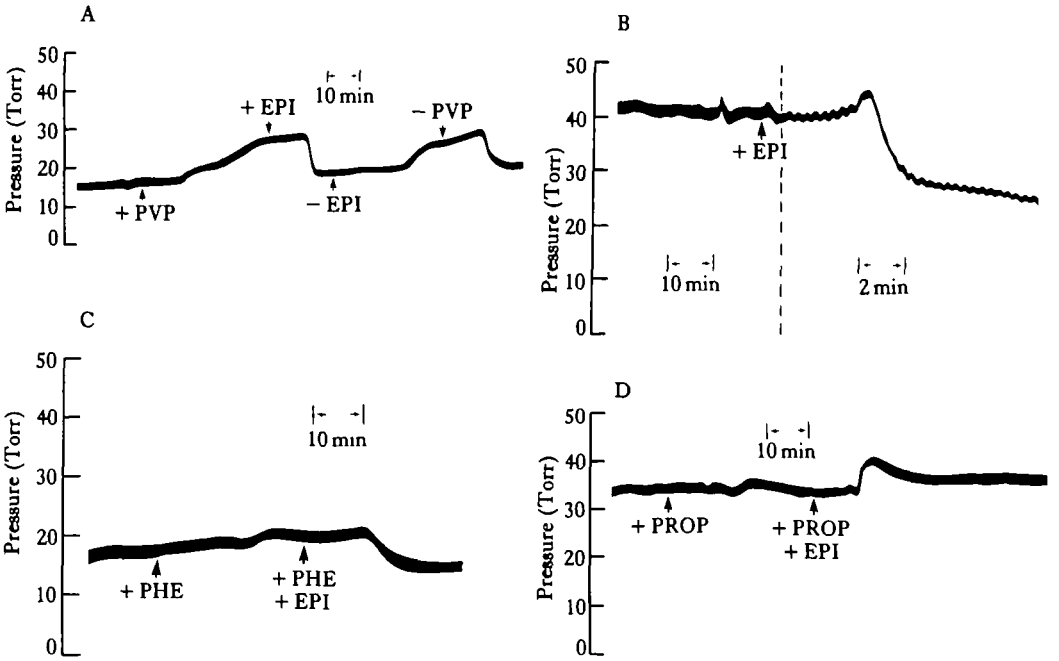

Fig. 1. (A) The effect of 3 % polyvinylpyrrolidone (PVP) and 10^{-5} M adrenaline (EPI) on the afferent pressure. –EPI and –PVP indicate switch to perfusate free of either EPI or PVP (and EPI). (B) Effect of 10^{-5} M adrenaline (EPI) on afferent pressure. Note change in time scale. (C) Effect of 10^{-4} M phentolamine (PHE) and phentolamine plus 10^{-5} M adrenaline (EPI) on afferent pressure. (D) Effect of 10^{-5} M propranolol (PROP) and propranolol plus 10^{-5} M adrenaline (EPI) on afferent pressure. Perfusate flow = 0·75 ml min^{-1} throughout.

Effect of α and β adrenergic blocking agents

To characterize the receptors for the adrenaline response we tested the effects of the α-adrenergic blocker phentolamine, and the β-adrenergic blocker propranolol. Addition of phentolamine to the perfusate (10^{-4} M) led to a slight increase in afferent pressure (Fig. 1C) some 12–20 min after the drug reached the head (i.e. the dead time was subtracted). In four heads this increase averaged 3·1 ± 1·2 Torr. At the same time the DA/BCV fell slightly (66 ± 35 % of the control), but not significantly ($P > 0·1$). When 10^{-4} M phentolamine was added along with 10^{-5} M adrenaline the afferent pressure fell by 5·0 ± 1·5 (4) Torr some 7–12 min after the two drugs reached the tissues (Fig. 1C). At the same time, the DA/BCV increased to 307 ± 129 % (4) of the control, not a significantly ($P > 0·1$) greater increase than that produced by adrenaline alone. Thus blocking α-adrenergic receptors did not change the observed pressure or flow response to adrenaline.

Addition of the propranolol (10^{-5} M) alone to the perfusate produced no significant change in the afferent pressure (–0·43 ± 2·8 Torr, four animals), or in DA/BCV (114 ± 33 % of control, four animals) (Fig. 1D). However, addition of propranolol plus adrenaline (10^{-5} M) produced an initial increase (+4·3 ± 2·0 Torr, four animals) in afferent pressure 1–4 min after the drugs reached the tissue, followed by a slight decline after 10–15 more minutes to a value still above the control (2·3 ± 0·4 Torr, four animals) (Fig. 1D). The final DA/BCV (after the second plateau had be

reached) was 75 ± 32 % of the control (four animals). Thus blockade of the β-adrenergic receptors inhibited both the pressure decline and increase in DA/BCV normally seen when adrenaline was applied to the perfused head. Importantly, β-blockade produced an increase in afferent pressure when adrenaline was applied, presumably secondary to the α-adrenergic action of adrenaline.

DISCUSSION

Viability and general haemodynamics of the preparation

It is obvious that the perfused shark 'pup' head does not suffer from the rapid haemodynamic degeneration which has characterized some other perfused head systems (Wood, 1974; Girard, 1976; Pettersson & Nilsson, 1979). We have also found recently that the perfused heads of three marine teleosts (*Myoxocephalus octodecimspinosus, Opsanus beta* and *Fundulus similis*) maintain consistent afferent pressures (under the conditions of a constant perfusion inflow) for periods extending from 3 h to 8 h (Claiborne & Evans, 1980; Oduleye *et al.* 1982; D. H. Evans & K. More, unpublished). The *in vivo* cardiac output of adult *Squalus acanthias* has been determined to be 2·7 ml $100g^{-1}$ min^{-1} by Murdaugh, Robin, Millen & Drewry (1965) using the dye-dilution method and approximately 1·4 ml $100g^{-1}$ min^{-1} by Kent & Peirce (1978) using an electromagnetic flow probe. Since the intact 'pups' weighed approximately 50 g we perfused the heads at 0·75 ml min^{-1} which translates into a weight specific inflow of approximately 1·5 ml $100g^{-1}$ min^{-1}. The afferent pressure (approximately 17 Torr) measured at this perfusion flow was nearly identical to the ventral aortic pressure measured in the intact adult (Kent & Peirce, 1978; Opdyke *et al.* 1979). Thus it appears that the perfused 'pup' head is maintaining vascular tone equivalent to that in the intact adult fish. This is especially interesting considering that post-branchial pressures in our preparation are nearly zero. This indicates that post-branchial resistance plays little role in determining the total resistance to blood flow through the gill of the 'pup'. We have recently found that near *in vivo* pressure: flow relationships are displayed by the perfused heads of the marine teleosts *M. octodecimspinosus* and *O. beta* (Claiborne & Evans, 1980; Oduleye *et al.* 1982), despite the lack of significant post-branchial back pressure. These findings are to be contrasted to the situation in the perfused trout head (Wood, 1974) where a lack of post-branchial pressure produced branchial resistances significantly above those found *in vivo*. Since this trout preparation appears to deteriorate rapidly, (Wood, 1974; Girard, 1976) it is apparent that there may be a correlation between near-*in vivo* pressure: flow relationships of a perfused head system and its longevity for experimentation.

There have been no systematic studies of the effects on a perfused head preparation of the addition of a colloid to the perfusate. We found that the presence of 3 % PVP did not appear to alter the longevity of the perfusion system, nor its response to adrenaline (unpublished results), but it did significantly increase (in a reversible manner) the afferent pressure (Fig. 1A). Whether this increase in afferent pressure is secondary to viscosity changes or changes in the distribution of extracellular fluids remains to be seen. Pettersson & Nilsson (1979) found that the increase in branchial

resistance with time in the cod head was unaffected by the presence of a colloid (RMI Dextran). These findings are to be contrasted with the data of Wood (1974) which showed that the persistent increase in gill resistance of the perfused trout head could be slowed somewhat by the presence of 4% PVP. Davie (1981) has recently shown that the baseline pressure exhibited by the isolated, perfused tail of an eel (*Anguilla australis schmidtii*) was increased when 5% human serum was added to the perfusate.

Inflow *vs* outflow data are not usually given for perfused head systems despite their importance as indicators of vascular integrity. The perfused 'pup' head obviously displays an outflow equivalent to the perfusion inflow, indicating persistent vascular integrity.

Effect of adrenaline

Adrenaline has been shown to produce a net reduction in branchial resistance in at least seven species of teleosts (Belaud, Peyraud-Waitzenegger & Peyraud, 1971; Rankin & Maetz, 1971; Wood, 1974, 1975; Payan & Girard, 1977; Pettersson & Nilsson, 1979; Claiborne & Evans, 1980; Farrell, 1981; Oduleye *et al.* 1982). Published data on elasmobranchs are much rarer and equivocal: Kent & Peirce (1978) found that adrenaline did not affect the branchial resistance in perfused intact *S. acanthias*, while Davies & Rankin (1973) had previously found that the drug reduced the branchial resistance of perfused gills from *S. canicula*, and Capra & Satchell (1974) found a biphasic response when adrenaline was applied to isolated, perfused prebranchial arterial strips from *S. acanthias*. Our data indicate quite clearly that adrenaline does significantly reduce the branchial resistance of the perfused 'pup' head and increases the flow of perfusate into the dorsal aorta. The concentration of adrenaline used (10^{-5} M) in our studies was substantial, but corresponds to that used in many studies in teleosts. Moreover, the observation that the response was reversible (Fig. 1A) and inhibited by the β-adrenergic receptor blocker propranolol (Fig. 1D) indicates that the response to 10^{-5} M adrenaline was specific. It is interesting that only some 50% of the perfused 'pup' heads displayed the transient, initial increase in branchial resistance shown in Fig. 1B, and described for the effects of adrenaline on the branchial vasculature of many species of teleosts (see Claiborne & Evans, 1980; for relevant references) as well as the isolated, perfused arteries of *S. acanthias* (Capra & Satchell, 1974).

It is difficult to reconcile our results with an earlier study which showed that the calculated branchial resistance of intact, but perfused, adult *S. acanthias* did not change when adrenaline was added to the perfusate (Kent & Peirce, 1978). It is unlikely that the lack of response was due to the extremely low concentrations which they used ($1{\cdot}6$ to 52×10^{-8} M) since Davies & Rankin (1973) found that the perfused gill of *S. canicula* was sensitive to extremely small concentrations of adrenaline (10^{-11} to 10^{-9} M). It is more likely that, in the study of Kent & Peirce (1978), a fall in branchial vascular resistance was masked by a dominant increase in the resistance of the systemic blood vessels. Indeed, Kent & Peirce (1978) showed that both ventral and dorsal aortic pressure increased when adrenaline was applied, and Wood & Shelton (1975) found that adrenergic constrictory receptors predominate in the systemic vasculature of the trout. However, they found that this dominant vasoconstrictory action of adrenaline on the systematic vasculature was much less sensitive to the dr

than the vasodilatory response of the branchial vasculature. One would have to propose that the systemic vasculature of *S. acanthias* is at least equally sensitive to adrenaline as the branchial vasculature in order to invoke systemic vasoconstriction as an explanation for the results of Kent & Peirce (1978). It may also be that their inability to detect an effect of adrenaline on the branchial vasculature was secondary to high, stress-induced levels of catecholamines which precluded any further stimulation by exogenous hormone.

Effect of α and β adrenergic antagonists

The slight increase in afferent pressure observed when phentolamine was added alone (Fig. 1D) indicates that relatively high concentrations of this α-antagonist may produce vasoconstriction. [Preliminary experiments using lower concentrations of phentolamine (10^{-5} and 2×10^{-5} M) in conjunction with 10^{-5} M adrenaline indicated that slight, transitory increases in afferent pressure were sometimes still present.] J. B. Claiborne and D. H. Evans, (unpublished results) found that phentolamine alone also produced drastic vasoconstriction in the perfused head of the teleost, *M. octodecimspinosus*. In the present experiments, when phentolamine was added in conjunction with adrenaline (i.e. allowing the expression of only the β-mediated affects of adrenaline, since the α receptors were blocked), the afferent pressure decline was identical to that produced by adrenaline alone. These data are to be contrasted with those of Payan & Girard (1977) and Claiborne & Evans (1980) which indicated that, with perfused teleost heads, blockade of α-adrenergic receptors or perfusion with the β-adrenergic agonist isoproterenol led to a greater expression of the β-mediated fall in afferent pressure. This indicates that under adrenaline stimulation, the β-mediated fall in afferent pressure displayed by those teleosts was reduced by a concomitant increase in pressure, mediated by α-receptors. Our data show that in the perfused head of the dogfish 'pup' the expression of the β-stimulated fall in pressure is not reduced by the presence of α-stimulated effects of adrenaline. This would account for our finding that many of the preparations did not show an initial, transitory increase in afferent pressure subsequent to the addition of adrenaline. Payan & Girard (1977) found that blockade of α-adrenergic receptors in the perfused trout head inhibited the normal stimulation of perfusate flow through the dorsal aorta produced by adrenaline. Moreover, Claiborne & Evans (1980) showed that isoproterenol alone did not stimulate flow of perfusate into the dorsal aorta of the perfused sculpin head. In the present work, the DA/BCV was increased when the α-adrenergic receptors were blocked with phentolamine indicating that β-adrenergic receptors, rather than α-adrenergic receptors, control flow into the dorsal aorta in *S. acanthias*.

That both the adrenaline-mediated fall in afferent pressure and the preferential flow of perfusate into the dorsal aorta are controlled *via* β-adrenergic receptors is supported by our further finding that blockade of β-adrenergic receptors with propranolol inhibits both phenomena (Fig. 1D and Results). In fact, the afferent pressure actually increased, indicating some α-adrenergic mediated effects of adrenaline. Thus, it seems clear that the fall in afferent pressure and increase in dorsal aortic perfusate flow produced by adrenaline are both mediated by β-adrenergic receptors, contrary to the apparent situation in teleosts where pressure changes are

β-mediated, but dorsal aortic flow changes are mediated *via* α-receptors (Payan & Girard, 1977; Claiborne & Evans, 1980).

The actual site of action of these pressure and flow effects of epinephrine on perfused 'pup' heads remains to be delineated. In teleosts it is generally assumed (Payan & Girard, 1977; Claiborne & Evans, 1980) that prelamellar arteriolar constriction is reduced under the β-mediated stimulation of adrenaline, which produces an increase in lamellar perfusion (e.g., Booth, 1979; Holbert, Boland & Olson, 1979) and a concomitant fall in the resistance of the gill vasculature. The α-mediated preferential flow into the dorsal aorta is thought to be *via* vasoconstriction of the anastomoses between the postlamellar arterioles and the venous drainage from the gills (Payan & Girard, 1977; Claiborne & Evans, 1980). Olson & Kent (1980) have recently described anastomoses between both pre- and postlamellar arteriolar vessels and the extensive sub-lamellar network of vessels in the gills of *S. acanthias*, which they refer to as interlamellar, collateral and nutrient circulatory pathways. We have no direct evidence of the role of these anastomoses and/or the non-lamellar filamental pathways in the pressure and circulatory changes described in the present work. However, Wright (1973) described smooth muscle in the prelamellar arterioles of *S. acanthias* so our data are consistent with the proposition that prelamellar vasoconstrictory sites can be dilated under β-adrenergic stimulation by adrenaline, thereby increasing lamellar recruitment, decreasing afferent pressure, and increasing blood flow to the dorsal aorta. Presumably, these sites are distal to pre-lamellar anastomoses into the sublamellar circulatory system, thereby allowing control of arterial *vs* venous drainage as well as pressure. The site of the α-adrenergic-mediated vasoconstriction (which is not always seen, unless propranolol is present) is probably also on a prelamellar (and post-anastomoses) arteriole. Of course, more definite statements regarding the site of action of adrenergic drugs on the elasmobranch vasculature await a more thorough study using vascular casting subsequent to adrenergic drug treatment similar to that recently published by Olson (1980) for the freshwater catfish.

The isolated perfused 'pup' head displays long-term viability and responses to vasoactive drugs characteristic of both *in vivo* and *in vitro* studies of other elasmobranchs and teleosts. Its ease of preparation and handling may allow its use for studies of the mechanisms of solute and water transfer across the elasmobranch branchial epithelium.

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REFERENCES

BELAUD, A., PEYRAUD-WAITZENEGGER, M. & PEYRAUD, C. (1971). Étude comparée des réactions vasomotrices des branchies perfusées de deux téléostéens: la Carpe et le Congre. *C. r. Séanc. Soc. Biol.* **165**, 1114–1118.

BOOTH, J. H. (1979). The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. *J. exp. Biol.* **83**, 31–39.

CAPRA, M. F. & SATCHELL, G. H. (1974). Beta-adrenergic dilatory responses in isolated, saline perfused arteries of an elasmobranch fish, *Squalus acanthias*. *Experientia* **30**, 927–928.

CLAIBORNE, J. B. & EVANS, D. H. (1980). The isolated, perfused head of the marine teleost fish, *Myoxocephalus octodecimspinosus*: Hemodynamic effects of epinephrine. *J. comp. Physiol.* **138**, 79–85.

Claiborne, J. B. & Evans, D. H. (1981). The effect of perfusion and irrigation flow rate variations on NaCl efflux from the isolated, perfused head of the marine teleost, *Myoxocephalus octodecimspinosus*. *Mar. Biol. Letters* **2**, 123–130.

Colin, D. A., Kirsch, R. & Leray, C. (1979). Hemodynamic effects of adenosine on gills of the trout (*Salmo gairdneri*). *J. comp. Physiol.* **130**, 325–330.

Davie, P. S. (1981). Vascular resistance responses of an eel tail preparation: alpha constriction and beta dilation. *J. exp. Biol.* **90**, 65–84.

Davies, D. T. & Rankin, J. C. (1973). Adrenergic receptors and vascular responses to catecholamines of perfused dogfish gills. *Comp. gen. Pharmac.* **4**, 139–147.

Evans, D. H. (1982). Mechanisms of acid extrusion by two marine fishes: the teleost, *Opsanus beta*, and the elasmobranch, *Squalus acanthias*. *J. exp. Biol.* **97**, 289–299.

Evans, D. H., Oikari, A., Kormanik, G. A. & Mansberger, L. (1982). Osmoregulation by the prenatal spiny dogfish, *Squalus acanthias*. *J. exp. Biol.* **101**, 295–305.

Farrell, A. P. (1981). Cardiovascular changes in the lingcod (*Ophiodon elongatus*) following adrenergic and cholinergic drug infusions. *J. exp. Biol.* **91**, 293–305.

Forster, R. P., Goldstein, L. & Rosen, J. K. (1972). Intrarenal control of urea reabsorption by renal tubules of the marine elasmobranch, *Squalus acanthias*. *Comp. Biochem. Physiol.* **42**A, 3–12.

Gilbert, P. W. (1958). The ability of yolk-sac dogfish pups to survive outside the uterus. *Bull. Mt Desert Isl. biol. Lab. 1958*, 68.

Girard, J. P. (1976). Salt excretion by the perfused head of the trout adapted to sea water and its inhibition by adrenaline. *J. comp. Physiol.* **111**, 77–91.

Girard, J. P. & Payan, P. (1977*a*). Kinetic analysis of sodium and chloride influxes across the gills of the trout in fresh water. *J. Physiol., Lond.* **273**, 195–209.

Girard, J. P. & Payan, P. (1977*b*). Kinetic analysis and partitioning of sodium and chloride influxes across the gills of the sea-water adapted trout. *J. Physiol., Lond.* **267**, 519–536.

Holbert, P. W., Boland, E. J. & Olson, K. R. (1979). The effect of epinephrine and acetylcholine on the distribution of red cells within the gills of the channel catfish, *Ictalurus punctatus*. *J. exp. Biol.* **79**, 135–146.

Kent, B. & Peirce, II., E. C. (1978). Cardiovascular responses to changes in blood gases in dogfish shark, *Squalus acanthias*. *Comp. Biochem. Physiol.* **60**C, 37–44.

Kent, G., Levy, M. & Opdyke, M. B. (1980). Effect of acetylcholine on oxygen uptake in the gills of *Squalus acanthias*. *Bull. Mt Desert Isl. biol. Lab.* **20**, 109–112.

Murdaugh, H. V., Robin, E. D., Millen, J. E. & Drewry, W. F. (1965). Cardiac output determinations by the dye-dilution method in *Squalus acanthias*. *Am. J. Physiol.* **209**, 723–726.

Nilsson, S. & Pettersson, K. (1981). Sympathetic nervous control of blood flow in the gills of the Atlantic cod, *Gadus morhua*. *J. comp. Physiol.* **144**, 157–163.

Oduleye, S. O. & Evans, D. H. (1982). The isolated, perfused head of the toadfish, *Opsanus beta*. II. Effects of vasoactive drugs on unidirectional water flux. *J. comp. Physiol.* **148**B, 115–120.

Oduleye, S. O., Claiborne, J. B. & Evans, D. H. (1982). The isolated, perfused head of the toadfish, *Opsanus beta*. I. Vasoactive responses to cholinergic and adrenergic stimulation. *J. comp. Physiol.* **148**B, 107–113.

Olson, K. R. (1980). Application of corrosion casting procedures in identification of perfusion distribution in a complex microvasculature. *Scanning Elec. Microsp./1980/III*, 357–372.

Olson, K. R. & Kent, B. (1980). The microvasculature of the elasmobranch gill. *Cell Tiss. Res.* **209**, 49–63.

Opdyke, D. F., Holcombe, R. F. & Wilde, D. W. (1979). Blood flow resistance in *Squalus acanthias*. *Comp. Biochem. Physiol.* **62**A, 711–717.

Payan, P. (1978). A study of the Na^+/NH_4^+ exchange across the gill of the perfused head of the trout (*Salmo gairdneri*). *J. comp. Physiol.* **124**, 181–188.

Payan, P. & Girard, J. P. (1977). Adrenergic receptors regulating patterns of blood flow through the gills of trout. *Am. J. Physiol.* **232**, H18–H23.

Pettersson, K. & Nilsson, S. (1979). Nervous control of the branchial vascular resistance of the Atlantic cod, *Gadus morhua*. *J. comp. Physiol.* **129**, 179–183.

Rankin, J. C. & Maetz, J. (1971). A perfused teleostean gill preparation: vascular actions of neurohypophysial hormones and catecholamines. *J. Endocrinol.* **51**, 621–635.

Wood, C. M. (1974). A critical examination of the physical and adrenergic factors affecting blood flow through the gills of the rainbow trout. *J. exp. Biol.* **60**, 241–265.

Wood, C. M. (1975). A pharmacological analysis of the adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* **53**, 1569–1577.

Wood, C. M. & Shelton, G. (1975). Physical and adrenergic factors affecting systematic vascular resistance in the rainbow trout: a comparison with branchial vascular resistance. *J. exp. Biol.* **63**, 505–523.

Wright, D. D. (1973). The structure of the gills of the elasmobranch, *Scyliorhinus canicula* (L.). *Z. Zellforsch. mikrosk. Anat.* **144**, 489–509.