wound healing, the animals were again anesthetized and their forelimbs amputated through mid-radius. The stump created presents a "half surface" from which regeneration might proceed. The animals were subsequently observed for at least 60 days during which time progress of regenerates was observed.

The results confirm the earlier observations that although split-limbs can regulate to produce distally complete normal regenerates, a majority of the amputated half-limbs produced deficient regenerates. The range of regenerate expression was from completely arrested outgrowth (no regeneration) to a normal, 4-digit hand. Most of the reduced regenerates reflected their origins from the pre-axial half of a split forelimb. These were seen to arise directly from the apical end of the amputated limb. The record for distally complete regenerates indicated an oblique origin reflecting a base of outgrowth other than the intended "half-stump". By employing an oblique base, the regenerate has access to complete circumferential "positional information" and is thus able to regulate completely. Allometric growth also enables the oblique outgrowth to subsequently rectify its relationship with the axis of the limb. This latter phenomenon has been described during urodele tail regeneration from oblique amputation surfaces (Spallanzani, 1768; Todd, 1823 and personal observations). From the description offered by Bryant (vide supra), the complete regulation she observed is related both to the operative technique employed and the consequent oblique outgrowth induced. While the explanation offered was that "intercalation" of positional values occurred restoring a complete circle of information, the present study has shown that 1) complete regulation of distal limb regeneration from a split forelimb employs the oblique mode of outgrowth to provide a normal base of positional information and 2) regeneration of a half-limb from the apex of an amputated split-limb can indeed occur in the absence of a "complete-circle" of positional information.
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HEMODYNAMICS OF THE ISOLATED, PERFUSED HEAD OF THE DOGFISH "PUP"

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In recent years various laboratories have utilized perfused head preparations to investigate both hemodynamic and osmoregulatory parameters of marine and freshwater teleost fish (see Claiborne and Evans, J. Comp. Physiol., 138: 79-85, 1980, for relevant citations). Similar techniques have not been utilized with elasmobranchs. However, at least the hemodynamics of the elasmobranch gill have been studied using both isolated, perfused gill arches (Davies and Rankin, Comp. Gen. Pharmacol., 4: 139-147, 1973) and perfused intact fish (Kent and Peirce, Comp. Bioch. Physiol. 60c: 37-44, 1978). Unfortunately, these studies disagreed about the effect of epinephrine on gill resistance, with the former showing that the drug produced a transient fall in gill resistance, and the latter study finding no effect on gill resistance. Importantly, no studies of ionic transport across the elasmobranch gill have utilized perfused heads. This would be of great interest since it has recently been shown that the gill of Squalus acanthias excretes both NH_4 and H in exchange for sea water Na and possibly also functions in extrusion of both Na and Cl (Evans, J. Exp. Biol., 1982, in press; Evans and Mansberger, Bull. MDIBL 19: 101-103, 1979). The present study was initiated to examine the hemodynamics of the isolated, perfused head of S. acanthias "pups" in an effort to test this preparation for further use in investigations of elasmobranch gill transport, as well to test directly the effect of epinephrine, phentolamine, propranolol, carbachol and atropine on gill resistance and pattern of blood flow.

Perfused heads were prepared as described previously (Claiborne and Evans, 1980, op. cit.) with the exception that the cannula into the conus arteriosus was a short (1-2 cm) length of flared PE 50 tubing. Irrigation (sea water) flow rates were usually 175 ml. min^{-1}, but in some experiments were varied between zero and 350 ml min^{-1}. Perfusion flow rates were usually 0.75 ml·min^{-1}, but were varied between 0.4 and 1.75 ml·min^{-1} in

other experiments. Perfusion fluid was elasmobranch Ringer's solution (Forster et al., Comp. Bioch. Physiol., 42A: 3-13, 1972). Perfusion and irrigation fluids were maintained between 15-20°C with water baths. Post-branchial perfusate was collected from both a cannula (PE 50) in the dorsal aorta (DA) and the cut muscle mass in tared vials for comparison of inflow and outflow rates, and outflow partitioning. The drainage from the cut muscle mass was probably a combination of branchial and cephalic venous flows and was therefore termed BCV (branchial/cephalic venous).

We found that the perfused head could maintain relatively stable afferent pressure (±2 torr) for at least three hours; however most experiments were performed in the period 1-2 hours post preparation. Variation of the perfusion flow rate demonstrated that linear increases in afferent pressure were produced as the inflow varied between 0.4 and 1.25 $ml \cdot min^{-1}$; above this rate it appeared that the pressure was less affected by increases in inflow. The pressure:flow relationship was usually reciprocal if inflows were first reduced and then subsequently increased. At a perfusion inflow rate of 0.75 $ml \cdot min^{-1}$ (equivalent to in vivo cardiac output of the adult--Kent and Peirce, 1978, op. cit.) the afferent pressure was 16.8 ±4.9 torr (27 animals; $\bar{X} \pm$ S.D.) in the same range as that described for the in vivo adult (K&P, ibid). Since ourpreparation does not allow in vivo post-branchial resistances, our data indicate that gill resistances are not affected substantially by systemic resistance distal to the gills. Addition of 3% polyvinylpyrrolidone reversibly increased the afferent pressure and was added to the perfusate thereafter. When outflow (sum of DA and BCV flows) was compared with inflow we found that the two were equal (outflow of 0.79 ± 0.07 $ml \cdot min^{-1}$, 22 fish, at an inflow of 0.75 $ml \cdot min^{-1}$), indicating that perfusate was not leaking out of the branchial vessels. Variation of the irrigation rate from zero to 350 $ml \cdot min^{-1}$ resulted in changes of afferent pressure of less than 2 torr over the entire range, indicating that transmural pressures play little role in gill resistance, and that deformation of the gill lamellae or filaments during irrigation did not limit the pattern or pressure of perfusate flow through the branchial vasculature of the perfused head. We concluded from these preliminary studies that isolated, perfused "pup" heads could maintain in vivo hemodynamics for prolonged periods of time, despite post-branchial systemic pressures below in vivo levels.

Addition of 10^{-5} epinephrine (epi) to the perfusate resulted in a relatively rapid (3-5 min after the drug reached the tissue) fall in afferent pressure to some 5.1 ± 1.6 (4) torr below the control. The effect was reversible when epi-free Ringer's was re-introduced and was sometimes preceeded by a rapid (lasting less than 1 min) increase in afferent pressure. Epi also resulted in a significant increase (to 188 ± 4 (4) % of the control) in the ratio of the DA vs BCV outflows (i.e., increased perfusate flow into the dorsal aorta at the expense of flow into the cephalic and gill venous systems). Addition of phentolamine alone to the perfusate (10^{-4}M) led to a slight increase in afferent pressure, and a slight, but not significant fall in DA/BCV. When this alpha-blocker was added along with 10^{-5} epi the afferent pressure fell by 5.0 ± 1.5 torr (4) and the DA/BCV increased to 307 ± 129% (4) of the control. Thus, blocking alpha-adrenergic receptor sites did not affect the tissues response to epi. Addition of propranolol alone (10^{-5} M) had no affect on either the afferent pressure or the DA/BCV--addition with 10^{-5} epi resulted in a slight increase in pressure (2.3 ± 0.3 torr above the control, 4 animals) and a fall in DA/BCV to 75 ± 32% (4) of the control. Thus, blockade of beta-receptors resulted in inhibition of both the pressure decline and increase in perfusion of the dorsal aorta normally seen after epi stimulation. Importantly, this blockade produced a slight increase in afferent pressure, presumably secondary to a slight alpha-mediated vasoconstriction--this would account for the occasional, preliminary and transitory increase in pressure when epi is added alone. The actual site(s) of action of the beta-mediated effects of epi are unknown, but our data are consistant with a preiamellar site of action, probably on the prelamellar arterioles at a site distal to any anastomoses into the sublamellar circulatory system as described

by Olsen and Kent (Cell Tiss. Res. 209: 49-63, 1980). Our findings of a beta-adrenergic control of both branchial resistance and pattern of flow is to be contrasted with the general pattern in teleosts where afferent pressure is controlled by a beta-adrenergic site, presumed to be on the prelamellar arteriole, while perfusate flow into the dorsal aorta is controlled by alpha-adrenergic sites, presumably at the postlamellar arteriovenous anastomoses (Payan and Girard, Am. J. Physiol., 232:1718-1723, 1977; Claiborne and Evans, 1980, op. cit.).

When the acetylcholine agonist carbachol (5×10^{-6}M) was added to the perfusate the afferent pressure increased by 7.5 ± 4.5 torr (3) some 12 to 20 minutes after the drug reached the tissue. The response was reversible. Perfusion with atropine (5×10^{-5}M) alone did not alter either parameter. Thus it appears that at least gill resistance is also controlled by muscarinic, cholinergic inputs.

Our data demonstrate that the isolated, perfused head of the dogfish "pup" is a viable preparation for the study of the hemodynamics of the branchial vasculature. It has shown clearly that gill resistance can be increased by stimulation of alpha-adrenergic or muscarinic-cholinergic receptors or decreased by stimulation of beta-adrenergic receptors. In addition, the pattern of blood flow is controlled by beta-adrenergic sites. Usefulness of this preparation for studies on branchial transport mechanisms of elasmobranchs await further experiments, but these preliminary hemodynamic investigations indicate that this may be a suitable vehicle for such studies. This research was supported by NSF PCM 81-04046.

THE TRANSEPITHELIAL POTENTIAL ACROSS THE GILLS OF THE ISOLATED, PERFUSED HEAD OF MYOXOCEPHALUS OCTODECIMSPINOSUS

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Recently, we have shown that the isolated, perfused head preparation (IPHP) of the long-horned sculpin, M. octodecimspinosus, exhibits a long term viability and response to epinephrine (Claiborne and Evans, Bull. MDIBL 19:96-101, 1979; J. Comp. Physiol., 138:79-85, 1980), and a unidirectional NaCl efflux which may be via separate rate-limiting pathways (Claiborne and Evans, Bull. MDIBL 20:50-52, 1980; Marine Biol. Letters 2:123-130, 1981). Since the assessment of transepithelial potential (TEP) changes are important during branchial ion transport experiments (see review by Evans, Amer. J. Physiol. 7(2):R224-R230, 1980), the present study delineates the effects of external ion substitutions on the TEP across the gills of the sculpin IPHP.

The IPHP of M. octodecimspinosus was prepared as described previously (Claiborne and Evans, 1980, J.C.P., op.cit.). Transepithelial potential (TEP) measurements were accomplished by inserting 4% agar Ringer's bridges into the afferent perfusion line and the peritoneal cavity of the IPHP. The bridges were also connected to a voltmeter (Keithley, Model 616) via two matched calomel electrodes. For comparison purposes, the in vivo TEP in the sculpin was measured according to the methods of Evans, et al. (J. Exp. Biol. 61:277-283, 1974).

Table 1.--Comparison of TEPs in M. octodecimspinosus during seawater ion substitutions in vivo and in vitro

	seawater	-Na	seawater	-Cl
in vivo (N = 6)	7.15 ± 0.64	-0.78 ± 1.43 ($P<0.001$)	5.47 ± 0.91	8.68 ± 0.84 ($P<0.01$)
IPHP (N = 4)	9.28 ± 2.29*	1.03 ± 1.49 ($P<0.01$)	8.95 ± 2.38*	8.34 ± 1.98 (NS.)

All values represent TEP in mV; Mean ± S.E. with the serosal side of the gills taken as reference. Comparisons of differences between seawater and -Na or -Cl seawater were made utilizing paired differences and Student's t-test.

*A comparison of average seawater TEPs in vivo and in vitro indicated no significant difference between these values.