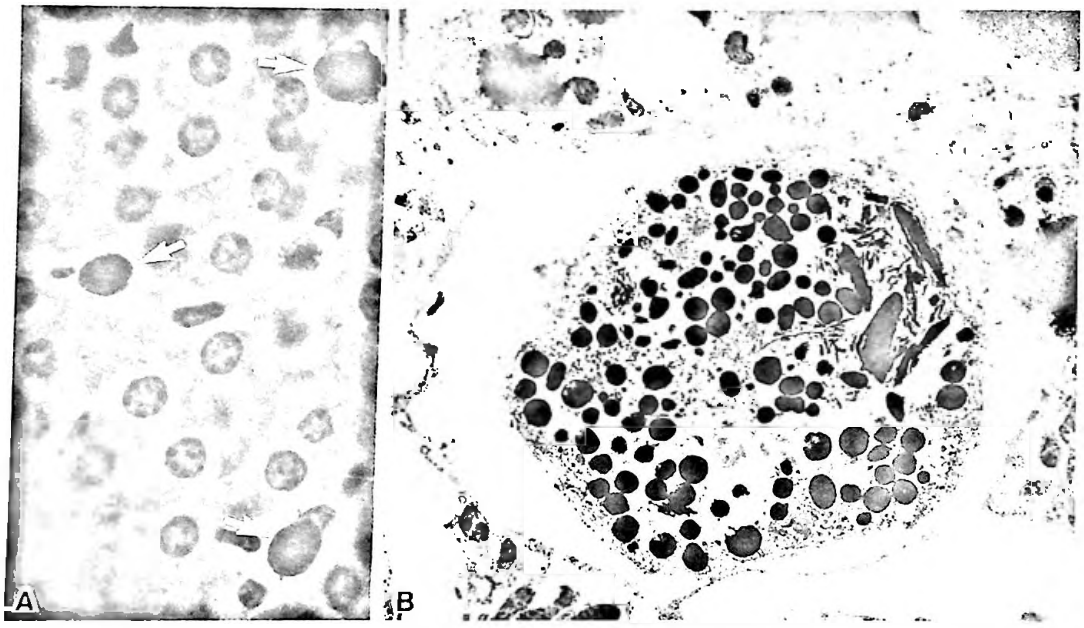

Figure 1. A. H & E stained section of skate liver. Magnification x 150. White arrows point to pigmented cells in hepatic sinusoids. B. Transmission electron micrograph of pigmented cell. Final magnification x 6500.

2.5 cm H_2O of hydrostatic pressure for approximately 10 minutes. Dissecting microscopic examination of raxor thin slices revealed pigmented cells localized predominantly to zone 3 of the hepatic lobule (central regions), a lobular distribution which was particularly notable in lightly pigmented livers. This zonal distribution was less prominent when darker livers containing a greater number of pigment cells were examined. Liver tissue was then examined by transmission electron microscopy after post fixation in osmium tetroxide (Fig. 1B). Occasionally, the cells were recessed into the space of Disse or the intracellular space between adjacent hepatocytes. Plasmalemma from pigment containing cells contacted adjacent endothelial cells or the surface of the hepatocytes. The cells were easily identified by the large number of small pigment granules which numbered more than 100 per cell section and which were densely osmophilic and occupied the majority of the cytoplasm. Characteristically, a large nucleus and prominent nucleoli were also observed. In occasional cells, an osmophilic crystalline material was also observed within the cytoplasm. Although the nature of the pigment and its origin are not clear, the morphologic characteristics of these cells closely resemble those of melanocytes observed in the skin of vertebrate species. Histologic examination of liver tissue from hagfish, fresh water eels, flounders, and goosefish fail to reveal pigmented sinusoidal lining cells. However, the darkly pigmented liver of the amphibian mudpuppy, Necturus, demonstrated dense sinusoidal infiltrates of similar pigmented cells. On the basis of these morphologic studies, we believe that pigment cells populate the sinusoidal lining cells of the hepatic sinusoid in elasmobranchs and in Necturus and account for the variable pigmented appearance of these livers. The origins of the pigment and the function of these pigment cells remains to be determined.

THE ISOLATED, PERFUSED HEAD OF THE LONG-HORNED SCULPIN: ADRENERGIC RECEPTORS CONTROLLING THE EFFECT OF EPINEPHRINE ON THE AFFERENT PERFUSION PRESSURE AND EFFERENT FLOW RATES

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Investigations of cardiorespiratory and osmoregulatory physiology of fishes has often involved the use of intact

dividuals. However, results are often marred by the inability to control stress or the chemical composition of the ood. In recent years, various in vitro techniques have been investigated to avoid these problems, but isolated, erfused gills do not allow the proper external irrigation of the branchial epithelium necessary for ionic flux studies .g., Shuttleworth, J. Comp. Physiol. 124:129-136, 1978; Maetz, Rankin, Shuttleworth, Olsen, Personal ommunications). The isolated perfused head of the trout, Salmo gairdneri, has provided a preparation which has een very useful in the investigation of various hemodynamic and osmaregulatory parameters (Payan and Girard, Am. . Physiol. 232: H18-H23, 1977; Payan, J. Comp. Physiol. 124:181-188, 1978).

Payan and Girard (op. cit.) found that the response to epinephrine displayed by the isolated, perfused head of he trout (Salmo gairdneri) could be dissected into alpha and beta receptor responses which controlled both branchial ascular resistance and the partitioning of efferent blood between the dorsal artery and a "venous" component. The atter consisted of drainage from the post-lamellar filamental sinus and, presumably, also from various cephalic rteries exiting the dorsal aorta anterior to the implanted cannula. These studies were complicated by the fact that he isolated, perfused trout head has a functional viability limited to approximately 30 minutes (e.g., Girard J. Comp. hysiol. 111:77-91, 1976).

Preliminary experiments in our laboratory have indicated that the gulf toadfish, Opsanus beta, provides an isolated, erfused head whose viability (measured by consistant afferent perfusion pressure and response to epinephrine) extends eyond 8 hours. The present study investigates the viability of the isolated, perfused head of the long-horned sculpin, Myxocephalus octodecimspinosus and the role of alpha and beta adrenergic receptors regulating the response to pinephrine by this preparation.

Specimens of M. octodecimspinosus (100-250g) were prepared in much the same way as described by Payan and Matty (J. Comp. Physiol. 96:167-184, 1975). The animals were injected intraperitoneally with 1500 U.S.P. K Units of ammonium heparin, 30 minutes prior to use in the experiment. Before surgery, the specimens were anesthesized for 5 minutes in MS-222 (2:10,000). A small tube was then inserted and sutured into the buccal cavity, the mouth was sutured shut, and an incision was made in the ventral body wall and the esophagus ligated. A Ringer's filled cannula was then inserted into the ventral aorta and sutured in place. The animal was decapitated at the level of the initial incision behind the pectoral fins and a small circular, plastic "ring" sutured into the open end of the body cavity to give support to the ventral body wall. The head was then placed into a plastic (PVS pipe) cylinder, ventral side up, with the cut end held in place at one end of the cylinder with an elastic rubber dam (prophylactic) stretched around the head and over the end of the cylinder. Drains in the bottom of the cylinder provided for the effluent of the irrigation solution which was pumped across the gills and exited the head through the opercular openings. The volume of efferent perfusate (post branchial) was collected and measured in tared vials, and consisted of dorsal arterial flow out of the cannulated (PE 50) dorsal aorta and "venous" flow from the peritoneal cavity and the cut muscle mass.

Both sea water and perfusate were maintained at 11-13°C. The sea water irrigation of the gills was carried out at a rate of 500 ml/min by a solid-state Varistalic pump, and was fed by the MDIBL running sea water. Afferent perfusion was monitored with an in-line pressure transducer (Statham P23AC) connected through a "home-made" D.C. preamplifier to a chart recorder (Bausch and Lomb VOM 7). The perfusion flow rate (approximately 1.4 ml/min) was adjusted, by means of a Gilson Miniplus 2 pump, to allow afferent perfusion pressures of 40 to 60 torr. The sea water teleost Ringer's solution was composed of the following (mM): 148 NaCl, 2.6 KCl, 2.7 NaH_2PO_4, 1.26 $CaCl_2$, 1.24 $MgSO_4$, 14.9 $NaHCO_3$, glucose (lg/L), polyvinylpyrrolidone (3%), and heparain (2500 U.S.P. KUnits/L). The isolated head was initially perfused with Ringer's solution for 30 minutes to clear the gill and cephalic vessels of blood. In the case of epinephrine response and viability experiments, a second Ringer's solution perfusion was then carried out from 0.5 to 2.5 hours to establish control perfusion pressures and efferent flow rates. Epinephrine (1×10^{-5} M) was then added to the perfusate. Adrenergic receptor experiments were divided into four 20 minute

periods. During the first period, drug-free Ringer's solution was again perfused into the head to establish control perfusion pressures and efferent flow rates. During the second period, either the beta blocker, propranolol (2×10^{-5}M), or the beta mimetic, isoproterenol (1×10^{-4}M), were added to the perfusion solution. Epinephrine (1×10^{-5}M), in combination with the previously added beta mimetic or blocker was then perfused through the head in the third experimental period. Finally, the head was perfused with Ringer's solution once again. Initial afferent pressure control values are taken at the moment of drug addition to the perfusate. For all experiments, pressure change values are the differences between the initial perfusion pressures for each period and the pressure taken 10 minutes after the start of the next period. All flow values were calculated by the collection of efferent Ringer's solution throughout the last 10 minutes of each period. Flow rate change values for each period are the differences between the flow rate for each period and the flow rate of the preceeding period.

The afferent perfusion pressure of the isolated sculpin head was found to remain relatively stable (±4.3 torr, N=21) for a period of one hour during the initial Ringer's solution control period. In three cases, perfusions were carried out for over three hours, and the preparation still exhibited a constant afferent pressure and an epinephrine response (Fig. 1). Figure 2 and Table 1 show the effects of epinephrine on the afferent perfusion pressure and

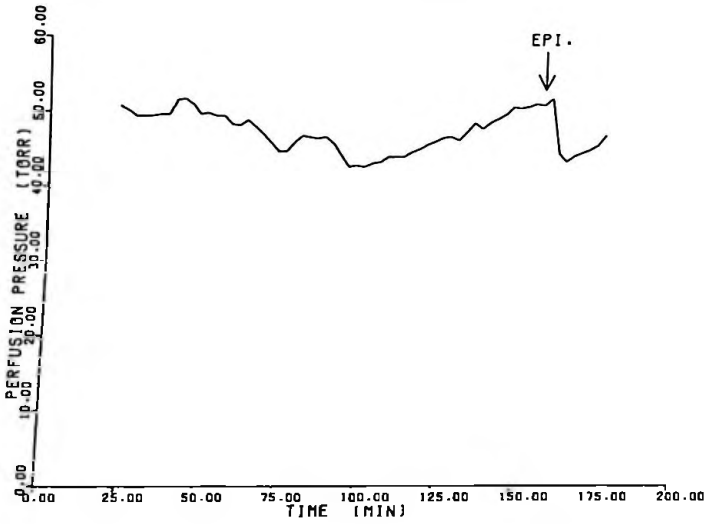

Figure 1. Afferent perfusion pressure over a three hour period, redrawn from the original recording. EPI.= time of epinephrine application. Time scale, minutes after start of perfusion.

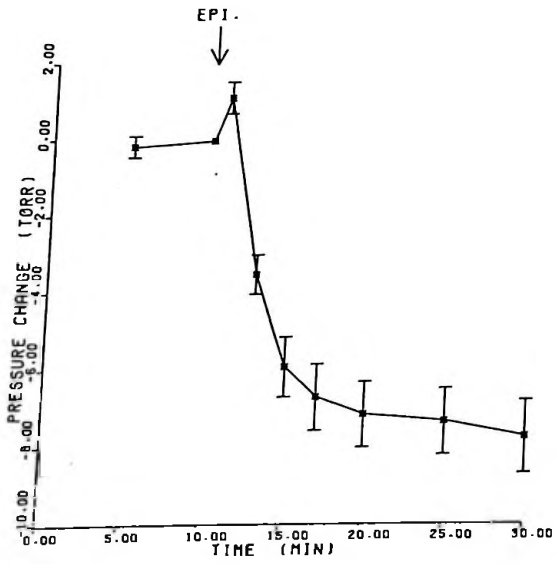

Figure 2. Afferent perfusion pressure before and after epinephrine application. Each point represents the mean difference ± S.E. (n=13) between the measured pressure and the pressure at the moment prior to epinephrine addition (EPI.). All differences after epinephrine addition are significant ($p<0.02$ for initial pressure increase; $P<0.001$ for all subsequent decreases).

Table 1. Effect of epinephrine on afferent perfusion pressure and efferent dorsal aortic and venous flow rates

	Control	Epinephrine
Pressure (Torr)	46.22 ± 1.57 (13)	-7.15 ± 0.86
Dorsal A. ($\mu l. min^{-1}$)	393 ± 58 (8)	+560 ± 57
Venous ($\mu l.min^{-1}$)	1090 ± 77 (8)	-575 ± 42

All values are expressed as mean ± S.E. All changes occurring with epinephrine application are significant ($p<0.001$). Statistical analysis was made by paired differences using two-tailed analysis.

arterial and "venous" efferent flow rates. As seen in Fig. 2, the initial response to epinephrine under constant flow conditions was an increase in perfusion pressure, followed by a larger decrease. Arterial flow rates increased significantly along with a reciprocal decrease in "venous" flow (Table 1).

Table 2 shows that propranolol alone had no effect on the afferent pressure or efferent flow parameters; however, when isolated heads were treated with propranolol plus epinephrine, a significant increase in perfusion pressure along with an increase in dorsal arterial flow and a reciprocal decrease in the "venous" flow was seen. Upon reapplication of Ringer's solution, the relative efferent flow rates returned towards control levels, but the perfusion pressure increased again. Isoproterenol produced a large decrease in pressure but no change in efferent flow rates (Table 3). When epinephrine was applied with isoproterenol, no change in perfusion pressure was observed, but dorsal arterial and "venous" flow rates changed in the same manner as a preparation treated with propranolol and epinephrine or epinephrine alone. When Ringer's was reapplied, pressure and relative efferent flow rates returned to control levels.

Table 2. Effect of propranolol and epinephrine on the afferent perfusion pressure and efferent flow rates

	Control	Prop. Change	Prop. $\bar{X}$	Prop. + Epineph. Change	Prop. + Epineph. $\bar{X}$	Control Change	Control $\bar{X}$
Pressure	45.67 ± 1.39 (N=4)	-2.36 ± 1.42 (NS.)	42.35 ± 2.67	+5.27 ± 1.26 (P<0.05)	45.63 ± 2.35	+5.0 ± 1.17 (P<0.05)	50.72 ± 2.58
Dorsal A ($\mu l.min^{-1}$)	337 ± 108 (N=4)	-14 ± 31 (NS.)	323 ± 93	+415 ± 91 (P<0.025)	739 ± 181	-361 ± 121 (P<0.01)	378 ± 132
Venous	1124 ± 97 (N=4)	+57 ± 57 (NS.)	1181 ± 76	-449 ± 61 (P<0.01)	732 ± 13	+171 ± 121 (NS.)	903 ± 138

All values are expressed as the mean ± S.E. The test of significance of the differences between each value and that of the preceeding period was made utilizing paired differences and two-tailed analysis.

Table 3. Effect of isoproterenol and epinephrine on the afferent perfusion pressure and efferent flow rates

	Control	Isoprot. Change	Isoprot. $\bar{X}$	Isoprot + Epineph. Change	Isoprot + Epineph. $\bar{X}$	Control Change	Control $\bar{X}$
Pressure (Torr)	59.62 +3.34 (N=6)	-12.65 +2.20 ($\bar{P}$<0.01)	46.11 +3.23	+0.76 +0.85 (NS.)	47.68 +2.58	+8.51 +2.09 ($\bar{P}$<0.01)	58.09 +3.74
Dorsal A. ($\mu l.min^{-1}$)	355 +58 (N=5)	-56 +21 (NS.)	299 +64	+409 +70 ($\bar{P}$<0.01)	709 +373	-361 +83 ($\bar{P}$<0.025)	347 +71
Venous ($\mu l.min^{-1}$)	1162 +56 (N=5)	+68 +29 (NS.)	1230 +67	-396 +80 ($\bar{P}$<0.01)	834 +120	+282 +84 ($\bar{P}$<0.05)	1116 +67

The above responses to epinephrine corroborate the data from Payan and Girard (op. cit.) on the isolated, perfused trout head but differ in some details. The initial increase and secondary fall in afferent pressure produced by epinephrine addition is similar to that of the trout (+6.2 torr and -7.4 torr, respectively, for S. gairdneri); though the trout exhibited an initial pressure increase of greater magnitude. Under control conditions, the dorsal arterial flow for S. gairdneri was 75% greater than the "venous" flow, and became 3.8 times as great when 10^{-5}M epinephrine was added to the perfusate. In M. octodecimspinosus, under control conditions, the dorsal aortic flow was only 36% of the "venous" flow; after epinephrine addition it became 85% greater than the "venous" flow.

Our data also indicate that, as Payan and Girard (op. cit.) found for S. gairdneri, the response of the isolated, perfused head of M. octodecimspinosus to epinephrine is mediated via both alpha and beta adrenergic receptors. In the present case, the increase in afferent pressure (under conditions of constant perfusion rate) seen when epinephrine is added in conjunction with the beta-blocker propranolol (Table 2) is presumably under alpha control. The fact that the beta mimetic isoproterenol lowers afferent pressure (Table 3) indicates that the delayed (but longer lasting) fall in afferent pressure produced by epinephrine (Payan and Girard, op. cit.) is mediated via a beta receptor. The bimodal pressure response appears to be the sum of the vasoconstrictive and vasodilatory effects induced by this drug (Fig. 3). It is now generally accepted that these pressure changes mediated by epinephrine

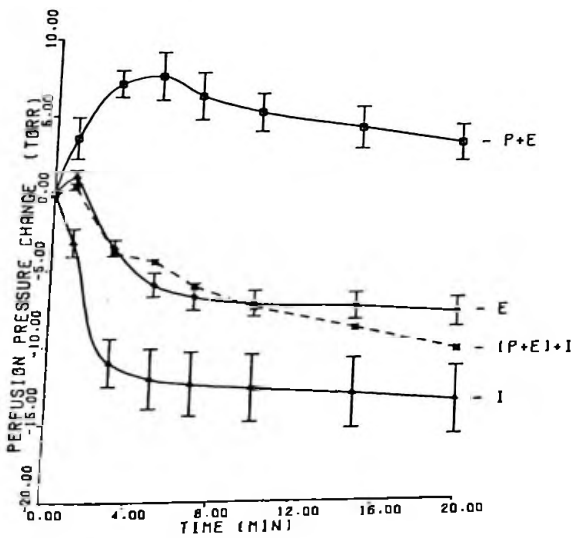

Figure 3. Effects of propranolol plus epinephrine (-P+E; 2 x 10^{-5}M and 10^{-5}M, respectively, N=4), epinephrine alone (-E; 10^{-5}M, N=13), and isoproterenol alone (-I; 10^{-4}M, N=6) on the afferent perfusion pressure. Each point is the mean ± S.E. of the differences between the pressures measured after drug addition and the control pressures recorded immediately prior to drug addition. The theoretical curve (dashed line) represents the sum of the points from the propranolol plus epinephrine and isoproterenol curves.

ld ition are controlled by prelamellar vaso-constriction or vasodilation which alter the number of lamellae which 'e perfused and, hence, the cross sectional area (and resistance) of the branchial vasculature (Girard and Payan, m. J. Physiol. 230:1555-1560, 1976). Payan and Girard (op. cit.) found that the alteration in efferent flow ltes partitioned between the dorsal artery and a "venous" pathway were also controlled by both alpha and beta :eptors but proposed that alpha control predominated. Our results support this conclusion. Tables 2 and 3 show le arly that perfusate is preferentially shunted into the dorsal arterial system when either epinephrine is added in ne presence of the beta-blocker propranolol or in the presence of the beta-stimulant isoproterenol. Importantly, timulation of beta receptors (addition of isoproterenol alone) does not alter the pattern of perfusate flow. Thus, he shunting of blood to the dorsal artery, subsequent to the addition of epinephrine is mediated entirely by an lpha receptor which presumably vasoconstricts the post-lamellar arterio-venous anastomoses between the efferent ranchial arteriole and the central filamental venous sinus (Girard and Payan, op. cit.). It is important to note hat our (and presumably S. gairdneri's) efferent flow responses to epinephrine are probably underestimated because of arterial contamination of the "venous" pathway. Finally, the fact that readdition of Ringer's perfusate after ome 40 minutes of isoproterenol treatment resulted in a return of control afferent pressures and control partitioning of efferent flow indicates that the tissue is quite viable for long periods of time. It therefore appears that the isolated, perfused head of M. octodecimspinosus provides a preparation in which the solutions bathing both the serosal and mucosal sides of the gill can be controlled. This system exhibits a prolonged viability and responsiveness to epinephrine through both alpha and beta adrenergic receptors, and will be useful for kinetic studies of osmo-regulation as well as branchial hemodynamics. This research was supported by NSF PCM 77-03914 to DHE.

FURTHER STUDIES ON THE OSMOREGULATION OF PREMATURE "PUPS" OF Squalus acanthias

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Preliminary investigations on various aspects of osmoregulation by the premature "pups" of Squalus acanthias indicated that they "are able to maintain blood Na and Cl levels below uterine fluids (and sea water) because of low ionic permeability and cloacal (presumably rectal gland) extrusion of these ions (the typical adult pattern)" (Kormanik and Evans, Bull. MDIBL 18:65-69, 1978). The present investigation examines Cl effluxes from the "pups" and the effect of extirpation of the rectal gland on blood Na and Cl concentrations.

To determine the rate of ^{36}Cl efflux, individual "pups" were injected with 0.5 μCi (20-40μl) of the isotope and treated as described previously for ^{22}Na efflux determinations (Kormanik and Evans, Ibid.). In some cases, after a few hours in control sea water, the "pups" were transferred to artificial sea water in which Cl had been replaced with benzenesulfonate. Extirpation of the rectal gland was made via a ventral incision while the branchial chamber of the "pup" was irrigated with 0.05% MS222 in sea water. A ligature was placed between the proximal end of the rectal gland and its vascular supply before the gland was removed. Sham operations consisted of the same anesthesia and ventral incision, followed by exteriorization of the gland. In both cases the incision was then sutured closed. After 48 hours, blood was collected in heparinized microhematocrit tubes from the caudal vessels after tail transection; the animal was then sacrificed by spinal transection. The blood was centrifuged, the plasma frozen and shipped to Miami where it was analyzed for Na (via flame photometry) and Cl (via amperometric titration).

The rate constant (fraction of exchangeable Cl) of Cl efflux from the 8 "pups" was $21.0 \pm 3.5 \times 10^{-3}$ .hr^{-1} ($\bar{X} \pm$ S.E.). This is seven times the rate constant of Na efflux (Kormanik and Evans, op. cit.). If we assume that the exchangeable Cl space is approximately the same as the exchangeable Na space and the blood Cl concentration is approximately 21 0mM/l (Table 1 and Kormanik and Evans, Ibid.), we can calculate the apparent Cl efflux to be approximately 278 μM.100 g^{-1}.hr^{-1}. This indicates that these animals maintain a much higher Cl efflux than Na