In order for a Cl^-/HCO_3^- exchange to contribute to the Cl^- secretion, HCO_3^- would first have to gain access to the cell interior and then be exchanged for external Cl^- in the serosal bathing medium. This could be accomplished by CO_2 diffusion across the basolateral membranes and its subsequent hydration and dissociation to H^+ and HCO_3^- in the cell interior. Carbonic anhydrase would not contribute significantly to this sequence of events, since its inhibition by acetazolamide (Degnan et al., J. Physiol. 271:155, 1977) or methazolamide (unpublished observations) has no inhibitory effect on the Cl^- secretion across the opercular epithelium. The fact that only part of the $J_{sm}^{Cl^-}$ is sensitive to serosal HCO_3^- (or CO_2), suggests either another Cl^- entry step, such as a Na^+-coupled Cl^- transport (Silva et al., Am. J. Physiol. 233:F298, 1977), or that the intracellular CO_2 generation is sufficient to maintain a Cl^-/HCO_3^- exchange at a significant level. The known presence of a HCO_3^--ATPase in gill epithelia (Kerstetter & Kirschner, Comp. Biochem. Physiol. 48:581, 1974), and the recent demonstrations of the presence of this enzyme in uncontaminated microsomal fractions (Kinne-Saffran & Kinne, J. Memb. Biol. 49:235, 1979; Bornancin et al., Am. J. Physiol. 238:R251, 1980), makes the possible involvement of this enzyme in Cl^- transport more plausible than previously believed. This work was supported by NIH grants GM25002 and EY 01340 and the Commissariat a l'Energie Atomique, France.

THE EFFECT OF PERFUSION AND IRRIGATION FLOW RATE VARIATIONS ON NaCl EFFLUX FROM THE ISOLATED, PERFUSED HEAD OF *MYOXOCEPHALUS OCTODECIMSPINOSUS*

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Previous investigations of the isolated, perfused head of the long-horn sculpin, *Myoxocephalus octodecimspinosus*, have demonstrated that it displays prolonged viability when compared to the perfused head of the trout (Payan and Girard, Am. J. Physiol. 232:H18-H23, 1977) and, like the trout, responds to epinephrine with a transitory, alpha-mediated vasoconstriction and longer-term, betamediated vasodilation (Claiborne and Evans, Bull. MDIBL 19:96-101, 1979, J. Comp. Physiol. 138-79-85, 1980). The present study examines the effect of variation of either perfusion or irrigation flow rates on Na and Cl effluxes, determines *in vivo* afferent blood pressures and compares *in vivo* Na and Cl effluxes with those from the isolated, perfused head.

The isolated, perfused head of *M. octodecimspinosus* was prepared as described previously (Claiborne and Evans, ibid.). To determine the unidirectional effluxes, either ^{22}Na or ^{36}Cl was added to the afferent perfusate at approximately 3 uCi/500 mls. Effluxes were monitored by taking 5 ml samples of the irrigation bath at various time intervals, mixing the sample with 5 mls of Aquasol-2 and counting in a Packard Tricarb Liquid Scintillation System. Quenching was corrected by internal standardization. The rate of efflux ($uM \cdot 100g^{-1} \cdot hr^{-1}$) was calculated by dividing the appearance of radioactivity per unit time in the irrigation solution by the specific activity of the perfusate and correcting for the weight of the animal. To monitor the effect of alterations of perfusion (and pressure) on the ^{22}Na or ^{36}Cl efflux, the efflux was measured during an initial control rate period (afferent pressure approximately 30 torr), followed by one period (^{22}Na) or two periods (^{36}Cl) at reduced afferent flow rates. ^{22}Na efflux periods were 20 minutes long while ^{36}Cl efflux periods were 15 minutes in length. In all cases an equilibration period (at the new pressure) of the same length was interspersed between the experimental periods. The effect of variations of the irrigation flow rates was determined for either isotope during 4 successive, 15 minute efflux periods during which the irrigation rate was varied over the range of 138 to 980 mls/min. The sequence of irrigation rate changes was different for each perfused head. In these experiments the afferent perfusion pressure was maintained at approximately 25 torr. *In vivo* ^{22}Na and ^{36}Cl efflux rates were determined by injecting approximately

1 uCi intraperitoneally (10 uls). After a 1 hour equilibration period to allow mixing of the isotope in the body fluids, the rate of efflux over a 5 hour period was determined in the same manner as described previously (Kormanik and Evans, Bull MDIBL 18:65-69, 1978). *In vivo* afferent branchial blood pressure was determined by inserting a heparinized Rinser's filled cannula into the afferent branchial artery on the second gill arch of anesthetized sculpins. The cannula was connected to a pressure transducer and the pressure was recorded continuously, as described previously (Claiborne and Evans, op. cit.). The fish were allowed to recover from the anesthesia and stable pressures were recorded for up to 1 hour starting approximately 30 minutes after implantation of the cannula.

The *in vivo* afferent blood pressure of *M. octodecimspinosus* was found to be 31.7 ± 3.1 torr (5) (X ± S.E., N), which is in the same range as that described for other marine teleosts (Ngan et al., Jap. J. Ichthyol. 22:1-8, 1974; Kicenuik and Jones, J. Exp. Biol. 69:247-260, 1977). The *in vivo* unidirectional ^{22}Na and ^{36}Cl effluxes were 1472 ± 140 (5) and 1210 ± 259 uM·100g^{-1}-hr^{-1} (5), respectively, again in the same range as Na and Cl effluxes described for other marine teleosts of similar weight (Evans, in Comparative Physiology of Osmoregulation in Animals, ed. by B.M.O. Maloiy, 305-390, 1979). Table 1 shows that, with the isolated perfused head, reduction of the afferent flow rate (and the concomitant reduction in afferent pressure) has a pronounced effect on the rate of efflux of ^{22}Na; however, the efflux of ^{36}Cl is unaffected (Table 2). Variations of the irrigation rate (with

Table 1.--Effect of Changing Perfusion Flow Rate on the Afferent Perfusion Pressure and Unidirectional ^{22}Na Efflux

	Perfusion rate Control	Low
Flow rate (mls/min)	2.53 ± 0.17 N=7)	0.66 ± 0.10 (p<.001)
Pressure (torr)	30.6 ± 2.2 (N=7)	13.3 ± 2.6 (p<.001)
J_{out}^{Na} (uM·100g^{-1}·hr^{-1})	1999.6 ± 220.9 N=7)	685 ± 217.8 (p<.001)

All values are expressed as the mean ± S.E. The test of significance of the differences between each control and low perfusion rate value was made utilizing paired differences and Student's t-test.

Table 2.--Effect of Changing Perfusion Flow rate on the Afferent Perfusion Pressure and Unidirectional ^{36}Cl Efflux

	Period 1	Period 2	Period 3
Flow rate (ml/min)	3.65 ± 0.38 (N=8)	2.14 ± 0.26 (p<.001)	0.90 ± 0.15 (p<.001)
Pressure (torr)	30.2 ± 3.0 (N=7)	24.6 ± 3.5 (p<.02)	15.9 ± 1.9 (p< .005)
J_{out}^{Cl} (uM·100g^{-1}·hr^{-1})	270.8 ±51.3 (N=8)	296.0 ±50.6 (NS.)	228.5 ±70.4 (NS.)

All values are expressed as mean ± S.E. The test of significance of the differences between each value of periods 1 & 2 and periods 2 & 3 was made utilizing paired differences and Student's t-test.

afferent pressure maintained constant at approximately 25 torr) did not effect either the rate of ^{22}Na or ^{36}Cl efflux from the perfused head (Table 3). Importantly, using afferent perfusion pressures equivalent to *in vivo* pressures, the perfused head displayed *in vitro* Na effluxes similar to the *in vivo* fluxes, but Cl effluxes distinctly below the *in vivo* values ($p < .025$) (Tables 1 and 2).

Table 3.--Effect of Changing Irrigation Flow Rate on the Unidirectional Efflux of ^{22}Na and ^{36}Cl

	Irrigation flow rate (mls/min)			
	138	556	732	980
J_{out}^{Na} ($uM \cdot 100g^{-1} \cdot hr^{-1}$)	615.67 ± 183.57	707.43 ± 174.84	636.27 ± 108.90	542.26 ± 89.89
	perfusion flow rate = 1.61 ± 0.11 mls/min perfusion pressure = 24.1 ± 1.4 torr			
J_{out}^{Cl} ($uM \cdot 100g^{-1} \cdot hr^{-1}$)	192.77 ± 83.14	193.56 ± 43.59	177.53 ± 33.7	240.30 ± 101.04
	perfusion flow rate = 3.45 ± 0.44 mls/min perfusion pressure = 26.8 ± 1.5 torr N = 5			

All values are expressed as mean ± S.E. The efflux values for either ion did not vary significantly when irrigation flow rates were altered.

Our data demonstrate that when the isolated head of *M. octodecimspinosus* is perfused at *in vivo* afferent branchial pressure, the isotopically determined Na efflux is equivalent to measured *in vivo* rates, but is distinctly dependent upon afferent perfusion pressures. In contrast, *in vitro* ^{36}Cl effluxes are less than *in vivo* rates, and are not affected by substantial alterations of afferent perfusion rate and pressure. Neither Na or Cl effluxes from the isolated, perfused head are effected by gross alterations in irrigation rate.

We conclude from the present study that: 1. *In vitro*, and presumably, *in vivo* branchial irrigation is not rate-limiting to either Na or Cl efflux, 2. The efflux of Na is flow/pressure dependent and the perfused head provides the proper control of these parameters to result in Na effluxes equivalent to *in vivo* levels, 3. The efflux of Cl is not flow/pressure dependent and therefore is presumably via some pathway distinct from the pathway for Na efflux, 4. The perfused head somehow limits the efflux of Cl because either the pathway for Cl efflux is not properly perfused in this preparation or some stimulatory factors are not present in the perfusate. Whether maintenance levels of hormones such as corticosteriods or catecholamines are necessary for eliciting Cl effluxes from the perfused head equivalent to those demonstrated *in vivo* remains to be seen. However it appears obvious from the present study that Na and Cl effluxes have different rate-limiting steps. This conclusion supports the conclusions of Silva et al (J. Exp. Zool. 199:419-426, 1977) but is at odds with the recent proposition that NaCl is moved down pressure gradients across the leaky tight junctions between the Cl cells (Sargent et al., In: Physiology and Behavior in Marine Organisms., D.S. McLusky & A.J. Berry, eds., Pergamon Press, Oxford, 123-132, 1978). This research was supported by NSF grant PCM80-08366 to DHE.