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The Fish Gill: Site of Action and Model for Toxic Effects of Environmental Pollutants

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The gill epithelium is the site of gas exchange, ionic regulation, acid-base balance, and nitrogenous waste excretion by fishes. The last three processes are controlled by passive and active transport of various solutes across the epithelium. Various environmental pollutants (e.g., heavy metals, acid rain, and organic xenobiotics) have been found to affect the morphology of the gill epithelium. Associated with these morphological pathologies, one finds alterations in blood ionic levels, as well as gill Na,K-activated ATPase activity and ionic fluxes. Such physiological disturbances may underly the toxicities of these pollutants. In addition, the epithelial transport steps which are affected in the fish gill model resemble those described in the human gut and kidney, sites of action of a variety of environmental toxins.

Introduction

The increasingly obvious effects of pollution of the biosphere in general and aquatic ecosystems in particular are known to everyone and are the subject of daily accounts in the popular press as well as textbooks (*1*) and scientific monographs (*2,3*). It is the intent of this short review to: (a) examine the morphology and physiology of the fish gill epithelium and its underlying vasculature, (b) briefly review selected data which indicate that a variety of toxicants in the aquatic environment can affect gill structure and function, (c) delineate specific sites of action in the gill which may account for the toxic effects, and (d) suggest that the fish gill may be used as a model system for study of the more generalized effects of toxicants on ion transport across cellular and epithelial membranes, as well as vasoactivity of blood vessels. The literature review and discussion will be limited to the gills of teleost (bony) fishes and is meant to be more representative than exhaustive. Cited references will emphasize review papers or chapters in books in many cases in order to facilitate entry into a vast literature and to save space.

Structure and Function of the Fish Gill Epithelium

The gill epithelium is the dominant site of gas exchange, ionic-regulation, acid-base balance, and nitrogenous waste excretion for fishes (*4,5*), thereby serving a multitude of vital functions for these aquatic animals. The evolutionary and morphological development of the gill has recently been reviewed by Hughes (*6*) and need not be detailed here. Suffice it to say that the epithelium covers four (in rare cases, two or three) branchial arches plus, when present, the pseudobranch (remnant of the gill on the mandibular arch) and, in some cases, the inner surface of the operculum and buccal cavity. The epithelium on each branchial arch is subdivided into two dorsoventral columns of filaments (flattened extensions running at right angles to the branchial arches), with dorsal and ventral rows of secondary lamellae being further subdivisions on each filament, lying at right angles to the filamental plane (Fig. 1). The secondary lamellae are the site of gas exchange, with blood to water diffusion distances of less than one micrometer in active species and 1 to 10 μm in more sluggish fish species (*6*). The epithelium is supplied with blood directly from the heart, through the ventral aorta, with afferent and efferent branchial arteries in the arches. The pattern of blood flow through the filaments and secondary lamellae is relatively complex, with unoxygenated blood flowing through afferent filamental arteries and afferent lamellar arterioles into the secondary lamellae. Oxygenated blood leaves the lamellae and returns to the efferent branchial artery via efferent lamellar arterioles and efferent filamental arteries. Anastomoses between the efferent filamental artery and the central venous sinus of the filament provide for a parallel drainage of oxygenated blood directly back to the branchial vein, and subsequently, the heart (Fig. 2) (*7*). In addition, some species display anastomoses between the afferent filamental arteries and the central venous sinus, providing for a potential shunt around the lamellae (*8*). Blood flow into these afferent and efferent lamellar pathways is controlled by catecholamines. There is now abundant evidence (*9,10*) that epinephrine, via β-adrenoceptors, produces a fall in gill vascular resistance by opening

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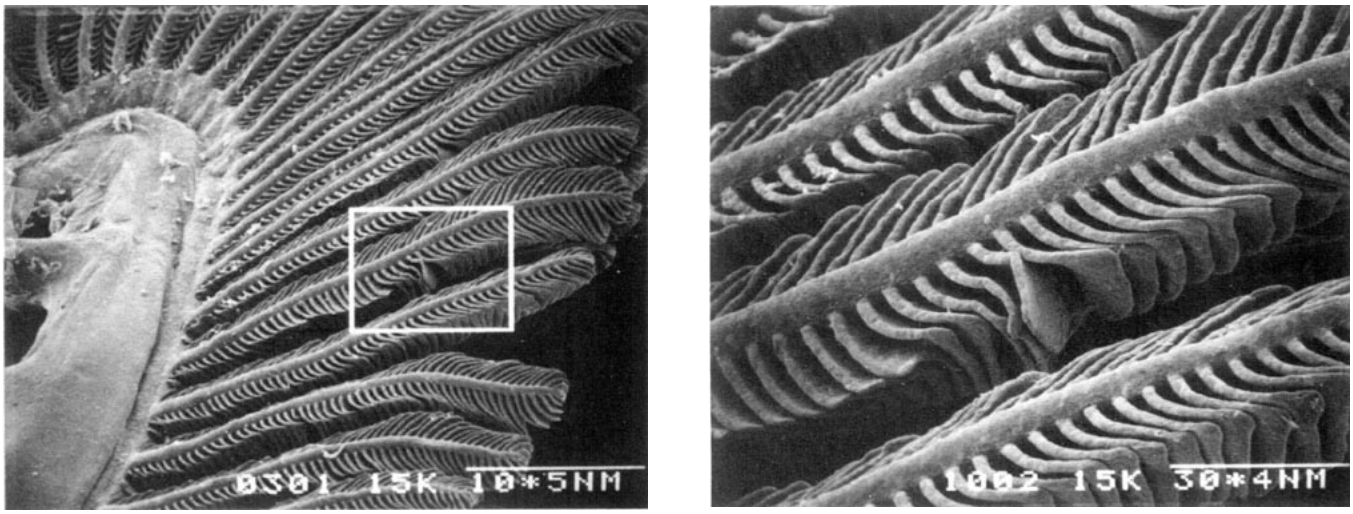

a *b*

FIGURE 1. Scanning electron micrographs of (*a*) branchial arch and filaments of gill from the teleost, *Opsanus beta*; calibration line is 1 mm (10×10^5 nm); (*b*) filament and secondary lamellae of gill from the teleost, *Opsanus beta*; calibration line is 300 μm (30×10^4 nm).

prelamellar arterioles, and an increase in flow into the efferent filamental artery (at the expense of the venous flow into the central venous sinus) via α-adrenoceptors. Alterations in the pattern of blood perfusing the filaments vs. the secondary lamellae theoretically could have profound effects on osmoregulation because of the relative distributions of "leaky" tight junctions and chloride cells on the filaments and secondary lamellae (see below). The relative role of blood-borne hormones vs. catecholamines from nerve terminals in the gill tissue is still open to some debate (*10,11*).

The cellular composition of the branchial epithelium is usually divided into a filamental epithelium and a much simplified lamellar epithelium, although recent evidence indicates that at least one cell type (chloride cell, see below) formerly thought to be unique to the filamental epithelium is also found in the lamellar epithelium in some species (*8*) (Fig. 3). The filamental epithelium is composed of five major cell types, including squamous pavement cells which characteristically possess microridges; mucous (goblet) cells; heavily innervated, neuroepithelial cells which contain biogenic amines; accessory cells (which may be precursors of chloride cells) (*12*); and chloride cells, whose role in ion transport is now well established (*13–16*). The epithelium of the secondary lamellae is much more simple and thinner (see above) than that of the filament, and consists of two major cell types: the superficial cells and the basal cells, the latter thought to be cells differentiating to replace the former. The basal cells might also differentiate into chloride cells when they are found in the lamellar epithelium (*17*). Despite its relative thinness, the lamellar epithelium is considered to be relatively impermeable to ions, water, and organic molecules because of extensive intercellular strands forming "tight junctions" (*18*). This is to be contrasted with the filamental epithelium, where relatively diffuse strands between chloride cells, or between chloride and accessory cells, (especially in seawater-acclimated fishes) indicate leaky "tight junctions," and therefore relatively high solute permeability (*14,19*). It is generally considered that these leak pathways represent the site of the dissipative ion and water movements which must be countered by active osmoregulatory systems by fishes in both sea water and fresh water (*20*).

Since, like all vertebrates except the hagfishes, teleost fishes maintain the NaCl content of their body fluids at approximately 40% that of sea water, it is clear that they face a net diffusional loss of salt into fresh water and net diffusional uptake of salt from sea water. In the marine teleosts the diffusional salt load is exacerbated by salt influx across the gut dictated by the need to absorb ingested water to balance the water lost osmotically to the hyperosmotic marine environment (*20*). The molecular pathways which allow the gill of marine teleosts to extrude unwanted salts (*14,21*) are basically the same as those utilized by the salt-excretory, rectal gland of elasmobranchs (*22*) as well as those described for NaCl uptake across the marine fish gut epithelium, subsequent to ingestion of sea water (*23,24*). Figure 4 depicts the current working model for the steps for NaCl extrusion by the marine fish gill epithelium. Recent microprobe analysis demonstrated conclusively that this transport step resides in the chloride cells of the teleost gill epithelium (*25*). Importantly, numerous studies have demonstrated that the basolateral aspect of the chloride cells contains high concentrations of the transport enzyme Na,K-activated ATPase (*13,14*). Freshwater teleosts (and presumably all gilled, freshwater vertebrates) extract needed NaCl from the me-

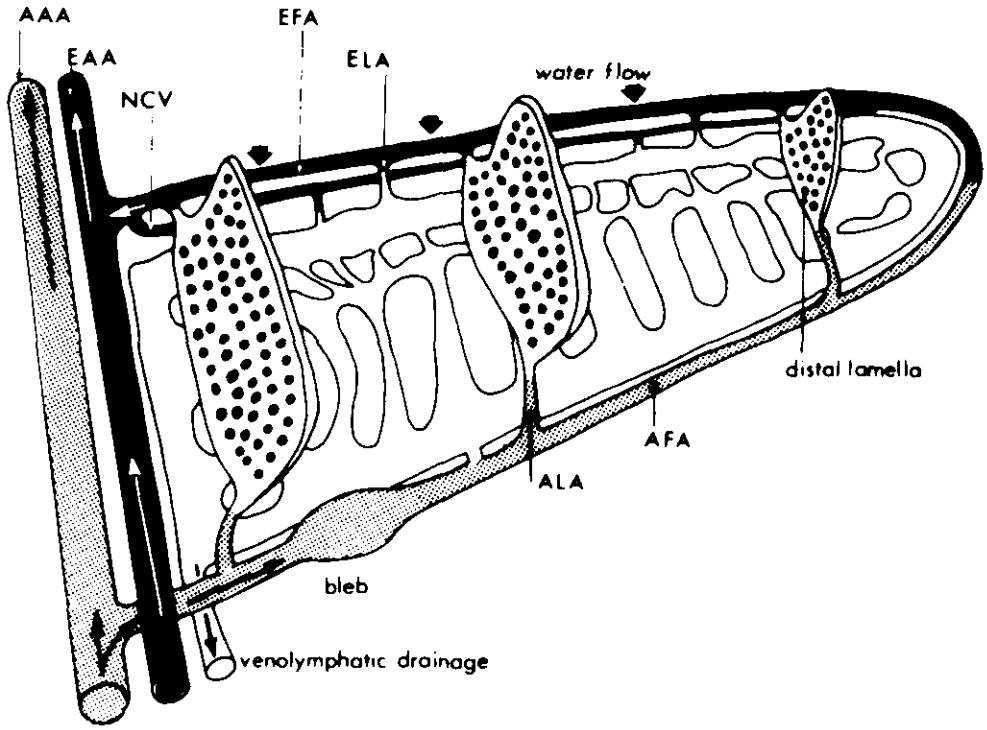

FIGURE 2. Diagram of vascularization of gill arch, filament, and lamellae of a typical teleost fish. (AAA) afferent arch artery; (AFA) afferent filament artery; (ALA) afferent lamellar artery; (EAA) efferent arch artery; (EFA) efferent filament artery; (ELA) efferent lamellar artery; (NCV) nutritive companion vessel; (CVS) central venolymphatic sinus. From Randall et al. (*7*) with permission.

dium via parallel, antiport systems involving exchange of intracellular H^+ or NH_4^+ for external Na^+ and internal HCO_3^- (or possibly OH^-) in exchange for external Cl^- (Fig. 5) (*26*). The actual cell involved in these transport steps is unknown, but is generally assumed to be the chloride cell, despite its usually reduced population in freshwater species (*13*). Moreover, the energizing step for these antiports is also presently unknown, but the chloride cell does contain substantial concentrations of Na,K-activated ATPase even in freshwater species (*13*). In addition, intracellular carbonic anhydrase may play a role, supplying H^+ and HCO_3^- for the apical ionic exchanges (Fig. 5). In the past several years it has become apparent that both the Na^+/H^+ and Cl^-/HCO_3^- antiports are also present in the gill epithelium of marine teleosts (*27,28*), being involved in acid-base regulation and nitrogenous waste excretion in both marine and freshwater forms (*26,29*). Recent evidence indicates that the antiports evolved first in the hagfishes for acid-base regulation, even before the vertebrates entered fresh water (*30*). Finally, despite the potential for extrusion of unwanted ammonia (fish are ammonotelic) via the gill Na^+/NH_4^+ exchange, it is apparent that diffusive pathways for both NH_4^+ and NH_3 exist across the fish gill epithelium (*31*). It is important to note that these branchial pathways for extrusion of ammonia and acid/base equivalents predominate over renal extrusion pathways, even under conditions of experimental manipulation of blood pH (*29*).

Effects of Selected Pollutants on Gill Structure and Function

One need only consult a recent review chapter on histopathology of tissues from aquatic organisms to determine that gill pathologies are common symptoms of toxic effects on fishes of a wide variety of aquatic pollutants, including organochlorines, petroleum compounds, organophosphates, carbamates, miscellaneous herbicides, acidification, nitrogenous compounds, heavy metal salts, and chemotherapeutic agents (*32*). The morphological anomalies commonly include "hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia (marked dilation of terminal blood vessels), edema with epithelial separation from basement membranes, general necrosis, and/or epithelial desquamation" (*32*). It is important to note that a recent statistical analysis of common gill histopathologies produced by a variety of toxicants indicates that, rather than toxicant-specific responses, these gill structural damages may merely be reflections of generalized stress responses, often secondary to a failure of gill cellular osmoregulation in freshwater species (*33*). Unfortunately, morphological pathologies are often described without concomitant description of physiological changes, and physiological studies often do not include data on morphological changes. Nevertheless, examination of some selected studies can provide insights into the effects of at least three classes of environmental pollutants on fish gill

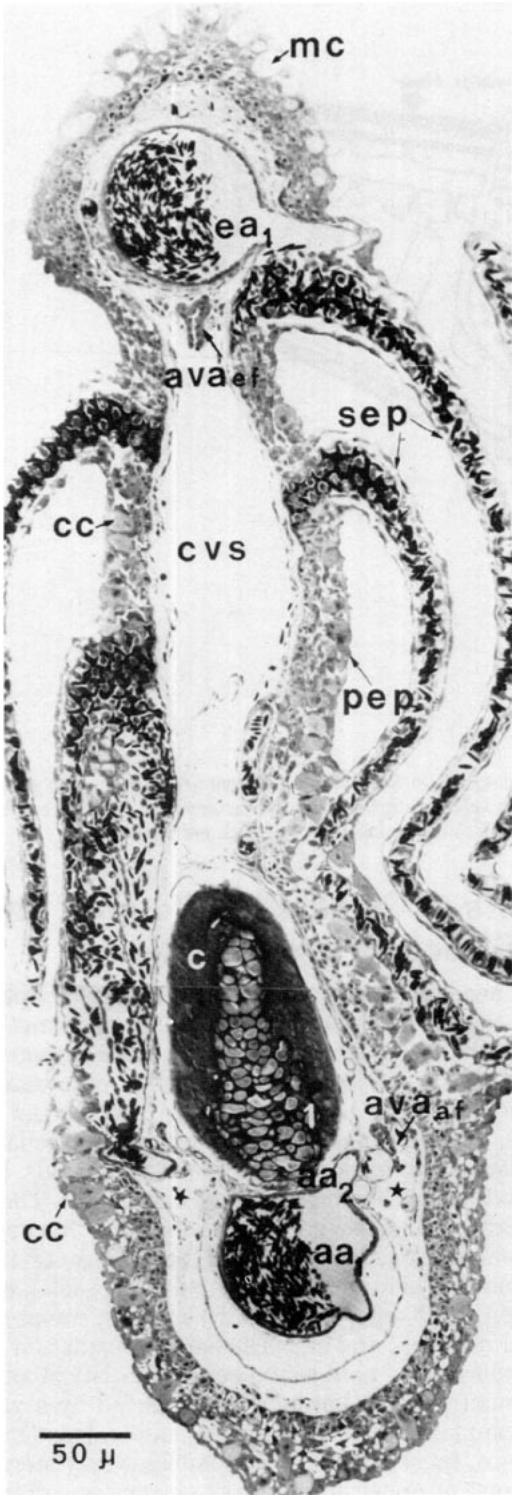

FIGURE 3. Cross section of filament and lamellae of the eel, *Anguilla rostrata*. (pep) primary (filamental) epithelium; (sep) secondary (lamellar) epithelium; (cvs) central venus sinus; (aa_1) afferent filamental artery; (ea_1) efferent filamental artery; (ava_{af}) arteriovenous anastomosis between aa_1 and cvs; (ava_{ef}) arteriovenous anastomosis between ea_1 and cvs; (c) cartilage; (cc) chloride cell; (mc) mucous cell. From Laurent and Dunel (*17*) with permission.

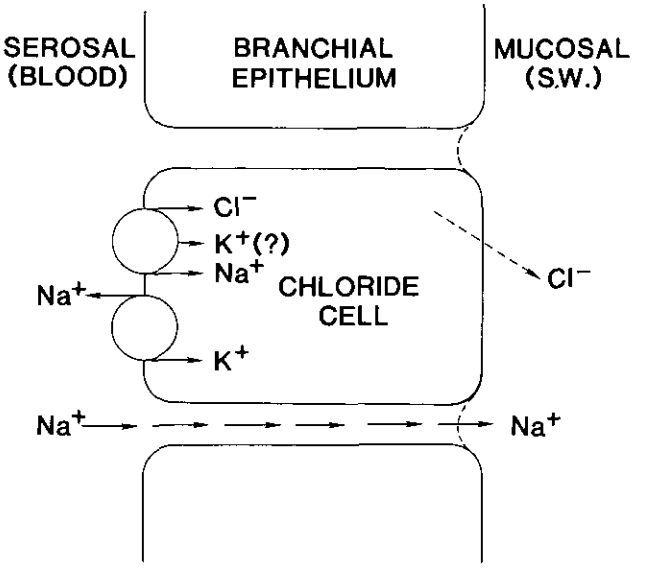

FIGURE 4. Current model of pathways for Na^+ and Cl^- extrusion by the marine teleost branchial epithelium. Single dotted lines at the apical junctions of adjacent cells denote leaky "tight junctions" characteristic of sea water gills. K^+ (?) indicates that the basolateral carrier has been shown to transport both KCl and NaCl in many tissues (*114*), but K-sensitivity in the fish gill has not yet been demonstrated. See text for details.

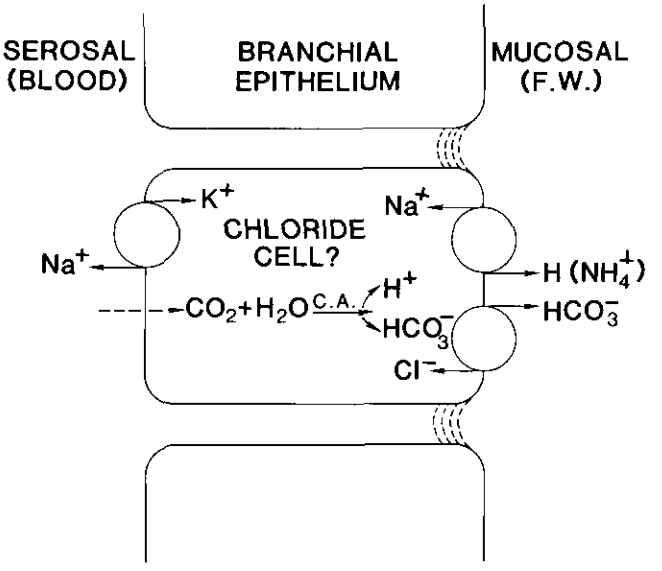

FIGURE 5. Current model of pathways for Na^+ and Cl^- transport by the freshwater teleost branchial epithelium. Four dotted lines at the apical junction between adjacent cells denote "tight junctions" characteristic of freshwater gills. C.A. indicates intracellular carbonic anhydrase. See text for details.

structure and function. We will be concerned only with perturbations of solute transport across the gill epithelium and gill hemodynamics. Satchell (*34*) has recently reviewed the literature on the effects of various pollutants on gas exchange in fishes.

Heavy Metals*

It is clear that exposure of various species of fishes to heavy metals in the environment is associated with obvious structural damage to the gill epithelium. For example, Skidmore and Tovell (*36*) demonstrated that exposure of rainbow trout (*Salmo gairdneri*) to 40 ppm Zn^{2+} for approximately 3 hr resulted in severe curling and edema of the secondary lamellae, with the epithelium lifted away from the basement membrane. Chloride cells were also partially detached and swollen. Olson et al. (*37*) described less severe morphological changes (decreased height of lamellar cell ridges, appearance of vacuolated epithelial cells, and chloride cell degeneration) after exposure of rainbow trout to either mercuric chloride or methylmercury (approximately 50 ppb for 1 week or 0.25 ppb for 6–8 weeks). Matthiessen and Brafield (*38*) examined the effect of exposing sticklebacks (*Gasterosteus aculeatus*) to 0.5 to 1.0 ppm Zn^{2+} for 1 to 3 days in distilled water (usually fatal) or 2 to 6 ppm Zn^{2+} for up to 29 days in hard water (not fatal). In the distilled water exposures the most characteristic response was "detachment and sloughing of epithelial cells and coalescing of adjacent secondary lamellar epithelia." Gill responses in the hard water experiments were characterized by the appearance of chloride cells on the secondary lamellae. These authors suggest that, at least in hard water, survival of Zn^{2+} exposure may be associated with excretion of the unwanted cation via activated chloride cells. This conclusion was supported by their finding that if fish were allowed to recover in Zn^{2+}-free hard water for 9 days after exposure to 1 ppm Zn^{2+} in distilled water for 16 hr the gill epithelium was often characterized by the appearance of chloride cells on the secondary lamellae. One might argue (*33*) that this epithelial detachment was secondary to increased osmotic influx of water in this hyperregulating, freshwater species, but Baker (*39*) found swelling and clumping of secondary lamellae in the gills of the marine, winter flounder (*Pseudopleuronectes americanus*) exposed to copper. Since this species normally is faced with an osmotic loss of water, it is clear that copper is not merely increasing osmotic permeability of the gill epithelium in this case.

Given these structural changes, it is clear that heavy metals should produce profound effects on gill solute and water transport. Acute exposure of rainbow trout to copper (12.5 to 200 ppb) for 12 to 24 hr is correlated with a decline in blood Na^+ and Cl^- concentrations (*40*), corroborating earlier studies with copper (*41,42*). This Cu^{2+}-induced decline in blood NaCl was secondary to an inhibition of uptake of both ions at lower Cu^{2+} concentrations (12.5 to 50 ppb); at higher Cu^{2+} concentrations ionic effluxes were also stimulated (*40*). The authors propose that this latter effect on ionic permeability may have been secondary to displacement of Ca^{2+} from anionic sites of the intercellular cement, thereby opening "tight junctions" and allowing Na^+ and Cl^- to diffuse across the gill epithelium, down their respective electrochemical gradients. Cu^{2+}, as well as other heavy metals, can potentially displace Ca^{2+} from biological ligands (*35*). Inhibition of ionic uptake, at least in the case of Na^+, may have involved reduction of Na^+/NH_4^+ exchange since blood ammonia levels also rose under copper treatment (*40*). It is important to note that these authors found that the threshold concentration for these effects of copper on trout osmoregulatory pathways was 12.5 ppb, only twice the water quality level set by the International Joint Commission (*43*).

Exposure of killifish (*Fundulus heteroclitus*) to 125 ppb of mercuric chloride for 24 hr completely blocked net Na^+ uptake. Interestingly, similar exposure to methylmercury resulted in only transient inhibition of uptake, followed by uptake at control rates after 30 min (*44*). The authors suggested that this recovery from methylmercury toxicity may have been secondary to rapid redistribution of the toxicant from the gill tissue to the liver and kidney. They also found that both mercury compounds significantly reduced the gill Na,K-activated ATPase in exposed killifish (*44*). In these experiments, the isotopically measured Na^+ efflux from the killifish was unchanged by exposure to either mercury compound, consistent with the proposition that the passive ionic permeability of the gill was unaffected. More recently Lock et al. (*45*) have found that exposure of rainbow trout to either mercuric chloride (1–2 ppm for 4 hr or 100 ppb for 1 week) or methylmercury (100 ppb for 4 hr or 5 ppb for 1 week) was associated with a significant reduction in blood Na^+ and Cl^- concentrations. However, gill Na,K-activated ATPase levels were reduced only at concentrations of either mercury compound at twice these concentration/exposure levels. Since the authors also measured increased water uptake by isolated gills, they suggest that ionic reduction under mercury stress may be due merely to increased water influx, rather than inhibition of ionic uptake mediated by Na,K-activated ATPase. However, passive ionic losses were not monitored in this study, and could have played a role in the blood concentration decline which was noted.

The effects of zinc exposure are less well defined. Lewis and Lewis (*46*) found that exposure of channel catfish (*Ictalurus punctatus*) to lethal zinc concentrations (12–30 ppm) was associated with a decline in the serum osmolality, which was reversed by addition of NaCl to an osmotic pressure of 235 mOsm. However, Spry and Wood (*47*) found no significant changes in blood Na^+, K^+, or Cl^- concentrations of rainbow trout exposed to either 0.8 or 1.5 ppm Zn^{2+} for up to 12 hr, despite significant, and fatal, mixed acidosis (increased PCO_2 and blood lactate levels) and hypoxia, at least at 1.5 ppm Zn^{2+}. These data are at odds with an earlier study on rainbow trout which demonstrated that exposure to 40 ppm Zn^{2+} resulted in a slight fall in blood

*I will use the standard notation of "heavy metals" for ions such as Cu^{2+} Hg^{2+}, and Zn^{2+}, but they are probably more properly referred to as "borderline" (Cu^{2+} and Zn^{2+}) and "class B" (Hg^{2+}) metals based upon their respective reactivities with various ligands. See Nieboer and Richardson (*35*) for an interesting discussion of the validity of the term "heavy metals."

osmotic and Na^+ concentrations (*48*). Nevertheless, the author proposed that respiratory stress was the critical parameter affected by Zn^{2+} exposure. In a more recent study, Spry and Wood (*49*) found that exposure of rainbow trout to much lower levels of Zn^{2+} (0.8 ppm for 72 hr) resulted in a mixed acidosis, normoxia, and reversal of branchial net uptake of both Na^+ and Cl^- to net loss. Interestingly, blood Na^+ and Cl^- concentrations did not change, despite 53% mortality over the 3-day experimental period, and a substantial stimulation of passive NaCl losses across the gills. This may have been due to the fact that branchial uptake of both ions was also stimulated, especially after 48 hr of Zn^{2+} exposure. The authors proposed that some of the metabolic component of the acidosis observed was secondary to a stimulation of net base loss (equivalent to acidic equivalent uptake) produced by stimulation of Cl^-/HCO_3^- exchange vs. Na^+/H^+ exchange (Cl^- influx increased more than Na^+ influx, at least initially). The authors also suggested that these acid-base disturbances were insufficient to account for the observed mortality, and hence, the causes of death may be disruption of cellular events, such as oxygen delivery and/or utilization (*49*). It is important to note that, despite the uncertainty of osmoregulatory disruption produced by low concentrations of Zn^{2+} (less than 1 ppm), 1 to 100 ppm Zn^{2+} significantly inhibited both gill Na,K-activated ATPase (*50*) and carbonic anhydrase (*51*). Since both enzymes are probably intimately involved in gill NaCl transport (Figs. 4 and 5), it is rather surprising that more definitive changes in blood NaCl concentrations have not been demonstrated in fishes exposed to environmental Zn^{2+}.

Heavy metals also have the potential to affect fish osmoregulation in sea water. Stagg and Shuttleworth (*52*) demonstrated that exposure of the marine flounder (*Platichthys flesus*) to 170 ppb Cu^{2+} for 42 days resulted in significant increases in blood Na^+ and Cl^- concentrations. In a subsequent study (*53*) they showed that acute exposure of perfused flounder gills to Cu^{2+} (in the perfusate) resulted in a concentration-dependent (1 to 100 μM = 65 to 6500 ppb) reduction of the electrical potential across this tissue. Since this electrical potential has been shown to be a direct measurement of the active ionic extrusion mechanisms of the perfused gill (*54*), it is clear that the heavy metal must be interfering with one of the transport steps outlined in Figure 4. Since the ouabain-sensitive component of the oxygen consumption of isolated gill tissue was reduced in the presence of Cu^{2+}, as was the Na,K-activated ATPase, the authors proposed that the site of action of the heavy metal was the enzyme itself. In support of this conclusion, Crespo and Karnaky (*55*) found that acute application of 2.6 ppm (4×10^{-5} M) Cu^{2+} or Zn^{2+} to the serosal side of the isolated, short-circuited opercular membrane (rich in chloride cells and accepted as a model for the seawater ionic extrusion systems of marine fishes) (*16*) of the killifish reduced the short-circuit current (I_{sc}) and electrical potential significantly, indicating a direct effect on active transport mechanisms. Concomitant measurements of Na,K-activated ATPase activities indicated substantial inhibition at Cu^{2+} or Zn^{2+} concentrations above 325 ppb (*55*). Importantly, the electrical resistance of the isolated tissue was unaffected by this treatment, and neither I_{sc} nor resistance was affected by addition of the heavy metals to the mucosal side of the epithelium, supporting the proposition that the effect was directly on the basolateral transport steps. Given the known effects of heavy metals on the ionic permeability of the branchial epithelium in freshwater fish species, and the leaky "tight junctions" in the gill epithelium of marine fishes (*28*), it is surprising to find that heavy metals did not also increase the passive ionic permeability of the marine fish gill. One might argue that this is due to the fact that the permeability is already high; however, studies have shown that its permeability is still sensitive to external Ca^{2+} concentrations (*56,57*), and therefore presumably to heavy metal effects. A more likely explanation is that the *in vitro* studies of Crespo and Karnaky (*55*) involved acute doses, while metal-induced changes in permeability take longer to be effected.

The foregoing indicates that heavy metals (only Cu^{2+}, Hg^{2+}, and Zn^{2+} have been described here) do produce toxic effects on the fish gill, both morphological and physiological. Physiological effects involve a reduction in blood ionic levels or acidosis, associated with increased ionic permeabilities (produced probably by displacement of Ca^{2+} from paracellular channels) and inhibition of enzymes (e.g., Na,K-activated ATPase and carbonic anhydrase) involved in transport of ions. Indeed, it is now clear that a variety of gill transport ATPases may be affected by heavy metals (*53*).

Aquatic Acidification

In light of current concerns over acid precipitation, there are surprisingly few published studies of the effects of low environmental pH on fish gill morphology. Daye and Garside (*58*) found separation of epithelial layers of the secondary lamellae on gills of brook trout (*Salvelinus fontinalis*) exposed for 7 days to pH 5.2. Yearling Sunapee trout (*Salvelinus alpinus oquassa*) Exposed to pH 4.5 for 192 hr showed slight swelling of secondary lamellae, which became more pronounced at pH 4.0 (lethal limit) (*59*). Leino and McCormick (*60*) demonstrated a significant increase in the number of chloride cells, an increase in the number of chloride cells on the secondary lamellae, and a striking increase in the number of chloride cells with apical pits [usually indicative of acclimation to increased salinities (*61,62*)] in the gills of fathead minnows (*Pimephales promelas*) exposed for 129 days to pH 5.0. Most recently, Chevalier et al. (*63*) found that brook trout (*Salvelinus fontinalis*) residing in acidified lakes (pH 5.5) in the Canadian Shield (Quebec) displayed "extensive epithelial damage, mainly separation of the epithelial layer from underlying tissue, deformation of secondary lamellae, and degeneration of chloride cells, which was accompanied by pronounced hyperplasia of undifferentiated epithelial cells in the primary lamellae." Importantly,

Bolis et al. (*64*) have recently shown that phospholipid composition of gill tissue from rainbow trout exposed to pH 4.0 to 4.5 for 4 to 5 days changes significantly and is accompanied by a substantial increase in the percentage composition of unsaturated fatty acids. The authors propose that such changes could affect the bilayer structure and fluidity of the gill epithelial cell membranes, and hence their osmotic and ionic permeabilities. Finally, mucus production on both the gills and skin is significantly enhanced by acid exposure (*58,59,65,66*); indeed, it is one of the best characterized responses to acid stress.

It is now abundantly clear that exposure of freshwater fishes to environmental pHs below approximately 5.0 is associated with a pronounced decline in blood Na^+ and Cl^- concentrations (*26,67–71*). The magnitude of the acid-induced perturbation of blood ionic levels is correlated with ambient Ca^{2+} concentrations (at least in rainbow trout), with fish in softer waters showing more significant falls in blood NaCl concentrations than those in higher Ca^{2+} waters (*72,73*). It is clear from Figure 4 that such a reduction could be the result of either decreased active uptake or increased diffusional loss of both ions. Studies on a variety of species (*26,71*) have demonstrated that both transport pathways are affected. The increased efflux may be, at least in the case of Na^+, partially due to changes in the electrical potential across the gill (*74*), but it is more likely that the generalized NaCl loss is secondary to increased leakiness of the branchial epithelium produced by acid titration of the Ca^{2+} on the gill membrane. This view is supported by the fact that low Ca^{2+} solutions exacerbate the effect of low external pH (see above), and low pH solutions significantly increase the rate of efflux of bound Ca^{2+} from gills isolated from brown trout, *Salmo trutta* (*75*) and increase the electrical conductance, as well as the Na^+, Cl^-, and mannitol effluxes, across the isolated opercular epithelium of *S. fontinalis* (*76*). The precise locus and mechanism(s) of inhibition of Na^+ and Cl^- uptake remain unknown. External H^+ could certainly interfere with an apical Na^+/H^+ exchanger directly, either by reversing the direction of exchange or by noncompetitive inhibition, thereby producing both the fall in blood Na^+ and blood pH which is normally seen (*69,73*). The mode of inhibition of Cl^- influx which is usually also seen (*69,73*) is less easy to explain. It could actually be a rapid response to the fall in blood pH, producing a compensatory decline in Cl^-/HCO_3^- exchange, or a generalized, noncompetitive inhibition of the exchanger by the high external acidity.

Acidification of fresh waters is usually associated with mobilization of aluminum from the substrate (*65*), and compounds of this heavy metal may produce some of the ionoregulatory symptoms of acid poisoning in salmonids, even at relatively higher pHs (such as 5.0) (*77*), where acid stress alone is rather slight (*65*). Under these conditions inhibition of transport enzymes may play a role, since Staurnes et al. (*77*) found that exposure of young specimens of Atlantic salmon, *Salmo salar* and *S. gairdneri,* to pH 5 and 200 ppb of $AlCl_3$ was correlated with significant reductions in the activities of both carbonic anhydrase and Na,K-activated ATPase. However, it appears that at least Na,K-activated ATPase can be inhibited by acid stress alone, since Saunders et al. (*78*) have shown that the branchial enzyme activity is more than halved by rearing *S. salar* parr in pH 4.2 to 4.7 fresh water. In addition, blood Na^+ and Cl^- concentrations were reduced in the individuals reared at low pH. Inhibition of the basolateral Na,K-activated ATPase could presumably disrupt the electrochemical gradients favoring Na^+/H^+ exchange, thereby inhibiting Na^+ influx and H^+ efflux. Inhibition of intracellular carbonic anhydrase would presumably interfere with both Na^+/H^+ and Cl^-/HCO_3^- exchange, thereby resulting in inhibition of both Na^+ and Cl^- uptake.

It is interesting to note that the acid-induced secretion of mucus by the fish gill may actually be adaptive, providing for a reduction in epithelial ionic permeability by binding to environmental Ca^{2+} (although the effects of fish mucus on ionic diffusion are controversial) (*79,80*), as well as binding to external Na^+ to provide more substrate for Na^+/H^+ exchange (*81*). However, if increased external H^+ concentrations have already titrated the polyanionic sites on the mucus, these effects may be minimal. In fact, Miller and MacKay (*82*) found that the ability of fish mucus to bind to copper was completely abolished at pH 3.5. Thus, the role of mucus secretion in acid stress remains unclear.

The foregoing indicates that low environmental pHs can affect fish osmoregulation by either increasing passive ionic loss (secondary to displacement of Ca^{2+} from the gill epithelium) or by direct effects on the apical ionic exchange systems or basolateral Na,K-activated ATPase (especially if aluminum species are present). In both cases, the most common symptom is a reduction of blood ionic concentrations, as well as reduced blood pH.

However, the actual cause of death in acid-exposed fishes may be much more complicated than merely ionoregulatory failure. Cardiovascular collapse may be the final cause, secondary to increased erythrocyte volume and shift of extracellular fluids into cells, both of which lead to increased hematocrit and blood viscosity. Concomitant catecholamine mobilization, producing increased cardiac output and vasoconstriction, along with the increased blood viscosity, lead to increased arterial blood pressure, and eventually circulatory failure may occur (*83,84*).

Organic Xenobiotics

Gross morphological anomalies are seen in the gill epithelium of yearling coho salmon (*Oncorhynchus kisutch*) exposed to the herbicides dinoseb (100 ppm for 114 hr), paraquat (100 ppm for 120 hr), and atrizine (15 ppm for 140 hr), including necrosis, desquamation, hypertropy and hyperplasia, and telangiectasia (*32*). Similar morphological changes characterize exposures of fishes to fumigants such as methyl bromide (*85*); pyrethroid insecticides such as permethrin (*86*); detergents

such as sodium lauryl sulfate (*87,88*); organochlorines such as DDT, endrin, and dieldrin; petroleum compounds such as phenol and naphthalene; organophosphates such as malathion and methylparathion; and carbamates such as sevin (*32,89*). Interestingly, injection of *Tilapia aurea* (adapted to either ⅔ sea water or fresh water) every 3 days with 10 mg/kg DDT for 30 days produced no abnormalities in the chloride cells (*90*).

One would expect that the usual morphological changes would be correlated with osmoregulatory malfunction, and this appears to be the case, despite rather depauperate literature. For example, Grant and Mehrle (*91*) demonstrated that exposure of goldfish (*Carassius auratus*) to high concentrations of endrin (430 μg/kg body weight) for 157 days did result in a slightly reduced blood Cl^- concentration; however, blood Na^+ levels were unchanged at this exposure and were actually increased at lower doses. Leadem et al. (*92*) did not find any significant change in either blood osmolality or Na^+ in *S. gairdneri* dosed orally with 2.75 mg/kg or 8.30 mg/kg DDT every 48 hr for 2 weeks (both doses were "environmentally realistic" according to previous studies of pesticide residues in fishes) (*93*). These findings are especially puzzling since Leadem et al. (*92*) did demonstrate a significant inhibition of gill Na,K-activated ATPase in their studies, and McBride and Richards (*94*) found that aldrin (180 ppb) inhibited Na^+ uptake by the isolated, perfused carp (*Cyprinus carpio*) gill. Moreover, Davis and Wedemeyer (*95*) showed that the organochlorines, DDT, dicofol, and endosulfan inhibited rainbow trout gill Na,K-activated ATPase by 60 to 100% *in vitro* at concentrations between 10^{-5} and 10^{-4} M. However, these *in vitro* concentrations were 1 to 2 orders of magnitude greater than acutely toxic levels *in vivo*. It therefore remains unclear whether these xenobiotics affect gill solute transport in freshwater fishes, despite apparently clear effects of gill Na,K-activated ATPase.

The picture seems to be somewhat clearer for marine species. Eisler and Edmunds (*96*) demonstrated hypernatremia in the marine, northern puffer (*Sphaeroides maculatus*) exposed to endrin, and Kinter et al. (*97*) found that exposure to the polychlorinated biphenyl Aroclor 1221 (75 ppm for 24 hr) produced significant increases in blood Na^+ concentrations in seawater-adapted killifish, while exposure to DDT (0.25 ppm for 6 hr or 0.075 ppm for 24 hr—both of which produced approximately 50% mortality) did not alter blood Na^+ concentrations. However, in companion experiments, exposure of the seawater-adapted eel (*Anguilla anguilla*) to 1 ppm DDT for 6 hr did result in a significant increase in blood Na^+ concentrations (*97*). In a subsequent study, Miller and Kinter (*98*) found that exposure of killifish to either 0.1 ppm or 1 ppm DDT for 4 to 24 hr resulted in an increase in blood Na^+ concentrations (however, only 0.1 ppm for 24 hr was statistically significant due to extreme variability) concomitant with some 30% mortality in the exposed fishes. Again, the site of action appears to be the transport enzyme Na,K-activated ATPase, since Janicki and Kinter (*99*) showed that the enzyme isolated from gill tissues from *P. americanus* was inhibited 54% when incubated with 50 ppm DDT and a subsequent study (*97*) found that 50 ppm DDT also inhibited gill Na,K-activated ATPase from *A. anguilla*.

Detergents represent another class of xenobiotic compounds that produce gill structural pathologies (see above). A single study showed that the diffusional inflow of water across perfused rainbow trout gills was enhanced when linear alkylate sulfonate (LAS) was added to the irrigate at a concentration of less than 100 ppm (exclusive of vascular effects) (*100*). However, other published studies have shown that a major site of action of detergents may be adrenoreceptors on vessels controlling the perfusion of various regions of the gill epithelium, rather than on cellular ionic transport *per se*. Of course, changes in the pattern of blood flow (e.g., increased lamellar perfusion or increased flow into the central venous sinus, which underlies the majority of the chloride cells, see above) could have profound secondary effects on gill transport. Bolis and Rankin (*101*) demonstrated that perfusion of isolated gills from various salmon species (*Onchorhynchus* sp.) with perfusate containing 0.6 to 3 ppm LAS produced a concentration-dependent vasodilation that was blocked by the β-adrenoceptor antagonist propranolol, and was therefore presumably via direct interaction with a β-adrenoceptor. In a subsequent study they extended these findings to both *S. trutta* and *A. anguilla* and also found that LAS (1 ppm in acclimation medium or 2×10^{-7} M in perfusate) actually interfered with norepinephrine-induced vasodilation (*102*). More recently, Stagg et al. (*103*) found that 2×10^{-8} M (6 ppb) sodium lauryl sulfate (SLS) in the perfusate reversibly (and noncompetitively) inhibited the vasodilatory action of noradrenaline on the perfused gills of *A. anguilla*. They proposed that the detergent interacted with the vascular membrane, thereby producing direct or indirect (reversible and noncompetitive) changes in the β-adrenoceptor that produced vasodilation and/or inhibition of the sensitivity of the system to norepinephrine. They also propose that such subtle hemodynamic effects may have severe deleterious effects on fish gas exchange and ion balance, at concentrations well below those known to produce visible changes in the gill morphology (18 ppm for 45 hr) (*87*).

Thus, it appears that a wide variety of organic xenobiotics are capable of affecting fish gill morphology, and that pesticides, such as DDT, are toxic because of inhibition of gill Na,K-activated ATPase. Surprisingly, the concomitant osmoregulatory effects are rather unclear. Detergents appear to produce severe hemodynamic effects on the gill vasculature, which could certainly affect osmoregulation indirectly, via alterations in perfusion of specific areas of the gill epithelium.

Use of the Fish Gill as a Model System for Cellular Modes of Action of Toxicants

It is obvious from the foregoing that the fish gill is morphologically and physiologically affected by a variety of environmental pollutants. The specific, subcellular sites of action on the fish gill have been proposed, but not proven, for many pollutants, and it is clear that toxicant interaction with various gill transport steps or perfusion patterns could have profound effects on the ability of fishes to osmoregulate in either fresh water or sea water. These potentially sensitive steps include: (a) carrier-based ionic exchanges such as apical Na^+/H^+, Na^+/NH_4^+, or Cl^-/HCO_3^- exchanges, basolateral Na^+/K^+ or Na^+/NH_4^+ exchanges, and basolateral NaCl + KCl cotransport; (b) transport enzymes such as basolateral Na,K-activated ATPase and intracellular carbonic anhydrase; (c) paracellular pathways (affected by mucus and/or external Ca^{2+}); and (d) vascular hormone receptors, such as those for catecholamines.

More important, in general terms, is the fact that these potentially sensitive transport and hemodynamic sites in the fish gill are common to many human tissues and organs that are known sites of action of a wide variety of environmental pollutants. In fact, various authors have proposed that membrane effects may account for many of the pathological responses to these substances in the "membrane theory of toxicity" (*104–106*). For example, because the human kidney receives 20 to 25% of the resting cardiac output, is capable of actively extracting and concentrating various blood solutes in renal cells and tubular lumina, and may concentrate filtered and secreted solutes in the distal tubules passively because of water reabsorption, relatively high concentrations of toxicants are presented to renal cells. In addition, renal tubular contents may become acidified in some segments, which may provide for interactions with toxic substances not taking place at the normal cellular pHs in other tissues. It is abundantly clear that the kidney is one of the major foci of the toxic effects of a wide variety of environmental pollutants (*107–109*). Since the renal epithelia contain high concentrations of transport enzymes such as Na,K-activated ATPase and carbonic anhydrase, ionic-permeable paracellular pathways, as well as the ionic carriers mediating Na^+/H^+ and Cl^-/HCO_3^- exchanges, and the NaCl + KCl cotransport system (*110*), it is clear that human renal pathophysiologies in response to environmental pollutants could be mediated via perturbations in transport pathways which can be modeled by the gill epithelium of fishes.

In addition, many of the transport pathways outlined in Figures 4 and 5 are present in the human gut and liver (*111–116*), organs which are sites of absorption and concentration of toxins as well as toxin-induced pathologies (*117–119*).

Finally, some pollutants (especially heavy metals) (*119*) affect the cardiovascular system by interfering with the vasoactivity of peripheral vessels, including coronary arteries (*120,121*). The fish gill vasculature has been shown to possess a variety of receptors for vasoactive substances: adrenergic and cholinergic (*9*), purinergic (*122*, and Evans, unpublished), and peptidergic, such as those sensitive to glucagon, vasoactive intestinal peptide, and somatostatin (*123*) and, most recently, those sensitive to atrial natriuretic factor (*124,125*). The fish gill vasculature is, in fact, the evolutionary precursor of the coronary vessels of mammals (*126*). Thus, hemodynamic studies of the vasculature of the fish gill may give us access into the stimulus-response coupling processes which may be underlying hemodynamic pathologies produced by environmental pollutants.

Summary

The teleost fish gill is covered by a complex epithelium whose function is controlled by perfusion through a rather intricate vascular system. In addition to being the site of gas exchange for these aquatic animals, the gill epithelium possesses transport steps which mediate active and passive movements of ions, counteracting dissipative movements down electrochemical gradients between the fish's blood and either fresh water or sea water. Finally, these same transport steps play major roles in acid-base regulation and excretion of unwanted nitrogen in the form of ammonia. It is clear that a variety of aquatic pollutants produce gross histopathologies of the gill epithelium, which are often associated with osmoregulatory, acid-base, or hemodynamic malfunction. It is proposed that such symptoms are secondary to toxin interaction with specific transport steps or membrane-bound receptors. Since similar pathways and receptors are common to a variety of human tissues, which are affected by environmental pollutants (e.g., kidney, intestine, liver, and blood vessel), the fish gill presents a model system which may be used to more carefully investigate general epithelial pathologies produced by toxic substances.

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