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Novel Route of Toxicant Exposure in an Ancient Extant Vertebrate: Nickel Uptake by Hagfish Skin and the Modifying Effects of Slime

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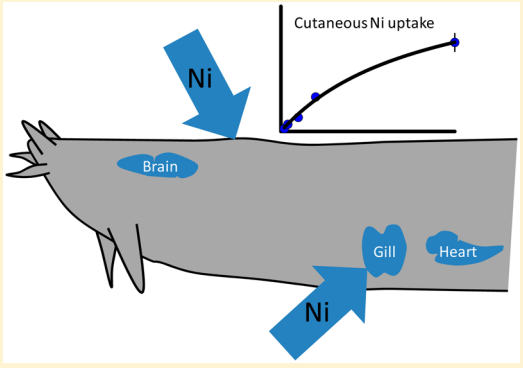
ABSTRACT: Utilizing an *in vitro* technique, the skin of Pacific hagfish (*Eptatretus stouti*) was shown to take up nickel from the water via a high affinity, low capacity transport pathway. Uptake was biphasic, with saturation occurring at low nickel exposure concentrations, superseded by linear, diffusive uptake at levels greater than 50 μM. *In vivo* exposures showed that nickel accumulated mainly in the gill, heart, and brain, representing a tissue distribution distinct from that found in teleosts. Slime on the epidermal surface was shown to significantly reduce the uptake of low concentrations (10 μM) of the metals zinc and nickel, but slime had no effect on organic nutrient (the amino acid L-alanine) absorption. At a higher metal exposure concentration (1 mM), slime was no longer protective, indicating saturation of metal-binding sites. This is the first study to show that metals can be taken up by the integument of hagfish. The ability of the skin to act as a transport epithelium may be of particular importance for a burrowing, benthic scavenger, such as hagfish, which are likely to be exposed to relatively enriched levels of metal toxicants through their habitat and lifestyle, and this may have consequences for human health through hagfish consumption.



INTRODUCTION

The Pacific hagfish (*Eptatretus stouti*) can absorb organic nutrients directly from the water by utilizing the gill and skin as transport surfaces.[1,2] This strategy, unique among vertebrates, is suggested to be an adaptation to a scavenging lifestyle, allowing hagfish to maximize nutritive uptake from a food source that is temporally and spatially dispersed. This phenomenon is facilitated by a feeding behavior that involves hagfish entering the internal cavities of dead and dying animals,[3] where its epithelial surfaces will be exposed to enriched levels of dissolved organic nutrients. More recent evidence suggests that this is not a phenomenon restricted to organic nutrients, as inorganic phosphorus has also been shown to be taken up across the skin and the gill of this species.[4]

The use of the skin as a transport epithelium is inconsistent with the major role of this tissue in the homeostasis of all other vertebrates. The primary function of the skin is to isolate an animal from its external environment, minimizing exchange and therefore facilitating processes such as osmotic regulation.[5] Hagfishes, however, are osmoconformers, and although they do regulate the levels of some ions and acid−base equivalents,[6] the barrier role of the skin is clearly less important than in other aquatic vertebrates. This allows the hagfish to use the skin as an exchange surface. However, the modifications that enhance nutrient uptake across hagfish skin might also increase the permeability of this surface to toxicants.[5]

Trace metals are an important class of toxicants in aquatic settings. At low levels, some trace metals have important nutritive roles (e.g., copper, zinc), and consequently, there are specific transporters that allow these to be absorbed via the diet or from the water via the gill.[7] Nonessential metals (e.g., cadmium, lead) may be absorbed across epithelia via mimicry of important ions.[8] Irrespective of the specific route, an enhanced environmental level of metal exposure can lead to higher accumulated levels of metals and, therefore, enhanced toxic effects. If the skin of hagfish is a transport pathway for trace metals, then this could increase their toxicological risk. Hagfish are likely to be particularly vulnerable to metal exposure given their habitats and lifestyles. They scavenge on carrion from higher trophic levels,[9] where metal levels may be elevated due to bioaccumulation and bioconcentration. Furthermore, as a benthic animal that may burrow into substrates,[3] hagfish may also be exposed to metals via sediments. As a consequence of these exposure routes, the skin could be a vector for significant metal accumulation in hagfish.

Nickel is one metal of environmental concern. Entering waters from anthropogenic processes such as incineration of

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fossil fuels and urban and industrial wastewaters,[10] nickel can reach levels where toxicity occurs.[11] Of relevance to benthic scavengers like hagfish, nickel has been shown to accumulate to high levels (95 μg/g wet weight) in tissues of whales exposed to crude oil,[12] while marine sediments near sewage outflow into the Pacific have recorded levels of nickel as high as 50 μg/g dry weight.[13] Depending on the exact nature of the sediment and the exposed organism, nickel has a high capacity for bioaccumulation,[14] suggesting that if the skin of hagfish is capable of absorption this could be a significant route of nickel exposure.

Little is known regarding the importance and toxicological significance of the skin as a route of exposure. However, it is well established that the route of exposure has an important influence on metal handling and the eventual toxicity of the accumulated metal in teleost fish. For example, in the fathead minnow, waterborne nickel exposure results in a significantly greater proportion of nickel accumulation in the heat-denatured protein subcellular fraction relative to dietary exposure,[15] a cellular location thought to contribute significantly toward toxicity as this is the fraction that contains potentially metal-sensitive enzymes.[16] Metal handling of hagfish is of toxicological relevance to hagfish themselves, but metals in hagfish tissues are also a potential source of human exposure,[17,18] so an understanding of how these animals handle environmental metal exposure is also of importance to food safety.

The current study investigated nickel transport across the integument of the Pacific hagfish (*E. stouti*). Concentration-dependent kinetics of nickel uptake were determined using an *in vitro* modified Ussing chamber technique, and the tissue distribution of absorbed nickel was examined via whole animal exposures. Hagfishes are renowned for their ability to excrete copious amounts of slime which has documented roles in predator defense[19] and as a deterrent toward other competitors for carrion.[20,21] Given the association of slime with feeding, the effects of slime on the epidermal transport of nickel, the nutritive metal zinc, and an amino acid (L-alanine) were also investigated.

MATERIALS AND METHODS

Animals. Pacific hagfish (*E. stouti*) were collected by baited trap from Barkley Sound (Vancouver Island, Canada) and held in covered 500 L plastic tanks receiving flow-through seawater, at 12 °C, for at least 2 weeks prior to experimentation. Hagfish were not fed during this period. All animal procedures were approved by the Bamfield Marine Sciences Centre Animal Care Committee.

***In Vitro* Skin Transport Assays.** Following euthanasia (3-aminobenzoic acid ethylester (MS222); 2 g L^{-1}), dorsal medial skin sections were removed and concentration-dependent transport kinetics of mucosal to serosal nickel uptake were assessed using a modified Ussing chamber method, as described previously.[2] "Cold" nickel (1, 2, 5, 10, 50, 100, 500, or 1000 μM) was added to the mucosal solution from a stock of $NiCl_2 \cdot 6H_2O$ with radiolabel (^{63}Ni; PerkinElmer; ~6.4 μC_i per 500 mL) added as a tracer. Temperature was maintained at 12 °C by conducting the exposure in a wet-table supplied with flowing seawater, pumped from Bamfield Inlet. Uptake assays were conducted for 2 h, and samples were processed for scintillation counting as described previously.[2] Two components of mucosal to serosal transport were assessed: transport of nickel from the water into the skin tissue and subsequent movement of nickel from the skin into the serosal compartment.

Role of Slime in Modifying Transport. To investigate the role of slime on transport, dorsal skin sections were investigated for their ability to transport one of three substrates in the absence or presence of slime covering the epidermal surface. Sections without slime were set up in an identical manner to those above, whereas sections with slime had the slime stretched across the mucosally exposed surface of the skin. The slime was freshly sourced from a single hagfish that had exuded into clean seawater. A small subsample (~3−5 g) of the exuded slime bolus was stretched by hand until it formed a thin layer, glassy in appearance. This was then gently pressed against the skin so that it adhered to the surface.

Three substrates were investigated. The transport of nickel was examined using ^{63}Ni as described above, at two nickel concentrations (10 and 1000 μM). The cutaneous uptake of the nutrient metal zinc was also examined at the same two concentrations (10 and 1000 μM), using radiolabeled ^{65}Zn as a tracer (~3 μC_i per 500 mL; PerkinElmer). Finally, the impact of slime on the transport of the amino acid L-alanine at 10 μM was also assessed (^{3}H-L-alanine; ~3 μC_i per 500 mL; PerkinElmer). Exposures and sample processing were conducted as previously described.[2]

***In Vivo* Nickel Exposure.** Hagfish were placed individually in lidded, aerated, 600 mL plastic exposure chambers with 500 mL of seawater and one of three nickel exposure concentrations (1, 10, or 1000 μM nickel as $NiCl_2 \cdot 6H_2O$). Each exposure chamber was spiked with ^{63}Ni (~6.4 μC_i), and the hagfish were left covered on a wet-table that maintained water temperature at ~12 °C, for 24 h.

After 24 h, hagfish were removed from the exposure chambers and placed in an anesthetic bath (2 g L^{-1} MS222) until euthanasia was complete (~5−10 min). A blood sample (~1 mL) was withdrawn from the caudal sinus and separated into red blood cells and plasma by centrifugation (3000*g*, 5 min). The hagfish was dissected via a longitudinal incision down the ventral surface, exposing the digestive tract and allowing bile to be withdrawn from the gallbladder via syringe. A section of the liver (~0.2−0.5 g) was removed, along with a section of gut (~2 cm, immediately distal to the bile duct), before dissection of the heart. Gills (the 3−4 most distal on one side) were also removed, along with a section of skin (~1 cm^2) and underlying muscle (~0.2−0.5 g) from the dorsal midsection of the animal. Finally, the brain was dissected. All tissues were then acid-digested in 3−5 volumes of 2 N HNO_3 (based on weight) and incubated for ~48 h at 65 °C. To each digest, 5 mL of scintillation fluor (UltimaGold) was added, and the radioactivity was measured via scintillation counting (LS6500). Quenching was accounted for by application of the external standards ratio method. Tissue accumulation was calculated as

$$\text{tissue accumulation (nmol/g)} = \frac{\text{cpm/SA}}{\text{mass}}$$

where cpm are the quench-corrected counts per minute, SA is the specific activity (cpm/nmol) of the exposure medium, and mass is tissue weight in g.

Data Analysis. Concentration-dependent uptake kinetics were modeled using SigmaPlot (ver. 11.2). Prior to statistical testing, data were assessed via tests of normality and equal variance, and if these tests were passed, parametric analysis was performed. For differences in tissue distribution following *in*

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vivo nickel exposure, this constituted a one-way ANOVA followed by a Tukey *posthoc* test, and for testing the effect of slime on substrate transport, a two-way ANOVA, followed by Tukey analysis, was used. Determination of proportional distribution of nickel in the skin and serosal compartments failed normality and equal variance tests and was therefore analyzed via nonparametric Kruskal–Wallis ANOVA, followed by a Dunn's *posthoc* test. All statistical analyses were performed in Sigmaplot.

RESULTS

***In Vitro* Skin Transport Assays.** Analysis of the concentration-dependent nickel uptake across the skin of the Pacific hagfish revealed two patterns of uptake (Figure 1). At

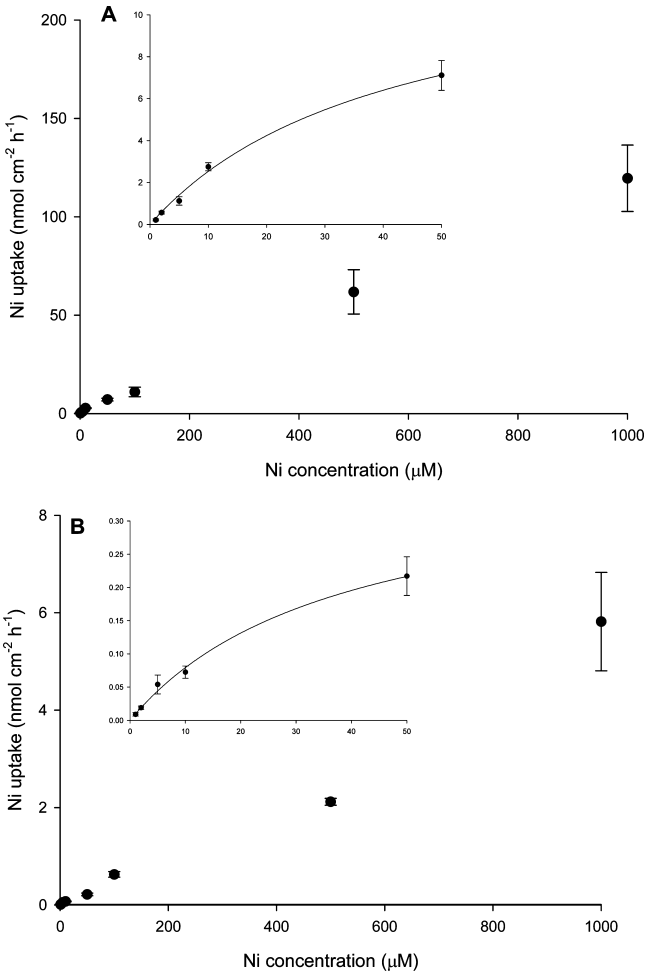

Figure 1. Concentration-dependent nickel uptake into the skin (A) or serosal (B) compartment of Pacific hagfish as determined *in vitro* across the full range of tested nickel levels or across the range where saturable uptake could be modeled (<50 μM; insets). Plotted points represent means ± SEM of 5–6 preparations. In the insets, curves were fitted using SigmaPlot (ver. 11.2).

low concentrations (<50 μM), nickel uptake was characterized as saturable into both the skin (Figure 1A inset) and serosal (Figure 1B inset) compartments. Calculation of Michaelis–Menten kinetic constants revealed an uptake affinity (nickel concentration required to give half the maximal uptake; K_m) of 42 ± 13 μM and a maximal rate of transport (J_{max}) of 13 ± 2 nmol cm^{-2} h^{-1} for saturable uptake into the skin. The K_m for saturable transport into the serosal compartment was similar to that for skin uptake at 38 ± 15 μM but with a considerable smaller J_{max} of 0.4 ± 0.1 nmol cm^{-2} h^{-1}. At exposure concentrations of nickel in excess of 50 μM, a linear transport component fitted the data with a higher r^2 value than other, nonlinear (i.e., sigmoidal or hyperbolic), curves (Figure 1A,B).

The comparatively lower maximal rate of transport into the serosal compartment was reflected in the proportional distribution of nickel between the two uptake compartments (Figure 2). Regardless of the tested nickel concentration, the

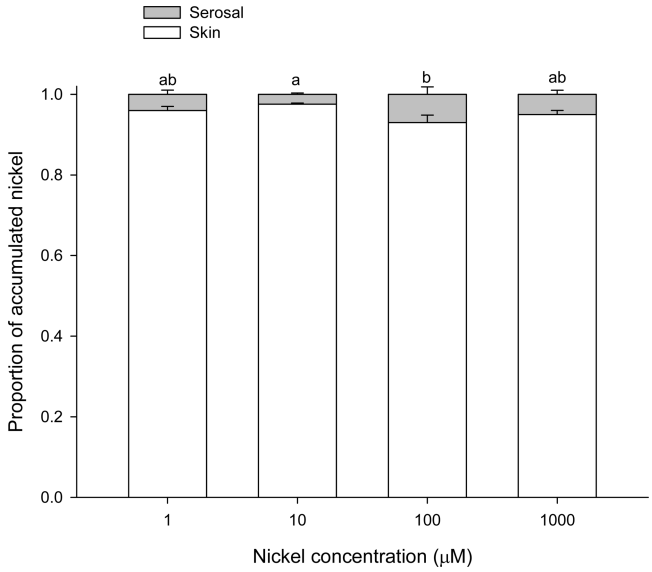

Figure 2. Proportional distribution of nickel accumulation in skin (white) or serosal (gray) compartments as determined *in vitro*. Plotted points represent means ± SEM of 5–6 preparations. Bars sharing letters are not significantly different ($p < 0.05$) as determined by Kruskal–Wallis ANOVA, followed by a posthoc Dunn's test.

vast majority of nickel accumulated in the skin (93–98%). Although there was some variation in the magnitude of the proportional skin accumulation with nickel level (e.g., 100 μM showed a significantly lower value than 10 μM), there was not a consistent pattern with exposure concentration.

***In Vivo* Nickel Transport.** A 24 h immersion period in nickel led to the accumulation of this metal in hagfish tissues. Across the three tested concentrations, approximately 2–6% of the total nickel that was present in the 500 mL exposure volume was taken up by the animal (data not shown).

At the lowest nickel exposure concentration (1 μM), the highest levels of nickel accumulated in the gill and the brain (Figure 3A). The level of nickel in these tissues was approximately 4-fold higher than that of the next most concentrated tissue, the heart. Concentrations of nickel were low (<0.05 nmol g^{-1}) in all other tissues. As nickel exposure level increased, tissue nickel concentrations did too with values in all tissues increasing approximately 10-fold with a 10-fold increase in nickel exposure concentration from 1 to 10 μM (Figure 3B). At this exposure level, the gill accumulated the highest concentration of nickel, followed by the brain and the heart. All other tissues exhibited nickel concentrations of less than 0.3 nmol g^{-1}. At the highest tested nickel exposure level (1000 μM; Figure 3C), levels of accumulation were greater than those at lower nickel exposures, with the highest levels found in the heart and brain (~150 nmol g^{-1}), respectively,

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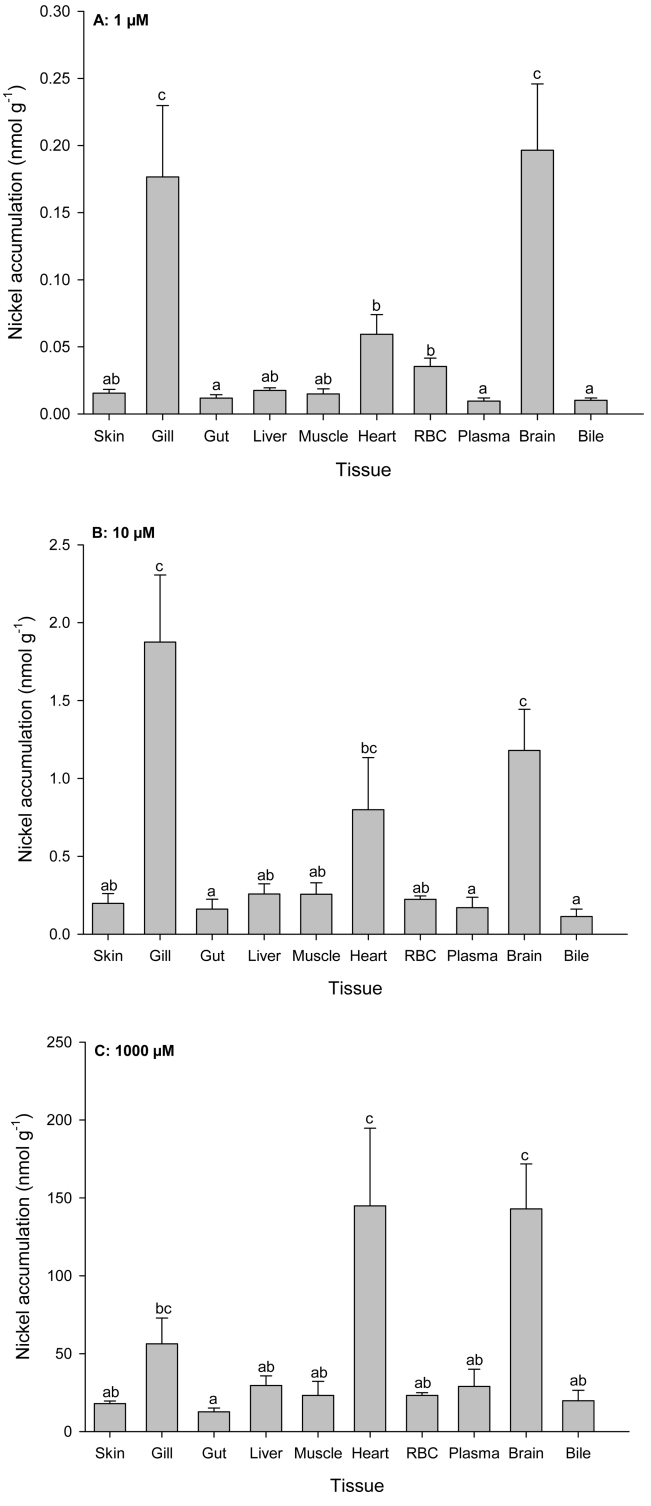

Figure 3. Accumulation of nickel in tissues of the Pacific hagfish following *in vivo* exposure to 1 μM (A), 10 μM (B), or 1000 μM (C) nickel for 24 h. Plotted points represent means ± SEM of 5−6 replicates. Bars sharing letters are not significantly different ($p < 0.05$) as determined by one-way ANOVA, followed by a posthoc Tukey test.

180- and 120-fold higher than corresponding levels at 10 μM. At this highest exposure level, the gill accumulated 56 nmol g^{-1} a value only 30-fold of that which accumulated at the nickel exposure level of 10 μM. All other tissues accumulated nickel to concentrations between 13 (gut) and 29 (plasma) nmol g^{-1}.

***In Vitro* Effect of Slime on Skin Transport.** Slime application to the mucosal surface of the skin was shown to have a significant substrate-dependent effect on epidermal transport. At low metal concentrations (10 μM), slime significantly reduced the transport of zinc (50% decrease) and nickel (72% decrease) across the skin (Figure 4A). There

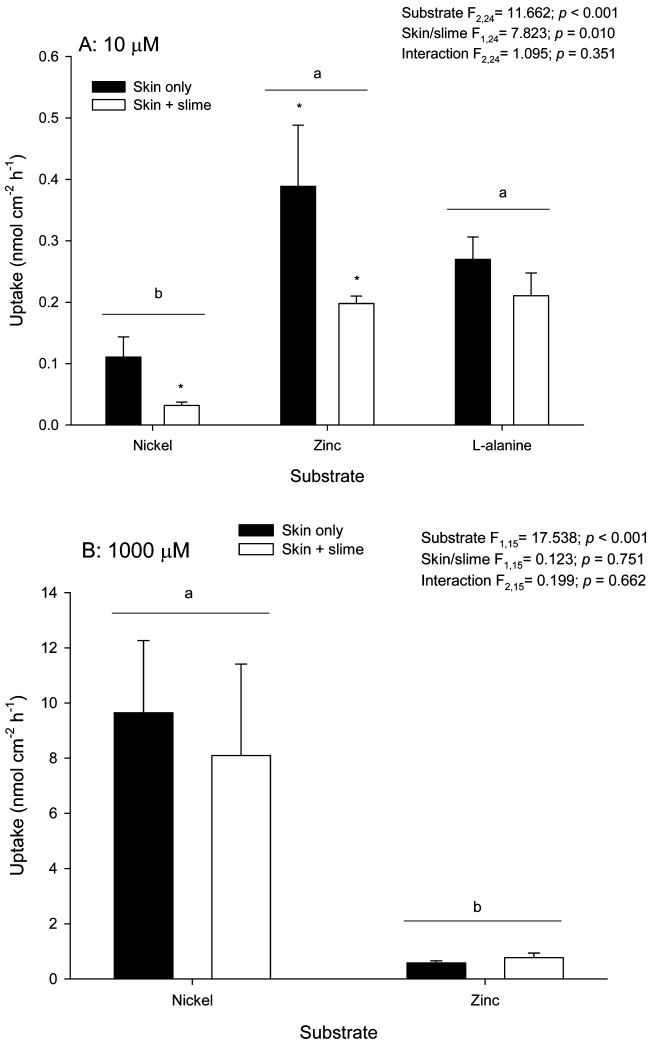

Figure 4. Effect of slime on skin substrate transport at concentrations of 10 μM (A) or 1000 μM (B) as determined *in vitro*. Plotted points represent means ± SEM of 4−5 replicates. Bar groupings (transport substrates) sharing letters are not significantly different ($p < 0.05$), while asterisks indicate differences in uptake owing to the presence of slime. Statistical significance was determined via two-way ANOVA, followed by a posthoc Tukey test.

was, however, no effect of slime on the uptake of the amino acid L-alanine. At substrate concentrations of 10 μM, nickel was transported less effectively than the other two tested substrates. At elevated zinc and nickel levels (1000 μM), the presence of slime did not impair transport, and it was notable that the uptake of nickel at this concentration was significantly greater (17-fold in the "without slime" preparations) than that of zinc (Figure 4B).

DISCUSSION

Cutaneous Nickel Uptake *in Vitro*. Nickel transport across the skin of hagfish was biphasic, with a saturable

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

component prevalent at low nickel concentrations (<50 μM) and a linear, diffusive component prominent at higher levels. This pattern of uptake was seen with respect to accumulation into the skin and also in terms of passage of nickel into the serosal medium. The affinity constant for skin accumulation and serosal uptake were very similar (42 and 38 μM, respectively); however, the rate of uptake differed substantially. The rate of nickel accumulation in the skin was 13 nmol cm^{-2} h^{-1}, greatly in excess of the rate of serosal nickel uptake (0.4 nmol cm^{-2} h^{-1}), suggesting that transport into the serosa was rate-limiting. This difference in transport rate constants was also reflected in data showing that the vast majority of "absorbed" nickel remained trapped in the skin (Figure 2). This buildup of nickel in the skin at the expense of the true uptake into the serosal medium suggests limitations in cellular-to-serosal transport. Either there are a relative small number of basolateral transporters available for nickel or nickel is being chelated by intracellular ligands as it traverses the cell. Metallothionein, the cysteine-rich metal-binding protein, has been shown to bind nickel in the gills of teleost fish where it is purported to reduce nickel toxicity,[22] while dietary nickel exposure induces metallothionein in lake whitefish.[23] There are, however, no studies that have examined metallothionein expression in the skin of hagfish, so the role of metallothionein in trapping nickel in the skin remains speculative.

The kinetic characteristics of nickel uptake across hagfish skin are similar to those that have been described for intestinal nickel uptake in rainbow trout.[24] At nickel concentrations less than 60 μM, a saturable process with a K_m of 42 μM and a J_{max} of 0.2 nmol cm^{-2} h^{-1} was described, similar to values for nickel transport into the serosal compartment in the current study (K_m = 38 μM; J_{max} = 0.4 nmol cm^{-2} h^{-1}). As for hagfish skin, at higher levels, intestinal nickel uptake was also diffusive.[24] Affinity constants for waterborne nickel uptake into the gills of freshwater fish are similar to those for hagfish skin and trout gut, with values between 18 and 87 μM being measured.[25] These values are also similar to those reported for nickel uptake in mammalian intestine (e.g., 38 μM)[26] and rainbow trout kidney (18 μM).[27] This hints that the mechanism of nickel transport across epithelia may be conserved, although much work remains to characterize the specific transport pathway involved.

The characteristics of cutaneous nickel uptake do, however, differ significantly from other substrates known to traverse the skin of the Pacific hagfish. As for nickel, the transport of glycine, L-alanine, and inorganic phosphate are all saturable. However, the affinity of uptake ranges from 262 μM (L-alanine)[2] to 930 μM (inorganic phosphate),[4] an order of magnitude higher than that of nickel. However, while nickel transport affinity is relatively high, its transport capacity is relatively low. The J_{max} for other tested substrates varies from ~10 nmol cm^{-2} h^{-1} for inorganic phosphate to 22 nmol cm^{-2} h^{-1} for L-alanine, substantially greater than that of nickel (0.4 nmol cm^{-2} h^{-1}). This pattern is reminiscent of the general pattern of uptake for amino acids versus metals in the gut of fish, where low affinity, high capacity pathways exist for the former and high affinity, low capacity pathways mediate uptake of the latter.[28] This is likely a reflection of the relative concentrations of the transport substrates in the feeding environment.

Tissue Distribution of Nickel. While *in vitro* data indicated that nickel is capable of being transported across the skin of hagfish, gill nickel accumulation following *in vivo* exposure (Figure 3A–C) suggests that branchial uptake of waterborne nickel may also contribute significantly toward nickel body burden. This is consistent with data from teleost fish that show the gills to be an important route of dissolved nickel uptake.[29] We were not able to determine the relative importance of the gill versus the skin in terms of nickel uptake in the current study, as the *in vivo* technique employed did not allow isolation of these two transport epithelia. Nevertheless, the higher levels of nickel in the gills relative to the skin indicate that branchial uptake is of significant importance. The low levels of skin accumulation *in vivo* may reflect mechanisms that limit accessibility of nickel to the epidermal surface, such as skin surface mucus that might be expected to have a similar effect on uptake as slime (see below). In natural exposure settings, however, the skin surface will be in direct contact with sediment nickel when hagfish burrow, a scenario that may increase the importance of the skin as an uptake surface relative to the gill, because the latter will likely be able to access only dissolved metal.

On the basis of regulated uptake, it has been suggested that nickel may be essential to fish.[30] In the current study, the distribution of nickel into three tissues (gill, heart, and brain) not generally considered to play a role in metal detoxification, is suggestive of specific handling. It remains to be determined whether this is a consequence of physicochemical similarity to key nutritive ions (see below) or whether nickel might have a specific biological role in these tissues.

The pouched gills of hagfish, like the gills of teleosts, are exposed directly to the environment and exhibit high surface areas and small diffusive distances.[31] These properties are ideal for transport, and thus, it was unsurprising that this tissue demonstrated relatively high nickel accumulation. Nickel has been previously shown to accumulate to levels as high as 400 nmol g^{-1} in the gills of round goby after a 48 h exposure to 11 μM nickel,[22] a value more than 7-fold greater than that of hagfish gills after a 24 h exposure to a similar waterborne concentration (10 μM) in the current study. Although the specific mechanism by which nickel is taken up across the gill of fish has yet to be established, evidence to date shows that enhanced levels of divalent cations such as calcium and magnesium reduce nickel toxicity, presumably mediated in part by competition at the gill uptake site.[32,33] It is therefore likely that the higher cation levels in seawater versus freshwater explain much of the difference in the magnitude of accumulation between hagfish and freshwater teleosts. Confirming this, significantly lower levels of nickel accumulation are found in the gills of seawater versus freshwater killifish exposed to the same concentrations of waterborne nickel.[34]

The accumulation of nickel in the heart and brain of Pacific hagfish was a more surprising finding. In teleost fish, nickel accumulates preferentially in plasma, bone, gills, and kidney, and accumulation is mostly a consequence of plasma trapping.[29,35] For example, there was no accumulation in the brain of fish exposed to waterborne nickel (e.g., goldfish;[36] round goby and rainbow trout[25]). Conversely, Alsop and colleagues,[37] showed that the zebrafish brain increased nickel accumulation by more than 40% following an 80 day dietary exposure to 116 μg g^{-1} nickel. Although zebrafish and hagfish both accumulate nickel in the brain, the magnitude of this response is quite different. The mean value of 143 nmol g^{-1} for the brain of hagfish exposed to waterborne nickel is 15-fold higher than the level of nickel measured in the brain of zebrafish exposed to dietborne nickel.[37] These differences in nickel tissue accumulation patterns could reflect pathways of

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

exposure or differences in handling between species. For example, nickel is known to be taken up via the olfactory epithelium into the brain of mammals,[38] and if similar pathways exist for lower vertebrates, then differences in neuroanatomy and nasal perfusion could contribute toward differences in brain nickel accumulation.

An explanation for the accumulation of nickel in the brain could be provided by the recent finding that hagfish have a remarkable capacity to reallocate glycine toward the brain under hypoxia.[1] Nickel is known to have a strong affinity for glycine,[39] and previous studies have shown a marked effect of nickel chelation on nickel tissue distribution. For example, a lipophilic nickel-binding ligand, sodium diethyldithiocarbamate, caused a 230-fold increase in brain nickel when included in a waterborne nickel exposure to brown trout.[40] Nickel is known to inhibit respiratory function in mammals and in fish,[41,42] which could lead to hypoxemia and thus a shift of glycine-chelated nickel to the brain.

A further possible explanation for the observed tissue distribution patterns of nickel in hagfish, which may apply to both heart and brain, is that accumulation relates to the presence of voltage-gated calcium channels. The ability of nickel to inhibit low voltage T-type calcium channels is well-described,[43] and it is intriguing that the heart and brain are the two tissues that most heavily express T-channels in vertebrates.[44] However, it should be noted that, on the basis of a relative insensitivity to nickel, recent studies suggest a limited role for T-channels in the heart of *E. stouti*.[45] Further studies are required to understand the mechanisms underlying the tissue-specific accumulation of nickel in hagfish.

Effect of Slime. Feeding hagfish are known to produce slime, thought to be a mechanism that discourages other scavengers from feeding on a spatially and temporally dispersed source.[3,20] The presence of hagfish slime on the epidermal surface was shown to impair the uptake of nickel at low concentrations (10 μM). However, at high levels (1000 μM; representing a concentration where diffusive uptake dominates; Figure 1), nickel uptake was not impaired. This is indicative of nickel binding to a chelating ligand in the slime which prevents uptake at low levels, but which becomes saturated at higher concentrations, leading to nickel "spillover" and transport by the skin. The nature of this metal-binding ligand remains unknown, but elevated levels of glycine (see above) have been measured in hagfish slime exudate supernatant.[46] It is important to note, however, that the highest nickel level tested here is unlikely to be encountered in nature.

This study was the first to examine zinc uptake by hagfish skin and showed that, at a concentration of 10 μM, zinc was taken up at a level equivalent in magnitude to the organic nutrient L-alanine (Figure 4A). This could reflect the essentiality of zinc, which is a known micronutrient in fish.[47] However, like nickel, zinc transport across the skin was impaired by slime. A similar effect on zinc uptake has been observed for intestinal mucus, which is secreted in response to zinc in the gut of fish and which is proposed to have an important role in modifying toxicity.[48,49] It was notable that, at the higher tested concentration (1000 μM), zinc uptake across the skin was significantly less than that for nickel, reversing the relative rates of uptake at 10 μM. This suggests that the pattern of zinc uptake across the skin may be distinct from that of nickel, a phenomenon that requires further investigation.

In contrast to its effects on nickel and zinc, slime did not impair uptake of L-alanine. In this regard, slime appears to act as a selective filter, allowing the passage of key nutrients, such as amino acids, across the skin, but impairing the transport of trace elements that may cause toxicity at relatively elevated levels. L-Alanine is neutrally charged at physiological pH, and it is likely this property that allows it to proceed past the moieties that would be likely to bind the positively charged metal ions. This indicates, however, that slime may not be an effective barrier against small, neutral organic toxicants to which hagfish may be exposed via burrowing into carrion or via contact with sediment.

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Notes

The authors declare no competing financial interest.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX