EDITORIAL FOCUS

Heads you gain, tails you lose

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THE FIELD OF TRANSPORT PHYSIOLOGY was fundamentally changed by the discovery of transport proteins that are specific for small neutral molecules such as urea, water, ammonia, and CO₂. The identification of first urea transporters (12), then aquaporins or water channels (1), and most recently ammonia transporters (8) (that may also facilitate the movement of CO₂), challenged the long-held view that membranes were relatively permeable to these small neutral molecules, which were thought to traverse a lipid bilayer directly, driven by a diffusion gradient. By contrast, the involvement of specific transport proteins suggests a far higher capacity for control over the movement of these molecules, as is the case for ions that move across membranes via protein transporters or channels. The ammonia-transporting Rhesus-associated (Rh) glycoproteins are particularly interesting in this context because they function in the transport of either or both ammonia gas NH₃, a small neutral molecule, and the ammonium ion NH₄⁺, the two forms of ammonia that are in equilibrium with each other (“ammonia” here is used to denote total ammonia, i.e., NH₃ + NH₄⁺). The mammalian Rh glycoproteins belong to solute transporter family SLC42 and comprise the red blood cell-specific Rhag, as well as Rhbg and Rhcg, which exhibit broader tissue distributions including prominent expression in the kidney (9). In the kidney, Rhbg and Rhcg play a vital role in ammonia secretion and hence in the maintenance of acid-base balance, which in mammals is largely dependent on renal bicarbonate ion reabsorption and net acid excretion. Net acid excretion, in turn, reflects renal ammonia metabolism, the balance between ammoniagenesis and ammonia excretion at the kidney (9).

The primary role of the kidney in maintaining acid-base balance in mammals is in fish played by the gill (6). Moreover, the multifunctional gill also serves as a key site of ionic and osmotic regulation and nitrogen waste excretion, with most fish (the sharks, skates, and rays being notable exceptions) excreting nitrogen waste predominantly as ammonia. With the identification of Rh glycoproteins in fish (7), the scene was set for a paradigm shift in our understanding of nitrogen excretion in fish and its integration with ion transport pathways and acid-base balance (10), a shift in which epithelial transport of ammonia is viewed as a regulated process rather being dependent on passive diffusion. In the ensuing years, increasing research effort has focused on the branchial expression and regulation of Rh glycoproteins in a range of fish species and in response to changes in environmental conditions. But what happens to ammonia excretion when gill function is compromised, for example in species that emerge from water onto land with a resultant collapse of gill surface area? Study of such species has revealed interesting and often unexpected solutions to the problem of ammonia excretion in terrestrial conditions, including induction of urea production and excretion of ammonia across the skin, with localization of Rh glycoproteins to the skin (11). Hagfish present another situation in which ammonia excretion at the gill may be compromised. These jawless fish are perhaps best known for their ability to produce copious quantities of slime as an antipredator strategy, but they are also well known as scavengers that burrow into carrion on the ocean floor. Clifford et al. (4) recognized the difficulties of this lifestyle with respect to branchial ammonia excretion; inserting the head and branchial region into decomposing tissue high in ammonia is likely to result in ammonia accumulation via gill Rh glycoproteins. They hypothesized that hagfish could compensate for such ammonia accumulation by favoring cutaneous ammonia excretion across the posterior region of the animal, which often remains exposed to seawater while the animal feeds.

In support of this hypothesis, Clifford and colleagues (4) reported greater abundance of Rhcg in the skin from the middle and posterior regions of Pacific hagfish (Eptatretus stoutii) than from the anterior region. Accompanying these regional differences in expression of ammonia transport proteins were differences in the ammonia flux measured across excised pieces of skin; ammonia excretion measured in vitro increased linearly for skin sections sampled from the anterior to the posterior region of the animal. These data indicate that the capacity for cutaneous ammonia excretion increases along the length of the animal, from snout to tail. In addition, ammonia excretion across excised skin was increased by prior exposure of the animal to high ambient ammonia levels, suggesting regulation of the cutaneous ammonia excretion pathway in response to conditions where it might be favored. Unfortunately, the authors were unable to collect data on Rhcg protein expression in skin samples from animals exposed to high ammonia. At the whole animal level, a divided chamber approach was used to collect independent measurements of ammonia flux for the anterior skin and gills versus the posterior skin. These measurements revealed that while overall ammonia excretion was dominated by excretion via the gills (and anterior skin), the contribution of posterior skin to overall ammonia excretion was disproportionately increased in animals that had been exposed to high ambient ammonia levels. Collectively, these data suggest a scenario in which loss of ammonia gained across the anterior end of the animal when it burrows into decaying carrion is favored by posterior excretion of ammonia via the skin, with the additional possibility that cutaneous ammonia

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transport capacity is enhanced by exposure to high ammonia conditions.

Solidifying this hypothesis will require additional study, in particular, to quantify branchial versus cutaneous surface area; a limitation of the study by Clifford and colleagues (4) was the inability to partition ammonia flux into branchial and cutaneous components in a quantitative fashion. Assessment of cutaneous and branchial Rh glycoprotein expression in response to feeding and ambient ammonia exposure both individually and in combination also should be explored. Enhanced cutaneous ammonia transport capacity in response to high ambient ammonia levels could be a disadvantage if the entire surface of the hagfish (rather than just the anterior end) is surrounded by carrion, a situation that requires consideration. Hagfish would presumably benefit from mechanisms that limit ammonia gain when the anterior end of the animal is embedded in decaying carrion, and some indications support this possibility (3, 4). For example, plasma ammonia concentrations stabilize over time in hagfish exposed to high ambient ammonia levels (3) and exposure of the anterior end of the animal to high ammonia levels has little impact on plasma ammonia relative to exposing the posterior end of the animal (4). These observations, coupled with reports of ammonia excretion occurring against an inwardly directed ammonia gradient (3), suggest the existence of an active ammonia excretion mechanism that has yet to be identified. Finally, unanswered questions remain with respect to the transport capacity and/or permeability of hagfish skin for gas transfer. The data of Clifford and colleagues (4) suggest that a small but physiologically relevant component of ammonia excretion occurs across the skin of Pacific hagfish. At the same time, however, the skin in this species does not appear to play a significant role in oxygen uptake, likely owing to its thickness (70–100 μm) and the potential for the formation of boundary layers depleted in oxygen (5). Localization of Rhcg to the basal portion of the epidermis (4) may facilitate ammonia movement out of the blood into the epidermis, but does not address its movement from there to the surrounding water. Measurements of cutaneous CO2 excretion, which would also provide insight into the practicality of the skin as a gas exchange site, appear to be lacking for hagfish. In short, the observations of Clifford and colleagues (4) that hagfish may gain ammonia across the gills when the head is buried in carrion only to lose it across the skin at the tail end of the animal are intriguing not least because of the many questions that remain to be addressed.

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