Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

Classics

THE HUMBLE BEGINNINGS OF THE STUDY OF NITROGEN EXCRETION IN FISH

Tamara Rodela discusses Homer William Smith's classic paper 'The excretion of ammonia and urea by the gills of fish', published in the *Journal of Biological Chemistry* in 1929.

Homer William Smith (1895–1962) was best known for his work as a renal physiologist. His extensive research on the mammalian kidney greatly enhanced our understanding of the functional properties of the glomeruli, tubules and renal vascular bed, and his examination and comprehension of functional alterations in renal disease garnered him a great deal of respect from clinical physicians and physiologists alike (Pitts, 1967). While the majority of his time was focused on clinical studies in mammals, Smith spent his summers at the Mount Desert Island Biological Laboratory (MDIBL) in Salisbury Cove (ME, USA) 'dabbling' in comparative physiology (Evans, 2008). Dabbling should be a term used lightly as Smith's early experiments in comparative physiology built the foundation for our current knowledge of nitrogen excretion in fish. In his seminal 1929 paper in the *Journal of Biological Chemistry*, Smith became the first researcher to report that the gills are the major site of nitrogen excretion in freshwater fish (Smith, 1929).

In vertebrates, amino acids cannot be stored in the tissues without detrimental effects to the physiology of an animal. Excess amino acids that are not shuttled towards growth and maintenance are catabolized to ammonia (the sum of NH_3 and NH_4^+) (reviewed by Wilkie, 2002), a potentially toxic nitrogenous product when concentrated in the blood and body tissues of vertebrates. Therefore, ammonia must be either eliminated or modified (to urea or uric acid) to avoid adverse effects on normal physiological function and homeostasis. Although the precise pathologies of ammonia toxicity were not elucidated until over 50 years later (reviewed by Ip et al., 2001), in the 1920s Smith and his colleagues were aware that mammals excreted large amounts of nitrogenous compounds (urea >> ammonia > uric acid > creatinine > creatine) in their urine, leading to the idea that the kidneys were the 'master builders' of the internal environment. In contrast, the limited literature available on fish at the time indicated that teleosts excreted only small amounts of nitrogenous compounds in their urine (Denis, 1913; Grollman, 1929). Smith was intrigued that the main urinary constituent in these studies was reportedly creatine and all other nitrogenous products were unusually low. Therefore, Smith hypothesized that the most probable route for the loss of nitrogenous waste (i.e. urea and ammonia) in fish would be *via* the gills (Smith, 1929).

To test his hypothesis, Smith pioneered a simple yet innovative experimental setup that partitioned the anterior end of the fish from the posterior end. These 'classic' divided chamber experiments enabled the discrete measurement of compounds excreted from branchial routes and the simultaneous collection of urinary excretions. Common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) were individually placed in a box divided into two chambers by two superimposed layers of rubber dam. Sufficient water was put into the chamber to immerse the fish and both chambers were aerated to ensure oxygen levels remained normoxic. The collection of urine was accomplished by the implantation of a glass retention catheter sewn into the urinary papilla and the catheter drained into a light rubber bag: this technique allowed a continuous collection of urine with minimal disturbance to the fish. Following a chemical analysis of the collected samples, Smith reported that the amount of ammonia excreted from the gills, as measured by the accumulation in the anterior chamber, was approximately 6–10 times higher than the urinary output during the same time interval. In addition, a more detailed examination of branchial and urinary excretions by Smith revealed that ammonia accounted for the greatest fraction (55–63%) of nitrogenous waste, followed by urea (6–7%), creatine (4–6%) and amines (3–10%): a significantly different composition from nitrogenous excretion in mammals (where urea >> ammonia > uric acid > creatinine > creatine). Collectively, these findings were the first not only to unequivocally identify the gills as the primary site of nitrogen excretion in fish but also to demonstrate the differential patterns of nitrogenous waste handling between aquatic and terrestrial animals.

In the decades following the publication of Smith's paper in the *Journal of Biological Chemistry*, other studies confirmed Smith's basic conclusions on nitrogen excretion in teleosts (Fromm, 1963; Goldstein and Forster, 1961; Wood, 1958). In more recent experiments, Smith's classic divided chamber setup was rarely employed, with researchers opting to minimize stress in individual fish by examining nitrogen excretion in individual fish confined to small volumes of water or in more realistic 'bulk in-tank' experiments where groups of fish are kept in their normal feeding and holding tanks. Both these approaches were useful for generating a large amount of excellent data during the 1970s and 1980s. The results from these studies revealed that ammonia excretion was variable and could be influenced by the fasting *versus* fed state of the animal, activity levels, and numerous environmental conditions (e.g. ambient pH, temperature, ambient ammonia, levels of CO_2) (for reviews, see Ip and Chew, 2010; Randall and Wright, 1987; Wood, 2001). Moreover, researchers began to discover notable exceptions to the observation that teleosts excrete the majority of their waste in the form of ammonia. The Lake Magadi tilapia (*Oreochromis alcalicus graham*), Gulf toadfish (*Opsanus beta*), Singhi catfish (*Heteropneustes fossilis*) and slender lungfish (*Protopterus dolloi*) have all adopted unconventional patterns of urea excretion in response to various environmental constraints that typically limit ammonia excretion (reviewed by Ip and Chew, 2010; McDonald et al., 2012; McDonald et al., 2007).

Less than two decades ago, the simultaneous discoveries and subsequent characterization of Rhesus (Rh) glycoproteins and facilitated urea transporters (UT) in mammals led to a renaissance in the study of ammonia and urea excretion, respectively, in fish. A detailed discussion on these ammonia and urea transport paradigms in fish is beyond the scope of this article; however, the updated piscine model on ammonia excretion has been thoroughly and elegantly summarized by Wright and Wood, establishing a role for Rh glycoproteins as NH_3 conduits in freshwater fish (Wright and Wood, 2009; Wright and Wood, 2012). McDonald and colleagues have compiled comprehensive reviews on the mechanisms of urea transport in fish gills and summarized the compelling data that demonstrate that UTs play a crucial role in the removal of urea in both ureotelic (urea-excreting) and ammoniotelic (ammonia-excreting) teleosts (see McDonald et al., 2007; McDonald et al., 2012). From this literature, it is clear there has been a shift from quantitative studies on nitrogen excretion to in-depth characterization and analyses of the regulation of transport mechanisms controlling both branchial ammonia and urea excretion in fish.

In the 80 years since the initial publication of Smith's paper entitled 'The excretion of ammonia and urea by the gills of fish', over 400 scientific articles have been written on the topic of nitrogen excretion in fish. It is humbling to consider that such a simple experiment by a man that 'dabbled' in fish research had such a profound effect on the establishment of an entire branch of comparative physiology. In the many biographical memoirs written since Smith's death in 1967, colleagues and fellow researchers have sung Smith's praise for his contribution to mammalian renal physiology (see Berliner, 1993; Pitts, 1967); however, it is also clear that comparative physiologists likewise owe a great debt of gratitude toward Smith's scientific curiosity and genius.

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