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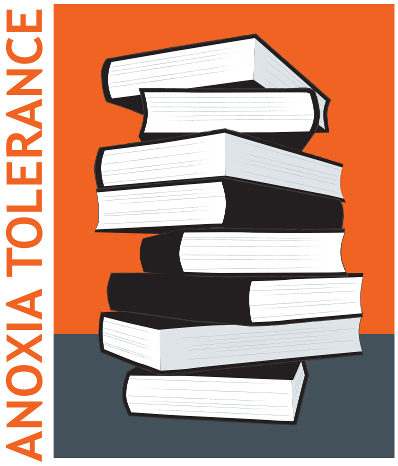
CLASSICS

Revealing how goldfish defy anoxia



Jean-Michel Weber discusses the impact of Eric Shoubridge and Peter Hochachka's classic paper 'Ethanol: novel end product of vertebrate anaerobic metabolism', published in *Science* in 1980.

Just a few rare vertebrates – three carp relatives and two species of turtles – show the astonishing capacity to survive without any oxygen for days, while all others die within minutes (Bickler and Buck, 2007; Nilsson, 2010). By the end of the 1970s, the ability of these anaerobic champions had already been known for decades, but the physiological mechanisms underlying their survival were not understood. Unexplainably, these remarkable fish had been shown to sustain respiratory quotients (the ratio of CO_2 produced to O_2 consumed) above 2 during hypoxia and to continue generating CO_2 under anoxic conditions (see van Waarde et al., 1993). In a landmark paper, Eric Shoubridge and Peter Hochachka (1980) reported their discovery that the goldfish, one of these exceptional carp species, uses a truly unexpected strategy to cope with this metabolic challenge. It tolerates a prolonged, normally lethal, lack of oxygen by using large glycogen stores to generate ethanol as a by-product of energy metabolism. More surprisingly, its most vulnerable tissues, such as the brain and the heart, simply use conventional glycolysis to provide ATP: the only form of chemical energy that cells can use. Lactate is also produced by these tissues in the process, but the end-product is then transported to muscles, where its ultimate catabolism to ethanol and CO_2 takes place exclusively. To prevent intoxication, the highly diffusible ethanol is eventually moved to the gills for excretion.

In the introduction to the paper, the authors pointed out a study published more than 20 years earlier by Ladd Prosser and his colleagues that came close to elucidating the same secret (Prosser et al., 1957). This inspiring article had reported that anoxic goldfish release an unknown product – different from lactate – but the mysterious metabolite remained unidentified. Interestingly, psychologists studying the effects of alcohol on behaviour had been using the goldfish as a model vertebrate for years before this discovery. They had noticed that it equilibrates ethanol (and bourbon!) particularly quickly between the external medium and the central nervous system (Ryback, 1969). This characteristic had conveniently eliminated the need for invasive measurements of alcohol levels directly within the brain of the tested animals.

For his PhD research, Shoubridge had subjected goldfish to complete anoxia by treating them with carbon monoxide before placing them in N_2-saturated water for 12 h. Remarkably, this simple experiment was sufficient to provide the most crucial information for the discovery. Hochachka recounted that after sniffing the anoxic water of the tank Shoubridge had said: 'there is booze in here' (Somero and Suarez, 2005). In a scientific era in which incredibly sophisticated tools are usually needed for success, it is particularly refreshing to realize that having a nose for innovation was as essential in 1980 as it will ever be. Shoubridge then analyzed the bodies of the fish and their tank water, which allowed him to determine that, in addition to CO_2, anoxic goldfish generate two other end-products – ethanol and lactate – and that most of the ethanol was released in the environment. In another experiment, he measured lactate-to-ethanol ratios, but this time in individual tissues. The results suggested that lactate was both produced and metabolized by the fish and that its ultimate fate was excretion as ethanol. To test this hypothesis, Shoubridge and Hochachka injected ^{14}C-labeled lactate in anoxic goldfish and were able to recover one main radiolabeled compound in the tank water. Then, after concentrating it, they used gas chromatography to identify unequivocally that the released metabolite was ethanol. To complete the study, they searched for the presence of alcohol dehydrogenase (ADH), the key enzyme necessary for ethanol synthesis during fermentation, and found high ADH activities in skeletal muscle, but almost none in all of the other tissues. Together, these complementary approaches led them to conclude that the goldfish relies on a network of two interacting pathways to sustain ATP supply in the absence of oxygen: (1) classic glycolysis in tissues lacking ADH, such as the brain and the heart, and (2) alcoholic fermentation in muscle.

Before this important contribution, cellular synthesis of alcohol with release to the environment was largely considered the prerogative of microorganisms, some plants and various invertebrates (Lutz and Storey, 1997; van Waarde, 1991). The work of Shoubridge and Hochachka demonstrated that the goldfish is able to match the biochemical capacity of yeast, and it characterized the popular aquarium pet as the first vertebrate known to do so. It also provided a much needed explanation for the miraculous spring revival of household goldfish inadvertently left to overwinter in the backyard pond…where they had turned anoxic under thick ice!

More importantly, this study prompted physiologists to investigate all aspects of the cellular and molecular mechanisms that allow the brain and the heart of a vertebrate to tolerate prolonged anoxia. In the tradition of the Krogh principle, researchers started to ask whether these amazing fish and turtles could provide useful medical lessons to deal with stroke and myocardial infarction, or to improve the outcome of organ

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transplants. Because it tolerates anoxia better than goldfish, the crucian carp has been used as a preferred model to identify the biochemical adaptations that are necessary for ethanol production (e.g. see Nilsson, 1988). More recently, transcriptomics and metabolomics have provided exciting new insights into vertebrate adaptations for anoxia tolerance (Krivoruchko and Storey, 2015; Lardon et al., 2013). In particular, the key roles played by nitric oxide and nitrite in orchestrating all the responses needed to sustain vertebrate life without O_2 have been characterized (Fago and Jensen, 2015). Finally, global warming and eutrophication are causing an unprecedented expansion of oxygen minimum zones in the world oceans (Diaz and Rosenberg, 2008; Seibel, 2011). A recent study of ADH activity in the muscles of mesopelagic fish (Torres et al., 2012) suggests that many more species will have to rely on the now classic metabolic strategy of the goldfish to avoid extinction in a warmer future.

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