AMMONIA, AMMONIUM, NITRITE, AND NITRATE: Causes of disease in axolotls and prevention of their toxic effects

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When maintaining aquatic animals, four nitrogen compounds can be a source of disease. These four compounds are ammonia, ammonium, nitrite and nitrate. In aquatic systems at least one of these four will normally be found. When any of these compounds is present at a low concentration, they have little or no effect on the animals. However, if their concentration becomes too high, these nitrogen compounds can cause adverse reactions.

Ammonia and nitrite are the two most toxic and are normally converted by bacteria to a much less toxic product, nitrate, in aquatic systems. Ammonium, a relatively non-toxic product, is in a pH dependent equilibrium with ammonia, such that the amount of ammonia formed increases as the pH becomes more alkaline above 6.0. When the pH of the water is above 6.5, ammonia is oxidized by *Nitrosomas* to nitrite which is then oxidized by *Nitrobacter* to nitrate. These bacteria can only function when there is sufficient oxygen and when the oxygen level falls, other bacteria can convert nitrate back to nitrite. Algae and aquatic plants can also absorb ammonia and nitrate from the water although the amount absorbed is not large. Ammonia (or ammonium) is formed by decaying organic material and as a waste product from the animals (1,2). Another source of these compounds is apparently pure water supplies where these compounds may be present as pollutants (3).

When excess amounts of ammonia, ammonium, nitrite, or nitrate build up in closed systems, any of these can cause toxic reactions. Ammonia acts as a caustic substance, attacking the skin and gills directly, and as a growth inhibitor. The maximum acceptable level of ammonia is less then 0.1 mg/liter (ppm) for fishes while 0.6 mg/liter can be toxic (2). The mechanism of nitrite toxicity is the oxidation of hemoglobin to the form methemoglobin which cannot bind oxygen. Nitrite is transported across the gills and is concentrated in the blood against a gradient. The maximum acceptable level of nitrite is 0.1 mg/liter while the 96 hr LC-50 (lethal concentration where 50% die) for the larvae of *Ambystoma texanum* is 1.09 mg/liter in water of low chloride and calcium contents. This compares with a range in fishes of 0.7 mg/liter for trout to 27.0 mg/liter for channel catfish (4,5). Although axolotls were not tested, they would probably be at least as sensitive as *A. texanum* since *A. texanum* inhabit warm ponds of stagnant, low oxygen water (4,5). Although ammonium and nitrate are not very toxic, they can cause problems by ionic imbalance similar to the concentration of salt by evaporation. Also if the pH rises or the oxygen level falls, these could be converted into more toxic forms. The maximum acceptable concentration for ammonium is at least 40 mg/liter (2) and for nitrate is at least 100 mg/liter for fishes (1).

These symptoms of toxicity have been reported in other aquatic animals and are similar to those reported in axolotls. They include: increased skin mucus, destruction of skin and gills, cloudy or protruding eyes, rapid gulping of air, internal hemorrahaging, reduced growth rate and stunting (1,2). Secondary infections by bacteria and fungus will often follow after the initial toxic effects (2). Treatment by excess salt or 100% Holtfreter's solution, which often gives relief, is consistent with toxicity. When symptoms such as these appear, the water should be tested for ammonia, nitrite, and nitrate. This can be done quite inexpensively with test kits available from most pet shops.

The manner in which the axolotls are maintained determines which forms will probably be present. Axolotls maintained in pans or bowls which are frequently scrubbed will be subjected to excesses of ammonium and ammonia. In a test in our lab, 10 three-month-old axolotls were kept in pans containing 4 liters of 20% Steinberg's solution at pH 7.2, which were scrubbed daily. The ammonia level far exceeded the acceptable level in less than 12 hours. The ammonium level was well below the maximum while nitrite and nitrate were not detectable. The bacteria necessary to convert the ammonia could not establish in sufficient numbers to be effective. When the animals are maintained in aquariums and bowls which are not scrubbed frequently, any form can be in excess. When a system is newly set up or cleaned, ammonia will accumulate; then as the *Nitrosomas* populate, nitrite will build up. Once the *Nitrobacter* establish, nitrate will accumulate. A test in our lab showed that it can take as much as three weeks for this cycle to establish when the aquarium has been completely cleaned and restocked with animals.

These toxic effects can be decreased or eliminated by a number of methods. First, lowering the pH will reduce the amount of ammonia present. At pH 6.0 no ammonia is present, at pH 7.0 1%, and at pH 8.0 5% (1,2). Second, by increasing the amounts of monovalent ions, competition will apparently prevent most of the nitrites from entering the gills. Tests on *A. texanum* showed that when chlorides were present at level of 300 mg/liter, no mortality was found at 10 times the LC-50 (5). Ca_2^+ showed a similar result in trout (4). It should be noted that the chloride concentration of 25% Holtfreter's solution is 500 mg/liter and 650 mg/liter for 20% Steinberg's solution. Third, maintaining a high level of oxygen will allow the bacteria to convert the toxic forms to nitrate. An interruption of as little as one hour can cause a lethal rise in ammonia and nitrite (2). Fourth, disturb the filter media as little as possible, since the nitrifying bacteria are slow to establish. When cleaning filters or aquaria, reduce the amount of food and "seed" the clean filter with bacteria if possible. An alternate method would be to use two filters, one of which could be a "sponge" filter. These sponge filters support an excellent bacteria flora, are cleaned by rinsing, are inexpensive, and can be used in both small bowls and large tanks (1,2). Fifth, exercise caution when treating with antibiotics since many of these will destroy the nitrifying bacteria (2). When using antibiotics, it is usually best to clean the aquarium, increase the salt concentration, and/or lower the pH. Sixth, change part of the aquarium water regularly to prevent a buildup of ammonium and nitrate. Changing half the water weekly is best in crowded aquariums (1,2,3,6). However, use caution if the new water has a higher pH than the old; this can cause the less toxic ammonium present to be converted into ammonia (1). Finally, the water supply should be "conditioned". This can be done by holding the water in tanks (trash cans, fiberglass pools, etc.), aerating it, and exposing it to light for a day or more. Water treated this way will not only be chlorine free but will also support an algal and nitrifying bacteria flora and have a bacteria-bacteriophage equilibrium. In this way the ammonia will be consumed and the necessary bacteria to establish a good flora will be present (6).

Good maintenance techniques can prevent toxic effects of ammonia, ammonium, nitrite, and nitrate. If the animals are stressed by raw water, overcrowding, or disease treatment, monitor the ammonia and nitrite levels and counteract by increasing the salt concentration and maintaining the pH near or below 7.0 by the use of buffers. When maintaining axolotls in bowls, pans, or boxes, where a balanced flora of sufficient size is not usually possible, adding sodium chloride or a balanced salt mixture to conditioned water and keeping the pH near or below neutral is advised. When maintaining axolotls in aquaria, where using high salt concentrations can be harmful to aquatic plants and can be increased by evaporation, disturbing the bacteria flora as little as possible, growing plants or algae, and replacing half the water with conditioned water once a week will yield the best results.

References

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