Glutamate Dehydrogenase and the Changing Pattern of Excretory Ammonia and Urea in *Heteropneustes fossilis*

Shuvasish Roy Choudhury, Rita Mahanta, Aparajita Borkotoki

**Abstract**—Fishes, in general, follow ammonotelic mode of excretion. However, certain stress factors may provoke them to excrete urea. In the present study, the possible role of ureogenesis to avoid accumulation of toxic ammonia under water-restricted condition was tested in *Heteropneustes fossilis*. A total of hundred fishes were collected and sacrificed. Excretory urea and ammonia were estimated in the water of the aquarium and glutamate dehydrogenase activity was measured in the hepatic tissue. During the experimental period, excretory ammonia in *Heteropneustes fossilis* was found between 931% to 16% above the baseline ammonia and excretory urea was found between 112% to 898% above the baseline urea. A high degree of correlation with r (coefficient of correlation) above 0.9 is observed between excretory ammonia and urea. A high degree of correlation with r (coefficient of correlation) above 0.9 is observed between excretory ammonia and urea. However, only a moderate degree of correlation is observed between the activity of glutamate dehydrogenase and excretory ammonia and urea.

**Keywords**—Ammonia, aquarium, glutamate dehydrogenase, urea, ureogenesis.

I. INTRODUCTION

Excretion of nitrogen is a necessary consequence of protein breakdown; when proteins are converted to carbohydrates to provide energy, the amino group is removed and must be dealt with. In the body, the amino group is quickly oxidized to form ammonia (or, at high body pH, the ammonium ion). Ammonia is highly toxic and highly soluble in water. Many freshwater organisms, having sufficient source of water, excrete ammonia in water.

In any event, ammonia must be dealt quickly because of its toxicity. Urea is commonly used as an excretory product in vertebrates, and is rarely used in invertebrates. Some organisms, such as sharks and snails, allow urea to accumulate in their blood to help with overall osmotic balance.

Sometimes conditions like exposure to exogenous ammonia, water limitations, or alkaline conditions hamper the release of ammonia. In such conditions, some teleosts detoxify ammonia through synthesis of urea by urea cycle in liver [12].

The majority of teleost fishes are ammonotelic, i.e., ammonia is simply excreted directly across the gills into the surrounding aqueous environment. But, a functional urea cycle and ureotelism have been documented in a few adult species as adaptations to unusual environmental circumstances as stress, air exposure, high pH, exposure to high concentration of ammonia. Fishes are highly individualistic in the mechanisms they employ for adapting to varying environmental challenges, i.e., expression of the urea cycle is only one of the several possible strategies [1].


Reference [6] studied the sub cellular localization of different urea cycle enzymes in the liver and kidney of a freshwater air-breathing teleost *Heteropneustes fossilis*.

Glutamate dehydrogenase (GLDH) is an important enzyme, linking nitrogen elimination with utilization of amino acid carbons for energy metabolism. NAD-linked glutamate dehydrogenase catalyzes the major, but not sole, pathway for generation of ammonia from glutamate. In liver, excessive glutamate dehydrogenase activity results in increased ammonia production. The endogenous ammonia production in different fishes has a significant role in glutamate catabolism [8], [11], [7].

Reference [4] suggested that NADH-glutamate dehydrogenase was involved in the detoxification of high nitrogen levels. Adaptation of nitrogen metabolism is one of the important prerequisites for any vertebrate species while migrating from aquatic to the terrestrial habitat [17]. In African lung fishes, *Protopterus annectens* and *Protopterus aethiopicus*, a greater part of waste nitrogen is converted to urea via the urea cycle, when they undergo aestivation [10].

The presence of a functional urea cycle has recently been reported in some Indian air-breathing teleosts [13], [14]. It has been demonstrated that at least in two Indian air-breathing cat fishes (*Heteropneustes fossilis* and *Clarias batrachus*) ureogenesis is stimulated under hyper-ammonia stress [15], [16].

Hence, the present study was aimed to investigate the changing pattern of excretory ammonia and urea and the possible role of glutamate dehydrogenase in hepatic tissue of *Heteropneustes fossilis*.
II. MATERIALS AND METHODS

A. Specimen

*Heteropneustes fossilis* were collected from a local pond and were kept in the aquarium for acclimatization.

B. Method

Total hundred fishes were collected. Those hundred fishes were divided in ten sets, each set comprising ten fishes. Every day, one fish from one aquarium was sacrificed for the experiment. The experiment was continued till tenth day. Enzyme activity was measured in the liver tissue of the freshly killed fishes of normal and experimental group.

C. Processing of the Collected Sample

The water of the aquarium was used for excretory ammonia and urea analysis. The liver tissue from the normal and experimental fishes was weighed and homogenized using distilled water. The homogenized tissue was centrifuged and the supernatant was used for enzyme assay.

D. Estimation of Ammonia and Urea

Ammonia was estimated by following the method of [18]. Urea was estimated by following Crest Biosystems Modified Berthelot method by [19].

E. Estimation of Glutamate Dehydrogenase

Glutamate dehydrogenase activity was determined by following the method [20].

III. RESULTS

**TABLE I**

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<tbody>
<tr>
<td>% deviation of excretory ammonia</td>
<td>931.25</td>
<td>898.11</td>
<td>528.57</td>
<td>511.84</td>
<td>256.48</td>
<td>331.94</td>
<td>144.14</td>
<td>62.66</td>
<td>18.75</td>
<td>16.56</td>
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<tr>
<td>% deviation of excretory urea</td>
<td>112.50</td>
<td>126.82</td>
<td>288.17</td>
<td>470.58</td>
<td>586.41</td>
<td>878.31</td>
<td>823.07</td>
<td>834.56</td>
<td>848.23</td>
<td>898.80</td>
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**TABLE II**

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<tr>
<td>% deviation</td>
<td>0.83</td>
<td>18.69</td>
<td>29.92</td>
<td>8.06</td>
<td>40.00</td>
<td>45.45</td>
<td>22.68</td>
<td>28.68</td>
<td>34.16</td>
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IV. DISCUSSION

The major nitrogenous waste product in fishes is ammonia. However, under some circumstances as reduced water level in the surrounding, fishes are reported to change their nitrogen excretion mechanism by excreting urea instead of ammonia [5]. In the present study, changes in the activity of glutamate dehydrogenase in *Heteropneustes fossilis* in relation to ammonotelic and ureotelic nitrogen excretion is tried to probe with monitoring the excretory nitrogen forms as urea and ammonia in the rearing medium.

In case of *Heteropneustes fossilis* there is sharp increase in the excretory ammonia with percent deviation of 930% above normal on the very first day of the experiment which gradually declines and finally touches the baseline level on the tenth day of experiment (Table I). The trend of decreasing excretory ammonia in this fish species is highly correlative with duration signifying a persistent and definite decrease in ammonia excretion with increase in duration of experiment.

In *Heteropneustes fossilis*, gradual and persistent increase in excretory urea with increasing duration of the experimental period with fairly high degree of correlation ranging from \( r = 0.936 \) (Fig. 1) is observed.
present study basically shows that there is daily fluctuation in the glutamate dehydrogenase activity (Table II). In *Heteropneustes fossilis* the fluctuating glutamate dehydrogenase activity results in a gradual increase in activity with increase in number of days of experiment followed by a decrease in the activity.

However, on the simultaneous interpretation of the trends of glutamate dehydrogenase activity with trends of changing excretory ammonia and urea under the same experimental setup it is observed that there is no any definite and appreciable relationship between the trends of this fluctuation (Figs. 2 and 3).

From the experimental outcome with determination of nitrogen excretion of ammonia and urea and their relationship with hepatic glutamate dehydrogenase (GLDH), it has been observed that excretory ammonia and urea are interrelated with each other (r=0.9362) and the relationship with glutamate dehydrogenase is quite pronounced in *Heteropneustes fossilis*. The findings of the present study may suggest ureotelism as the possible strategy of survival in *Heteropneustes fossilis*, living in a water-restricted condition.

**REFERENCES**


