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Ethological and Haematological Response of Catfish, *Heteropneustes fossilis*, Exposed to Exogenous Urea

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Abstract Exposure of the airbreathing catfish, *Heteropneustes fossilis* to a sublethal concentration (200 mg/*l*) of urea resulted in blanching of skin, increased mucus secretion, hyperexcitability and higher rate of surfacing and gill ventilation. These changes were more pronounced during the first eight days of exposure as compared to the later period. A progressive increase in haematocrit, erythrocyte count, percentage of immature erythrocytes, mean corpuscular volume and erythrocyte sedimentation rate was observed during the 20 day exposure period. White blood cell-thrombocyte count registered a sharp decline initially upto 6 days but steadily increased afterwards. Initial decrease in the total leucocyte count was found to be mainly due to the decline in the number of lymphocytes and thrombocytes while the percentage of granulocytes (neutrophils and eosinophils) and monocytes showed a reverse trend.

Urea [$CO(NH_2)_2$] is most widely used in agriculture as an economical and effective fertilizer. Its efficacy in promoting growth of plants and increasing production is unchallenged. However, in animals it is responsible for manifestations ranging from inhibition of growth to death. In aquaculture practices where it is used as a fertilizer, it is frequently reported to diminish fish production and in some cases even mortality (Jhingran, 1982). Meehean and Marzulli (1945) while using different fertilizers in fish ponds reported a negative correlation between fish production and level of organic nitrogen. Urea is also known to bring about pernicious physiological changes in fish (Sriwastawa and Shivastava, 1977; Narain and Srivastava, 1979; Srivastava and Narain, 1982). Although known for its harmful effects, the interaction of urea with fishes and other commercially important animals inhabiting the water masses which are vulnerable to large-scale contamination with this nitrogenous product has received little attention. For understanding the response of fishes to exogenous urea certain aspects requiring attention include behavioural reaction, osmoregulation and haematology, although evaluation of other influences must not be disregarded.

Haematological parameters of fish are known to respond quickly to change in environmental conditions and that is why in recent years they have been increasingly used in toxicological studies. They provide important information about the clinical status of the organism. Haematological changes that are proving to be useful as indices include erythrocyte numbers, haematocrit, haemoglobin concentration and particularly the total and differential leucocyte numbers (Gardner and Yevich, 1970; McLeay and Gordon, 1977; Esch and Hazen, 1980; Wedemeyer and McLeay, 1981; Mustafa and Murad, 1984; Ellsaesser et al., 1985; Pickering and Pottinger, 1985; Ellsaesser and Clem, 1986; Fries, 1986; Heath, 1987).

The present work was designed to examine the effect of a sublethal concentration of exogenous urea on behaviour and some of the haematological characteristics (haematocrit, erythrocyte count, percentage of immature erythrocytes, mean corpuscular volume, erythrocyte sedimentation rate and total and differential white blood cell-thrombocyte count) of airbreathing catfish, *Heteropneustes fossilis* (Bloch). The species of fish selected, *H. fossilis*, is found commonly in ponds, lakes and rivers and constitutes a substantial part of the inland fish catch in the Indian subcontinent.

Materials and methods

Live and healthy specimens of *H. fossilis* were procured from the local fish supplier. Fish showing injuries or emaciation were discarded.

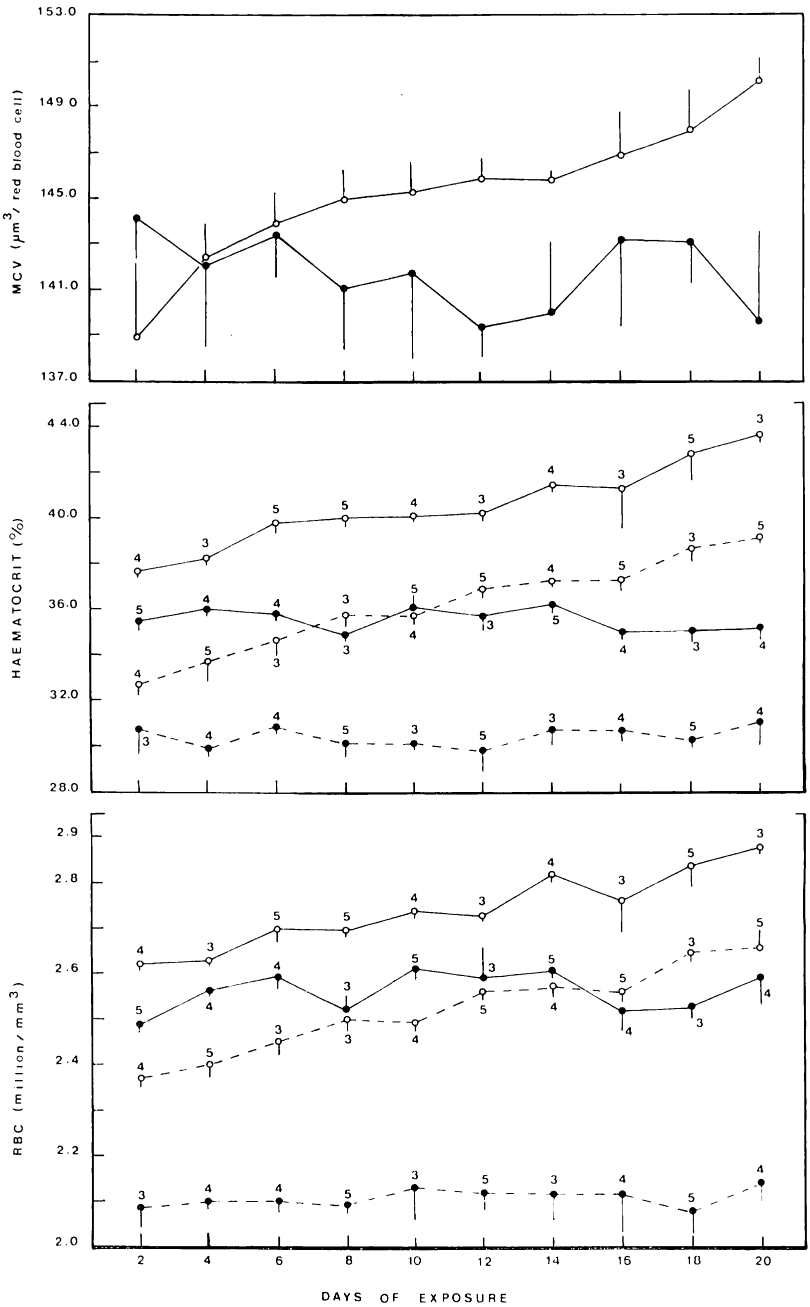

Fig. 1. RBC numbers, haematocrit and mean corpuscular volume (MCV) in control (male: ●—●; female: ●---●) and urea exposed (male: ○—○; female: ○---○) specimens of *Heteropneustes fossilis*. Vertical lines indicate one standard error. Unless indicated, the number of observations at each sampling interval is eight.

The average weight of 160 fish used in the present investigation which included both sexes was 24.35±1.49 g (range: 13.0–56.0 g) and mean total length was 16.61±0.34 cm (range: 13.0–23.2 cm). The specimens were reared in 100 *l* glass aquaria. During acclimation for two weeks fish were fed with minced buffalo meat to satiety at an interval of 24-hours. Uningested food was siphoned off

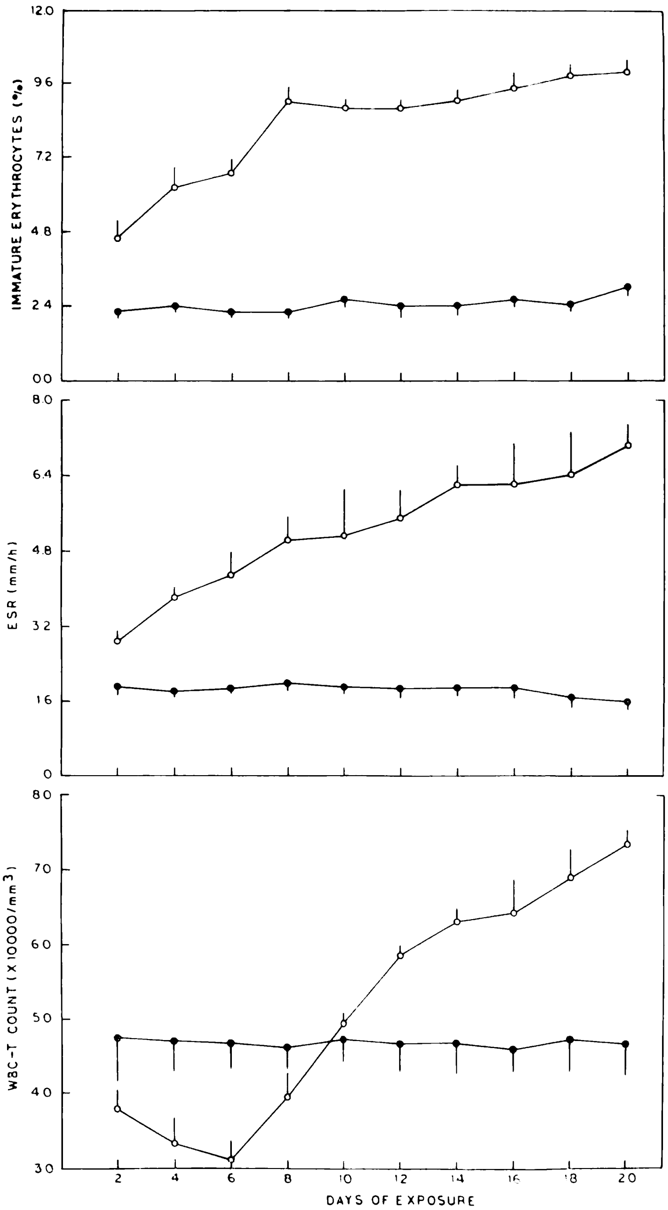

Fig. 2. WBC-T, ESR and immature erythrocytes abundance in control (●—●) and urea exposed (○—○) specimens of *Heteropneustes fossilis*. Vertical lines indicate one standard error. Number of observations at each sampling interval is eight.

and water was changed daily. The photoperiod followed the normal daylight hours of Aligarh in the month of September. Physico-chemical conditions of the aquaria water was as follows: temperature 25±1°C, dissolved oxygen 7±1 mg/*l*, carbon dioxide, not detected, carbonate 8±2 mg/*l*, bicarbonate 50±4 mg/*l*, chloride 34±2 mg/*l* and pH 7.6–7.9.

Fish were exposed to a sublethal concentration of urea (200 mg/*l*) for the period of 20 days. Medium was renewed at 24 hours interval. Control sets were run under similarly simulated conditions except that the water supplied was completely free of urea.

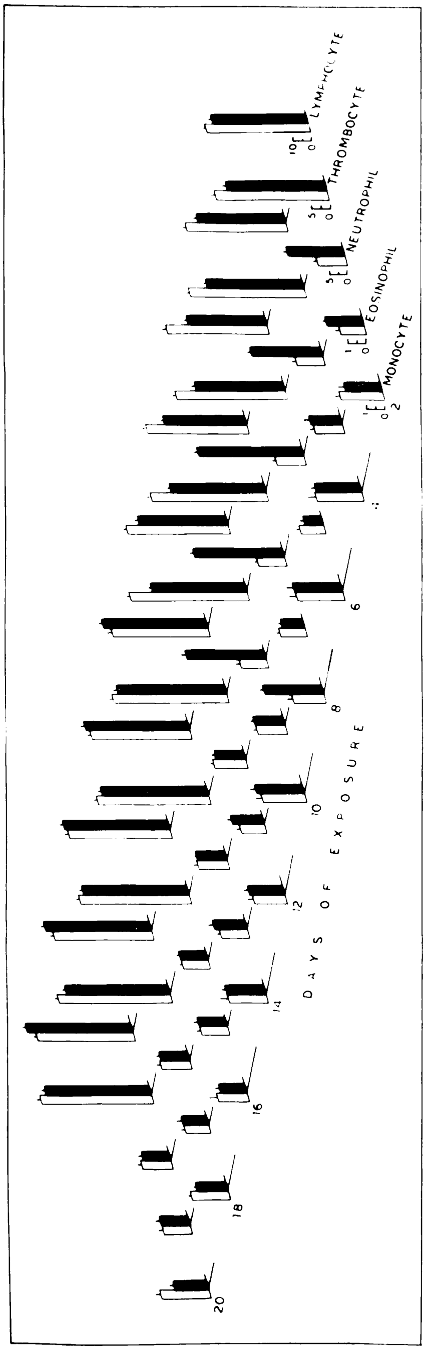

Fig. 3. Differential leucocyte analysis of control (white bars) and urea exposed (black bars) specimens of *Heteropneustes fossilis*. Vertical lines indicate one standard error.

Eight specimens from urea treated and control batches each were removed from the aquaria after 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days for haematological examination.

Methods for collection of blood and for the determination of various blood characteristics viz., total erythrocyte count, percentage of immature erythrocytes, haematocrit, mean corpuscular volume (MCV), total and differential white blood cell-thrombocyte (WBC-T) count and erythrocyte sedimentation rate (ESR) were the same as described by Mustafa and Murad (1984) and Murad and Mustafa (1988). The percentage of immature erythrocytes was computed by randomly selecting 10 microscopic fields (1,000×) from the stained slides and counting the number of basophilic erythroid cells present in those ten fields. The total number of erythrocytes counted from the ten fields varied from 1,500 to 2,000. For differential leucocyte count the cells identified and enumerated were lymphocytes (both small and large), thrombocytes, neutrophils, eosinophils and monocytes.

Results and discussion

Ethological changes. Exposure of *H. fossilis* to sublethal concentration of urea resulted in blanching of the skin, increase in mucus secretion, hyperexcitability and fast swimming activity. Fish, however, were more restless during the first eight days of exposure than during the later period. Rate of surfacing increased from 12–24/hour (normal condition) to 42–54/hour in treated specimens. Likewise gill ventilation rate assessed by the count of the opercular beats appeared to rise from 56–62/minute to 108–120/minute.

Increase in mucus secretion is commonly observed in fish exposed to pollutants (Mustafa and Murad, 1984) and other stressful conditions, i.e. handling and transportation. This stress related phenomenon may be in response to provide a protective coating to reduce exposure to toxicant through epidermis and (or) to expedite discharge of the pollutant via epidermal mucus as suggested by Varanasi and Markey (1978). Color fading of the skin as observed in the urea exposed fish signified physiological change and may be caused by inhibition of the release of acetylcholine (which effects pigment dispersal in chromatophores) by endplates of cholinergic nerve fibres;

stimulation of adrenaline production by adrenergic axon terminals, or else the disruption of normal output of melanophore stimulating hormone(s) and (or) the W-substance (melanophore-concentrating hormone) by the hypophysis (Brown, 1957; Fujii, 1969; Matty, 1985).

Haematological changes. Some of the haematological parameters monitored during the course of the study have been indicated in Figs. 1 to 3. Exposure of fish to urea of sublethal concentration for 20 days resulted in increase in haematocrit, MCV, total erythrocyte count, percentage of immature erythrocytes and ESR. WBC-T count, however, seemed to decline initially upto 6 days but registered appreciable steady increase afterwards. Differential leucocyte count also changed due to urea exposure (Fig. 3). Pronounced increase in the RBC count and haematocrit can be attributed to stress-mediated release of the cells by the erythropoietic tissues. Findings of McLeay and Gordon (1977) support this view and regard the phenomenon as a compensatory adjustment to deal with possible tissue hypoxia. Yamamoto et al. (1980, 1983, 1985) while working on yellowtail, *Seriola quinqueradiata*, demonstrated the release of erythrocytes into the circulating blood from the spleen in response to exercise and hypoxia. According to Erslev (1977) the release of larger number of erythrocytes is appropriate and part of compensatory effect to minimize a threatening tissue hypoxia which improves the oxygen-carrying capacity of the blood. The hypoxia may result from hyperexcitability and strenuous muscular activity which can not be sustained by normal supply of oxygen to the cells. Interference of urea with oxygen uptake by the cells can not be ruled out but merits investigation before anything is said about this aspect with certainty. Observations of Sriwastawa and Shivastava (1977) on increase in the metabolic activities of Golgi bodies and mitochondria under urea stress in a carp *Puntius sophore* relate the change to hightened exercise and enhanced bioenergetics. The modifications in the internal environment tend to enable the fish to withstand the stressful condition that makes heavy demands of oxygen, fuel oxidation and energy.

Increase in the MCV in urea-treated fish points to swelling of the erythrocytes. Nikinmaa (1988) also found swelling of erythrocytes during stress and hypoxia in *Salmo gairdneri* and considered such a variation as physiological adjustment induced by changes in blood oxygen tension.

Marked decrease in WBC-T count of the urea exposed fish seemed mainly a consequence of sharp reduction in the number of circulating lymphocytes and thrombocytes. This haematological change could be correlated with increased activity of pituitary-interrenal axis. Enhanced output of adrenocorticotrophic hormone (ACTH) results in higher blood titers of corticosteroids which bring about the lysis of lymphocytes and thrombocytes. Earlier studies by Ball and Slicher (1962) and Ellis (1977) leave no scepticism about this process. Donaldson and Dye (1975), Fryer (1975), Singley and Chavin (1975), Schreck et al. (1976) and Mazeaud et al. (1977) emphasized that pituitary-interrenal system responds to different stimuli in non-specific manner; the qualitative response is identical and takes the form of increased output of ACTH and corticosteroids, whereas the quantitative response depends upon the nature of stress. It is well established that these hormones are catabolic and work towards protein mobilization. Elevation of ESR can be explained in the light of such changes in the body characterized by proteolysis, lymphocytolysis and thrombocytolysis. Present interpretation finds favour with the version of Blaxhall and Daisley (1973). Recovery in these cells and also in the WBC-T count can be attributed to a number of factors, namely, feedback inhibition of ACTH production, decrease in body's response to corticosteroids, increased resistance of fish to environmental toxicants. Wedemeyer (1970) has concluded that repeated application of stressors and prolonged exposure lead to lowered response of stress hormones in vertebrates.

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ナマズの一種 *Heteropneustes fossilis* の水中尿素に対する行動学的ならびに血液学的反応

Ajmal Murad・Saleem Mustafa

空気呼吸をするナマズの一種 *Heteropneustes fossilis* を致死濃度以下 (200 mg/*l*) の尿素液中におくと，皮膚が白っぽくなり，粘液分泌が増し，興奮しやすくなり，浮上と鰓蓋運動の頻度が上昇した．これらの変化は，最初の 8 日間の方がそれ以後に比べて著しかった．20日間の暴露期間中にヘマトクリット，赤血球数，幼若赤血球の百分比，平均血球容積，および赤血球沈降速度の漸増が観察された．白血球—栓球数は，当初 6 日目までは急減し，その後は一様に増加した．全白血球数の初期の減少は，主にリンパ球と栓球の減少によるものであったが，顆粒球（好中球と好酸球）および単球の百分比は逆の傾向を示した．