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Lipid peroxidation and ascorbic acid status in respiratory organs of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature increase

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Lipid peroxidation and ascorbic acid (AsA) contents were measured in the gill and air sac of male and female catfish, *Heteropneustes fossilis*, after exposure to temperatures (25–37°C) at various times. Lipid peroxidation in gill and air sac biomembranes was enhanced on increasing the temperature from 25 to 37°C for 60–240 min. In gill, the significant decline in AsA was observed only at 240 min exposed with different temperature range. In other exposure periods, the decline was nonsignificant. Air sac AsA was decreased significantly by exposure of 32 and 37°C temperatures at various times. Lipid peroxidation and AsA contents after temperature exposure in gill and air sac of male and female fish showed no significant difference. The findings indicated an increased oxidative stress in gill and air sac of male and female fish after increased temperature exposure. The decline in AsA level supports its antioxidant role in relation to oxygen radicals.

Key words: Lipid peroxidation; Ascorbic acid; Heteropneustes fossilis.

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Introduction

Different biochemical approaches have been taken to understand the pro-oxidant and antioxidant mechanisms in fish and other aquatic organisms (DiGiulio *et al.*, 1989; Winston *et al.*, 1990; Winston and DiGiulio, 1991; Livingstone *et al.*, 1992, 1993; Lemaire and Livingstone, 1994). However, oxygen radical generation and antioxidative defenses in fish respiratory organs have received relatively little attention. Gills are normally red, and hyperemia is common in low oxygen or excessively warm water. Swim bladder and gas gland in many teleosts operate under conditions of hyperoxia and low pH. It appears that hyperoxia enhances generation of oxygen radicals (Jones, 1985), which in aerobic cells play a crucial role in lipid peroxidation. To tolerate the high oxygen tension and oxygen radicals, the gas gland of fish has been reported to possess effective antioxidant defenses (Lemaire *et al.*, 1993).

In normal metabolism, a balance exists between the generation of free radicals and antioxidant defense mechanisms (Halliwell, 1987). However, when normal enzymatic defenses are stressed, secondary defenses, such as vitamins A, C and E, prevent the chain reaction of autoxidation. Ascorbic acid (AsA) and tocopherol compounds have long been linked as essential factors to ameliorate some of the toxic effects of oxygen radicals (Meister and Anderson, 1983; Burton and Ingold, 1989; Krinsky, 1989; Geesin *et al.*, 1990; Aten *et al.*, 1992; Carlson *et al.*, 1993). The effectiveness of the antioxidant defense system in relation

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to the lipid peroxidation process is of particular interest in the case of aquatic animals, which show seasonal metabolic variations related to fluctuation of environmental factors such as temperature and to the physiological status of the animals. The oxidative damage and relationship of antioxidants to temperature exposure in fish respiratory organs are much less studied. The present study deals with lipid peroxidation and AsA status in gill and air sac of male and female catfish (*Heteropneustes fossilis*) during temperature exposure at various times.

Materials and Methods

Animals and experimental design

Two hundred fifty-six adult freshwater Indian catfish (*Heteropneustes fossilis*, average weight 42 ± 2.5 g and length 17 ± 0.5 cm) were captured in April from the vicinity of Ujjain, India, and acclimated for 2 weeks in a glass aquarium to laboratory conditions before the start of the experiment. Animals were maintained at a water temperature of 25°C and a photoperiod of 12-hr light–12-hr dark cycle. Four main groups of 64 animals each were used. The first group was kept as control. The second, third and fourth groups were kept for temperature exposure. Sixteen fish (eight male and eight female) were held in properly aerated aquariums.

Temperature exposure

Experimental fish were exposed to temperatures ranging from 25 to 37°C for 60, 90, 180 and 240 min (using a thermostatic heater). Control fish were kept at 25°C.

Tissue homogenization

The animals were killed by a blow to the head, and gill and air sac samples were dissected. Particular care was taken to minimize disturbance of the fish in other aquariums. The tissues were homogenized in a polytron homogenizer in 50 mM phosphate buffer for tissue peroxidation measurements and in ice-cold 0.25 M perchloric acid for AsA analysis. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant kept for analysis.

Total protein content measurement

Total protein content of the supernatant was determined in a Perkin Elmer Lambda 3A double-beam spectrophotometer by the Folin-phenol reaction as described by Lowry *et al.* (1951) using bovine serum albumin as a standard.

Lipid peroxide assay

Lipid peroxide of the supernatant was measured in the terms of malonaldehyde (MDA) by the thiobarbituric acid test (Ohkawa *et al.*, 1979). The amount of MDA formed was measured by the absorbance of the upper organic layer at 532 nm using appropriate controls. The results were expressed as nmol MDA/mg protein. Thiobarbituric acid assay is a generally used method for the detection of lipid peroxidation but is limited because other reactive aldehydes may not be detected and the malonaldehyde produced *in vivo* may react with other cellular constituents (Kosugi and Kikugawa, 1989; Janero, 1990; Valenzuela, 1991).

AsA assay

AsA was assayed by a modification of the dinitrophenylhydrazine technique of Terada *et al.* (1978) described by Thomas *et al.* (1982). All analyses were carried out in duplicate.

Statistical analysis

All data are expressed as means ± SEM, and control and treatment values were compared by Student's *t*-test. The 0.05 level was selected as the point of minimal statistical significance in every comparison.

Results and Discussion

To estimate the degree of oxidative stress imposed on the animals by temperatures at various times, the levels of MDA (tissue peroxidation product) was estimated in gill and air sac of male and female fish. The MDA levels were significantly increased ($P < 0.05$) in gill and air sac with the increase in temperature from 25 to 37°C (Figs 1 and 2). Exposure of each temperature range was extended for periods of 60, 120, 180 and 240 min. In both gill and air sac, increase of exposure of each temperature range for 180 and 240 min caused a significant increase in MDA. However, at 60 and 120 min of exposure, increasing the temperature did not increase the MDA level significantly. No extensive studies on oxidative stress in respiratory organs and tissues under temperature exposure have previously been reported for fish. Nevertheless, our data on lipid peroxidation that increased significantly by temperature exposure are indicative of oxidative damage in gill and air sac.

Gills of fish are well equipped with phagocytic cells that lie between adjacent capillaries and exhibit phagocytic response in warm water (Chilmonczyk and Monge, 1980). This is consistent with the fact that temperature exposure could increase the phagocytic activity

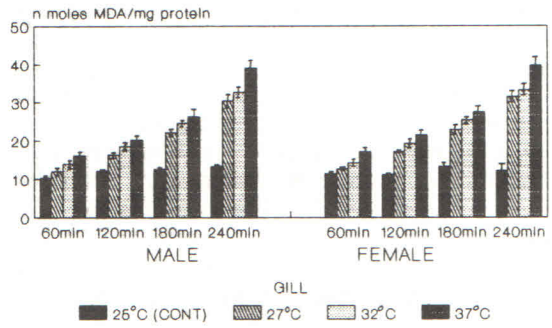

Fig. 1. Changes in the concentration of MDA during temperature exposure from 25°C (control) to 37°C at various times in gill of male and female catfish, *H. fossilis*.

(Cheville, 1983), which results in internalization of oxidases on the surface of the membranes (Robinson, 1978). Involvement has been suggested of microsomes (Winston and Cederbaum, 1983, 1986; Livingstone *et al.*, 1989; Winston *et al.*, 1990) and active phagocytes (Higson and Jones, 1984; Secombes *et al.*, 1988) in oxygen radical production in aquatic animals, including fish. The present finding, together with views of these authors, suggest temperature-mediated phagocytic activity in superoxide anion radical ($O_2^{\cdot-}$) production and subsequently lipid peroxidation in gill.

Air sacs are always hyperoxic, and temperature exposure could further increase oxygen tension because of high oxygen uptake through air (Brett, 1964). The increasing oxygen tension can increase the basal rate of oxygen radical production (Jones, 1985). A mechanism thought to be important against lipid peroxidation in air sac is the presence of considerable amounts of oxygen during temperature exposure, increasing the cellular generation of highly reactive oxygen species, including $O_2^{\cdot-}$, hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) (Halliwell and Gutteridge, 1986), utilizing either NADH or NADPH as reducing equivalents (Lemaire *et al.*, 1993).

Figures 3 and 4 show the AsA level in gill and air sac after exposure of temperatures for varying periods. The AsA level was depleted at each temperature exposed for 240 min. The decline at other exposures was not significant. In air sac, the AsA content was significantly declined ($P < 0.05$) at 32 and 37°C exposed for varying periods. The depletion in AsA at 27°C was not significant. When exposure of each temperature was extended for varying periods, the further significant decline in AsA was noted only for 240 min at 37°C.

On the whole, the marked depletion of AsA with the increase in lipid peroxidation in air sac seems to be due to the acute generation of reactive oxygen species. Because AsA functions as an antioxidant (Bendich *et al.*, 1986), in conditions of its deficiency, symptoms of oxidative stress, possibly including cell damage, might be expected to appear. Nevertheless, oxidative stress reflected to AsA deficiency under temperature exposure has been indicated in our data and in those reported by Henning *et al.* (1991) by an increase in lipid peroxidation. AsA has been reported to reduce organic peroxide (Jenkins *et al.*, 1988). The reducing power of AsA can be helpful in preventing oxidative stress.

In gill, only maximum exposure (240 min) resulted in depletion of AsA. At other exposures, the AsA level remained more or less the same. Relative maintenance of constant levels of AsA in gill after 60, 120 and 180 min could be due to its role in phagocytic activity to acclimatize in response to temperature. Studies with human subjects showed that elevated levels of AsA increase phagocytic activity (Goetzl *et al.*, 1974; Kraut *et al.*, 1978) and enhance the humoral immune response, antibody production and complement activity in channel catfish (Li and Lovell, 1985).

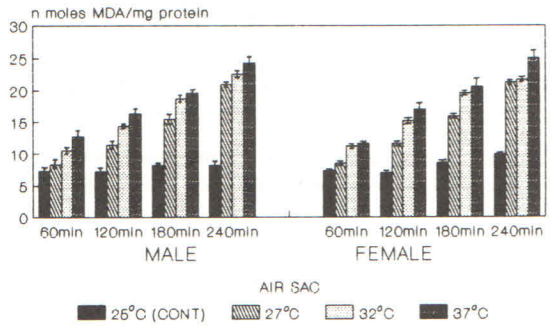

Fig. 2. Changes in the concentration of MDA during temperature exposure from 25°C (control) to 37°C at various times in air sac of male and female catfish, *H. fossilis*.

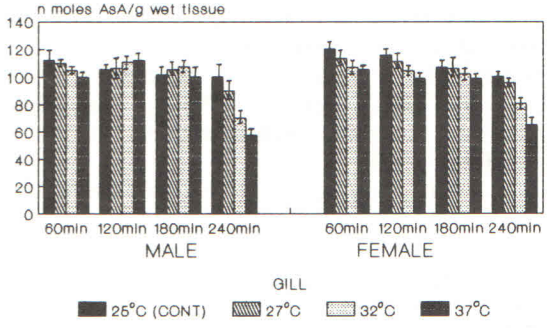

Fig. 3. Changes in the AsA content in gill after temperature exposure from 25°C (control) to 37°C at various times of male and female catfish, *H. fossilis*.

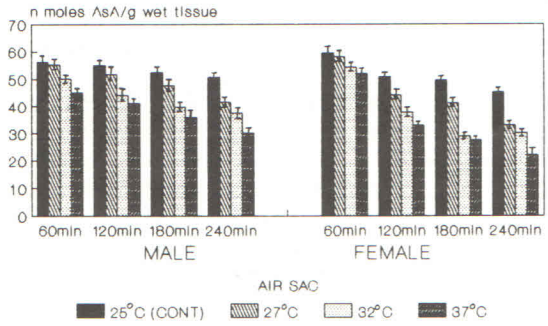

Fig. 4. Changes in the AsA content in air sac after temperature exposure from 25°C (control) to 37°C at various times of male and female catfish, *H. fossilis*.

Temperature exposure resulted in hypersecretion of a gel-like mucoid substance, continuously dissipating into the surrounding medium. High molecular weights of glycoconjugants are secreted in granules and packed in goblet cells of the gill. Rapid expulsion of mucus under appropriate stimulus is primed by fusion of membranes of goblet cell mucous granules that are packed together. Cell membrane fusion has been shown to depend on increase in membrane fluidity (Batzri and Korn, 1975; Kantor and Prestegard, 1975). This increase in fluidity of phospholipid bilayer appears to involve oxygen radicals (Hochstein and Jain, 1981). In view of these studies and the present findings, the possible mechanism of mucous secretion in goblet cells of gill could be explained. Temperature exposure could increase the oxygen radical production as has been evidenced by an increase in lipid peroxidation in the present study. The enhanced oxygen radical production could increase the fluidity of the membrane and consequently affect the fusion of mucous granule membranes for rapid expulsion of mucus. Although no direct evidence appeared in the literature indicating involvement of oxygen radicals in mucus secretion in fish, observation of oxygen radical stimulated mucus secretion from epithelial cells was obtained in mammalian lung tissue culture after purine/xanthine oxidase exposure (production of superoxide anions) (Adler *et al.*, 1990). Mucus hypersecretion is also a clinial finding in several human lung disorders such as asthma (Doelman and Bast, 1990).

A comparison of results of lipid peroxidation and AsA contents after temperature exposure in gill and air sac of male and female fish showed no significant difference. In summary, significantly increased lipid peroxidation in gill and air sac with increase in temperature at various times are indicative of oxidative damage. The decline in AsA level in relation to endogenous potential for oxygen radical production in air sac supports the antioxidant role of AsA as has been reported by Leung *et al.* (1981) and Machlin and Bendich (1987). Possible mechanism of mucus secretion may concern the relationship between oxygen radicals and mucus exudation, which have been discussed for mammalian lung by Martin (1984) and Adler *et al.* (1990). However, this augments further studies on this aspect. These findings are considered important not only from a physiological point of view but also in helping to elucidate the mechanism of regulation of mucus exudation during temperature exposure.

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