Copper Intoxication—a Possible Biochemical Homologue

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Research on the mechanism of metal toxicity to a particular fish species has been limited to date. WESTFALL (1945) reported goldfish, exposed to solutions of metals at different pHs, responded by increasing flow of mucous to the gills, thus reducing the functional respiratory surface area due to an increase in gaseous diffusion distance. CARPENTER (1927), ELLIS (1937) and LLOYD (1960) concluded that mortality resulting from metal exposure was due to an effect at the gill surface sometimes increasing, sometimes decreasing mucous secretion. BROWN (1968), SKIDMORE and TOVEL (1972) and BURTON et al. (1972) reviewed metal toxicity data and proposed several theories to explain the mechanism of action. Consensus of opinion points to the gill as being the major organ affected by metal toxicants (BURTON et al., 1972; SKIDMORE, 1964, 1970; LIFE, 1970), but the exact site and mode of action upon this complex organ is still unclear.

Recent research (COURTOIS and MEYERHOFF, 1975) has demonstrated a transient inhibition of osmoregulatory homeostasis following ambient exposure of freshwater acclimated adult striped bass (Roccus saxatilis) to copper. Acute exposure resulted in expansion of the plasma volume (based on dye analyses) within minutes following introduction of the metal. Further research was needed to clarify this response to answer questions as to where and how toxicity affects normal homeostasis. The purpose of the present research is to further define the mechanism of action of copper ion upon juvenile striped bass. Two different methods are used to assess mode of action: First, the ability of test fish acclimated to different environmental salinities to osmoregulate following exposure to copper ion is monitored; and secondly, acetazolamide, a compound which has well established chemical properties, is used for comparative purposes to further define the mode of action of copper ion.

Materials and Methods

The juvenile striped bass (52.43 ± 3.10 gm) used in this investigation were obtained via hook and line from the Sacramento River near Knight's Landing, Yolo County, California. All fish were held in the laboratory for one week prior to being used in experiments to ensure a healthy condition. During this holding period the fish were trained to eat Oregon test diet (COURTOIS,

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1974) via daily ad libitum feedings. Photoperiod controlled by an electric timer was set to provide 14 L:10 d (hours), which corresponded to ambient conditions during August-September. All acclimation tanks were flushed out with a volume of water equal to the tank capacity once per day with the appropriate water supply to prevent buildup of waste materials within the tanks. Water temperature was held constant at 17.5 ± 0.5°C and aeration maintained dissolved oxygen levels at or above 7.5 mg/l in all tanks.

Acclimation conditions--test fish were acclimated to either fresh water or sea water. Fresh water was city water and sea water was filtered San Francisco Bay water. Freshwater fish were acclimated for ten days to the described conditions prior to testing. A second group of fish were slowly adapted to sea water over a three-day period by additions of sea water to their holding tank. Salinity was maintained at 30.0 ± 1.0 ppt for the twenty-four day acclimation period prior to testing this group.

Test procedure--osmoregulatory ability was determined by monitoring the gross body weight change and serum Na+ content of control, copper and acetazolamide-exposed fish by the following procedures: individual fish were removed from their respective acclimation tanks, blotted dry, weighed to the nearest hundredth of a gram and placed in the test chamber. The test chamber contained 2.0 l of test solution of equal osmotic strength to that of the acclimation media. Five different solutions were used (7 to 8 fish were tested in each solution):

1. Distilled water
2. Distilled water plus 1.0 mg/l $CuSO_4$ (0.38 mg/l Cu++)
3. Distilled water plus 30 gm/l NaCl (30.0 ppt)
4. Distilled water plus 30 gm/l NaCl (30.0 ppt) plus 1.0 mg/l $CuSO_4$ (0.38 mg/l Cu++)
5. Distilled water plus 30 gm/l NaCl (30.0 ppt) plus 1.0 mg/l Acetazolamide.

Each fish was exposed to one of the test solutions for a five-minute period and then stunned via a five-second pulse of 110 volts A.C. This enabled rapid capture and ensured every fish was terminated after the same exposure periods. The stunned fish was then blotted dry, reweighed and a 0.5 ml blood sample removed via cardiac puncture. The syringe used for blood sampling had been previously heparinized with ammonium heparin (1000 USP units/ml, aqueous solutions) and air dried. Three microhematocrit tubes were prepared from the heparinized sample (BLAXHALL, 1972) and centrifuged at 3600 rpm for fifteen minutes. The clear serum layer was separated from the packed cell mass by snapping the tube in half. A 20 ul serum sample was mixed with 3.98 ml 15 meg/l lithium dilution solution (Becton-Dickinson unopette system No. 5861), sealed and stored at 4°C until quantitative analyses were performed. An IL Flame Photometer (Model 143) was used for determination of serum Na+ content.

Results

Acute exposure of freshwater acclimated striped bass to copper ion resulted in significantly higher weight gain and a lower serum Na+ content then control fish (at the 80% level, Students t test). Acute exposure of seawater acclimated striped bass to copper ion caused the reverse situation--significantly lower weight gain and a higher serum Na+ content then control fish (at the 80% and 95% levels, respectively, Students t test). Seawater acclimated striped bass acutely exposed to acetazolamide responded in a similar manner to those exposed to copper ion and displayed significantly lower weight gain and higher serum Na+ content then control fish (at the 90% and 95% levels, respectively, Students t test). The results from all three of these experiments are graphically compared (Figure 1).

Discussion

STEVENS (1972) reported transient weight changes in Tilapia mossambica caused by the stresses of handling or exercise. The direction (increase or decrease) of change was dependent upon previous acclimation conditions--freshwater acclimated fish gained weight and saltwater acclimated fish lost weight, the magnitude of change being the same in both situations. The present results display similar changes in weight in response to acute copper exposure. The magnitude of weight change in exposed striped bass acclimated to different environments was identical (+0.34% for freshwater fish and -0.34% for seawater fish). STEVENS (1972) suggested that the elevated transfer factor across the gills in response to increased oxygen needs to meet handling and exercise stress resulted in increased water flow across the gill, the degree and direction dependent upon acclimation conditions. Copper ion could be affecting the gill in similar manner.

Analysis of serum Na+ content gives further support for copper exposure causing breakdown in normal water balance. If water was moving down its osmotic gradient (inward in freshwater fish and outward in seawater fish) there should be a corresponding decrease or increase in serum Na+ levels. The present results confirm this hypothesis. Freshwater acclimated striped bass exposed to copper ion displayed a lower serum Na+ and seawater acclimated striped bass exposed to copper ion displayed a higher serum Na+ then control fish. Previous research (LEWIS and LEWIS, 1971) reported a decrease in blood osmolality following ambient exposure of freshwater acclimated channel catfish (Ictalurus punctatus) and golden shiner (Notemigonus crysoleucas) to solutions of copper or zinc. This decrease in serum osmolality parallels the drop in serum Na+ reported here and demonstrates a similar pattern of response to metals for other species. LEWIS and LEWIS (1971) also reported test fish exposed to solutions of NaCl in addition to the metals had higher serum osmolalities then control fish. Again, present results show good agreement, saltwater acclimated bass having elevated serum Na+ following copper exposure. Certainly serum

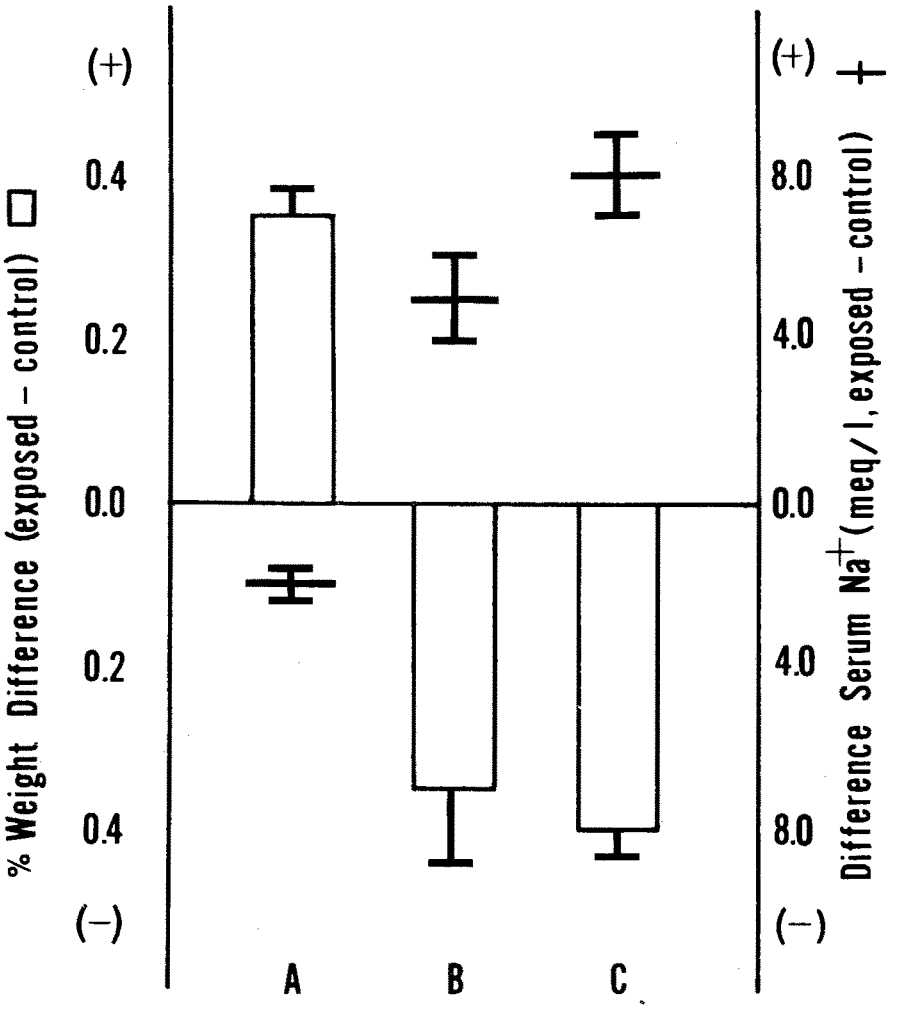

Figure 1. Differences in Percent Weight Change and Serum Na+ Between Exposed and Control Fish Tested In Three Different Situations: A = Freshwater Acclimated Copper Exposed; B = Seawater Acclimated Copper Exposed; C = Seawater Acclimated Acetazolamide Exposed.

osmolality is not identical to serum Na+ but the similarity of response in the two parameters cannot be overlooked. The concentration of copper used in the present research (0.38 mg/l Cu^{++} or 1.0 mg/l $CuSO_4$) is much lower than that used by LEWIS and LEWIS (1971) (1.90 mg/l Cu^{++} or 5 mg/l $CuSO_4$).

Ambient exposure of striped bass to copper ion caused a breakdown in normal osmotic homeostasis. The mechanism and site of copper toxicity was further investigated by use of acetazolamide, a biochemical with well defined physiological properties. MAETZ (1953, 1956) had previously reported acetazolamide to be a potent carbonic anhydrase inhibitor, stopping both Cl- and Na+ uptake through goldfish gills. KERSTETTER et al. (1970) showed acetazolamide to inhibit Na+ uptake at the trout gill membrane. They presented data which indicated that H+ is the exchanging cation--based on pH shift data. When acetazolamide was injected I.P. there was a downward pH shift and inward Na+ movement was inhibited. Present results also demonstrates this compound to affect the gill membrane of the striped bass. Seawater acclimated bass responded similarly in solutions of either acetazolamide or copper, increasing serum Na+ and decreasing weight compared to control fish (Figure 1). Since the Na+-H+ exchange site has been previously shown to be blocked by acetazolamide (KERSTETTER et al., 1970), the higher exterior osmotic gradient could cause the animal to lose water down its higher interior concentration gradient (less salt) to the less concentrated exterior solution (more salt); thus the animal would lose weight and display dehydration (increased serum Na+) compared to controls. Since the percent weight change and serum Na+ values show similar changes for both copper and acetazolamide-exposed fish, the proposed breakdown in water balance at the Na+-H+ exchange site on the gill could be the mode and site of action of copper ion. The observed weight increases and serum Na+ decreases for freshwater acclimated striped bass above and below those for control fish seem to parallel the plasma expansion reported earlier (COURTOIS and MEYERHOFF, 1975). The magnitude of change in plasma volume (+0.95%) however is greater than that of weight gain reported here (+0.34%), but the direction of change (increase over control values) in response to copper exposure is identical in both situations. Perhaps the difference between these values is due to the shorter exposure period (five minutes as opposed to 6-15 minutes) or to the smaller size of the test fish used in the present study. Further work is currently being completed to further clarify these areas.

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