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VARIATION IN THE BLOOD CHEMISTRY OF JUVENILE *Clarias lazera* SUBSEQUENT TO ACUTE AND SUBACUTE COPPER EXPOSURE

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ABSTRACT

This study has delineated the effect of acute and subacute copper exposure on a fresh water fish, **Clarias lazera.** Five different non-cellular parameters in the blood including serum total proteins, serum chloride, sodium, potassium and glucose were investigated. Mean values of serum total protein for all groups of fish exposed to different copper concentrations were higher than the corresponding control value. A decline in serum chloride levels were reported in both acute and subacute tests. The concentrations of both sodium and potassium observed during the acute and subacute copper exposure were higher than anticipated.

Serum glucose levels in the acute test were elevated above the corresponding control levels. In the subacute test, the magnitude of disturbance to glucose levels was considerably less than that caused by acute exposure to copper.

INTRODUCTION

Copper is surprisingly widespread pollutant of water. Numerous studies have shown that the addition of copper salts to natural water can seriously threaten aquatic life. The frequent introduction of copper into the aquatic environment and the sensitivity of aquatic life to copper has prompted considerable toxicological research as summarized by McKee and Wolf (1963) and Doudoroff and Katz (1953). However, the information on its toxicity still warrants further study.

The increasing need to determine accurately the physiological state of fishes exposed to environmental stress had led to extensive investigation of various biochemical indicators that might vary in a predictable manner in respose to different levels of stress. For such a purpose and to provide a background for future forensic diagnosis several physiological characteristics of juvenile **Clarias lazera** have been monitored to be used as indicators of stress resulting from acute and subacute exposure to copper.

MATERIAL AND METHODS

The origin, maintenance, and acclimation of **Clarias lazera** stocks used in this study have been previously noted by El-Domiaty (1987).

Blood was obtained from unanesthetized fish by puncture of the caudal vessel, and each fish bled only once. During sampling, fish were captured as rapidly and with little disturbance as possible. Fresh samples were centrifuged for five minutes and the serum was stored at -15°C until analyzed.

Initially the 96 hr TLm was determined to establish the nominal test concentrations of cooper for the subacute exposure.

Both acute (96 hr) and subacute (4 weeks) toxicity tests were done in a static system. Copper was added to test chambers as copper sulphate solution 30 min. before the introduction of test fish. Copper concentration corresponding to the 96 hr TLm value (3.2 mg/L) was used throughout the 96 hr exposure period (acute test). For the subacute test, copper concentrations were set at 0.5, 1.0, 1.5 and 2.0 mg/L.

Serum total proteins were determined by the use of biuret reaction as described by Barnet and Youden (1970).

Serum chloride level was titrimetrically determined using the method of Scales and Schales (1941).

The Lytetek type flame photometer was used for the determination of sodium and potassium.

Glucose level in serum was measured by the colorimetric method of Clerch and Miale (1971).

Mean and standard deviation values were calculated for each experimental group of fish. Students t-test was used to compare treatment values for each variable and significance was reported at $P<0.05$ level.

RESULTS

Serum total proteins

When compared with control value (4.25 mg/dL), exposure of **Clarias lazera** to 3.2 mg Cu^{+2}L for 96 hr resulted in a significant elevation of serum total proteins during the first 24 hr (Fig. 1) after which the values showed a general downward trend but still remained higher than that of the corresponding control level.

In the subacute test (Fig. 2), the data of serum total proteins showed an increase after one week exposure to all concentrations of copper tested, followed by a decrease in the values during the second and third weeks. Another elevation takes place during the fourth week.

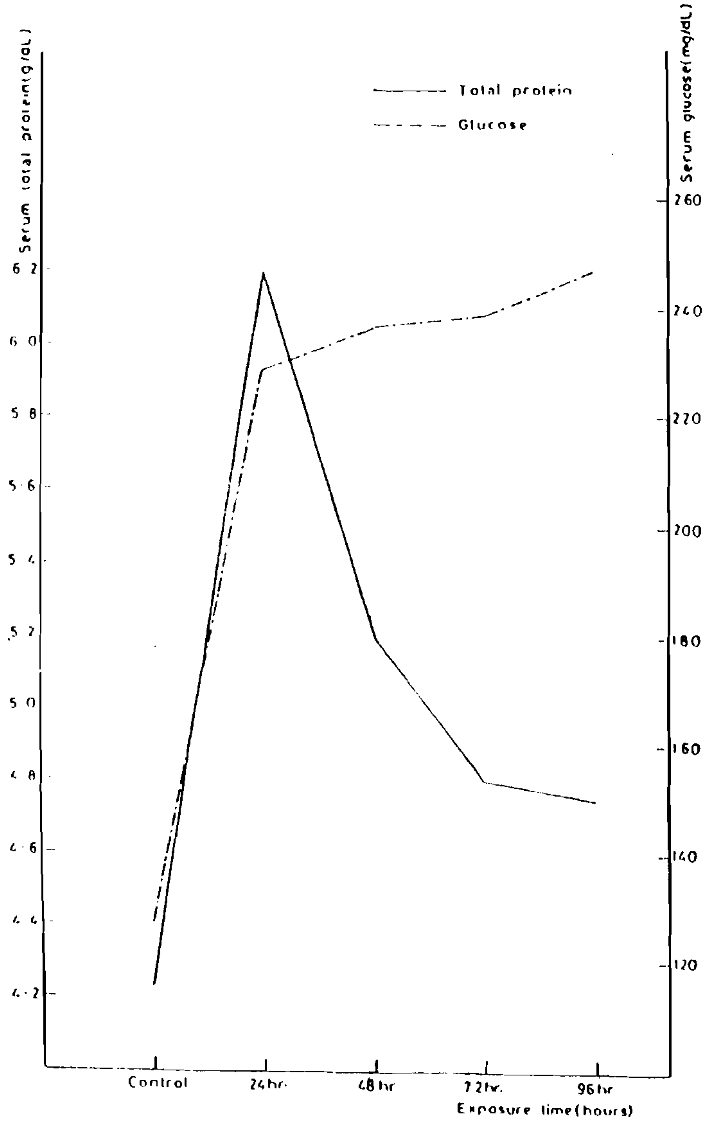

Fig. (1)
Serum total protein and glucose values of juvenile
Clarias lazera exposed to 3.2 mg/l cu^{+2}
(as copper sulphate) for 96 h.

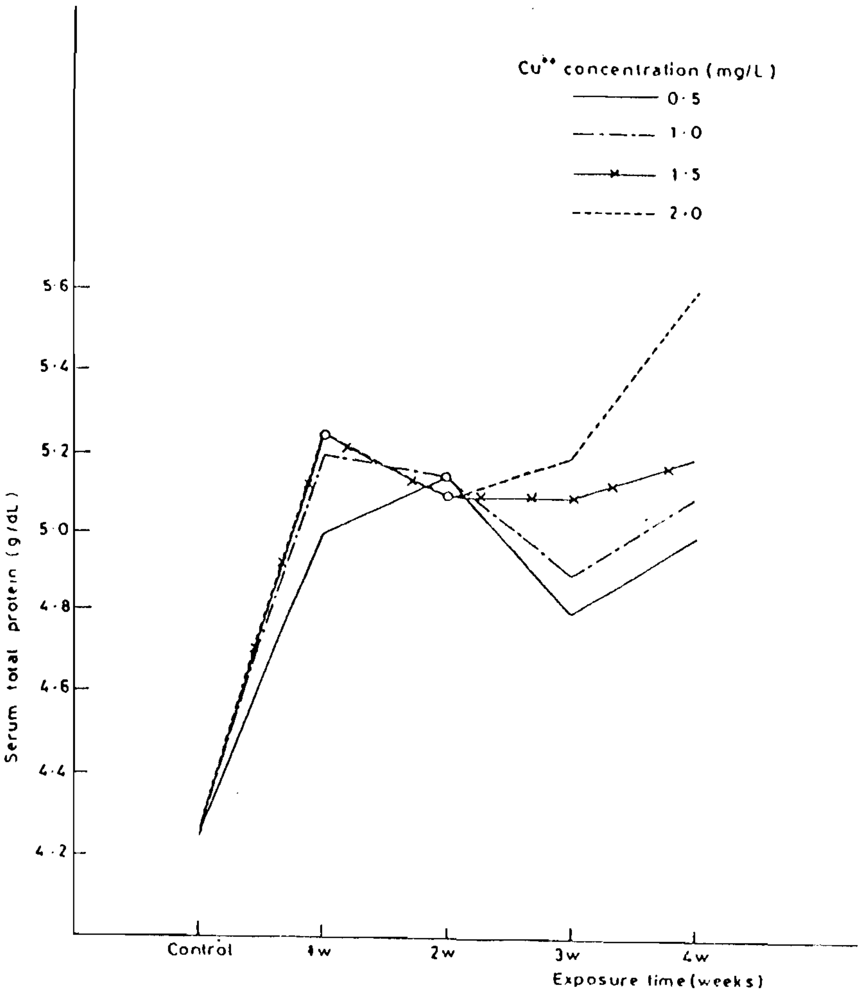

Fig. (2)
Serum total protein concentration of juvenile **Clarias lazera** exposed to elevated concentrations of cu^{+2} (as copper sulphate) for 4 weeks.

Serum electrolytes

Several distinct trends were encountered among the three ions tested. As shown in Fig. (3 and 4), serious hypochloremia developed in both acute and subacute tests allover the exposure period.

By contrast, serum sodium concentration in the acute test (Fig. 3) increased significantly from 102.5 (control value) to 130 meq/L after 48 hr. A decrease to 109.5 meq/L was observed after 96 hr, but this value still higher than that seen in the control fish. The pattern with respect to this ion, therefore, appears to be one of elevation and depression.

The same pattern of change was also observed during the subacute test for fish groups exposed to the higher copper concentrations (1.5 and 2.0 mg Cu^{+2}/L). Although fluctuations in the serum sodium level were recorded in the case of 0.5 and 1.0 mg Cu^{+2}/L exposed groups, the serum level of this ion tended to remain above control value (Fig. 5).

On the other hand, potassium concentrations were, with the exception of the sample taken after 24 hr (1.75 meq/L), in excess of control level (2.25 meq/L) in both acute and subacute tests (Figs. 3 and 6).

Serum glucose

Marked hyperglycemia with a peak concentration of 247 mg/dL was recorded after 96 hr during the acute exposure (Fig. 1).

In the subacute test, mean serum glucose levels at the first two weeks were consistently higher than values for the corresponding control sample (118 mg/dL), particularly at the higher concentration of copper. The trend over the two week period of observation was clearly toward a decrease in the serum level of this parameter (Fig. 7).

DISCUSSION

Mean values of serum total protein levels for all groups of fish exposed to different copper concentrations were higher than the corresponding control value. This is in agreement with Bouck (1972) who stated that increases in plasma protein concentration of fish are associated with stress. McKim et al. (1970) also reported similar increase in serum total protein levels in book trout (**Salvelinus fontinalis**) after short term exposure to copper.

Baker (1969) found that copper resulted in necrosis of the kidney and destruction of the haemopoietic tissue of winter flounder (**Pseudopleuronectes americanus**). McKim et al. (1970) reported an increase in plasma glutamic oxaloacetic transaminase (PGOT) in brook trout after short term exposure to copper, an indication of cellular degradation. Therefore, tissue destruction caused by copper, releasing cellular proteins into the blood may be one cause of the observed elevation in serum total proteins. The damage may be from trauma or from physiological degradation. Another possible explanation for the observed hyperproteinemia may be due to the effect of metal-binding proteins. Kagi and Nordberg (1979) found that the low

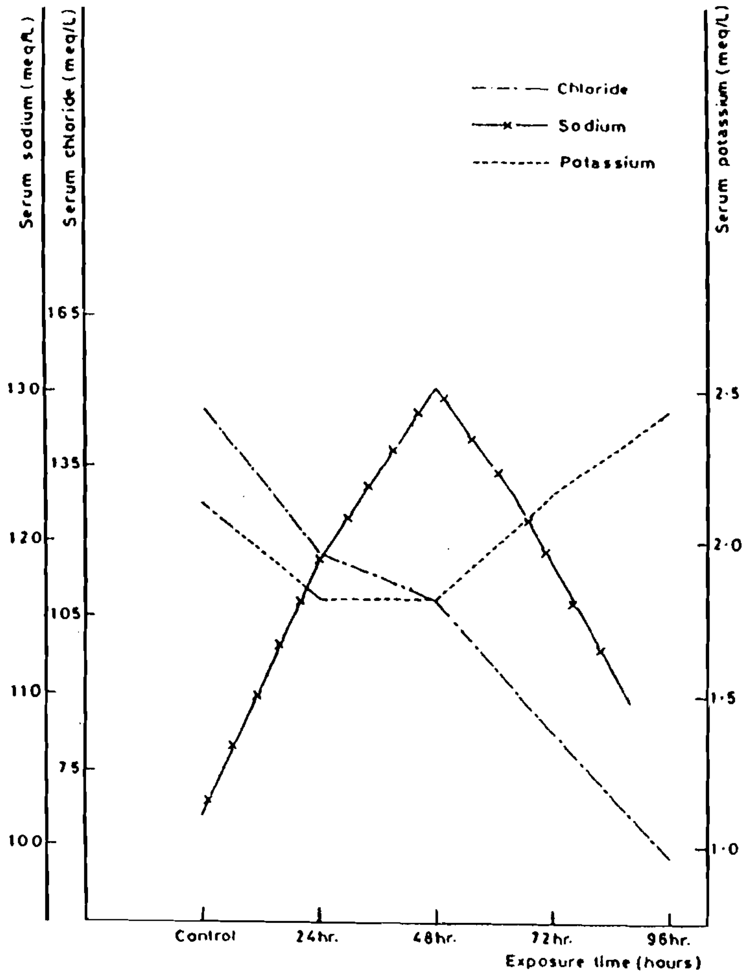

Fig. (3)
Serum sodium, potassium and chloride values of juvenile
Clarias lazera exposed to 3.2 mg/l cu^{+2}
(as copper sulphate) for 96 h.

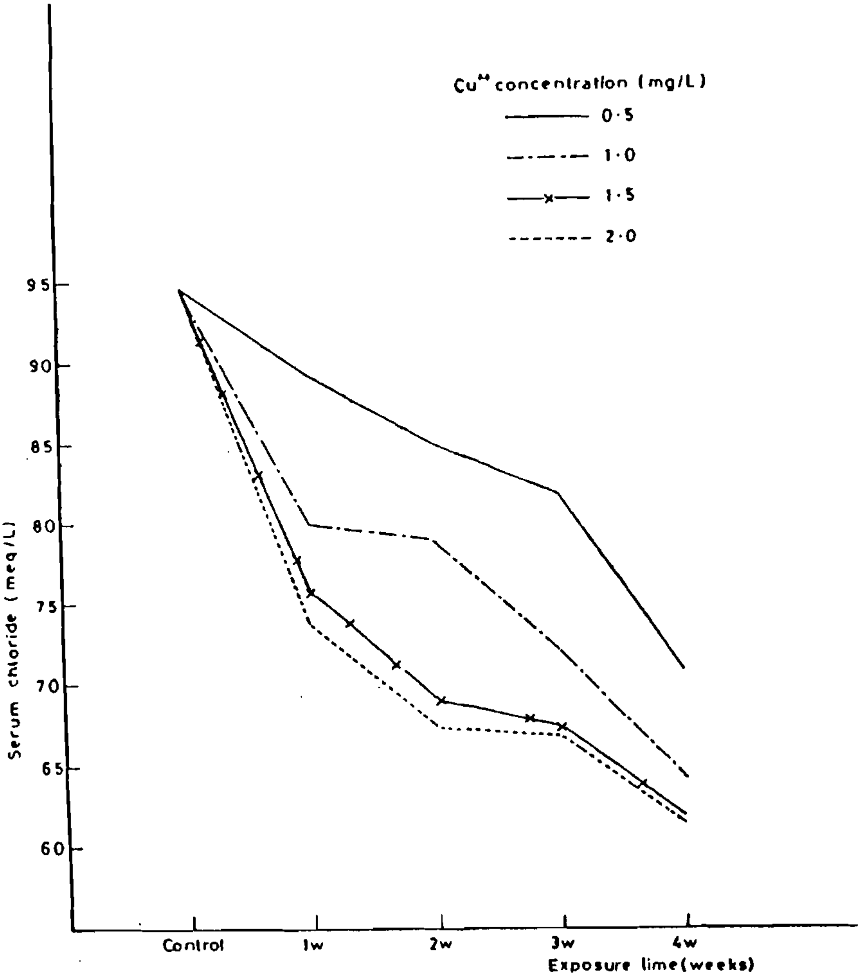

Fig. (4)
Serum chloride concentration of juvenile **Clarias lazera** exposed to elevated concentrations of cu^{+2} (as copper sulphate) for 4 weeks.

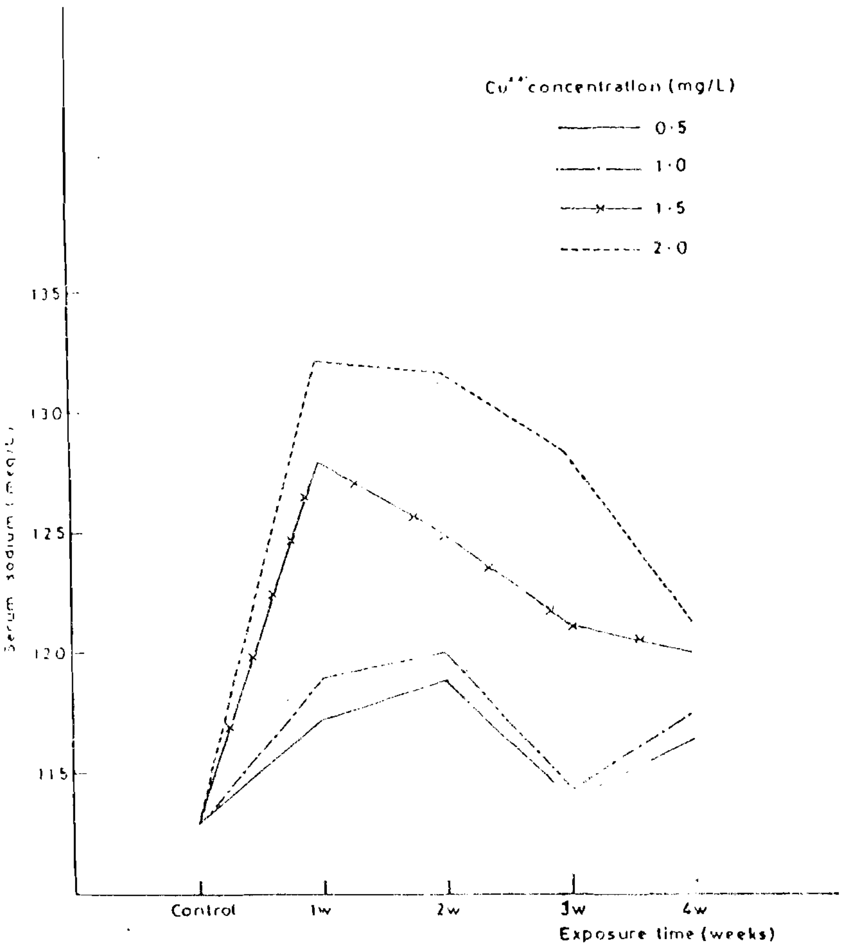

Fig. (5)
Serum sodium concentration of juvenile **Clarias lazera** exposed to elevated concentration of cu^{+2} (as copper sulphate) for 4 weeks.

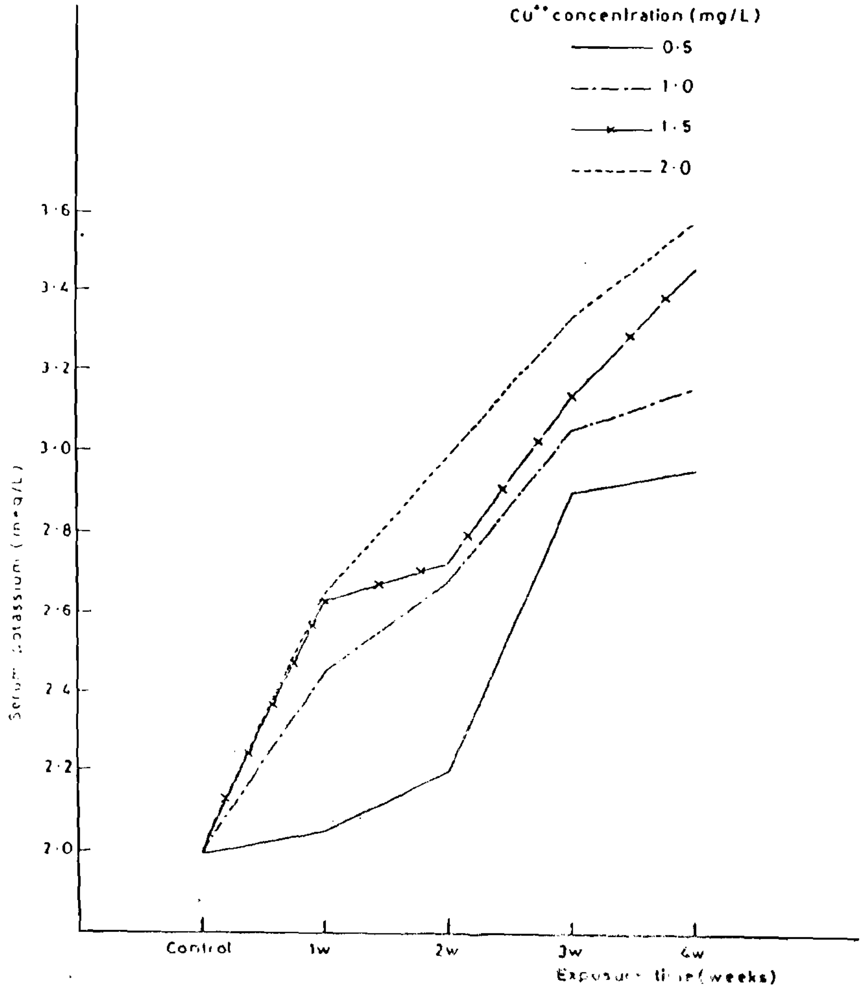

Fig. (6)
Serum potassium concentration of juvenile **Clarias lazera** exposed to elevated concentrations of cu^{+2} (as copper sulphate) for 4 weeks.

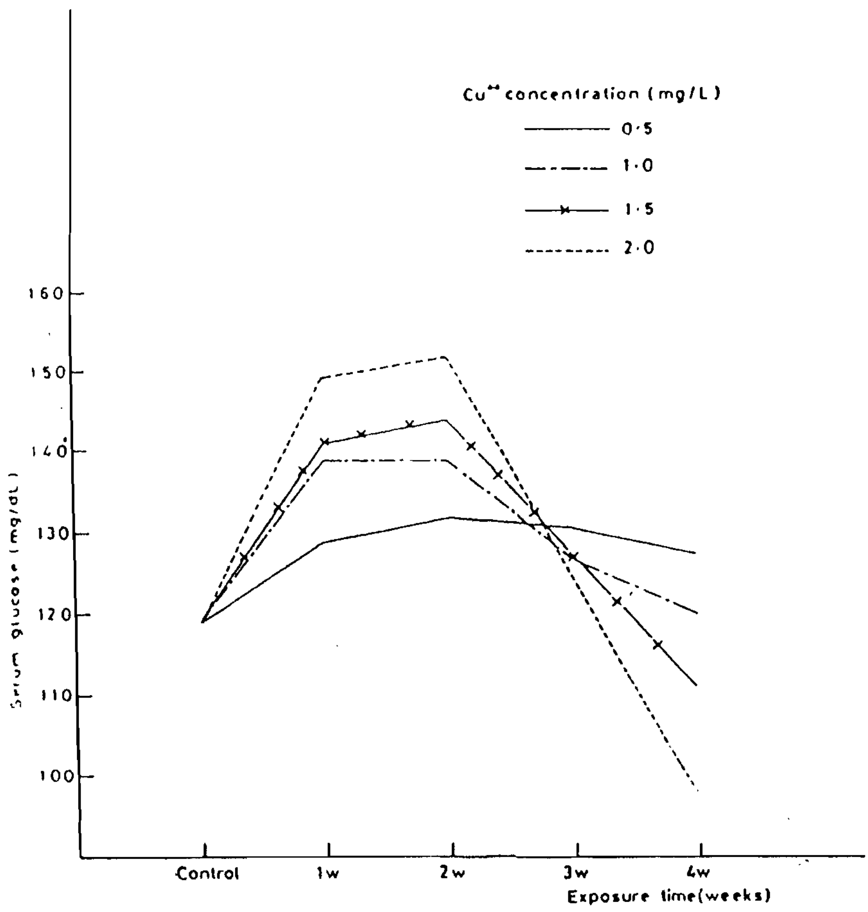

Fig. (7)
serum glucose concentrations of juvenile **Clarias lazera** exposed to elevated concentrations of cu^{+2} (as copper sulphate) for 4 weeks.

molecular weight protein metallothionein is synthesized in livers of mammals on exposure to cadmium, copper, zinc and mercury; Metallothionein - like proteins have also shown to exist in teleosts, and the quantity of this protein increases following administration of cadmium (Overnell and Coombs, 1979), mercury (Bouquegneau et al., 1975), zinc (Marafante, 1976) and copper (McCarter et al., 1982).

The decline observed in serum total protein concentration after three weeks exposure to copper probably represents an adaptation response to the stressor. However, the secondary rise (after four weeks) may indicate that the fish is unable to adapt successfully.

A decrease in serum chloride levels of **Clarias lazera** acutely and subacutely exposed to copper was observed in this study. Similar hypochloremia was obtained by Christensen et al. (1972) in brown bullhead (**Ictalurus nebulosus**) and McKim et al. (1970) in brook trout (**Salvelinus fontinalis**) after short term exposure to copper.

Hypochloremia was found to be usually associated with the stress response of fresh water fish (Mazeaud et al., 1977).

That exposure of fishes to chemical or physical stresses prompts adrenal responses is well established. Exposure to stress results in the release of ACTH from the pituitary gland which stimulates the synthesis and release of cortisol from the inter-renal gland, the homologue of mammalian adrenal. Donaldson and Dye (1975) working on sockeye salmon (**Oncorhynchus nerka**) reported a rapid corticosteroid stress response to lethal and sublethal concentrations of copper. The hypochloremia reported here, is therefore a stress response which may result from increasing levels of cortisol elicited by copper.

Injection of cortisol was found to decrease both plasma sodium and potassium in fresh water rainbow trout (Holmes and Butler, 1963), and goldfish (Uminger and Gist, 1973). Conversely, the concentrations of both sodium and potassium during acute and subacute exposure of **Clarias lazera** to copper were higher than anticipated. We may suggest that **Clarias lazera** possess an active mechanism to compensate for the losses of sodium and potassium. An increased influx of ions through the gills (Randall et al., 1972), or mobilization of these ions from body stores (Stanley and Colby, 1971) is a possible mean.

Blood sugar levels are elevated in fish during acute exposure to a variety of environmental alterations considered as stressful (Miles et al., 1974; Strange, 1980) including exposure to toxicants (Watson and McKeown, 1976; Hilmy et al., 1980; Farouzia, 1982).

Stressful stimuli elicit rapid secretion of glucocorticoids (Fagerlund, 1967; Wedemeyer, 1969) and catecholamines (Nakano and Tomlinson, 1967) from the adrenal tissue of fish. Both of these groups of hormones produce a rapid hyperglycemia (Young and Chavin, 1965; Oguri and Nace, 1966).

It is speculated that acute exposure of **Clarias lazera** to copper caused a stress induced, hormonally mediated elevation of serum glucose levels.

In all the subacute studies, the magnitude of disturbance to glucose levels was considerably less than that caused by acute exposure of **Clarias lazera** to copper.

Selye (1950) has categorized the mammalian stress response into three stages: the alarm stage, the stage of resistance and the stage of exhaustion.

It appears that the initial increase in serum glucose levels during the first two weeks of exposure to copper typifies the alarm stage in Selye's pattern of response to stress. The fish did not reach the stage of exhaustion, rather, they were able to accomodate and adapt to the stressor during the third and fourth weeks.

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