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Copper Induced Metabolic Changes in *Sunetta scripta* (Bivalvia): Oxygen Uptake and Lactic Acid Production

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Physiological parameters such as oxygen consumption, filtration rate, ciliary activity, etc. have been recognized as measures of metabolic change (Manley 1983) but such measurements may be incomplete in themselves in that bivalves respond to stress by complete/partial closure of shell valves and temporary shift to anaerobic metabolic pathways (de Zwaan 1983). The switching of these pathways occurs at phosphoenolpyruvate (PEP), which is broken down to pyruvate by pyruvate kinase aerobically, but to oxaloacetate by PEP carboxykinase during anaerobic metabolism (Ebberink et al. 1979). Alanine, volatile fatty acids such as succinate, propionate and acetate, and an entirely new class of end products - alanopine and strombine have also been shown to accumulate during air exposure and anoxia (Schulz et al. 1982; Nichitta and Ellington 1983). These pathways are characterised by a much reduced Pasteur effect and when compared with the "classical lactate pathway", energy yield is increased but the rate of energy production is decreased (de Zwaan 1983).

The objective of this investigation was to study the extent of artificial anoxia/hypoxia experienced by *Sunetta scripta* on exposure to sub-lethal concentration of copper and to relate oxygen consumption rates to lactic acid production in selected tissues of the clam through 8d of exposure and subsequently 7d of depuration in clean water.

MATERIALS AND METHODS

The specimens of *S. scripta* for experimental use were collected from the clam bed off Fort Cochin, S.W India (10°00'N, 76°31'E) transported to the laboratory, and acclimated for 4d in recirculating seawater tanks fitted with biological filters (salinity 30ppt,

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Send reprint requests to K Y Mohammed Salih at the above address temperature 28±1°C). Clams of 35-40-mm shell length were selected for the experiment. On the 5th day, 55 clams were transfered to each of eight 15-litre tanks. The tanks were filled with filtered seawater and two tanks each were dosed with 0.3, 0.5 and 1.0 mg/L Cu. Analar grade copper sulphate(Glaxo Labs., Bombay) was used. The concentrations (viz., 0.3, 0.5, 1.0 mg/L Cu) were such that they were close to realistic levels as possible and yet able to elicit measurable responses in the clam. Two tanks were the controls and contained clean filtered seawater. The test medium was renewed daily with Cu dosed seawater during the first 8d and subsequently with toxicant free seawater till the termination of the experiment. Feeding was stopped during the experimental period and the tanks were monitored continuously to ensure that non-experimental parameters viz., temperature, salinity, oxygen etc., were at or near optimum level.

Six clams each, pre-exposed to the respective Cu concentrations for periods of 24, 72, 120 and 168 hr and subsequently 24, 72, 120 and 168 hr after transfer to clean water, were used to record oxygen consumption. Each animal was placed in a 250-mL beaker containing 200 mL of test solution, along with a control group. To avoid gaseous exchange the water column of the respiratory chamber was sealed with inert liquid paraffin. A 10-mL glass syringe was used to draw an initial water sample 15 min after the introduction of the clam and a final sample was drawn from the chamber 30 min (in the case of active clams) to 3hr later (in the case of less active clams) as the case may be. Oxygen content of the water was determined using the Winkler method (Welsh and Smith 1953). After the experiment the animals were dissected and the fresh weight (FW) of the soft tissues determined. Oxygen consumption was expressed as μLO_2/g FW/hr.

In a parallel experiment and at the same time intervals, adductor muscle and digestive gland from 6 clams of each experimental group and control were dissected out, weighed and homogenized in 3 mL of 5% cold trichloroacetic acid. The homogenate was centrifuged at 1040 g for 15 min and the supernatant was used for lactic acid determination following the method of Barker (1957). Lactic acid was converted quantitatively into accetaldehyde and the purple color formed with p-hydroxydiphenyl was measured colorimetrically. The results were expressed as μg lactic acid/g FW of tissue.

Analysis of variance test (Zar 1974) was employed to compare all mean values of the experimental bivalves and control group and the 'two-tailed t-test' (Zar 1974) was employed to determine statistical significance of difference in values of lactic acid in tissues and oxygen consumption, under normal and experimental conditions.

RESULTS AND DISCUSSION

In 1.0 mg/L exposed clams, siphonal activity and shell valve movements were terminated almost immediately on introduction to the Cu dosed seawater, and oxygen consumption (Table 1) showed a significant ($P<0.05$) reduction in comparison with clams of the control and 0.3 mg/L Cu dosed groups. The clams exposed to 0.5 mg/L Cu showed a significant ($P<0.05$) reduction of oxygen consumption at 120 and 168 hr, as compared to the control and 0.3 mg/L Cu exposed clams. At 24 hr after the start of depuration, oxygen consumption of clams previously exposed to 1.0 mg/L of Cu was significantly higher ($P<0.05$) than that of clams previously exposed to lower concentrations and the control. Thereafter at 72, 120 and 168 hr of depuration, oxygen consumption of control and experimental clams showed no significant differences.

Lactic acid in the adductor muscle of 0.5 mg/L Cu dosed clams showed a significantly higher value (27.29 μg/g FW ; $P<0.05$) than control clams (21.59 μg/g FW) at 24 hr of exposure. A statistically significant ($P<0.05$) increase in lactic acid levels was also noticed in the digestive gland (37.84 μg/g FW) and adductor muscle (29.16 μg/g FW) of clams previously exposed to 1.0 mg/L Cu, 24 hr after the start of depuration (levels in controls being 30.62 μg/g FW and 22.74 μg/g FW respectively). At all other sampling times during exposure and recovery, levels of lactic acid in experimental clams were not significantly different from the control.

Thampuran (1986) reported 100% survival of *S. scripta* under identical conditions at 6.0 mg/L Cu but physiological and biochemical responses are evidently initiated at much lower concentrations. Shell valve adduction to overcome stress is ubiquitous among the bivalves (Manley 1983). Copper ions are also known to precipitate respiratory and vascular depression (Scott and Major 1972). Mclusky and Philips (1975) from studies on *Phyllodace maculata* suggested that the rate of uptake of a toxicant rather than the actual amount accumulated may be the deciding factor determining toxicity of a solution. At the lowest concentration used, Cu entering into the tissues may have been easily metabolized and detoxified. At 0.5 mg/L added Cu, the detoxification mechanism may probably have been saturated at the later exposure times and hence reduced ventilation effectively prevents toxic influx of Cu into the tissues. It is also probable that at 1.0 mg/L added Cu, the rate of influx

Table 1. Sequential changes in oxygen uptake of the clam *Sunetta scripta* during sublethal exposure to copper and recovery (N = 6)

Concentration of copper ions	Oxygen uptake μL O_2 /g FW/hr*							
	Exposure time				Recovery time			
	24hr	72hr	120hr	168hr	24hr	72hr	120hr	168hr
Control	55.33 ±23.85	63.52 ±27.26	57.61 ±35.23	48.43 ±37.16	57.72 ±29.41	51.73 ±32.77	62.85 ±19.29	54.68 ±24.11
0.3ppm	58.25 ±29.16	74.76 ±32.16	61.54 ±32.88	52.17 ±21.36	59.47 ±26.19	49.45 ±21.32	57.62 ±23.70	59.76 ±27.80
0.5ppm	49.47 ±25.28	57.24 ±26.19	10.91** ±8.70	7.43** ±2.11	55.29 ±34.84	55.43 ±26.37	57.24 ±27.66	62.58 ±30.59
1.0ppm	7.21** ±2.77	6.94** ±2.16	6.87** ±1.82	6.91** ±2.29	74.24** ±31.16	64.91 ±29.36	55.32 ±21.15	55.32 ±27.64

* Each value is a mean ± 1 S.D

** All values significantly different from the control ($P < 0.05$)

is greater than the detoxifying capacity and hence shell closure is the immediate response. Any oxygen consumption at the higher concentration may be ascribed to "testing behaviour", (Manley 1983), when shell valves show intermittent movement and the animal samples the ambient water. Prolonged shell valve adduction in the 1.0 mg/L Cu exposed clam results in an increase in oxygen consumption rates on introduction to clean water. This increase probably facilitates repayment of the "oxygen debt" (de Zwaan 1977) incurred by the animal during shell closure. In the 0.5 mg/L pre-exposed clams, increased oxygen consumption may not have been detected, the "oxygen debt" being compensated within 24 hr of recovery.

A reduction in oxygen availability caused by the shell closure response would necessitate a conversion to anaerobic pathways of energy metabolism. In this study, metal induced hypoxia did not result in a significant increase in lactic acid levels in digestive gland or adductor muscle of *S. scripta* at all exposure concentrations, except in the adductor muscle of 0.5 mg/L exposed clams. The reason for this exceptional value is not clear. Analytical error cannot be ruled out though care was taken to adhere to analytical specifications.

The absence of lactate accumulation and the capacity for long term anaerobiosis supports the theory of the alternative glycolytic pathway in *S.scripta*. High concentrations of carbohydrate reserves in *S.scripta* (unpublished) may be an adaptation to anaerobiosis. Glycolysis proceeds till phosphoenolpyruvate (PEP) which acts as the branch point for pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEP-CK). de Vooys (1980) suggested that a low ratio of PK to PEP-CK activity may indicate increased anaerobic capacity. A drop in pH together with other factors effectively inhibits PK acitivity and results in a switch to the PEP-CK pathway (de Zwaan 1977). Cu ions also inhibit PK activity by binding to NH_2 or SH ligands at or near the active site or by direct competition with Mg or Mn ions for ADP (Cortesi et al. 1985). The funneling of carbon through the PEP-CK pathway may be a dose dependent response to Cu by PK.

Propionate production (6.71 moles of ATP, de Zwaan 1983) is intermediate in energy yield between lactate (2 moles) and aerobic respiration (37 moles). Despite the lower energetic efficiency there was no clear indication of increased carbohydrate utilization in a companion experiment. A possible explanation is that under oxygen stress ATP utilization in bivalves may be as low as 5% of the aerobic level of metabolism (de Zwaan 1983).

Increased ventilation and muscular activity on removal of the stress factor can be considered a possible explanation for the significant lactic acid level in the tissues of the 1.0 mg/L pre-exposed clams at 24 hr of depuration. de Zwaan et al.(1983) suggest that elevated energy demands during recovery outstrip the aerobic capacity of the tissue and consequently there is elevated glycolytic flux. Coupling of pyruvate reductases with glyceraldehyde 3-phosphate dehydrogenases results in NAD being constantly replenished while lactate or opines accumulate (Gade and Grieshaber 1986). Lactic acid from other sites transported for reutilization may also account for the higher values in digestive gland.

Capacity for mobilisation of carbon via anaerobic pathways depending upon the tissue oxygen supply may represent an evolutionary process of adaptation, enabling the animal to occupy unfavorable environments with variable oxygen content. Muley et al. (1987) found the wedge clam *Donax cuneatus* to respond initially to pesticide exposure by valve closure, but later the valves were opened leading to mortality. The onset of the summer monsoon considerably lowers the salinity of *S. scripta* beds from the optimum at 30 ppt for extended periods. Larger clams show less survival at the lowered salinities than juveniles. It is interesting to speculate that the latter may have a more efficient mechanism to survive anaerobiosis. Such a surmise is well worth investigation.

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