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CHANGES IN RESPIRATION AND IONIC CONTENT IN TISSUES OF FRESHWATER MUSSEL EXPOSED TO METHYL PARATHION TOXICITY

(Key words not submitted)

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SUMMARY

Oxygen consumption and sodium, potassium and calcium concentration were determined in the freshwater mussel, *Lamellidens marginalis*, and its tissues during methyl parathion (MP) toxicity. A transient increase followed by decrease in whole animal or tissue respiration as a function of time was observed. Greater loss of calcium by the mantle, sodium by the gill, and calcium and sodium by the hepatopancreas and foot was observed. As compared to calcium and sodium, the loss of potassium by the tissues was less.

INTRODUCTION

A certain degree of water pollution resulting from application of pesticides will continue into the foreseeable future [1]. OP pesticides are widely used because of their biodegradability [2]. Inhibition of AChE by OP pesticides was found to lead to a series of physiological changes in animal systems [3]. Also, they were found to alter protein, nucleic acid, carbohydrate and lipid metabolism, adrenocortical and respiratory functions in non-target organisms [4–8]. Inhibition of oxygen uptake by tissues was found to decrease mitochondrial oxidation of succinate, malate and glutamate during OP toxicity in fish and rat [6, 9, 10]. Decrease in ciliary activity and oxidative metabolism as a consequence of OP pesticide toxicity prompted the

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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; MP, methyl parathion; OP, organophosphorus.

investigators to study the changes in respiration and ionic contents in the tissues of freshwater mussels exposed to MP [11]. These mussels are suitable indicators for toxic assessment because they are stationary, hence their exposure levels can be easily measured. As filter feeders, they can potentially accumulate large amounts of dissolved or suspended materials. Mussels are particularly relevant test species because of their economic value as staple food in certain localities of South India and other areas of the world.

MATERIALS

Freshwater mussels, *Lamellidens marginalis* (30 ± 5 g), were collected from local freshwater ponds and fed ad lib with plankton. Prior to use they were acclimatized to laboratory conditions for 1 week and starved 24 h prior to experimentation [12]. The LC_{50} (25 ppm) was determined by the method of Bayne et al. [13]. Mussels exposed to sublethal concentration (8 ppm) of MP for 6, 12, 24, 36, 48 or 72 h were used for the determination of oxygen (O_2) consumption. Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) levels were estimated in hepatopancreas, foot, mantle and gill tissues of the mussels exposed to MP for 24, 48 or 72 h.

METHODS

Whole animal O_2 consumption was estimated by Winkler's iodometric method [14] and tissue respiration was measured by a manometric method [15] in a Warburg respirometer. The cations, Na^+, K^+ and Ca^{2+} were estimated by the method of Dall [16] in a flame photometer.

TABLE I

CHANGES IN RESPIRATION OF MUSSEL (ml O_2 CONSUMED/h) AND ITS TISSUES (μl CONSUMED/g WT WEIGHT/h) EXPOSED TO SUBLETHAL CONCENTRATION OF METHYL PARATHION

Animal/ tissue	Control	Time of exposure (h)						
		6	12	18	24	36	48	72
Whole animal	3.38 ± 0.26	3.53 ± 0.31 (+4.44)	3.78 ± 0.28[c] (+11.8)	3.27 ± 0.28 (−3.25)	2.96 ± 0.29[c] (−12.4)	2.70 ± 0.25[a] (−20.1)	2.51 ± 0.26[a] (−25.7)	2.28 ± 0.21[a] (−32.5)
Hepato-pancreas	267 ± 10.1	282 ± 19.6 (+5.62)	290 ± 16.8[c] (+8.61)	263 ± 18.6 (−1.50)	244 ± 14.9[c] (−8.61)	226 ± 9.93[a] (−15.4)	207 ± 10.1[a] (−22.5)	185 ± 9.32[a] (−30.7)
Foot	248 ± 12.6	250 ± 20.1 (+0.81)	258 ± 19.8 (+4.03)	243 ± 20.2 (−2.02)	229 ± 19.6 (−7.66)	222 ± 15.6[b] (−10.5)	197 ± 10.3[a] (−20.6)	186 ± 11.3[a] (−25.0)
Mantle	328 ± 24.6	346 ± 15.0 (+5.49)	339 ± 19.5 (+3.35)	325 ± 25.6 (−0.91)	308 ± 26.5 (−6.10)	295 ± 19.6[c] (−10.1)	270 ± 19.8[a] (−17.7)	254 ± 15.5[a] (−22.6)
Gill	382 ± 10.9	409 ± 20.8[c] (+7.07)	420 ± 25.6[b] (+9.95)	374 ± 26.4 (−2.10)	331 ± 26.9[b] (−13.3)	311 ± 20.9[a] (−18.6)	289 ± 19.9[a] (−24.3)	255 ± 15.9[a] (−30.6)

Each value is ± SD of six individual observations. Values in parentheses are % changes over control. [a]$P<0.001$; [b]$P<0.01$; [c]$P<0.05$.

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/h) AND ITS TISSUES (μl CONSUMED/g WT
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	36	48	72
5 ± 0.29[c]	2.70 ± 0.25[a]	2.51 ± 0.26[a]	2.28 ± 0.21[a]
12.4)	(−20.1)	(−25.7)	(−32.5)
± 14.9[c]	226 ± 9.93[a]	207 ± 10.1[a]	185 ± 9.32[a]
3.61)	(−15.4)	(−22.5)	(−30.7)
± 19.6	222 ± 15.6[b]	197 ± 10.3[a]	186 ± 11.3[a]
7.66)	(−10.5)	(−20.6)	(−25.0)
± 26.5	295 ± 19.6[c]	270 ± 19.8[a]	254 ± 15.5[a]
.10)	(−10.1)	(−17.7)	(−22.6)
± 26.9[b]	311 ± 20.9[a]	289 ± 19.9[a]	255 ± 15.9[a]
3.3)	(−18.6)	(−24.3)	(−30.6)

theses are % changes over control. [a]$P<0.0$

RESULTS AND DISCUSSION

Rates of O_2 consumption by whole mussel or tissue obtained during MP exposure are presented in Table I. During 6 to 12 h exposure to MP, there was a slight increase in O_2 consumption by the treated mussels or the tissues studied. Consumption gradually declined to values lower than those of time-matched controls with maximum relative decreases observed at 72 h. When percent deviations in rates of O_2 consumption by the tissues were compared, maximum changes were observed in gill tissue, followed by hepatopancreas, mantle, and foot. In general, alterations in O_2 consumptions due to MP exposure followed the same pattern in all tissues studied.

TABLE II

CHANGES IN IONIC CONTENT (μmol/g WET WT. TISSUE) IN TISSUES OF MUSSELS EXPOSED TO SUBLETHAL CONCENTRATION OF METHYL PARATHION

Tissue	Control	Time of exposure (h)		
		24	48	72
Hepatopancreas				
Calcium	0.137 ± 0.009	0.129 ± 0.008 (−5.84)	0.121 ± 0.004[b] (−11.7)	0.102 ± 0.009[a] (−25.6)
Sodium	0.128 ± 0.009	0.120 ± 0.008 (−6.25)	0.109 ± 0.009[b] (−14.8)	0.094 ± 0.008[a] (−26.6)
Potassium	0.095 ± 0.008	0.088 ± 0.066 (−7.37)	0.083 ± 0.006[c] (−12.6)	0.078 ± 0.006[b] (−17.9)
Foot				
Calcium	0.082 ± 0.005	0.081 ± 0.005 (−1.22)	0.073 ± 0.005[c] (−11.0)	0.065 ± 0.004[a] (−20.0)
Sodium	0.089 ± 0.006	0.084 ± 0.005 (−5.62)	0.077 ± 0.006[b] (−13.5)	0.068 ± 0.006[a] (−23.6)
Potassium	0.053 ± 0.003	0.048 ± 0.003[c] (−9.43)	0.047 ± 0.004[c] (−11.3)	0.047 ± 0.003[b] (−11.3)
Mantle				
Calcium	0.158 ± 0.012	0.149 ± 0.011 (−5.70)	0.132 ± 0.011[b] (−16.5)	0.115 ± 0.008[a] (−27.2)
Sodium	0.058 ± 0.005	0.055 ± 0.003 (−5.17)	0.050 ± 0.003[b] (−13.8)	0.045 ± 0.003[a] (−22.4)
Potassium	0.085 ± 0.005	0.080 ± 0.004 (−5.88)	0.076 ± 0.005[c] (−10.6)	0.070 ± 0.005[a] (−17.6)
Gill				
Calcium	0.067 ± 0.004	0.064 ± 0.005 (−4.48)	0.062 ± 0.004 (−7.46)	0.059 ± 0.003[b] (−11.9)
Sodium	0.169 ± 0.012	0.157 ± 0.011 (−7.10)	0.141 ± 0.009[b] (−16.6)	0.117 ± 0.009[a] (−30.8)
Potassium	0.064 ± 0.003	0.062 ± 0.003 (−3.13)	0.058 ± 0.004[c] (−9.37)	0.055 ± 0.003[a] (−14.1)

Each value is ± SD of six individual observations. Values in parentheses are % changes over control. [a]$P<0.001$; [b]$P<0.01$; [c]$P<0.02$; [d]$P<0.05$.

Levels of Na^+, K^+ and Ca^{2+} were estimated in tissues of mussels exposed to MP for 24, 48 or 72 h (Table II). No changes were observed following 24 h exposure, however, 48-h and 72-h groups had significant decreases in levels of Na^+, K^+ and Ca^{2+}. Decreases in Na^+ were more pronounced in gills, whereas K^+ and Ca^{2+} decreased primarily in mantle and hepatopancreas, respectiveiy. The changes in ionic content in general were parallel with the changes in O_2 consumption as a function of time during MP exposure.

Oxygen consumption of an animal reflects basal metabolic status, which may be altered by environmental stress. A slight transient increase in O_2 consumption for 6–12 h followed by gradual decrease as a function of time is a finding peculiar to the present study. The initial increase in O_2 consumption may be an indication of mobilization of metabolic reserves in response to immediate toxic effects. Decrease in O_2 consumption after 12 h may be due to the increased flow or accumulation of MP as a function of time. Based on earlier studies and the present study, rates of O_2 consumption inhibition by fish and molluscs exposed to various OP compounds are as follows: malathion > foschlar > MP > dichlorvos [17].

Oxygen consumption of tissues which account for the decreased respiratory activity of the whole animal indicate that compared to hepatopancreas and gill, the response of the mantle or foot in initial periods of exposure was less and the latter was found to develop edema rapidly. Similar decreases in O_2 uptake in vitro were observed in livers of fish exposed to parathion and malathion [9]. Responses of hepatopancreas and gill in general were found to be similar. Maximum inhibition in O_2 uptake by gills may be related to intimate contact with MP in water. The possibility of altered permeability of gill tissue following MP exposure should also be considered. As reported earlier, formation of a coagulation film as a consequence of pesticide exposure results in the changes in differential characteristics of respiratory epithelium [18]. O'Brien [3] proposed inhibition of AChE by OP compounds results in accumulation of ACh with in turn increases the vascular resistance thereby directing the blood away from the secondary lamellae resulting in reduced O_2 transport capacity.

Apart from O_2 consumption, osmoregularity of tissues plays an important role in regulation of cellular metabolism and its imbalance will lead to various physiological changes. Decreases in ionic content of tissues as a consequence of MP toxicity suggest changes in permeability properties of membranes and/or inhibition of Na^+, K^+ and Ca^{2+} ionic pumps. The greater loss of Ca^{2+} by mantle, Na^+ by gill, and Ca^{2+} and Na^+ by hepatopancreas and foot indicate differential responses of tissues to the induced MP stress. Compared to Ca^{2+} and Na^+, the loss of K^+ ions was minor. Decreases in subcellular Ca^{2+} in the present study correlate with the structural and functional changes in mitochondria which are the sinks for this ion [19]. The maximum decrease in the Ca^{2+} of mantle as compared to other tissues could be attributed to increased decalcification.

Changes in ionic contents could be correlated to the O_2 consumption of tissues

during MP stress. Decreased O_2 consumption results in reduced energy production which in turn affects the energy dependent transport of ions and the water permeability system, as evidenced by edema in the foot of mussels during MP exposure.

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