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In vivo sub-acute physiological stress induced by phosphamidon on carbohydrate metabolism in phasic and tonic muscles of penaeid prawn, *Penaeus indicus* (H Milne Edwards) during acute and chronic exposure

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Abstract. Penaeid prawn, *Penaeus indicus* (H Milne Edwards), was subjected to acute (2 day) and chronic (15 day) exposure to sublethal concentration (0·2 ppm) of phosphamidon, and changes in the carbohydrate metabolism of phasic and tonic muscles were investigated. Differential patterns in carbohydrate metabolism were witnessed. The glycogen content of phasic muscle was elevated, whereas that of tonic muscle was depleted in response to acute exposure. During chronic exposure, both muscle tissues had elevated glycogen content. In general, both phasic and tonic muscles exhibited low-level of glycolysis and elevated oxidative pathway operation, leading to elevated glycogen content. Both, phasic and tonic muscle metabolisms were oriented towards conservation of carbohydrates and lesser production of organic acids during chronic exposure, as a possible adaptive metabolic mechanism of this prawn enabling it to counteract the toxic stress imposed by phosphamidon.

Keywords. *Penaeus indicus*; phosphamidon; carbohydrate metabolism; glycolysis.

1. Introduction

Despite concern over environmental contamination by pesticides, man continues to depend on the use of pest-control chemicals for increase in agricultural productivity and disease control. Increased use of organophosphate (OP) pesticides due to suspended or cancelled registration of chlorinated hydrocarbon pesticides, generates a need for information on their effects on the aquatic environment (Bookhout and Monroe 1977; Nimmo 1979). OP pesticides may enter surface and estuarine water through industrial effluent, run off, or direct application (Coppage and Matthews 1974). OP insecticides are believed to be potent inhibitors of the enzyme acetylcholinesterase (AchE), which modulates the neurotransmittor substance, acetylcholine, at the nerve cell junctions, i.e., synapses. Inhibition of AchE by OP insecticides and also by their metabolites causes accumulation of acetylcholine and disruption of normal neurotransmission (O' Brien 1967) which will further lead to some physiological and metabolic changes in several non-target organisms, including a variety of crustaceans (Couch 1979). Phosphamidon, like many other OP insecticides, is widely used to control crop pests presumably because it is biodegradable and leaves residues, but for a short time. Eventhough phosphamidon decomposes rapidly in the environment, it may be toxic to target and non-target organisms alike. Since it is relatively a non-persistent insecticide, repeated applications may be necessary for control of pests which may result in cumulative reduction of AchE. Hence, increasing concentrations of phosphamidon have to be used to control pests and the danger to non-target organisms becomes greater (Nagabhushanam *et al* 1982; Srinivasulu Reddy *et al* 1985a, b). The present investigation is aimed at understanding the toxic impact of phosphamidon in phasic

and tonic muscle carbohydrate metabolism of a penaeid prawn, *Penaeus indicus* (H Milne Edwards), after acute and chronic exposure to a sublethal concentration. This prawn is considered to be a sensitive indicator of marine and estuarine pollution (Butler 1966) and also forms one of the commercially important fishery of India.

2. Materials and methods

P. indicus (10 ± 1 g) were collected from Buckingham canal, Thummalapenta seacoast, near Kavali. They were kept in large aquaria with continuous aeration to acclimatize to laboratory condition for 1 week under constant salinity 15 ± 1‰, pH 7·2 ± 0·1 and temperature 23 ± 2°C. They were fed *ad libitum* with oil cake powder. Technical grade phosphamidon [92% w/v; 0, 0–dimethyl–0–(1-methyl–2–chloro–2–diethyl–carbomoyl–vinyl) phosphate] obtained from CIBA-GEIGY, Bombay was used as test chemical. The preparation of test solution was described earlier (Srinivasulu Reddy *et al* 1985a). LC_{50} value (computed as per Finney 1964) was found to be 1·2 ppm for 48 h. The laboratory-acclimated intermoult prawns were divided into 3 groups viz control and acutely and chronically exposed. The control prawns were maintained in seawater without test chemical. Prawns were exposed to 0·2 ppm for acute (2 day) and for chronic (15 day) exposure, respectively. After 50 prawns were sacrificed, phasic and tonic muscles were isolated and rapidly chilled by placing them in cold crustacean ringer. These tissues were used for biochemical analysis.

Glycogen (Carrol *et al* 1956), pyruvic acid (Friedmann and Hangen 1942), and lactic acid (Barker and Summerson 1941, modified by Huckabee 1956) were estimated in phasic and tonic muscles of control and phosphamidon exposed (PE) prawns. The activities of phospharylase '*a*' and '*ab*' were estimated in the direction of glycogen synthesis (Cori *et al* 1955). Homogenate (5%) was prepared in an aqueous medium containing 0·037 on ethylene diamine tetraacetic acid (EDTA) and 0·1 M sodium fluoride, pH 6·5, as recommended by Guillary and Mammaerts (1962). The homogenate was centrifuged for 15 min at 2500 rpm and the supernatant was diluted 4 times with cysteine (0·03 M), B-glycerophosphate (0·015 M) buffer, pH 6·5. The diluted enzyme (0·4 ml) was added to 0·2 ml of 2% glycogen and incubated for 20 min at 37°C. The reaction was started by the addition of 0·2 ml of 0·016 M glucose-1-phosphate (G-1-P) to one tube (Phosphorylase '*a*'), 0·2 ml of G-1-P and 0·004 M adenosine-5-monophosphate to the other (phosphorylase '*ab*'). After incubation for 15 min for phosphorylase '*ab*' (total) and 30 min for phosphorylase '*a*' (active) activities, the reaction was stopped by the addition of 10% sulphuric acid. The inorganic phosphate (Pi) liberated was estimated by the method of Fiske and Subba Row (1925) and Protein by the method of Lowry *et al* (1951). Aldolase activity was estimated by the method of Bruns and Bergmayer (1965). The activity levels of succinate dehydrogenase (SDH), NAD-glutamate dehydrogenase (GDH), and NAD-lactate dehydrogenase (LDH) were estimated by the method Nachlas *et al* (1960). The activity level of NADP glucose-6-phosphate dehydrogenase (G-6-PD) was estimated by the method of Georg and Waller (1965). The statistical correlations were conducted using Students '*t*' test as described by Bailey (1965).

3. Results and discussion

The data presented in tables 1 and 2 illustrate the metabolic variations in phasic and tonic muscles of prawn subjected to acute exposure to a sublethal concentration of phosphamidon. With acute exposure, glycogen content was depleted (23%) in tonic muscle; phasic muscle had (28%) elevation in glycogen content. Phosphorylase '*ab*' was depleted in both phasic (26%) and tonic (45%) muscles. Phosphorylase '*a*' was inhibited (28%) in tonic muscle, with no significant change (3%) in phasic muscle. Phosphorylase '*b*' was depleted by 47% and 39% in tonic and phasic muscles, respectively. Aldolase activity was depleted in phasic (23%) and tonic (52%) muscle, whereas NAD–LDH was elevated by 36% in phasic and 50% in tonic muscles. In tonic muscle, pyruvic acid content was elevated by 24% and lactic acid content showed no significant change. Phasic muscle showed no significant change in pyruvic acid content, whereas lactic acid content was depleted (27%). Glucose-6-phosphate dehydrogenase (G-6-PDH) activity levels were decreased by 12% in tonic muscle and 23% in phasic muscle. GDH activity was elevated in both the muscles. Both SDH and malate dehydrogenase (MDH) activities were elevated in tonic and phasic muscles after acute exposure to phosphamidon.

The tonic and phasic muscles of chronically-exposed prawns (tables 3 and 4) showed significant elevation in the glycogen content, the maximum being in phasic muscle (64%). The activity levels of Phosphorylase '*ab*' and '*b*' were significantly depleted in both the muscles, but phosphorylase '*a*' was significantly elevated. Aldolase activity was inhibited by 70% in tonic muscle, but was elevated by 28% in phasic muscle. The levels of pyruvic acid and lactic acids were decreased in both PE

Table 1. Levels of glycogen and lactic acid and the activity levels of phosphorylase and aldolase in tonic and phasic muscles of control prawns and those acutely exposed to phosphamidon.

Parameter	Control	Tonic muscle	Control	Phasic muscle
Glycogen[1]	6·14 ± 0·38	4·71 ± 0·22 (−23)	10·18 ± 0·65	13·03 ± 0·68 (+28)
Phosphorylase '*a*'[2]	3·12 ± 0·18	2·25 ± 0·11 (−28)	3·84 ± 0·25	3·71[a] ± 0·22 (−3)
Phosphorylase '*ab*'[2]	10·85 ± 0·46	5·91 ± 0·22 (−46)	12·05 ± 0·65	8·86 ± 0·51 (−26)
Phosphorylase '*b*'[2]	6·92 ± 0·38	3·68 ± 0·21 (−47)	7·78 ± 0·51	4·72 ± 0·34 (−39)
Aldolase[3]	3·89 ± 0·23	1·91 ± 0·10 (−51)	4·13 ± 0·27	3·19 ± 0·20 (−23)
Pyruvic acid[4]	5·94 ± 0·48	7·35 ± 0·49 (+24)	6·25 ± 0·56	5·83[a] ± 0·39 (−7)
Lactic acid[5]	1·33 ± 0·21	1·49[a] ± 0·10 (+12)	1·41 ± 0·20	1·03 ± 0·11 (−27)

Each value is mean of 6 individual observations.
Mean ± SD; + and − indicates per cent increase or decrease over control.
All values are statistically significant at $P < 0{\cdot}001$ except *a*: Not significant.
Values are expressed in 1: mg/gm wet tissue; 2: μ mol pi formed/mg protein/h; 3: μ mol FDP cleaved/mg protein/h; 4: μ mol/g wet tissue; 5: mg/g wet tissue.

Table 2. Levels of glycogen and lactic acid and the activity levels of phosphorylase and aldolase in tonic and phasic muscles of control prawns and those chronically exposed to phosphamidon.

Parameter	Control	Tonic muscle	Control	Phasic muscle
Glycogen[1]	6·23 ± 0·29	9·84 ± 0·31 (+58)	10·32 ± 0·71	16·32 ± 0·83 (+64)
Phosphorylase '*a*'[2]	3·28 ± 0·21	3·91 ± 0·17 (+19)	3·91 ± 0·28	4·69 ± 0·33 (+20)
Phosphorylase '*ab*'[2]	11·21 ± 0·49	4·02 ± 0·13 (−64)	12·71 ± 0·63	3·92 ± 0·26 (−69)
Phosphorylase '*b*'[2]	7·09 ± 0·28	1·85 ± 0·10 (−74)	8·03 ± 0·58	1·44 ± 0·12 (−82)
Aldolase[3]	4·08 ± 0·21	1·22 ± 0·11 (−70)	4·45 ± 0·31	5·69 ± 0·38 (+28)
Phravic acid[4]	6·18 ± 0·31	4·50 ± 0·18 (−27)	6·89 ± 0·33	3·51 ± 0·22 (−49)
Lactic acid[5]	1·45 ± 0·20	1·08 ± 0·09 (−26)	1·52 ± 0·23	0·72 ± 0·08 (−53)

Each value is mean of 6 individual observations.
Mean ± SD; + and − indicates per cent increase or decrease over control.
All values are statistically significant at $P < 0·001$.
Values are expressed in 1: mg/g wet tissue; 2: μ mol pi formed/mg protein/h; 3: μ mol FDP cleaved/mg protein/h; 4: μ mol/g wet tissue; 5: mg/g wet tissue.

Table 3. Activity levels of G-6-PDH, NAD-LDH, GDH, SDH and MDH in tonic and phasic muscles of control prawns and those acutely exposed to phosphamidon.

Enzyme	Control	Tonic muscle	Control	Phasic muscle
G-6-PDH	1·31 ± 0·15	1·15[a] ± 0·10 (−12)	1·40 ± 0·19	1·08 ± 0·09 (−23)
LDH	0·103 ± 0·019	0·155 ± 0·010 (+50)	0·121 ± 0·012	0·165 ± 0·019 (+36)
GDH	0·651 ± 0·049	1·080 ± 0·105 (+66)	0·784 ± 0·056	1·120 ± 0·130 (+43)
SDH	0·375 ± 0·032	0·465 ± 0·041 (+24)	0·856 ± 0·069	0·992[b] ± 0·071 (+16)
MDH	0·068 ± 0·007	0·092 ± 0·005 (+35)	0·135 ± 0·011	0·195 ± 0·014 (+44)
SDH : GDH	0·576	0·431 (−25)	1·09	0·886 (−19)
SDH : LDH	3·641	2·994 (−18)	7·07	6·01 (−15)

Each value is mean of 6 individual observations.
Mean ± SD: + and − indicate per cent increase or decrease over control.
All values are statistically significant at $P < 0·001$ except *a*: $P < 0·02$; *b*: $P < 0·01$.
Values are expressed in μ mol formazan formed/mg protein/h.

Table 4. Activity levels of G-6-PDH, NAD-LDH, GDH, SDH and MDH in tonic and phasic muscles of control prawns and those chronically exposed to phosphamidon.

Enzyme	Control	Tonic muscle	Control	Phasic muscle
G-6-PDH	1·43 ± 0·12	2·32 ± 0·25 (+62)	1·45 ± 0·21	2·28 ± 0·32 (+57)
LDH	0·114 ± 0·007	0·210 ± 0·015 (+84)	0·128 ± 0·014	0·244 ± 0·029 (+91)
GDH	0·662 ± 0·031	1·220 ± 0·109 (+84)	0·803 ± 0·049	1·630 ± 0·321 (+103)
SDH	0·389 ± 0·038	0·595 ± 0·049 (+53)	0·889 ± 0·061	1·190 ± 0·305 (+34)
MDH	0·075 ± 0·006	0·137 ± 0·018 (+83)	0·149 ± 0·014	0·242 ± 0·028 (+62)
SDH:GDH	0·588	0·113 (−81)	1·10	0·73 (−34)
SDH:LDH	3·412	2·833 (−17)	6·94	4·88 (−30)

Each value is mean of 6 individual observations.
Mean ± SD: + and − indicates per cent increase or decrease over control.
All values are statistically significant at $P < 0·001$.
Values are expressed in μ mol formazan formed/mg protein/h.

prawn muscles. NAD–LDH and G-6-PDH activity levels were significantly elevated in both PE prawn muscles. The activity levels of GDH, SDH and MDH were significantly elevated in both PE prawn muscles after chronic exposure.

Phasic and tonic muscles of *P. indicus*, exhibited differential patterns of carbohydrate metabolism following exposure both acutely and chronically to a sublethal concentration of phosphamidon. The glycogen content might have been actively mobilized towards the haemolymph glucose under this pesticide stress to provide maximum energy, similar to observations in different crustacean groups applied to other environmental stress conditions (Bhagyalakshmi *et al* 1983, 1984). Phosphamidon-induced stress may lead to depleted glycogen levels in tonic muscle on acute exposure to phosphamidon. Decreased activities of phosphorylase '*ab*' (total), '*a*' (active) and '*b*' (inactive) in tonic muscle were suggestive of regulation imposed at the metabolic levels to prevent further glycogen degradation. Depletion of aldolase activity was also suggestive of a decreased rate of glycolysis in this tonic muscle. However, the pyruvic acid level in tonic muscle tissues increased in prawns acutely exposed to phosphamidon. The elevated activity level of NAD–LDH, observed in the present study might be responsible for the increased pyruvic acid content (Srinivasulu Reddy *et al* 1985c). Increased activity of alanine amino-transferase (AlAT) in the muscle exposed to phosphamidon, however might also suggest mobility of amino acids towards the formation of pyruvic acid (Srinivasulu Reddy *et al* 1985d). Significantly lower G-6-PDH activity may be attributed to decreased mobilization of glycogen into the hexose monophosphate (HMP) pathway. Activity levels of SDH and MDH were elevated, suggesting stepped up oxidative metabolism in tonic muscle tissues of pesticide exposed prawn. This elevation in the oxidative phase might be due to mobilization of amino acids as revealed by elevated GDH and pyruvic acid and as indicated by increased LDH

(Bhagyalakshmi *et al* 1984). SDH:GDH and SDH:LDH ratios were decreased in these muscles indicating that the extent of elevation in SDH did not correlate with elevation of either GDH or LDH, suggesting the possibility of diversion of citric acid intermediates (Huggins and Munday 1968). Hence, in response to acute exposure to a sublethal concentration of phosphamidon, the tonic muscle had inhibited glycolysis with depleted glycogen content.

Phasic muscle however, showed a different trend in that it had a significant elevation in the glycogen content on a acute exposure to phosphamidon. Similar kind of observations were also made by Sreenivasulu Reddy *et al* (1983) in *Oziotelphusa senex senex* during chronic sumithion exposure. Since NAD–LDH and GDH activities were significantly elevated, active mobilization of lactic acid and amino acids into the oxidative metabolism can be envisaged. Phasic muscle metabolism was also oriented towards inhibited glycolysis (Hochachka *et al* 1962) as indicated by decreased aldolase activity leading to elevated glycogen content, (Sreenivasulu Reddy *et al* 1983). In spite of the operation of similar metabolic events in tonic and phasic muscle, the phasic muscle had elevated glycogen content, which might be due to participation of tissue lipid components in metabolism (Huggins 1966; Bhagyalakshmi 1981). The free fatty acid content was depleted in the muscle suggesting the participation of free fatty acids in tissue oxidations. Such a possibility of fatty acid oxidations might be responsible for the suppressed glycolysis leading to elevation in glycogen content (Bhagyalakshmi 1981; Chang and O' Connor 1983).

Muscle metabolism seems to be different in acutely and chronically-exposed prawn, since with chronic exposure to phosphamidon both tonic and phasic muscles had elevated glycogen content with the maximum increase in phasic muscle. Most probably phasic muscle forms a reserve site for nutrients and its increased glycogen content suggests storage of glycogen reserves for general muscular activity (Hohnke and Scheer 1970). In tonic muscle, inhibited aldolase activity in the presence of glycogen suggests metabolic shifts towards pathways other than glycolysis (Dean and Vernberg 1965). Since the activity level of G-6-PDH was considerably elevated, the possible mobilization of glycogen and glucose towards the hexose monophosphate pathway can be visualized. This might be an adaptation toward decreasing formation of lactic and pyruvic acid, aiming for decreased acid production in the phasic and tonic muscles. Hence this suggests tissue compensation at the metabolic level in response to the stress imposed by phosphamidon exposure. Elevated activity levels of LDH and GDH were suggestive of increased mobilization of lactic acid and amino acids into the oxidative metabolism. Consequent on such changes, there were not only depleted lactic acid levels in PE prawn muscles, but also an elevated oxidative phase of metabolism as indicated by SDH and MDH activities. Metabolism of phasic muscle was oriented towards mobilizing glycogen into hexosemonophosphate shunt pathway, since phosphorylase, aldolase and G-6-PDH activities are elevated. Hence both phasic and tonic muscles in chronically exposed prawns have regulated the carbohydrate metabolism towards decreasing the production of metabolic acids.

From the present investigation it can be concluded that during chronic exposure of penaeid prawn to a sublethal concentration of phosphamidon, phasic and tonic muscle metabolism was oriented toward lower production of metabolic acids and increased carbohydrate reserves with switching over to aerobic phase. These modifications in tissue metabolism might represent compensatory mechanisms

leading to adaptive changes which provide positive survival chances in phosphamidon-polluted aquatic environments.

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References

Bailey N T J 1965 *Statistical methods in biology* (Great Britain: The English language book society and the English University Press Ltd.)

Barker S B and Summerson W H 1941 The colorimetric determination of lactic acid in biological material; *J. Biol. Chem.* **138** 535–554

Bhagyalakshmi A 1981 *Physiological studies on the freshwater field crab Oziotelphusa senex senex (Fabricius) in relation to pesticide impact*, Ph.D. thesis, S V University, Tirupati

Bhagyalakshmi A, Sreenivasula Reddy P and Ramamurthi R 1983 Changes in hemolymph glucose, hepatopancreas glycogen, total carbohydrates, phosphorylase and aminotransferases of sumithion stressed freshwater rice field crab, *Oziotelphusa senex senex*; *Toxicol. Lett.* **18** 277–284

Bhagyalakshmi A, Sreenivasula Reddy P and Ramamurthi R 1984 Subacute stress induced by sumithion on certain biochemical parameters in *Oziotelphusa senex senex*, the fresh water rice field crab; *Toxicol. Lett.* **21** 127–134

Bookhout C G and Monroe R J 1977 Effects of Malathion on the development of crabs; in *Physiological responses of marine biota to pollutants* (ed) F J Vernberg, A Calabrese, F P Thunberg and W B Vernberg (New York: Academic Press) pp 3–19

Bruns S H and Bergmeyer H U 1965 in *Methods of enzymatic analysis* (ed) H U Bergmeyer (New York: Academic Press) pp 724–728

Butler P A 1966 The problems of pesticides in estuaries; in *A symposium on estuarine fisheries*; *Am. Fish. Soc.* pp 110–115

Carrol N V, Longlev R W and Roe J H 1956 Glycogen determination in liver and muscle by the use of anthrone; *J. Biol. Chem.* **220** 583–593

Chang E S and O' Connor J D 1983 Metabolism and transport of carbohydrates and lipids; in *Internal anatomy and physiological regulation.* (ed) L H Mantel (New York: Academic Press) vol. 5, pp 163–201

Coppage D L and Mathews E 1974 Short term effects of organophosphate pesticides on cholinesterases of estuarine fishes and Pink Shrimp; *Bull. Environ. Contam. Toxicol.* **11** 483–488

Cori G T, Illingworth B and Keller P G 1955 in *Methods in Enzymology* (eds) S P Colowick and N O Kaplan (New York: Academic Press) **1** 200–213

Couch J A 1979 Shrimps (Arthropoda Crustacea Penaeidae); *Pollution ecology of estuarine invertebrates.* (eds) C W Hart Jr and L H Samuel (New York: Academic Press) 235–255

Dean J M and Vernberg F J 1965 Variations in blood glucose level of crustacea; *Comp. Biochem. Physiol.* **14** 29–39

Finney D J 1964 *Probit analysis* (London: Cambridge University Press)

Fiske C H and Subba Row M 1925 The colorimetric determination of phosphates; *J. Biol. Chem.* **66** 375–379

Friedemann T E and Hangen G E 1942 Pyruvic acid. I. Collection of blood for the determination of pyruvic and lactic acid; *J. Biol. Chem.* **144** 67–77

Georg W L and Waller H D 1965 Glucose-6-Phosphate dehydrogenase (zwischeriferment); in *Methods of enzymatic analysis.* (ed) H U Bergmeyer (New York, London: Academic Press) 744–751

Guillary R J and Mammaerts W F H M 1962 The state of activity of phosphorylase in frog sartorius muscle; *Biochim. Biophys. Acta* **65** 316–325

Hochachaka P W, Teal J M and Telford M 1962 Pathways of carbohydrate metabolism in labster hepatopancreas; *Can. J. Biochem. Physiol.* **40** 1043–1050

Honkhe L and Scheer B T 1970 Carbohydrate metabolism in crustaceans; in *Chemical Zoology* (eds) M Florkin and B T Scheer (New York: Academic Press) Vol. 5, 147–165

Huckabee W E 1956 Control of concentration gradients of pyruvic and lactate across cell membrane in blood; *J. Appl. Physiol.* **9** 163–170

Huggins A K 1966 Intermediary metabolism in *Caricinus maenus*; *Comp. Biochem. Physiol.* **18** 283–290

Huggins A K and Munday K A 1968 Crustacean metabolism; in *Advances in comparative physiology and Biochemistry* (ed) O Lowenstein (New York: Academic Press) Vol. 3, 271–377

Lowry O H, Rosebrough N J, Farr A L and Randall R H 1951 Protein measurements with the folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Nachlas M M, Margulies S I and Seligman A M 1960 A colorimetric method for the determination of succinic dehydrogenase activity; *J. Biol. Chem.* **235** 499–504

Nagabhushanam R, Reddy T S N and Sarojini R 1982 Impact of organophosphates on neurosecretory cells in the cerebral ganglia of freshwater prawn, *Caridena weberi*; in *Proc. All. Ind. Symp. Phy. Res. Ani. Pollut.* (eds) R Sarojini, V R Awad and R Nagabhushanam (Aurangabad: Marathwada University Press) pp 133–138

Nimmo D R 1979 Pesticides: Their impact on the estuarine environment; in *Marine pollution: Functional responses.* (eds) W B Vernberg, A Calabrese, F P Thunberg and F J Vernberg (New York: Academic Press) pp 259–269

O' Brien R D 1967 *Insecticides and metabolism* (New York: Academic Press)

Sreenivasula Reddy P, Bhagyalakshmi A and Ramamurthi R 1983 In vivo subacute physiological stress induced by sumithion on carbohydrate metabolism in hepatopancreas of *Oziotelphusa senex senex* (Fabricius); *Toxicol. Lett.* **13** 179–182

Srinivasulu Reddy M, Narasimha Murthy B and Ramana Rao K V 1985a Toxicity of phosphamidon to palaemonid shrimp *Macrobrachium malcomsonii* – a time course study; *Environ. Ecol.* **3** 278–279

Srinivasulu Reddy M, Narasimha Murthy B, Venkateswarlu Y and Ramana Rao K V 1985b Toxicity of insecticide phosphamidon on tissue carbohydrate catabolism of penaeid prawn, *Penaeus indicus* (H Milne Edwards); *Indian J. Mar. Sci.* **14** 224–225

Srinivasulu Reddy M, Venkateswarlu Y, Narasimha Murthy B and Ramana Rao K V 1985c Alterations in the glycolytic pathway of penaeid prawn, *Penaeus indicus* under phosphamidon induced stress; *Environ. Ecol.* **3** 342–344

Srinivasulu Reddy M, Venkateswarlu Y, Surendranath P and Ramana Rao K V 1985d Changes in the nitrogen metabolism in the selected tissues of a penaeid prawn, *Penaeus indicus* exposed to phosphamidon; *Environ. Ecol.* **3** 500–503