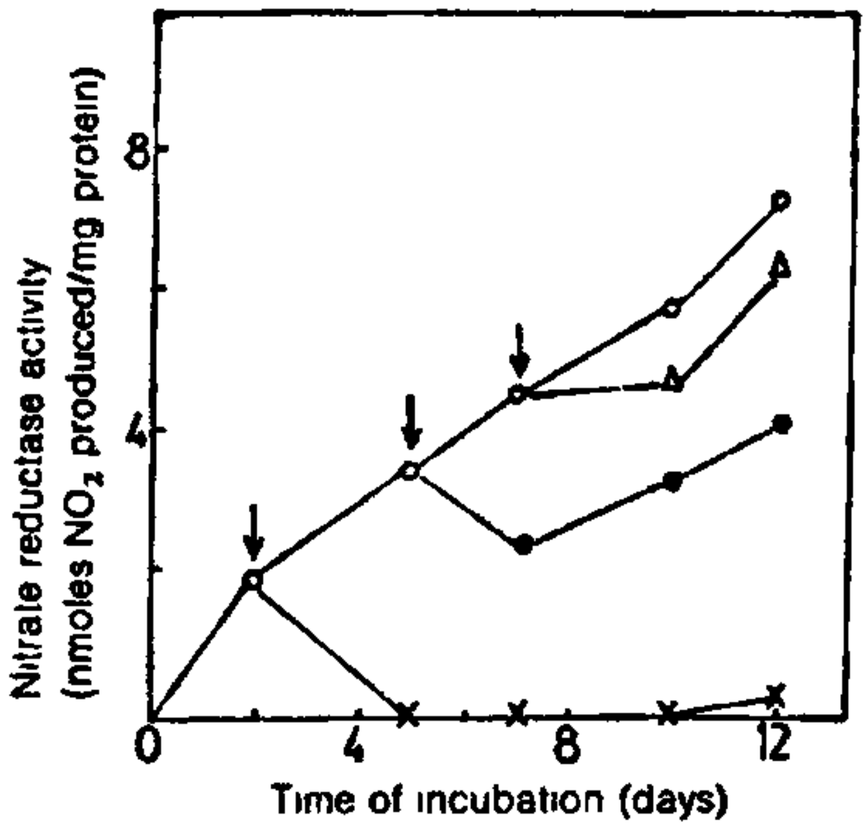

Figure 2. Effect of chloramphenicol on nitrate reductase activity in *O. laetevirens*: chloramphenicol absent (○–○), or added on day 2 (×–×), day 5 (●–●) or day 7 (△–△) of culture.

to zero by day 5 and was totally absent up to day 12; and then only 3.57% of the activity of the control culture could be observed (figure 2). When the antibiotic was added to five-day-old and seven-day-old cultures, about 55% and 85% enzyme activity respectively could be detected on day 12. These results indicate that most of the enzyme protein might be synthesized in the early stage of incubation, i.e. within the first five days. During this period the enzyme was sensitive to antibiotic-mediated inhibition. Once the rise in enzyme activity commenced, severity of inhibition was less. The results are similar to those for the eukaryotic *Chlorella vulgaris*, where cycloheximide inhibits the development of nitrate reductase activity only when added immediately after the transfer of cells from ammonia to nitrate medium, but not when added after the rise in enzyme activity has commenced[17].

The present results show that nitrate reductase of *O. laetevirens* is an inducible enzyme.

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METABOLIC RESISTANCE TO METHYL PARATHION TOXICITY IN A BIVALVE, *LAMELLIDENS MARGINALIS*

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INDISCRIMINATE use of pesticides is causing serious ecological imbalance, particularly in freshwater

Table 1 *Metabolic changes in mussels exposed to methylparathion (MP) for one to four weeks*

		1 week		2 weeks		3 weeks		4 weeks	
		Control	MP	Control	MP	Control	MP	Control	MP
Total protein (mg/g wet weight)	Foot	59.045 ± 3.73	43.397* ± 6.82 (−26.50)	59.412 ± 9.81	34.317* ± 9.52 (−42.23)	57.647 ± 13.11	49.60 ± 7.88 (−13.95)	57.831 ± 11.82	33.837* ± 5.99 (−41.48)
	Mantle	41.910 ± 14.77	35.930 ± 12.07 (−14.26)	42.687 ± 11.62	38.647 ± 9.26 (−9.46)	41.972 ± 11.45	32.872 ± 3.28 (−21.68)	40.201 ± 7.79	29.281* ± 5.30 (−27.16)
Free amino acids (μmoles of tyrosine equivalents/g wet weight)	Foot	79.15 ± 13.52	106.49* ± 10.49 (+34.54)	81.67 ± 7.38	127.24* ± 19.97 (+55.79)	83.88 ± 11.92	85.53* ± 11.49 (+1.967)	69.64 ± 2.77	115.66* ± 10.95 (+66.08)
	Mantle	82.44 ± 15.59	72.95 ± 6.79 (−11.51)	79.86 ± 10.09	72.29 ± 7.76 (+9.48)	82.29 ± 7.76	81.82 ± 6.39 (−0.57)	75.04 ± 6.43	102.27* ± 22.21 (+36.28)
Glutaminase (μmoles of NH_3 formed/mg protein/h)	Foot	0.0918 ± 0.064	0.1088* ± 0.008 (+18.51)	0.0958 ± 0.008	0.1139* ± 0.008 (+18.89)	0.0979 ± 0.009	0.1186* ± 0.005 (+21.14)	0.0992 ± 0.012	0.1258* ± 0.006 (+26.81)
	Mantle	0.1327 ± 0.007	0.1486* ± 0.006 (+11.98)	0.1378 ± 0.008	0.1646 ± 0.003 (+19.44)	0.1379 ± 0.011	0.1755* ± 0.006 (+27.26)	0.1347 ± 0.009	0.1539* ± 0.007 (+14.25)
Catalase (μmoles of H_2O_2 formed/mg protein/h)	Foot	5.890 ± 0.43	6.633* ± 0.47 (+12.61)	6.106 ± 0.43	9.923* ± 0.28 (+62.51)	5.626 ± 0.71	10.529* ± 0.46 (+87.14)	5.175 ± 0.40	11.371* ± 0.43 (+119.73)
	Mantle	8.449 ± 0.30	9.476* ± 0.46 (+12.15)	8.816 ± 0.30	12.818* ± 0.34 (+45.39)	8.844 ± 0.25	16.819* ± 0.48 (+90.17)	8.633 ± 0.40	10.713* ± 0.38 (+24.09)
Xanthine oxidase (μmoles of formazone formed/mg protein/h)	Foot	0.1387 ± 0.010	0.1808* ± 0.09 (+30.35)	0.1432 ± 0.04	0.2159* ± 0.01 (+50.76)	0.1323 ± 0.02	0.2347* ± 0.01 (+77.39)	0.1421 ± 0.03	0.1658* ± 0.08 (+10.67)
	Mantle	0.1063 ± 0.08	0.1259* ± 0.02 (+18.43)	0.1090 ± 0.01	0.1522* ± 0.08 (+39.63)	0.1129 ± 0.01	0.1930* ± 0.01 (+70.94)	0.1025 ± 0.09	0.1731* ± 0.02 (+68.87)

Values are mean ± SD ($n = 8$); figures in parentheses are per cent change over control.
*Significant difference, $P < 0.05$.

ecosystems. Invertebrates constitute a very large proportion of the fauna in aquatic ecosystems. Freshwater mussels have been commonly employed in toxicity evaluation studies and water quality management programmes[1-3]. In the present study the biochemical and physiological responses to methylparathion (MP) toxicity with reference to ammonia detoxification has been evaluated in the tissues of a bivalve to assess the tissue-specific metabolic compensatory mechanisms that aid survival chances of mussels.

Bivalves *Lamellidens marginalis* (25 ± 5 g) were acclimated to laboratory conditions in aquaria under 12:12 light:dark periods. The physico-chemical characteristics of water were: temperature 27 ± 2°C, pH 7.1–7.3, hardness 61 mg/l (HCO_3^-), dissolved oxygen 5.3 ± 0.72 ml/l.

Based on dose–mortality and tolerance data, a pesticide level of 15 ppm (sub-lethal concentration) was selected. The animals were divided into two batches of 20 each and one batch was exposed to 15 ppm of MP (obtained from Pesticides India Ltd, Bombay) for four weeks. The water in the troughs was changed every 24 h. A second group of animals maintained under identical conditions in freshwater (without MP) served as control.

After the stipulated time (1, 2, 3 and 4 weeks) foot and mantle from both control and MP-exposed mussels were excised and chilled to 0°C. Total protein[4], free amino acids (FAAs)[5], catalase[6], xanthine oxidase[7,8] and glutaminase[9] were estimated.

Table 1 gives the results of the study. MP was reported to trigger acid and alkaline protease activity of the tissues[3,10]. Seshagiri Rao[11] reported histopathological alterations in foot during pesticide stress. These observations point to augmented tissue protein degradation, which might be responsible for the reduction in tissue proteins under MP toxicity. The unique metabolic potential of mantle[12] and its defensive action, viz. secretion of mucus[13], might be responsible for the smaller decrease in total protein content compared to foot (table 1).

Increased proteolysis is always associated with high FAA levels. The FAAs may be used for the synthesis of specific enzymes[14]. In order to cope with the toxic condition, they may be channelled to the TCA cycle to meet the energy demand[15] or to the regulation of ionic and osmotic balance. The FAAs may also contribute to gluconeogenesis and keto acid formation. The rise in tissue FAA levels during MP stress might be a compensatory mechanism by which these FAAs provide energy through transdeamination during stress to meet the high energy demand.

The deamination of FAAs results in the production of peroxides[16]. The increase in catalase activity in the tissues of MP-exposed mussels indicates possible elevation of amino acid oxidase activity and predominance of deamination resulting in more peroxide formation. Such a metabolic situation warrants enhanced catalase activity to mobilize peroxides.

Glutamine oxidation was found to increase in response to the energy demand in the tissues under stress conditions. Since metabolic acidosis was reported during MP stress in the tissues of freshwater mussels[17], stepped-up ammonia production by increased glutaminase activity may be an adaptive measure to maintain the acid–base balance. The high glutaminase activity is indicative of rapid turnover of glutamine, a major product of ammonia. During pesticide stress the enzyme has to deal with increasing quantities of transported ammonia to maintain metabolic homeostasis.

Xanthine oxidase plays an important role in ammonia detoxification and nitrogen balance of the tissue. The increased activity of this enzyme in the tissues of MP-exposed mussels indicates increased synthesis of uric acid under toxic stress.

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IN VITRO DIGESTIBILITY OF CASHEW KERNEL PROTEIN

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PROTEIN content of defatted kernel flour of some cashew varieties has been shown to vary between 32.1 and 43.7%[1]. Cashew kernel protein contains all the essential amino acids[2,3]. Protein efficiency ratio of cashew-nut extraction meal (3.23) compares well with that of casein[4] (3.12). Lysine content of cashew kernel protein was significantly different among varieties[5]. Detailed studies were initiated to compare the varieties for *in vitro* digestibility of kernel protein by proteolytic enzymes such as trypsin, α-chymotrypsin and pepsin.

Trypsin (from bovine pancreas, 13,000 BAEE units/mg protein), α-chymotrypsin (from bovine pancreas, 47 units/mg protein) and pepsin (from porcine stomach mucosa, 3165 units/mg protein) were obtained from Sigma Chemical Co., USA. All the buffer salts used were of analytical reagent grade. Cashew kernel flour, after defatting with a chloroform–methanol mixture (2:1 v/v), was extracted with double-distilled water (1:20 w/v) at pH 10 and 4 for 60 min at 25 C and the supernatant was precipitated with ammonium sulphate (90% saturation). The precipitate was taken in a suitable buffer (0.1 M potassium phosphate, pH 7.6, for trypsin and α-chymotrypsin; 0.2 M KCl HCl, pH 2.0, for pepsin) and dialysed overnight at 25 C against the same buffer. Protein in the extract was estimated by Bradford's dye method[6].

In vitro digestibility of cashew kernel protein by trypsin, α-chymotrypsin and pepsin was studied as described earlier[7]. The ratio of enzyme to protein was 1:100. Protein for the assay varied between 3 and 3.5 mg/ml, and the enzyme concentration was 35 μg/ml. For pepsin, kernel protein and enzyme were taken separately in 1 ml of 0.2 M KCl–HCl buffer (pH 2.0), while for trypsin and chymotrypsin, enzyme and kernel protein were taken separately in 1 ml of 0.1 M potassium phosphate buffer (pH 7.6). Digestion was carried out at 37°C for 15 min and terminated by addition of 2 ml of 20% trichloroacetic acid (TCA). Absorbance at 280 nm of the TCA supernatant after centrifugation was read against reagent blank.

Cashew kernel protein extracted at pH 10.0 was denatured by heating for 10 min at different temperatures and *in vitro* digestibility by trypsin was studied. Denaturation was also done using SDS by heating the protein extracted at pH 10.0 with SDS and β-mercaptoethanol (SDS 4% and β-mercaptoethanol 10% final concentrations) for 2 min in a boiling water bath. SDS-denatured protein was

Table 1 *In vitro digestibility of cashew kernel protein by trypsin*

Incubation time (min)	Digestibility*	
	Tr. No. 1 kernel protein	BSA
5	0.07	0.036
15	0.084	0.082
30	0.11	0.119
60	0.134	0.201
90	0.144	0.223
120	0.137	0.232

*Expressed as increase in absorbance at 280 nm of TCA supernatant (see text for details).

Table 2 *Comparison of in vitro digestibility of cashew kernel protein, haemoglobin and BSA*

Protein	Digestibility (%)	
	Trypsin	α-Chymotrypsin
Haemoglobin	100 (0.088)*	100 (0.094)*
BSA	100	73
Cashew kernel protein		
i) pH 10.0 extract	133	62
ii) pH 4.0 extract	37	14

*Figures within parentheses are actual increase in absorbance at 280 nm of TCA supernatant. *In vitro* digestibility of BSA and cashew kernel protein is expressed as a percentage, taking digestibility of haemoglobin as 100. Kernel protein from cashew variety H-3-17 was used. Values are mean of three estimations.