Aminotransferases in the bivalve mollusc *Mytilus edulis* L. and short term effects of crude oil in brackish water

M. Narvia and P. Rantamäki

Transaminases are among the crucial enzymes in amino acid metabolism, which in aquatic organisms is known to be affected by exposure to oil hydrocarbons. The transamination reactions in *Mytilus edulis* L. were studied to estimate their adequacy to indicate short term oil exposure in mussels. The transamination reactions were measured using paper chromatography and spectrophotometry. A high degree of transamination was observed between 2-oxoglutarate and alanine, aspartate and ornithine. A slight degree of transamination was shown with methionine, leucine, isoleucine, phenylalanine, serine, tryptophan, threonine, tyrosine and valine. No transamination was observed between 2-oxoglutarate and glycine, arginine, histidine, lysine, proline, citrulline and β-alanine. The effect of the water-accommodated fraction (WAF) of crude oil on selected transaminase reactions was measured. The highest changes during the WAF exposure were mostly observed in the gills and mantle. Alanine aminotransferase (EC 2.6.1.2) activity in the mantle was, at its highest, 55% over the control. Aspartate aminotransferase (EC 2.6.1.1) activity increased in the gills by 52%. For ornithine transamination, in the gills the highest increase was by 75% and in the mantle by 50%. The metabolic pathways involved in the alterations of aminotransferase activities are discussed. It is concluded that ornithine transamination in gills is a potential indicator for short term crude oil exposure in *Mytilus edulis*. More studies are needed to evaluate the effects of other organic pollutants on ornithine transamination.

Keywords: *Mytilus edulis, aminotransferases, crude oil.*

Introduction

The activity of aminotransferases in aquatic animals has been shown in several cases to respond to chemical stress. The first studies date from the 1960s. In fish the altered activity of aminotransferases has been caused by bromobenzene/carbon tetrachloride (Bell 1968), phenol and its derivatives (Kristofferson *et al.* 1974, Gupta *et al.* 1983, Tiedge *et al.* 1986, Casillas and Myers 1989), carbon tetrachloride (Racicot *et al.* 1975), benzo(*a*)pyrene (Oikari and Jimenez 1992), and the insecticides Kepone, fenvalerate and cypermethrin (Rice and Mills 1987, Reddy and Yellamma 1991, Reddy *et al.* 1991). Both serum and various tissues in fish have shown alterations in their enzyme activity.

The common mussel (*Mytilus edulis* L.) has been used as a sentinel organism in large monitoring programmes such as Mussel Watch in the USA (Goldberg *et al.* 1978). In tissues of *Mytilus edulis* both aspartate aminotransferase (ASAT) (EC 2.6.1.1) and alanine aminotransferase (ALAT) (EC 2.6.1.2) have been demonstrated. Several other transaminase reactions have also been observed even though the activities were low (Read 1962). Oysters (*Crassostrea virginica*) showed significantly elevated ALAT levels when exposed to emulsified crude oil (Heitz *et al.* 1974). In bivalves the metabolism of nitrogen compounds is also linked to other environmental challenges such as changes in salinity and temperature or nutritive stress. According to Bayne (1973) temperature and nutritive stress increase the excretion of α-amino-N in *Mytilus edulis*. Hammen (1968) has proved that the excretion of amino acids correlates closely to the activity of aminotransferases when various species of bivalve molluscs are compared with each other.

The main object of the present study was to investigate transaminase reactions in *Mytilus edulis* of the Baltic Sea and to examine how the transaminase activities react to the water-accommodated fraction (WAF) of crude oil. *Mytilus edulis* in the northern part of the Baltic Sea lives in extreme conditions of low temperature and low salinity of water. Mussels have to reserve part of their energy budget to adapt to the unfavourable environment which results in the reduced size of the organisms. Therefore in this situation they might be especially sensitive to stress.

The work was carried out in the Department of Biochemistry and Food Chemistry, University of Turku, FIN-20500 Turku, Finland. Correspondence should be addressed to P. Rantamäki at the Agricultural Research Centre of Finland, Food Research Institute, FIN-31600 Jokoinen, Finland.

MATERIALS AND METHODS

Animals

Mussels (*Mytilus edulis*) were collected with an Agassiz net in the northern Baltic Sea in the Turku Archipelago between November and February. The salinity of the water fluctuated between 6.6‰ and 7.0‰. In November the water temperature at the bottom of the sea was 6°C and in February 0°C. The mussels were acclimated to the laboratory conditions and maintained in aerated sea water (9°C) taken from the collection area. The mussels for the demonstration of aminotransferases in the tissues were collected in December and the specimens for the oil exposure test in February.

Exposure to oil

A water-accommodated fraction (WAF) of crude oil was prepared daily according to the method of Widdows *et al.* (1982). Russian crude oil, delivered by Neste, was used. The experiments were conducted in glass aquaria, each of which contained 3.0 l of sea water (salinity 6.8‰) and 30 mussels (shell length, 3.0±0.1 cm (SD); shell thickness 1.2±0.1 cm (SD)) at the beginning of the experiment. The temperature of the aquaria was kept at 9°C with a thermostat. The aquaria were covered with aluminium foil and aeration was carried out with aeration pumps. The mussels were acclimated for a week in the laboratory and for 2 days in the aq

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Every 24 h 80 ml WAF was dosed to one of the aquaria and 240 ml to another one. Eighty ml WAF was equal to 1.0 ml of crude oil and contained about 0.3 mg hydrocarbons, 51% of which were aromatic (Ahlgren, personal communication). One aquarium was kept as a control. With the addition of sea water at the same time as the WAF, the volume of water was kept constant in all three aquaria. Mussels were not fed during the experiment. WAF was dosed to the aquaria for four successive days.

Samples

Before the first WAF dosing, four mussels were taken from all three aquaria and pooled in one sample. Thirteen mussel samples were taken from all aquaria after an exposure time of 1 day and 4 days.

Tissue preparation

Tissue homogenates were prepared and aminotransferase activities determined according to a modified method of Rowsell (1962). For the demonstration of transamination reactions all the tissues of mussels were homogenized together. When measuring the effects of oil, the gills, mantle and the rest of the animal, except the foot, were studied separately. The rest of the animal is referred to later in this paper as the digestive gland although other tissues were present. The pooled samples were used throughout the experiment. The tissues were weighed and homogenized in a glass homogenizer in ice cold 0.1 M phosphate buffer (pH 7.4), the amount of which was five times the weight of the tissue. The homogenate was filtered through cotton gauze and dialysed against the same phosphate buffer for 20 h at 9°C. After dialysis, the homogenates were stored at –20°C.

Transaminase assays

The reaction mixture contained 9.5 mM 2-oxoglutarate, pH 7.4, 9.5 mM L-amino acid (neutralized), pyridoxal phosphate (9.5 μg ml^{-1}) and dialysed tissue homogenate (0.5 ml) in a volume of 1.05 ml. In the blank test, the amino acid was replaced with distilled water. The control contained boiled homogenate. The homogenate was added last and the reaction mixture was immediately mixed and gassed with nitrogen. The test tube was sealed with a rubber bung and transferred to a 37°C water bath. According to the results obtained in preliminary tests, a reaction time of 60 min was used for alanine and 120 min for other amino acids. After incubation, the reaction was stopped by keeping the tube in boiling water for 5 min. The denatured protein was centrifuged down (1900*g*, 15 min) and the supernatant was deep-frozen (–20°C) to await analysis.

Paper chromatography

Reaction extracts (5–20 μl, depending on the amino acid) were applied to 25 × 23 cm Whatman No. 1 paper. A standard of 0.01 M glutamate was included. Chromatograms were developed by the ascending method. The solvent for alanine, methionine, valine, leucine, phenylalanine, tryptophan, tyrosine and isoleucine was *n*-butanol–methanol–water (10 : 10 : 5) (Rowsell 1962), for aspartate and ornithine, pyridine–acetic acid–water (Decker and Riffart 1950) and for glycine, arginine, histidine, lysine, proline, serine, threonine, β-alanine and citrulline, phenol–water (115 : 10) (Clark and Switzer 1977). After development, the chromatograms were dried in a current of warm air. Papers were sprayed with 0.5% ninhydrin in 71% ethanol and heated at 65°C for 20 min. When glutamate deriving from 2-oxoglutarate was observed on the chromatogram, it was regarded as evidence of transamination. The glutamate spots were cut out of the paper, and as a blank a chromatogram rectangle of the same size which no amino acid had permeated was used. Rectangles cut into small pieces were extracted with 5 ml of 70% ethanol in test tubes. Absorbance was measured at 575 nm using a spectrophotometer and the concentration of glutamate was calculated per mg protein. Samples were spotted on chromatograms as duplicates. Protein was estimated according to the method of Rowsell (1962).

Statistics

The 95% confidence intervals were calculated for all test groups and they were compared with the mean of controls at 0, 1 and 4 days in each transamination measurement. The pooled samples were used throughout the experiment. Therefore, the standard deviation of the controls (between 0, 1 and 4 days) was also used to describe the standard deviation of every test sample.

Results

The results obtained on the specificity of various transamination reactions using L-amino acids and 2-oxoglutarate are summarized in Table 1. A high degree of transamination was observed between 2-oxoglutarate and alanine, aspartate and ornithine. A slight degree of transamination was shown with methionine, leucine, isoleucine, phenylalanine, serine, tryptophan, threonine, tyrosine and valine. No transamination in the prevailing test conditions was observed between 2-oxoglutarate and glycine, arginine, histidine, lysine, proline, citrulline and β-alanine.

The effect of WAF exposure was studied on six transamination reactions. In the experiments, all high degree transaminations with alanine, aspartate and ornithine, and in addition three aliphatic branched chain amino acids, leucine, isoleucine and valine, were observed. The results are presented in Figure 1. The most noticeable changes during the WAF exposure were observed in the gills and mantle. When the dose was 80 ml of WAF per day, the activity of ALAT in the gills decreased by 77% in 1 day against the mean of the control values. After three more days the decline compared with the control was only 30%. ALAT activity in the mantle increased above the control value with 80 ml of WAF in 4 days by 55% compared with the control. ASAT activity increased in the gills after 4 days with both doses, by 52% with 80 ml and by 45% with 240 ml of WAF compared with the control. Ornithine transamination achieved a higher level compared with the control in 4 days with both doses in the gills and in 1 day in the mantle. In the gills, the highest change was 75% with the 80 ml dose and 50% in the mantle with 240 ml. In the digestive gland, the highest increase was 94% with the

Amino acid	Transamination with 2-oxoglutarate
Alanine	+++
Aspartate	+++
Ornithine	+++
Glycine	–
Methionine	+/–
Leucine	+/–
Isoleucine	+/–
Arginine	–
Histidine	–
Lysine	–
Phenylalanine	+/–
Proline	–
Serine	+/–
Tryptophan	+/–
Threonine	+/–
Tyrosine	+/–
Valine	+/–
Citrulline	–
β-Alanine	–

Table 1. Specificity of transaminations in *Mytilus edulis* between L-amino acids and 2-oxoglutarate.

Key: +++ indicates high degree of transamination, +/– slight degree and – no detectable degree of transamination.

In the case of leucine, isoleucine and valine transamination the changes caused by WAF exposure were mostly negligible. With isoleucine the highest change was observed in the digestive gland, i.e. a 39% decrease in 4 days with a dose of 240 ml. With leucine transamination the most remarkable change was to be seen in the gills. After 4 days with the dose of 80 ml, leucine transamination had increased by 64% compared with the control. The activities of the three aminotransferase in the digestive gland followed the same pattern, i.e. the WAF exposure decreased the activities consistently with the dose.

Discussion

In the present study, there were three high degree transaminations in the tissues of *Mytilus edulis* in the Baltic Sea: alanine, aspartate and ornithine transamination. Read (1962) demonstrated ALAT and ASAT activities in tissues of *Mytilus edulis* in the Atlantic Ocean, but ornithine transamination was not studied. In this study no attempt was made to obtain more information on ornithine transamination. Ornithine transaminase activity may be involved or alternatively the specificity of other transaminases is so overlapping that ornithine can also be used as a substrate. Also, other transaminations were observed by Read although the activities were negligible. The amino acids which reacted with α-ketoglutarate were glycine, methionine, leucine, isoleucine, histidine, phenylalanine, serine, tryptophan, threonine, tyrosine and valine. In the present study, no transamination was found between α-ketoglutarate and glycine or histidine, otherwise the same slight activities were observed. With ALAT and ASAT, alanine, aspartic acid and glutamate are transformed to with the corresponding keto acid intermediates of the citrate cycle. According to Read (1962) both ALAT and ASAT reactions are freely reversible. In many organisms ornithine can also enter the citrate cycle by transamination via glutamate. In bivalves the pathway has not been studied.

In our former study, the accumulation of ornithine, urea and NH_3 was observed in the gills and mantle of *Mytilus edulis* as a consequence of crude oil exposure (Soini and Rantamäki 1985), which suggests enhanced protein catabolism. The ratio between urea and NH_3 concentrations in the gills and the mantle fluctuated mostly between 10 and 100. As a marine bivalve *Mytilus edulis* would be expected to be an ammonotelic species, and according to Bayne (1973), *Mytilus edulis* in the North Sea excreted the majority of their nitrogen as ammonia or amino-N. In our study *Mytilus edulis*, adapted to the brackish water of the northern Baltic Sea (salinity 5–7‰), was observed to be ureotelic. Andrews and Reid (1972) were able to detect all of the enzymes of the urea cycle, except carbamylphosphate synthetase, in the digestive gland of *Mytilus californianus*. According to them, low sensitivity in the analytical technique possibly caused the inability to demonstrate carbamylphosphate synthetase. None of the enzymes were present in the mantle, and the gill tissues were not analysed. For interpreting the results of the present study, information about the functioning of the urea cycle would be useful. Our study demonstrated a high degree of ornithine transamination in the gills, mantle and digestive gland and the exposure to WAF increased the transamination in the gills and mantle and also, in some cases, in the digestive gland. The elevated ornithine level in the gills and mantle might be due to the inhibition of ornithine transcarbamylase in the digestive gland and the subsequent transportation of ornithine via haemolymph, to the local inhibition of ornithine transcarbamylase in gills, or to the increased release of arginine in the consequence of protein catabolism and its subsequent degradation to ornithine by arginase even without the functioning urea cycle. By elevated ornithine transaminase activity, ornithine could be more effectively directed to the citrate cycle to produce energy.

The activities of ALAT and ASAT also reacted to WAF. An exposure of 1 day reduced markedly the activity of ALAT in gills. In the mantle, the activity of ALAT increased. According to Heitz *et al.* (1974) the activity of ALAT in oysters was significantly stimulated by the exposure to crude oil. The activity of soluble ALAT in the combined homogenates of all tissues of oysters was elevated on the second day of the experiment to 130% of the control, after which it returned to the control level. The mitochondrial enzyme level was elevated significantly on day 7. Racicot *et al.* (1975) observed that CCl_4, given as one injection, caused the lower level of ALAT activity in the liver of rainbow trout (*Salmo gairdneri*). The activity was at its lowest on the third day. At the end of the 10-day period the activity had returned to near the control level. According to Santos *et al.* (1990) kraft mill effluent significantly lowered ALAT and ASAT activity in the liver of yellow eel (*Anguilla anguilla* L.) in 3

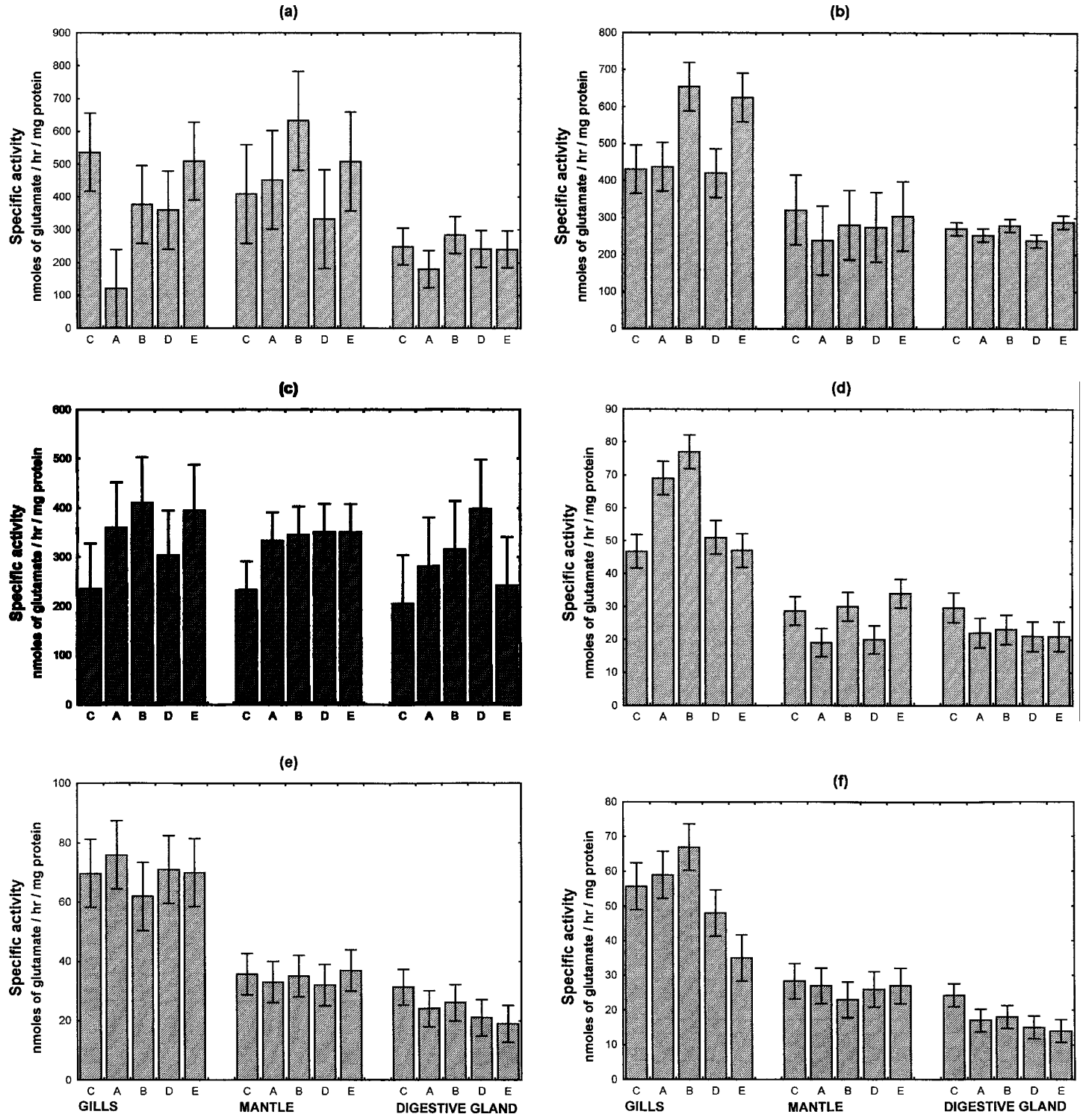

Figure 1. Effect of WAF exposure on transamination reactions in the gills, mantle and in the digestive gland of *Mytilus edulis*. Activity is expressed as nmoles of glutamate h^{-1} mg^{-1} protein. (a) ALAT, (b) ASAT, (c) ornithine transamination, (d) leucine transamination, (e) isoleucine transamination and (f) valine transamination. C = mean of controls of 0, 1 and 4 days, A = WAF 80 ml, 1 day (total dose 93 μg^{-1}), B = WAF 80 ml, 4 days (total dose 303 $\mu g\ l^{-1}$), D = WAF 240 ml, 1 day (total dose 278 $\mu g\ l^{-1}$), E = WAF 240 ml, 4 days (total dose 909 $\mu g\ l^{-1}$). *N* in controls = 38, *N* in test samples = 13. Activities with 95% confidence intervals are shown.

found that phenolic compounds increased the activity of ALAT by 17%–121% in the gills of the fish *Notopterus notopterus* compared with the control and of ASAT by 17%–109%, respectively after 15 and 30 days' exposure. The only marked change of ASAT activity in the present study was on the fourth day in gills when the activity increased by 52% and 45% over the control level with 80 ml and 240 ml doses, respectively. The striking feature in th

activity was the lack of response to a change in dose. The same feature is noticeable in several other cases in this study. Livingstone (1985) reported the phenomena to be ubiquitous in the response of organisms to pollutants. When the changes in activities of enzymes are used as a measure of pollution, the reaction may be independent of the concentration of a pollutant. Oikari and Jimenez (1992) thoroughly discussed the lack of a dose–response relationship in connection with enzymes of fish as indicators of complex mixtures of pollutants. Some of the compounds may have an inducible effect on several enzyme activities and others act as inhibiting factors. Crude oil may have the same potency. Reddy and Yellamma (1991) observed elevated ALAT and ASAT activity in liver, brain and gill tissues of the fish *Tilapia mossambica* following exposure to cypermethrin, a pyrethrin-type insecticide. They concluded that transamination was used to increase the production of keto acids of the citrate cycle to cope with the elevated energy demand created by the toxicity of cypermethrin. They also noticed a change in the ratio of ALAT and ASAT activity during the exposure and suggested a selectivity between pyruvate and oxaloacetate formation in tissues. Probably the elevated transaminase activities observed in this study are correspondingly in connection with energy production. The decreased ALAT activity in the gills after 1 day exposure may be due to the season and the nutritional state of the mussels. In the late winter mussels have to use protein as their energy source. The growing demand for energy, caused by chemical stress, may have changed the relative importance of reaction pathways. It is also possible that ALAT in the gills is more exposed to the toxic compounds of crude oil in water than the same enzyme in the mantle or the digestive gland. After 4 days when the enzyme activity had returned to near the control level, the repairing mechanisms against cellular damage were probably effectively recruited.

The branched amino acids leucine, isoleucine and valine formed 20% (molar percentages) of the amino acid content of proteins in gills (Soini and Rantamäki 1985). However, these amino acids were not among the free amino acids in the gills (when 94% of the total was calculated) after the oil exposure although the total amount of free amino acids markedly increased. The increase is supposed to be due to degradation of cytosolic proteins. Xenobiotics such as phenanthrene, a component of crude oil, induce the destabilization of lysosomal membranes which increase the protein catabolism in short-term exposure in *Mytilus edulis* (Viarengo *et al.* 1992). The rearrangements of the free amino acid pool seem to proceed by reactions other than transaminations, because the exposure to oil mostly seemed to reduce these transaminations. The distinct exception was leucine transamination in gills. Livingstone *et al.* (1979) suggested that in the case of an abrupt increase in salinity, when the concentration of free amino acids increases, the change of the amino acid composition could occur not through initial transamination but by a process of deamination and reamination. The most important enzymes could be either alanine dehydrogenase or a combination of glutamate dehydrogenase and ALAT.

The interpretation of the results is complicated by the fact that oil in water may make mussels temporarily close their valves and change over to anaerobic metabolism. Thus the changes in enzyme activities are a combination of the direct effect of chemicals and anaerobic metabolism. The major end-products of anaerobic metabolism in marine bivalves are succinate and alanine (Stokes and Awapara 1968; Hammen 1969, De Zwaan and Zandee 1972, De Zwaan and Van Marrewijk 1973). Organic toxicants have also been found to disturb the anaerobic metabolism. Wang *et al.* (1992) have shown that pentachlorophenol and tributyltin caused a shift from succinate to lactate anaerobic pathways in *Mytilus edulis*.

As a summary, the following statements can be made in relation to processes involved in aminotransferase response to oil pollution. It is not possible to find any comprehensive information in the literature. In the present study, no histological work has been performed, which makes it difficult to evaluate the involved processes, some suggestions could be made, however. Chemical stress results in the stimulation of metabolic rate and protein catabolism and possibly a change in the relative importance of the reaction pathways. Hydrocarbons are also known to promote protein catabolism by the destabilization of lysosomal membranes (Bayne 1989, Viarengo *et al.* 1992), by which action hydrolytic enzymes are released inside the cell. The transformation of released amino acids to keto acids in the citrate cycle to produce energy demands transaminase activity and so does repairing cell damage. The elevated ASAT activity in the gills and the changed ASAT/ALAT activity ratio could be connected to this suggestion. Elevated ornithine transamination could be associated with dealing with protein catabolism end products, though the mechanism is not yet known.

As regards the use of transaminases as an indicator of oil spill in sea water, most changes are relatively small and some enzyme activities rapidly return to normal level. The most promising indicator could be considered to be the ornithine transamination in gills. Because several authors have reported that pesticides and other chemicals also affect transaminase activities, further research would be needed to discover the typical pattern of the effects of different pollutants.

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References

ANDREWS, T. R. AND REID, R. G. B. (1972) Ornithine cycle and uricolytic enzymes in four bivalve molluscs. *Comparative Biochemistry and Physiology*, **42B**, 475–491.

BAYNE, B. L. (1973) Physiological changes in *Mytilus edulis* L. induced by temperature and nutritive stress. *Journal of the Marine Biological Association of the United Kingdom*, **53**, 39–58.

BAYNE, B. L. (1989) Measuring the biological effects of pollution: the mussel watch approach. *Water Science and Technology*, **21**, 1089–1100.

BELL, G. R. (1968) Distribution of transaminases (aminotransferases) in the tissues of Pacific salmon (*Oncorhyncus*), with emphasis on the properties and diagnostic use of glutamic–oxaloacetic transaminase. *Journal of the Fisheries Research Board of Canada*, **25**, 12

CASILLAS, E. AND MYERS, M. S. (1989) Effect of bromobenzene and *o*-bromophenol on kidney and liver of English sole (*Parophrys vetulus*). *Comparative Biochemistry and Physiology*, **93C**, 43–48.

CLARK, J. M. AND SWITZER, R. L. (1977) *Experimental Biochemistry* (W. H. Freeman and Company, New York, San Francisco).

DECKER, P. AND RIFFART, W. (1950) Papierchromatographie mit wassermischbaren Lösungsmitteln. *Chemiker-Zeitung*, **74**, 261.

DE ZWAAN, A. AND ZANDEE, D. I. (1972) The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* L. *Comparative Biochemistry and Physiology*, **43B**, 47–54.

DE ZWAAN, A. AND VAN MARREWIJK, W. J. A. (1973) Anaerobic glucose degradation in the sea mussel *Mytilus edulis* L. *Comparative Biochemistry and Physiology*, **44B**, 429–439.

GOLDBERG, E. D., BOWEN, V. T., FARRINGTON, J. W., HARVEY, G., MARTIN, J. H., PARKER, P. L., RISEBROUGH, R. W., ROBERTSON, W., SCHNEIDER, E. AND GAMBLE, E. (1978) The Mussel Watch. *Environmental Conservation*, **5**, 101–125.

GUPTA, S., DALELA, R. C. AND SAXENA, P. K. (1983) Effect of phenolic compounds on *in vivo* activity of transaminases in certain tissues of the fish, *Notopterus notopterus*. *Environmental Research*, **32**, 8–13.

HAMMEN, C. S. (1968) Aminotransferase activities and amino acid excretion of bivalve molluscs and brachiopods. *Comparative Biochemistry and Physiology*, **26**, 697–705.

HAMMEN, C. S. (1969) Metabolism of the oyster, *Crassostrea virginica*. *American Zoologist*, **9**, 309–318.

HEITZ, J. R., LEWIS, L., CHAMBERS, J. AND YARBROUGH, J. D. (1974) The acute effects of Empire Mix crude oil on enzymes in oysters, shrimp and mullet. In *Pollution and Physiology of Marine Organisms*, F. J. Vernberg and W. B. Vernberg, eds (Academic Press, New York), pp. 311–328.

KRISTOFFERSSON , R., BROBERG, S., OIKARI, A. AND PEKKARINEN, M. (1974). Effect of a sublethal concentration of phenol on some blood plasma enzyme activities in the pike (*Esox lucius* L.) in brackish water. *Annales Zoologici Fennici*, **11**, 220–223.

LIVINGSTONE, D. R. (1985) Biochemical measurements. In *The Effects of Stress and Pollution on Marine Animals*. B. L. Bayne, D. A. Brown, K. Burns, D. R. Dixon, D. R. Ivanovici, D. R. Livingstone, D. M. Lowe, M. N. Moore, A. R. D. Stebbing and J. Widdows, eds (Praeger Publishers, New York), pp. 81–132.

LIVINGSTONE, D. R., WIDDOWS, J. AND FIETH, P. (1979) Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: adaptation to abrupt and fluctuation changes in salinity. *Marine Biology*, **53**, 41–55.

OIKARI, A. AND JIMENEZ, B. (1992) Effects of hepatotoxicants on the induction of microsomal monooxygenase activity in sunfish liver by naphthoflavone and benzo(a)pyrene. *Ecotoxicology and Environmental Safety*, **23**, 89–102.

RACICOT, J. G., GAUDET, M. AND LERAY, C. (1975) Blood and liver enzymes in rainbow trout (*Salmo gairdneri* Rich.) with emphasis on their diagnostic use: study of CCl_4 toxicity and a case of *Aeromonas* infection. *Journal of Fish Biology*, **7**, 825–835.

READ, K. R. H. (1962) Transamination in certain tissue homogenates of the bivalved molluscs *Mytilus edulis* L. and *Modiolus modiolus* L. *Comparative Biochemistry and Physiology*, **7**, 15–22.

REDDY, A. T. AND YELLAMMA, K. (1991) Cypermethrin induced changes in nitrogen metabolism of fish, *Tilapia mossambica*. *Biochemistry International*, **23**, 649–654.

REDDY, P. M., PHILIP, G. H. AND BASHAMOHIDEEN , M. (1991) Fenvalerate induced biochemical changes in the selected tissues of freshwater fish, *Cyprinus carpio*. *Biochemistry International*, **23**, 1087–1096.

RICE, C. D. AND MILLS, R. R. (1987) Effects of Kepone tolerance on liver alkaline phosphomonoesterase and glutamic–oxaloacetic amino transferase from *Fundulus heteroclitus*. *Bulletin of Environmental Contamination and Toxicology*, **39**, 655–662.

ROWSELL, E. V. (1962) Transaminases in animal tissue homogenates. In *Methods in Enzymology*, Vol. 5, N. O. Kaplan and S. P. Colowick, eds (Academic Press, NY, London), pp. 685–697.

SANTOS, M. A., PIRES, F. AND HALL, A. (1990) Metabolic effects of kraft mill effluents on the eel *Anguilla anguilla* L. *Ecotoxicology and Environmental Safety*, **20**, 10–19.

SOINI, J. AND RANTAMÄKI, P. (1985) Free amino acid pattern in blue mussel (*Mytilus edulis)* exposed to crude oil. *Bulletin of Environmental Contamination and Toxicology*, **35**, 810–815.

STOKES, T. M. AND AWAPARA, J. (1968) Alanine and succinate as endproducts of glucose degradation in the clam *Rangia cuneata*. *Comparative Biochemistry and Physiology*, **25**, 883–892.

TIEDGE, H., NAGEL, R. AND URICH, K. (1986) Effect of substituted phenols on transaminase activity in the fish, *Leuciscus idus melanotus* L. *Bulletin of Environmental Contamination and Toxicology*, **36**, 176–180.

VIARENGO, A., MOORE, M. N., PERTICA, M., MANCINELLI, G. AND ACCOMANDO, R. (1992) A simple procedure for evaluating the protein degradation rate in mussel (*Mytilus galloprovincialis* Lam.) tissues and its application in a study of phenanthrene effects on protein catabolism. *Comparative Biochemistry and Physiology*, **103B**, 27–32.

WANG, W. X., WIDDOWS, J. AND PAGE, D. S. (1992) Effects of organic toxicants on the anoxic energy metabolism of the mussel *Mytilus edulis*. *Marine Environmental Research*, **34**, 327–331.

WIDDOWS, J., BAKKE, T., BAYNE, B. L., DONKIN, P., LIVINGSTONE, D. R., LOWE, D. M., MOORE, M. N., EVANS, S. V. AND MOORE, S. L. (1982) Responses of *Mytilus edulis* on exposure to the water-accommodated fraction of North Sea oil. *Marine Biology*, **67**, 15–31.

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