Once formed, wax esters are conserved by calanoid copepods largely for use in reproduction. When calanoids are starved their wax ester reserves are slowly depleted, whereas the much smaller reserves of triacylglycerols are rapidly lost (Lee et al., 1974). In some but not all species, wax esters in the adult female appear as triacylglycerols in the egg. This lipid transformation, whose pathway has yet to be investigated in zooplankton, may represent a means of labilizing the wax ester energy reserve.

A major site of conversion of wax esters into triacylglycerols in the marine food chain occurs at the trophic level zooplankton—fish. The major fishes preying on calanoid copepods (herring, anchovy, sprats, pilchards, sardines etc.) readily convert dietary wax esters into triacylglycerols in their intestinal mucosae (Lee & Puppione, 1972; Patton et al., 1975). Our recent experiments with herring intestine demonstrate that this tissue catalyses the oxidation of fatty alcohol to fatty acid concomitant with glycerogenesis from glucose or glyceroneogenesis from amino acids. Zooplankton generally are deficient in reserve carbohydrate and it is postulated that the oxidative conversion of fatty alcohol into fatty acid in the intestine of fish such as the herring is coupled to the reductive formation of glycerol from dietary amino acids. The extent to which such a mechanism for labilizing wax esters occurs during reproduction in zooplankton remains to be assessed.


Octopine Dehydrogenase in the Cockle Cardium edule

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Octopine was first isolated from the muscle tissue of the cephalopod Octopus octopodia by Morizawa (1927). Later octopine and/or the enzyme octopine dehydrogenase was also found in some marine lamellibranchs and gastropods (Moore & Wilson, 1937; Roche et al., 1952; Regnouf & Thoai, 1970), in one species of Sipunculidae (Haas et al., 1973) and in the muscle tissue of freshwater bivalves (Gäde & Zebe, 1973; Gäde, 1974). Thoai & Robin (1959, 1961) demonstrated the biosynthesis of octopine in muscle extracts of some marine molluscs; it is synthesized from arginine and pyruvate by an NADH-dependent octopine dehydrogenase (arginine+pyruvate+NADH = octopine +NAD*). Thoai et al. (1969) purified this enzyme from Pecten maximus and reported its properties (see below). Studies by Olomucki and co-workers have shown that octo-

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pine dehydrogenase from *P. maximus* has a single polypeptide chain (Olomucki et al., 1972), and steady-state kinetic studies have shown that the enzyme obeys a Bi-Ter sequential mechanism, in which the coenzyme binds first to the enzyme (Doublet & Olomucki, 1975; Doublet et al., 1975).

Our experimental animal is the cockle *Cardium edule*. Like many bivalves this intertidal species is a facultative anaerobe, which uses oxygen when available (during high tide), but is also capable of surviving for some time in the absence of oxygen (during low tide).

Some years ago when we started work on the anaerobiosis of the cockle we assayed activities of key enzymes of the anaerobic pathway in the adductor muscle of *Cardium* (Gäde & Zebe, 1973). We found not only a higher activity of octopine dehydrogenase than of lactate dehydrogenase, indicating the possibility of octopine fermentation instead of lactate production, but also a high ratio (13.5) of pyruvate kinase/phosphoenolpyruvate carboxykinase, which, according to Bueding & Saz (1968), should favour accumulation of lactate and not of succinate.

The next step was the determination of the end products in whole mussel after 15 h of experimental anoxia (Gäde, 1975). l-Lactate and octopine increased very little (from 5 to 10 μmol/g dry weight and from 0.17 to 0.70 μmol/g dry weight respectively), but alanine and succinate increased markedly (from 40 to 58 μmol/g dry weight and from 0.8 to 21 μmol/g dry weight respectively), and together accounted for about 70% of all accumulated compounds. To some extent propionate was also produced. Thus the anaerobic metabolism of the cockle was similar to that of *Mytilus edulis* (de Zwaan et al., 1976) and *Anodonta cygnea* (Gäde et al., 1975).

These results suggested that octopine dehydrogenase of *C. edule* required study in some detail. We wanted to obtain more information about the properties of the enzyme and to compare our data with that for the purified enzymes from other species. Has octopine dehydrogenase, which occurs only in a few species in the whole animal kingdom, the same physicochemical and kinetic properties in all species? In addition we hoped to get some information from the kinetic studies as to the exact role of the enzyme in the energy metabolism of the cockle, especially during anaerobiosis.

The purification of the adductor-muscle octopine dehydrogenase from *C. edule* followed the standard procedures. After (NH₄)₂SO₄ precipitation the preparation was further purified by gel chromatography on Sephadex G-100 and by ion-exchange chromatography on DEAE-Sephadex A-50. Octopine dehydrogenase activity always appeared as a single peak. The final preparation had a specific activity of 500 units (μmol/min)/mg of protein and the purification was about 200-fold. Polyacrylamide-gel electrophoresis showed five distinct bands, when stained for protein with Amido Black. The same pattern of bands was found when the gels were stained for enzyme activity (Fig. 1).

This agrees with our results on the octopine dehydrogenase of *A. cygnea*, where we demonstrated seven bands with octopine dehydrogenase activity (Gäde & Grieshaber, 1975), but is in contrast with the findings of Thoai et al. (1969) for *P. maximus*, which

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**Fig. 1. Bands of octopine dehydrogenase after electrophoresis on polyacrylamide gel**

Gels were stained for enzyme activity with NAD⁺ (1.5 mm), phenazine methosulphate (0.25 mm), Nitro Blue Tetrazolium (2.5 mm) and octopine (0.4 mm). The black band to the right was the front (Bromophenol Blue). Purified octopine dehydrogenase from *P. maximus* was a gift from Dr. A. Olomucki (Paris).
had only a single band. A re-investigation, in our laboratory, of the enzyme from *P. maximus* showed the presence of two isoenzymes (Fig. 1). A molecular-weight estimation of the purified octopine dehydrogenase from *C. edule* on Sephadex G-100 gave a value of about 37000, which is in good agreement with that of the enzyme from *P. maximus* (38000), *Sipunculus nudus* (Haas et al., 1973) and *A. cygnea* (both 40000).

The pH–activity curve for octopine formation exhibits a maximum at pH 6.8 for *C. edule*, which is in the same range as for *P. maximus* (6.4), *S. nudus* (6.2) and *A. cygnea* (6.3). The reverse reaction shows a maximum at pH 8.7; the corresponding values for *P. maximus* (9.8), *S. nudus* (9.0) and *A. cygnea* (10.1) are also in the alkaline region. All kinetic studies followed the classical Michaelis–Menten pattern. From Lineweaver–Burk plots, apparent Michaelis constants of 2.0 mM for arginine and about 1.0 mM for pyruvate were obtained. The apparent $K_m$ values of the octopine dehydrogenase preparations from *P. maximus* (1.5 and 1.5 mM), *S. nudus* (1.9 and 1.7 mM) and *A. cygnea* (1.0 and 0.4 mM) are in the same millimolar range.

The data from the purification of octopine dehydrogenase from *C. edule* and the properties of the enzyme are similar to those of the enzyme from other species. Thus octopine dehydrogenase, which is restricted to only a few species in the whole animal kingdom, probably has the same physicochemical and kinetic properties in all these animals. Probably Nature only developed octopine dehydrogenase once, and it was not changed during evolution. But what is the biological role of octopine dehydrogenase?

Thoai & Robin (1961) suggested that production of octopine was a modification of classical glycolysis; arginine is liberated by hydrolysis of phosphoarginine, and pyruvate results from glycolysis. But this was only speculation and not proven. We have determined octopine after anaerobiosis, but found very little accumulation in the cockle *C. edule*. This result became clear when we studied the inhibitory effects on octopine dehydrogenase. None of the end products of anaerobic metabolism, such as alanine, lactate or succinate, had any effect on octopine dehydrogenase at concentrations from 0.5 to 10 mM. Octopine, however, inhibited the condensation of arginine and pyruvate even at low concentrations (Fig. 2). The same effect was also obtained with octopine dehydrogenase from *A. cygnea*. Even after 5 h incubation of adductor muscle with $^{14}$C-labelled pyruvate no significant difference in the amount of labelled octopine could be observed under aerobic or anaerobic conditions. Sakaguchi et al. (1973), however, found that radioactivity from labelled pyruvate was rapidly incorporated into octopine during post-mortem ice storage of the adductor muscle of *Placopecten magellanicus.*
Table 1. End products in the mantle muscle of Loligo vulgaris and Octopus vulgaris in rest, after hunting and after electrical stimulation

Values are given in μmol/g dry weight.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Condition</th>
<th>Arginine phosphate</th>
<th>Arginine</th>
<th>Octopine</th>
<th>Arginine phosphate + arginine + octopine</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. vulgaris</td>
<td>At rest</td>
<td>185</td>
<td>140</td>
<td>8</td>
<td>333</td>
</tr>
<tr>
<td></td>
<td>Hunted</td>
<td>7</td>
<td>205</td>
<td>123</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td>Unstimulated control</td>
<td>123</td>
<td>168</td>
<td>18</td>
<td>309</td>
</tr>
<tr>
<td></td>
<td>Stimulated</td>
<td>36</td>
<td>212</td>
<td>86</td>
<td>334</td>
</tr>
<tr>
<td>O. vulgaris</td>
<td>At rest</td>
<td>96</td>
<td>87</td>
<td>20</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>Hunted</td>
<td>20</td>
<td>73</td>
<td>100</td>
<td>193</td>
</tr>
</tbody>
</table>

These findings suggest that octopine dehydrogenase is not important in the slow-moving sedentary bivalves (such as C. edule and A. cygnea), in which it is maybe only an evolutionary relic, but that it plays a metabolic role in Pecten, which is capable of fast swimming movements. Another group of molluscs, the cephalopods, exhibit high activities of octopine dehydrogenase and are capable of rapid locomotion or jet propulsion. After hunting and after electrical stimulation of the nerve ganglia we found octopine accumulation in the mantle muscle of Octopus vulgaris and the squid Loligo vulgaris (Grieshaber & Gade, 1976). The concentration of phosphoarginine decreased, so that the sum of arginine phosphate plus arginine plus octopine remained the same (Table 1). We believe octopine dehydrogenase takes over the role of lactate dehydrogenase during short-time anaerobiosis in normally aerobic working muscles in Pecten and some cephalopods, and has a parallel development to the function of lactate dehydrogenase in most vertebrate tissues.

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Gäde, G. & Grieshaber, M. (1975) J. Comp. Physiol. 102, 149-158


