Methods. Animals and diet. Male albino guinea-pigs (Dunkin-Hartley strain) maintained on a semi-synthetic scorbutogenic diet[8] were used as a source of liver material and in the age-enzyme activity experiment.

Enzyme purification and assay. Approximately 4 g of tissue, immediately on removal from the animal, were homogenized with 20 ml 0.25 M sodium phosphate-sucrose buffer (pH 7.4) and centrifuged for 1.25 h at 0 °C and 105,000 × g in an MSE Automatic Superspeed 50 refrigerated centrifuge. The supernatant was fractionated with ammonium sulphate and the 0.52–0.68 saturation fraction further purified by elution with 0.05 M tris buffer (pH 7.4) on Sephadex G75. Preparations from both rat and guinea-pig liver showed the same elution pattern and the use of marker proteins indicated a mol.wt of 25,000 for the dehydroascorbatase fraction.

The enzyme was assayed by measuring the rate of destruction of dehydroascorbic acid. 1 ml of dehydroascorbic acid (5.75 mM)[9] was incubated at 37 °C with 0.5 ml $MgSO_4$ (0.167 M), 0.5 ml of the enzyme preparation and 5 ml of 0.2 M trismaleate buffer (pH 5.8)[7]; activity was measured at pH 5.8 to minimize non-enzymic delactonization of dehydroascorbic acid (table 1). After 8 min, 0.5 ml of the incubation mixture was removed and added to 6 ml homocysteine solution (4.93 mM) in 0.2 M trismaleate buffer (pH 6.8) to convert undestroyed dehydroascorbic acid to AA[9]. After 12 min at room temperature the reaction was stopped by the addition of 5 ml 10% metaphosphoric acid and the AA determined by the 2,6 dichlorophenolindophenol method[9,10]. An enzyme unit was defined as 1 µmole of dehydroascorbic acid destroyed enzymically in 1 min at pH 5.8 and 37 °C.

Results and discussion. Livers of rats and guinea-pigs were potent sources of the enzyme: no activity was detected in brain, spleen or eye lens[7]. Magnesium ions were essential for full activity but not glutathione - the latter finding being in contrast with results obtained by Kagawa and Takiguchi[7]. Activity was lost by heating at 100 °C for 30 sec. Dehydro*iso*ascorbic acid (dehydro-D-araboascorbic acid) had substrate potency equivalent to dehydroascorbic acid - a finding in agreement with results reported for other enzymes involved in AA metabolism[11]. Blackcurrant anthocyanins, quercetin and fluoride, administration of which increases the concentration of tissue AA in vivo[12,13], were without any in vitro effect on the dehydroascorbatase activity at 10^{-3} M concentrations. There was no significant difference between the liver dehydroascorbatase in young and old guinea-pigs (table 2). Thus, even in animals with strong dehydroascorbatase activity changes in this system are unlikely to account for the modifications in tissue ascorbic acid concentration associated with the administration of fluoride, anthocyanins and flavonoids and with age.

It has been questioned whether enzymic delactonization of dehydroascorbic acid is of any significance in human nutrition[4]; diketogulonic acid, the immediate product of DHAA delactonization, occurs at relatively high concentrations in human blood[14] but this could be a consequence of the non-enzymic breakdown of DHAA which is a relatively rapid reaction at physiological conditions of pH and temperature (table 1).

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A rapid method for the purification of octopine dehydrogenase for the determination of cell metabolites[1]

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Summary. Unlike other NAD^+-dependent dehydrogenases, octopine dehydrogenase was not bound by blue Sepharose. A rapid 2-step purification procedure (gel filtration on Sephadex G-100 followed by affinity chromatography on blue Sepharose) resulted in a final preparation of octopine dehydrogenase which had a sp. act. of 65 units/mg protein and was free of contaminating NAD^+-oxidoreductases. This preparation has been used successfully for the estimation of phospho-L-arginine, L-arginine and octopine in perchloric acid extracts.

Octopine dehydrogenase (octopine: NAD^+ oxidoreductase, E.C. 1.5.1.11) has been used for the determination of phospho-L-arginine, L-arginine and octopine in biological extracts[3–5]. In these determinations, it is important that the enzyme preparation should be free from other NAD^+-oxidoreductase activities, and that it should be of a reasonably high specific activity.

Crude homogenates of *Pecten maximus* adductor muscle contain appreciable amounts of lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, E.C. 1.1.1.27), malate dehydrogenase (L-malate: NAD^+ oxidoreductase, E.C. 1.1.1.37) and octopine dehydrogenase (see Table 1). Lactate dehydrogenase may be eliminated from the preparation, together with other high mol.wt dehydrogenases, by chromatography on Sephadex G-100[3]. This procedure does not, however, completely remove malate dehydrogenase, or other NAD^+ oxidoreductases of low mol.wt comparable to octopine dehydrogenase (approximately 40,000 daltons[6–8]). Octopine dehydrogenase has been purified to homogeneity from *P. maximus*[9] and *Loligo vulgaris*[5]. The procedures are

complicated and time consuming, finally involving preparative electrophoresis[9] or isoelectric focussing[5]. In an attempt to devise an alternative chromatographic procedure, we have looked at the behaviour of octopine dehydrogenase on blue Sepharose[10].

Purification of octopine dehydrogenase. Cibacron blue F3G-A Sepharose derivatives bind enzymes having a 'dinucleotide-fold' common to most NAD^+ and $NADP^+$-dependent dehydrogenases[11,12]. Cross-linked Sepharose 4-B was coupled with Cibacron blue F3G-A[13] and a 4× 1.5 cm column of the blue Sepharose was equilibrated with 10 mM Tris HCl buffer, pH 7.5 containing 100 mM NaCl. An aliquot of a preparation of octopine dehydrogenase made by the method of Gäde and Grieshaber[3] from *P. maximus* adductor muscle was added to the column (2 mg protein/ml gel). Under these conditions, octopine dehydrogenase was surprisingly not bound by the blue Sepharose. The eluate was collected together with about 5 ml of washings. The table shows that the final preparation contained all of the octopine dehydrogenase but none of the malate dehydrogenase activity. The column was regenerated by washing with 3M NaCl (3 column volumes) and 10 mM Tris HCl buffer, pH 7.5 containing 100 mM NaCl. The eluates from several batches were pooled and the octopine dehydrogenase activity concentrated by precipitation with 80% saturated ammonium sulphate and then resuspended in 10 mM Tris HCl buffer, pH 7.5 containing 100 mM NaCl. This rapid 2-step procedure resulted in a preparation of octopine dehydrogenase which was free from malate dehydrogenase and had a sp. act. of 65 units/mg protein.

Contaminating activities. A prerequisite for the use of octopine dehydrogenase to determine metabolite levels in tissue extracts is the absence of contaminating NAD^+-oxidoreductase activities. The mol.wt of octopine dehydrogenase (40,000 daltons) is one of the lowest known for NAD^+-dependent dehydrogenases, so that high mol.wt (100,000 daltons) contaminating enzymes will be removed by gelfiltration on Sephadex G-100. Also Cibacron blue F3G-A is a group specific adsorbent for NAD^+ and $NADP^+$-dependent dehydrogenases.

Purification of octopine dehydrogenase from adductor muscle of *Pecten maximus*. Specific activities: Units/mg protein

Fraction	Lactate dehydrogenase	Octopine dehydrogenase	Malate dehydrogenase
Crude homogenate	0.12	1.7	2.3
Sephadex G-100 preparation	0	19.5	9.2
Blue Sepharose preparation	0	65.0	0

Unexpectedly, octopine dehydrogenase does not bind to blue Sepharose and this has been exploited to separate octopine dehydrogenase from other NAD-dependent dehydrogenases, although we have only assayed for malate dehydrogenase (mol.wt 70,000 daltons). There is still a 10-fold difference in the specific activity of the final preparation and the pure enzyme from *P. maximus* (1000 units/mg protein[9]). The most likely contaminating proteins are soluble sarcoplasmic proteins which may constitute up to 25% of the total protein in the adductor muscle[14].

An experimental check on the possible interference of contaminating activities in the enzymatic assay for octopine was carried out, using extracts of the adductor muscle from *Ostrea edulis* which does not contain octopine[15]. Under standard assay conditions[16], no increase in absorbance at 340 nm was recorded on addition of the octopine dehydrogenase preparation to the oyster extracts. This preparation has been used successfully for the measurement of changes in metabolite levels in the adductor muscle of *P. maximus*, during the escape response[16].

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Proteolytic enzymes in the *Rhodnius prolixus* midgut[1]

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Summary. Sequential chromatographic fractionation of *Rhodnius prolixus* midgut homogenate yielded only one endopeptidase, but revealed the presence of carboxypeptidase-A and B-like enzymes, di- and tripeptidases, as well as aminopeptidase activities.

R. prolixus is a blood-sucking insect and one of the known transmissors of *Trypanosoma cruzii*, the agent causing Chagas disease. The bug requires a single and very large blood meal for ecdysis and oogenesis[2]. In a previous report we have described a SH-dependent endopeptidase[3], which seems to initiate the digestion of proteins in the insect's midgut. This enzyme is confined to the midgut and is present in all instars of the insect[4,5]; its production seems to be controlled by a secretagogue mechanism[6]. The present paper describes other midgut enzymes related to the proteolytic hydrolysis of ingested proteins.

In order to characterize different proteolytic activities, the