recently that thyroid hormones acted discriminatively on different types of MAO in the rat heart[12], and that selective effects of oestradiol benzoate to ovariectomized rats on type A MAO were found in the basomedial hypothalamus and the corticomedial amygdala[13]. Similarly, present results suggest that thyroidectomy induced selective changes on the multiple forms of MAO in the discrete circumventricular nuclei. Further studies with a specific substrate for type B MAO, such as β-phenylethylamine, will be needed to ascertain the selective effects on type B MAO in the circumventricular nuclei, since tyramine is deaminated by both types of MAO.

It is known that all these nuclei are located close to the 3rd ventricle, and that the ependymal cells are distributed on the surface of these nuclei[5-7]. Furthermore, it is suggested that some hypothalamic hormones in the CSF are transported to hypophyseal portal blood mediated by the ependymal cells[6-8]. If it is assumed that the high amounts of type B MAO in these circumventricular nuclei are contained in the ependymal cell layer, the significant changes of type B MAO in the nucleus arcuatus and the nucleus periventricularis may be related to the changes of the transport function of the ependymal cells in these circumventricular nuclei.

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Tissue ascorbic acid and a liver dehydroascorbatase in guinea-pigs

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Summary. The extraction, partial purification and assay of a dehydroascorbatase from guinea-pig liver is described. There was no evidence that changes in dehydroascorbatase activity could account for the modified tissue ascorbic acid concentrations associated with ageing or with the ingestion of fluoride, flavonoids or anthocyanin material.

Ascorbic acid (L-xyloascorbic acid, AA) is a labile component of most tissues and its concentration, in those species unable to synthesize it, is primarily a reflection of its intake in the diet. Tissue concentrations of AA may also, however, be substantially modified by a number of metabolic and physiological factors such as dietary composition, growth rate, metabolic activity, age, sex etc.[2,3]. The immediate determinants in the modification of tissue AA are largely uncharacterized but it would appear that a) the retention capacity of tissues and b) the rate of AA metabolism and breakdown are significant factors.

The catabolic pathways for tissue AA have not been completely elucidated and it would appear that considerable interspecies differences exist[4,5]. Ascorbic acid, at physiological conditions of pH and temperature, is rapidly converted to dehydroascorbic acid. Dehydroascorbic acid however is unlikely to attain any significant concentration in the tissues because of a) the ability of tissues to reduce it back to AA[6] and b) the presence of factors catalysing its further breakdown.

The enzyme glutathione: dehydroascorbate oxidoreductase (E.C. 1.8.5.1) which catalyzes the reduction of dehydroascorbic acid to AA in the presence of thiol compounds has been characterized in plants and there is evidence that a similar system exists in animal tissues[6]. A dehydroascorbatase catalyzing the delactonization of dehydroascorbic acid to diketogulonic acid, has been characterised in animal tissues[7]. The relative activities of these 2 systems could, in part, determine the concentration of AA in tissues; inhibition of dehydroascorbic acid breakdown would presumably result in elevated tissue AA concentrations and vice versa.

This note describes studies designed to determine whether nutritional and environmental factors shown to change AA concentrations in guinea-pig tissues were likely to operate by modifying a dehydroascorbatase system.

Table 1. Stability of dehydroascorbic acid (10^{-3} M) in phosphate buffer at 37 °C (% remaining)

pH	Time (min)				
	1	2	4	8	16
5.8	99	97	95	92	87
6.2	98	96	92	88	76
6.4	96	92	82	66	32
7.0	94	86	75	55	11
7.4	91	80	64	34	0

Dehydroascorbic acid was prepared by bromine oxidation of AA[9] and assayed by the homocysteine method as described in the text.

Table 2. Liver dehydroascorbatase activity in guinea-pigs of different ages

	Age (days)		
	20	250	880
Dehydro-ascorbatase activity*	0.358 ± 0.021 (8)	0.359 ± 0.028 (8)	0.285 ± 0.094 (7)

* Activity expressed as µmoles of DHAA destroyed/g tissue/min (mean values with SE); numbers in brackets refer to the animals in the group.

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