to inhibit the spontaneous oxidation of epinephrine to adrenochrome at pH 10.2. The total volume was 1.0 ml and at 30 °C the rate of autooxidation was found to be 0.025 OD units/min at 480 nm. Protein was assayed according to LOWRY et al.[10] Human brain controls used here had no known neurological abnormalities.

Results and discussion. The distribution of SOD activity for various brain regions is presented in Table I and shows a remarkably homogeneous pattern in human brain. The activity was present in all areas examined (mean value for all areas: 40.69 ± 9.69 (SD) units/mg protein), in both gray and white matter, and the enzyme was stable for at least 6 months when stored at −70 °C. The distribution of this enzyme has also been reported recently in rat telencephalon, cerebellum and medulla oblongata, and similar activities were found in these regions in both male and female animals[3]. These observations would suggest that the enzyme is widely distributed in brain and is very likely present in both neurons and glia. It was particularly interesting to note that brains taken at autopsy from patients afflicted with Huntington's Chorea had apparently normal levels in both the cerebral cortex, caudate nucleus and putamen (Table I). This autosomal dominant disease of unknown etiology strikes the basal ganglia (caudate nucleus and putamen) hardest, leading to loss of smaller interneurons and infiltration of this region by glial cells. In patients with this disease, this region can be so adversely affected that it is reduced to only 10% of the gross weight of normal controls. It has previously been established[11] that a number of other enzymes are dramatically reduced in Huntington's Chorea, including choline acetyl transferase and glutamic acid decarboxylase, which are involved in the synthesis of the neuronal putative transmitters, acetylcholine and γ-aminobutyric acid. It appears likely then that there is no reduction in the level of SOD in the basal ganglia in this disease state.

The subcellular distribution of SOD is given in Table II. In both guinea-pig and human cortical gray matter, the highest specific activity was found in the supernatant fraction, where 26–35% of the total activity was recovered after differential centrifugation. After fractionation of the crude mitochondrial fraction on a sucrose gradient over half of the recovered SOD activity was in the synaptosomal fraction. These experiments would appear to show that the brain enzyme is widely distributed, but we could release up to 80–85% of the SOD in soluble form by hypoosmotic treatment or brief sonic irradiation of the homogenate. In liver up to 84% of the SOD can be recovered in soluble form from homogenates without the latter treatments[4]. The liver and brain enzymes appear as 2 isozymes, A and B, and in studies with KB cells the former was found in soluble form, while the latter was enriched in mitochondria[12]. However, unlike the situation in liver[4], no clear-cut bimodal distribution of SOD was observed in brain in the present studies. Moreover, the purified mitochondrial fraction from guinea-pig brain had relatively low specific activity and we were not able to demonstrate enzyme latency susceptible to sonic irradiation. The present work suggests differences between liver and brain tissue in both the distribution and association of SOD with subcellular components.

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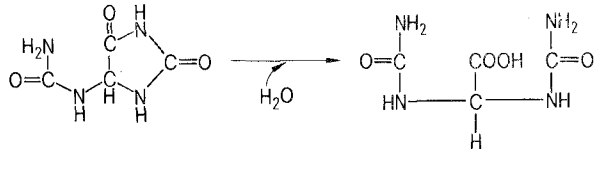
Allantoinase in the Marine Polychaete *Eudistylia vancouveri*[1]

DORA R. M. PASSINO[2] and G. W. BROWN, JR.

Laboratory of Biochemical Ecology, College of Fisheries, University of Washington, Seattle (Washington 98195, USA), 10 May 1976.

Summary. Allantoinase, an enzyme in the purine-urea cycle, was found in *Eudistylia vancouveri* (Polychaeta). The enzyme had a pH optimum at 7.6. The K_m was 0.012 *M* allantoin, and the Arrhenius energy of activation was 12.6 to 14.6 kcal/mol.

Allantoinase (allantoin amidohydrolase, EC 3.5.2.5) catalyzes the hydrolysis of allantoin to allantoic acid[3,4]:



This reaction is part of the purinolytic pathway for degradation of uric acid to glyoxylic acid and urea. Allantoinase is commonly present in Amphibia[3,4], fish[4,5], green plants[6], and microorganisms, e.g., *Escherichia coli* and *Streptococcus allantoicus*[7]. Both the presence and absence of allantoinase have been reported for polychaetes[8,9]. Because of our interest in the comparative biochemistry of uricolytic enzymes and their evolutionary significance for the polychaetes, we assayed preparations of the common sabellid worm *Eudistylia vancouveri* (Kinberg) for allantoinase activity.

Specimens were collected off docks of San Juan Island, Puget Sound, Washington. Homogenates of entire worms and also water extracts of an acetone powder[10] (mean = 5.4 mg protein/ml) prepared from abdomens of the polychaete were assayed at 37 °C by estimating the amount of allantoic acid produced. Allantoic acid was converted to glyoxylic acid which was measured spectrophotometrically (470 nm) as the 2,4-dinitrophenylhydrazone in alkaline solution[11]. The reaction mixture contained 28.6 μmole allantoin (adjusted to pH 7.5 with NaOH), 50 μmole *Tris*-HCl buffer (pH 8.2), 0.4 ml of extract or homogenate, and water to a final volume of 2.0 ml. The pH measured in situ was 7.6. Protein concentrations were determined[12] with purified bovine serum or purified egg albumin as standards.

The activity found for allantoinase is shown in the Table. The pH optimum of the enzyme was 7.6. The Michaelis constant, K_m, was found to be 0.012 *M* for allantoin, as determined by either a Lineweaver-Burk plot or by a weighted Hofstee plot[13]. An Arrhenius plot of log

activity vs. reciprocal of absolute temperature yielded energies of activation of 14.6 kcal/mole and 12.6 kcal/mole for the whole homogenate and acetone powder, respectively. While linearity of the Arrhenius plot was obtained over the range of 14–38 °C for the whole homogenate, the plot for the acetone extract deviated from a straight line above 22 °C, a feature noted previously[7] with some purified allantoinases from other sources. This is thought not to be an effect of denaturation of the enzyme at the higher temperatures[7] but may represent a conformational change in the enzyme.

Two classes of the phylum Annelida, the Oligochaeta and Hirudinea, are purinostatic and lack enzymes of uricolysis[14]. For the third class, the Polychaeta, allantoinase has been found in 4 of the 6 species of Sedentaria tested[8,9], but only 'traces' have been found in one of the 6 Errantia tested[8]. This report provides evidence for the occurrence of allantoinase in another species of Sedentaria. These discoveries of purinolytic activity in the polychaetes give evidence of a biochemical link with the related phylum Sipunculida, which has a complete system of purinolysis[14].

Polychaetes of the Sedentaria may be one of the few groups of organisms possibly possessing both complete purinolysis and ornithine-urea cycle enzymes as mechanisms for the production of urea. It would be of interest to determine whether or not L-ornithine-ketoacid aminotransferase (EC 2.6.1.13) is present in the Sedentaria since this enzyme provides a link between the ornithine-urea cycle and the purine-urea cycle. The question of why errant polychaetes lack enzymes of uricolysis and some sedentary polychaetes possess them might be answered by further examination of the adaptive significance of this pathway for polychaetes.

Allantoinase activity in extract of acetone powder of *Eudistylia vancouveri* for two typical experiments

System	Glyoxylic acid produced (μmoles)
Experiment 1[a]	
Complete	0.245, 0.249[c]
Complete (boiled extract)	0.035
Complete (zero time control)	0
Experiment 2[b]	
Complete	0.257, 0.266, 0.295[c]
Complete (boiled extract)	0.032
Complete (zero time control)	0

[a] 30 min incubation time, 7.70 mg protein/ml. [b] 45 min incubation time, 5.09 mg protein/ml. [c] Replicates.

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[2] Present address: U. S. Fish and Wildlife Service, Great Lakes Fishery Laboratory, 1451 Green Road, Ann Arbor, Michigan 48105, USA.

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Evidence of an Essential Histidyl Residue in Arylsulphatase B[1]

A. A. FAROOQUI[2]

Centre National de la Recherche Scientifique, Centre de Neurochimie, 11, rue Humann, F-67085 Strasbourg Cedex (France), 30 April 1976.

Summary. Diazotization and carbethoxylation studies of arylsulphatase B have indicated that a histidine residue is essential for arylsulphatase B activity.

Arylsulphatase B has recently been obtained in a homogeneous form from ox tissues[3,4] and human liver[5]. This enzyme differs from the corresponding arylsulphatase A both by its higher isoelectric point and lower molecular weight[6]. Although some information is available on the functional groups in the active site, and on the physiological role of arylsulphatase A[7–10], very little is known about the active site and the physiological importance of arylsulphatase B[11,12]. Recent studies of AGOGHUA and WYNN[5] have indicated that probably a histidine residue is involved in the reaction catalyzed by arylsulphatase B. The present study was undertaken to gain more information about the participation of a histidine residue in the hydrolysis of arylsulphates by arylsulphatase B.

Materials and methods. The homogeneous preparations of ox liver arylsulphatases $B1_\alpha$ and $B1_\beta$ were prepared

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