Table II. Effect of temperature on the kinetic constants of the reaction catalyzed by the citrate synthase from *Bacillus stearothermophilus*.

Temperature (°C)	Apparent K_m for acetyl-CoA (μM)	Apparent K_m for oxaloacetate (μM)	Turnover number (min^{-1})
30	3.7	1.1	4000
37	4.4	1.3	7000
43.5	6.1	2.1	11100
50	6.9	2.9	21200
55.4	9.1	3.3	28200
62	13.5	3.3	38800

The reaction mixtures were as previously described[17], except that 20 mM potassium phosphate buffer (pH 7.6) was used instead of *Tris*-HCl, 0.1 M KCl was present, and the concentrations of acetyl-CoA or oxaloacetate were varied. When used as the fixed substrate, the concentrations of acetyl-CoA and oxaloacetate were 0.05 and 0.25 mM, respectively. The turnover number values were calculated from the V_{max} values obtained from the double-reciprocal plots for acetyl-CoA at a saturating concentration of oxaloacetate, employing the molecular weight of 100,000 estimated from gel filtration experiments.

several concentrations of the co-substrate intersected at the abcissa, showing that the K_m values were independent of the concentration of the co-substrate. As in the case of the citrate synthases isolated from other Gram positive bacteria[20,21], the thermophilic enzyme was not inhibited by NADH or α-oxoglutarate concentrations up to 0.5 mM or 5 mM, respectively, but was inhibited by ATP and less effectively by ADP; AMP was ineffective at concentrations up to 5 mM. The inhibition by 5 mM ATP or ADP (62% and 28%, respectively) was the same either in the presence or in the absence of KCl (0.1 M), at 30 °C or at 62 °C. As in the case of other bacterial citrate synthases, with the exception of the enzyme from *B. subtilis* HS 2A2[4], the inhibition by both nucleotides at 30 °C was strictly competitive towards acetyl-CoA, and non-competitive towards oxaloacetate. The apparent K_m for acetyl-CoA (27 μM oxaloacetate) was increased from 3.7 μM in the absence of inhibitors to 10.0 μM and 31.2 μM in the presence of 3 mM ADP or ATP, respectively, without change in the apparent V_{max}. The apparent K_m for oxaloacetate (50 μM acetyl-CoA), on the other hand, was not changed in the presence of 3 mM ADP or ATP, whereas the apparent V_{max} decreased by 19% and 45%, respectively.

The citrate synthase from *B. stearothermophilus* showed considerably greater thermal stability than the enzyme from pig heart, which has similar kinetic and regulatory properties. The latter, as a 20 μg/ml solution in 20 mM potassium phosphate buffer (pH 7.6) and 1 mM EDTA, was completely inactivated after heating for 2 min at 50 °C. A 10 μg/ml solution of the thermophilic enzyme in the same buffer, on the other hand, decayed only by 32% after heating for 20 min at 74 °C. Half-lives were not calculated, since the decay of the thermophilic enzyme did not follow first-order kinetics, being considerable within the first 3 min of incubation, and becoming much slower afterwards. A similar finding has been reported for the 5,10-methylenetetrahydrofolate dehydrogenase from *Clostridium tetanomorphum*[22], and has been interpreted as a possible conversion of the enzyme into a more stable, but less active, form during heating.

The apparent K_m values for both substrates increased about 3-fold when the temperature was raised from 30 °C to 62 °C (Table II). The turnover number of the thermophilic enzyme at 62 °C, 38,800 min^{-1} (Table II) was 3 times higher than the value reported for the rat heart enzyme at 28 °C, 13,000 min^{-1} (ref. [8]).

The Arrhenius plot of the natural logarithm of the turnover number values given in Table II as a function of the reciprocal absolute temperature values was linear from 30 °C to 62 °C; at higher temperatures (not shown), a decrease was observed. The activation energy calculated from the Arrhenius plot was 15 kcal/mol. The corresponding value for the pig heart enzyme was 9.7 kcal/mol at temperatures higher than 20 °C, and 13.7 kcal/mol at temperatures lower than 20 °C (ref. [23]).

The results described in this paper show that the citrate synthase from *B. stearothermophilus* shares the kinetic and regulatory properties and the molecular weight of the enzymes isolated from suitable mesophilic counterparts; only its thermal properties, particularly its considerable stability, seem to differ from those of the citrate synthases from mesophilic Gram positive bacteria and eucaryotes.

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Regional and Subcellular Distribution of Superoxide Dismutase in Brain

T. C. LOOMIS, G. YEE and W. L. STAHL[1]

Neurochemistry Laboratory, Veterans Administration Hospital and Departments of Physiology and Biophysics and Medicine (Neurology), University of Washington School of Medicine, Seattle Washington 98108, USA), 17 May 1976.

Summary. Superoxide dismutase has been found to be widely distributed and of approximately the same specific activity in all regions of human brain examined. It is not reduced during degeneration of the basal ganglia in Huntington's Chorea. After subcellular fractionation of human and guinea-pig cerebral cortex, the highest specific activity of the enzyme was found in the soluble fraction.

Superoxide dismutase (SOD) has been demonstrated in a variety of tissues and cell types and appears to protect against the toxic effects of the O_2-radical[2]. Recent studies[3–6] indicate that very high levels of activity are present in liver, while the adrenals, kidney and red blood cells have intermediate activity and lower activities were found in most other tissues including brain. We have been interested in possible changes in SOD in nervous tissue in certain neurological diseases, and we report here the distribution of SOD in guinea-pig and human brain.

Materials and methods. Guinea-pigs were killed by a blow to the neck followed by exsanguination. Brains were immediately excised and separated into gray and white matter with a dull spatula and were then homogenized and separated into subcellular fractions by a modification of the method of GRAY and WHITTAKER[7] as previously described[8]. Human brains were obtained at autopsy and tissue from various regions was either immediately homogenized and separated into subcellular fractions or was placed in vials, quickly frozen in dry ice-acetone and stored at −70 °C. In certain cases, analyses were carried out on unfrozen tissue, immediately after dissection. In most cases a 10% homogenate of tissue in 0.32 *M* sucrose was prepared for SOD assay. SOD was assayed according to the procedure of MISTRA and FRIDOVICH[9], which is based on the ability of SOD

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Table I. Regional distribution of superoxide dismutase in human brain

Region	Specific activity (units SOD/mg protein)		
	Controls ($n = 3$) [a]	Huntington's Chorea 1	Huntington's Chorea 2
Medulla	32.9–40.4	–	–
Pons	32.7–38.0	–	–
Cerebellar cortex	46.4–61.3	–	–
Cerebellum, dentate nucleus	35.8–38.9	–	–
Parietal Cortex			
Gray	27.9–45.9	47.5	–
White	27.9–38.9	36.3	27.0
Frontal cortex			
Gray	30.9–34.8	23.2	28.0
White	47.9–53.1	22.3	–
Temporal cortex, gray	27.8–39.5	–	–
Hippocampus	41.6–46.8	–	–
Substantia nigra	42.4–64.1	–	–
Midbrain without sub. nigra	39.2–52.6	–	–
Thalamus	33.2–60.5	–	–
Hypothalamus	37.2–55.5	–	–
Putamen	34.7–47.8	39.9	37.4
Caudate nucleus	36.6–51.4	39.9	37.4

[a] Controls: 1. female, 71 yr (34 h); 2. male, 62 yr (29 h); 3. male, 86 yr (18 h). Huntington's Chorea: 1. female, 80 yr (7 h); 2. female, 33 yr (6 h). Numbers in parentheses refer to time between death and autopsy.

Table II. Subcellular distribution of superoxide dismutase

Fraction	Guinea-pig gray matter ($n = 5$)		Human temporal cortex gray matter ($n = 2$)	
	Specific activity [a]	Distribution (%)	Specific activity	Distribution (%)
Homogenate	24.30 ± 5.44 [b]	100	27.40	100
Nuclear	24.26 ± 3.63	46.36 ± 2.95	23.80	54.2
Crude mitochondrial	26.04 ± 3.87	17.00 ± 4.30	24.70	6.9
Myelin	22.58 ± 2.41	15.80 ± 4.69 [c]	–	–
Synaptosomal	26.16 ± 5.18	31.58 ± 3.96 [c]	–	–
Purified mitochondrial	15.54 ± 0.98	11.70 ± 2.51 [c]	–	–
Microsomal	19.48 ± 3.04	6.72 ± 1.89	19.00	4.4
Supernatant	46.60 ± 9.61	34.65 ± 3.09	68.90	25.6

[a] Specific activity = units/mg protein at 30 °C. [b] Mean value ± SD. [c] Relative to the crude mitochondrial fraction.

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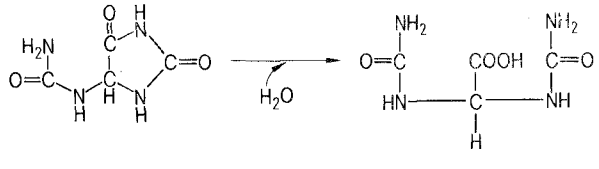
Allantoinase in the Marine Polychaete *Eudistylia vancouveri*[1]

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