The question whether the formation of oximes by *E. lipolytica* is only a way of detoxication or constitutes a route for direct utilization of not fully reduced nitrogen, represents points of interest. If the latter is of significance 2-oximinopropanoic acid could be expected to be present as a metabolite in cells grown on more oxidized nitrogen sources. Experiments were therefore run with some representative procaryotic and eucaryotic microorganisms, which unlike *E. lipolytica* are able to assimilate nitrate. Nitrate was substituted for HA and glucose for glycerol in the culture media. Cells from the middle log phase were analysed for oxime content using the acetonitrile method. 2-oximinopropanoic acid was found in all microorganisms tested, although in varying amounts (Table).

Analysis of the listed cells grown on ammonium sulphate always gave negative results. No oximes could be traced in chloroplasts from etiolated or non-etiolated wheat seedlings grown on nitrate.

The oxime content as a function of growth was studied in cells of *Cryptococcus albidus* grown on nitrate medium (Figure 2). It is obvious that maximum oxime content was reached to in middle log phase of growth (A_{610} about 0.8). At later stages the amount of oximino-acid decreased rapidly and became negligible when growth entered stationary phase (A_{610} about 1.7). The findings support the suggestion that the oximino-acid is used as a source of not fully reduced nitrogen in the metabolism.

The further metabolic transformation of 2-oximinopropanoic acid is not known. It has been shown that oximes may become enzymatically reduced to amino-compounds[7]. Transoximases[8] presumably play an important role.

It appears important to decide whether the formation of oximinoacids represents a bypass of minor importance or an indispensable pathway in the regular metabolic reduction of nitrogen. Work in the field is being continued at this institute.

Zusammenfassung. Es wird gezeigt, dass in Zellen von *Cryptococcus albidus* und in Kulturlösung von *Endomycopsis lipolytica* die Oxime bis zur Mitte der exponentiellen Zuwachsphase in steigenden Konzentrationen vorkommen, während sie in Ammonium-kultivierten Zellen fehlen.

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Glutamine Synthetase, an Enzyme Characteristic of Vertebrate Systems in Invertebrate Tissues

The central nervous system and sensory structures of coleoid cephalopods (e.g., squid, cuttlefish, octopi) are highly organized, and much has been made of the dramatic convergent evolution shown between these structures and those of higher vertebrates (Young[1]; Wells[2,3]; Barber[4]). It was of considerable interest to us, therefore, whether glutamine synthetase (GS), which is a characteristic enzyme of vertebrate nervous and digestive systems (Wu[5]), would also be present in large amounts in coleoid and other invertebrate tissues. Because of the intimate relationship between the structural organization of cells in the chick neural retina and the possible precocious induction of GS during development of this tissue (Piddington and Moscona[6]; Morris and Moscona[7]), we were particularly interested in knowing whether the coleoid eye, which is remarkably histotypically similar to the vertebrate eye, would also show GS activity and whether an eye of an entirely different structural type such as the typical crustacean eye would lack the enzyme.

Specimens of mature *Octopus vulgaris* and *Rossia pacifica* were obtained near the Marine Science Center of Oregon State University at Newport, Oregon. The organs were dissected in sea water and following a brief rinse in 0.01 *M* phosphate buffer (pH 7.1) were lyphilized and stored at −20 °C until assayed. For comparison, tissues of adult specimens of the commercial crab, *Cancer magister*, and the purple sea urchin, *Stronglyocentrotus purpuratus* were prepared in the same manner. Crab eyes (6 per assay) with or without eye stalks were homogenized in a porcelain mortar and pestle; all other tissues were homogenized in a glass tube with a motor-driven Teflon pestle. Assays for GS specific activity depended on the γ-glutamyltransferase properties of the enzyme and were performed according to the method of Rudnick et al.[8], as modified by Kirk[9]. The specific activity of GS was calculated as the number of micromoles of γ-glutamylhydroxamate produced per hour per mg protein in the homogenate. Protein was determined according to Lowry et al.[10].

It was found (Table) that coleoid nervous tissue in general showed relatively high GS specific activity with the optic ganglia showing the highest activity while non-nervous tissue activity was low and skin had no activity at all. This situation was analagous to that found in several vertebrates (Wu[5]). All tissues examined in the crab were notably low in GS activity, but sufficiently large amounts of tissue were used in these assays to demonstrate that the enzyme was, in fact, present. The neural ganglia, which lacked pigments, showed significantly lower GS specific activity in the crab than either coleoid species. Also, the retina of the crab was seen to show much less activity than the neural (thoracic) ganglia of this animal. It should be noted, however, that owing to the considerable amount of non-nervous tissue in the crab compound eye the specific activity of GS in the individual retinula cells may well have been considerably higher. It was also seen that the crab digestive gland revealed much less activity than the generalized nervous tissue as was the situation with coleoid tissues. The Table also

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Average specific activity of glutamine synthetase in invertebrate tissues

Phylum	Animal	Tissue							
		Optic ganglia	Neural retina	Brain	H.P. digestive gland	Digestive gland	Thoracic ganglia	Gut	Skin
Mollusca	*Octopus vulgaris* (2)[a]	11.9	5.3.	5.0	1.24				0.0
	Rossia pacifica (3)	6.9	2.3						
Arthropoda	*Cancer magister* (3)		0.066[b]			0.14	0.66		
			0.21[c]						
Echinodermata	*S. purpuratus* (3)								0.16

[a] Number of specimens; [b] 6 eyes without stalks; [c] 6 eyes with stalks.

shows the presence of very low GS activity in the gut of the sea urchin. Other tissues of this species were not assayed.

We have presented evidence here that glutamine synthetase is present in certain adult tissues of organisms representing 3 major invertebrate phyla. Enzyme specific activity was relatively high in coleoid nervous tissue and less so in digestive tissues. Levels of activity were considerably lower in tissue from functionally comparable crab organs. The high GS specific activity in both the vertebrate and the coleoid nervous systems correlates with the well known convergence concerning their anatomy, electrophysiology, and behavior. It is not possible at present to suggest what functional significance this correlation may have. The role of large amounts of GS in vertebrate nervous systems has been a topic of debate, but may be associated with the peculiar transport problems of nervous tissue. Also, glutamate has recently been implicated as a excitatory neuro-transmitter in certain animals (KRNJEVIC[11]). Consequently, the presence of GS would be functionally indispensable in the synaptic physiology of any system.

Zusammenfassung. Nachweis eines Invertebraten-Enzyms, das bei Vertebraten insbesondere im Nervengewebe eine wichtige Rolle spielt. Diese Glutamin-Synthetase wurde vor allem im optischen Ganglion in der Retina und im Gehirn zweier Tintenfischarten gefunden und überdies in sehr geringer Menge bei Crustaceen und Echinodermen.

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Asymmetrical Activation by Ca^{2+} of the Erythrocyte Membrane K^+-Dependent Phosphatase

It is known that in the presence of Mg^{2+} the ($Na^+ + K^+$)-independent and ouabain-insensitive fraction of the red cell membrane ATPase activity is markedly enhanced by low concentrations of Ca^{2+}, Sr^{2+} or Ba^{2+}[1-3]. We have shown elsewere that in the presence, but not in the absence, of ATP, Ca^{2+}, Sr^{2+} or Ba^{2+} are also able to activate the K^+-dependent fraction of the red cell membrane phosphatase activity, which under these conditions loses its sensitivity to ouabain[4]. Since activation by divalent cations of the membrane phosphatase and ATPase follows very similar curves for each cation, it has been suggested that a single mechanism may be the cause of the activation of both enzymes[4]. If this were the case, membrane ATPase and phosphatase would share the same ATP and Ca^{2+} sites. One of the main predictions that this assumption leads to is that activation of the K^+-dependent phosphatase by Ca^{2+} and ATP should take place, as in the ATPase[5], only when ATP and Ca^{2+} are at the inner surface of the cell membrane.

In this communication we wish to report experiments designed to test this prediction on reconstituted ghosts of human red cells.

Two kinds of ghosts were prepared by a modification of the procedure of SCHATZMANN and VINCENZI[5]. Singly hemolyzed ghosts were prepared lysing 1 volume of packed red cells in 800 volumes of an ice-cold solution containing 5 m*M* $MgCl_2$, 40 m*M* *Tris*HCl, 0.5 m*M* ethyleneglycol-*bis*(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 0.3 m*M* ATP (*Tris* salt), pH 7.2 at 25 °C. Doubly hemolyzed ghosts were prepared lysing one volume of singly hemolyzed ghosts in 40 volumes of an icec-old solution containing 5 m*M* $MgCl_2$, 10 m*M* *Tris*-HCl, 2 m*M* ATP (*Tris* salt), 0 or 1 m*M* $CaCl_2$, pH 7.2 at 25 °C. For both kinds of ghosts the hemolysate was allowed to stand at 0 °C for 10 min. After this step the ghosts were washed 3 times at 5 °C with about 30 volumes of a solution containing 5 m*M* $MgCl_2$ and 150 m*M* *Tris*-HCl, pH 7.4 at 25 °C. The second hemolysis was found to be necessary because the presence of Ca^{2+} in the lysing solution yielded ghosts retaining a large amount of the intracellular soluble phosphatase normally present in red cells. The volume fraction of ghosts occupied by ghosts sealed to Ca^{2+} or ATP was estimated according to the procedure already described[6]. About 50% of the singly hemolyzed

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