the absorbance at 240 nm. Specific activity of each enzyme was expressed as μmoles of product per mg protein per h. Protein was determined by the method of Lowry et al.[23].

Results and discussion. Activities of pyruvate carboxylase and the two mitochondrial enzymes, citrate synthase and fumarate hydratase, in various preparations from the *Neurospora* cell are shown in the table. Most of the pyruvate carboxylase activity was found in the cytosolic fractions, C1S and C18S, whereas no activity was detected in the mitochondrial fractions, M1P, M1P-S and M18S. Pyruvate carboxylase of animals and some bacteria requires acetyl-CoA[3,5,6]. Pyruvate carboxylase of *Neurospora* shows its activity in the absence of acetyl-CoA[11,12]. Although the present assay system contains acetyl-CoA as a component of the reaction mixture, the mitochondrial fractions did not show the activity of pyruvate carboxylase. On the other hand, citrate synthase and fumarate hydratase activities were found in both the mitochondrial and the cytosolic fractions. Although some of this activity could have been due to disruption of mitochondria during the preparation, it is obvious that mitochondria contain citrate synthase and fumarate hydratase. Mitochondrial inner membrane is permeable to pyruvate, malate and aspartate, but not to oxalacetate. In animal cells, a shuttle mechanism works for the transmission of oxalacetate out of the mitochondria for gluconeogenesis[24], because pyruvate carboxylase is localized only in the mitochondrial matrix[18]. While predominance of the gluconeogenic role of the enzyme in vertebrates is indicated[3,25], the primary role of the enzyme in microorganisms is believed to be anaplerotic, that is the production of oxalacetate from pyruvate for both the operation of the TCA cycle and the biosyntheses of carbon compounds[15,26]. The present study shows that the pyruvate carboxylase of *Neurospora crassa* is localized outside the mitochondria. Therefore oxalacetate is formed outside the mitochondria and a shuttle mechanism is not necessary for the gluconeogenesis. Rather, oxalacetate must be transferred from the cytosol into mitochondria for supplementation of the substrate of the TCA cycle. The fact that pyruvate carboxylase of *Neurospora* and yeast exists outside the mitochondria indicates that the regulation of organic acid metabolism in fungi is different from that in animal cells.

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Purification and properties of ornithine aminotransferase from rat brain

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Summary. Ornithine aminotransferase (E.C. 2.6.1.13) from rat brain was purified 100-fold by ammonium sulphate fractionation, DEAE cellulose chromatography, calcium phosphate gel and alumina C γ gel. Pyridoxal phosphate was essential for maximum activity of the enzyme. The brain enzyme did not differ from liver and kidney enzymes in properties such as pH optimum, K_m, substrate specificity and the inhibition by branched chain amino acids. Unlike rat liver enzyme, brain ornithine aminotransferase was able to catalyze the reaction between L-lysine and 2-oxoglutarate. Spermidine and spermine inhibited brain ornithine aminotransferase activity.

Ornithine aminotransferase (OAT; E.C. 2.6.1.13) is a mitochondrial enzyme present in many tissues including liver, kidney and brain[2]. It is the only enzyme known to catalyze the reversible step in the metabolic pathway interconverting arginine, proline and glutamate[3]. In the rat liver, OAT is mainly involved in ornithine catabolism[4]. The metabolic role of this enzyme in other tissues has not been completely identified. Comparative data obtained from the purified enzymes from rat liver and kidney indicated that the two proteins are identical[5,6]. Although OAT from liver and kidney has been characterized in detail, very little is known about the properties of OAT from brain. In the present

communication the purification and the characterization of OAT from rat brain is described.

Materials and methods. Animals. Adult male rats weighing approximately 200 g, of Charle's Foster strain, were used in all experiments. Rats were sacrificed by cervical dislocation and their brains were quickly removed. A 10% (w/v) homogenate was prepared in chilled 0.1 M Tris-HCl, pH 7.5 containing 0.25% Triton-X 100.

Enzyme assay. OAT activity was assayed as described by Peraino and Pitot[7]. The assay system consisted of Tris-HCl, pH 8.0, 50 µmol; L-ornithine, 20 µmol; 2-oxoglutarate, 10 µmol; pyridoxal phosphate, 0.1 µmol and enzyme 0.5 ml (10% homogenate) or 0.2 ml purified enzyme in a total volume of 4.0 ml. Reaction was carried out at 37 °C for 30 min. To stop the reaction, 10% TCA was added followed by 0.1 ml (10 mg/ml) 0-aminobenzaldehyde. After centrifugation, the optical density of the quinazolium complex was measured at 430 nm. In control tubes ornithine was added after the incubation.

One enzyme unit is defined as the amount of enzyme required to form 1 µmol of Δ^1-pyrroline-5-carboxylate per h under assay conditions. The molar extinction coefficient of 2710/ml/cm was used to calculate Δ^1-pyrroline-5-carboxylate formed[7]. Proteins were measured according to the method of Lowry et al.[8].

Purification of ornithine aminotransferase: The homogenate prepared as described above was centrifuged at 10,000 × g for 40 min. The supernatant was then adjusted to pH 5.8 with 0.1 N acetic acid, allowed to stand for 1 h (4 °C) and then centrifuged at 10,000 × g for 30 min. The supernatant was readjusted to pH 7.5 with 0.1 N sodium hydroxide. Solid ammonium sulphate (24.3 g/100 ml) was added to the enzyme with constant stirring. It was then centrifuged at 10,000 × g for 30 min. Ammonium sulphate (10 g/100 ml) was added with constant stirring to the supernatant, and after keeping for 30 min the mixture was centrifuged at 10,000 × g for 30 min. The resudue was dissolved in 0.01 M sodium phosphate, pH 7.5 and dialyzed overnight against the same buffer. After dialysis, pyridoxal phosphate (5 µg/ml) was added to the enzyme and it was put on a DEAE-cellulose column equilibrated with 0.01 M sodium phosphate, pH 7.0. The column was washed with 5 5 volumes of 0.01 M NaCl and the enzyme was eluted with 0.2 M NaCl. Fractions showing enzyme activity were combined and put on calcium phosphate gel (18 mg/ml dry wt, in a centrifuge tube) in a gel to enzyme volume ratio of 2:5. The enzyme adsorbed on the gel and was eluted with 0.1 M sodium phosphate, pH 7.5 containing 5 µg/ml pyridoxal phosphate. Solid ammonium sulphate (35.1 g/100 ml) was then added with constant stirring and after 30 min was centrifuged at 10,000 × g for 20 min. The residue was again dissolved in 0.01 M sodium phosphate, pH 7.5 and dialysed against the same buffer. After dialysis pyridoxal phosphate (5 µg/ml) was added and the enzyme was put on alumina Cγ gel (22 mg/ml dry wt, in a centrifuge tube) in a gel to enzyme volume ratio of 1:5. The adsorbed enzyme was eluted with 0.2 M sodium phosphate, pH 7.5 containing 5 µg/ml pyridoxal phosphate.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was carried out as described by Davis[9]. The enzyme was applied using 5% (w/v) sucrose on the top of the gel. Electrophoresis was carried out at 10 °C using 2.5 mA per tube in 0.01 M Tris-glycine, pH 8.3 for 3 h. The gel columns were removed from the tubes, frozen and sliced into 3-mm sections. Each section was gently homogenized in 1 ml of Tris-HCl, pH 7.5 and assayed for enzyme activity.

Table 1. Purification of ornithine aminotransferase from rat brain

	Volume (ml)	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Purification (fold)	Yield (%)
Crude extract	290	267	2985	0.09	1.0	100
Supernatant	260	239	1845	0.13	1.4	98
PH fractionation	260	224	1483	0.15	1.8	84
First ammonium sulphate fraction after dialysis	43	145	132	1.11	12.0	54
DEAE eluate	43	88	31	2.80	31.0	33
Calcium phosphate gel eluate	32	62	14	4.40	49.0	23
Second ammonium sulphate fraction after dialysis	20	33	6	5.50	61.0	12
Alumina C_γ gel	20	27	3	9.00	100.0	10

Table 2. Effects of in vitro addition of various compounds on brain ornithine aminotransferase activity

Addition	Concentration (mM)	% of control value	Addition	Concentration (mM)	% of control value
None	–	100			
L-Valine	2.5	72	Spermidine	5.0	70
	5.0	59		10.0	52
L-Leucine	2.5	92	Spermine	5.0	67
	5.0	78		10.0	44
L-Isoleucine	2.5	71	$HgCl_2$	0.025	3
	5.0	71		0.05	3
Cystein	2.5	52	$COCl_2$	5.0	29
	5.0	34		10.0	28
N-Acetyl-	2.5	76	$CaCl_2$	2.5	44
ornithine	5.0	73		5.0	11
L-Arginine	2.5	100	$NiCl_3$	2.5	28
	5.0	100		5.0	28
Histamine	5.0	58	$AlCl_3$	2.5	36
	10.0	50		5.0	11
Putrescine	5.0	100	PCMB	0.01	17
	10.0	92		0.02	14
			Iodoacetate	2.5	36
				5.0	6

Results are means of 3 or more separate experiments. PCMB = p-chloromercuribenzoic acid.

Results and discussion. Ornithine aminotransferase was purified 100-fold with 10% recovery by fractionation with ammonium sulphate, DEAE-cellulose chromatography, calcium phosphate gel and alumina Cγ gel (table 1). Purified enzyme migrated as a single protein on disc gel electrophoresis. pH optimum for the purified enzyme using Tris-HCl was 8.0. Enzymes from rat liver, kidney and small intestine[5–10] and from *T. pyriformis*[11] also showed similar pH optima.

K_m, determined by primary and secondary plots, for L-ornithine and 2-oxoglutarate were 1.67 mM and 0.5 mM respectively. Initial velocity studies carried out by varying either of the substrates showed that the enzyme follows a ping-pong mechanism.

The brain enzyme was able to catalyze the reaction between L-lysine and 2-oxoglutarate to about 40% of that with ornithine. This is in contrast to rat liver enzyme, where no activity was obtained with lysine[7]. The rate of reaction was not different when either DL or L-ornithine was used as a substrate. The enzyme, however, appeared to be specific for 2-oxoglutarate, because the rate of reaction was low (10%) when 2-oxoglutarate was replaced by either pyruvate, oxaloacetate or glyoxylate. OAT from rat liver, kidney and small intestine also showed similar substrate specificity[5].

Pyridoxal phosphate was essential for the enzyme activity. At all the stages of purification, it was necessary to add pyridoxal phosphate. As in the case of OAT from other sources[12-14], the brain enzyme was completely inhibited by 1 mM hydroxylamine confirming the requirement of pyridoxal phosphate.

L-Valine, L-leucine and L-isoleucine inhibited the brain enzyme (table 2). As with OAT from other sources[14-16], the inhibition by L-valine was competitive with ornithine. Cysteine and N-acetylornithine also inhibited the brain enzyme. Similar to the rat liver[15] and chick liver[14] enzymes, the brain enzyme was not inhibited by L-arginine. Significant inhibition of the enzyme activity was seen when either histamine, spermidine or spermine was included in the assay system (table 2). Putrescine did not have a significant effect on the enzyme activity.

Heavy metal ions like Hg^{++}, Co^{++}, Ni^{+++}, and Al^{+++} inhibited the enzyme activity (table 2). Iodoacetate and p-chloromercuribenzoic acid (PCMB) also inhibited the enzyme activity demonstrating the requirement of $-SH$ groups for the enzyme activity. Similar results have been reported for rat liver and chick liver enzymes[7,14].

The metabolic function of OAT in the brain is not known. Brain tissue contains appreciable amounts of arginase[18]. Ornithine thus formed from arginine cannot be converted to citrulline because ornithine carbamyl transferase (E.C. 2.1.3.3) is absent from the brain[19]. Ornithine formed by the action of arginase may either be decarboxylated to form putrescine (a precursor of the polyamines spermidine and spermine) or transaminated to glutamic γ semialdehyde (a precursor of GABA). Both spermine (table 2) and GABA[20] inhibited OAT from rat brain.

In conclusion, the properties of the brain enzyme were comparable to those of OAT from rat, fish and chicken liver and rat kidney. Brain OAT showed a pH optimum, substrate specificty and K_m similar to those of liver, kidney and small-intestinal enzymes. In other studies such as inhibition by branched chain amino acids or the effect of p-chloromercuribenzoic acid and heavy metals, the brain enzyme did not differ from kidney and liver enzyme. Since brain has a very low level of activity of carbamyl phosphate synthetase[17], and lacks ornithine carbamyl phosphate transferase[19] activities, it is possible that brain OAT, like that of rat kidney and Chang's liver cells, is mainly used for ornithine degradation.

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A rat mutant unable to synthesize vitamin C[1]

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Summary. A colony of Wistar rats with a hereditary defect in L-ascorbic acid-synthesizing ability was established. This rat, like primates and guinea pigs, lacks L-gulonolactone oxidase (EC 1.1.3.8) which catalyzes the last step of L-ascorbic acid biosynthesis. When L-ascorbic acid was added to their drinking water, the rats grew almost normally and were fertile. These mutant rats should be useful not only for nutritional and parmacological studies on vitamin C, but also for genetic studies on the lack of this enzyme.

Most animals can synthesize L-ascorbic acid from D-glucose via the D-glucuronic acid pathway[3-5], in which the last step is the conversion of L-gulono-γ-lactone into L-ascorbic acid, catalyzed by L-gulonolactone oxidase (EC 1.1.3.8)[6]. This step is missing in primates, guinea-pigs and a few other animals which require a dietary supply of this vitamin. In our laboratories, a rat strain with a hereditary osteogenic disorder controlled by a single autosomal recessive gene with the gene symbol *od* was established from Wistar rats[7]. This paper reports that the homozygotes of this strain, like primates and guinea-pigs, do not