Intracellular Distribution of Pyruvate Carboxylase in Mammalian Brain Cortex[1]

Although the avian liver mitochondria are the better source of pyruvate carboxylase[2], the enzyme is largely diffused in the animal kingdom; it has been shown to be present in the ox, frog, rat and sheep liver, and in the rabbit kidney and liver[2,3].

The pyruvate carboxylase has been purified from lyophilized chicken liver mitochondria and its characteristics have been described by UTTER et al.[4-7]; the enzyme catalyses the overall reaction of synthesis of oxaloacetate from pyruvate and CO_2. Oxaloacetate synthesis is dependent on the presence of pyruvate, $MgCl_2$ and acetyl CoA.

Evidence has been presented in an earlier paper suggesting the presence of the pyruvate carboxylase in the ox-brain cortex mitochondria[8].

Some properties, such as inhibition by avidin, and by ATP concentration higher than 2 m*M*, the narrow range of ATP optimal concentration, and the requirement of ATP and $MgCl_2$ are very similar to those of the same enzyme present in liver.

The present work was carried out to study the diffusion of the brain cortex pyruvate carboxylase in the animal kingdom and the intracellular distribution of pyruvate carboxylase in the mammalian brain cortex.

The enzyme assay, the preparation of the condensing enzyme and of the enzyme extracts, the citrate isolation, and the measurement of the enzymatic activity have been reported in a previous paper[8].

Subcellular fraction preparation. All the operations were carried out in the cold. Ox-brain cortex was homogenized in 10 volumes (ml/g of tissue) of 0.44 *M* sucrose. Cellular debris, heavy and light mitochondria were fractionated by differential centrifugation at 600 and 12,000 *g*. All fractions were suspended in cold water and lyophilized. The 'soluble fraction' was obtained at 105,000 *g*, subjected to extensive dialysis and stored lyophilized.

The extracts obtained as described before[8] from the various cellular fractions, have been tested for the pyruvate carboxylase activity. Table I shows that the cerebral pyruvate carboxylase is widely diffused in the animal kingdom. With some differences in the specific activity and in the activity per g of tissue, we determined the pyruvate carboxylase activity in the central nervous system of rat, guinea-pig, rabbit and cat.

Table II shows that the activity is mainly located in the brain cortex mitochondria, while the soluble fraction has no activity. A further fractionation of mitochondria in light and heavy components has shown that the latter fraction is the richest source of pyruvate carboxylase. The same subcellular location has been reported for chicken liver[2], and the heavy mitochondria represent the usual material for the extraction and purification of the enzyme from this tissue.

Liver mitochondria from many, although not all, species can synthesize phosphoenolpyruvate from pyruvate, yet these mitochondria contain only negligible amounts of malate enzyme, since this enzyme is in the soluble cytoplasm. The same argument holds for pyruvate kinase, which also is found in the soluble portion of the cell.

Evidence has been presented[2] suggesting that pyruvate carboxylase and phosphoenolpyruvate carboxykinase functioning in sequence may catalyse reactions constituting a major pathway for phosphoenolpyruvate synthesis from pyruvate or lactate in the overall process of gluconeogenesis in avian liver mitochondria.

The recent studies of KREBS[9] on gluconeogenesis in kidney slices implicate pyruvate carboxylase, since glucose synthesis is accelerated by precursors of the acyl CoA compounds which activate this enzyme[10]. HENNING et al.[11], PRINZ et al.[12] and WAGLE[13] have also reported that

Table I. Pyruvate carboxylase in the brain cortex of several mammalian species

Species		μmoles of pyruvate incorporated/mg of mitochondrial protein
Rat	(2)	107
Guinea-pig	(2)	119
Rabbit	(2)	54
Cat	(2)	118
Ox	(6)	362

The numbers in parentheses indicate the number of mitochondrial preparations obtained as described in the text from brains of 5 rats, 5 guinea-pigs, 3 rabbits, 2 cats and 1 ox. The numbers are the average of 3 separate determinations carried out with each preparation by the radiochemical assay described elsewhere[8].

Table II. Intracellular distribution of pyruvate carboxylase in mammalian brain cortex

Fraction		μmoles of pyruvate incorporated/mg of protein
Mitochondria	(4)	420
Soluble fraction	(4)	70
Cellular debris	(4)	non detectable
Light mitochondria	(2)	150
Heavy mitochondria	(2)	200

The numbers in parentheses indicate the number of fraction preparations obtained as described in the text from ox-brain cortex. The numbers are the average of 3 separate determinations carried out with each preparation by the radiochemical assay described elsewhere[8].

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liver pyruvate carboxylase activity is elevated in fasted and in alloxan diabetic rats and after administration of corticosteroids; these findings are consistent with a role for this enzyme in gluconeogenesis.

The presence of the phosphoenolpyruvate carboxykinase has been previously demonstrated in cat, rat, guinea-pig and ox brain cortex mitochondria[14].

The very high similarity of some characteristics of the cortex and liver pyruvate carboxylase and the common intramitochondrial location of the brain cortex pyruvate carboxylase and phosphoenolpyruvate carboxykinase support the hypothesis that the 2 enzymes, functioning in sequence, may catalyse reactions constituting a pathway for phosphoenolpyruvate synthesis from pyruvate or lactate in mammalian brain cortex mitochondria.

Riassunto. La presenza della piruvico carbossilasi è stata evidenziata nella corteccia cerebrale di ratto, cavia, coniglio, gatto, bue. È stata studiata la localizzazione subcellulare della piruvico carbossilasi cerebrale; l'enzima risulta essere particolato: il citoplasma solubile non presenta alcuna attività mentre i mitocondri pesanti costituiscono la frazione più attiva. Viene discusso il possibile ruolo fisiologico della piruvicocarbossilasi nella corteccia di mammifero.

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Production of 4-Imidazole Ethanol from Histamine by *Saccharomyces rouxii*[1]

Histamine is converted in mammalian tissue by diamine oxidase to 4-imidazole acetaldehyde. Xanthine oxidase or aldehyde dehydrogenase converts this to 4-imidazole acetic acid[2], which is found in the urine[3]. Nakajima and Sano[4] found that the urine of patients treated with an aldehyde dehydrogenase inhibitor contained small amounts of 4-imidazole ethanol. They also found traces of this compound in the urine of normal subjects. The concentration is low; we calculate their results to indicate an excretion by normal man of less than 200 µg/day. Such low amounts suggest that the study of its formation in animal tissue will be difficult.

In a study of histidine metabolism by yeasts[5] we have found several strains which produce imidazole ethanol from histamine in readily detectable amounts, and one strain which produces this compound as the major product of histamine degradation. This paper describes the identification and estimation of 4-imidazole ethanol produced by *Saccharomyces rouxii*.

Methods. Imidazole ethanol was prepared as the chloroplatinate, as described by Wrede and Holtz[6]. *S. rouxii* PRL 411–64 was isolated from a bumblebee's nest from Melfort, Saskatchewan.

The growth medium used was Yeast Carbon Base (Difco) containing 3 m*M* histamine or 3 m*M* L-histidine. Yeasts were grown aerobically in 125 ml shake flasks on a rotary shaker at 150 rpm at 25 °C. The substrates were completely metabolized after 3 days. The yeast cells were removed by centrifugation. 2 or 10 µl of supernatant were applied to Whatman No. 1 paper, chromatographed in 8 solvents, and imidazole derivatives detected with diazotized sulphanilic acid (DAS) and sodium carbonate[7]. The Rf of the product was compared with those of imidazole ethanol and imidazole acetic acid.

The amount of imidazole ethanol in 0.02 ml of supernatant was determined by the formation of a coloured compound with diazotized *p*-nitro aniline[8]. The optical density at 550 nm was measured with a Beckman DU Spectrophotometer.

Results and discussion. The only detectable product of histidine metabolism had the same Rf as imidazole acetic acid. When 2 µl of supernatant from the culture grown on histamine was chromatographed as described above, only one imidazole derivative reacting with DAS could be observed. The Rf values of this product in the 8 solvents were indistinguishable from those of authentic imidazole ethanol. When 10 µl of sample were chromatographed, a faint spot with the same Rf as imidazole acetic acid was also detected.

Chromatographic identification of imidazole-4-ethanol

Solvent	Rf · 100	
	Imidazole-4-ethanol	Histamine product
n-Propanol-N-acetic acid, 3:1	52	54
Butanol-acetic acid-pyridine-H_2O, 4:1:1:2	28	30
Methanol-*n*-butanol-benzene-H_2O, 2:1:1:1	88	87
Butanol-acetic acid-H_2O, 4:1:1	35	34
Butanol-pyridine-H_2O, 1:1:1	75	75
Ethanol-H_2O, 77:23	73	71
iso-Propanol-conc. NH_4OH-H_2O, 80:5:15	88	86
tert-Butanol-formic acid-H_2O, 70:15:15	64	63

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