Carbamyl transfer with mammalian and bacterial enzymic preparations*

The reaction ornithine → citrulline has been shown to be composed of two main enzymic steps[1,2,3] reactions 1 and 2.

$$1.\ AG^{**} + CO_2 + NH_3 + ATP \xrightarrow[Mg^{++}]{\text{Enzyme I}} \text{Compound X} + ADP$$

$$2.\ \text{Compound X} + \text{Ornithine} \xrightarrow{\text{Enzyme II}} \text{Citrulline} + CG + PO_4^{---}$$

The purification of Compound X as well as partial identification showing Compound X to be a carbamyl phosphate or related derivative of AG or CG has been reported elsewhere[1,4].

A most important contribution has been made recently by JONES *et al.*[5], who prepared synthetic carbamyl phosphate and found it to react enzymically with ornithine to form citrulline, thus confirming the carbamyl phosphate nature of Compound X. CP and Compound X react equally well with partially purified preparations of E II from calf liver or with bacterial extracts as shown in Fig. 1. Fig. 2 shows that CP is inactivated enzymically as is Compound X by the splitting enzyme already described[6]. Fig. 2 shows also that the spontaneous decomposition rate is the same for both compounds.

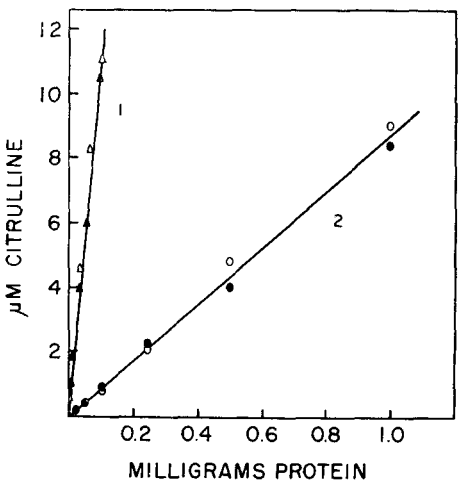

Fig. 1. The enzymic synthesis of citrulline from Compound X and CP. Each tube contained the following expressed in micromoles per 2 milliliters: tris buffer, pH 7.5, 100; Compound X, potassium salt, 12; CP, lithium salt[5], 12; ornithine, 15. Curve 1, bacterial extract; curve 2, liver E II preparation. Open signs are experimental points obtained using CP, solid signs are experimental points obtained using Compound X. Incubation at 26° for 10 minutes.

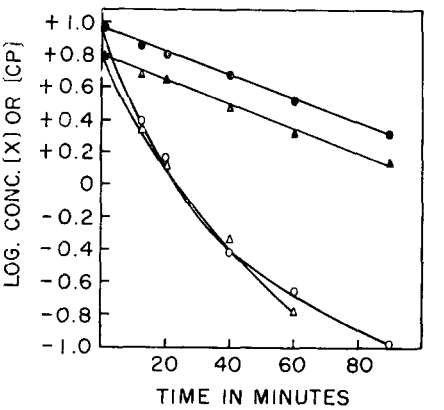

Fig. 2. Enzymic and non-enzymic decomposition of Compound X and CP. Each tube contained the following expressed in micromoles per 2.5 milliliters: tris buffer pH 7.5, 100; Compound X, potassium salt, 6.6; CP, lithium salt, 9.6. Experimental points marked with solid signs represent the values obtained without splitting enzyme; open signs represent values obtained in the presence of a water extract of acetone dried rat brain, 18 milligrams protein per tube; circles, CP as substrate; triangles, Compound X as substrate. Compound X and CP were measured in all cases as previously described[2]. Temperature 38°.

It is clear from the data presented here that Compound X and CP are very closely related compounds, however they do not appear to be identical on the basis of analytical data[4] of highly

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** The following abbreviations are used throughout this paper: AG, Acetyl glutamate; CG, Carbamyl glutamate; Compound X, the active intermediate for citrulline synthesis formed from AG or related compounds; CP, Carbamyl phosphate[5]; tris, tris(hydroxymethyl)aminomethane. E II, 20–40% acetone fraction of acetone powder extracts of calf liver treated as previously described for Enzyme II[2]; Enzyme I, as previously described[2]. Bacterial extracts prepared according to OGINSKY AND GEHRIG[7] from Strain D 10, Group D streptococci, kindly given by Dr. SLADE and grown according to his procedure[8]. All analytical methods and procedures have been described in preceding papers[1,2,3].

purified Compound X and on the basis of the molar relationship shown between CG added to incubation mixtures and Compound X synthesis[2].

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A simple method for the estimation of glutamine in brain extracts*

During investigations concerning the synthesis of glutamine by brain cells[1], a micro-method for the estimation of glutamine in brain extracts has been developed which was found to be simpler than other methods described in the literature[2,3,4].

The method depends on the hydrolysis of the amide-N of glutamine by means of half-saturated potassium hydroxide solution; the ammonia split off is determined by the microdiffusion method of CONWAY[5]. The hydrolysis is carried out at room temperature in a Conway dish, the ammonia being trapped in a dilute solution of acid contained in the central chamber of the dish. Thus the hydrolysis of the glutamine and the distillation of the ammonia split off are carried out in one operation.

The procedure as used by the author for the estimation of glutamine synthesised by brain cells *in vitro* is as follows. Brain cells, prepared by passing the cerebrum through a 40 mesh sieve, are shaken in Warburg flasks at a constant temperature of 37.5° C. At the end of the incubation period the flasks, containing 100 to 150 mg brain tissue suspended in 3 ml Ringer solution, are removed from the bath and 0.2 ml of 16% phosphotungstic acid is added to the contents of each flask. The Ringer solution plus tissue is then poured into centrifuge tubes and centrifuged at 1800 *g* for 5 minutes. A 1 ml aliquot of the clear supernate is placed in the outer chamber of a standard size Conway dish, the central chamber of which contains 1 ml of 0.002 *M* potassium biiodate solution. 1 ml (approximately) of saturated potassium hydroxide solution is then added to the fluid in the outer chamber, and the dish is sealed with a greased glass plate. (It is also advisable to place a small amount of grease on the top of the wall of the central chamber, as the potassium hydroxide solution has a tendency to "creep".) The time allowed for hydrolysis of the glutamine and diffusion of ammonia is 90 minutes (2 hours in cold weather), at the end of which time the potassium biiodate solution is titrated with dilute barium hydroxide solution (approximately 0.004 *M*), using a 0.25 ml microburette. The indicator used is the one recommended by CONWAY[5], *i.e.* a mixture of methyl red and methylene blue.

Since some of the ammonia determined in this way will be free NH_3, a second 1 ml aliquot is analysed for free NH_3, using half-saturated potassium carbonate solution to liberate the ammonia from the tissue extract[5]. The difference between the first and the second determination gives the amount of amide-N present.

A small quantity of ammonia is split from glutamine by half-saturated potassium carbonate solution, amounting to 3.2% of the amide-N of glutamine in 90 minutes at 27° C, under the conditions of the CONWAY method. For ordinary purposes this quantity may be considered negligible, but a correction can be applied if considered necessary.

Experiments with glutamic acid showed that the amino-N of glutamic acid (and presumably

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