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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therefore of glutamine) was relatively stable under the conditions used. Over a period of 17 hours, half-saturated potassium carbonate and half-saturated potassium hydroxide solution split respectively 0.15% and 0.23% of the amino-N of glutamic acid.

Table I shows the recovery of glutamine from pure glutamine solutions and from solutions containing known amounts of brain tissue and glutamine. It may be noted that the amount of glutamine found in the tissue is of the same order as the amounts found in brain tissue by other workers[6,7].

Table II presents the results of an experiment in which the amount of glutamine synthesised by brain cells *in vitro* was estimated by (a) the present method and (b) the method of KREBS[2], in which the glutamine is hydrolysed under acid conditions. The results of both methods are similar to those of KREBS[2] and of WEIL-MALHERBE[8].

TABLE I

RECOVERY OF GLUTAMINE FROM PURE GLUTAMINE SOLUTIONS AND FROM TISSUE SUSPENSIONS

For recovery of glutamine from tissue suspension, guinea pig brain tissue was passed through a 40-mesh sieve immediately after excision of the organ from the animal, and dispersed in ice-cold solutions containing glutamine and phosphotungstic acid.

Glutamine added (μg amide-N)	*Brain tissue (mg)*	*Free NH_3 (μg N)*	*Glutamine (μg N)*	*% Recovery of glutamine*
20.0	-	0.3	19.4	97
5.0	-	0.1	4.9	98
-	100	1.8	7.4	-
20.0	100	2.0	27.2	99
5.0	100	1.8	12.6	104

TABLE II

GLUTAMINE SYNTHESIS BY BRAIN CELLS: GLUTAMINE DETERMINED USING (a) ACID HYDROLYSIS, (b) ALKALINE HYDROLYSIS

Guinea pig brain tissue incubated for 2½ hours in phosphate Ringer solution containing glucose (0.01 *M*) and sodium glutamate (0.02 *M*). Temperature 37.5° C.

Method	*Free NH_3 (μg N per 100 mg tissue)*	*Total NH_3 (μg N per 100 mg tissue)*	*Amide-N (μg N per 100 mg tissue: by difference)*
Acid hydrolysis	4.5	25.5	21.0
	4.2	26.1	21.9
Alkaline hydrolysis	4.3	25.5	21.2
	3.9	25.8	21.9

Since potassium hydroxide solution will hydrolyse other non-protein amino-compounds contained in brain tissue besides glutamine (such as asparagine and nicotinamide), the present method cannot be used when the amount of glutamine to be analysed is small in relation to the amount of tissue; for such determinations the more specific method of KREBS[3] is to be preferred. However, for serial determinations of the relatively large amounts of glutamine synthesised by brain cells *in vitro*, the method, owing to its simplicity, may serve a useful purpose.

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