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The Enzymic Hydrolysis of Glutamine and its Spontaneous Decomposition in Buffer Solutions

By H. G. BRAY, SYBIL P. JAMES, ISABEL M. RAFFAN AND W. V. THORPE

Department of Physiology, Medical School, University of Birmingham

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The investigation of glutaminase activity reported in the preceding paper (Bray, James, Raffan, Ryman & Thorpe, 1949) led us to consider the nature of the products of the action of glutaminase upon glutamine. The evidence hitherto presented for these being glutamic acid and ammonia is indirect and based upon ammonia and amino-nitrogen estimations (e.g. Krebs, 1935; Archibald, 1944; Archibald & Hamilton, 1945). As far as we are aware, glutamic acid has not yet been isolated from the digests and characterized. Early in our present investigation it seemed possible that pyrrolidonecarboxylic acid might be a product of the reaction since there was a discrepancy between the percentage hydrolysis of glutamine brought about by tissue extracts as measured by formol titration (corrected) and by ammonia estimation. Such a result could be accounted for by the formation of pyrrolidonecarboxylic acid, which would give rise to the liberation of ammonia without an equivalent increase in formol acidity:

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This would have been in accord with the hypothesis of Leuthardt (1940), which was, however, withdrawn owing to failure to isolate pyrrolidonecarboxylic acid from digests.

The present paper shows that, under certain conditions, pyrrolidonecarboxylic acid may be formed in glutamine digests as a result of spontaneous decomposition.

EXPERIMENTAL

The methods and materials used were the same as those described in the preceding paper (Bray *et al.* 1949).

Estimation of amino N. Samples from the digests were deproteinized with sodium tungstate and H_2SO_4 , and the amino N estimated in portions of the protein-free solution in the Van Slyke micro apparatus (reaction time 4 min.). Under these conditions 90% of the N of glutamine was liberated and 40% of the N of ammonia. (Hamilton (1945) found 38% under similar conditions.) Ammonia N was estimated on another portion and the appropriate correction made to the amino-N values.

RESULTS

Formol titration of glutamine, glutamic acid and pyrrolidonecarboxylic acid. The compounds were dissolved in phosphate buffer pH 7.4. Glutamine and

pyrrolidonecarboxylic acid in concentrations up to 200 mg./100 ml. gave the theoretical titrations corresponding to one carboxyl group/mol. Glutamic acid, however, in concentrations up to 300 mg./100 ml. gave consistently low titrations corresponding to $77 \pm 3\%$ of the theoretical, calculated as equivalent to two carboxyl groups. In enzyme studies the conversion of glutamine to glutamic acid is measured, so that the increase in titration corresponds to the formation of a second carboxyl group. The first carboxyl group of glutamic acid can be taken as titrating normally, as in glutamine, so that the second carboxyl group only titrated to an extent of $54 \pm 6\%$. This value has been used to 'correct' the formol titrations. A higher percentage of the theoretical titration could be obtained by increasing the pH of the end point by using thymolphthalein instead of thymol blue as indicator, but the end point with the digests was so difficult that consistent duplicates could not be obtained. The ethanol titration method (Harris, 1923) was not practicable, owing to the large dilution with ethanol required.

Incubation of glutamine with tissue extracts. Extracts of various tissues were incubated with glutamine in phosphate buffer at pH 7.4, and the extent of hydrolysis of the substrate estimated by both ammonia and formol methods. Table 1 shows some typical results. The formol values have been 'corrected' as described above. It can be seen that, in experiments in which glutamine was rapidly and almost completely hydrolyzed by the very active brain extracts (Exps. 1 and 2), there was, considering the unprecise nature of the correction, reasonable agreement between the formol and ammonia values. In short-term experiments with less active extracts (Exps. 3 and 4), in which hydrolysis was far from complete, there was an indication that the ammonia values exceeded the formol values after 4–5 hr. to a significantly greater extent than after 1 hr. This is shown more clearly in the long experiments (nos. 5 and 6). In Exp. 5 almost complete decomposition was attained after 23 hr. as judged by the ammonia value, although the formol method indicated only 63% conversion. In Exp. 6

with a less active extract the ammonia value after 22 hr. was more than twice the formol value, although the extent of the discrepancy in the two experiments was the same (equivalent to 28% of the glutamine). This amount was the same as that obtained when glutamine was incubated with boiled tissue extract (Exp. 7), and corresponded closely with the extent of the spontaneous decomposition of glutamine in phosphate buffer as described below.

Table 1. *Results of incubation of glutamine with extracts of various tissues in phosphate buffer pH 7.4*

Exp. no.	Tissue	Time (hr.)	Percentage hydrolysis determined by	
			Formol ('corrected') method	Ammonia method
1	Rabbit brain	0.75	76	86
		4	76	88
2	Rabbit brain	0.5	61	60
		2.5	81	90
3	Rabbit kidney	0.5	11	23
		1.5	39	43
		4	46	66
4	Rabbit liver	0.67	9	14
		5	26	46
5	Rabbit kidney	3.5	54	61
		23	63	91
6	Rat liver	2	19	23
		4	19	29
		22	19	47
7	Rat liver (extract as in Exp. 6, heat-inactivated)	2	0	5
		22	0	30

Spontaneous decomposition of glutamine in buffer solutions. It is known that glutamine in neutral, weakly acid or alkaline solution is readily converted to pyrrolidonecarboxylic acid by heating at 100° (Chibnall & Westall, 1932; Vickery, Pucher, Clarke, Chibnall & Westall, 1935; Melville, 1935). This reaction at pH 6.5 and 100° is accelerated by the presence of phosphate (Hamilton, 1945). It seemed desirable, therefore, to examine the effect of keeping glutamine in a phosphate solution at 37°.

Table 2. *Spontaneous decomposition of glutamine in buffer solutions*

pH	Buffer	Glutamine in substrate (M)	Percentage decomposition after				
			2.5 hr.	5 hr.	10 hr.	20 hr.	40 hr.
7.4	Phosphate	0.005706	7.0	11.0	17.0	26.5	40.5
7.4	Phosphate	0.01141	7.0	9.2	12.4	26.0	41.0
7.4	Phosphate	0.02282	4.5	9.2	16.0	28.0	—
7.4	Phosphate	0.01364	7.0	12.5	20.3	32.0	46.5
6.8	Phosphate	0.01141	2.5	5.0	10.0	19.6	39.5
8.0	Phosphate	0.01141	7.0	12.5	22.0	37.0	56.8
7.4	Veronal-HCl	0.01370	0.0	0.1	1.2	3.8	10.4
7.4	Veronal-HCl	0.02740	2.0	3.0	5.0	8.8	14.8
Average for phosphate buffer, pH 7.4			6.5	10.5	18.5	28.0	43.0
Average for veronal buffer, pH 7.4			1.0	1.5	3.1	6.4	12.6

Solutions of glutamine were incubated in buffer solutions at 37.4°. Samples were withdrawn at intervals, and the extent of decomposition measured by estimation of ammonia; there was no increase in formol acidity. The results are given in Table 2.

It is clear that on long incubation there is appreciable decomposition of glutamine. This is greater in phosphate buffer, in which approximately 28 % of glutamine is decomposed in 20 hr., than in a phosphate-free buffer (veronal), in which only 6 % is decomposed. The absence of increase in formol acidity suggests that pyrrolidonecarboxylic acid is the product. This we have confirmed by isolation of the compound from a solution of glutamine (400mg.) incubated in phosphate buffer (100 ml.) for 42 hr. The acid was extracted with ethyl acetate (cf. Woodward & Reinhart, 1942) and had m.p. 157–160°: melting point mixed with authentic pyrrolidonecarboxylic acid, 157–160°.

Attempted isolation of end product of the action of liver extracts on glutamine. For these experiments short-term digests were used so as to avoid the complication of the formation of considerable amounts of pyrrolidonecarboxylic acid by spontaneous decomposition of glutamine.

We were unable to detect any pyrrolidonecarboxylic acid in our digests by the method used successfully

by Woodward & Reinhart (1942) for its isolation from digests of glutathione. Several estimations of the loss of amino nitrogen supported Krebs's (1935) findings that glutamic acid and not pyrrolidonecarboxylic acid is formed. Attempts to isolate glutamic acid from glutamine digests have so far been unsuccessful, but paper chromatography (cf. Consden, Gordon & Martin, 1944) of the material extracted by *n*-butanol from such digests suggests that the product is indeed glutamic acid. We thus have no reason to dispute the conclusion of the earlier workers that glutamic acid is the principal product of the enzymic hydrolysis of glutamine.

SUMMARY

The nature of the products of the action of tissue extracts on glutamine has been examined. Whilst glutamic acid appears to be the main product, significant amounts of pyrrolidonecarboxylic acid may be present in long-term digests, owing to spontaneous decomposition of glutamine.

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The Effect of Various Diets on the Metabolism of Nicotinic Acid and Nicotinamide in the Rabbit

By P. ELLINGER AND M. M. ABDEL KADER, *Lister Institute of Preventive Medicine, London*

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It has recently been shown (Ellinger & Abdel Kader, 1948, 1949) that nicotinic acid and nicotinamide are not metabolized in an identical manner by various mammalian species. Man, dog, cat and rat aminate nicotinic acid to nicotinamide and methylate the latter to nicotinamide methochloride, whilst the rabbit and guinea pig deaminate nicotinamide to nicotinic acid and eliminate small amounts of an

unidentified compound which was provisionally called 'pseudo nicotinamide methochloride'. Whilst the guinea pig eliminates nicotinic acid unchanged, the rabbit methylates part of it to trigonelline. It appears that the species which aminate nicotinic acid are omnivorous or carnivorous, while those which deaminate nicotinamide are strictly herbivorous. It appeared possible that these differences