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Stability of Glutamine *in vitro*.* (30294)

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Glutamine is considered an unstable compound with two pathways of breakdown: 1) Formation of ammonia and glutamic acid; 2) Formation of ammonia and a cyclical compound, pyrrolidone carboxylic acid(1,2). In modifying the resin chromatographic methods of Hirs, Stein and Moore(3) for quantitative separation of the dicarboxylic amino acids, the impression was obtained that glutamine was stable. The early peaks that emerged from the resin column remained unchanged regardless of age of the specimens. The following experiments were performed to evaluate this observation.

Materials and methods. A modified resin chromatography method was employed to analyze quantitatively glutamic and aspartic acids(4). Glutamine could readily be separated from glutamic acid with this weakly basic anionic resin (Fig. 1). Initially the amino acid† analyses were performed by collecting 100 one ml effluent fractions on a Technicon drop-counter fraction collector and analyzing the samples with ninhydrin(5,6). However, recently the method has been adapted to the Technicon AutoAnalyzer, employing the same Rohn and Haas 100-200 mesh CG-4B anionic resin and 0.2 N sodium acetate buffer, pH 4.5(4,7).

The analyses of ammonia in glutamine standards were also performed by the ion exchange method of Hutchinson and Labby(8), but the colorimetric analysis was carried out by the technique outlined by Chaney(9).

Experiments. 1) One milliliter of 1 mM aqueous and 0.1 N sodium citrate buffer pH 5 solutions of glutamine were repeatedly analyzed on the resin columns during an 8-week period. The solutions were stored at 4°C. Other specimens were repeatedly analyzed after being left at room temperature for 3 to 4 weeks. 2) Pyrrolidone carboxylic acid emerged after aspartic acid as was shown by analyzing the effluent samples with ninhydrin after alkaline hydrolysis. 3) The effluent fractions obtained on chromatography of the glutamine solutions were subjected to alkaline hydrolysis, to search for a peak in the position of emergence previously demonstrated for pyrrolidone carboxylic acid. 4) Fresh solutions of glutamine and glutamine stored at 4°C for several weeks were alkalinized and allowed to distill into the Nessler's reagent *in vacuo* for 30 minutes. After this period the solution was brought back to pH 5 and analyzed with ninhydrin(5,6). 5) When the method for analyzing quantitatively glutamic and aspartic acids was adapted to the AutoAnalyzer, an ammonia peak could be clearly separated from glutamine (Fig. 1). Therefore, chromatograms of fresh and stored glutamine solutions were reanalyzed to observe whether there would be an increase in quantity of ammonia and a decrease in concentration of glutamine with increasing age of the specimens. 6) Two milliliters of glutamine stock solutions were added to a Na K

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† Amino acids were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

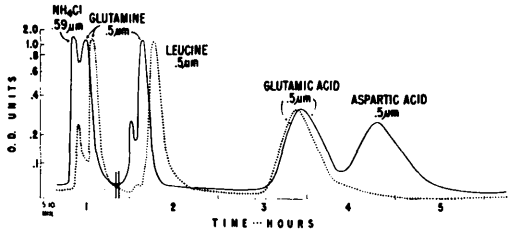

FIG. 1. Standard amino acid curves obtained by the AutoAnalyzer employing 2 separate columns as noted by dotted and solid lines.

1) To the left of the black parallel lines (1 hr 20 min) the separation of ammonia from glutamine is demonstrated. The quantitative recovery of glutamine is noted from a standard stored at 4°C.

2) To the right of the black parallel line is a new series of analyses demonstrating quantitative separation of leucine and glutamic acid as noted by the dotted curves and glutamine, glutamic and aspartic acids as noted by solid lines. No additional glutamic acid is noted in solid line curves indicating absence of glutamic acid in the stored glutamine.

Dowex 50W-X12 cation exchange resin(8) and analyzed by the Chaney reagents(9).

Results. 1) Fresh solutions of glutamine and solutions stored for 8 weeks at no time showed any significant quantitative alteration in the peak which is known to be glutamine. No glutamic acid was ever recovered (Fig. 1).

2) When a chromatographed glutamine standard was subjected to alkaline hydrolysis no pyrrolidone carboxylic acid peak emerged after aspartic acid.

3) Fresh and stored solutions of glutamine were distilled through Nessler's reagent *in vacuo* for 30 minutes. At no time did the Nessler's reagent change color to indicate the absorption of ammonia. When the original solution was analyzed with ninhydrin, there was no loss of ninhydrin positive material—showing again that there was no ammonia in the stored glutamine.

4) With the use of the AutoAnalyzer, the observations that the area of the ammonia recovery remained unchanged with time, as did the glutamine peak, demonstrate that there was no alteration of the original glutamine solution (Fig. 1).

5) No increase in the quantity of ammonia was noted when stored glutamine standards were analyzed by the ion exchange method(8,9).

Discussion. In the studies presented, the original observation that glutamine appears to be stable seems to be confirmed. There was no evidence of breakdown of glutamine to glutamic acid, and there was no suggestion that ammonia was liberated with the formation of pyrrolidone carboxylic acid.

The fact that glutamine is stable *in vitro* would indicate that once plasma is deproteinized, the analysis of glutamic acid would be quantitative. Glutamine values would be expected to be recovered quantitatively, as it is analyzed, as glutamic acid, after acid hydrolysis (Table II). It has been observed that urine or plasma stored at 4°C have a significant alteration in the glutamic acid and glutamine concentrations.

TABLE I. Repeated Plasma Determinations.

No.	Date specimen obtained and prepared	Date of analysis (1964)	Method of storage	Glutamic acid Plasma conc, mg/100 ml
1	July 17, 1964	7/17		.635
2	Pooled normal	8/ 3	4°C filtrate	.620
3	plasma	"	−70°C "	.635
4		9/28	4°C "	1.55
5		30	4°C P. acid*	1.27
6		10/20	4°C filtrate	1.12
7		26	−70°C "	1.23
8		27	−70°C "	1.53
1	Nov. 13, 1964	11/18	4°C filtrate	.95
	Normal plasma			.95
2		20	4°C "	1.27
				1.18
3		23	4°C "	1.02
				1.04
4		24	4°C "	1.06
				.92
5		30	4°C P. acid	.85
				.85
6		12/ 3	4°C filtrate	1.37
7		3	4°C P. acid	1.21
8		4	4°C filtrate	1.22
9		4	4°C P. acid	1.07
10		7	−70°C filtrate	.94
11		8	−70°C "	1.09
1	Dec. 16, 1964	12/16		.92
	Normal plasma			.84
2		17	4°C filtrate	.95
				.85
3		18	4°C "	.87
4		22	4°C "	1.03
				1.01
5		23	4°C "	1.14
				1.03

* P. acid = Picric acid.

TABLE II. Analyses of Hydrolyzed Pooled Plasma.

No.	Date specimen obtained and filtrate made	Date of analyses (1964)	Method of storage, °C	Glutamine	Asparagine
				Plasma conc, mg/100 ml	
1	July 17, 1964	7/20	4	11.9 12.0	2.83 2.89
2		31	−70	11.2 11.3	2.97 3.48
3		9/29	4	11.3	3.41
4		30	−70	11.3	3.36
5		20	4	12.1	3.48
6		10/20	−70	11.4	3.06
7		27	−70	11.6	3.51

Plasma filtrate stored at 4°C and −70°C for about 3 to 4 weeks revealed no increase in glutamic acid, confirming the suggestion that deproteinized physiologic solutions are stable (Table I). Studies have not been performed to evaluate whether these stored filtrates contain pyrrolidone carboxylic acid and ammonia.

Summary. The data presented indicate that glutamine remained stable when prepared in citrate or aqueous solution, stored at 4°C for at least 8 weeks or at room temperature for 3 to 4 weeks. Analyses of deproteinized plasma indicate that the glutamic acid concentrations were constant and stable for approximately 4 weeks. Plasma glutamine determinations were also quantitatively reproducible with time. These results would also suggest that with deproteinization of blood, the values obtained for glutamic acid are not due to glutamine breakdown but are true glutamic acid values.

ADDENDUM: Since submitting this manuscript, it has been demonstrated that there is no significant increase in ammonia concentration in samples analyzed for one week from a plasma filtrate.

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Inhibition of Proline-C^{14} Incorporation into Rat Liver Ribosomes by Thiazolidine-4-Carboxylic Acid in a Cell-Free System.* (30295)

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The hydroxyproline in collagen-like proteins synthesized *in vitro* has been shown to originate from free proline(1). The question then arises as to whether proline is being converted to hydroxyproline by way of a transfer RNA (t-RNA) bound proline inter-

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