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BLOOD AND SPINAL FLUID, INCLUDING A METHOD FOR ITS
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STUDIES REGARDING A GLUTAMINE-LIKE SUBSTANCE IN BLOOD AND SPINAL FLUID, INCLUDING A METHOD FOR ITS QUANTITATIVE DETERMINATION

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It has been pointed out by several investigators (1, 2) that glutamic acid is the only amino acid that produces an increase in oxygen consumption by brain tissue *in vitro*. It has also been suggested that glutamic acid may act as an ammonia carrier in cellular metabolism (2, 3). The *in vitro* experiments of Krebs (4) demonstrated that the enzyme glutaminase is present in various organs, such as brain, liver, kidney, and retina. He also showed that tissues containing the enzyme will synthesize glutamine, *in vitro*, from ammonia and glutamic acid in the presence of a source of energy, such as glucose. This does not occur if a source of energy, such as glucose, is absent from the substrate.

In view of our finding that the level of the amino acids in the blood is depressed during insulin hypoglycemic shock therapy in patients with mental disorders, we suggested (5) that the above mentioned mechanisms may be involved in this phenomenon. This and the following paper are reports of studies in support of this suggestion. They indicate that a substance with the properties of glutamine is present in blood and spinal fluid, and that it is affected by insulin hypoglycemia and also glucose administration. While this work was in progress, Hamilton (6), in a short communication, reported on the presence of a glutamine-like substance in blood plasma.

Earlier investigators have reported on some of the characteristics by hydrolysis of glutamine, glutamine derivatives, asparagine, and urea. Thierfelder (7) and his co-workers showed that gliadin, dipeptides and tripeptides of glutamine, and various acetyl derivatives of glutamine, split off ammonia at a much slower rate than glutamine in $N H_2SO_4$, both on boiling and at $20^\circ C.$ for 18 to 24 hours.

Chibnall and Westall (8) also investigated the extent of hydrolysis at $100^\circ C.$ at different hydro-

gen ion concentrations of asparagine as compared with glutamine, together with the effect of hydrolysis on the change in the quantity of alpha amino acids as determined by the Van Slyke nitrous acid method, since, when the amide group of glutamine is hydrolysed, ring formation takes place between the amino group and the carboxyl group which prevents the decomposition by nitrous acid. They suggested conditions in which a combination of both determinations would be suitable for the quantitative determination of glutamine and asparagine in a mixture of these substances in plant extracts. They pointed out that the presence of urea would introduce a certain amount of error which, however, could be corrected for if the quantity of urea were known.

Later, Vickery and his co-workers (9) also investigated the characteristics of hydrolysis of these two acid amides and developed a quantitative method for the determination of glutamine in plant extracts by the amount of ammonia liberated by hydrolysis at pH 6.5 at $100^\circ C.$ for 2 hours. They pointed out that, although asparagine does not split off ammonia under these conditions, urea does and they also stated that other compounds might be present in crude plant extracts which might hydrolyze under the specified conditions and that therefore the results of such determinations should be interpreted with caution.

Krebs (4), in his studies on the formation of glutamine by glutaminase in various tissues, used the amount of ammonia liberated in $2N H_2SO_4$ (approximately) at $100^\circ C.$ for 5 minutes as a measure of the amount of glutamine formed in his *in vitro* experiments. Since Chibnall (8) had shown that urea liberates 22.9 per cent of its total nitrogen content as ammonia in 2 hours in $N H_2SO_4$ at $100^\circ C.$, it is obvious that a small amount of urea must have decomposed under the

conditions of Krebs' experiments although the amount was too small to vitiate his results.

Since the body fluids which we wished to investigate contained urea as one of the interfering substances and perhaps other compounds which might liberate ammonia, a more detailed study of the rate of ammonia formation from substances in some of the body fluids and of solutions of glutamine, urea, asparagine, and other compounds on hydrolysis in various acidities and at different temperatures was undertaken.

PROCEDURE AND METHODS

Ammonia was determined by Conway's method (10) of micro-diffusion analysis. The micro-diffusion cells used were the standard glass units as recommended by him. Standard acids of the proper ranges of normality containing Tashiro's indicator were prepared according to his directions. Kirk's standardized capillary burette (11) with a capacity of 0.1 ml. was used for titration. The method permits the determination of 2 to 5 gamma per ml. of ammonia nitrogen with an accuracy of about 2 per cent and 0.4 gamma with an accuracy of about 5 per cent. Although Conway found that diffusion at 37° C. for 1 hour allowed practically complete distillation of the ammonia contained in 1 ml. of solution, we found that where strong acid had been used in the preparation of the material and then neutralized with sodium hydroxide, 2 hours at 37° C. was preferable, and this period of diffusion was therefore used in all determinations. One ml. of the various solutions investigated was used for each analysis. Each determination was done in duplicate or triplicate.

The free ammonia present in the fluid and the reagents was determined prior to hydrolysis by addition of saturated K_2CO_3 and distillation at 37° C. for 2 hours and titrating. By adding fresh standard acid to the central chamber of the Conway dish after the 2-hour period and allowing the distillation to continue for an additional 2-hour period, titrating, and repeating this procedure several times, it was found that very small amounts of ammonia were given off both by aqueous glutamine solutions, and also the body fluids, due to decomposition of the amide by alkali. It was found that this continued at a slow constant rate in tests carried out at 2-hour intervals for 6 to 8 hours. Therefore, corrections for the ammonia, liberated by such decomposition, were made by adding fresh standard acid to the central chamber after each ammonia determination and allowing the distillation to continue for an additional 2 hours at 37° C. The amount contributed from this source gradually decreases as the acid hydrolysis progresses and no additional ammonia is obtained by this procedure where the glutamine solution or the labile compound in the body fluid being analyzed has been completely hydrolyzed.¹ Urea solution in saturated K_2CO_3 at 37° C. does not liberate ammonia.

¹ The maximum correction required due to the decomposition of unhydrolyzed glutamine or glutamine-like

Spinal fluid, 10 per cent trichloroacetic acid filtrates of plasma and serum, ultrafiltrates of serum and plasma, and solutions of glutamine, asparagine, and urea were investigated in this study. Ultrafiltrates of serum and plasma were prepared by filtration through Number 600 cellophane membranes at 30 lbs. pressure.

The rate of ammonia liberation on hydrolysis in 10 per cent trichloroacetic acid, 0.7N HCl or H_2SO_4 , N HCl or H_2SO_4 , and 2N HCl or H_2SO_4 , with and without 10 per cent trichloroacetic acid, at temperatures of 40° C., 50° C., 60° C., 70° C., and 100° C., at various time intervals, was investigated, as indicated in the tables and figures.

In comparing the rate of hydrolysis of the body fluids with that of the aqueous glutamine solutions, corrections were made for the slight amount of ammonia liberated by urea decomposition during acid hydrolysis, as will be indicated in the experiments.

OBSERVATIONS

Ammonia liberated on acid hydrolysis by urea and spinal fluid

In Figure 1 is shown the result of a preliminary study of the rate of liberation of ammonia nitrogen by normal spinal fluid heated at 60° C., 70° C., 80° C., and 100° C., in 0.7N HCl and 2N HCl, and also by that of urea (33 mgm. and 40 mgm. per 100 ml.), dissolved in similar acid solutions and heated at 80° C. and 100° C. Ammonia determinations were made every half hour for 4 to 6 hours. The ammonia liberated is calculated as milligrams of ammonia nitrogen per 100 ml. of original solution. It will be noted, Figure 1 (curve V), that at 70° C., the ammonia liberated rises rapidly and tends to approach a plateau level in $1\frac{1}{4}$ to $1\frac{1}{2}$ hours. At 80° C., the rise is somewhat more rapid than at 70° during the first hour, and no similar plateau level is reached, the liberated ammonia rising slowly in practically a straight line over a 3-hour period beyond the first hour (Figure 1, curve IV). At 100° C., the liberated ammonia rises most rapidly in the first half hour and then at a slightly slower but steady rate during the subsequent 4 to 6 hours of observation. At 60°, not shown in the figure, ammonia was liberated more slowly and reached a plateau level in 3 to 4 hours.

It is obvious that above 70° C., some substance besides that readily decomposed at 70° C. or below, decomposes in appreciable amounts, this effect increasing with the increase in temperature.

substance by the strong alkali was about 0.06 mgm. of ammonia nitrogen per 100 ml. of solution.

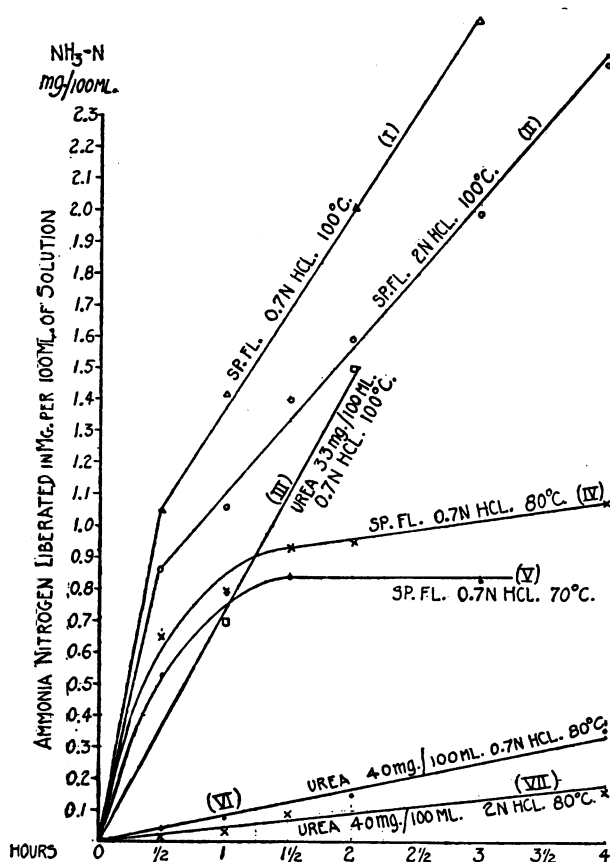


FIG. 1. SHOWS THE LIBERATION OF AMMONIA ON ACID HYDROLYSIS BY UREA SOLUTIONS AND SPINAL FLUID IN 0.7N AND 2N HCL AT 70°C., 80° C., AND 100° C.

The urea solution liberates ammonia at a slow steady rate at 80° C. (curve VI) but at 100° C. (curve III) the rate is very much more marked.² At 70° C. the amount of ammonia liberated by urea in 1¼ hours is slight (Table I).

Since the spinal fluid contained 26 mgm. of urea per 100 ml., the steady slow liberation of ammonia at 80° beyond the 1½-hour period could

² It will be noted that the decomposition of urea is somewhat less in 2N HCl than in 0.7N HCl. This is in keeping with the findings of Kreible and Holst (12) and Hammett (13) that in strong inorganic acid solutions, the decomposition of amides decreases with increase in strength of the acid, after a certain maximum point has been reached. The acidity at which the maximum rate of decomposition occurs was found to vary for different amides. The parallel behavior of glutamine and the glutamine-like substance in this regard is added evidence that the latter is probably glutamine. The findings of Vickery, *et al.* (9) that at pH 4, at 100° C., urea liberates very much more ammonia than at pH 6.5, is in contrast, but is not incompatible with the above findings.

practically be accounted for by the slow decomposition of its urea (compare curve IV with ammonia liberation by urea solution curve VI at 80° C.). This also is true at 100° C. (compare curves I and III of Figure 1) beyond the ½-hour period.

TABLE I

Decomposition of aqueous solutions of urea in 10 per cent trichloroacetic acid at 70° C.

Ammonia liberated, calculated as nitrogen per 100 ml. of original solution			
Time		Urea solution (30 mgm. per 100 ml.)	Urea solution (60 mgm. per 100 ml.)
hours	minutes	mgm. N per 100 ml.	mgm. N per 100 ml.
1	15	0.05	0.08
2	30	0.09	0.16
3	45	0.12	0.24
Average per 1 hour and 15 minutes		0.043	0.08 or (0.04 mg. N per 30 mg. of urea in 100 ml.)

Rate of ammonia liberation on hydrolysis in 10 per cent trichloroacetic acid

Since it was planned to use trichloroacetic acid to deproteinize blood serum and plasma for investigation, the following studies were carried out.

(A) Ammonia liberated by urea in 10 per cent trichloroacetic acid solution at 70° C.

In Table I is shown the amount of ammonia nitrogen liberated by 30 mgm. and 60 mgm. of urea per 100 ml. of solution, in 10 per cent trichloroacetic acid, at 70° C. It will be seen that, under these experimental conditions, 0.04 mgm. of ammonia nitrogen per 100 ml. is liberated in 1 hour and 15 minutes, per 30 mgm. of urea in 100 ml. of solution. This decomposition occurred at this steady rate for 3 hours and 45 minutes of observation. This datum will be used later for making corrections for the ammonia liberated by the urea present in the body fluids. It is obvious that this correction, which will be required for spinal fluid and blood from individuals without nitrogen retention, will be very small, since the urea present is usually below 30 mgm. per 100 ml.

(B) Rate of hydrolysis of glutamine and spinal fluid in 10 per cent trichloroacetic acid at temperatures of 50° C., 60° C., and 70° C.

One part of an aqueous 40 per cent solution of trichloroacetic acid was added to 3 parts of an

aqueous glutamine solution (freshly prepared) and to 3 parts of spinal fluid, in order to obtain 10 per cent trichloroacetic acid in the reaction mixtures without too great a dilution of the material being hydrolyzed. The protein precipitated in the spinal fluid was removed by centrifuging. The nitrogen liberated as ammonia on acid hydrolysis was calculated per 100 ml. of the original solution.

The results of experiments at 60° C. and 70° C. are shown in Table II. It will be seen that at 70° C., the ammonia liberated both by glutamine and spinal fluid approaches a plateau level in 1 hour and 15 minutes. At 60° C., a plateau level is not reached until about 3 to 4 hours.

In Figure 2 are shown the results of additional experiments of the rate of hydrolysis of glutamine and spinal fluid carried out at 50°, 60°, and 70° C., in 10 per cent trichloroacetic acid and 0.7N H_2SO_4 . $\log(C_0 - X) = \log C$ has been plotted against time (t), in which (C_0) represents the total amount of labile "amide" nitrogen at $t = 0$, and X represents the amount hydrolyzed in time (t) of the glutamine or the labile substance in the spinal fluid, expressed in millimoles per liter. The latter figure was obtained from the ammonia liberated in 1 hour and 15 minutes at 70° C. in 10 per cent trichloroacetic acid, and was corrected for the slight decomposition of urea and for free am-

monia present prior to hydrolysis, as indicated earlier in the paper. The figures have also been corrected for the slight decomposition of glutamine or glutamine-like substance due to the strong alkali used to distill off the ammonia, as indicated previously in the section on methods. This correction (maximum about 0.06 mgm. N per 100 ml.) decreases, as indicated previously, and approaches zero as the hydrolysis proceeds.

It will be seen from Figure 2 that glutamine and the substance in the spinal fluid decompose as a first-order reaction and that the slopes are practically identical for the 2 substances.

The reaction rate has also been studied in 10 per cent trichloroacetic acid plus N HCl and 2N HCl. These also gave practically identical rates for the 2 substances under similar conditions of acidity and temperature. It should be noted, however, that the rate was found to be somewhat more rapid in the stronger acid solutions. This is similar to the increase in rate of acid hydrolysis of formamide, acetamide, and propionamide, observed with increase in strength of the inorganic acid solutions within certain ranges. A discussion of this effect is given by Kreibel and Holst (12) and Hammett (13).³

The values of k (specific reaction rate), as determined from the slopes of the lines from Figure

³ See also footnote 2.

TABLE II
Comparison of hydrolysis of glutamine solution and spinal fluid in 10 per cent trichloroacetic acid at temperatures of 60° and 70° C.

Ammonia liberated calculated as NH_3 -Nitrogen in mgm. per 100 ml. of original solution							
60° C.				70° C.			
Time		Spinal fluid	Glutamine solution	Time		Spinal fluid	Glutamine solution
hours	minutes	mgm. N per 100 ml.	mgm. N per 100 ml.	hours	minutes	mgm. N per 100 ml.	mgm. N per 100 ml.
0		0	0			0	0
0	15	0.14	0.16	0	10	0.25	
0	30	0.28	0.34	0	20	0.36	0.52
0	45	0.34	0.46	0	30	0.48	0.65
1	0	0.42	0.55	0	45	0.59	0.80
1	15	0.47	0.61	1	0	0.69	0.89
1	30	0.54	0.65	1	15	0.70	0.91*
2	0	0.60	0.77	1	30	0.70	0.92
3	0	0.67	0.89	2	0		0.92
4	0	0.73	0.91	3	0		

* Nitrogen analysis (Kjeldahl) of the glutamine used yielded 18.7 per cent N. Half of this would correspond to a maximum of 9.35 per cent of amide N (theoretical = 9.6 per cent). Since a solution of 10 mgm. of glutamine per 100 ml. was used, 0.91 represents 97.3 per cent of the maximum amide nitrogen which might be expected to be liberated as ammonia, or 95 per cent of the theoretical content of glutamine. The glutamine was prepared from beet root by the method of Vickery, *et al.* It was recrystallized twice and had a melting point of 187°C. (uncorrected).

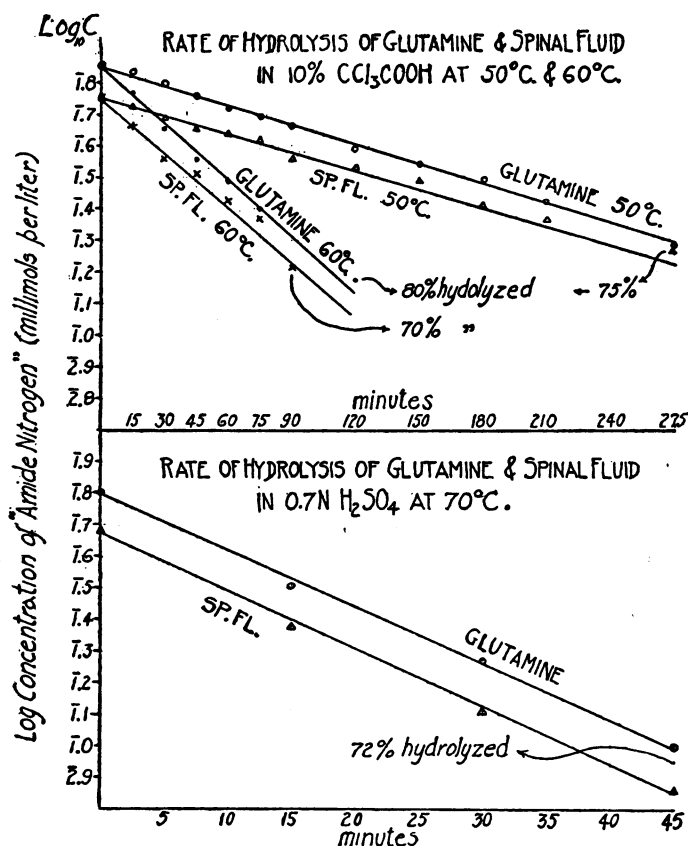


FIG. 2. RATE OF HYDROLYSIS OF GLUTAMINE AND SPINAL FLUID IN 10 PER CENT CCl_3COOH AT 50° C. AND 60° C., AND IN 0.7N H_2SO_4 AT 70° C.

2, are shown in Table III ($\log_{10} C_0 - \log_{10} C = 0.434 kt$ (t in seconds) was used in the calculations).

Hydrolysis rates of ultrafiltrates and trichloroacetic acid filtrates of serum and plasma compared with glutamine at 70° C. in 0.8N trichloroacetic acid

One part of a 40 per cent trichloroacetic solution was added to 3 parts of serum or plasma in a 15 cc. graduated centrifuge tube. The mixture was centrifuged and the clear protein-free supernatant fluid was pipetted off. The protein precipitate was washed once with an amount of 20 per cent trichloroacetic acid approximately equal to $\frac{3}{4}$ of the volume of the compact protein precipitate, as measured in the graduated centrifuge tube. This was again centrifuged and the washing was added to the above supernatant fluid. This was made up to volume and aliquots were used for

TABLE III
Values of (k) calculated from slopes of lines in Figure 2
($\log_{10} C_0 - \log_{10} C = 0.434 kt$)
(t in seconds)

Acidity	Temperature	Substance	$k \times 10^4$
10 per cent CCl_3COOH	50° C.	Glutamine	0.84
	50° C.	Spinal fluid	0.74
0.7N HCL	60° C.	Glutamine	2.30
	60° C.	Spinal fluid	2.22
0.7N HCL	70° C.	Glutamine	6.91
	70° C.	Spinal fluid	6.91

study. A part of this material was titrated with methyl red as an indicator and the normality determined. This was found to be 0.8 normal. The glutamine was dissolved in trichloroacetic acid of the same normality. An aqueous solution of trichloroacetic acid was added to the ultrafiltrate to give the same acidity, the dilution of the ultrafiltrate being recorded. The ammonia liber-

ated during the various stages of hydrolysis was calculated as nitrogen per 100 ml. of serum, serum ultrafiltrate, or glutamine solution, respectively. The necessary corrections for urea decomposition, etc., were made as previously described.

In Figure 3, $\log_{10}(C_0 - X)$ has been plotted against time, as previously described for spinal fluid. It will be seen that the decomposition is that of a first order reaction and that the slopes of the lines for glutamine, ultrafiltrate, and trichloroacetic acid filtrate are practically parallel.

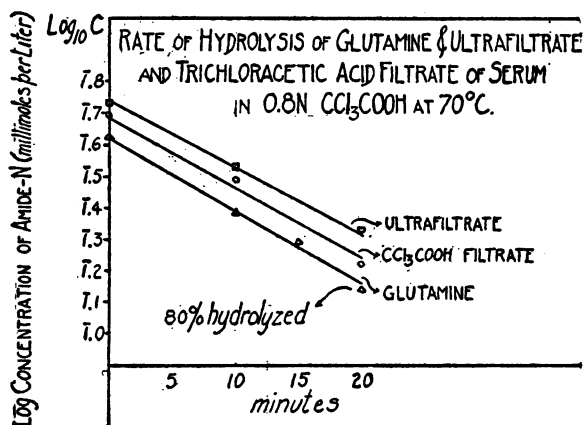


FIG. 3. RATE OF HYDROLYSIS OF GLUTAMINE AND ULTRAFILTRATE AND TRICHLOROACETIC ACID FILTRATE OF SERUM IN 0.8N CCl_3COOH AT 70°C .

It will also be noted that the amounts of ammonia nitrogen liberated by the trichloroacetic acid filtrate of serum and by its ultrafiltrate in 75 minutes at 70°C . are almost identical (0.70 mgm. N per 100 ml. of serum and 0.75 mgm. N per 100 ml. of ultrafiltrate), thus showing that the substance yielding the ammonia is present in solution in the serum and does not result from the chemical treatment of the serum for protein precipitation.

Other experiments carried out in 10 per cent trichloroacetic acid and in $2\text{N H}_2\text{SO}_4 + 10$ per cent trichloroacetic acid, at 50°C ., 60°C ., and 70°C . showed the same parallel tendency in rate of hydrolysis between glutamine solutions, ultrafiltrates, and trichloroacetic acid filtrates of serum or plasma, for similar conditions of acidity and temperature. The data are not included since the findings are essentially the same as for spinal fluid.

Comparison of hydrolysis of glutamine and asparagine solutions and spinal fluid in 10 per cent trichloroacetic acid and in $2\text{N H}_2\text{SO}_4$ plus 10 per cent trichloroacetic acid at 70°C . for $1\frac{1}{2}$ hours

Asparagine solutions in 3 different concentrations, containing the equivalents of 8.0 mgm., 3.0 mgm., and 1.0 mgm. of amide nitrogen per 100 ml. of solution respectively, a glutamine solution with an amide nitrogen equivalent of 1.0 mgm. per 100 ml. of solution, and spinal fluid were hydrolyzed in 10 per cent trichloroacetic acid and in 10 per cent trichloroacetic acid plus $2\text{N H}_2\text{SO}_4$ at 70°C . for $1\frac{1}{2}$ hours. The results are shown in Table IV.

TABLE IV

Comparison of hydrolysis of glutamine, asparagine, and spinal fluid in 10 per cent trichloroacetic acid and 10 per cent trichloroacetic acid plus $2\text{N H}_2\text{SO}_4$ at 70°C . for $1\frac{1}{2}$ hours.

Ammonia liberated, calculated as NH_3 -Nitrogen in mgm. per 100 ml. of original solution				
Substance	10 per cent CCl_3COOH		10 per cent $\text{CCl}_3\text{COOH} + 2\text{N H}_2\text{SO}_4$	
	mgm. N per 100 ml.	per cent of T.A.N.	mgm. N per 100 ml.	per cent of T.A.N.
Glutamine solution 1.00 mgm. amide N per 100 ml.	1.00	100*	0.99	99*
Asparagine solutions				
8.0 mgm. amide N per 100 ml.	1.19	15	3.40	42
3.0 mgm. amide N per 100 ml.	0.36	12	1.25	42
1.0 mgm. amide N per 100 ml.	0.15	15	0.39	39
Spinal fluid	0.73†	100	0.74	102

* Per cent of T.A.N. = Per cent of the total amide-N present in the original solution which was liberated as ammonia.

† 0.73 is considered as the total amide-N due to the glutamine-like substance in the spinal fluid.

It will be noted that there is a marked difference in behavior of the asparagine solutions as compared with that of the glutamine solution and spinal fluid in the two acidities. While the amide nitrogen of glutamine and of the glutamine-like substance in the spinal fluid is completely liberated as ammonia under both conditions, that of asparagine is split off to the extent of 12 to 15 per cent in trichloroacetic acid alone, and 39 to 42 per cent in trichloroacetic acid plus $2\text{N H}_2\text{SO}_4$ during the same period of hydrolysis. It is apparent from the figures that if asparagine were present in the spinal fluid in an amount equivalent to the lowest concentration used in the experiment, it

would have been readily detected by this procedure.⁴

Comparison of the determination of amide nitrogen of glutamine solutions determined by different methods

Two solutions of glutamine, solution I containing 10 times as much glutamine as solution II, were used to compare the quantitative determina-

TABLE V

Comparison of various methods for quantitative determination of glutamine in terms of amide nitrogen

Amide nitrogen calculated as ammonia nitrogen liberated per 100 ml. of solution		
Method	Solution I*	Solution II*
	mgm. N per 100 ml.	mgm. N per 100 ml.
Vickery <i>et al.</i> (Buffer solution pH 6.5 at 100° C. for 2 hours)	8.60	0.86
Krebs (2N H ₂ SO ₄ at 100° C. for 5 minutes) (2N H ₂ SO ₄ at 100° C. for 10 minutes)	8.80	0.88
	9.05	0.89
Hydrolysis in 10 per cent CCl ₃ COOH 70° C. for 1 hour 15 minutes 70° C. for 2 hours 30 minutes	8.85	0.89
	8.95	0.90

* Solution I contained 10 times as much glutamine as solution II. For percentage of nitrogen recovered, see legend for Table II.

tion of glutamine by the amount of ammonia liberated in 1 hour and 15 minutes at 70° C. in 10 per cent trichloroacetic acid, with the method of Vickery (9), and the method used by Krebs (4). Vickery states that his method gave from 96 per cent to 103 per cent recoveries. The results show

⁴ Another procedure which was adopted in the quantitative determination of the glutamine-like substance in studies to be reported in the next paper was as follows: The amount of ammonia liberated in 10 per cent trichloroacetic acid at 70° C., in 1 hour and 15 minutes and in 2 hours and 30 minutes, was determined. Since the ammonia liberated from glutamine or glutamine-like substance is practically complete in 1 hour and 15 minutes, under these conditions, the increase in ammonia occurring during the longer heating period is due to some other substance in the body fluid hydrolyzing at a slower rate than glutamine. This served as a check on the determination. Also, the difference in ammonia liberated between the 2 heating periods can be used to correct the figure obtained in the shorter heating period. This small correction can be accounted for, in whole or in very large part, by the slight decomposition of urea.

a very close agreement between the various procedures, the method of Krebs and our method giving slightly higher results (Table V).

DISCUSSION

Although the final proof that the glutamine-like substance present in blood serum and plasma and spinal fluid is glutamine will be chemical isolation and identification of the substance (a task which Hamilton (6) states he is pursuing), various facts indicate strongly that it is glutamine. These facts are as follows:

(1) Certain tissues in the body contain the enzyme glutaminase which can synthesize glutamine. (2) McIlwain (14) has isolated glutamine from horse meat. (3) The parallel behavior under the various conditions of hydrolysis of glutamine and the glutamine-like substance. (4) The observations of Hamilton (6) of the probable formation of pyrrolidone carboxylic acid by the glutamine-like substance in blood plasma.

We were so strongly inclined to the view, as a result of our studies, that the substance is glutamine and is probably of considerable importance, that we had proceeded to investigate the behavior of this substance in various clinical and experimental conditions. These data will be reported in subsequent communications.

Our findings are in agreement with those of Hamilton (6) that the amount of ammonia liberated on acid hydrolysis by this compound in blood plasma is equivalent to about 5 to 10 mgm. of glutamine per 100 ml. This we have found is also true not only for man but for the blood of the rabbit. This level is also found in human spinal fluid which may be considered as being, in a way, an ultrafiltrate of the blood. The level in blood and spinal fluid is, however, not fixed, but has been found to change with certain physiological and experimental conditions. Some of these phases of the work are still under investigation. The effect of insulin hypoglycemia and glucose administration are reported in the next paper.

SUMMARY AND CONCLUSIONS

1. Studies were made which indicate that blood plasma, serum, and their ultrafiltrates, and also spinal fluid, contain a substance which has the physicochemical properties of glutamine.

2. Supporting evidence that the glutamine-like substance is probably glutamine is discussed.

3. A micro method for its quantitative determination is indicated.

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