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Received Nov. 13, 1968. P.S.E.B.M., 1969, Vol. 131.

Amino Acids, Including Asparagine and Glutamine, in Plasma and Urine of Normal Human Subjects (33859)

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A recent development in the technology of amino acid assay via ion-exchange column chromatography was the separation of asparagine and glutamine by employing lithium citrate buffer systems (1) instead of the sodium citrate buffer systems usually used for determination of the acidic and neutral amino acids (AN) (2). We recently reported (3) our evaluation and modifications of the use of lithium buffers at flow rates employed in the Beckman/Spinco model 120 amino acid analyzer. Our major changes of the original method of Benson *et al.* (1) were the correction of an inadvertent interchange of the elution positions of citrulline and α-aminoadipic acid, and an improvement in the resolution in the cystine-to-leucine region by lowering the pH of the second buffer of the new, two-stage, lithium buffer system from 4.15 to 3.82.

The present report presents comparative results of the application of both the new and previous techniques for measuring free amino acids in plasma and urine of normal subjects. Also included are results of the determination of total amino acids found after acid hydrolysis of protein-free extracts of plasma and urine. The measurements of free and total aspartic and glutamic acids, as well as their respective amides, provided an opportunity to evaluate the contributions of these acids, their amides, and other conjugates to the total hydrolyzable aspartic and glutamic acids in plasma and urine.

Materials and Methods. Collection of samples for analysis. Fasting (overnight) blood samples (10 ml) were taken by venipuncture at three 6-month intervals from two female and three male, healthy, mature volunteers (wt range, 53–77 kg; age range, 24–42 years). The blood was immediately mixed with dry sodium heparinate (10 units/ml of blood), cooled in an ice bath, and centrifuged at 1500 rpm for 10 min. The plasma was removed and recentrifuged, and a portion was deproteinized immediately. The remainder was stored at —20° for later direct determination of tryptophan and creatinine.

Concurrent with the last two blood samplings, we also collected urine from the volunteers. The collection period was from 6:30 to 10:30 a.m., during which time the overnight fast was continued. Only water *ad libitum* was allowed. At the midpoint of the urine collection time, the blood sample was withdrawn. These conditions were dictated by what was considered to be the most practicable in the clinical situation for studies in uremic patients (4, 5), since the samples from these normal subjects were to serve as bases for comparison. The urine was refrigerated immediately after voiding. Following completion of the collection, a portion was deproteinized and the remainder was stored at —20°.

Deproteinization. Protein-free filtrates of plasma or urine for analysis of amino acids were prepared by the picric acid method (6). Urine was deproteinized in anticipation that samples from uremic patients would require

this procedure. A 4-ml aliquot of plasma or urine was mixed with 20 ml of 1% picric acid. After centrifugation, the excess picric acid was removed by passing the supernatant liquid through a column (2 × 1.9 cm) of an anion exchange resin, AG-2, X10, chloride form (Bio-Rad Laboratories). The effluent liquid was then lyophilized, reconstituted in 5.0 ml of 0.02 *N* HCl, and stored at −20°. Plasma and urine (0.2 ml) for analysis of tryptophan were deproteinized with 4 vol of 10% aqueous trichloroacetic acid. Plasma and urine for analysis of creatinine were deproteinized with sulfuric acid–sodium tungstate solutions: 0.2 ml of plasma or of a 1:100 dilution of urine was efficiently mixed with 0.2 ml of water, 0.2 ml of 0.67 *N* H_2SO_4, and 0.2 ml of 5% aqueous sodium tungstate solutions; the mixture was then centrifuged.

Analysis for amino acids. Amino acids in the protein-free filtrates of plasma or urine were determined on an automatic analyzer (Beckman/Spinco model 120) which had been upgraded to yield sensitivities comparable to those of currently available models and modified to provide continuous automatic operation after sample addition (3). The accelerated two-column chromatographic method (2, 7) was used. Sodium citrate buffers (7) were used for analysis of basic amino acids (B) and for AN of plasma obtained at the first and second bleedings (Groups 1 and 2) as well as in the urine collected concurrently with the plasma from the second bleeding (Group 2). These buffers were also used for analysis of B of plasma and urine obtained at the third bleeding (Group 3), but lithium citrate buffers were employed for AN in the plasma and urine of this group. The lithium citrate buffer formulations and details of their use were as described previously (3). An amount of plasma or urine corresponding to 0.33 ml of original material was used for each analysis of free amino acids, and the equivalent of 0.10 ml of original material was used for each analysis of hydrolysates. Unknowns from analyses using either lithium or sodium buffers were estimated by reference to corresponding integration constants obtained by running standard solutions of amino acids with appropriate additions (3). These constants were redetermined weekly.

Tryptophan was measured by separate assay, as it is lost during the picric acid deproteinization procedure. Creatinine was also measured directly since amounts of this compound were too small to be analyzed in plasma by the analyzer, and its peak was not well resolved in urine samples.

Analysis for total amino acids after hydrolysis. The total amino acids after hydrolysis of the protein-free plasma or urine were determined on the analyzer, using the standard sodium citrate buffers. Using 0.5 ml of the reconstituted protein-free solution and 0.5 ml of 12 *N* HCl, hydrolysis was carried out in a 10-ml hydrolysis tube (Kontes, K-89685). The mixture was shell-frozen in a dry ice–acetone mixture and the tube was evacuated ($\leq$ 50 μ) for 15 min. The evacuated tube was heated at 110 ± 1° for 22 hr. After cooling, the hydrolysate was transferred quantitatively to a round-bottom flask and evaporated to dryness on a rotary evaporator at a maximum bath temperature of 55° under reduced pressure. A few milliliters of water were then added and evaporated to ensure removal of the excess acid. The sample was reconstituted with 1.0 ml of 0.02 *N* HCl and stored at −20° before analysis. The total amino acids were determined on the analyzer, using the sodium citrate buffers.

When standard calibration mixtures (Beckman/Spinco type 1) containing 18 amino acids were hydrolyzed, all but cystine and methionine gave 90–100% recoveries. Asparagine, citrulline, creatine, creatinine, glutamine, and 3-methylhistidine were hydrolyzed individually with calibration mixtures. Asparagine and glutamine were recovered quantitatively as aspartic and glutamic acids, respectively; 50% of the citrulline was hydrolyzed to ornithine; creatine, which is not ninhydrin-positive, was completely converted to creatinine; and 80% of the creatinine was recovered.

Tryptophan assay. Tryptophan was determined in 0.1-ml aliquots of the protein-free filtrates of plasma or urine, obtained as described above, by modifications of the fluoro-

TABLE I. Acidic and Neutral Amino Acids Measured in the Plasma of Fasting Normal Subjects.[a]

Compound	Plasma concentration (mg/100 ml; mean ± SD)		
Group:	1 and 2[b]	3[c]	1, 2 and 3[d]
Alanine	2.94 ± 0.48	2.85 ± 0.68	2.91 ± 0.53
α-Amino-*n*-butyric acid	0.19 ± 0.09	0.19 ± 0.08	0.19 ± 0.08
Asparagine		0.63 ± 0.10	0.63 ± 0.10[c]
Asparagine + glutamine	8.33 ± 1.04		8.33 ± 1.04[b]
Aspartic acid	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
Citrulline	0.51 ± 0.20	0.50 ± 0.18	0.51 ± 0.19
Cystine, ½	0.80 ± 0.43	1.17 ± 0.10	0.93 ± 0.39
Glutamic acid	0.48 ± 0.18	0.44 ± 0.14	0.46 ± 0.16
Glutamine		8.14 ± 0.72	8.14 ± 0.72[c]
Glycine	1.67 ± 0.42	1.76 ± 0.38	1.70 ± 0.40
Isoleucine	0.73 ± 0.13	0.79 ± 0.11	0.75 ± 0.12
Leucine	1.44 ± 0.24	1.56 ± 0.19	1.48 ± 0.23
Methionine	0.30 ± 0.07	0.37 ± 0.07	0.33 ± 0.07
Phenylalanine	0.80 ± 0.08	0.84 ± 0.03	0.81 ± 0.07
Proline	1.65 ± 0.39	1.98 ± 0.51	1.76 ± 0.45
Serine	1.11 ± 0.16	1.31 ± 0.13	1.18 ± 0.18
Taurine	0.63 ± 0.14	0.58 ± 0.07	0.61 ± 0.12
Threonine	1.70 ± 0.34	1.75 ± 0.31	1.72 ± 0.32
Tryptophan	1.06 ± 0.13[c]	1.13 ± 0.17	1.10 ± 0.15[b]
Tyrosine	0.89 ± 0.11	0.99 ± 0.15	0.92 ± 0.13
Urea	30.8 ±10.7	29.4 ± 5.6	30.3 ± 9.1
Valine	2.31 ± 0.59	2.83 ± 0.38	2.48 ± 0.58

[a] Comparison of levels found using buffers containing Na^+ (Groups 1 and 2) and Li^+ (Group 3).

[b] Mean of 10 observations in five subjects, unless otherwise indicated.

[c] Mean of single observations in five subjects.

[d] Mean of 15 observations in five subjects, unless otherwise indicated.

metric method of Hess and Udenfriend for analysis of tryptamine (8). The method is based on the conversion of tryptophan to the fluorophore norharman in a two-step reaction: (a) the cyclization of tryptophan to a tetrahydronorharman derivative with 18% formaldehyde by heating at 100° for 20 min; and (b) the subsequent oxidation to norharman with hydrogen peroxide by another heating period of 20 min at 100°.

Fluorescence was measured in an Aminco-Bowman spectrophotofluorometer equipped with enhancing mirrors (9) at 365 and 450 mμ, as excitation and fluorescence wavelength maxima, respectively. The major changes from the original method (8) were: (a) the hydrogen peroxide concentration was reduced from 5 to 3%; (b) the heating times were controlled by cooling the tubes immediately in an ice-water mixture at the end of each heating period; and (c) the fluorescence was read at a constant temperature range of 24 ± 2°. In addition, to correct for inherent and variable quenching of the fluorophore by endogenous plasma and urinary constituents, an internal standard was prepared by adding tryptophan, 0.2 μg for plasma and 1.0 μg for urine, to another aliquot of each of the protein-free filtrates being analyzed. Each experimental sample was then corrected for the extent of quenching observed. Control studies demonstrated that the percentage decrease of fluorescence due to quenching was constant in the range of 0.1–2.5 μg of tryptophan/sample. Protein-free filtrates of plasma exhibited a mean quenching of 19.7 ± 3.8% (SD) and similar filtrates of urines, 55.9 ± 6.5% (SD). A complete assay system without

formaldehyde or tryptophan served as a blank.

Creatinine assay. Creatinine in plasma and urine was measured by the alkaline picric acid method of Martinez and Doolan (10). Their procedure was modified by reduction of all volumes of reagents by one-half and by extending the time of color development from 30 to 45 min. Absorbancies were determined at 500 mμ on a Gilford spectrophotometer using 1-ml cuvettes.

Results and Discussion. Table I presents the mean values of the AN found in the first two bleedings (Groups 1 and 2) and the third bleeding (Group 3) of our five volunteers. Since no sex difference was noted, we have combined the data from these bleedings. Comparison of the means of these amino acids obtained using the sodium or lithium buffers shows that both systems gave essentially the same results. In addition, the sum of the means for asparagine and glutamine measured separately in Group 3 (8.77 mg/100 ml) was within the range of the mean ± SD for these two compounds measured together in Groups 1 and 2 (8.33 ± 1.04). In Column 4 all determinations are combined to obtain a normal mean for AN in this study. It is apparent that the lithium buffer systems provide the advantage of measuring asparagine and glutamine separately and still retain the ability to resolve the other AN. The mean values for plasma asparagine and glutamine obtained in the present work agree with those reported by Benson *et al.* (1) for plasma, using similar analytic conditions. Stein and Moore (6) indicated that the concentration of asparagine in normal plasma was less than one-tenth that of glutamine. In the current study, the ratio of asparagine to glutamine was approximately 1:13.

The results of the determination of B in these plasma samples are shown in Table II. Again, Groups 1 and 2, and 3 are reported separately even though they were analyzed by the same system. As in the case of the AN, comparison of mean levels found at the different bleeding times shows little difference and therefore a mean of all available values is presented in Column 4 of Table II. The means of both the AN and B found in these subjects agree closely with those reported previously by other authors using ion-exchange column chromatographic procedures (6, 11, 12).

TABLE II. Basic Amino Acids Measured in the Plasma of Fasting Normal Subjects.

Compound	Plasma concentration (mg/100 ml; mean ± SD)		
	Groups 1 and 2[a]	Group 3[b]	Groups 1, 2 and 3[c]
Arginine	1.38 ± 0.25	1.51 ± 0.14	1.42 ± 0.22
Creatinine	1.4 ± 0.2[b]	1.2 ± 0.3	1.3 ± 0.2[a]
Histidine	1.21 ± 0.14	1.27 ± 0.08	1.23 ± 0.12
Lysine	2.41 ± 0.36	2.58 ± 0.20	2.46 ± 0.32
Ornithine	0.66 ± 0.16	0.84 ± 0.11	0.72 ± 0.17

[a] Mean of 10 observations in five subjects unless otherwise indicated.

[b] Mean of single determinations in five subjects.

[c] Mean of 15 observations in five subjects unless otherwise indicated.

The results of analyses for AN in urine collected concurrently with the second and third bleedings are presented in Table III. Since the total volumes of urine excreted by the subjects were quite variable (92–314 ml), the results are expressed in micrograms of constituent per milligram of creatinine excreted during the 4-hr collection period. The mean excretion of creatinine was 264 ± 88 (SD) mg by Group 2 and 208 ± 67 mg by Group 3.

Comparison of the results of Group 2 (sodium buffers) with Group 3 (lithium buffers) again shows that similar results were obtained, although there was much more variation between individuals in these urines than in the plasma samples. Proline was not found in any urine sample. The sum of the separately determined asparagine and glutamine in Group 3 was 56.3 μg/mg of creatinine, within the range of the mean ± SD of the two amides measured together in Group 2 (39.5–59.1). The ratio of asparagine to glutamine in the urine was 1:7 quite unlike the 3:2 ratio stated by Benson *et al.* (1). However, our estimations of this ratio from the sample chromatogram presented by these authors (Fig. 2 of Ref. 1) indicated a ratio of

TABLE III. Acidic and Neutral Amino Acids Measured in the Urine of Fasting Normal Subjects.[a]

Compound	Urinary excretion (μg/mg of creatinine; mean ± SD)		
Group:	2[b]	3[b]	2 and 3[c]
Alanine	12.8 ± 3.7	12.9 ± 5.6	12.8 ± 4.5
α-Amino-*n*-butyric acid	2.6 ± 0.6	0.8 ± 0.2[d]	1.8 ± 1.1[e]
β-Amino-*i*-butyric acid	10.0 ± 3.0	9.9 ± 2.6[f]	10.0 ± 3.7[g]
Asparagine		7.0 ± 3.6	7.0 ± 3.6[b]
Asparagine + glutamine	49.3 ± 9.8		49.3 ± 9.8[b]
Aspartic acid	4.7 ± 0.9	2.1 ± 0.9	3.4 ± 1.6
Citrulline	3.1 ± 1.7	2.0 ± 0.6	2.6 ± 0.9
Cystine, ½	7.6 ± 2.5	9.1 ± 4.0	8.3 ± 3.5
Glutamic acid	2.1 ± 0.4	3.9 ± 1.1	2.9 ± 1.2
Glutamine		49.3 ± 24.9	49.3 ± 24.9[b]
Glycine	71.1 ± 40.5	60.6 ± 21.0	65.9 ± 31.0
Isoleucine	1.0 ± 0.2	2.8 ± 1.6	1.9 ± 1.4
Leucine	5.3 ± 0.9	2.9 ± 1.5	4.1 ± 1.7
Methionine	Not resolved	4.6 ± 1.4	4.6 ± 1.4[b]
Phenylalanine	7.4 ± 1.7	5.7 ± 2.0	6.5 ± 2.0
Serine	23.0 ± 3.4	30.4 ± 15.5	26.7 ± 11.2
Taurine	51.8 ± 23.7[d]	65.1 ± 40.0	59.2 ± 32.6[e]
Threonine	13.1 ± 4.8	16.1 ± 10.0	14.6 ± 9.0
Tryptophan	Not determined	11.1 ± 3.4	11.1 ± 3.4[b]
Tyrosine	11.7 ± 3.1	13.8 ± 7.3	12.8 ± 5.4
Urea	12,000 ± 777	9620 ± 463	10,800 ± 534
Valine	2.7 ± 0.3	4.8 ± 1.4	3.8 ± 1.7

[a] Comparison of levels found using buffers containing Na^+ (Group 2) and Li^+ (Group 3).
[b] Mean of five subjects unless otherwise indicated.
[c] Mean of 10 observations in five subjects unless otherwise indicated.
[d] Mean of four subjects.
[e] Mean of nine observations.
[f] Mean of three subjects.
[g] Mean of eight observations.

asparagine to glutamine of approximately 2:9. In the analysis of Group 2 using sodium buffers, methionine could not be calculated because of poor resolution from unidentified materials. This difficulty was overcome in the lithium system by the spreading out of peaks in the cystine-to-leucine region, as described earlier (3). The values for B in the two groups are shown in Table IV. Again, both urine collections gave very similar values and, therefore, in both cases, means were calculated for all observations (Column 4 in Tables III and IV). These values cannot be compared directly with earlier reports since in the past it was a general practice to collect 24-hr urines from nonfasting subjects (13–15). Soupart (15) reported his data by expressing individual amino acids in molar percent of the total excretion of amino acids in 24 hrs. By treating our 4-hr mean values in the same way, we can compare our results with those of Soupart. On that basis, as shown in Fig. 1, good general agreement between our 4-hr excretions and the 24-hr excretions of amino acids is apparent. Only lysine excretion is markedly different in the two groups of data.

An estimation of the conjugated or bound amino acids in protein-free filtrates of plasma and urine was obtained in this study by measuring the total amino acids produced by hydrolysis. The increases of amino acids over the amounts measured before hydrolysis are interpreted as bound or conjugated amino acids; their mean values are presented in Table V. It is clear that these values were

TABLE IV. Basic Amino Acids Measured in the Urine of Fasting Normal Subjects.

Compound	Group:	Urinary excretion (μg/mg of creatinine; mean ± SD) 2[a]	3[a]	2 and 3[b]
Arginine		1.5 ± 0.6	2.0 ± 0.8	1.7 ± 0.7
Histidine		91.3 ± 41.1	87.4 ± 39.3	89.3 ± 38.0
Lysine		17.8 ± 11.0	24.3 ± 23.0	21.0 ± 17.3
1-Methylhistidine		16.9 ± 12.1[c]	28.5 ± 12.6[d]	21.8 ± 12.8[e]
3-Methylhistidine		25.1 ± 2.2	28.4 ± 5.5	26.8 ± 4.4
Ornithine		1.2 ± 0.5[c]	1.4 ± 0.5	1.3 ± 0.5[f]

[a] Mean of five subjects unless otherwise indicated.
[b] Mean of 10 observations in five subjects unless otherwise indicated.
[c] Mean of four subjects.
[d] Mean of three subjects.
[e] Mean of 7 observations.
[f] Mean of 9 observations.

extremely variable between individuals, as previously reported by Stein (13). The major share of the total bound amino acids in either plasma or urine was due to only a few amino acids. Thus, 70.6% of the total μmoles of bound amino acids in the plasma was accounted for by the four compounds, glutamic acid (49.7%); glycine (10.6%); aspartic acid (5.2%); and alanine (5.2%). Similarly, in the urine, 78.9% of the total μmoles of bound compounds was due to glycine (35.3%); glutamic acid (24.8%); aspartic acid (11.9%); and proline (6.9%). Other single amino acids in plasma or urine contributed less than 5% to the respective totals.

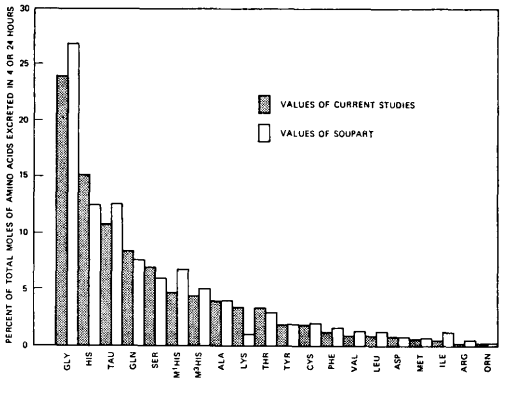

FIG. 1. Comparison of excretion of amino acids in the urine during 4 and 24 hr. Our mean values were from 10 samples of 4-hr fasting urines from 5 normal subjects (2 ♀, 3 ♂). The mean values given by Soupart (15) were from 24-hr urines obtained from 15 normal subjects (9 ♀, 6 ♂) not being fasted.

The availability of data on the total and free aspartic and glutamic acids and their

TABLE V. Bound or Conjugated Amino Acids Measured in the Plasma and Urine of Fasting Normal Subjects.

Compound	Plasma (mg/100 ml; mean ± SD)[a]	Urine (μg/mg of creatinine; mean ± SD)[b]
Alanine	0.68 ± 0.97[c]	15.9 ± 3.8
α-Amino-n-butyric acid	0.12 ± 0.10[c]	4.4 ± 2.1
Arginine	0.48 ± 0.54[c]	11.8 ± 2.6
Aspartic acid	1.02 ± 0.24[c]	87.1 ± 24.1
Glutamic acid	10.8 ± 3.5	200 ± 58
Glycine	1.18 ± 0.86	145 ± 66
Histidine	0.33 ± 0.41	15.3 ± 7.5[d]
Isoleucine	0.17 ± 0.22	3.7 ± 1.2
Leucine	0.35 ± 0.43	9.4 ± 3.8
Lysine	0.84 ± 0.79	26.6 ± 19.2
Ornithine	0.48 ± 0.30	7.4 ± 1.6[d]
Phenylalanine	0.29 ± 0.25	4.8 ± 2.2
Proline	0.83 ± 0.70	43.6 ± 12.2[e]
Serine	0.37 ± 0.37	15.4 ± 4.2
Taurine	0.17 ± 0.23	2.8 ± 2.2[f]
Threonine	0.50 ± 0.91	14.1 ± 3.6
Tyrosine	0.21 ± 0.26	3.2 ± 1.4[e]
Valine	0.57 ± 0.87[c]	12.2 ± 4.2

[a] Mean of 15 observations in five subjects unless otherwise indicated.
[b] Mean of 10 observations in five subjects unless otherwise indicated.
[c] Mean of 14 observations.
[d] Mean of 9 observations.
[e] Mean of 8 observations.
[f] Mean of 4 observations.

TABLE VI. Contribution of the Respective Amino Acids, Amides, and Unknown Conjugates to the Total Aspartic and Glutamic Acids Measured after Hydrolysis in Plasma and Urine.

Constituents	Plasma[a] (mg/100 ml)	Urine[a] (μg/mg of creatinine)
Total aspartic acid after hydrolysis	0.94 ± 0.11	106 ± 25
Aspartic acid before hydrolysis	0.05 ± 0.02	2.1 ± 0.9
Asparagine before hydrolysis	0.63 ± 0.10	7.0 ± 3.6
Net aspartic acid derived from unknown aspartyl conjugates	0.26 ± 0.10	96.8 ± 20.6
Total glutamic acid after hydrolysis	9.47 ± 0.73	230 ± 73
Glutamic acid before hydrolysis	0.44 ± 0.14	3.9 ± 1.1
Glutamine before hydrolysis	8.14 ± 0.72	49.3 ± 24.9
Net glutamic acid derived from unknown glutamyl conjugates	0.89 ± 0.21	177 ± 55

[a] Values are the means ± SD from five fasting normal subjects. Blood was obtained at the midpoint of the 4-hr urinary collection.

amides now makes possible an estimation of the contributions of the acids and their amides to the total acids measured. The data in Column 2 of Table VI show that in the plasma, 75% of the total aspartic acid found after hydrolysis was due to aspartic acid and its amide, while 25% was derived from unknown aspartyl conjugates. Similarly, 91% of the total glutamic acid of the plasma was due to glutamic acid and its amide; unknown glutamyl conjugates contributed only 9%. In the urine, as shown in Column 3, a markedly different pattern is apparent since 91 and 77% of the total aspartic and glutamic acids were derived from unknown aspartyl and glutamyl conjugates, respectively. It is reasonable to suggest that these unknown conjugates in the urine are β-aspartyl and γ-glutamyl peptides since other workers have reported the isolation of numerous peptides of this type in normal urine (16–18). Their occurrence in plasma has not as yet been demonstrated.

Summary. The use of lithium citrate buffer systems for the analyses of acidic and neutral amino acids in plasma and urine was tested and found superior to the usually employed sodium citrate buffer systems. The lithium systems have the advantage of resolving asparagine and glutamine without loss of resolution of other acidic and neutral amino acids. Normal levels of these amides in plasma and urine were established, and the relative contributions of the aspartic and glutamic acids, their amides, and unknown conjugates (peptides?) to the total measurable acids in protein-free filtrates after hydrolysis were determined. The general pattern of excretion of amino acids in urine collected for 4 hr from fasting individuals was shown to be quite similar to the pattern found in 24-hr urines collected from nonfasting subjects.

This work was supported in part by Contract PH 43-66-1132 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

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Received Jan. 16, 1969. P.S.E.B.M., 1969, Vol. 131.

Abolition of Milieu-Induced Hyperlipemia in the Rat by Electrolytic Lesion in the Anterior Hypothalamus* (33860)

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The postprandial lipemia of the rat fed fat was observed in our previous studies to be exaggerated when this animal was exposed to white noise before and after ingestion of oil (1) or was placed in the central area of the general laboratory after oil ingestion (2). We designated this phenomenon as milieu-induced hyperlipemia and from the nature of the excitant we concluded that the initial stimulus for this elevation of the postprandial plasma triglyceride (PTG) is received and acted upon by the central nervous system (CNS) of the animal. However, the exact locus in the CNS where this stimulus initiates a lipid elevating mechanism and the final pathways and tissues or organs participating in this mechanism remains unknown.

In our last study (2), the removal of the adrenals and hypophysis did not abolish the milieu-induced postprandial hyperlipemia of the rat fed fat. In view of these findings, we decided to study what effect hypothalamic lesions might exert upon milieu-induced hyperlipemia. The results herein described, indicate that electrolytic lesions involving the anterior hypothalamic nuclei abolish milieu-induced hyperlipemia.

Methods. A. Induction of lesions in anterior and midhypoyhalamic areas. Young (12–14 weeks) male rats (Long-Evans strain) were employed in this study. Under pentobarbital anesthesia, two separate electrolytic lesions on each side were produced in the midhypothalamic or tuberal area, by means of the Kopf stereotaxic instrument. The first bilateral lesion was produced by an electrode placed 1.4 mm caudal to Bregma, 0.7 mm lateral to the midline, and 9.5 mm beneath the dorsal surface of the brain. The second bilateral lesion was induced with the electrode placed in the above same lateral and vertical coordinates but 1.7 mm caudal to Bregma. A direct current (2 mA for 10 sec) was used for the electrolytic injury. Control animals were treated exactly the same except the electrode was not activated.

A single lesion was produced on each side in the anterior or rostral hypothalamic area of rats of similar age, sex, and strain by an electrode inserted only 0.2 mm caudal to Bregma and with the same lateral and vertical coordinates described above. After operation, the rats were housed in groups of five and fed Simonsen laboratory chow.

At autopsy the brain of each rat was removed, fixed, embedded in paraffin, and serial sections (every 200 μ) were obtained. These were stained with Luxol fast blue and cresyl violet and then examined.

B. Pre- and postprandial chemical studies. After 2 weeks of convalescence, groups of experimental and control rats were starved for 15 hr and then brought into the central

* Aided by grants from the National Institutes of Health, National Heart Institute, HE-00119 and HE-03429.