Comparison Between Free Amino Acid Levels in Plasma Deproteinated with Picric Acid and with Sulfosalicylic Acid.* (31333)

WALTER D. BLOCK, MARA E. MARKOVS, AND BETTY F. STEELE
Department of Dermatology, Biochemical Research Laboratories, University of Michigan Medical Center, Ann Arbor

The presence of protein interferes with the chemical determination of compounds containing amino or amido nitrogen(1). Hamilton and Van Slyke(2) chose 1% picric acid to precipitate the proteins in plasma because it gave lower CO_2 blanks in the Van Slyke manometric procedure than other precipitating agents. When Stein and Moore(3) introduced their method of ion-exchange chromatography for determination of free amino acids in plasma, they modified the deproteination procedure to remove the excess picric acid by passing the supernatant fluid through a resin column. The eluate was concentrated to a known volume before a portion of it was subjected to chromatography. The method is time-consuming, and chances for error are inherent because of the many manipulations involved.

Other investigators(4-6) have used sulfosalicylic acid in concentrations varying from 3% to 20% to precipitate plasma proteins and have applied the supernatant fluid directly to the ion-exchange column for chromatography without removing the remaining precipitating agent. This latter method is less time-consuming, and few manipulations are involved.

In the present investigation, a comparison is made between the levels of free amino acids in human plasma after precipitation of the proteins by picric acid(3), and by 20% sulfosalicylic acid(5).

Materials and methods. Subjects and collection of plasma. Blood was withdrawn from the cubital vein of 17 healthy human adults in a fasting state, and from one subject 2 hours after the ingestion of free L-methionine; plasma was separated by centrifugation. A pool was made from the plasma collected from 11 subjects and frozen until deproteinated; the other 7 plasma samples were deproteinated before freezing.

Plan of experiment. Pooled plasma. Picric acid deproteinated samples of plasma, and of plasma plus a standard solution† containing 17 amino acids were prepared in triplicate as follows: 1) 10 ml of plasma plus 2 ml of H_2O plus 48 ml of 1% picric acid were prepared for amino acid analysis(3); 2) 10 ml of plasma plus 2 ml of standard solution† plus 48 ml of 1% picric acid were treated as above. Sulfosalicylic acid deproteinated samples of plasma, and of plasma plus a standard solution† were prepared in triplicate as follows: 1) 8 ml of plasma plus 4 ml of H_2O plus 4 ml of 20% sulfosalicylic acid were centrifuged. The supernatant fluid was filtered and a portion applied to the ion-exchange column used for amino acid analysis; 2) 8 ml of plasma plus 2 ml of standard solution† plus 2 ml of H_2O plus 4 ml of 20% sulfosalicylic acid were treated as above.

Plasma from individual subjects. Plasma from each of 6 subjects was divided into two portions. One portion was treated with picric acid, and the other with sulfosalicylic acid as described under *pooled plasma.* Plasma from the subject who received free L-methionine was divided into 2 portions, each of which was deproteinated with picric acid.

Method of amino acid analysis. Free amino acids and other ninhydrin-reacting substances were determined by the automated ion-exchange chromatographic method of Spackman *et al*(7). The Beckman-Spinco accelerated technique(8) was used to analyze one portion of the protein-free plasma (7th plasma sample) from the subject receiving L-methionine.

Results. Table I lists the amount of the

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† Beckman-Spinco Standard (2 ml contains 1 μmole of each of the amino acids), Spinco Division, Beckman Instruments, Inc., Stanford Industrial Park, Palo Alto, Calif.

TABLE I. Free Amino Acids in a Pooled Plasma Sample After Deproteination with Picric Acid and with Sulfosalicylic Acid (mg/100 ml).

Amino acid	Method of deproteination: Picric acid	Sulfosalicylic acid
Taurine	1.31 ± .00 (.0)*	1.46 ± .02 (1.4)
Aspartic acid	.21 ± .00 (.0)	.22 ± .01 (4.5)
Threonine	1.92 ± .00 (.0)	2.04 ± .01 (.5)
Serine	1.52 ± .00 (.0)	1.63 ± .02 (1.2)
Asparagine + glutamine	6.22 ±1.62 (26.0)	6.87 ± .41 (6.0)
Proline	2.12 ± .05 (2.3)	2.30 ± .09 (3.9)
Glutamic acid	2.96 ± .12 (4.0)	3.46 ± .10 (2.9)
Citrulline	.73 ± .03 (4.1)	.82 ± .08 (9.8)
Glycine	1.65 ± .02 (1.2)	1.78 ± .00 (.0)
Alanine	3.26 ± .01 (.3)	3.48 ± .02 (.6)
Valine†	2.72	3.00
Cystine†	.16	.21
Cystathionine	.02 ± .00 (.0)	.02 ± .00 (.0)
Methionine	.42 ± .01 (2.4)	.48 ± .00 (.0)
Isoleucine	1.01 ± .00 (.0)	1.08 ± .00 (.0)
Leucine	1.92 ± .00 (.0)	2.03 ± .01 (.5)
Tyrosine	1.11 ± .01 (.9)	1.22 ± .01 (.8)
Phenylalanine	1.04 ± .01 (.9)	1.16 ± .02 (1.7)
β-Alanine	>.00	>.00
Ethanolamine	.08 ± .00 (.0)	.08 ± .00 (.0)
Lysine	2.76 ± .06 (2.2)	3.14 ± .17 (5.4)
Histidine	.83 ± .12 (14.5)	1.23 ± .02 (1.6)
Arginine†	1.18	1.49

* Mean ± S.D. of 3 replicate samples, and coefficient of variation expressed as percent(9).
† Single analysis (accelerated technique(8)).

free amino acids and other ninhydrin-reacting substances in the pooled plasma sample after deproteination with picric acid or with sulfosalicylic acid. The precision of both methods is reflected in the low standard deviations from the mean, and the coefficients of variation, a statistical indicator of the relationship between the standard deviation and the mean value(9). The coefficients for most of the amino acid values, regardless of the method of plasma protein precipitation ranged from 6.0% to 0.0%. Exceptions were asparagine plus glutamine (26.0%) and histidine (14.5%) in the picric acid deproteinated plasma, and citrulline (9.8%) in the sulfosalicylic acid deproteinated plasma (Table I).

The amounts of all amino acids were greater in the sulfosalicylic acid deproteinated plasma than in the picric acid deproteinated plasma (48.2% histidine to 4.8% aspartic acid) except for cystathionine and ethanolamine which were the same in both extracts.

The recovery of added amounts of aspartic acid, threonine, glycine, valine, isoleucine, leucine, tyrosine, phenylalanine, and arginine from the picric acid deproteinated plasma pool ranged from 93.1% to 107.5%. Proline, glutamic acid, alanine, methionine, lysine, and histidine recovery values ranged from 85.2% to 119.2%. Recovery values for serine were 123.9%, and for cystine, 69.1%.

After sulfosalicylic acid deproteination of plasma plus added amount of amino acids, recovery values for proline, glycine, alanine, valine, cystine, methionine, isoleucine, tyrosine, and phenylalanine ranged from 92.7% to 108.8%. Aspartic acid, threonine, serine, glutamic acid, leucine, lysine, histidine, and arginine recovery values ranged from 82.7% to 116.9%.

The results from the 6 individual subjects whose plasma was deproteinated by sulfosalicylic acid or picric acid were essentially the same as those found for the pooled plasma.

Table II gives the values for the free amino acids determined by the method of Spackman *et al*(7), and the Beckman-Spinco accelerated technique(8) in a single sample of picric acid deproteinated plasma. The values for all but 3 of the amino acids agree within 10%. Aspartic acid, threonine, and serine values were lower (60% to 19%) by the accelerated technique than by the non-accelerated procedure.

Discussion. Precision and accuracy for free amino acid values determined by ion-exchange chromatography in replicate samples of plasma deproteinated with 20% sulfosalicylic acid were similar to those obtained in aliquots of the same plasma deproteinated by the conventional 1% picric acid method(3). The sulfosalicylic acid extract was less time-consuming to prepare, and required fewer manipulations than the preparation of the picric acid extract. The former extract gave higher values than the latter extract, but the differences were not statistically significant.

Scharff *et al*(5) used 20% sulfosalicylic acid to deproteinate plasma from rats before amino acid analysis, but no comparative studies with picric acid deproteination were

TABLE II. Plasma Free Amino Acid Levels (mg/100 ml) Determined by Non-Accelerated and by Accelerated Automated Ion-Exchange Chromatography.

Amino acid	Non-accelerated	Accelerated
Taurine	1.06	.98
Aspartic acid	.10	.04
Threonine	1.79	1.46
Serine	1.04	.85
Asparagine + glutamine	8.65	8.73
Proline	2.34	2.38
Glutamic acid	.84	.78
Citrulline	.38	.37
Glycine	1.59	1.47
Alanine	3.08	3.37
Valine	*	2.15
Cystine	*	1.04
Cystathionine	.04	.04
Methionine	6.20	6.36
Isoleucine	.49	.44
Leucine	1.27	1.19
Tyrosine	.86	.80
β-Alanine	>.00	>.00
Phenylalanine	.72	.68
Lysine	2.96	3.01
Histidine	.92	.83
Arginine	1.51	1.44

* Not resolved by the ion-exchange column.

done. Hamilton(4) compared the levels of a few amino acids in protein-free filtrates from the same serum deproteinated with 3% sulfosalicylic acid and with picric acid and found no significant differences.

Gerritsen *et al*(6), in a more comprehensive study found that 13 of the 19 amino acids and other ninhydrin-reacting substances were higher in pooled serum deproteinated by picric acid than by sulfosalicylic acid.

In their study, serum was deproteinated with 3% sulfosalicylic acid; in the present investigation, plasma deproteinated with 20% sulfosalicylic acid was used. The difference in results between the 2 investigations possibly could be due to these factors.

Summary. A comparative study of 20% sulfosalicylic acid and of 1% picric acid in precipitating plasma proteins before analysis for free amino acids by ion-exchange chromatography showed that both methods give values comparable in precision and accuracy. The sulfosalicylic acid method is less time-consuming, and requires fewer manipulations.

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Studies on a Murine Lymphoma Induced by Reovirus Type 3: Some General Aspects of the Lymphoma 2731/L.* (31334)

D. KEAST AND N. F. STANLEY

Department of Microbiology, University of Western Australia, Perth

The clinical and histopathological pictures of acute and chronic disease of mice following neonatal infection with types 1, 2 and 3 reovirus have been fully reported(1,2,3). The development of the long-term chronic disease following reovirus type 3 infection of neonatal mice indicates chronic immunological damage and associated premalignant changes(4). One

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