A study of familial aspects of human urinary amino acid excretory patterns utilizing paper chromatography [1]

Arnold R. Kaplan [2]

Department of Zoology, University of Texas, Austin

Individuals have been observed to generally maintain relatively characteristic urinary amino acid excretory patterns (Williams et al., 1951). The present study was designed to investigate the possible occurrences of correlations between genetic relationship and quantitative similarities in as many urinary amino acids as was routinely practicable.

Methods

The utilization of paper chromatography for analyzing urinary amino acids has been largely limited by the fact that the concentrations of amino acids are very low in comparison to the concentrations of salts, urea, etc. The latter substances cause chromatographic distortion and interference in the resolutions of the various amino acids. The objective of making a preliminary investigation of a maximal number of urinary amino acids necessitated the utilization of the largest practicable aliquots of urine in the chromatograms. This practice, in turn, necessitated the utilization of a procedure for the routine 'purification' and desalting of the urine specimens prior to their chromatographic analysis, so that minimal amounts of salts and other interfering substances would be present.

Each specimen was added to a sample of activated charcoal (Darco, Grade G-60), filtered, concentrated, and then desalted by ion-exchange with a basic preparation of the resin, Dowex-2 (Dow Chemical Co.). Aliquots of processed urine were standardized to 50.0 gamma alpha-amino acid nitrogen for each chromatogram, the standardization techniques having been based on methods by Fister (1950). Chromatograms were prepared on Whatman No. 1 filter paper rectangles, 23.0 by 28.5 cm. in size. In each case, a standard quantity of the processed, concentrated specimen was applied to the paper in the form of a circular spot not more than 1.0 cm. in dia-

[1] From a dissertation (Kaplan, 1956) completed in partial fulfillment of the requirements for the Ph. D. degree at the University of Texas, Austin.

[2] Present address: Dept. of Medical Genetics, N. Y. State Psychiatric Institute, New York 32, N. Y.

Ricevuto il 16. IX. 58.

meter, and with the center located 5.0 cm. from each of two edges of the rectangle. Each sample was applied as a series of 5.0-microliter amounts with the use of a capillary pipette, and the process was expedited by heating the area of application with an infrared lamp. The papers were placed in a phenol-water solvent (72.5 percent phenol by volume) for 16 hours, dried thoroughly, and then placed in a lutidine-water solvent (3 parts 2,4-lutidine to 2 parts water by volume) for 18 hours. After being dried again, the papers were dipped in a solution of 0.5 percent ninhydrin in absolute ethanol, then dried for 20 minutes in an oven at 80.0 degrees C. Densitometric index values were obtained with a densitometer (Photovolt Corp., No. 501A), by directing the machine's light beam through the hub of each chromatogram spot.

The data

The data of the present study were based on investigations of first morning urine samples from 32 female and 23 male normal white subjects. Attempts were made to obtain 3 samples from each individual, but some subjects donated only 2 or 1 specimen each, for a total of 120 specimens (71 from females, 49 from males). The analyses of the data were based on comparative densitometer readings of the chromatogram spots. In the case of each subject, the average values for the respective variables were employed in the statistical calculations.

One series of analyses compared the (semiquantitative) densitometric index values of the males with those of the females — the probability determination of a significant sex difference for each variable having been based on the quotient value of the difference between the averages divided by the standard error of the differences. Tables 1 and 2 contain the average densitometric index values for the males and females, respectively. The males' values were higher for serine and valine, at the one and five percent levels of significance, respectively. The females' values were higher for B-alanine and B-aminoisobutyric acid, each at the five percent level of significance; and for " C " at the one percent level. The intraindividual variabilities and the ranges of variation, in general, were greater in the females than in the males.

Two series of statistical analyses were based on determinations of variances of the differences within pairs of 'experimental' and control groups. The probability value in each case in the latter series was based on an 'F' value obtained by dividing the mean square between samples by the mean square within samples. Two groups of genetically-related pairs, parent-child and sibling pairs, and their respective genetically-unrelated control pairs, were examined. Statistical analyses were also made for same-sex pairs only, in the cases of those variables which indicated significant sex differences. The control subjects were members of the same population as the 'experimental' subjects, corrected for age and sex. In each case in which significant deviation of intrapair differences was observed, the intrapair difference within the genetically-unrelated control pairs exceeded that within the genetically-related pairs. Statistically significant deviations between the parent-child pairs and their controls

were observed at the one percent level for B-alanine, glutamine, methylhistidine, leucine, " A, " and " C "; at the five percent level for arginine and histidine. Significant deviations between the sib pairs and their controls were observed at the one percent level for " G "; at the five percent level for glutamine and methylhistidine, and for alanine when only the same-sex sib pairs and their controls were considered.

Table 1 - Densitometric Index Values of 23 normal males

Subject		Average Densitometer Reading												
Code	Age	Ala	Arg	BAla	BAib	Glu	MHis	His	Leu	Ser	Val	« A »	« C »	« G »
A1	49	.79	.87	.40	.45	.04	.20	.04	.41	.73	.36	x	x	.19
B1	34	1.02	x	.27	.41	.13	.17	.25	.51	1.00	.43	x	x	.05
D1	64	1.64	.25	.47	.46	x	.33	x	.55	.92	51	x	x	x
D2	34	1.38	x	x	.57	x	.19	.09	.50	.96	.43	x	.01	x
D5	7	1.27	x	x	.46	x	.28	.39	53	1.07	.48	.04	x	.11
F1	51	.58	.07	.14	.30	.15	.18	.38	.14	.81	.23	.10	.05	.06
F3	22	.72	.05	x	.51	.26	.21	.26	.41	.78	.39	x	x	x
F4	21	1.21	.30	x	.63	.16	.21	.45	.73	1.06	.55	x	x	x
F6	11	.92	.03	.17	.31	x	.21	x	.23	.98	.20	.07	x	.05
G4	53	1.13	.20	x	x	x	.96	.88	.88	1.04	.62	.43	x	.31
G5	48	.81	x	.27	.51	x	.70	x	.24	.63	.24	x	x	x
G6	29	.80	.24	.23	.62	x	.47	.86	.40	.99	.50	.29	x	.33
G10	7	.82	x	.42	.18	x	1.23	x	.25	93	.26	x	x	.27
H2	32	1.47	.29	.21	.63	x	.39	.70	.57	1.33	.50	.17	x	.26
H4	10	.87	.20	.44	.56	x	.32	.27	.37	.70	.33	.12	x	.32
H5	7	.87	.12	.38	.51	x	.05	x	.27	64	.30	x	.03	.27
H6	2	.83	.08	.53	.52	.20	.38	.45	.31	.78	.44	x	x	.34
I1	28	.91	.16	.22	.45	.12	.08	.12	.39	.94	.41	x	x	.07
J1	38	.84	.08	.16	.32	.47	.18	.47	.30	.98	.34	x	x	.18
J4	7	1.05	x	.22	.67	.38	.33	.38	.43	1.08	.42	x	x	.07
K1	55	1.14	x	x	1.35	x	x	x	.32	.82	.40	x	x	x
L2	63	1.25	.21	.23	.46	.39	.73	.80	.46	1.01	.35	.22	x	.22
L5	23	1.05	.24	.47	.75	.67	.73	.41	.67	1.33	.51	.44	x	.23

In addition to the thirteen substances discussed above, which average densitometric readings are listed in Tables 1 and 2, sixteen other substances were identified in some of the urine chromatograms. Statistical studies of their average densitometric values yielded no significant deviations between the sexes or between genetically-related and control pairs. The sixteen substances have been identified as follows: alpha-aminobutyric acid, asparagine, aspartic acid, cysteic acid, gamma-aminobutyric acid, glutamic acid, glycine, hydroxyproline, lysine, proline, taurine, threonine, tyrosine, ethanolamine-phosphoric ester, ethanolamine, and an unknown substance designated " K ".

Statistical comparisons of intrapair differences between 12 genetically-unrelated individuals living together (10 husband-wife pairs and 2 stepfather-stepdaughter pairs) and genetically-unrelated controls not living together indicated no significant deviations. These negative results support the hypothesis that environmental differences, alone, did not cause the significant deviations which were observed to occur between genetically-related pairs of subjects and genetically-unrelated control pairs.

Table 2 - Densitometric Index Values of 32 normal females

Subject		Average Densitometer Reading												
Code	Age	Ala	Arg	BAla	BAib	Glu	MHis	His	Leu	Ser	Val	« A »	« C »	« G »
A2	46	.97	.05	.31	.34	.13	.20	.35	.17	.27	.18	.10	.03	.08
A3	25	.99	.07	.33	.62	.50	.15	.53	.24	.80	.34	.03	.05	.03
A4	21	.97	.07	.41	.28	.04	.20	.04	.21	.39	.20	x	.04	.04
B2	34	1.18	.23	.11	.67	.15	.14	.15	.13	1.17	.49	x	x	x
B3	7	.88	.05	.06	.38	.23	x	x	.50	.87	44	x	x	x
D3	33	.91	x	x	.64	.17	x	.17	.37	.79	.44	.13	x	x
D6	3	1.32	x	x	.55	.37	.56	.37	.57	1.10	.55	.x	x	.25
F2	46	1.16	.09	x	.60	x	.23	x	.34	.27	.21	x	x	x
F5	12	1.11	.04	.49	.49	x	.22	.30	.45	1.21	.40	.06	x	.05
G1	82	1.15	.18	x	1.35	47	.40	.47	x	x	x	x	.11	x
G2	56	.61	x	x	x	x	1.13	.39	.35	.76	.21	x	x	.5
G3	50	.76	.21	x	1.27	x	.49	32	.12	.16	.25	.26	x	14
G7	29	.84	x	.34	31	x	.66	x	.30	.91	.30	.26	x	.15
G8	25	1.03	.18	x	.84	x	.72	.71	.70	1.29	.56	.70	.05	.25
G9	10	.40	x	.20	.65	x	.78	1.06	.78	.63	.48	.34	x	.32
G11	5	.60	x	.42	.76	x	.29	x	.27	.41	.33	x	x	x
H1	35	.47	x	.27	.49	.18	.15	.18	.32	.76	.29	x	.07	x
H3	12	1.11	.17	.24	.85	x	.24	.45	.42	1.11	.36	x	.03	.30
J2	38	.82	x	.11	1.37	.22	x	x	.25	.79	.25	.25	.09	x
J3	8	1.15	.18	.46	.56	.28	.18	.48	.24	.61	.24	.13	.07	.06
J5	5	1.18	x	x	.70	.38	.16	.38	.27	1.19	.42	x	x	.21
K2	55	1.14	x	.21	.35	.27	.33	.27	.22	.71	.38	x	x	x
K4	28	.95	x	x	.56	x	.23	x	.02	.51	.02	x	x	x
K5	19	.86	.03	.29	1.29	.26	.27	.26	.23	1.05	.27	.08	.01	.07
K6	19	.97	x	x	1.65	.24	.38	.24	.41	1.11	.41	x	x	x
K7	17	1.20	.20	.46	.56	x	.27	x	.28	.28	.13	x	x	.10
K8	15	1.15	.16	.17	.57	.50	.43	.50	.41	.74	.37	x	x	.37
L1	70	1.44	.26	.56	.67	x	.38	.12	.18	.39	.34	.17	x	.26
L3	28	1.34	.41	.60	.85	.33	.44	.42	.80	1.22	.66	.18	x	.30
L4	29	1.47	.20	.56	.56	.34	.50	.62	.19	.10	.31	.27	x	.11
E2	23	1.19	.06	.30	.48	x	.42	x	.19	x	.33	.11	.12	.41
E6	30	.72	.03	.38	.27	x	.34	.18	.48	.91	.40	x	.05	.03

Discussion

Much of the glutamine was lost. Also, tyrosine and the other aromatic compounds were largely lost from the urine during the processing procedure with activated charcoal — although the loss was partly inhibited by the addition of several drops of chloroform.

The 'leucine' spot was primarily a manifestation of the leucine in the specimen, but varying amounts of isoleucine, norleucine, phenylalanine, and tryptophan also occurred in the same region. The 'valine' spot contained mostly valine, but apparently included smaller and varying amounts of methionine and or norvaline. Substance " A " occurred in approximately the same position as, and may have been identical with, " Spot 22 " previously described by Gartler et al. (1955). Substances " A, " " C, " and " G " have not yet been qualitatively identified.

Summary

A method for the preliminary analysis of a maximal number of urinary amino acids has been devised, which is based on preliminary 'purification' and desalting of each specimen prior to its subjection to two-dimensional paper chromatography. Aliquots of processed and concentrated specimens are standardized to quantities of alpha-amino acid nitrogen as determined colorimetrically.

Densitometric index values were analyzed for sex differences and for intrapair differences comparing genetically related and unrelated pairs. Significant sex differences were observed for five substances. Significant deviations between intrapair differences of genetically-related and control pairs were observed for ten substances. The results are compatible with the hypothesis that quantitative differences in the respective excretions involve genetic factors.

Acknowledgments

Sincere gratitude is hereby expressed for support from a grant by the R. A. Welch Foundation, and for the cooperation of the administration and staff of the M. D. Anderson Hospital and Tumor Institute, Houston; to Dr. C. P. Oliver for his helpful and patient guidance during the study and in preparation of the manuscript; to Dr. R. Fuerst for his guidance in chromatographic techniques; and to Dr. F. J. Kallmann for his active accommodation in preparation of the manuscript.

Reference

Fister, H. J.: 1950. Manual of Standardized Procedures for Spectrophotometric Chemistry. Easton, Pa.: Mack Ptg. Co.

Gartler, S. M., DobzHansky, T., and Berry, H. K.: 1955. Chromatographic studies of urinary excretion patterns in monozygotic and dizygotic twins. II. Heritability of the excretion rates of certain substances. *Am. J. Human Genet.*, 7: 108-121.

Kaplan, A. R.: 1956. Chromatographic studies of genetic aspects of human urinary amino acid excretory patterns. Ph. D. dissertation, University of Texas, Austin.

Williams, R. J., et al.: 1951. Biochemical Institute Studies. IV. Univ. Tex. Pub. No. 5109. Austin: Univ. Texas Press.

RIASSUNTO

È stato ideato un metodo per l'analisi preliminare del massimo numero di aminoacidi urinari, metodo che si basa su di una purificazione e desalinizzazione preliminare di ogni campione prima di sottoporlo alla cromatografia bidirezionale su carta. Aliquote di campioni preparati e concentrati vengono standardizzate rispetto a quantità di azoto alfa-aminoacido mediante determinazione colorimetrica.

I valori dell'indice densimetrico sono stati analizzati rispetto alle differenze di sesso ed alle differenze intracoppia confrontando coppie geneticamente imparentate e non imparentate. Sono state trovate differenze significative tra i sessi per cinque sostanze. I risultati sono compatibili con l'ipotesi che le differenze quantitative nelle rispettive escrezioni comportino fattori genetici.

RÉSUMÉ

On a suggeré une méthode pour l'analyse préalable du plus grand nombre d'aminoacides urinaires, méthode qui est fondée sur une purification et un dessalage préalable de chaque spécimen avant de le soumettre à la chromatographie bidirectionnelle sur papier. Des aliquotes des spécimens préparés et concentrés sont standardisés par rapport à quantités d'azote alfa-aminoacide par détermination colorimétrique.

Les valeurs de l'index densimétrique ont été analisées, pour les différences de sexe et les différences intracouple, par la comparaison de couples génétiquement apparentées et non-apparentées. On a trouvé de différences significatives entre les sexes pour cinq substances.

Les résultats sont compatibles avec l'hypothèse que les différences quantitatives des excrétions respectives comportent des facteurs génétiques.

ZUSAMMENFASSUNG

Es wurde eine Methode erdacht für die Voranalyse der Höchstzahl an Aminosäuren im Urin. Dabei wurd zuerst jedes Muster, bevor es mittels Papierchromatographie in zwei Richtungen untersucht wird, gereinigt und entsalzt. Aliquoten präparierter und konzentrierter Muster werden durch Kolorimetrie auf ihren alpha-aminosauren Stickstoffgehalt standardisiert.

Die Werte des Densimeterindexes wurden auf Geschlechtsunterschiede und auf die Differenzen zwischen genetisch verwandten und nicht-verwandten Paaren untersucht. Für fünf Stoffe wurden bezeichnende Unterschiede zwischen den Geschlechtern gefunden. Die Ergebnisse decken sich mit der Annahme, dass die quantitativen Unterschiede in den verschiedenen Ausscheidungen genetische Faktoren enthalten.