

# Amino Acid Excretion Patterns in Diabetic Children

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As part of a program for study of metabolic disorders utilizing primarily paper chromatographic methods, amino acid excretion patterns in a large number of children were examined. We have previously reported results of investigations of amino acid excretions by normal children<sup>1</sup> and a summary of amino acid excretion patterns in approximately 700 children,<sup>2</sup> both normal and those hospitalized for a variety of disorders.

The purpose of the present report is to present results obtained from study of a group of children with diabetes mellitus. The subjects were forty-one diabetic children, twenty-three boys and eighteen girls, ranging in age from one month to fifteen years. They were enrolled either as private or clinic patients under the care of Dr. George M. Guest. Each child was receiving insulin therapy and was being managed according to the unmeasured normal dietary regimen of the clinic.<sup>3</sup> The children selected were free from major infections, ketonemic acidosis or other illness at the time of obtaining the urine specimens.

## PROCEDURES AND METHODS

Procedures for collection of urine specimens and the paper chromatographic methods for quantitation of amino acids were the same as those described in previous publications.<sup>1-4</sup> Fasting morning urine specimens were collected, preserved with thymol and refrigerated immediately. Creatinine was determined by the alkaline picrate method and was used as an internal reference standard for quantitative expression of amino acids, thereby allowing comparison of amino acid excretion levels independent of the urine volume.

Most of the amino acids were determined on duplicates of two-dimensional chromatograms using Whatman No. 1 filter paper, eleven inches square. Volumes of urine containing approximately 40 ug. of creatinine, or less if the sample was dilute, were applied in increments of 5 ul. The first solvent mixture was water-saturated, buffered phenol. The second solvent mixture

was 65 per cent 2,6-lutidine. Resolved chromatograms were thoroughly dried, sprayed with 0.2 per cent ninhydrin in butanol, and heated for eight to ten minutes at 100° C. for color development of the amino acids. A Photovolt 525 Densitometer was used for reading of maximum optical density and quantitation was made from reference standard curves prepared for each amino acid. Note was made of the entire map configuration of ninhydrin-positive spots obtained on the two-way chromatograms. Decomposition of the glutamine of a specimen was suspected if there was an apparent increase in excretion of glutamic acid and decrease in glutamine while excretions of other amino acids were reasonable.

Confirmation of the presence of proline, phenylalanine, tryptophane, leucine, histidine and cystine was obtained by one-dimensional chromatography in selected solvent mixtures using specific reagents for characteristic color development.<sup>4</sup>

## RESULTS AND DISCUSSION

In table 1 the urinary excretions of twelve commonly found amino acids by forty-one individual diabetic children are presented. Wide variations in excretion levels and totals were found within the group. Glycine and alanine were excreted by 98 per cent of the children, glutamine by 92 per cent, glutamic acid by 85 per cent, valine by 85 per cent, serine by 76 per cent, lysine by 71 per cent, taurine by 61 per cent, B-AIB by 59 per cent, tyrosine by 56 per cent, threonine by 56 per cent, and leucine by 37 per cent. Totals of the twelve amino acids ranged from 18 to 755 ug./mg. creatinine per child, the mean being 359. There was a gradual decrease in these means with increasing age.

Urine specimens from the children with diabetes were also examined for proline, hydroxyproline, homocitrulline, aspartic acid, methionine, tryptophane, cystine, phenylalanine, arginine, ornithine, phosphoethanolamine, alpha-aminobutyric acid, histidine and methylhistidine. The findings appear in table 2. Proline, hydroxyproline and homocitrulline were found only in Case No. 1, an infant. These are characteristically found in the urine of

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AMINO ACID EXCRETION PATTERNS IN DIABETIC CHILDREN

TABLE 1  
Urinary excretion of amino acids by diabetic children (ug./mg. of creatinine)

Case no.	Age yrs.	Sex	Creat. mg. %	Glut. A.	Ser.	Glyc.	Thr.	Ala.	Glut.	Val.	Leuc.	BAIB	Lys.	Tyr.	Taur.	Total†	Mean
1	1 month	M	43	12	68	90	28	34	11	—	—	1	140	—	—	384	
2	under 2	M	66	5	44	68	34	46	10	36	13	5	13	20	—	294	} 525
3		M	72	40	42	119	—	114	78	62	*	52	20	52	170	755	
4	2	F	21	50	120	*	112	116	72	16	—	4	26	—	—	603	} 426
5	to 4	M	53	50	66	120	28	100	13	25	13	20	8	12	33	488	
6		M	22	32	5	26	—	80	35	26	15	—	—	—	—	219	
7		F	40	20	44	126	56	30	7	—	—	—	75	40	—	398	
8		M	70	30	120	123	—	146	23	57	28	—	30	—	—	557	
9		M	31	*	—	173	—	22	*	8	18	—	—	27	—	293	
10	5	M	41	38	75	50	—	28	19	22	23	32	27	—	17	331	
11	to 6	M	28	—	—	35	—	30	—	—	—	—	—	—	—	65	
12		F	70	10	15	76	—	42	5	—	—	—	50	—	—	198	
13		F	54	30	60	160	—	52	58	8	—	30	70	20	80	568	
14		M	75	13	21	63	40	70	42	12	5	4	—	—	—	270	
15		M	18	30	—	20	16	20	4	8	—	4	—	—	—	102	
16		F	30	11	—	213	—	106	32	10	3	37	68	—	126	606	
17	7	F	30	25	—	159	—	44	8	35	—	21	57	13	173	535	
18	to 8	M	40	5	48	72	34	44	48	16	—	3	—	35	240	545	
19		M	83	4	23	50	40	90	52	35	—	40	35	47	100	516	
20		F	60	37	—	107	38	55	38	32	—	—	17	—	74	398	
21	9	M	113	—	35	57	8	18	18	6	—	9	6	15	26	198	
22	to 10	M	75	*	45	53	30	—	*	5	5	—	5	30	70	288	
23		M	42	—	—	27	—	17	—	—	—	—	—	—	—	44	
24		F	63	—	97	109	—	78	—	21	—	—	52	30	—	387	
25	11	F	109	21	15	52	8	20	10	6	—	—	—	15	70	217	
26	to 12	F	72	21	55	97	59	44	21	9	5	12	—	—	27	350	
27		F	87	4	39	114	44	60	45	12	24	14	32	22	34	444	
28		M	64	33	25	66	56	74	60	18	30	24	28	41	98	553	
29		M	78	13	64	34	61	28	17	10	—	21	12	9	5	274	
30	13	M	79	5	28	56	31	8	4	7	—	—	9	12	78	238	
31	to 15	M	79	22	59	114	57	75	40	20	—	17	22	15	125	566	
32		F	57	—	—	—	—	16	2	—	—	—	—	—	—	18	
33		M	113	—	—	*	—	58	10	8	—	5	13	20	75	276	
34		F	105	—	19	8	—	55	29	22	20	—	13	10	17	193	
35		F	130	10	16	85	—	18	15	4	—	5	42	10	17	222	
36		M	83	26	*	140	39	53	—	14	58	19	57	40	72	556	
37		F	88	*	*	58	—	63	*	—	—	11	—	—	35	250	
38		F	49	55	160	55	78	38	46	16	—	—	75	—	56	579	
39		F	69	52	*	40	32	32	42	10	—	55	26	—	—	327	
40		M	43	—	—	42	—	20	43	—	—	—	—	—	—	105	
41		M	62	15	30	83	73	83	52	24	—	—	51	61	66	538	
Mean			64	19	38	87	24	52	26	15	6	11	27	15	47	359	
Std. Dev.			27	17	39	63	28	33	22	14	12	15	30	17	57		

—Below minimum detectable amount.

\*No determination, interference.

†In arriving at total, group mean assumed when no determination (\*) was made.

normal infants. Cystine, aspartic acid and phenylalanine were found in the urine of the diabetic children in amounts less than 10 ug./mg. creatinine. Tryptophane, ornithine, arginine, methionine, phosphoethanolamine, alpha-aminobutyric acid and methylhistidine were not detected. Histidine was commonly excreted in large amounts and was considered to be influenced by diet. The above-mentioned findings are consistent with amino acid excretion patterns observed in the normal children.

Glycine, alanine and glutamine are prominent in the urine of normal children. Data from the diabetic children subjected to analysis of variance failed to show significant correlations of these three amino acids and insulin dosage, age of onset of symptoms, or duration of treatment.

Gross "amino aciduria" was not encountered in any of the children studied. Further examination of the data from diabetics for a borderline increase or de-

TABLE 2

Urinary excretion of amino acids by diabetic children

Proline	Excreted only by Case No. 1, an infant (140 ug./mg. creatinine)
Hydroxyproline	Excreted only by Case No. 1, an infant (180 ug./mg. creatinine)
Homocitrulline	Excreted only by Case No. 1, an infant (25 ug./mg. creatinine)
Cystine	Less than 10 ug./mg. creatinine
Aspartic acid	Less than 10 ug./mg. creatinine
Phenylalanine	Less than 10 ug./mg. creatinine
Tryptophane	0
Ornithine	0
Arginine	0
Methionine	0
Phosphoethanolamine	0
Alpha-aminobutyric acid	0
Methylhistidine	0
Histidine	Excreted by 100 per cent (range 20 to 400 ug./mg. creatinine)

crease in excretions was made in the effort to determine whether a quantitative relationship could be identified when screening large numbers of patients. Figure 1 shows the mean values of selected amino acid excretions by the forty-one diabetic children compared with quartile levels of excretions by a heterogeneous group of approximately 700 nondiabetic children.<sup>2</sup> In general, the mean values of the diabetic group fell at approxi-

mately the twenty-five percentile with the exception of taurine which was excreted in amounts greater than the seventy-five percentile level. Mean glutamine excretion fell approximately at the fifteen percentile level.

Mean values of amino acid excretions of the diabetic children according to age groups were compared to mean values obtained from normal children of comparable ages (table 3). Either none or large excretions of certain amino acids have been reported within the same age groups in normal young children.<sup>1</sup> For this reason, calculated mean values may be misleading, particularly in dealing with small numbers of young children. The diabetic subjects below five years of age in the present study are too few to justify separate comment. In general, the wide variations in normal excretion levels of normal young children and the narrower range of distribution of normal older children's levels were reflected in the data from diabetic children. The trends indicated in figure 1 could not be demonstrated when data from the diabetics were compared to data from normal subjects according to age groups. Mean values of taurine excretion bore an inconsistent relationship to those of control subjects. Diminished excretion of the remaining amino acids was not a consistent finding. Mean glutamine levels were somewhat lower than control levels in each age group. The ratios of glutamine plus glutamic acid to alanine excretion are shown in table 4. Again the results were not consistent for all age groups.

The majority (thirty-two patients) of the diabetic children studied were five to fifteen years of age. For the purpose of this study it was felt that the most valid conclusions could be made by examining data from this group separately (table 5). The mean values of amino acid excretions compare closely with mean values of the normal control group.

Other investigations of amino acid excretions in patients with diabetes mellitus have yielded somewhat contradictory results. Significant hyperaminoaciduria has been reported by Gray and Illing,<sup>5</sup> Hall,<sup>6</sup> Schreier and Szybko,<sup>7</sup> Müting,<sup>8</sup> Galambos and Tausz,<sup>9</sup> and Luetscher.<sup>10</sup> In most cases these findings were in association with acidosis. Conti et al.<sup>11</sup> found no elevation of amino acid excretions in patients under insulin control. The above reports included no studies of juvenile diabetes. Quantitative data on diabetic children are lacking for comparison with results of the present study. However, using qualitative methods, Bauza<sup>12</sup> observed no elevation of amino acid excretions in four diabetic children under good control, in eight under fair control and in five under poor control.

AMINO ACID EXCRETION IN DIABETIC CHILDREN

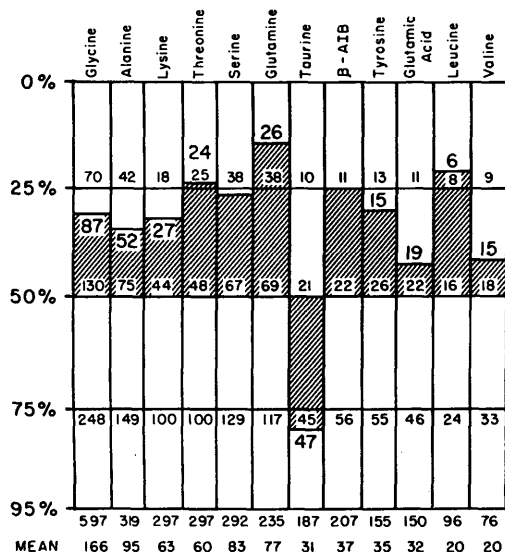


FIG. 1. Mean excretion values of forty-one diabetic children superimposed on grid depicting percentile levels of excretions by a heterogeneous group of 700 nondiabetic children.

AMINO ACID EXCRETION PATTERNS IN DIABETIC CHILDREN

TABLE 3

Mean excretions of amino acids in diabetic children by age groups (ug./mg. of creatinine)

Age group		Glut. A.	Ser.	Glyc.	Thr.	Ala.	Glut.	Val.	Leuc.	BAIB	Lys.	Tyr.	Taur.	12 A.A.
1 month	n=1	12	68	90	28	34	11	—	—	1	140	—	—	
Controls	n=15	81	214	447	169	256	156	23	13	16	157	33	145	
1 to 2 yrs.	n=2	28	43	94	17	80	44	49	7	29	17	36	85	525
Controls	n=15	35	78	113	54	70	83	24	—	58	33	70	70	688
2 to 4 yrs.	n=6	36	59	152	33	82	30	22	12	4	23	13	6	426
Controls	n=10	21	52	110	51	78	78	12	8	23	67	43	47	590
5 to 10 yrs.	n=15	15	28	83	14	46	23	14	2	12	27	14	62	336
Controls	n=50	7	30	71	25	38	41	2	3	5	52	19	18	318
11 to 15 yrs.	n=17	20	36	65	32	41	26	11	8	11	22	15	45	335
Controls	n=12	5	33	85	25	58	31	9	11	—	42	12	46	357

TABLE 4

Ratio of excretion of glutamine plus glutamic acid vs. alanine in diabetic children compared with normal controls (Mean value in ug./mg. creatinine)

Age group	Glut. plus Glut. A.	Ala-nine	Glut. plus Glut. A./Ala.	Difference in per cent
1 to 2 yrs.	72	80	0.90	
Controls	118	70	1.69	
				-79
2 to 4 yrs.	66	82	0.81	
Controls	99	78	1.27	
				-46
5 to 10 yrs.	38	46	0.83	
Controls	48	38	1.26	
				-43
11 to 15 yrs.	46	41	1.12	
Controls	36	58	0.62	
				+50
5 to 15 yrs.	41	45	0.91	
Controls	42	48	0.88	
				+3

Low glutamine excretion observed in some of the children in the present study bears further examination on an individual basis. Whether changes in glutamine, taurine, and sugar concentrations of the urine can be correlated is of interest.

SUMMARY

Urinary excretion levels of twenty-six amino acids were studied by quantitative paper chromatographic methods on forty-one diabetic children under insulin

treatment. Wide variations in excretion levels and patterns were found within the group. Hyperaminoaciduria was not present in any of the specimens studied. Histidine was excreted by all of the children studied. Glycine and alanine were excreted by 98 per cent, glutamine by 92 per cent, glutamic acid by 85 per cent, valine by 85 per cent, serine by 76 per cent, lysine by 71 per cent, taurine by 61 per cent,  $\beta$ -aminoisobutyric acid by 59 per cent, tyrosine by 56 per cent, threonine by 56 per cent, and leucine by 37 per cent. Proline, hydroxyproline and homocitrulline were excreted by only one child, an infant. Cystine, aspartic acid and phenylalanine were excreted in amounts less than 10 ug./mg. creatinine. Tryptophane, ornithine, arginine, methionine, phosphoethanolamine, alpha-aminobutyric acid and methylhistidine were not detected in any of the specimens from diabetic children.

Excretion levels of glycine, alanine and glutamine, commonly found in the urine of normal children, showed no significant correlations with insulin dosage, age of onset of symptoms or duration of treatment among the diabetic group.

Gross comparison of mean excretion levels of the diabetic children to a heterogeneous group of 700 non-diabetic children showed levels below the 50 percentile with the exception of taurine which fell at the 75 percentile level. Glutamine excretions in the diabetic group fell at the 15 percentile.

TABLE 5

Mean excretions of amino acids in diabetic children five to fifteen years of age (ug./mg. of creatinine)

		Glut. A.	Ser.	Glyc.	Thr.	Ala.	Glut.	Val.	Leuc.	BAIB	Lys.	Tyr.	Taur.	12 A.A.
Diabetics	n=32	16	32	74	23	45	25	12	5	11	25	15	54	337
Controls	n=62	6	32	78	25	48	36	6	7	3	47	16	32	336

When the diabetic children were compared to groups of normal children, selected according to age, no significant differences in mean excretion levels or patterns could be demonstrated consistently. The five- to fifteen-year-old age group of diabetics studied contained thirty-two patients. The mean values of amino acid excretions from this group compared closely with mean values of the normal group.

#### SUMMARIO IN INTERLINGUA

##### *Le Excretion de Amino-Acidos in Juveniles Diabetic*

Le nivellos del excretion urinari de vinti-sex amino-acidos esseva studiate per medio de quantitative methodos de chromatographia a papiro in quaranta-un juveniles con diabete durante que illes esseva sub tractamento con insulina. Extense variationes in le nivellos e schemas de excretion esseva trovate intra le gruppo. Hyperaminoaciduria non esseva incontrate in ulle del specimens studiate. Histidina esseva excernite per omne le juveniles in le studio. Glycina e alanina esseva excernite per 98 pro cento, glutamina per 92 pro cento, acido glutamic per 85 pro cento, serina per 76 pro cento, lysina per 71 pro cento, taurina per 61 pro cento, acido B-aminoisobutyric per 59 pro cento, tyrosina per 56 pro cento, threonina per 56 pro cento, e leucina per 36 pro cento. Prolina, hydroxyprolina, e hemocitrullina esseva excernite per solmente un del subjectos, un infante. Cystina, acido aspartic, e phenylalanina esseva excernite in quantitates de minus que 10 µg per mg de creatinina. Tryptophano, ornithina, arginina, methionina, phosphoethanolamina, acido alpha-aminoisobutyric, e methylhistidina non esseva detegite in ulle del specimens ab iste juveniles diabetic.

Le nivellos del excretion de glycina, alanina, e glutamina, que es communmente trovate in le urina de juveniles normal, monstrava nulle significative correlationes con le dosage de insulina, con le etate del subjecto al tempore del declaration de symptomias de diabete, o con le duration del tractamento.

Un grossier comparison del nivellos medie de excretion in le juveniles diabetic con le valores pro un gruppo heterogenee de 700 juveniles sin diabete monstrava que le valores pro le diabeticos esseva infra le cinquantesime percentiles in le normales pro omne le amino-acidos con le exception de taurina. Pro isto, ille puncto esseva al septanta-quinte percentil. Le excre-

tiones de glutamina in le gruppo diabetic esseva al dece-quinte percentil del normales.

Quando le juveniles diabetic esseva comparate con gruppos de juveniles normal intra categorias selectionate secundo le etates, nulle significative differentias in le valores medie o in le schemas del excretion poteva esser demonstrate con continuitate. Le gruppo de etates de inter cinque e dece-cinque annos in le serie de diabeticos studiate consisteva de trenta-duo patientes. Le valores medie del excretion de amino-acidos in iste gruppo esseva proximemente comparabile con le valores medie del gruppo normal.

#### ACKNOWLEDGMENT

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