A FAMILIAL STUDY OF A HUMAN ENZYME DEFECT, ARGININOSUCCINIC ACIDURIA*

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The human enzyme defect in which considerable amounts of argininosuccinic acid (ASA) are excreted in the urine was first described in two children by Allen, Cusworth, Dent and Wilson (1958). Westall (1960) determined that the compound was argininosuccinic acid, a known intermediate in urea formation. Levin, Mackay and Oberholzer (1960) studied a third child with the defect. All three children were similar in certain characteristic physical features and all suffered from various physical disabilities and mental retardation. Each excreted about 3 grams of ASA per day. Recently Carson and Neill (1962) found 2 additional cases in northern Ireland, and Van Pilsum and Halberg (1962) make mention of a case, to make a total of 6 reported.

The child with argininosuccinic aciduria who has been under investigation here for the past 2 years, was retarded in physical and mental development and appeared malnourished and chronically ill. This child, now 4 years old, resembled in appearance and clinical detail the 3 children described by previous investigators. A full clinical report will be published elsewhere.

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The appearance of malnutrition and failure of development suggested the possibility that the continual loss of dietary arginine through excretion of ASA together with the block in the usual path for synthesis of arginine had resulted in a relative arginine deficiency. When the child was fed a good, high-protein diet spread over 13 hours of the day for 2 months, improvement was dramatic. The child gained 2.6 kilos in the 2 month period as compared to 2 kilos in the previous 15 months and began to appear more like a healthy child -- even her hair improved in quality and quantity.

The ASA in the urine was identified by procedures similar to those used by Westall (1960). For quantitative determination with the 150 cm. column of the Beckman-Spinco amino acid analyzer, the ASA was changed to its two cyclic anhydrides by standing at room temperature at a pH of 2.2 for 48 hours. Values were corrected for analytical recovery (Cusworth and Westall, 1961) and calculated from comparison with leucine standards (Westall, 1960).

In the use of the amino acid analyzer to determine small amounts of the "C" isomer of ASA the peak "C" is close to but not identical with the β alanine peak on the chart. Since the areas covered by the two peaks overlap, a small part of what was calculated as ASA actually may be β alanine.

Twenty-four hour excretion of argininosuccinic acid determined as "B" and "C" anhydrides was found to be 2.8 grams on analysis of a urine sample collected by catheterization when the child was 36 months old and weighed 8.6 kg. Considerable amounts of histidine (1.95 mg. per kilo body weight) as well as the two methyl histidines were excreted in the urine in contrast to the very low values found by Cusworth and Dent (1960) for one of the original cases studied. Furthermore, the excretion of anserine (1.95 mg. per kilo) and especially of carnosine (3.87 mg. per kilo) by this child was unusually high.

On investigation, the child (propositus) was found to be a part of a large kindred who had lived in a mountainous section of northwestern Georgia and southeastern Tennessee for several generations. The family believe themselves to be of Irish ancestry and the name is of Irish origin. Three generations of the kindred were available and random urine samples were collected from 50 members of the family. What appears to be a recessive trait for this enzyme defect has been found in 3 generations of the family, although the investigation of the kindred is not yet complete. As shown in Table I, 10 apparently heterozygous relatives of the propositus were found who excreted from 4 to 29 mg. of ASA (determined as the "C" anhydride) per gram of creatinine. Two half-brothers of the propositus, the mother and 2 of her 3 brothers, 2 brothers (but not a sister) of the maternal grandfather and 3 of 4 first cousins of the propositus were all found to excrete small amounts of ASA.

Table I

Argininosuccinic Acid Excretion of Propositus, Relatives, Normal Subjects

Identification	Sex, Age	Argininosuccinic acid excretion, mg. per gm. creatinine.
Propositus	F- 3	18.6×10^3 *
Half-brothers	M- 9	29
	M- 2	20
Mother	F-28	12
Brothers of mother	M	0
	M-30	17
	M	12
Cousins of propositus	F-12	6
	M-10	0
	M- 8	18
	F- 6	14
Uncles of mother	M-66	4
	M-63	5
Aunt of mother	F-69	0
Six normal subjects, 3 males, 3 females		0

* Based on 24 hour excretion of 2.8 gm. of ASA and 0.15 gm. creatinine.

Since Westall (1960) found administered citrulline increased ASA excretion in a child with this enzyme defect, a similar loading test was performed on a trait-carrier (the mother of the propositus) and 2 normal subjects. Each was given 74 mg. of L-citrulline per kilo of body weight with each of 3 meals per day for 2 days. The ASA excretion figures in Table II show that on the second day the trait-carrier excreted 3 times as much ASA per gm. creatinine as the average excretion of the 2 normal females.

Table II

Argininosuccinic Acid Excretion on Second Day of Citrulline Loading Test; 74 mg. L-Citrulline Per Kilo Body Weight Given With Each of Three Meals for Two Days.

Subject	L-Citrulline Gm. per Day	Mg. ASA Control Day		Mg. ASA Second Day of Loading Test	
		Per 24 hours	Per Gm. Creatinine	Per 24 hours	Per Gm. Creatinine
Mother of propositus	9	8	12	88	114
Normal female	12.7	0	0	33	26
Normal female	11.8	0	0	55	42

It seems likely considering the rarity of argininosuccinic aciduria that the father of the propositus was a heterozygous carrier of the trait, perhaps distantly related, but from the same large kindred as the mother.

Chromosome studies on the propositus were done by culture of peripheral blood by a modification of the technic of Moorehead et al. (1960). Counts were made in 76 apparently undamaged cells in mitotic metaphase and detailed analysis was made of 10 cells with the aid of enlarged photomicrographs. The modal chromosome number was 47 rather than the usual 46 as there were 17 chromosomes in group C instead of 16. The extra chromosome, which was metacentric, had almost the same length as No. 12. The extra chromosome probably was not an extra X (sex) chromosome as typical sex chromatin was found in 30-40% of the interphase nuclei of oral mucosal cells.

One cell with 45 chromosomes was analyzed and it was found that both small metacentrics in No. 20 position were missing but the extra metacentric chromosome in group C was present. In a cell with 46 chromosomes similarly analyzed, an extra metacentric group C chromosome was again found but there were only 3 acrocentric chromosomes in group G.

No attempt was made to determine the per cent drumsticks in the polymorphonuclear leukocytes in the peripheral blood.

The results of the chromosome analysis are summarized in Table III.

Table III

Summary of Chromosome Analyses of the Propositus

Chromosome Counts Found in Leukocytes							
						TOTAL NO. OF CELLS	
44 or less	45	46	47	48	49 or more	Cells Counted	Analyzed
0	2	3	70	1	0	76	10

In chromosomal studies of the mother now in progress, 77 cells were counted. Thirteen were found to have 45 chromosomes, 52 to have 46 chromosomes and 12 to have 47. The 12 cells with 47 chromosomes had the extra metacentric chromosome in group C as described above for the propositus. Detailed analysis of the cells with 45 and 46 chromosomes has not been completed.

The enzyme defect involving the enzyme for splitting argininosuccinic acid appears to be inherited as a recessive trait detectable through a small excretion of argininosuccinic acid. Interpretation of the significance of the chromosomal anomaly found in the propositus and mother must await further detailed chromosome studies of members of the kindred.

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