Qualitative Study of Aminoaciduria in Muscular Dystrophy and Myotonia Dystrophica.* (22142)

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Studies by Ames and Risley(1) indicated that increased amounts of amino acids were excreted in the urine of muscular dystrophy patients. However, no identifications of individual amino acids or excretion pattern studies were done. More recently Hurley and Williams(2), by means of quantitative paper chromatographic analyses, have reported higher leucine, taurine, threonine, valine and arginine levels in the urine of male patients. Glycine, alanine, serine and citrulline levels were not significantly higher than in normal urines. Urines of females were not analyzed.

In the present investigation an attempt has been made to discern the various amino acids excreted in the urine of male and female patients with muscular dystrophy and myotonia dystrophica by means of 2-dimensional filter paper chromatography. Siblings and parents of the muscular dystrophy patients were studied whenever possible.

Procedures and methods. The subjects were 43 muscular dystrophy patients ranging in age from 14 to 60 years, 9 male patients with myotonia dystrophica and 53 normal subjects. In addition, a group of 13 parents and 16 uninvolved siblings were investigated. Samples for analysis were taken from the first voided morning urine. All samples were analyzed the day of voiding or stored temporarily in the frozen state. The amino acids were determined by descending 2-dimensional filter paper chromatography, essentially by the methods of Levy and Chung(3) and Fink *et al.*(4). Aliquots of 70 microliters of urine applied to Whatman No. 1 paper in 3-microliter portions were used for analysis. The developing solvents were (1) phenol-0.1 M borate buffer, pH 8.3 (85.6:20. w/v) and (2) butanol-glacial acetic acid-water (4:1:5) — organic phase. Development in phenol was carried out at 25 ± 1°; and butanol at room temperature. Ninhydrin was used as the spray reagent(3). Because of similar R_F values, the following groups of amino acids could not be resolved: isoleucine and leucine; glutamine, citrulline and arginine; methionine and valine; and methionine sulfoxide and sarcosine. β-Aminoisobutyric and γ-amino-n-butyric acids if present would travel in the methionine-valine band. Taurine under these conditions is obscured by glycine in most instances. Further separations of these groups have not been attempted.

Results. There is a qualitative increase in the excretion of amino acids and certain related unidentified substances in muscular dystrophy patients, as shown by an increased number of spots on the urine chromatograms. The amino acids which appeared most frequently in the urine of dystrophy patients were methionine or valine, isoleucine or leu-

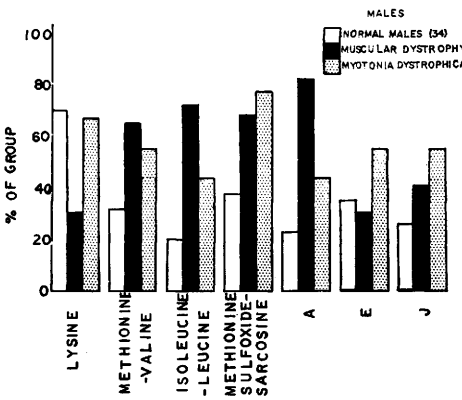

FIG. 1. Urinary amino acid excretion patterns in normal males and male patients with muscular dystrophy and myotonia dystrophica.

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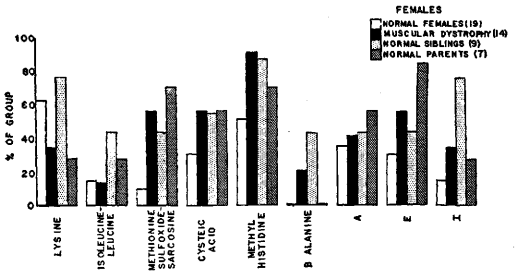

FIG. 2. Urinary amino acid excretion patterns in normal females, normal siblings, parents, and female muscular dystrophy patients.

cine, methionine sulfoxide or sarcosine, methyl histidine, cysteic acid and unidentified substances A and E (Fig. 1 and 2).‡

Nine subjects with myotonia dystrophica exhibited increased numbers of urinary amino acids. Lysine, methionine sulfoxide-sarcosine and unidentified substances E and J‡ appeared more frequently in the urine of these patients than in the urine of muscular dystrophy patients (Fig.1).

The amino acid excretion of a small group of clinically uninvolved male siblings and male parents was studied. There was a significant increase in the former group. Marked elevations of isoleucine or leucine, β-alanine, cysteic acid, and unidentified substances A and I‡ were observed. In the male parent group, methionine-valine and cystine were increased (Fig. 3).

A group of uninvolved female siblings and female parents manifested marked increases in the number of amino acids excreted (Fig. 2). The aminoaciduria was as extensive in these latter groups as in the female muscular dystrophy patients.

A relatively large number of unidentified spots were observed in the chromatograms of muscular dystrophy patients and their relatives. Spots designated A and E were most consistently present.

Discussion. The data presented indicate that a qualitative increase in urinary amino acids occurs in patients with muscular dystrophy and myotonia dystrophica and certain related normal individuals. Whether there is an associated quantitative increase in amino acid concentration in the urine of such individuals is currently under investigation.

The increased numbers of amino acids observed in the urine of normal siblings and parents of muscular dystrophy patients suggests the possibility of an inherited metabolic abnormality. The particularly large numbers of amino acids found in the urine of the normal female sibling and the maternal parent are striking evidence in favor of this thesis. Furthermore, genetic studies seem to indicate maternal transmission of the disease(6).

The presence of the relatively large group of unidentified ninhydrin staining substances, found in the urine of dystrophy patients and related individuals, is of interest. These components may be unidentified amino acids or peptides. It seems likely that these are normal constituents of urine, excreted at much lower levels in normal individuals since the urines of controls often contain these components. Boulanger, Biserte and Courtot(5) have described peptides in normal urines, some of which may be identical to those observed here. Two spots, A and E, are most consistently present in dystrophic individuals and their immediate relatives. The nature of these unknown substances is currently under study.

Summary. 1. An increase in the number of urinary amino acids and certain related unidentified substances was observed in muscular dystrophy and myotonia dystrophica patients. 2. Increased numbers of amino acids were found in the urine of normal sib-

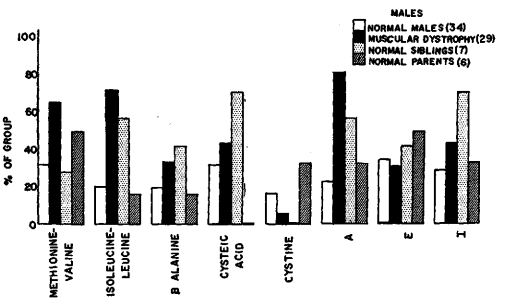

FIG. 3. Urinary amino acid excretion patterns in normal males, normal siblings, parents, and male muscular dystrophy patients.

‡ The R_F values of these unknown ninhydrin-reacting substances in solvents 1 and 2 respectively are— A: 0.75, 0.29; E: 0.50, 0.29; I: 0.85, 0.17; and J: 0.85, 0.11.

lings and the maternal parents of muscular dystrophy patients.

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Effect of a High Fat Fructose and Casein Diet on Diabetic Cataracts.* (22143)

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Two or 3 months after the chemical induction of diabetes in rats, the lenses of the animals become opaque(1,2). The rate at which these cataracts develop is directly related to the height of the blood sugar(2). Decreasing the hyperglycemia by partial starvation(3) or the administration of phlorizin(4) delays or prevents cataracts to the extent that the blood sugar is lowered. These results suggest that hyperglycemia may play a direct role in the production of cataracts. If the high blood sugar level acts directly on the lens to produce cataracts, then the amount of sugar reaching the eye by way of the bloodstream should be of importance. Altering the blood supply by unilateral carotid ligation influences the development of galactose cataracts but does not affect the development of diabetic cataracts(5). This finding raises a question regarding the direct role of hyperglycemia and leads to the suggestion that the prevention of cataracts might be due to the provision of an alternate energy supply by the ketosis that accompanies phlorizin administration or starvation. Diets containing large amounts of fat or protein provide non-carbohydrate substrates for energy and delay or prevent cataracts(1,6). However, these diets also lower the blood sugar and an interpretation of the results is difficult. Experiments in this laboratory indicate that a high-fat diet containing fructose rather than glucose, can be fed to diabetic rats without producing a depression in the blood sugar level. The use of this diet makes it possible to determine the effect of the dietary constituents in the prevention of cataracts. This paper reports the results obtained with a diet containing large amounts of fructose, fat and protein.

Methods. Male Sprague-Dawley rats weighing 76-173 g were made diabetic by the intravenous injection of 40 mg per kilo of alloxan monohydrate. The animals were maintained with unlimited amounts of water and ground dog chow. They were weighed and the blood sugars(7) determined once or twice each week. At the end of the first four weeks the blood sugar determinations for each rat were averaged. Those animals with blood sugar levels greater than 350 mg per 100 cc of blood were retained in the experiment and divided into two groups. The control group was maintained on the same diet as had been used in the first 4-week period. The experimental group was placed on an unlimited amount of the special diet. The latter diet consisted of 50% fructose, 25% corn oil and

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