fessor P. C. Elmes, Dr. D. A. D. Montgomery, and Dr. Daphne Lowe, of the Royal Victoria Hospital, Belfast. Xg^a blood grouping was performed by Dr. Ruth Sanger, of the Lister Institute. We are indebted for technical assistance to the laboratory staff of Westminster Hospital and St. James' Hospital. Dr. A. W. G. Goolden, of the Royal Postgraduate Medical School, Hammersmith, offered helpful criticism of the manuscript.

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Preliminary Communications

Paper Chromatographic Survey of Urinary Amino-acids in Muscular Dystrophy

British Medical Journal, 1970, 3, 29-30

Summary: A paper chromatographic analysis in 60 patients with muscular dystrophy from 49 families and in 351 of their relatives showed a high incidence of generalized hyperaminoaciduria in both patients and relatives. There was a higher incidence of threoninuria in patients and their relatives than in controls. One family was found to have excess histidinuria and another excess lysinuria.

INTRODUCTION

The frequency and significance of abnormal amino-aciduria in muscular dystrophy is at present controversial. Partial or universal hyperaminoaciduria has been found in patients with muscular dystrophy (Ames and Risley, 1948; Schöenberg, 1955; Berger, 1962) and unaffected relatives (Blahd *et al.*, 1955; Beckmann and Ritter, 1967). A basic hyperaminoaciduria has been described in a family in which there were several members affected with facio-scapulo-humeral muscular dystrophy (Hurwitz *et al.*, 1965, 1967). A similar pattern of amino-aciduria has also been reported in four out of five siblings with severe congenital muscular hypotonia (Clara and Lowenthal, 1965). Some authors have found an excess aminoaciduria restricted to leucine and taurine and possibly threonine and valine (Hurley and Williams, 1955) and to arginine, threonine, and proline. There have, however, also been studies in which there was no excess amino-aciduria in muscular dystrophy (Bickel and Souchon, 1955; Carver *et al.*, 1961). The present survey attempts to determine the incidence and pattern of hyperaminoaciduria in patients with muscular dystrophy and in their relatives, using a paper chromatographic screening technique.

METHODS

Urinary amino-acids were analysed by paper chromatography, using a technique described by Carson and Neill (1962), in patients with muscular dystrophy residing in North-

ern Ireland and their relatives. Since dietary and environmental factors are known to have a profound effect on amino-acid excretion, written instructions were given to all subjects, stating that the urine sample should be produced in the morning before breakfast, and during the mid-menstrual cycle in the case of women. A health visitor collected the samples on the day on which they were provided. The subjects were instructed to notify her of any unusual dietary intake and medications but to endeavour to provide the specimen when there was no unusual food intake. Two or three samples were collected from subjects at about monthly intervals. The urine specimens were stored at -25°C . until analysis.

The chromatographic procedures were rigorously standardized. The volume of urine applied to the paper was that equivalent to a standard amount of nitrogen. In addition to the urine sample, 5 μ l. of β -amino-octanoic acid was applied at the origin of each chromatogram. The β -amino-octanoic acid migrated to a position well away from the naturally occurring amino-acids. This was used as a standard, and the colour intensities of the urinary amino-acids after the ninyhydrin reaction were visually compared with that of the standard and given arbitrary units of intensity with a range of 1 to 5. This attempt at semiquantitation made the assessment of hyperaminoaciduria less subjective.

SUBJECTS

The propositus in each family was examined clinically and a provisional diagnosis made of the type of muscular dystrophy, except in the three families in which the propositus was not alive. An attempt was made to examine clinically all first-degree relatives, and detailed information was sought of any other relatives who might have muscle disease, and they were then examined or hospital documents obtained.

The clinical classification of the dystrophy is given in Table I. There were 36 male patients aged 7 to 68 years, 24 females aged 18 to 58 years, and 157 male and 194 female

TABLE I.—49 Families with Muscular Dystrophy

X-linked Duchenne	..	..	..	..	..	..	3
Resembling Duchenne	..	..	..	..	..	..	11
Facio-scapulo-humeral	..	..	..	..	..	..	7
Autosomal recessive limb girdle	..	..	..	..	..	..	20
Dystrophia myotonica	..	..	..	..	..	..	6
Congenital myopathy	..	..	..	..	..	..	2

relatives. As controls 122 urine specimens from healthy subjects were examined; these included two or three specimens from members of three families.

RESULTS

Abnormalities in urinary amino-acid excretion were found in patients with different clinical types of myopathy and their relatives. The results so far cannot be analysed separately for statistical purposes for male and female parents and other relatives because of the relatively few abnormalities, but this will be done as the study proceeds. In subjects with hyperaminoaciduria the abnormal excretion pattern was often present in all samples though in 51% of the cases the abnormality was found in only one of the specimens. Multiple samples from controls were consistently normal.

Generalized Hyperaminoaciduria.—In the present context this term is used to describe a heterogeneous group of amino-acidurias of varying compositions. Instances of this condition could not be classified into groups of similar pattern since no consistent pattern of amino-acid grouping could be shown. The incidence of a generalized hyperaminoaciduria in patients and their relatives is given in Table II. The most frequently

TABLE II

	No.	Percentage with Generalized Hyperaminoaciduria	Percentage with Threoninuria
Normal controls	122		5.7
Patients	60	18.3	21.7
Parents	50	8.0	20.0
Siblings	66	9.1	21.3
Other relatives	235	8.9	20.0

increased amino-acid was glycine, and others commonly present in excess were serine, taurine, alanine, histidine, glutamine, glutamic acid, and, less commonly, lysine, cystine, aspartic acid, tyrosine, methionine, valine, and methyl histidine.

Threoninuria.—Threonine, which was not a feature of generalized hyperaminoaciduria, was found more often in the urine of muscular dystrophy patients and their relatives than of controls (Table II). Lee *et al.* (1967) reported a normal incidence of threoninuria of 2.8%. In the present series there was a high familial incidence of threoninuria, but no consistent pattern of inheritance could be demonstrated.

Histidinuria.—Histidinuria may occur as part of a more or less generalized hyperaminoaciduria. One family has been found, however, in which an increase in histidine was the dominant abnormality of urinary amino-acid excretion. The mother and four normal children in a sibship of seven had excess histidinuria, the three other male siblings having died with dystrophy before the start of this survey. The cousins of this family, though also possessing the trait for Duchenne type dystrophy, had no histidinuria in the affected or non-affected children, and their mother also had a normal amino-acid excretion pattern. The evidence suggests that the dystrophy and the excess histidinuria are inherited by different mechanisms as the amino-aciduria appears to be inherited as a dominant trait.

Lysinuria.—This was found in one family with myopathy. A detailed report of this family has been published (Hurwitz *et al.*, 1969).

DISCUSSION

This survey has shown a high percentage of abnormal patterns of urinary amino-acid excretion in patients and their relatives. This cannot be fully explained by muscle wasting, for not only were abnormal patterns found in normal relatives but there was no direct relation in patients between the degree of muscle wasting and the amino-aciduria. Also the myopathy and the amino-aciduria often segregated separately. Threonine, which is an essential amino-acid, was excreted in the urine more often in patients with muscular dystrophy and in their relatives than in controls. In addition to the generalized hyperaminoaciduria and threoninuria more specific patterns of abnormal excretion were found in two families. It is considered unlikely that the abnormal amino-aciduria is exclusively the result of dietary and environmental factors.

This study suggests the need for a more quantitative analysis of urinary amino-acids in muscular dystrophy. There is also value in the use of large-scale urinary screening by paper chromatography, to select those cases in which more detailed investigation should be performed.

The fact that amino-aciduria and muscular dystrophy were often found in the same family suggests some relationship. A direct relation is unlikely because of the occasionally different modes of the inheritance of the two traits in families and the separate segregation; also, similar amino-acidurias have been found in patients with other neurological diseases, and a detailed study of amino-aciduria in other diseases has not been attempted in this department. The amino-aciduria, however, may indicate a metabolic or cell transport disturbance which if defined could have considerable relevance to the understanding of the dystrophic process.

We are grateful to Dr. N. C. Nevin for advice on the genetics. We would also like to thank Miss E. McCrea, health visitor, for her invaluable help in arranging for the collection of the specimens and Mr. N. Gilmour and Mr. A. Bittles for technical assistance. The work was supported by grants from the Muscular Dystrophy Group of Great Britain and the Northern Ireland Hospitals Authority.

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