ALDOLASE ACTIVITY IN THE PLASMA OR SERUM OF NORMAL CHILDREN AND FAMILIES WITH MUSCULAR DYSTROPHY

BY

BARBARA E. CLAYTON, K. MARGARET WILSON and CEDRIC O. CARTER

From the Department of Chemical Pathology, The Hospital for Sick Children, and the M.R.C. Clinical Genetics Research Unit, Institute of Child Health, Great Ormond Street, London

(RECEIVED FOR PUBLICATION OCTOBER 25, 1962)

Aldolase catalyses the reversible splitting of fructose 1:6-diphosphate- to glyceraldehyde-3-phosphate and dihydroxy-acetone phosphate. Diagnostically it is of value in distinguishing primary muscular disease from neurogenic muscular atrophy.

Most investigators agree that levels are higher in normal children, especially the newly born, than in normal adults and that there is no sex difference (Schapira, Dreyfus, Schapira and Kruh, 1955; Evans and Baker, 1957; Soltan and Blanchaer, 1959; Pojerová and Továrek, 1959). However, Sibley and Fleisher (1954) found no variation with age, and Friedman and Lapan (1958) found higher levels in men than in women.

The determination is of value in the diagnosis of muscular dystrophy, and in the detection of this condition in children not yet affected clinically. Since the level is raised in affected children, the possibility of using this estimation to detect heterozygous carriers of genes responsible for the various forms of the disease has been considered. Results reported in the literature have been conflicting. Soltan and Blanchaer (1959) found no raised levels in 12 mothers and 11 sisters of patients with Duchenne type muscular dystrophy, but do not distinguish those who were known to be heterozygotes. Chung, Morton and Peters (1960) observed raised aldolase levels (at the 5% level of significance) on a single estimate in a small proportion, three of 21, of known heterozygotes for the Duchenne type muscular dystrophy, but in only one of 19 heterozygotes for the gene for the 'limb-girdle' type. Schapira, Dreyfus, Schapira and Demos (1960) found raised levels in seven of 18 known carriers of Duchenne type dystrophy. Leyburn, Thomson and Walton (1961) found no raised levels in any of six known carriers of Duchenne type dystrophy.

In the present investigation, aldolase levels have

been determined in an extensive series of normal children and in some adults. This was an essential preliminary to a study undertaken to see whether carriers could be detected.

Method

Cord blood was collected at the time of delivery. Capillary blood was collected by heel or finger prick (Wilkinson, 1960). Aldolase was determined by the method provided with the Biochemica Boehringer pack of reagents* and based on the work of Warburg and Christian (1943) and Racker (1947). A time interval of one to two minutes between the addition of serum or plasma and the first spectrophotometric reading was allowed. No significant difference was observed between serum or plasma. On a series of 20 normal adult plasmas, duplicate estimations varied by a maximum of 2.5 Bruns units/ml.

Separation of Red Cells and Haemolysis. Haemolysis results in a significant rise in aldolase activity of plasma or serum since it causes the release of the large concentration of aldolase present in the red blood cells. Before separation, blood should neither be refrigerated at +4° C. nor stored at -15° C., since this results in haemolysis. Provided room temperature does not exceed 22° C. blood may be separated up to 16 hours after collection, but at higher temperatures six hours is the limit. This is clearly shown in Fig. 1.

Storage of Serum or Plasma. Serum or plasma may be stored at +4° C. or -15° C. for two weeks without significant change in aldolase activity, but plasma must be centrifuged to remove fibrin clots before the estimation is performed.

Variations in Aldolase Level

No trend in the level of aldolase activity was

* Supplied by C. F. Boehringer and Soehne G.m.b.H., Mannheim, Germany.

FIG. 1.—Effect on the aldolase activity of (1) temperature and (2) length of time before separation of the blood. Blood was kept at 22° C. and 30–35° C. for varying periods before being separated. The ratio of the aldolase activity found, to the initial aldolase activity obtained when the blood was separated immediately after collection, has been plotted against the interval between collection and separation of the blood. - - - blood kept at 30–35° C.; — blood kept at 22° C.

observed during the day in three normal adults and three children with muscular dystrophy (see Table 1).

In Table 2, the variations from day to day in three normal adults and three children with muscular dystrophy are shown. No trend was observed from day to day, except that the 13-year-old boy on the third day had a single peak value which was greater than the mean + 2 standard deviations.

Aldolase estimations were also performed on four women at various stages of the menstrual cycle, but no significant variation was found (see Table 3).

Normal Series. Blood was collected from 271 well-nourished children and adults in whom there was no reason to suspect liver, muscle or neurological disease. The individual results are shown in Fig. 2 and the mean figures for various groups are given in Table 4.

It will be seen that the levels fall from birth to adolescence, but are fairly constant in adult life after the age of 25, with a mean of 9.02 with standard deviation 2.34 for men, and 9.17 with standard deviation 3.26 for women. There is no significant difference in aldolase levels between male and female in this series after the age of 2 years, though there is a significant difference, females being higher, in infancy.

Family Studies

Genetics of Limb-girdle Muscular Dystrophy in Children. At least three distinct mutant genes may cause a limb-girdle type of muscular dystrophy in children.

Much the commonest is the presumably X-borne recessive gene responsible for severe sex-linked muscular dystrophy (Duchenne type). In these families only boys are affected, the onset is in early childhood, patients are usually unable to walk by the age of 11 and are often dead by the age of 20 (Blyth and Pugh, 1959). A good example is given by family A shown in Fig. 3.

Less common is the autosomal recessive gene which gives a form of limb-girdle muscular dystrophy difficult to distinguish from the previous type; both sexes are equally affected, parental consanguinity is often found, patients less often show pseudohypertrophy, are rather less severely affected and are usually still ambulant at 11 years.

The third and least common gene is the presumably X-borne gene responsible for the rare mild form of sex-linked muscular dystrophy; in these patients the onset is usually in school age, but may be in the late 'teens, and patients may still be ambulant in middle age. This variant is not distinguished by some authorities from the Duchenne type; but it 'breeds true' within families (Becker, 1955; Blyth and Pugh, 1959), and so is almost certainly due to a distinct gene. A good example is given by family I in Fig. 5.

It is still possible that both 'sex-linked' forms are really due to an autosomal gene manifest only in male heterozygotes, but this is improbable.

Family Studies of Aldolase Levels

1. London Series. In this series three tests were made on each heterozygous woman.

(a) *Severe Sex-linked Type (Duchenne).* Four

TABLE 1
ALDOLASE ACTIVITY (Bruns units/ml. serum) AT VARIOUS TIMES OF DAY

Time of Collection	Normal Adults			Time of Collection	Boys With Pseudohypertrophic Muscular Dystrophy		
	1	2	3		1 (13 yrs)	2 (11 yrs)	3 (7 yrs)
10 a.m.	9.4	7.1	2.9	10 a.m.	15.4	21.7	64.5
12.30 p.m.	10.5	4.3	5.0	11.25 a.m.	15.3	19.8	
2 p.m.	8.7	6.8	6.9	12.50 p.m.	17.0	21.1	63.9
4.15 p.m.	10.7	3.7	7.1	2 p.m.	17.3		67.6
				3 p.m.	16.0		
				4 p.m.	18.5	20.9	
				5 p.m.	17.9		65.9
Mean level	9.8	5.5	5.5		16.8	20.9	65.5
Standard deviation ..	0.9	1.7	2.0		1.2	0.8	1.7

TABLE 2
VARIATION FROM DAY TO DAY OF ALDOLASE ACTIVITY (Bruns units/ml. serum) FOR THREE CHILDREN WITH PSEUDOHYPERTROPHIC MUSCULAR DYSTROPHY AND FOR THREE NORMAL ADULTS

Day	Normal Adults			Boys With Pseudohypertrophic Muscular Dystrophy		
	1	2	3	1 (13 yrs)	2 (11 yrs)	3 (7 yrs)
1	3.7	6.3	6.1	15.1	15.6	53.8
2	3.3	7.7	7.0	15.0		66.0
3	5.3	7.5	4.5	32.0	20.6	54.8
4	5.9	—	5.2	15.4	19.1	57.7
5	5.1	—	4.5	18.0	21.7	64.6
6	8.4	6.4	7.7	12.4	18.7	57.4
7	6.1	6.1	7.8	13.2		
8	4.6	5.1	6.5	12.7		
9				11.2		
10				13.5		
11				16.0		
12				16.7		
Mean	5.3	6.5	6.2	*15.9	19.1	59.1
Standard deviation ..	1.59	0.96	1.33	*5.42	2.32	2.24

Excluding Day 3 value, mean = 14.5, standard deviation = 2.1.

TABLE 3
VARIATION IN ALDOLASE ACTIVITY (in Bruns units/ml. serum) THROUGHOUT MENSTRUAL CYCLE OF FOUR ADULT WOMEN

Subject 1		Subject 2		Subject 3		Subject 4	
Day	Aldolase Activity	Day	Aldolase Activity	Day	Aldolase Activity	Day	Aldolase Activity
2	8.7	2	6.5	1	7.8	2	8.5
3	5.8	3	9.3	2	9.5	3	—
10	10.8	11	10.0	12	5.1	14	7.0
11	5.7	12	6.7	13	6.4	15	3.8
21	5.5	22	—	21	6.7	21	3.5
22	5.1	23	9.7	22	6.5	22	6.1
	Mean		Mean		Mean		Mean
	7.3		7.9		8.7		8.5
	8.2		8.4		5.8		5.4
	5.3		9.7		6.6		4.8

Days are numbered from the first day of menstruation.

As far as was possible aldolase activity was measured at nine-day intervals, thus dividing the cycle into three equal parts; the two values missing were due to a faulty spectrophotometer and absenteeism.

FIG. 2.—Aldolase activity in normal children and adults.

families were studied in which the mother of the index patient was certainly a carrier, since she had at least one other son or other male relative affected. The relevant parts of the four family trees are shown in Fig. 3, and the aldolase results in Table 5.

(b) *Autosomal Recessive Type.* Four families were studied in which there was a strong indication of autosomal recessive inheritance. In two families there were both an affected girl and parental consanguinity, in one family there was an affected girl, and in one family parental consanguinity. The relevant parts of the four family trees are shown in Fig. 4, and the aldolase results in Table 6. In these families both parents will be heterozygous for the gene concerned, but repeat estimations have been done on the mothers only.

(c) *Mild Sex-linked Recessive Type.* Two families were studied. The relevant parts of the family trees are shown in Fig. 5 and the aldolase results in Table 7.

2. Leeds Series. An additional series of single

tests was made on certain known heterozygotes of the families reported from Yorkshire by Blyth and Pugh (1959); the results are shown in Table 9 with a reference to the family and pedigree in the 1959 report. Three women did give high readings on a first estimation. It was realized that this was probably due to the effect of heat on unseparated blood, and repeat estimations which gave normal figures were substituted.

TABLE 4
NORMAL LEVELS OF ALDOLASE ACTIVITY
(Bruns units/ml. plasma or serum)

Age-group	Male		Female	
	No. of Persons	Mean Aldolase Activity $\pm$ S.D.	No. of Persons	Mean Aldolase Activity $\pm$ S.D.
Cord blood ..	22	13.5 $\pm$ 4.7	29	18.3 $\pm$ 7.3
0-24 months ..	15	12.5 $\pm$ 3.8	14	14.1 $\pm$ 5.7
2-5 years ..	14	10.9 $\pm$ 3.7	13	11.1 $\pm$ 2.9
5-10 years ..	14	10.6 $\pm$ 3.0	14	9.1 $\pm$ 2.4
10-15 years ..	15	10.3 $\pm$ 2.8	13	9.2 $\pm$ 3.2
15-25 years ..	14	10.0 $\pm$ 2.1	16	9.4 $\pm$ 6.1
25-40 years ..	14	8.8 $\pm$ 2.0	21	8.7 $\pm$ 3.0
40-60 years ..	17	9.3 $\pm$ 2.4	15	9.7 $\pm$ 3.8
60-over 70 years	7	8.8 $\pm$ 3.1	4	9.5 $\pm$ 2.8

FIG. 3.—Four pedigrees of severe sex-linked muscular dystrophy (Duchenne type).

FIG. 4.—Four pedigrees of autosomal recessive muscular dystrophy.

FIG. 5.—Two pedigrees of mild sex-linked recessive muscular dystrophy.

TABLE 5
ALDOLASE LEVELS IN LONDON FAMILIES WITH SEVERE SEX-LINKED
RECESSIVE MUSCULAR DYSTROPHY (Bruns units/ml. serum)

Family	Index Patient		Other Patients		Father	Heterozygous Mother (3 tests)			Mean
	Aldolase Level	Age (yrs)	Aldolase Level	Age (yrs)					
A	70	8	—		10	12	8	8	9.3
B	79	9	111	4	8	9	7.5	8.5	8.3
C	16	14	37	12	7	12	14	12	12.7
D	178	8	170	5	—	7	13	6	8.7

ALDOLASE ACTIVITY AND MUSCULAR DYSTROPHY

213

TABLE 6

ALDOLASE LEVELS IN LONDON FAMILIES WITH AUTOSOMAL RECESSIVE LIMB-GIRDLE MUSCULAR DYSTROPHY
(Bruns units/ml. serum)

Family	Index Patient		Other Patients		Hetero- zygous Fathers	Heterozygous Mothers (3 tests)			Mean
	Aldolase Level	Age (yrs)	Aldolase Level	Age (yrs)					
E	19	12	—	—	10	6	10	11	9.0
F	90	6	—	—	9	6	5	8	6.3
G	20	12	49	9	10	11	6	9	8.7
H	21	11	52	11	8	9	5	8	7.3

TABLE 7

ALDOLASE LEVELS IN LONDON FAMILIES WITH MILD SEX-LINKED RECESSIVE MUSCULAR DYSTROPHY
(Bruns units/ml. serum)

Family	Index Patient		Other Patients	Father	Heterozygous Mother (3 tests)			Mean
	Aldolase Level	Age (yrs)						
I	33	14	(see Table 8)	10	7	7	11	8.3
J	36	18	—	11	10	11	7	9.3

TABLE 8

ALDOLASE LEVELS IN AFFECTED MALES IN FAMILY WITH
MILD SEX-LINKED MUSCULAR DYSTROPHY

	Age of Patients (yrs)						
	8	14	16	17	32	36	38
Aldolase level (Bruns units/ ml.)	50	33	57	29	19	13	14

Discussion

The aldolase levels in the index patients are consistent with the usual findings. The highest levels are seen in the severe sex-linked recessive forms early in the disease (including the stage at which clinical symptoms have not yet appeared). The aldolase levels are constantly raised also in the early stages of the autosomal recessive and milder sex-linked recessive forms, but to a lesser degree. In the large family I (Fig. 5), with the mild sex-linked recessive type, the fall in aldolase level with increasing age of patient (and duration of disease) can be clearly seen in Table 8.

The findings in the heterozygous carriers in the London series were as follows: the two female heterozygous carriers of the gene for the mild sex-linked form have entirely normal levels; the eight heterozygous carriers of the gene for the autosomal recessive type, four female and four male, also had

TABLE 9

ALDOLASE LEVELS OF HETEROZYGOTES IN
LEEDS FAMILIES
(reference numbers refer to the pedigrees of Blyth and Pugh, 1959)

Severe Sex-linked Recessive	Autosomal Recessive		Mild Sex-linked Recessive
Mother	Mother	Father	Mother
M (II 12) 5	M7 (II 5) 9	(II 9) 10	S15 (II 5) 8
M21 (III 6) 7	M8 (II 9) 5	(II 18) 5	S12 (III 5) 12
	M10 (II 1) 10	—	S29 (II 3) 7
	M11 (II 3) 8	(II 13) 15	
	M15 (II 7) 13	(II 11) 12	

entirely normal levels; the four heterozygous carriers of the gene for the severe sex-linked type also had aldolase levels, on three separate tests, which were well within the normal range.

In the Leeds series the three women heterozygous for the gene for the severe and the two heterozygous for the gene for the mild sex-linked form, together with five women and four men heterozygous for the gene for the autosomal recessive form, also showed aldolase levels within normal limits, only one (a father) being beyond two standard deviations above the mean and this may well be a chance finding.

It is concluded that serum aldolase levels are not useful in distinguishing individuals who are heterozygous for any of the three genetic types of limb-girdle muscular dystrophy. They are, however, of great value in making a diagnosis of dystrophy in

the active phase of the disease in affected individuals, even before the onset of clinical signs.

Recent reports suggest that serum creatine phosphokinase estimations may be of more value in the detection of heterozygotes (Aebi, Richterich, Colombo and Rossi, 1961-62; Hughes, 1962).

Summary

A large series of estimates of serum aldolase levels on 271 normal children was used to establish the normal variation by age and sex. The levels fall from birth to adolescence, but are fairly constant in adult life. Females have significantly higher values in infancy, but not later in life.

Estimates of serum aldolase in individuals known to be heterozygous for one of the three genetic types of limb-girdle muscular dystrophy have shown normal values in all three types.

We thank the physicians of The Hospital for Sick Children for permission to investigate their patients. We are grateful to the Research Committee of the Hospital for financial assistance with this project; to the Department of Medical Illustration of the Hospital for the Figures; to the Muscular Dystrophy Society for an introduction to some of the families; to Mrs. K. Evans for much expert help with tracing and visiting the London families; to Dr. Helen Blyth for an introduction to the Leeds families and to Mr. A. F. Heeley for help with the collection of specimens for the Leeds families; to Miss J. Kendall for valuable technical assistance.

REFERENCES

- Aebi, U., Richterich, R., Colombo, J. P. and Rossi, E. (1961-62). Progressive muscular dystrophy. II. Biochemical identification of the carrier state in the recessive sex-linked juvenile (Duchenne) type by serum creatine-phosphokinase determinations. *Enzym. biol. clin.*, **1**, 61.
- Becker, P. E. (1955). Zur Genetik der Myopathien. *Dtsch. Z. Nervenheilk.*, **173**, 482.
- Blyth, H. and Pugh, R. J. (1959). Muscular dystrophy in childhood. The genetic aspect. A field study in the Leeds region of clinical types and their inheritance. *Ann. hum. Genet.*, **23**, 127.
- Chung, C. S., Morton, N. E. and Peters, H. A. (1960). Serum enzymes and genetic carriers in muscular dystrophy. *Amer. J. hum. Genet.*, **12**, 52.
- Evans, J. H. and Baker, R. W. R. (1957). Serum aldolase and the diagnosis of myopathy. *Brain*, **80**, 557.
- Friedman, M. M. and Lapan, B. (1958). Serum aldolase in the neonatal period: including a colorimetric determination of aldolase by standardisation with dihydroxyacetone. *J. Lab. clin. Med.*, **51**, 745.
- Hughes, B. P. (1962). Serum enzymes in carriers of muscular dystrophy. *Brit. med. J.*, **2**, 963.
- Leyburn, P., Thomson, W. H. S. and Walton, J. N. (1961). An investigation of the carrier state in the Duchenne type muscular dystrophy. *Ann. hum. Genet.*, **25**, 41.
- Pojerová, A. and Továrek, J. (1959). On enzymatic activity in the neonatal period. *Acta paediat. (Uppsala)*, **48**, 213.
- Racker, E. (1947). Spectrophotometric measurement of hexokinase and phosphohexokinase activity. *J. biol. Chem.*, **167**, 843.
- Schapira, F., Dreyfus, J. C., Schapira, G. and Demos, J. (1960). Étude de l'aldolase et de la créatine kinase du sérum chez les mères de myopathes. *Rev. franç. Étud. clin. biol.*, **5**, 990.
- Schapira, G., Dreyfus, J. C., Schapira, F. and Kruhl, J. (1955). Glycogenolytic enzymes in human progressive muscular dystrophy. *Amer. J. phys. Med.*, **34**, 313.
- Sibley, J. A. and Fleisher, G. A. (1954). The clinical significance of serum aldolase. *Proc. Mayo Clin.*, **29**, 591.
- Soltan, H. C. and Blanchaer, M. C. (1959). Activity of serum aldolase and lactic dehydrogenase, in patients affected with Duchenne muscular dystrophy and in their immediate relatives. *J. Pediat.*, **54**, 27.
- Warburg, O. and Christian, W. (1943). Isolierung und Kristallisation des Gärungsferments Zymohexase. *Biochem. Z.*, **314**, 149.
- Wilkinson, R. H. (1960). *Chemical Micromethods in Clinical Medicine*. Blackwell, Oxford.