J. Neurol. Neurosurg. Psychiat., 1964, 27, 181

Serum enzyme studies in muscle disease

Part III Serum creatine kinase activity in relatives of patients with the Duchenne type of muscular dystrophy

J. M. S. PEARCE[1], R. J. T. PENNINGTON, AND JOHN N. WALTON

From the Regional Neurological Centre, the General Hospital, Newcastle upon Tyne

In recent years it has been established that the measurement of serum enzymes is of value in the diagnosis of muscular dystrophy (Dreyfus, Schapira, and Schapira, 1958, Dreyfus, Schapira, and Demos, 1960; Aebi, Richterich, Stillhart, Colombo, and Rossi, 1961; Pearce, Pennington, and Walton, 1964b). The magnitude of the elevations found in serum enzymes when related to the clinical and genetic findings is of additional value in distinguishing between the various types of muscular dystrophy and in separating them from the acquired myopathies (Pearce *et al.*, 1964b).

Recent family studies (Pearson, 1962 a and b) have shown that studies of serum enzyme levels may be helpful in the detection of both early and preclinical cases of muscular dystrophy, enabling the subsequent development of the clinical disease to be predicted in infancy. The value of such studies in the detection of the carrier state is a controversial point. Leyburn, Thomson, and Walton (1961) were unable to identify the female carrier by means of estimations of serum aldolase and transaminases. Dreyfus and his co-workers (Dreyfus *et al.*, 1960, 1962b) have demonstrated increased activity of serum creatine kinase in some of the mothers of children suffering from the Duchenne type of muscular dystrophy. However, Okinaka, Kumagai, Ebashi, Sugita, Momoi, Toyokura, and Fujie (1961) found normal serum creatine kinase activity in the parents but increased activity in some female sibs of five cases of muscular dystrophy in childhood.

More recently the results of serum aldolase and serum creatine kinase estimations have been reported (Hughes, 1962, 1963) in known carriers and in possible carriers. The first of these studies has shown that seven of eight known carriers exhibited high serum creatine kinase levels, and that 50% of possible carriers showed raised serum enzyme activity. Hughes suggested that studies on a larger group of cases were necessary to assess the reliability of this method, and also showed that serum aldolase was a less useful tool in detecting carriers than serum creatine kinase activity.

The purpose of this paper, therefore, is to record the results we have obtained using serum creatine kinase activity in the diagnosis of preclinical muscular dystrophy of the Duchenne type, and in the detection of the carrier state. We have also measured the serum activity of creatine kinase in male and female sibs of affected patients. Previous communications from this centre (Pennington, Walton, and Barwick, 1964; Barwick, 1963) have reported the results of investigations of the carrier state, utilizing both serum creatine kinase activity and electromyography. Since these investigations our technique of measuring serum creatine kinase activity has been modified (Pearce *et al.*, 1964a) and a small but significant change in the values obtained has been found. This has made it impossible to integrate the results of previous investigations with the present one, as values now in the normal range would previously have been regarded as abnormal.

MATERIAL AND METHODS

The samples were taken from the relatives of known cases of Duchenne type muscular dystrophy when they attended as out-patients. Previous studies have shown that the serum creatine kinase activity does not vary significantly in normal or dystrophic individuals with physical exertion, the taking of food, or with menstruation (Pearce *et al.*, 1964a) and therefore these factors do not influence a casual estimation on an out-patient.

Serum creatine kinase activity was estimated according to the method previously described (Pearce *et al.*, 1964a). The upper limit of the normal range in adults is 3·5 units in this laboratory; slightly higher values are sometimes found in infancy and early childhood.

The patients studied were either brothers or female relatives of the patients. The latter were classified as follows:—

[1]Aided by research grants from the Muscular Dystrophy Associations of America, Inc., the Muscular Dystrophy Association of Canada, and the Muscular Dystrophy Group of Great Britain.

DEFINITE CARRIERS These are (1) women who have an affected son and an affected brother or maternal uncle; (2) women with an affected son and an affected nephew (son of a sister).

PROBABLE CARRIERS These are women with two or more affected sons but with no male sibs or uncles affected.

POSSIBLE CARRIERS These are (1) women with one affected son and no other affected male relatives; (2) female sibs of an affected male.

The reason why women with only one affected son are classified as 'possible carriers' is that in a proportion of these women the disease is presumed to have arisen as a result of gene mutation in one segment of an ovary; this would be unlikely to give rise to more than one affected son which is why we regard women with two or more dystrophic boys as 'probable carriers'.

RESULTS

The results are shown in Tables I to IV. Although the known carriers (classified on a genetic basis) are uncommon, it is noteworthy that 71% exhibited a high serum activity of creatine kinase. Five out of eight (62%) probable carriers had abnormally high levels of serum creatine kinase activity, varying between 4 and 52 units. Of 35 possible carriers, 18 (50%) had an elevated serum creatine kinase activity varying from 4 to 99 units.

TABLE I

SERUM CREATINE KINASE ACTIVITY IN KNOWN CARRIERS OF THE DUCHENNE TYPE OF MUSCULAR DYSTROPHY

Case	*Age (yr.)*	*Creatine Kinase Activity (units)*
M.M.	32	3·7
J.T.	38	4·7
R.L.	37	4·1
A.M.	74	5·7
N.M.	53	5·4
K.L.	42	1·8
L.D.	55	2·3

<3·5	>3·5	*Total No. of Cases*	*Carrier Detection Rate*
2	5	7	71%

TABLE II

SERUM CREATINE KINASE ACTIVITY IN PROBABLE CARRIERS OF THE DUCHENNE TYPE OF MUSCULAR DYSTROPHY

Case	*Age (yr.)*	*Creatine Kinase Activity (units)*
V.E.	36	51·8
J.B.	33	3·2
J.C.	32	2·3
M.W.	51	17·8
B.S.	42	14·8
M.R.	28	3·2
E.P.	40	6·0
M.J.	33	6·7

<3·5	>3·5	*Total No. of Cases*	*Carrier Detection Rate*
3	5	8	62%

TABLE III

SERUM CREATINE KINASE ACTIVITY IN POSSIBLE CARRIERS OF THE DUCHENNE TYPE OF MUSCULAR DYSTROPHY

Case	*Age (yr.)*	*Creatine Kinase Activity (units)*
M.J.	33	6·7
P.J.	3	4·9
M.J.	6	5·7
B.J.	5	3·9
D.C.	33	4·4
V.C.	35	2·0
E.H.	24	3·6
E.L.	17	2·2
F.C.	36	3·4
A.D.	34	2·5
K.S.	34	33·3
S.O.	31	5·6
D.N.	32	1·9
D.P.	36	4·8
L.L.	36	1·9
J.C.	34	2·2
L.T.	40	20·5
S.C.	27	1·8
D.S.	37	2·1
D.L.	38	2·7
M.W.	30	60·0
D.B.	26	99·0
M.I.	27	26·0
K.F.	15	45·0
D.L.	32	5·3
A.P.	17	11·0
M.B.	42	3·4
K.B.	6	2·2
M.B.	10	2·2
J.B.	26	1·9
F.M.	14	31·0
E.P.	2	2·1
A.P.	47	0·9
A.S.	36	0·9
A.P.	16	11·0

<3·5	>3·5	*Total No. of Cases*	*Carrier Detection Rate*
17	18	35	50%

TABLE IV

SERUM CREATINE KINASE ACTIVITY IN MALE SIBS OF PATIENTS WITH THE DUCHENNE TYPE OF MUSCULAR DYSTROPHY

Case	*Age (yr.)*	*Creatine Kinase Activity (units)*
V.J.	3	300·0
S.J.	5	800·0
F.C.	2	3·7
R.C.	3	3·3
R.C.	6	3·4
K.B.	3	452·0
I.L.	10	4·0
A.C.	4	4·0
E.R.	9 mth.	1,060·0
B.C.	6	3·6
G.D.	11	2·8
G.S.	2	870·0
A.N.	9	4·1
J.C.	2	9·6
M.M.	5	3·5
A.R.	7	4·7
M.R.	9	4·2
R.L.	5	1·8
K.W.	4	2·8
G.G.	14 mth.	792·0
J.B.	8	2·2
A.M.	10	2·6
T.P.	10	1·9

No. of Cases Examined	*Cases of Preclinical Dystrophy*
23	6

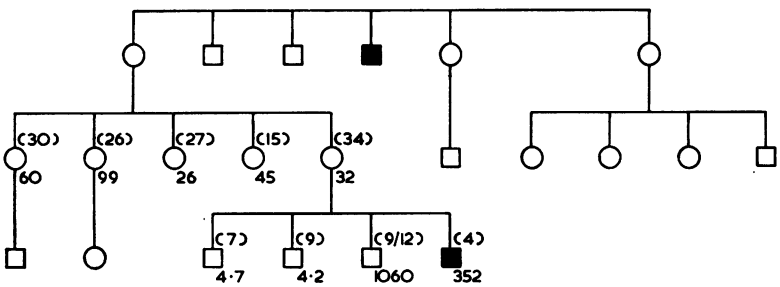

FIG. 1. *Creatine kinase activity in carriers and preclinical cases of the Duchenne type of dystrophy. The ages of the cases are set within brackets, and the figures below are the levels of serum creatine kinase activity.*

Twenty-three male sibs of boys with the Duchenne type of muscular dystrophy were studied; in this group six cases of preclinical muscular dystrophy were detected in young children who showed no detectable weakness, atrophy, or pseudohypertrophy of the muscles. Slightly higher values were found in some remaining sibs than are found in healthy adults, but this was attributed to the slight increase which normally occurs in childhood (Pearce *et al.*, 1964a). Figure 1 shows a family tree which includes female carriers and a case of preclinical muscular dystrophy, detected by means of estimations of serum creatine kinase activity.

DISCUSSION

The results obtained clearly indicate that the estimation of serum creatine kinase activity is of considerable value in the diagnosis of the carrier state in the Duchenne type of muscular dystrophy. The incidence of biochemical carriers as detected by this method is highest in those females who on genetic grounds are most likely to be carriers. The results in the three groups of carriers considered together indicate that the majority of carriers are detectable by means of estimations of the serum creatine kinase activity. The incidence of the carrier state judged by the biochemical abnormalities approximates to that expected on genetic grounds. There are a number of cases in which serum enzyme activity can be regarded as 'borderline'. We feel, however, that an activity of more than 4·0 units is strongly suggestive of the carrier state, and the patients with borderline elevations of 3·5 to 4·0 units who have had E.M.G. studies performed have shown 'myopathic' patterns (Barwick, 1963).

From the prognostic viewpoint, the presence of high serum creatine kinase activity in a female subject not suffering from any clinical disorder which could cause an elevation of creatine kinase activity indicates the likelihood that 50% of her male children will suffer from the Duchenne type of dystrophy and that half of her daughters will be carriers. These findings show that estimation of the serum creatine kinase activity is of more value in the detection of the carrier state than are serum enzymes previously studied.

Using serum lactic dehydrogenase, Brugsch and his colleagues (1960) were unable to detect the carrier state. Negative results have also been obtained using serum glutamic pyruvic transaminase and glutamic oxaloacetic transaminase (Brugsch, Brockmann-Rohne, and Fromm, 1960; Leyburn *et al.*, 1961). Dreyfus and his associates (1960) found elevated aldolase values in a small number of mothers of children with Duchenne type dystrophy, but contrary results were obtained by Evans and Baker (1957) and by Leyburn *et al.* (1961). Our own results utilizing serum creatine kinase activity correspond to those of other recent investigations with this enzyme (Dreyfus *et al.*, 1960; Hughes, 1962; Richterich, Rosin, Aebi, and Rossi, 1963), and are contrary to the findings of the Japanese workers (Okinaka *et al.*, 1961). It is concluded that serum creatine kinase estimations in the female relatives of patients suffering from Duchenne dystrophy offer a good method of detecting carriers, and are thus a sound basis for genetic counselling. If further refinements of the electromyographic method of detecting carriers (van den Bosch, 1963; Barwick, 1963) can be developed, it may eventually prove that the combined results of serum creatine kinase estimation and electromyography may be even more accurate in the identification of carriers than either alone.

In Table IV the results obtained in male sibs of affected patients are presented. Six children without clinically detectable muscle disease had grossly raised serum creatine kinase levels, and hence must be regarded as cases of preclinical muscular dystrophy. By means of estimations of serum creatine kinase in early infancy it is now clearly possible to decide whether a male child born into a family in which the Duchenne type muscular dystrophy is present will subsequently manifest the clinical stigmata of this crippling disease. On the other hand, it is also possible to give a good prognosis in the case of a clinically normal child with normal serum creatine kinase levels. Similar findings have been obtained with other

serum enzymes by Pearson (1962a), and his prognostications based on biochemical data had been proved to be correct by follow-up studies of up to four years.

The biochemical identification of preclinical muscular dystrophy sheds new light on the possible aetiology and pathogenesis of the disease. When correlated with the fact that histological changes in muscle biopsies obtained from preclinical cases have been seen by us (unpublished data) and reported independently by Pearson, Chowdhury, Fowler, Jones, and Griffith (1961), Pearson (1962a), it seems reasonable to conclude that the dystrophic process is active even in early infancy and long before clinical weakness is manifest. Indeed it seems likely that the disease process commences in foetal life, since the changes observed in early infancy appear too advanced to have developed in the brief interval since birth. Furthermore, our preliminary observations (to be published) suggest that histological and biochemical abnormalities may even be identified in the foetus.

The precise nature of the primary lesion in dystrophic muscle is still obscure. Muscle biopsy studies have revealed striking changes even in infancy, including variations in the diameter of muscle fibres, acidophilic hyalinization of fibres, interstitial fibrosis, and foci of regeneration. Such histological changes were seen in a 4-month-old child reported by Pearson (1962a) and were associated with high serum enzyme activity. Pearson concluded that before the clinical appearance of weakness, at least 50% of the muscle fibres are seriously involved in the dystrophic process. In such cases it seems probable that a defective muscle cell membrane may allow cellular enzymes to leak into the serum, and that the increased activity of these enzymes in the serum is a sequel to and not the cause of the dystrophic process. Nevertheless, their presence in increased quantity in the serum provides an invaluable aid in the early detection of this pathological process.

That the Duchenne type of muscular dystrophy is sex-linked in 90% of cases has been clearly shown in the past (Walton, 1956), and isolated cases may occur as a result of a mutation. Thus, in the majority of cases the disease is inherited as a consequence of an abnormality of the female X-chromosome. It has been shown that female carriers may rarely exhibit minor clinical stigmata of the disease (Chung, Morton, and Peters, 1960; Emery, 1963), and it is also evident that electromyographic and histological, as well as biochemical, abnormalities exist in carriers (Dreyfus and Schapira, 1962a; Barwick, 1963; Dubowitz, 1963). These findings are readily understood if it is assumed that as in other heterozygous states minimal manifestations of the disease process may be exhibited by carriers. It has also been demonstrated that occasionally the Duchenne type of muscular dystrophy can occur in females (Blyth and Pugh, 1959; Dubowitz, 1960). This occurrence is explained on the basis of occasional autosomal recessive mode of inheritance which accounts for up to 10% of cases (Dubowitz, 1960).

To explain the mottled coats of female mammals heterozygous for X-linked genes for coat colour, Lyon (1961, 1962) suggested that in a proportion of cells in the female one of the X-chromosomes is inactivated early in development. Either of the X-chromosomes in a cell might be inactivated, and all its descendant cells would then carry the same pattern. Genes with a localized action can, according to this hypothesis, produce a mosaic effect in the heterozygote. This explains the occurrence of clinical signs of muscular dystrophy in female carriers by assuming that the proportion of their cells in which the active X-chromosome carries the mutant gene is greater in number than in the majority of carriers.

The occurrence of raised creatine kinase levels in female carriers and the demonstration of histological abnormalities in their muscle biopsies (Walton, 1964) suggest that a subclinical form of dystrophy exists in the female carrier. The variability of the magnitude of the changes in serum creatine kinase activity, and the patchy changes observed in biopsy studies are explicable on the basis of the Lyon hypothesis. The female carrier's somatic tissues are a mixture of cells, some with a maternal X-chromosome which is active (the inactivated female X-chromosome forming the Barr bodies) and some with a paternal X-chromosome which is active. A female carrier, heterozygous for a sex-linked gene, will therefore exhibit variations in her tissues dependent upon the relative proportions of active normal X-chromosomes and active mutant-bearing X-chromosomes expressed. The mosaicism of the X-chromosomes thus achieved is associated with a dual population of muscle cells—one normal, one myopathic. The proportions of these two populations may govern the severity of the subclinical state. A carrier with a preponderance of normal cells may exhibit only a slight elevation of serum creatine kinase activity; a carrier with a preponderance of mutant X-chromosomal cells may exhibit a high serum creatine kinase activity, abnormal muscle biopsy findings, and even clinical evidence of muscular weakness. Recent work by Pearson, Fowler, and Wright (1963) has led to similar conclusions.

SUMMARY

Estimations of serum creatine kinase activity have been carried out in 74 close relatives of patients

suffering from the Duchenne type of muscular dystrophy. The results obtained indicate that by this method it is possible to detect the majority of 'carriers' as well as cases of preclinical muscular dystrophy of this type. The diagnostic implications of the results obtained are discussed. An attempt has been made to explain the findings in terms of the Lyon hypothesis of female X-chromosome mosaicism.

We are indebted to the patients for their cooperation in this survey. We would like to thank Miss M. Reid and Miss H. Caulfield for invaluable technical assistance, and Miss R. Allan and Miss M. Brown for their secretarial work.

REFERENCES

Aebi, U., Richterich, R., Stillhart, H., Colombo, J. P., and Rossi, E. (1961) *Helv. paediat. Acta*, **16**, 543.

Barwick, D. D. (1963). In *Research in Muscular Dystrophy*, ch. 1, p. 10. Proc. 2nd Symp. Muscular Dystrophy Group. Pitman, Medical Publishing Co., London.

Blyth, H., and Pugh, R. J. (1959). *Ann. hum. Genet.*, **23**, 127.

Brugsch, J., Brockmann-Rohne, K., and Fromm, H. (1960). *Z. ges. inn. Med.*, **15**, 891.

Chung, C. S., Morton, N. E., and Peters, H. A. (1960). *Amer. J. hum. Genet.*, **12**, 52.

Dreyfus, J. C., and Schapira, G. (1962a). *Klin. Wschr.*, **40**, 373.

——, —— (1962b). *Biochemistry of Hereditary Myopathies*, p. 74. Thomas, Springfield, Illinois.

——, ——, and Demos, J. (1960). *Rev. franç. Étud. clin. biol.*, **5**, 384.

——, ——, and Schapira, F. (1958). *Ann. N.Y. Acad. Sci.*, **75**, 235.

Dubowitz, V. (1960). *Brain*, **83**, 432.

——, (1963). *J. Neurol. Neurosurg. Psychiat.*, **26**, 322.

Emery, A. E. H. (1963). *Lancet*, **1**, 1126.

Evans, J. H., and Baker, R. W. R. (1957). *Brain*, **80**, 557.

Hughes, B. P. (1962). *Brit. med. J.*, **2**, 963.

—— (1963). In *Research in Muscular Dystrophy*, ch. 4, p. 167. Proc. 2nd Symp. Muscular Dystrophy Group. Pitman, Medical Publishing Co., London.

Leyburn, P., Thomson, W. H. S., and Walton, J. N. (1961). *Ann hum. Genet.*, **25**, 41.

Lyon, M. F. (1961). *Nature (Lond)*, **190**, 372.

—— (1962). *Amer. J. hum. Genet.*, **14**, 135.

Okinaka, S., Kumagai, H., Ebashi, S., Sugita, H., Momoi, H., Toyokura, Y., and Fujie, Y. (1961). *Arch. Neurol. (Chic.)*, **4**, 520.

Pearce, J. M. S., Pennington, R. J. T., and Walton, J. N. (1964a). *J. Neurol. Neurosurg. Psychiat.*, **27**, 1.

——, ——, —— (1964b). *Ibid.*, **27**, 96.

Pearson, C. M. (1962a). *Rev. canad. Biol.*, **21**, 533.

—— (1962b). *Brain*, **85**, 109.

——, Chowdhury, S. R., Fowler, W. M., Jones, M. H., and Griffith, W. H. (1961). *Pediatrics*, **28**, 962.

——, Fowler, W. M., and Wright, S. W. (1963). *Proc. nat. Acad. Sci. (Wash.)*, **50**, 24.

Pennington, R. J. T., Walton, J. N., and Barwick, D. D. (1964). *Biochem. Clin.*, in the press.

Richterich, R., Rosin, S., Aebi, U., and Rossi, E. (1963). *Amer. J. hum. Genet.*, **15**, 133.

Van den Bosch, J. (1963). In *Research in Muscular Dystrophy*, ch. 1, p. 23. Proc. 2nd Symp. Muscular Dystrophy Group. Pitman, Medical Publishing Co., London.

Walton, J. N. (1956). *Ann. hum. Genet.*, **21**, 40.

—— (1964). *Brit. med. J.*, in the press.