Diethylstilbestrol: Observations on Its Use in Duchenne's Muscular Dystrophy (DMD) (36562)

LOUIS COHEN, JULIET MORGAN, AND SIDNEY SCHULMAN
Department of Medicine, The University of Chicago, Chicago, Illinois 60637

Several observations suggest that the course of muscular dystrophy and other myopathies may be sex-influenced. Clinically, muscular dystrophy is less severe in women than in men (1), and the female carrier of the Duchenne gene suffers little muscle impairment though her serum enzymes may be higher than normal (2). Experimentally, dystrophic female mice grow better and live longer than do dystrophic male mice (3), and steroid-induced myopathy is less severe in the female than the male mouse (4). Finally, the synthetic estrogenic substance, diethylstilbestrol (DIES), appears to be an anabolic agent (5, 6) and has been found in skeletal muscle after administration to domestic animals (7, 8). These observations and others that will be discussed below suggested that estrogen administration might be useful in DMD. This report summarizes the effects on the serum enzymes and selected muscle parameters in three patients with DMD, treated for 6 months to 3 years with DIES.

Methods. The diagnosis of DMD was established in each patient by history, physical and neurological examinations, serum enzyme and lactate dehydrogenase (LDH) isozyme analyses, similar enzyme studies in family members, electromyography, and the histological examination and enzyme assay of several muscle biopsies. T.M., B.C. and D.S. were 13, 11 and 4 years of age, respectively, at the start of their studies. Their muscular disability was quantitated using a 0–10 grade scale (9) (0, normal; 10, totally disabled). T.M.'s disability was grade 6–7; B.C.'s was grade 6–7; and D.S.'s was grade 3.

Each therapeutic trial consisted of four periods. Period 1 was the control or pretreatment period. Period 2 was the period during which DIES was initially administered. During Period 3 DIES was discontinued; and during Period 4 DIES was readministered. The dose of DIES was 2 to 5 mg/day.

During each period, multiple venous blood samples were collected as atraumatically as possible, shortly after arising. Serum was analyzed spectrophotometrically, within 24 hr, for LDH by the method of Cohen and Larson (10) and creatine phosphokinase (CPK) by the method of Rosalki (11). Control serum was analyzed simultaneously, to insure reagent stability. The upper normal limit for LDH is 390 units/ml serum and for CPK 50 milli-International Units (mIU)/ml serum.

Muscle evaluations were performed at the start of Period 1 and end of each subsequent period by our chief physical therapist. He followed a specific protocol (12) and had no knowledge of the patient's therapy status. These evaluations included manual muscle testing, measurement of limb circumference, joint goniometry, and measurement of the time required to perform certain tasks.

Other tests performed at appropriate intervals included complete blood counts; urinalyses; X-ray examination of the chest; radiodensity of the fingers; creatinine clearance and blood urea nitrogen; electrocardiographic and vectorcardiographic examinations; electromyography; serum cholesterol and triglycerides; glucose tolerance and serum insulin levels after glucose loading; cytogenetic studies; and pulmonary function and esophageal motility studies. The last two tests were performed only in T.M., as the two younger boys were unable to cooperate adequately for these studies.

Results. The serum LDH and CPK activities for each period are depicted in Fig. 1, and the statistical analyses of these data are

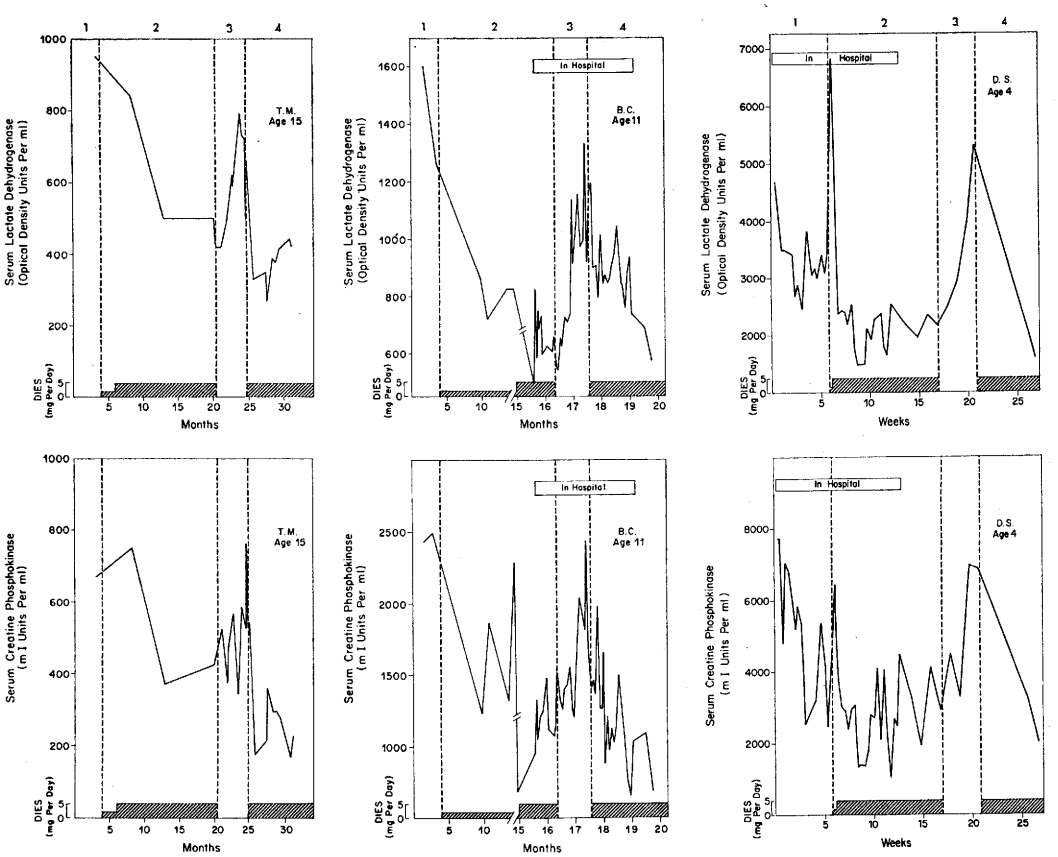


FIG. 1. Serum enzyme changes associated with the administration and withdrawal of diethylstilbestrol. The LDH activities (upper panels) and the CPK activities (lower panels) are shown for each patient studied. The periods of hospitalization for B.C. and D.S. are noted by an upper horizontal bar inset. The numbers at the top of the graph refer to the four periods of each therapeutic trial; these are further defined in the text. It should be noted that, prior to our first seeing him, T.M. had been originally diagnosed as having polymyositis, and was on prednisone, 6 mg every other day, for many years. This therapy was not altered during this study.

summarized in Table I.

In Period 1, the pretreatment serum enzyme activities in all three patients, as expected, were found to be many times higher than normal, and inversely related to age.

During Period 2, significant reduction occurred in the serum enzyme activities of each patient. These reductions persisted for as long as DIES was administered [11 weeks (D.S.); 15 months (B.C.); and 17 months (T.M.)].

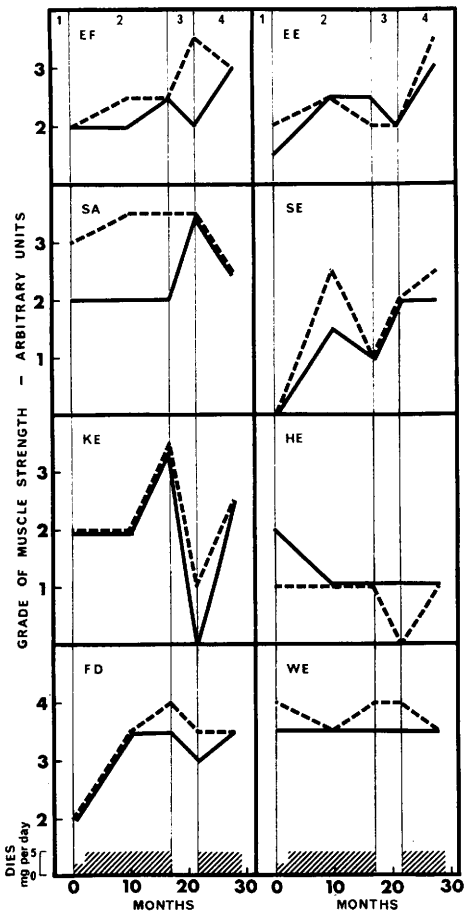
It is well known that as DMD progresses the serum enzyme activities decrease, and may be near normal at the end stage of this disease. To insure that the serum enzyme reductions associated with DIES administration were not merely those which would have occurred in the natural course of this disease, DIES was withdrawn during period 3. Within 3 to 12 weeks the serum enzyme activities rose to pretreatment levels, indicating that lowering the serum enzyme activities was related to the administered DIES and not the natural course of the disease. This was further supported by the observation that, within several weeks of restarting DIES (Period 4), the serum enzyme activities again fell to levels noted during Period 2. It should be noted that the *mean* serum enzyme values may not always reflect the changes which

TABLE I. Statistical Summary of Serum Enzymes Activities On and Off Diethylstilbestrol.[a]

Patient:	T.M.				B.C.				D.S.			
Period[b]:	1	2	3	4	1	2	3	4	1	2	3	4
					Lactate dehydrogenase (optical density units/ml)							
Mean	950	538	610	368	1430	685	870	886	3861	2414	3658	2086
SD		173	120	52	240	78	222	118	1389	1164	1249	381
(n)	(1)	(5)	(10)	(8)	(2)	(14)	(16)	(22)	(18)	(23)	(4)	(3)
p			$<<0.01$		<0.01		<0.9		$<<0.01$		<0.05	
					Creatine phosphokinase (mIU/ml)							
Mean	671	516	521	229	2460	1310	1586	1118	5193	2925	5386	3028
SD		205	25	82	42	233	272	381	1614	1213	1837	940
(n)	(1)	(3)	(12)	(8)	(2)	(14)	(14)	(22)	(18)	(25)	(4)	(3)
p			<0.01		$<<0.01$		<0.02		$<<0.01$		<0.05	

[a] SD, standard deviation; (n), number of enzyme assays; p, probability that difference was due to chance.

[b] Period 1. Control period before giving DIES; (2) Initial period of DIES administration; (3) DIES discontinued; (4) DIES restarted.



occur when DIES is administered or withdrawn. For example, if the manner of rise in Period 3 were inversely comparable to the rate of fall in Period 4, and the periods were of about equal duration, the mean values would be similar. This is precisely what occurred with the serum LDH in Periods 3 and

FIG. 2. Results of manual muscle testing. These graphs summarize the results of manual muscle testing in patient, T.M. The grading of muscle strength (vertical axis) is as follows: 5, normal muscle strength; 4, full range of motion against gravity with some resistance; 3, full range of motion against gravity without added resistance; 2, contraction able to move joint in absence of gravity; 1, perceptible contraction; and 0, absence of perceptible contraction. The four periods of the therapeutic trial are divided by vertical lines and numbered at the top of the graph. The muscles tested include those involved in elbow flexion (EF) and extension (EE); shoulder abduction (SA) and extension (SE); knee extension (KE); hip extension (HE); foot dorsiflexion (FD); and wrist extension (SE); the continuous line represents results in the right limb and the interrupted line results in the left limb. If the results are averaged, there is overall, a minimal improvement in muscle strength. In other words there was no deterioration in muscle function demonstrable by this means of testing over the 3-year period of study. Similar conclusions could be drawn from identical studies on patients B.C. and D.S.

4 in patient B.C., accounting for the absence of difference in mean values, despite a 250% rise in the serum LDH enzyme activity in Period 3, and a similar decline in Period 4.

Measurements of limb circumferences, the time required to perform certain tasks, goniometric measurements, and muscle testing showed little change during treatment; that is, no evidence of muscle deterioration was found by these parameters during DIES therapy. However, when DIES treatment was withdrawn (Period 3), some evidence of muscle deterioration was noted. The results of manual muscle testing in T.M. during each of the four periods are shown in Fig. 2.

During DIES treatment, it was also found that bone density increased in all three patients; and glucose tolerance and insulin levels after glucose loading improved in T.M. The other tests remained essentially unchanged.

Discussion. We have demonstrated that the administration of DIES to three boys with DMD resulted in significant lowering of the elevated serum LDH and CPK found in this disease. The fall in the serum enzyme activity required several weeks to reach its nadir. When the medication was stopped, the serum enzyme activities rose to pretreatment levels within 3 to 12 weeks, only to fall again when the medicine was restarted.

Since it is generally accepted that elevation of serum enzymes in muscular dystrophy is a consequence of degeneration of muscle, it seems reasonable to assume that this effect of DIES on serum enzyme levels in our patients was a reflection of a retarded rate of muscular degeneration. We are aware, however, that such an assumption may be incorrect, and that some secondary, more remote mechanism may underlie the effect of DIES on serum enzymes in muscular dystrophy. We also recognize that the lack of progression in weakness during therapy, and the evidence of increased weakness following cessation of treatment, may be accounted for by suggestion and motivational factors, and must therefore be interpreted with great caution.

Some of the reasons that DIES was selected for this therapeutic trial in DMD were mentioned earlier. In addition, it has had a long comparatively complication-free clinical use. [Although relatively safe, it should be noted that it has recently been implicated in the development of some cases of vaginal cancer (13, 14) in young women and may augment atherosclerotic complications in men over 60 years of age with prostatic cancer (15)]. However, the specific reasons for choosing DIES were related to the unusual LDH isozyme distributions found in the serum (16) and muscles (17) of children with DMD and our own findings, that LDH isozyme distributions could be manipulated by several stilbene derivatives including DIES.

The serum LDH abnormality found in DMD is an elevated LDH with an essentially normal LDH isozyme distribution; the serum is not enriched with the LDH isozymes (LDH 4 and 5) typically seen following, for example, muscle trauma. The LDH isozyme distribution in dystrophic muscle is also unusual and has been attributed to a lack of muscle differentiation in this disease, since it resembles that found in embryonic muscle (18). Other explanations (19) for the unusual serum and muscle LDH isozymes in this disease have been offered, but all are speculative.

Our own studies (20–23) have also offered a possible explanation for the unusual LDH isozymes in DMD. It was found that the distribution of LDH isozymes in normal human serum, red cells and skeletal muscle was sex-related. This led us to postulate (a) that certain estrogenic substances might normally be acting as intracellular organizers and stabilizers of LDH isozymes; and (b) their absence might result in the unusual LDH isozyme patterns seen in DMD. The first possibility was tested *in vitro* and indeed several synthetic estrogenic compounds were found which were effective as *in vitro* organizers and stabilizers of LDH isozymes. Of these, DIES and its chief metabolic product isodienstrol were the most effective (21). This suggested that the administration of DIES or isodienstrol to patients with DMD might be useful, and led to the study reported here. Despite this rationale for the use of

DIES in the treatment for DMD, the precise mechanism by which the salutary effects reported here were produced remains unknown.

Many therapies have been tried in this disease (24–26). None has stood the test of time. To our knowledge, with one exception, estrogenic therapy has never been tried in the treatment of *human* muscular dystrophy. In 1970, Yoshimatsu (27) reported that the administration of estradiol for 3 months to 3 patients with DMD had no effect on the serum CPK. However, review of his data indicated that a decrease in serum CPK comparable to that reported here occurred in one of these patients, after 11 weeks of therapy; 2 weeks after estradiol treatment was stopped, the serum enzymes in this patient returned to near pretreatment levels.

No toxic effect of DIES was noted in our patients. Breast enlargement occurred in all but was of some cosmetic concern only to T.M., the oldest boy, who was postpubertal when the treatment was started.

Before these children were treated, four adults with severe myotonic dystrophy were given DIES for several years. No change in the serum enzymes, which are normal in this variety of dystrophy, were noted, but the effects of muscle strength were sufficiently encouraging (23) that the trial with the children was undertaken. The dramatic responsiveness of the serum enzymes in the children was not anticipated. Rather, it was expected that the changes, if any, would be slow and gradual and best measured by assessing muscle strength. This explains the limited number of enzyme studies performed in the early phases of the studies of T.M. and B.C. Once these effects became apparent, more frequent sampling was performed.

This, then, is the first report which suggests that estrogenic therapy may be of at least palliative value in the treatment of DMD. The effects on the serum enzymes and muscle strength reported here may also have pertinence to the occurrence of normal serum enzymes in one-third of female carriers of this disease, and the relative protection from myopathy the female appears to enjoy. Further study of this therapeutic approach in DMD and other myopathies appears warranted.

Summary. Three boys with Duchenne's muscular dystrophy were treated with diethylstilbestrol for 0.5 to 3 years. The administration of this medication resulted in each instance in a considerable reduction in the serum enzymes, lactate dehydrogenase and creatine phosphokinase, which are characteristically elevated in this disease. The reduction was reversible when DIES was discontinued and reproducible when it was restarted. Tests of muscle function indicated that during administration of DIES there was no deterioration in muscle strength. These findings suggest that diethylstilbestrol may have a beneficial effect on Duchenne's muscular dystrophy, but a long-term, controlled trial will be required to establish this.

We are indebted to Dr. Irwin Siegel of the University of Illinois and Drs. Robert Cutler, Nicholas Lenn, Nicholas Vick, Jack Stevens, Scott Kleiman, and Mr. Robert Babbs for their respective and many contributions. The serum insulin assays were performed by Dr. Ann Lawrence and Mrs. Lydia Kirsten and Mrs. Gladys Rider helped enormously with the manuscript. Dr. John Morgan made possible the computerized statistical analyses reported here. This work was supported by the Muscular Dystrophy Association of America, the Chicago and Illinois Heart Association, the Fay Hunter Research Fund, and Grants RR 55 and 305 from the General Clinical Research Center's Program of the Division of Research Resources, National Institutes of Health. A special word of thanks is due to the families of these children.

1. Dreyfus, J-C., Schapira, G., and Schapira, F., Ann. N.Y. Acad. Sci. **75**, 235 (1958).

2. Emery, A. E. H., and Lee, C. S. N., Lancet **2**, 1966 (1964).

3. O'Steen, W. K., Proc. Soc. Exp. Biol. Med. **126**, 579 (1967).

4. Faludi, G., Gotlieb, J., and Meyers, J., Ann. N.Y. Acad. Sci. **138**, 61 (1966).

5. Kliewer, R. H., Kennick, W. H., and Church, D. C., J. Dairy Sci. **53**, 1766 (1970).

6. Preston, R. L., Proc. Soc. Exp. Biol. Med. **129**, 250 (1958).

7. Stob, M., Perry, T. W., Andrews, F. N., and Besson, W. M., J. Animal Sci. **15**, 977 (1956).

8. Wiberg, G. S., and Stephenson, N. R., Can. J. Biochem. Physiol. **35**, 1107 (1957).

9. Gardner-Medwin, D., and Walton, J. N., *in* "Disorders of Voluntary Muscle" (J. N. Walton,

ed.), Chap. 13. Little, Brown, Boston (1969).

10. Cohen, L., and Larson, L., N. Engl. J. Med. **275**, 465 (1966).

11. Rosalki, S. B., J. Lab. Clin. Med. **69**, 696 (1969).

12. Miller, J., Tex. Rep. Biol. Med. **22** (Suppl. No. 1), 871 (1964).

13. Greenwald, P., Barlow, J. J., Nasca, P. C., and Burnett, W. S., N. Engl. J. Med. **285**, 390 (1971).

14. Herbst, A. L., Ulfelder, H., and Poskanzer, D. C., N. Engl. J. Med. **284**, 878 (1971).

15. Veterans Administration Cooperative Urological Research Group, J. Urol. **98**, 516 (1967).

16. Thomson, W. H. S., Leyburn, P., and Walton, J. N., Brit. J. Med. **2**, 1276 (1960).

17. Wieme, R. J., and Lauryssens, M. J., Lancet **1**, 433 (1962).

18. Dreyfus, J-C., Demos, J., Schapira, F., and Schapira, G., C. R. Acad. Sci. **254**, 4384 (1962).

19. Pearson, C. M., and Kar, N. C., Ann. N.Y. Acad. Sci. **138**, 293 (1966).

20. Cohen, L., Block, J., and Djordjevich, J., J. Lab. Clin. Med. **68**, 865 (1966).

21. Cohen, L., Djordjevich, J., and Seckler, J., J. Lab. Clin. Med. **73**, 835 (1969).

22. Cohen, L., Wolfe, M., and Djordjevich, J., Clin. Res. **18**, 51 (1970).

23. Cohen, L., Lawrence, A., Schulman, S., Ugarte, F., and Jelinek, C., J. Lab. Clin. Med. **76**, 1008 (1970).

24. Milhorat, A. T., Med. Ann. D. C. **23**, 15 (1954).

25. Dowben, R. M., Tex. Rep. Biol. Med. **22** (Suppl. No. 1), 849 (1964).

26. Walton, J. N., and Gardner-Medwin, D., *in* "Disorders in Voluntary Muscle" (J. N. Walton, ed.), Chap. 14. Little, Brown, Boston (1969).

27. Yoshimatsu, J., Shikoku Acta Med. **26**, 182 (1970).

Received Mar. 6, 1972. P.S.E.B.M., 1972, Vol. 140.