A trial of therapy
by nucleosides and nucleotides
in muscular dystrophy

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The obscure nature of the disease process in progressive muscular dystrophy has restricted useful
treatment to supportive measures, such as attention
to general health, massage, cautious systematic
exercise, and orthopaedic assistance by protheses
or even corrective surgery; and these have been
the subject of many informative articles (Swinyard,
Deaver, and Greenspan, 1958; Vignos and

Though temporarily beneficial, none of these
procedures delays the progress of the disease, and
attempts at drug therapy have received increasing
attention. Many substances have been variously
administered, such as glycine (Armstrong and
Herbert, 1935), leucine (Edelschtein, 1957), multiple
vitamins and amino-acids (Wald and Lam, 1955),
vitamin E (Beneske, Butson, Gauld, and Levy,
1960), isoniazid (Kuberski and Sulat 1957), and
the anabolic steroids diandrobol and durabolin
(Bekeny, Kraft, and Lang, 1960; Brown and James,
1961; Barwick, Walton, and Newell, 1962) but none
has been found effective. Recently, however, it has
been reported (Beckmann, 1959; Coirault, Lévy,
Michel-Ber, Masbernard, Desclos de la Fonchais,
and Mazingant, 1960a, 1960b; Coirault, Herschberg,
Damario, Rigal, Alliot, and Bissière, 1961) that
administration of nucleotides may benefit muscular
dystrophy. Besides adenosine triphosphate (ATP)
(Krebs and Kornberg, 1957) other nucleotides may
be involved in energy transfer (Pinchart, 1957;
Burdette, 1957) and are present in healthy muscle
(Schmitz, 1961; Burdette, 1957; Jones and Murray,
1960) but in diminished amounts in diseased muscle
(Bot, Kövér and Varga, 1955; Ruskin and Wiley
1956; Pennington, 1962), and in dystrophic muscle
they may show an increased turnover rate (Zymaris,
Saifer, and Volk, 1961). Though ATP is removed
by muscle from the circulation (Hockerts and
Lamprecht, 1959; Mascitelli-Coriandoli and
Boldrini, 1957; Lamprecht, 1962), alone it seems
of little benefit in muscular dystrophy (Stelgens, 1957;
Goldsztajn, 1962). Demonstration of any effect must
depend on careful and systematic investigation
(Travell, 1955), including first, decisive evidence in
each case of the clinical type of muscular dystrophy
and its stage of evolution; second, numerical
measures for the dystrophic process free from
subjective variation by patient or investigator; and
third, careful interpretation of changes in these
measures after treatment.

In this investigation these conditions have been
observed and certain additional findings have been
recorded. By these means satisfactory conclusions
have been reached.

MATERIALS

THERAPEUTIC Simultaneous parenteral administration of
several nucleotides and precursors was achieved by
intravenous infusions in Ringer's solution of a nucleotide-
nucleoside preparation (Laedadosin) in sterile pyrogen-
free aqueous solution, each 10 ml ampoule of which
contained adenosine 50 mg., adenosine monophosphate
(AMP) 10 mg., adenosine diphosphate (ADP) 10 mg.,
ATP 30 mg., inosine 50 mg., guanosine 10 mg., guano-
sine monophosphate (GMP) 15 mg., and uridine 10 mg.
Buccal tablets for sublingual administration each con-
tained 1 mg. each of AMP, ATP, cytidine monophosphate
(CMP), GMP, and uridine monophosphate (UMP),
guanosine 5 mg., and adenosine 10 mg.

CLINICAL The subjects selected were 11 patients with
Duchenne-type muscular dystrophy at representative
stages of evolution, and one patient with the limb-girdle
form. All were treated in the Orthopaedic Unit of
Mearnskirk Hospital.

The diagnostic criteria fulfilled in every case were the
clinical appearances and history required by the classi-
fication of Walton and Nattrass (1954), histological
confirmation from muscle biopsy specimens, and bio-
chemical support by serum enzymology (Thomson,
1962). Clinical grading was by the eight-point system of Swinyard, Deaver, and Greenspan (1957).

METHODS

THERAPEUTIC On two successive occasions, with an interval of about 30 days between, each patient was kept in bed for five days while an intravenous infusion of Ringer's solution was maintained at a steady rate of 1 pint in 24 hours, venous patency being ensured by continuance, when necessary, with plain isotonic saline. Each successive pint contained an increased quantity of Laevadon; on the first occasion 5, 10, 15, 20, and 25 ml. (75 ml. in all), and on the second 10, 20, 30, 40, and 50 ml. (150 ml. in all). Too rapid or sudden administration had led to occasional febrile reactions, and sometimes to local venous thrombosis.

Immediately after each five-day treatment the patient was allowed up to go about as he pleased, and treatment was continued by daily intramuscular injections of 5 ml. Laevadon, given slowly.

After the conclusion of treatment and discharge from hospital, the patient was maintained by administration of three sublingual tablets thrice daily three times a week.

PROGRESS EVALUATION Electromyography and muscle biopsy histology were discounted because of their qualitative nature, and because repeated biopsies would be impossible. Satisfactory numerical measures were obtained by two independent methods.

Muscle strength measurement This was carried out in every patient by summation of the powers of defined muscle groups in each pair of limbs measured by two methods.

The first method was by grading according to M.R.C. recommendations (1943) from 0 to 5 for each muscle group, in which an additional intermediate point at 4.5 was taken. The muscle groups tested were: Upper limbs: shoulder abductors, elbows flexors and extensors, wrist flexors and extensors (maximum sum for both upper limbs 50), and lower limbs: hip flexors, extensors, and abductors, knee flexors and extensors, ankle dorsiflexors and planter flexors (maximum sum for both lower limbs 70).

The second method was by measuring maximal sustained effort against uniformly calibrated spring scales (Avery) exerted through a small sling and cord passing over a smooth pulley to the scale. Where muscles were very weak a weaker spring was used, but the same spring scale was used throughout, applied by the same methods, on the same pair of limbs in the same individual; the results are expressed as the percentage improvement of the sum of the readings from each pair of limbs. During testing gravity was eliminated where necessary, e.g., the right hip extensors were tested with the patient on his left side, his right leg supported in a broad, freely-swinging sling, and exertion made, with the knee fully extended, against the small sling and cord passing behind the ankle. The muscle groups tested were: Upper limbs: shoulder abductors and elbow flexors and extensors; lower limbs: hip flexors, extensors, abductors and adductors, and knee flexors and extensors.

Measurement by both methods was carried out before commencement of the first and after completion of the second treatments, since any improvement usually seemed greater after the second treatment, and since undue muscular effort on testing might have affected the validity of the serum enzyme assays.

There is inherent in each of these measures some possibility of subjective liability, though this is unlikely on the part of experienced observers.

Biochemical measurement Serial 24-hour urinary excretions of creatine and creatinine and their ratios in successive urine specimens were quite irregular, and thus failed to correlate with clinical changes or serum enzyme alterations, probably owing to difficulty in urine collection and the impossibility of continued imposition of the restricted diet advisable (Leyburn, Thomson, and Walton, 1961) and these assays were therefore abandoned.

A prolonged series of successive assays of the serum enzymes aldolase, GOT, and GPT was carried out in six patients with Duchenne-type dystrophy and in one with the limb-girdle form, as indicated in the Figures, using throughout the methods described earlier (Thomson, 1962) and the same units and normal ranges, i.e., serum aldolase 2.3 to 8.8 Bruns units/ml. (mean 5.7 units), SGOT 12 to 36 Sigma-Frankel units/ml. (mean 19 units), and SGPT 4 to 24 Sigma-Frankel units/ml. (mean 15 units).

Venepuncture was performed on the first, third, and fifth mornings of each five-day period of obligatory bed rest, on the morning of the next day just before getting up, on each successive morning of the next few days after ambulation, and thereafter on Mondays, Wednesdays, and Fridays, but always at the same time in the mornings and always, without exception, while the patient was still in bed before getting up. Only on isolated occasions during the whole series did venepuncture fail to secure a suitable specimen.

Further, in order that each patient should be his own control, an initial five-day bed rest, comparably restricted, followed by about a month of ambulation was arranged before starting any treatment at all, and serum enzyme studies were performed throughout exactly as if he had been receiving treatment. During this time the patient received no infusions of plain Ringer's solution, since every vein was needed, and since such slow infusions rapidly excreted, could have only the most minimal and transient effect, if any, on the serum enzymes.

The numerical measures thus obtained were quite objective; by making each patient his own control many variations due to extraneous causes, known and unknown, were eliminated, and subsequent statistical analyses were securely founded.

At no time during the entire investigation was physician therapeutic intervention of any kind permitted in any patient.

Other observations These included careful clinical assessment before and after therapy, with regrading of the eight-point system. In addition, the effect of treatments was noted on the intelligence quotient on the revised Stanford-Binet intelligence scale, on certain liver function tests, and on the marked halitosis and dislike for sweets, often found in patients with Duchenne-type dystrophy.
In each Figure the activity of serum aldolase is denoted by an uninterrupted line, of SGOT by long strokes, and of SGPT by short strokes. Period 1 represents preliminary bed rest without treatment, and periods 2 and 3 bed rest during therapeutic infusions; period A represents untreated ambulation after period 1, and periods B and C treated ambulation after rest during the successive therapeutic infusions.

**PATIENT 1** (Duchenne, age 4 years, Figure 1) Thought normal by parents until visited clinic with older brother (patient 2), when seen to have pseudo-hypertrophy of calves, slight lumbar lordosis, Gower's sign and inability to attain sitting from supine without hands, and slight difficulty in ascending stairs. After treatment Gower's sign absent, ascends stairs easily and runs about more; I.Q. and muscle strength could not be tested; was always alert. Grade 1 before and after treatment.

**PATIENT 2** (Duchenne, age 7 years 1 ½ months, Figure 2) Marked pseudo-hypertrophy calves with taut Achilles...
tendons, marked lordosis, waddling gait, Gowers' sign, ascends stairs using banisters; I.Q. 66, apathetic. After treatment waddles less and can run, physically stronger, and ascends stairs more easily; I.Q. 66, more alert and responsive. Grade 2 before and 1 after.

Mean S.D. Difference of Means Significance
Aldolase
Period A 126.72 27.69 A v B t = +2.51 +
Period B 101.94 28.13 A v C t = +4.61 +
Period C 87.35 19.22 B v C t = +1.77 N.S.
SGOT
Period A 180.27 41.44 A v B t = +2.15 +
Period B 146.41 42.78 A v C t = +4.33 +
Period C 126.47 25.98 B v C t = +1.51 N.S.
SGPT
Period A 147.20 38.98 A v B t = +2.59 +
Period B 116.94 24.35 A v C t = +5.60 +
Period C 85.82 17.99 B v C t = +4.24 +

Muscle Strength Testing
M.R.C. Scale
Arms 43 49† +32.4
Legs 56† 64‡ +57.5

Aldolase Correlation Coefficients

\[ v \text{SGOT} \]
Period A 0.89 (+ +) 0.61 (+)
Period B 0.95 (+ +) 0.67 (+ +)
Period C 0.81 (+ +) 0.54 (+)

\[ v \text{SGPT} \]
Period A 0.89 (+ +) 0.61 (+)
Period B 0.95 (+ +) 0.67 (+ +)
Period C 0.81 (+ +) 0.54 (+)

FIG. 2. Duchenne-type dystrophy, age 7 years 1½ months; ambulant and active.

PATIENT 3 (Duchenne, age 14 years 4½ months, Figure 3), Moderate pseudohypertrophy calves, moderate lordosis, Gowers' sign, rolling gait with hips braced by hands in pockets pressed on thighs (right hip much the weaker), frequent falls, can barely rise from chair unaided and needs help to ascend stairs; I.Q. 108, quiet reflective boy. After treatment physically stronger, gait improved with no need to brace hips, rarely falls and can stand on one leg; I.Q. 111, more alert. Grade 3 before and 2 after. Maintained by sublingual tablets; physical deterioration on withdrawal for a month restored by resumption of tablets, and improvement persisted though put on weight and right gluteals weakened somewhat.

Mean S.D. Difference of Means Significance
Aldolase
Period A 69.16 32.78 A v B t = +3.87 ++
Period B 32.70 10.04 A v C t = +2.08 +
Period C 46.51 25.47 B v C t = -2.12 +
SGOT
Period A 97.23 33.09 A v B t = +4.00 ++
Period B 57.56 15.11 A v C t = +2.15 +
Period C 71.78 31.80 B v C t = +1.69 N.S.
SGPT
Period A 83.46 23.47 A v B t = +6.38 ++
Period B 38.19 11.24 A v C t = +5.98 ++
Period C 40.00 13.69 B v C t = -0.42 N.S.

Muscle Strength Testing
M.R.C. Scale
Arms 45 46‡ +7.3
Legs 53 60 +30.1

% Increase in Strength
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PATIENT 3

Aldolase Correlation Coefficients

<table>
<thead>
<tr>
<th></th>
<th>v SGOT</th>
<th>v SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period A</td>
<td>0.96 (+ +)</td>
<td>0.66 (+)</td>
</tr>
<tr>
<td>Period B</td>
<td>0.95 (+ +)</td>
<td>0.54 (+)</td>
</tr>
<tr>
<td>Period C</td>
<td>0.96 (+ +)</td>
<td>0.90 (+ +)</td>
</tr>
</tbody>
</table>

FIG. 3. Duchenne-type dystrophy, age 14 years 4½ months; ambulant (patient 3).

PATIENT 4 (Duchenne, age 13 years 1 month, Figure 4)

Exceedingly lethargic and impassive temperament; pseudohypertrophy calves and slight bilateral talipes equinus, marked lordosis, rolling gait, generally weak and unable to rise from floor and only with great difficulty from chair; I.Q. 68, apathetic. After treatment arms and legs both stronger, yet gait unaltered due to natural hebetude, and remained idle and slow-moving throughout. I.Q. 66, more alert. Grade 4 before and 3 after.

FIG. 4. Duchenne-type dystrophy, age 13 years 1 month; ambulant but very lethargic.
PATIENT 4

<table>
<thead>
<tr>
<th>Muscle Strength Testing</th>
<th>M.R.C. Scale</th>
<th>Spring Scale Testing</th>
<th>% Increase in Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Arms</td>
<td>40</td>
<td>43</td>
<td>+36.8</td>
</tr>
<tr>
<td>Legs</td>
<td>49</td>
<td>52</td>
<td>+51.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aldolase Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>v SGOT</td>
</tr>
<tr>
<td>v SGPT</td>
</tr>
<tr>
<td>Period A</td>
</tr>
<tr>
<td>0.48 (N.S.)</td>
</tr>
<tr>
<td>0.81 (+)</td>
</tr>
<tr>
<td>Period B</td>
</tr>
<tr>
<td>0.88 (+)</td>
</tr>
<tr>
<td>0.37 (N.S.)</td>
</tr>
<tr>
<td>Period C</td>
</tr>
<tr>
<td>0.63 (+)</td>
</tr>
<tr>
<td>0.60 (+)</td>
</tr>
</tbody>
</table>

PATIENT 5 (Duchenne, age 9 years 5 months, Figure 5)
Referred to clinic for wheel-chair; atrophic stage, gross lordosis, feeble, high-stepping gait and early bilateral talipes equinus, very weak and unable to rise from floor or chair; I.Q. 68, dull and inattentive. After treatment slight increase in strength; I.Q. 87, more alert but still inattentive (hence muscle testing very difficult). Within three months both glutei failed, though quadriceps still stronger, and entered wheel-chair. Grade 4 before and after, later 5.

FIG. 5. Duchenne-type dystrophy, age 9 years 5 months; ambulant with difficulty.
PATIENT 6 (Duchenne, age 11 years 2½ months, Figure 6) Confined to wheelchair last nine months; slight pseudo-hypertrophy calves, flexion contractures hips and knees, and bilateral talipes equinus, stumps in chair, full use of hands but cannot abduct left arm against gravity; I.Q. 58, very apathetic. After treatment arms especially stronger, upright posture in chair; I.Q. 63, more alert. Grade 6 before and 5 after.

### Table: Serum Enzyme Activities

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference of Means</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aldolase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period A</td>
<td>56.39</td>
<td>9.77</td>
<td>A v B t = +1.50</td>
<td>N.S.</td>
</tr>
<tr>
<td>Period B</td>
<td>49.40</td>
<td>14.53</td>
<td>A v C t = +0.83</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

| **SGOT** |      |      |                     |             |
| Period A | 56.00 | 9.74 | A v B t = +1.93 | N.S.       |
| Period B | 56.00 | 10.10 | A v C t = +0.83 | N.S.       |
| Period C | 58.38 | 12.03 | B v C t = -0.45 | N.S.       |

| **SGPT** |      |      |                     |             |
| Period A | 52.71 | 7.48 | A v B t = +3.26 | +           |
| Period B | 42.57 | 4.90 | A v C t = +1.42 | N.S.       |
| Period C | 46.50 | 5.37 | B v C t = -1.71 | N.S.       |

### Table: Muscle Strength Testing

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>% Increase in Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M.R.C. Scale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arms</td>
<td>37½</td>
<td>40½</td>
<td>+40.0</td>
</tr>
<tr>
<td>Legs</td>
<td>39</td>
<td>43</td>
<td>+16.1</td>
</tr>
</tbody>
</table>

### Table: Aldolase Correlation Coefficients

<table>
<thead>
<tr>
<th></th>
<th>v SGOT</th>
<th>v SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period A</td>
<td>0.94 (+ +)</td>
<td>0.44 (N.S.)</td>
</tr>
<tr>
<td>Period B</td>
<td>0.67 (+ +)</td>
<td>0.16 (N.S.)</td>
</tr>
<tr>
<td>Period C</td>
<td>0.49 (N.S.)</td>
<td>0.33 (N.S.)</td>
</tr>
</tbody>
</table>

PATIENT 7 (limb-girdle, woman aged 29 years 2½ months, Figure 7) Increasing weakness lower limbs for eight years and of upper limbs for one year; ambulant but frequent falls due to right knee giving way, prefers support from nearby furniture; sister has similar condition. After treatment stronger in all four limbs, walked more confidently and seldom fell.
PATIENT 8 (Duchenne, age 9 years 3 months) Marked pseudo-hypertrophy calves with slight bilateral talipes equinus, marked lordosis, Gowers' sign, unsteady rolling gait and cannot run, generally weak and rises from chair with difficulty, can barely ascend stairs; I.Q. 38; mentally subnormal. After treatment physically stronger, less marked lordosis, and after plaster correction of equinus can run quite well; I.Q. 41, more alert, has learned to count. Grade 3 before and 2 after. Improvement maintained for 15 months by sublingual tablets.

\[
\begin{array}{|c|c|c|}
\hline
 & \text{SGOT} & \text{SGPT} \\
\hline
\text{Immediately before therapy} & 107 & 167 \\
\text{Ambulant during therapy} & 59 & 93 \\
\text{Fifteen months after therapy} & 54 & 18 \\
\hline
\end{array}
\]

**Muscle Strength Testing**

\[
\begin{array}{|c|c|c|}
\hline
 & \text{M.R.C. Scale} & \text{Spring Scale Testing} \\
\hline
\text{Before} & \text{After} & \% \text{Increase in Strength} \\
\hline
\text{Arms} & 39 & 40 & 4+4.8 \\
\text{Legs} & 47j & 58 & 9+29.6 \\
\hline
\end{array}
\]

PATIENT 9 (Duchenne, age 10 years 1½ months) Slight pseudo-hypertrophy calves with taut Achilles tendons, marked lordosis, Gowers' sign, waddling gait, some general weakness and difficulty ascending stairs; I.Q. 98, alert. After treatment physically stronger, less marked lordosis, ascends stairs more easily, I.Q. 101, alert. Grade 2 before and 1 after. Nineteen months later original improvement maintained.

\[
\begin{array}{|c|c|c|}
\hline
 & \text{SGOT} & \text{SGPT} \\
\hline
\text{Immediately before therapy} & 63 & 42 \\
\text{Ambulant during therapy} & 24 & 13 \\
\text{Nine months after therapy} & 16 & 17 \\
\hline
\end{array}
\]

**Muscle Strength Testing**

\[
\begin{array}{|c|c|c|}
\hline
 & \text{M.R.C. Scale} & \text{Spring Scale Testing} \\
\hline
\text{Before} & \text{After} & \% \text{Increase in Strength} \\
\hline
\text{Arms} & 42 & 45 & 4+17.7 \\
\text{Legs} & 62 & 65j & 2+25.7 \\
\hline
\end{array}
\]

PATIENT 10 (Duchenne, age 10 years 4½ months) Pseudo-hypertrophy calves, marked lordosis, Gowers' sign, waddling gait with frequent falls, some general weakness and ascends stairs bracing hips with hands on thighs; I.Q. 87, dull. After treatment physically stronger with improved gait and less frequent falls, ascends stairs more easily, can ride bicycle up gentle incline impossible before I.Q. 97, more alert. Grade 2 before and 1 after. Twenty-two months later original improvement fully maintained.

\[
\begin{array}{|c|c|c|}
\hline
 & \text{SGOT} & \text{SGPT} \\
\hline
\text{Immediately before therapy} & 370 & 317 \\
\text{Ambulant during therapy} & 62 & 134 \\
\text{Eleven months after therapy} & 50 & 40 \\
\hline
\end{array}
\]

**Muscle Strength Testing**

\[
\begin{array}{|c|c|c|}
\hline
 & \text{M.R.C. Scale} & \text{Spring Scale Testing} \\
\hline
\text{Before} & \text{After} & \% \text{Increase in Strength} \\
\hline
\text{Arms} & 45j & 47 & 6+3.6 \\
\text{Legs} & 60 & 63j & 6+16.2 \\
\hline
\end{array}
\]

PATIENT 11 (Duchenne, age 15 years 9½ months) Confined to wheel-chair last two years; some pseudohypertrophy calves with slight flexion contractures knees but no talipes equinus, general weakness, slumps in chair; I.Q. 52, apathetic. After treatment arms especially, erect posture in chair; I.Q. 63, more alert. Grade 7 before and 5 after. Sixteen months later original improvement fully maintained.

\[
\begin{array}{|c|c|c|}
\hline
 & \text{SGOT} & \text{SGPT} \\
\hline
\text{Immediately before therapy} & 62 & 77 \\
\text{During therapy} & 31 & 50 \\
\text{Two months after therapy} & 24 & 45 \\
\hline
\end{array}
\]

**Muscle Strength Testing**

\[
\begin{array}{|c|c|c|}
\hline
 & \text{M.R.C. Scale} & \text{Spring Scale Testing} \\
\hline
\text{Before} & \text{After} & \% \text{Increase in Strength} \\
\hline
\text{Arms} & 28½ & 36 & 1+38.1 \\
\text{Legs} & 32½ & 37 & 1+1.1 \\
\hline
\end{array}
\]

PATIENT 12 (Duchenne, age 15 years 7½ months) Confined to wheel-chair last four and a half years; general weakness and wasting, flexion contractures both knees and bilateral talipes equinus, slumps badly in chair; I.Q. 123, apathetic and depressed. After treatment arms, erect posture in chair; I.Q. not repeated, alert and more cheerful. Grade 7 before and 5 to 6 after. Twenty-two months later original improvement fully maintained.

\[
\begin{array}{|c|c|c|}
\hline
 & \text{SGOT} & \text{SGPT} \\
\hline
\text{Immediately before therapy} & 138 & 93 \\
\text{During therapy} & 41 & 60 \\
\text{Fifteen months after therapy} & 28 & 50 \\
\hline
\end{array}
\]

**Muscle Strength Testing**

\[
\begin{array}{|c|c|c|}
\hline
 & \text{M.R.C. Scale} & \text{Spring Scale Testing} \\
\hline
\text{Before} & \text{After} & \% \text{Increase in Strength} \\
\hline
\text{Arms} & 33½ & 37 & 1+33.3 \\
\text{Legs} & \text{Could not be tested} & \text{Not measured by second observer} \\
\hline
\end{array}
\]

**DISCUSSION**

It is not proposed at this stage to speculate on the mode of action of the therapy described but to present the evidence for its effects and the means by which this evidence was obtained.

Muscle strength before and after treatment was measured by two independent observers, each using a different technique. The first, using the M.R.C. grading from 0 to 5, was imprecise, there being no normal limb for comparison. A further grading of 4½ was used, as is now customary (Sharrard, 1955). Since this method also invites subjective error by the observer, the second method used mechanical measurement to eliminate that fault (Russell, 1952).

In the hands of careful and experienced operators the first gave rudimentary assessment but the second much more accurate and refined measures of change.
It is of interest that, using similar detailed clinical testing of muscular power before and after treatment in 98 patients receiving ineffectual therapies for a variety of dystrophies, Walton and Nattrass (1954) found that subjective improvement was sometimes claimed no patient showed any objective increase in strength.

For biochemical measurement continuous serial assays of serum aldolase, SGOT, and SGPT were carried out. In muscular dystrophy these serum enzyme activities are raised in proportion to the muscle mass remaining and to the severity of its involvement, and with an early onset (Pearson, Chowdhury, Fowler, Jones, and Griffith, 1961) and a constant rapid rate of muscle loss, as in Duchenne-type dystrophy, the elevations diminish as the patient grows older. This relationship is rectilinear among older immobilized patients but in younger active individuals is distorted by large variations due to physical activity (Thomson, 1962). The increased serum activities of these enzymes are held to be due to their profuse efflux from dystrophic muscle supported by rapid intracellular renewal, and this efflux is influenced by muscular movement, tending to be reduced by rest and increased by physical activity, with sudden discharge of accumulated intracellular enzymes. The evidence for these views is discussed elsewhere (Thomson, 1962), and in this study is further supported by the fall in serum enzyme activities during rest (period 1) and their steep rise during ambulation afterwards (period A), both features being most marked in younger patients with most muscle (Figs. 1 and 2), less in older patients (Figs. 3 and 5), and least in those with so little muscle left as to be unable to walk (Fig. 6).

During period A, when the untreated patient was freely ambulant, new findings were observed as large continuous variations in all three serum enzyme values, again in approximate proportion to the muscle mass remaining and to the activity of the patient, being very large in the youngest patients (Fig. 1 and 2), less in older individuals (Figs. 3 and 5), and least, but not absent, in the wheel-chair patient (Fig. 6). The differences in variation of individual enzymes may be explained since skeletal muscle contains abundant aldolase and GOT but 20 times less GPT than GOT (Sibley and Fleisher, 1954; Wróblewski and LaDue, 1956). Further, on intravenous injection in animals both aldolase (Sibley, 1958; Schapira, Dreyfus, and Schapira, 1962) and GOT are rapidly cleared but GPT much more slowly (Fleisher and Wakim, 1956) though the molecular weights of aldolase and GPT are similar and thrice that of GOT (Green, Leloir, and Nocito, 1945; Dixon and Webb, 1958). The somewhat lesser lability of SGPT in muscular dystrophy may thus be due to its slower clearance, slower efflux for the same reasons, and to its relative dearth in muscle.

These observations objectively described the individual dystrophic process during ambulation after rest, first without having had treatment (period A), then after two successive therapeutic infusions (periods B and C). Statistical analysis gave single characteristic measures for all the readings for each enzyme during each period, their arithmetic mean indicating total enzyme efflux and the standard deviation, a measure of their scatter about the mean, the effect on efflux of physical activity. The difference between the arithmetic means of two stated periods was then examined by a t test, and judged significant (+) where \( p < 0.05 \) (1 in 20) and highly significant (++) where \( p < 0.01 \) (1 in 100), \( p \) being the probability of such a difference occurring by chance. The correlation coefficient \( r \) between the readings for different enzymes in each period was also determined, subjected to statistical tests, and its significance denoted in the same manner.

Before discussing the results in particular patients, certain subsidiary matters arise. Hepatic function in dystrophic patients has been examined by a wide variety of tests, but with conflicting results (Morrell, 1959; Beckmann and Billich, 1962). Accordingly, the urinary urobilin and bilirubin, serum bilirubin, thymol and zinc sulphate turbidities, and alkaline phosphatase were examined in all Duchenne-type patients before therapy and on several occasions during and after therapy. Though all other tests were normal throughout, four patients had an initial slight hyperbilirubinaemia of between 1-1 and 1-6 mg./100 ml., three of whom had urobilinuria but only one of whom, on a single occasion before treatment, had also bilirubinuria and serum giving a positive direct van den Bergh test, indicating some hepatic regurgitation. All these findings rapidly became normal during treatment, however, and were not related to the stage of disability, but originally suggest a haemolytic origin (Maclagan, 1957), perhaps due to erythrocyte fragility, brought to mind by the sallow appearance of some patients with Duchenne-type dystrophy.

An unusual finding, for which there seems no ready explanation, was the presence of a persistent marked halitosis, often with a life-long dislike for sweets, in most of the 11 patients with Duchenne-type dystrophy. Four patients had no halitosis and enjoyed sweets, but seven had halitosis and three of these patients disliked sweets. No obvious relationship was noted between this condition, the results of liver function tests, or stage of disability. In each of the seven patients treatment abolished the halitosis, but only one of the three overcame his dislike for sweets.
In some children with Duchenne-type dystrophy the I.Q. is lower than normal in varying degree, but remains stable and is unrelated to age or physical disability (Allen and Rodgin, 1960; Worden and Vignos, 1962). In the present series the I.Q. ranged from 38 to 123 before treatment, with an average of 77, increasing to 82 after treatment due to a variable improvement in most patients, on each of whom the intelligence test was carried out at intervals not less than four months apart. These measurable observations may be related to the regular change from apathy to an alert attitude after therapy.

Patient 1 (Fig. 1) was only 4 years old, with early signs of characteristic muscular weakness. Though too young to cooperate for muscle testing, the signs of weakness were no longer apparent after the second therapeutic infusion. The effects on serum enzyme values of successive rest (period 1) and activity (period A) are very evident in this energetic child with the bulk of the dystrophic muscle still intact. The successively smaller diminutions on rest with therapy in periods 2 and 3, followed by rising means in periods B and C, significant by period C only in the case of SGPT, may indicate that therapy had increased intracellular enzyme renewal without as quickly stemming efflux. However, the diminutions of all three standard deviations after therapy indicate undoubted reductions in variations of efflux due to physical activity, despite the rising means, and by that measure efflux occurred less easily than before. Probably this child had too much muscle remaining for adequate treatment on the scale adopted, and several successive infusions might be necessary to secure more obvious results.

Patient 2 (Fig. 2), the older brother of patient 1, was just over 7 years old and showed well-marked signs of advancing disease. After treatment an apparently marked clinical improvement was accompanied by a large increase in measured muscle strength. He had much less muscle mass than patient 1, and the biochemical effects of treatment were correspondingly conspicuous. The diminutions due to rest with therapy were much greater in period 2 than in period 1, though a little less in period 3; and the means for each enzyme, especially SGPT, diminished very significantly in periods B and C. If therapy were increasing intracellular enzyme renewal, then efflux was declining at a greater rate, and this was confirmed by physical activity having progressively lesser effects as indicated by the marked diminutions of all three standard deviations by period C, after the second therapeutic infusion.

Patient 3 (Fig. 3) was nearly 14½ years old, but his deterioration had been less rapid than average, though all signs of the disease were now becoming gross. Apparent clinical improvement after therapy was again accompanied by an increase in measured muscle strength. His muscle mass was relatively much smaller than that of patient 2, however, and during rest with therapy there were notable diminutions of enzyme values in periods 2 and 3, with abolition in periods B and C of the immediate increases on ambulation seen in period A. Further, in period B there was a highly significant fall in all three means and standard deviations, indicating a diminution both of total enzyme efflux and of the effect on it of physical activity. During period C, however, some 10 days after period 3, the patient had become rather active, and a temporary enzyme efflux increased the means and standard deviations for that period, though all three means still remained significantly lower than in period A, especially that of SGPT, that of aldolase alone being significantly higher than in period B.

Patient 4 (Fig. 4), just over 13 years old, also had rather slowly progressive disease, yet was so very lethargic and resigned that he sat all day by his bedside, rarely walking though well able to do so, especially since after therapy his measured muscle strength increased. Because of this chronic inactivity, his serum enzyme values were lower from the start and showed far smaller variations than expected, with little change in the means or standard deviations of aldolase or SGOT, except for the standard deviation of the latter in period B due to a transient rise. However, despite the unexpected loss of information from significant changes in aldolase or SGOT due to physical activity, SGPT showed marked diminutions of the standard deviation in period C and of its means, significant in period C and highly significant in period B.

Patient 5 (Fig. 5), not quite 9½ years old, had deteriorated so rapidly that he could walk only with great difficulty. After therapy some clinical improvement seemed apparent, but testing muscle strength was very difficult due to his poor span of attention, and these results may be invalid. However, with such a small residual muscle mass marked biochemical responses followed treatment, apparent as large diminutions during rest with therapy in periods 2 and 3 and abolition in period B of the immediate increases seen in period A on ambulation after rest. Highly significant reductions of all three means occurred in periods B and C and of the standard deviations in period B, though they again increased in period C, but without significance and SGPT least of all, due to a transient high initial peak after physical activity. Unlike patient 3, this peak was very brief as physical activity was not well maintained, and, though both quadriceps remained stronger than before, the glutei failed within three months and a wheel-chair became obligatory. Possibly so little muscle remained
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that any response, however complete, would still have been ineffectual.

Patient 6 (Fig. 6), just over 11 years old, had been in a wheel-chair for nine months. Some apparent clinical improvement was accompanied by an increase of strength measured in the arms but less in the legs, possibly because the metabolism of idle muscle is depressed and may be slow to accept therapy. Because the remaining muscle mass of the lower limbs and pelvis was inactive, bed rest (period 1) and then self-propelled excursions by wheel-chair (period A) had little effect on serum enzyme values, their variations depending only on activity of the arm and trunk muscles. Rest with therapy caused marked diminutions in period 2 and rather less in period 3; and all three means diminished in period B, aldolase significantly and SGPT very significantly, but rose again, without significance, in period C together with the standard deviation of aldolase, probably due to some increase in physical activity. It should be noted how all three means and standard deviations throughout, especially of SGPT, are lower than in fully ambulant patients and how the same effect can be produced by wilful inactivity (patient 4).

Patient 7 (Fig. 7), a 29-year-old woman with moderately disabling limb-girdle dystrophy, could not, for family reasons, be studied for as long as the Duchenne-type patients, and the statistical conclusions are less securely founded. After treatment her apparent clinical improvement coincided with an increase in strength measured in both upper and lower limbs. Though rest with therapy in periods 2 and 3 caused increasing diminutions, especially of aldolase, the means in periods B and C diminished only slightly, that of aldolase alone showing a diminution which became highly significant only in period C. No conclusions were drawn from the standard deviations.

In each of the patients 8 to 12 the apparent clinical improvement was accompanied by increased measured muscle strength; and in each this improvement persisted, in some for nearly two years. Though none was subjected to continuous biochemical investigation, assays of SGOT and SGPT retained the much lower values to which they were rapidly brought from their original elevations.

Analysis of serial serum enzyme readings in patients 1 to 6 showed that in Duchenne-type dystrophy, though all three enzymes were informative, SGPT showed more significant changes with greater constancy in every case, possibly due to its relatively lesser sensitivity to trivial alterations in daily exertion by the patient. In active patients with a large residual muscle mass, serum aldolase and the transaminases correlated more closely than in those with greater muscle loss, when correlation of aldolase with SGPT seemed to decline more readily than with SGOT. These observations support the view that of the three serum enzymes SGPT is more individual, that serum aldolase and SGOT are approximately equivalent though aldolase is a little more sensitive, and that almost as much reliable information might have been obtained by using SGOT and SGPT assays alone. In the single limb-girdle dystrophy patient (7), however, only aldolase altered significantly and correlated indifferentily with the transaminases, so that here a sensitive enzyme seems more useful.

Using these methods, an active child with obvious signs of early dystrophy and some loss of muscle (patient 2) seems to provide clearer evidence of effects of treatment than a child in the earliest stages (patient 1), or one already immobile because of the disease (patient 6) or personal inclination (patient 4). Where any treatment offers hopeful prospects it should commence at the earliest possible age (patient 5).

SUMMARY AND CONCLUSIONS

In progressive muscular dystrophy the disease process may be measured by systematic testing of muscular power and independently by serial assays of the serum enzymes aldolase, GOT, and GPT continuously discharged by the diseased muscle mass into the blood stream.

The power of various muscle groups in each limb may be measured by the M.R.C. grading for basic information, or, with gravity eliminated, by mechanical testing against spring scales to give refined measures of change. The latter method is preferred for its consistent accuracy and freedom from observer error.

Characteristic objective measures of the individual dystrophic process may be obtained from serial serum enzyme assays of morning pre-ambulatory specimens for about a month. The arithmetic means of these values indicate the muscle mass remaining and its total enzyme efflux; their standard deviations measure their scatter about the means and thus the effects of ordinary physical activity on this abnormal efflux. Both measures diminish slowly as the disease progresses and the muscle wastes away. Statistically significant sudden diminutions, however, of either or both measures after treatment, with a corresponding measurable increase in muscle power, are taken as objective evidence suggesting some amelioration of the dystrophic process.

Such evidence has been obtained after parenteral administration of a solution of nucleosides and nucleotides in six patients with Duchenne-type muscular dystrophy and in one with the limb-girdle...
form. The six subjects were selected at various stages of the disease in order to explore the reliability of the test methods, and a system of statistical analysis is described to interpret the results. It is concluded that SGOT and SGPT assays are themselves capable of providing sufficient reliable information in Duchenne-type dystrophy, but that in the limb-girdle form serum aldolase assays may be necessary.

Certain additional evidence was obtained from five other patients with Duchenne-type dystrophy, but without the rigorous analysis to which the six had been subjected.

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