THE DIRECT INTERCONVERSION OF GLUCOSE AND FRUCTOSE IN HUMAN SKELETAL MUSCLE WITH SPECIAL REFERENCE TO CHILDHOOD MUSCULAR DYSTROPHY

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SUMMARY

1. Acid extracts of muscle-fibre preparations from human biopsies incubated with [U-14C]glucose were chromatographically analysed.

2. Radioactivity of fructose was significantly greater in muscle from childhood dystrophy patients and also in female carriers of the disease compared with normal, foetal, neurogenic muscle disease and polymyositis control groups.

3. Measurements of the activity of polyol–NADP oxidoreductase (EC 1.1.1.21) and L-iditol–NAD oxidoreductase (EC 1.1.1.14) in muscle extracts compared with the ability of extracts to dephosphorylate fructose 6-phosphate, indicated that the probable route of fructose formation from glucose is via glucitol. The mean activities of the two specified enzymes in dystrophic muscle showed 13- and 3.5-fold increases respectively compared with normal muscle, and smaller increases compared with other controls.

4. Direct comparison of labelling patterns following incubation of muscle-fibre preparations with D-[U-14C]glucose, D-[U-14C]fructose and D-[U-14C]glucitol showed that these three substances are rapidly interconvertible mutual major metabolic products, confirming the glucitol pathway as a major route of fructose formation.

5. [U-14C]fructose is readily metabolized by dystrophic muscle, excluding the possibility of its accumulation being the result of poor utilization.

6. Inhibition of polyol oxidoreductase in dystrophic muscle preparations by 3,3′-tetramethyleneglutарате drastically reduces the ability of the fibres to utilize [U-14C]-glucose, and prevents the formation of [14C]fructose.

7. Some implications of these results are discussed in relation to the pathogenesis of childhood muscular dystrophy.

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The muscular dystrophies are a group of genetically determined progressive wasting diseases, the commonest and most characteristic of which is Duchenne's childhood type. This is usually a sex-linked recessive trait, although a similar disease occurs more rarely in girls and is thought to be an autosomal recessive condition (Walton & Gardner-Medwin, 1969). Numerous investigations have brought to light many aspects of biochemical change in dystrophic muscle but none has convincingly related these to a primary genetic lesion (Pennington, 1969).

The present work arose from a survey of the pathways of metabolism in human muscle in health and disease, the object of which was to demonstrate some pathways or reaction altered characteristically for a given disease, showing thereby where detailed investigation might profitably be undertaken. In a preliminary report (Ellis & Eccleston, 1968) it was suggested that excised muscle from patients affected with dystrophy when incubated with [U-\(^{14}\)C]glucose yielded significantly more labelled fructose than did muscle from control subjects, indicating an increase in the size of the labelled fructose pool in the muscle fibre in childhood dystrophy. This difference between dystrophic muscle and controls offered the possibility of discovering changes arising from the genetic error. Fructose in mammalian tissues has been considered to arise from glucose chiefly by transhydrogenation involving D-glucitol (sorbitol) (Hers, 1956; Samuels, Harding & Mann, 1962; Ritter & Leuthardt, 1963; Britton, Huggett & Nixon, 1967) and part of the present investigation was concerned with whether or not this pathway was the route of fructose formation in human muscle. Increase in intramuscular fructose pool size might imply an enhanced rate of formation and/or a decreased rate of utilization. Therefore, experiments were carried out to compare potential rates of fructose production in dystrophic and other muscle preparations and attempts were made to compare the abilities of muscle to utilize both D-glucitol and fructose.

The results presented here form part of our survey of metabolism in muscular dystrophy, and relate to the possible significance of fructose formation in the pathology of the disease.

MATERIALS AND METHODS

Clinical material

Muscle biopsy specimens weighing 0.2–1.5 g were removed, usually from the deltoid (the site being determined by clinical considerations) under local anaesthetic, general anaesthesia being used for children. Biopsies were taken for diagnostic reasons, and in some families affected with childhood muscular dystrophy, were volunteered for research purposes by one or both parents as well as from their affected children. Normal control muscle was also obtained during excision of intervertebral prolapsed discs and other orthopaedic operations. Foetal muscle was removed immediately after hysterotomies performed during the 12th–18th week of pregnancy.

Neuromuscular disease was diagnosed by clinical, electromyographical, histological and histochemical examination, and by routine determination in serum of ATP-creatine phosphotransferase (EC 2.7.3.2.) activity. Female carriers were investigated by these same means and the histochemical test of Morris & Raybould (1971) and were classified according to the scheme of Walton & Gardner-Medwin (1969).

A summary of the clinical material used is given in Table 1.
Table 1. Summary of clinical material

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age range</th>
<th>Muscle biopsy sites</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>4-53 years</td>
<td>Latissimus dorsi (2), spinalis (1), scapularis (1), deltoid (5)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal foetuses (male)</td>
<td>6</td>
<td>13-19 weeks of gestation</td>
<td>Entire back and thigh musculature (6)</td>
<td>Therapeutic abortions of healthy foetuses</td>
</tr>
<tr>
<td>Childhood muscular dystrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Duchenne-type severe form affecting males</td>
<td>8</td>
<td>2-16 years</td>
<td>Rectus femoris (1), deltoid (7)</td>
<td>Ranging from almost normally active to severe disability (i.e. wheelchair-bound)</td>
</tr>
<tr>
<td>(b) Severe form affecting females</td>
<td>1</td>
<td>26 years</td>
<td>Deltoid (1)</td>
<td>In terminal pneumonia</td>
</tr>
<tr>
<td>(c) Becker type</td>
<td>2</td>
<td>14-22 years</td>
<td>Deltoid (2)</td>
<td>Both ambulant</td>
</tr>
<tr>
<td>(d) Abnormal male foetus from carrier mother of severe Duchenne-type dystrophy</td>
<td>1</td>
<td>18 weeks of gestation</td>
<td>Thigh (1)</td>
<td>Therapeutic abortion</td>
</tr>
<tr>
<td>Adult forms of muscular dystrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Facioscapulohumeral</td>
<td>2</td>
<td>14-29 years</td>
<td>Deltoid (2)</td>
<td>One ambulant, one wheelchair-bound</td>
</tr>
<tr>
<td>(b) Limb girdle</td>
<td>2</td>
<td>15-22 years</td>
<td>Deltoid (2)</td>
<td>One ambulant, one wheelchair-bound</td>
</tr>
<tr>
<td>(c) Dystrophia myotonica</td>
<td>4</td>
<td>29-57 years</td>
<td>Palmaris longus (2), deltoid (1), flexor carpi radialis (1)</td>
<td>All ambulant</td>
</tr>
<tr>
<td>Neurogenic muscular atrophy</td>
<td>4</td>
<td>4-61 years</td>
<td>Deltoid (1), flexor carpi ulnaris (1), flexor carpi radialis (1), palmaris longus (1)</td>
<td>All ambulant; childhood neuropathy (1), peroneal muscular atrophy (1), ulnar nerve trauma (1), cervical spondylitis (1)</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>6</td>
<td>7-52 years</td>
<td>Deltoid (6)</td>
<td>All ambulant</td>
</tr>
</tbody>
</table>
Reagents and radiochemicals

Enzymes, nucleotides and phosphate esters were obtained from Boehringer: most other biochemicals and reagents were from British Drug Houses. 1-Methylphenylhydrazine hydrochloride was obtained from Eastman–Kodak. 3,3'-Tetramethyleneglutaric acid, a product of Ayerst Research Laboratories, Montreal, was in the first instance a gift from Dr Jin Kinoshiita, and subsequently a gift from the Ayerst Company. D-[U-14C]Glucose, D-[U-14C]fructose and D-[U-14C]glucitol were purchased at the highest specific radioactivity from The Radiochemical Centre, Amersham, and were used without further purification. Samples of [U-14C]-glucose were subjected to the same chromatographic treatment as were muscle extracts followed by radioautography and counting as described, showed that at least 99% of the recovered 14C was present as [14C]glucose. Several other substances were present after this procedure, among them [14C]fructose, which contributed less than 1% to the total recovered 14C.

Incubation and subsequent treatment of muscle

Muscle specimens were placed on ice immediately after their surgical removal, and were kept moistened with Krebs–Ringer phosphate solution at 0°C.

Thin (0.1–0.5 mm) bundles of muscle fibres were peeled longitudinally from the specimen, using the dissecting microscope where necessary. Even with fibrous and fatty specimens it was possible to avoid contamination with gross debris, whilst preserving the maximum undamaged fibre length intact. Fibre-bundles were blotted dry and weighed; between 60 and 100 mg were transferred to 2 ml of cold Krebs–Ringer phosphate solution (Umbreit, Burris & Stauffer, 1957) in a 20 ml polystyrene beaker. The preferred weight of fibres used was 100 mg, but by reducing the scale as little as 10 mg has been successfully used. The beakers were transferred to a shaking incubator, allowed to equilibrate at 37°C, any further appropriate additions made, and 10–25 μCi of 14C-labelled sugar was added to a final volume of 2.1 ml. Final incubation concentrations have varied from 0.04 to 5 mM sugar.

After 30 min of incubation the fibre-bundles were washed rapidly with water, immersed in liquid nitrogen, pulverized, and extracted with 1 ml of 0.9 m-perchloric acid. After thawing, the mixture was neutralized with 5 m-potassium carbonate, and the supernatant extract was chromatographed on Whatman 54 paper (46 cm × 46 cm) in phenol–water and butanol–propionic acid–water as described by Moses (1969). After exposure to X-ray film for 100 days to locate radioactivity, the radioactive areas were carefully cut from the chromatogram into pieces small enough to lie flat in a standard scintillation vial. Ethanol–water (0.2 ml, 4:1, v/v) was added and left for 20 min approximately. Scintillation fluid (10 ml, containing PPO*, 4 g; POPOP, 0.5 g; xylene, A.R. grade, 1 litre) was then added, the shaken vial cleared if necessary with ethanol and counted using the external standard to determine efficiency, after calibration with an internal standard for quenching.

Assessment of radioactivities

Measured eluted radioactivities represent the order of magnitude of the pool(s) of a given metabolite susceptible to labelling with 14C derived from the incubation substrate. Changes observed will reflect the changed pool sizes of metabolites and, if consistently observed in a given state of muscle health, may signify some underlying metabolic abnormality. The labelled pools were found to vary only slightly relative to each other with the two parameters time and

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* Abbreviations: PPO, 1,4-diphenyloxazole; POPOP, 1,4-bis-(5-phenyloxazol-2-yl)benzene.
external sugar concentration, over the range 15–200 min and 0·02–20 mm. Absolute radioactivities were found to be very variable, and considerable relative variation within one type of muscle (e.g. normal deltoid) was found, presumably through uncontrolled factors (e.g. diet, exercise). There was no noticeable effect attributable to site of biopsy or age. Therefore radioactivities are expressed as a percentage of the total radioactivity found in thirty-six selected chromatogram spots (Fig. 1), excluding any unchanged substrate, and mean values for

Fig. 1. Diagram of a typical paper chromatogram showing the spots used for reference purposes. Neutralized perchloric acid extract corresponding to about 4 mg of muscle fresh weight was subjected to chromatography as described in the text, and radioactive spots were located with 100 days of exposure to X-ray film. Not every muscle contained all the spots depicted in the example shown, while occasionally as many as eighty spots have been detected in one chromatogram. The thirty-six numbered spots were of fairly general occurrence and are used for reference as described in the text; the others are unidentified.

**Identities**

1. Fructose diphosphate  
2. Uridinediphosphate glucose  
3. Glucose and fructose 6-phosphate+fructose 1-phosphate  
4. Glyceraldehyde 3-phosphate+2- and 3-phosphoglycerate  
5. Dihydroxyacetone phosphate  
6. Glyceraldehyde  
7. Phosphopyruvate  
8. Glycerol phosphate  
13. Aspartate  
15. Pyruvate  
16. Isocitrate  
17. Glutamate  
19. Oxoglutarate (probably)  
20. Citrate  
21. Malate  
23. Oxaloacetate  
24. Fumarate  
25. Succinate  
26. Lactate  
29. Glycerol  
31. Alanine  
32. Glyceraldehyde  
33. Fructose  
34. Histidine  
35. Glucitol  
36. Glucose
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a given metabolite are compared from group to group of patient material using the t-test.

Metabolites were identified by co-chromatography with authentic marker substances. Fructose was identified as the 1-methylphenylazomone (Neuberg & Mandl, 1946). Fructose, glucitol and glucose were separated in the solvent system of Rees & Reynolds (1958). Further solvents employed were the ‘semi-stench’ system of Crowley, Moses & Ullrich (1963) and others appropriately selected from those given by Smith (1969).

**Determination of enzymes**

Polyol oxidoreductase and iditol oxidoreductase were determined by similar spectrophotometric methods, designed to avoid interference from endogenous substrates and reaction competing for coenzymes (Samuels et al., 1962; Hayman & Kinoshita, 1965). About 300 mg of muscle was homogenized in 10 vol. of cold buffer (0-05 M-Tris, 0-02 M-KCl, pH 7-4). After centrifugation at 100000 g for 1 h at 4°C the supernatant was divided, each half being subjected to gel filtration on Sephadex G-25 (column dimensions 25 cm × 0-8 cm²) with the Tris-KCl buffer as solvent. Each effluent stream in turn was divided into two and was pumped by a multichannel ‘Delta’ pump (Watson-Marlow Ltd, Falmouth, Cornwall). One-half was mixed with ‘complete’ and the other half with ‘incomplete’ substrate solution (see below); each was segmented with air, mixed, incubated, de-bubbled and passed through the sample and reference beams, respectively, of a double-beam spectrophotometer. Flow rate through the column was 10–20 ml/h. Substrate solutions for the two enzymes were:

(i) (For polyol oxidoreductase determination) 200 mmol of dl-glyceraldehyde, 0-2 mmol of NADPH, 200 mmol of triethanolamine–HCl per litre, adjusted to pH 7-4, the NADPH being added just before use. ‘Incomplete’ substrate solution lacks glyceraldehyde.

(ii) (For iditol oxidoreductase determination) 20 mmol of d-glucitol, 0-2 mmol of NAD⁺, 20 mmol of glycine–HCl per litre, adjusted to pH 9-6. ‘Incomplete’ substrate solution lacks glucitol.

The difference in absorbance between the two streams was plotted and the area between the traces represents the activity of the enzyme: calibration was achieved by performing manual determination under otherwise identical conditions upon muscle extract prepared as described using the pooled protein peak eluted from the Sephadex column.

Phosphatase activity against fructose 6-phosphate was measured by release of inorganic phosphate under each of four different conditions at 37°C. Reaction mixtures contained either 2 or 20 µmol of fructose 6-phosphate in 3 ml (final volume) of 0-05 M Tris–maleate buffer (Gomori, 1955) at both pH 5-9 and pH 7-8. The reaction was started by addition of supernatant derived from 45 mg of muscle (fresh weight) by the procedure described for determination of polyol oxidoreductase. Samples (1 ml) were withdrawn at zero time and after a 2 h incubation for determination of inorganic phosphate by the method of Lowry & Lopez (1946).

**RESULTS**

*Labelled fructose pools in muscle incubated with [1⁴C]glucose*

The relative radioactivities of fructose spots eluted from paper chromatograms of muscle extracts are shown in Table 2. The radioactivity of fructose in extracts of muscles from subjects with Duchenne-type muscular dystrophy shows a significantly higher mean value than the normal control and is distinguished thereby from the normal foetal state, from polymyositis
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and from neurogenic muscle disease. There is a similar increase of [14C]fructose pool size in female carriers of Duchenne-type dystrophy. No correlation could be demonstrated between fructose pool size and severity or duration of the disease of these dystrophic patients.

TABLE 2. Relative labelling of fructose in extracted intermediary metabolites of human muscle incubated with [U-14C]glucose. Muscle fibres after incubation with [14C]glucose were extracted and chromatographed as described in the text. Radioactivity of the fructose spot was expressed as a percentage of the total counted radioactivity (± SD) in thirty-five regularly occurring radioactive spots. Glucose, the thirty-sixth spot, was excluded from this total.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No. of patients</th>
<th>Fructose (% of total radioactivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adult</td>
<td>9</td>
<td>14.4±10.1</td>
</tr>
<tr>
<td>Normal foetus</td>
<td>6</td>
<td>21.2±17.8</td>
</tr>
<tr>
<td>Childhood muscular dystrophy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Severe Duchenne-type</td>
<td>8</td>
<td>43.2±14.7</td>
</tr>
<tr>
<td>(b) Severe form affecting females</td>
<td>1</td>
<td>32.7</td>
</tr>
<tr>
<td>(c) Becker type</td>
<td>2</td>
<td>29.7, 34.1</td>
</tr>
<tr>
<td>(d) Myopathic male foetus, from carrier of Duchenne dystrophy</td>
<td>1</td>
<td>43.7</td>
</tr>
<tr>
<td>Female carriers of Duchenne dystrophy</td>
<td>7</td>
<td>48.7±21.2</td>
</tr>
<tr>
<td>Adult muscular dystrophy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Facioscapulohumeral type</td>
<td>2</td>
<td>5.56, 6.14</td>
</tr>
<tr>
<td>(b) Limb girdle type</td>
<td>2</td>
<td>11.8, 30.2</td>
</tr>
<tr>
<td>(c) Dystrophia myotonica</td>
<td>4</td>
<td>18.2±25.1</td>
</tr>
<tr>
<td>Neurogenic muscular dystrophy</td>
<td>4</td>
<td>9.8±9.3</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>8</td>
<td>20.9±20.0</td>
</tr>
</tbody>
</table>

Differences between these means were assessed by the t-test as follows:

Significant at the level P<0.001
Duchenne dystrophy and normal adult (t = 4.78); female carriers of Duchenne dystrophy and normal adult (t = 4.80).

Significant at the level 0.001<P<0.01
Duchenne dystrophy and all adult dystrophies (t = 3.31); Duchenne dystrophy and neurogenic atrophy (t = 4.46); female carriers of Duchenne dystrophy and all adult dystrophies (t = 3.36); female carriers of Duchenne dystrophy and neurogenic atrophy (t = 3.47).

Significant at the level 0.02<P<0.05
Duchenne dystrophy and polymyositis (t = 2.54); Duchenne dystrophy and normal foetus (t = 2.52); female carriers of Duchenne dystrophy and normal foetus (t = 2.58).

Route of fructose formation in human muscle

Table 3 gives the results of measurements of polyol–NADP reductase and L-iditol–NAD oxidoreductase activities in normal, dystrophic and otherwise diseased human muscle, and also of fructose 6-phosphatase activities measured at pH 6 and pH 8 in soluble fractions of muscle.
Dystrophic muscle shows a mean increase in polyol oxidoreductase activity of 13-fold compared with normal, and a much more variable increase in L-iditol oxidoreductase activity with a mean of about 3-5-fold. No hydrolysis of fructose 6-phosphate at physiological pH could be detected.

Table 3. Activities of polyol–NADP oxidoreductase, L-iditol–NAD oxidoreductase and phosphatase activity against fructose 6-phosphate in human muscle. Polyol oxidoreductase and iditol oxidoreductase activities were determined by a semi-automatic spectrophotometric method described in the text. Release of P$_i$ from fructose 6-phosphate was determined at two different substrate concentrations at each of two pH values. The source of enzyme for all these measurements was a de-salted supernatant of muscle homogenate, prepared as described in the text. 'Zero' activity implies a conversion of less than 0.001 µmol of substrate min$^{-1}$ g$^{-1}$ fresh weight.

<table>
<thead>
<tr>
<th>Substrate transformed (µmol min$^{-1}$ g$^{-1}$ of muscle fresh weight at 25°C)</th>
<th>Hydrolysis of fructose 6-phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polyol oxido-</td>
</tr>
<tr>
<td></td>
<td>reductase</td>
</tr>
<tr>
<td></td>
<td>2 mM</td>
</tr>
<tr>
<td>Normal</td>
<td>(12) 0.17 ± 0.53</td>
</tr>
<tr>
<td>Childhood dystrophy</td>
<td>(14) 2.17 ± 2.13</td>
</tr>
<tr>
<td>Other muscle disease</td>
<td>(14) 0.71 ± 1.45</td>
</tr>
</tbody>
</table>

Significance of difference between means, evaluated by t-test:

- **Polyol oxidoreductase:** Normal versus dystrophic $t = 3.21$; $0.001 < P < 0.01$
  
  Other muscle disease versus dystrophic $t = 2.56$; $0.02 < P < 0.05$

- **Iditol oxidoreductase:** Normal versus dystrophic $t = 1.91$; $0.05 < P < 0.1$
  
  Other muscle disease versus dystrophic $t = 1.83$; $0.05 < P < 0.1$.

d-[¹⁴C]Glucitol has been used as an indicator of extracellular space (Ling & Kromash, 1967), but we found that in several diseased muscle preparations glucitol was readily metabolized although it was excluded from normal muscle. Table 4 shows relative radioactivities of glucose, fructose and glucitol eluted from paper chromatograms prepared from recently denervated flexor carpi ulnaris muscle of a 41-year-old male. The muscle had been incubated with [¹⁴C]glucose, [¹⁴C]glucitol and [¹⁴C]fructose. All three substances, glucose, glucitol and fructose are present in each extract in differing proportions indicating that the three substances are interconvertible.

Utilization of fructose by normal and diseased muscle in vitro

Table 5 gives a typical comparison between the abilities of deltoid muscle from a normal man, deltoid muscle from his son (a patient with Duchenne-type dystrophy) and deltoid muscle from the (carrier) mother of this patient, to utilize [¹⁴C]fructose. The total radioactivities of the extracts, and of certain components of them are shown, and may be seen to remain in fairly constant relationship in the three types of muscle.
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Table 4. Comparison of radioactivities of glucose, glucitol and fructose from muscle incubated with [14C]glucose, [4C]glucitol or [14C]-fructose. Denervated muscle fibres from a 41-year-old male were incubated for 30 min with either [U-14C]glucose, [U-14C]glucitol or [U-14C]fructose (25 μCi in each case). Intermediary metabolites were chromatographed and counted. The total radioactivity occurring in thirty-six regularly occurring chromatogram spots was used as a reference basis for the individual radioactivities due to glucose, fructose and glucitol. Full experimental details are given in the text.

<table>
<thead>
<tr>
<th>Incubation substrate</th>
<th>% of total radioactivity</th>
<th>Glucose</th>
<th>Glucitol</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>[U-14C]Glucose</td>
<td></td>
<td>55.5</td>
<td>3.5</td>
<td>7.95</td>
</tr>
<tr>
<td>[U-14C]Glucitol</td>
<td></td>
<td>2.3</td>
<td>84.1</td>
<td>4.8</td>
</tr>
<tr>
<td>[U-14C]Fructose</td>
<td></td>
<td>8.4</td>
<td>7.8</td>
<td>31.2</td>
</tr>
</tbody>
</table>

Effect of inhibition of polyol oxidoreductase on [14C]fructose pool in dystrophic muscle incubated with [14C]glucose

The results of incubations of muscle preparations with [14C]glucose, with and without the specific inhibitor of polyol oxidoreductase 3,3'-tetramethylene glutaric acid (Kinoshita, Dvornik, Kraml & Gabbay, 1968), are shown in Table 6. The inhibitor does not appear to inhibit the utilization of [14C]glucose by normal deltoid muscle. However, in the dystrophic muscle, the total extracted radioactivity is decreased by the inhibitor to 16.3% of the value

Table 5. Comparison of fructose utilization by normal and dystrophic muscle and muscle from a female carrier of muscular dystrophy. Muscle fibre-bundles from deltoid biopsies of a boy aged 8 years suffering from Duchenne-type muscular dystrophy, of his 33-year-old mother (a definite carrier) and of his 35-year-old normal father were incubated for 30 min with 10 μCi of [U-14C]fructose. Intermediary metabolites were chromatographed and counted. Full experimental details are given in the text.

<table>
<thead>
<tr>
<th>(nCi per g of muscle fresh wt.)</th>
<th>Unchanged fructose</th>
<th>Extract total excluding fructose</th>
<th>Lactate</th>
<th>Glucose</th>
<th>Glycer-aldehyde phosphate+ phosphoglyceric acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal muscle</td>
<td>171</td>
<td>557</td>
<td>6.06</td>
<td>89.0</td>
<td>14.2</td>
</tr>
<tr>
<td>Dystrophic muscle</td>
<td>622</td>
<td>1552</td>
<td>8.90</td>
<td>598.0</td>
<td>9.55</td>
</tr>
<tr>
<td>Muscle from female dystrophy carrier</td>
<td>107</td>
<td>485</td>
<td>5.55</td>
<td>90.0</td>
<td>17.5</td>
</tr>
</tbody>
</table>
in its absence; the label of unchanged glucose is reduced to 11.7% and the fructose label to 3.1% of the control values in the absence of inhibitor. The total label of all detected metabolites, excluding glucose was decreased to 25.9% of that in the absence of inhibitor.

Table 6. Effect of 3,3'-tetramethyleneglutarate on metabolism of [14C]glucose by muscle from normal and dystrophic subjects. Muscle fibre-bundles from deltoid biopsies of a boy aged 8 years suffering from Duchenne-type muscular dystrophy and of his normal 35-year-old father were incubated for 30 min with 25 μCi of [U-14C]glucose in 2.1 ml (final volume) with or without 3,3'-tetramethyleneglutarate (TMG) at a final concentration of 4 mM. Intermediary metabolites were chromatographed and counted as described in the text.

<table>
<thead>
<tr>
<th></th>
<th>Extract total</th>
<th>Unchanged glucose</th>
<th>Total excluding glucose</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>601</td>
<td>173</td>
<td>428</td>
<td>123</td>
</tr>
<tr>
<td>Normal + TMG</td>
<td>1261</td>
<td>179</td>
<td>1082</td>
<td>123</td>
</tr>
<tr>
<td>Dystrophic</td>
<td>2759</td>
<td>1870</td>
<td>889</td>
<td>418</td>
</tr>
<tr>
<td>Dystrophic + TMG</td>
<td>448</td>
<td>218</td>
<td>230</td>
<td>12.8</td>
</tr>
</tbody>
</table>

**DISCUSSION**

**Route of formation of [14C]fructose from [14C]glucose by human muscle**

Two possible routes of fructose formation from glucose have received serious study in animal tissues; the first is dephosphorylation of a glycolytic intermediate such as fructose 6-phosphate (Ritter & Leuthardt, 1963) and the second is the direct two-stage transhydrogenation via D-glucitol (Hers, 1957; Samuels et al., 1962; Ritter & Leuthardt, 1963; Touster & Shaw, 1962; Britton et al., 1967). The results shown in Table 3 probably exclude the first pathway for practical purposes in human muscle, since no dephosphorylation of fructose 6-phosphate could be detected in extracts of either normal or dystrophic muscle. Conversely, direct evidence was obtained for the rapid interconversion of the three substances [14C]glucose, [14C]glucitol and [14C]fructose, and for the presence in muscle from health and disease of both enzymes necessary for the direct transhydrogenation pathway (Table 3). It is reasonable to conclude that this second pathway is likely to have the greater significance in human muscle.

**Importance of glucose conversion into fructose in muscular dystrophy**

The results of Tables 2 and 3 demonstrate the variability of the apparent importance of this conversion in muscle from different states of health. In all these measurements the scatter of individual values is wide. The parameters compared in Table 2 are the resultant effect of two processes, the formation of fructose on the one hand and its utilization on the other. It is therefore not surprising, given the varied diets and physical conditions of the patients examined, that the scatter of these pool-size results is large. Nevertheless, Duchenne-type muscular dystrophy and the carrier state in females heterozygous for this disease are both statistically
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associated with larger than normal pool sizes of $[^{14}\text{C}]$fructose in these experiments. In comparison, neurogenic atrophy, polymyositis and the adult muscular dystrophies are not found to show this increased $[^{14}\text{C}]$fructose pool; neither is normal foetal muscle, which suggests that whatever other attributes of the foetal state dystrophic muscle may exhibit (Pennington, 1969) this is not among them.

Comparison of the polyol oxidoreductase activities in muscle (Table 3) shows distinctly that Duchenne-type muscular dystrophy imposes upon skeletal muscle a requirement or an opportunity for strikingly elevated activities of this enzyme, and also for considerable elevation of iditol oxidoreductase activity. A proportion of normal muscle samples contain less than detectable quantities of both enzymes.

It is argued that polyol oxidoreductase cannot play a significant role in tissue metabolism of glucose except when glucose transport across the cell membrane is not the rate-limiting step in glucose utilization (Clements, Morrison & Winegrad, 1969). This argument derives from the high $K_m$ of polyol oxidoreductase for glucose (Hayman & Kinoshita, 1965), in comparison with the much lower $K_m$ for glucose of hexokinase (Sols, 1968). It is clear from the kinetic studies of these authors that glyceraldehyde is a far better substrate for the enzymes than is glucose. However, the more detailed analysis by Hästein & Velle (1969) shows that the reaction is by no means straightforward, involving possible allosteric effects, and no true $K_m$ values could be determined: Hayman & Kinoshita (1965) state that their values are approximate only. It is apparent that glucose is not a substrate of choice for this enzyme; nevertheless, under certain favourable conditions it may be expected that polyol formation may occur. These favourable conditions are: high rates of glucose transport, low rates of glycolysis or other situations restricting the activity of hexokinase (Sols, 1968). The case has been carefully argued by Pottinger (1967) for the analogous situation of the lens, in which a similar competition for glucose between these enzymes occurs. Her conclusion is that polyol oxidoreductase may be quantitatively significant, especially when the glucose concentration exceeds 0.5 mM. In diseased muscle, particularly dystrophic muscle, membranes generally become more permeable (Pennington, 1969), and our Tables 5 and 6 illustrate the increased tissue sugar concentrations which may result. It seems possible that diseased muscle may develop the enzymes of the polyol pathway partly as a response to abnormal intracellular glucose concentrations. It might be expected that all diseased muscle would show the polyol pathway to a degree proportional to the damage to the fibres: our results do not fulfil this expectation. There was no correlation between the dystrophic patient's age (Duchenne group only) and the labelled fructose pool size or polyol oxidoreductase activity. Further, in no other group of muscle diseases was anything approaching the value observed in Duchenne dystrophy attained. The demonstration of increased fructose pools in female carriers of Duchenne dystrophy, although in most cases these show no clinical muscular disease and only minimal local histological changes, is suggestive of a specific role for the polyol pathway in this particular disease entity.

When the initial glucose–glucitol step of the pathway is blocked by the inhibitor 3,3'-tetramethyleneglutarate (Table 6), dystrophic muscle becomes much less able to utilize glucose; i.e. dystrophic muscle appears dependent upon the conversion to fructose for metabolism of the glucose it uses in vitro. The inhibitor has no such effect upon normal muscle; it does not apparently restrict fructose formation, which suggests that it may not be penetrating inside the normal muscle fibre, or has more complex effects; it will be of interest to perform further experiments with this substrate.
Possible aetiological significance of the glucitol pathway in childhood muscular dystrophy

The increased conversion into fructose of [14C]glucose absorbed by muscle fibres from patients with, and carriers of, childhood muscular dystrophy might imply that the more usual pathway of utilization is hindered to some degree in this disease, and that the subsidiary alternative pathway of glucose utilization through glucitol has become enlarged to serve as the chief pathway.

The nature of any restriction is a matter of critical interest. It might be a direct modification of a glycolytic enzyme, producing a decrease in its maximum catalytic ability, or in its regulatory properties (Randle, 1969). Alternatively, it might be the specific result of a generalized change in the concentration of regulatory substances, e.g. the adenine and nicotinamide nucleotides.

The implications of a shunt of glucose metabolism via fructose in this disease are of some interest. Fructose has a metabolism in liver very distinct from that of glucose (Hers, 1957; Heinz & Weiner, 1969; Sillero, Sillero & Sols, 1969), and is largely converted into lipids, notably triglycerides (Zakim, Pardini, Herman & Sauberlich, 1967; Scherstén, Nilsson, Cahlin & Jilderos, 1970; Bruckdorfer, Khan & Yudkin, 1971). This important difference between glucose and fructose lies in the site of phosphorylation by the kinase concerned; in the special metabolism of fructose the product is fructose 1-phosphate. This ester is a fairly prominent substance among those derived from labelled glucose during metabolism of dystrophic muscle (D. A. Ellis, J. M. Strickland & J. F. Eccleston, unpublished results) and we suggest that this may indicate that fructose may, to some extent, be metabolized in dystrophic muscle by the special route followed in liver. Although no demonstration of the specific oxohexokinase has been achieved in muscle, Villar-Palasi & Sols (1957) and Cadenas & Sols (1960) showed that phosphofructokinase possesses oxohexokinase activity, which might play a role under the special circumstances we are considering. The appearance of fat in muscular dystrophy may thus prove to have an origin more specifically related to its biochemical genetics than is generally supposed. The accumulation of triglyceride is evidence only of defective control of triglyceride metabolism, which may be exerted not only at several enzymic steps in breakdown and synthesis but also at several sites in the body (see, e.g., Steinberg, 1963; Kohout, Kohoutova & Heimberg, 1971; Mayes & Felts, 1967; Lowenstein, 1968; Tzur, Tal & Shapiro, 1964; Ball, 1966). It is possible that diversion of glucose carbon from normal pathways in muscular dystrophy may contribute to the well-known fatty deposition in this disease.

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