Contracture of phosphorylase deficient muscle

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Three distinct enzyme disorders are now known to give rise to the syndrome initially attributed to a lack of phosphorylase in muscle. Although these basic abnormalities have now been defined, there is much still to be learnt as to the manner in which the individual signs and symptoms are brought about. Most of the main features of the syndrome were exemplified in the patient originally described (McArdle, 1951) and consisted of pain, stiffness, and weakness of any muscle on moderate exercise. More vigorous exercise led to shortening of the muscle groups concerned. This was especially noticeable in the forearm flexors, the fingers remaining partially clenched for several minutes after powerful gripping movements or after carrying heavy weights. Exercise such as climbing stairs or fast walking led to disproportionate ventilation and an even more disproportionate increase in heart rate. The latter was thought to be associated with a grossly abnormal increase in blood flow, found to occur in exercised muscles. It was shown that the exercising muscles failed to form any lactate even during ischaemic exercise. The condition was attributed to failure on the part of the muscle to break down glycogen. A gap of eight years elapsed before the description of the next two cases, one by Schmid and Mahler (1959), the other by Mommaerts, Illingworth, Pearson, Guillory, and Seraydarian (1959). Both groups made the very important observation that muscles obtained by biopsy lacked phosphorylase, an enzyme which (in association with amylo-1,6-glucosidase and oligo-1,4→1,4-glucan transferase) is responsible for the degradation of glycogen to glucose-1-phosphate. A characteristic feature of the condition is what Pearson, Rimer, and Mommaerts (1961) described as 'second wind'—that is, the occasional disappearance of symptoms on continued exercise provided this was of modest severity. This was later shown by Porte, Crawford, Jennings, Aber, and McIlroy (1966) to be associated with a rise in free fatty acids in the plasma which presumably were utilized within the muscle. Mommaerts et al. (1959) and Schmid and Mahler (1959) had earlier found that glucose and certain other intermediates in glycogen breakdown were also of some help in increasing exercise tolerance. Schmid and Mahler (1959) described the occurrence of myoglobinuria and muscular wasting in their patient. The very occasional occurrence of myoglobinuria has been subsequently reported in several cases, but frequent bouts are rare. Myopathy would appear to be uncommon, and it is not clear why it develops in some but not in others.

The chain of events leading to the shortening of muscle on exercise is also uncertain. That it is a contracture (McArdle, 1951) and not a cramp, has been confirmed by Rowland, Lovelace, Schotland, Araki, and Carmel (1966) and by Dyken, Smith, and Peake (1967). Depletion of adenosine triphosphate (ATP) giving rise to a contracture similar to rigor mortis would seem to be a likely possibility, but in some careful and well-controlled observations, Rowland, Araki, and Carmel (1965) showed that there was apparently little or no reduction in the ATP level of muscle in contracture. Also of interest, in view of the importance of phosphorylase in the breakdown of glycogen, are the relatively low glycogen levels commonly found in this condition as compared with some of the other muscle glycogenoses.

These clinical features are also characteristic of phosphofructokinase deficiency (Tarui, Okuno, Okura, Tanaka, Suda, and Nishikawa, 1965; Layzer, Rowland, and Ranney, 1967) and, to a rather less extent, of the phosphohexoseisomerase abnormality described by Satoyoshi and Kowa (1965).

An opportunity to study these problems was provided when a patient was referred to one of us by Professor Dornhorst of St. George's Hospital as a probable case of phosphorylase deficiency.

CASE REPORT

D.L., a 21-year-old medical student, had experienced...
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pain, stiffness, and weakness of his muscles on moderate exercise since childhood. His first definite recollection was, when aged 7 years, of trying to keep up with his parents when walking but being unable to do so because his legs were stiff and aching. His symptoms became more noticeable when he started football and other sports, but, none the less, since he was both athletic and keen, he was able to do quite well in a number of sports.

It is of interest that at the age of 17 he ran the 100 yards in 11 sec and was able to run the 320 yards as well as most other boys, though he was utterly unable to run any distance greater than this. He can still sprint 100 yards for a bus without pain or stiffness. Yet walking at a moderate pace may result after about 400 yards in pain, stiffness, and weakness. The pain precedes the stiffness and, if the pace is not too rapid, may be the only symptom.

It is noticed first in the calves and then, if he continues, successively in the thighs, buttoks, and lower back. The stiffness at times can be so severe that he is unable to bend his knee, not because of pain, but because the muscles are hard and contracted. If he stops, the pain goes within 1-5 min, but the stiffness usually takes longer, though, if the pain having gone, he then walks at a slower pace, it, too, may subside quite rapidly. He gives a clear history of 'second wind', though only when walking at a moderate pace on the flat; it never develops once stiffness has been noticed. Previous 'limbering up' noticeably improves his exercise tolerance.

The symptoms can occur in any muscles, including the jaw and abdominal muscles, and he gives the characteristic story of being unable to straighten his fingers after carrying heavy books. This may persist for 10 min, but the accompanying weakness will last a further 20 min. More vigorous exercise—even moderately fast walking on the flat—will give rise not only to these symptoms but also to dyspnoea and to pulse rates in the region of 200/min. His appetite is normally greater than that of other young men and after exercise becomes enormous.

In addition to these classical symptoms, he has noticed that persistence in vigorous exercise, especially of his arms, will be followed by progressive swelling and tenderness of the muscles involved, maximal 6-8 hr later, and then slowly subsiding over a period of 24-48 hr or longer. The swelling can be very marked—sometimes the muscles were 'twice their normal size'. On two or three occasions his urine has been dark red in colour. One of these episodes occurred the morning after a spell of rowing which had not resulted in particularly severe pain.

It has not been possible to trace any similar condition on either side of his family. His parents are alive and are not related. He has one brother, aged 18, who like their father is very athletic.

On routine examination he was a tall, intelligent, stable, and mature young man, giving a clear and objective account of his symptoms. His legs were well developed and powerful, but the muscles above the waist were noticeably less bulky and powerful, particularly those of the shoulders and upper arms. The most affected of these, the triceps muscles, were moderately weak, the right being rather weaker than the left. All reflexes were present and equal, with plantar responses flexor. There was no sensory loss and all other systems were normal. His blood pressure was 125/70 mm/Hg. Urinalysis was normal.

INVESTIGATIONS

BIOPSY TECHNIQUE AND ELECTRICAL STIMULATION OF MUSCLE. The left sartorius was exposed under general anaesthesia (N₂O, O₂, trichlorethylene and halothane) about midway along its length. (a) A slip of muscle (15 × 2 × 2 mm) was removed between ligatures attaching it to a length of stiff polyethylene tubing. This was for examination by the Natori technique. On the advice of Dr. R. Creese, it was immediately put into a polyethylene bottle containing freshly prepared Krebs-Ringer-phosphate at approximately 20°C and bubbled continuously and vigorously with 95% O₂ and 5% CO₂. (b) A slip of muscle was cut out (12 × 1.5 × 1.5 mm) between two ligatures attaching it to a sterile wooden applicator stick. This was put into cold 3% glutaraldehyde in 0.067-M cacodylate buffer pH 7.4. (c) A larger squarish piece of muscle was next removed from the vastus medialis. A small piece was immediately placed in liquid N₂ for histochemistry, a piece cut off for histology and the remainder plunged into liquid N₂ for chemical studies. The latter piece of muscle was later transferred from liquid N₂ and stored in solid CO₂ or at -20°C until analysis. (The sartorius was then directly stimulated by a battery-operated Minidyne Stimulator. The frequency of the trains of stimuli was rapidly increased to a maximum of 48/min. The duration of each train was 0.75 sec and consisted of pulses at a frequency of 45/sec with a maximum of 170 V.

At first the muscle contracted vigorously and relaxed well between contractions, but the contractions became less vigorous and the extent of relaxation diminished. After 2 min stimulation was stopped as the muscle appeared to be in contracture. Two pieces of muscle were then removed as in (a) and (b) above for examination by the Natori technique and by electron microscopy. At this point the contracture appeared to have partly subsided, the muscle was stimulated again for about 1 min and, the muscle being again in contracture, a piece was removed and dropped into liquid nitrogen. The wound was sewn up. A biopsy from the middle of the lateral aspect of his left triceps was then made, the piece being apportioned for histochemical, pathological and for biochemical studies as in (c) above.

BIOCHEMICAL Lactate was determined by the method of Barker and Summerson (1941), pyruvate by that of Friedemann and Haugen (1943), citrate
by that of Mc Ardle (1955), inorganic phosphate by that of Fiske and Subbarow (1925), Na and K by internal standard flame photometry, and glycogen both by the method of Hassid and Abraham (1957) using a modified Nelson reagent for the final sugar determination (Somogyi, 1945) and also by a specific method based on that of Lee and Whelan (1966). Anaerobic glycolysis was assayed by a slight modification of the method of Mommaerts et al. (1959), the bicarbonate being decreased by half and the substrates increased by 50-100%.


**PATHOLOGICAL.** Tissue fixed in formol-saline was embedded in paraffin and sections were stained with haematoxylin and eosin, PAS, and various other stains. For electron microscopy the tissue was fixed in cold buffered 3% glutaraldehyde for 4 hr, washed for 18 hr in buffered sucrose solution postfixed in 1% osmium tetroxide for 2 hr, dehydrated in graded alcohols, and embedded in Araldite. Thin sections were cut on a Huxley Cambridge microtome, stained with lead citrate, and viewed on an RCA EMU 3c electron microscope. Areas of muscle for thin sectioning were selected by light microscopical examination of 1-2 μ Araldite sections stained with toluidine blue.

**SPECIAL STUDIES**

**EFFECTS OF EXERCISE** The effects of exercise on D.L. differed in no way from those described in G.W. (McArdle, 1951) and subsequent cases. The effects of local ischaemic exercise were tested with the grip ergometer previously described (McArdle and Verel, 1956). This involved pulling at intervals of 1 sec a load of 13-6 kg, sufficient to cause fatigue in normal subjects with little or no pain. He accomplished (disregarding 'negative' work) 10-2 kg, a value below the normal range of 12-7-31-4 (mean 18-9 kg) found in 16 healthy control subjects. This test was repeated after an interval of 3 min with an unimpeded circulation, the work this time being 6-55 kg; that is a recovery of 64% and well within the normal range (36-79-6 mean 59%), but as an absolute value below the normal range of 6-9-16.0 (mean 11.1) kg. It is interesting that during the first bout of exercise, he noted stiffness after 22 sec but experienced scarcely any pain during the 40 sec of ischaemia. On release of the cuff he could straighten his fingers only to an angle of 30° to the horizontal and had to pull them straight with the other hand. He had much less stiffness on the second bout and, on release of the cuff, commented on the lack of residual stiffness. Fifteen to twenty minutes later he pulled, with the circulation unimpeded, a load of 5-4 kg once/sec for 82 pulls (8-6 kg) before fatigue forced him to stop. Pain was considerable but there was no stiffness.

These preliminary observations seemed, superficially at least, to point to a lack of a close association between pain, fatigue, and stiffness. It was thought advisable, however, not to pursue them further as his arm in the next few hours became swollen and tender and remained in this state for a further 36 hr. Clinical recovery took four days, but a serum CPK at this point was 1,230 i.u. (normal up to 70 i.u.), a value which in retrospect proved to be considerably higher than the majority of those encountered in the next six months, unless the blood had been taken within a week or less of exercise giving rise to similar swelling and tenderness of muscle. On three such occasions the CPK was 1,120, 1,930, and 2,270 i.u., whereas on eight other occasions it varied between 243 to 720 i.u. (mean 502 i.u.).

**BLOOD CHANGES FOLLOWING ISCHAEMIC WORK** The test of the ability of the forearm muscles to degrade glycogen to lactate during ischaemic exercise was carried out as described previously (McArdle, 1951) except that a standard amount of work (5 kg) was performed on a special grip type ergometer (McArdle and Verel, 1956). The levels of lactate and some other substances in blood and plasma are given in Table I. Changes in blood lactate and pyruvate, and also in plasma citrate, inorganic phosphate, and CPK are either negligible or limited to minor rises in the later part of the recovery period. The small rise in plasma Na following release of the cuff is normal, but the fall to a level 6 m-equiv/l. below the resting value at 9 and 24 min is unusual; a similar fall in plasma Na was observed in only one of three specimens from one of eight normal subjects, the mean maximum fall over this period being 1-2 m-equiv/l.

The absence of any rise in blood lactate during the 4 min following the release of the cuff and the negligible rise thereafter was clear evidence of a defect in glycogen breakdown. That this was due to a deficiency in phosphorylase was shown by the examination of muscle obtained by biopsy.
ELECTROMYOGRAPHY  We are indebted to Dr. R. G. Willison of the National Hospital, Queen Square, who carried out the electromyographic studies. He reported as follows:

'Both biceps muscles were sampled before exercise. There was a little spontaneous activity, mainly small fibrillation spikes. On needle movement there was a substantial amount of insertion activity.

On voluntary effort the pattern was full but individual motor units were polyphasic in appearance with brief spikes. Left biceps was fatigued by the patient repeatedly lifting a 5 kg weight, the muscle becoming stiff and painful. It was again sampled but there was no change in the spontaneous activity.

The left common extensors showed similar findings at rest and on volition. After squeezing a rubber ball until the muscle was weak and stiff there was no change in the amount of insertion activity, though the fingers could not be fully extended.

Conduction velocity was measured in the left ulnar nerve to abductor digiti minimi. Latency from wrist to muscle was 2.2 msec and velocity between elbow and wrist was 65 msec. The muscle action potentials measured with surface electrodes were 5 mV in amplitude.

COMMENT  Mild myopathic change in the muscle examined. The explanation of the spontaneous activity is not evident. The tightening of the muscle after exertion was not accompanied by increased spontaneous activity or by motor unit discharge.'

STUDIES ON BIOPSIED MUSCLE

BIOCHEMICAL STUDIES  The results of a number of enzyme and other estimations on the biopsied portions of muscle are given in Table II. It is evident that phosphorylase activity is completely absent, whereas the activity of all the other enzymes concerned with carbohydrate metabolism that have been examined are unimpaired. A possible exception is phosphofructokinase; its activity is below the normal range of Tarui et al. (1965), but this is almost certainly due to differences in method in view of the brisk rate of anaerobic glycolysis to lactate when substrates other than glycogen were added to the reaction mixture. As might be expected, virtually no breakdown of glycogen to lactate had occurred in the muscle before the start of the incubation; there was no further change during the 30 min period of incubation, nor did significant glycogenolysis occur when glycogen was added to the medium. The glycogen content of two pieces of muscle estimated by two different methods, though raised above the upper limit of normal (1:29 g %), was surprisingly low—that is, 2-1 and 1-35 g %, the latter value being obtained by a method highly specific for glycogen though with a lower upper limit of normal (0-5 g %).

Glycogen structure was also assessed by examining the absorption spectrum of the iodine complex formed with glycogen from biopsy material. Peak absorption lay between 460-480 mμ indicating normal structure in the outer chains of the glycogen molecules. In view of the relatively small increase in muscle glycogen, it is of interest that both gly-

### Table I

**BLOOD CHANGES FOLLOWING ISCHAEMIC WORK**

<table>
<thead>
<tr>
<th></th>
<th>Lactate (mg/100 g)</th>
<th>Pyruvate (mg/100 g)</th>
<th>Citrate (mg/100 g)</th>
<th>K (m-equiv/l.)</th>
<th>Na (m-equiv/l.)</th>
<th>PO₄ (mg/100 ml.)</th>
<th>CPK (iu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. arm before exercise</td>
<td>7-4</td>
<td>0-58</td>
<td>1-45</td>
<td>4-60</td>
<td>143</td>
<td>2-66</td>
<td>1230</td>
</tr>
<tr>
<td>R. arm 1 min after release of cuff</td>
<td>7-6</td>
<td>0-59</td>
<td>1-38</td>
<td>4-22</td>
<td>144</td>
<td>2-73</td>
<td>1310</td>
</tr>
<tr>
<td>4 min after release of cuff</td>
<td>7-0</td>
<td>0-67</td>
<td>1-55</td>
<td>4-30</td>
<td>145</td>
<td>2-62</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8-7</td>
<td>0-70</td>
<td>1-58</td>
<td>4-87</td>
<td>137</td>
<td>2-81</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>9-2</td>
<td>0-66</td>
<td>1-88</td>
<td>4-87</td>
<td>138</td>
<td>2-56</td>
<td>1340</td>
</tr>
<tr>
<td>L. arm 26 min after release of cuff</td>
<td>7-5</td>
<td>0-63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

### Table II

**MUSCLE GLYCOGEN, ANAEROBIC GLYCOLYSIS, AND ENZYME ACTIVITY IN MUSCLE HOMOGENATES**

<table>
<thead>
<tr>
<th>Determination and units</th>
<th>Patient</th>
<th>Normal range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerogen (g/100 g)</td>
<td>2:1</td>
<td>0-4-1:3</td>
</tr>
<tr>
<td>(Method of Hassid and Abraham)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Method of Lee and Whelan)</td>
<td>1-35</td>
<td>&lt;0-5</td>
</tr>
<tr>
<td>Anaerobic glycolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial lactate (mg/100 g)</td>
<td>24</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Lactate liberated (μ-mole/g/30 min) from added substrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No substrate</td>
<td>0</td>
<td>10-60</td>
</tr>
<tr>
<td>Glycogen</td>
<td>1-8</td>
<td>40-120</td>
</tr>
<tr>
<td>Glucose 1 phosphate</td>
<td>530</td>
<td>300-700</td>
</tr>
<tr>
<td>Glucose 6 phosphate</td>
<td>530</td>
<td>300-700</td>
</tr>
<tr>
<td>Fructose 6 phosphate</td>
<td>347</td>
<td>250-500</td>
</tr>
<tr>
<td>Phosphorylase (μ-mole/g/min)</td>
<td>1-8</td>
<td>50-90</td>
</tr>
<tr>
<td>Amylo-1,6-glucosidase</td>
<td>1-34</td>
<td>about 0-5†</td>
</tr>
<tr>
<td>(μ-mole/g/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid maltase (μ-mole/g/min)</td>
<td>0-05</td>
<td>0-03-0-11†</td>
</tr>
<tr>
<td>Phosphoglucomutase (μ-mole/g/min)</td>
<td>137</td>
<td>50-120</td>
</tr>
<tr>
<td>Glycogen synthetase</td>
<td>2-5</td>
<td>1-5†</td>
</tr>
<tr>
<td>Phosphofructokinase (μ-mole/min/mg protein)</td>
<td>0-06</td>
<td>0-13-0-25†</td>
</tr>
</tbody>
</table>

*Some of these limits are highly provisional and are those of McArdle (unpublished) unless otherwise stated.
†The normal range given is that of Ryman (unpublished).
‡The normal range given is that of Tarui et al. (1965).
cogen synthetase and acid maltase were normal. The latter enzyme is concerned with the degradation of glycogen within lysosomes. The activity of amylo-1, 6-glucosidase, the 'debranching enzyme' which normally acts in conjunction with phosphorylase, was apparently increased, but insufficient normal values using this technique are available.

**PHYSIOLOGICAL STUDIES** It is not clear why the muscles of these patients are liable to develop prolonged contractures on comparatively modest exertion. Rowland et al. (1965) have shown that the level of ATP, the immediate energy source for contraction, does not appreciably decrease in such patients after exercise. It is not possible, therefore, to ascribe the contracture to a state identical with that of rigor mortis, a condition in which the level of ATP is greatly reduced. The ultrastructural studies of Schotland, Spiro, Rowland, and Carmel (1965) indicated some damage to the sarcomatous system of those fibres in a state of contracture. Since this system and particularly the lateral vesicles are known to be associated with the binding and release of calcium, Schotland et al. (1965) suggested the contractures might be associated with a defect in this system. It seemed reasonable to suppose that the preparation described by Natori (1954), obtained by manually stripping the sarcolemma from single muscle fibres, might be usefully employed in the further study of the contracture. This preparation is well suited for the study of post-membrane excitation events, as it allows the manipulation of live muscle fibres without interference from events occurring at the sarcolemma. Such a preparation was used by Costantin and Podolsky (1965) to show that calcium mediates between membrane excitation and contraction of the myofibrils. More recently Gruener (1967) has shown that the Natori preparation may be used to demonstrate the sensitivity of mammalian muscle to caffeine.

Two pieces of sartorius muscle tied (as already described) on to stiff polyethylene tubing to avoid spontaneous contractures were transported in oxygenated Krebs solution, the interval before use being approximately 1 hr. One sample was from resting muscle; the other, before excision, had been electrically stimulated for 2 min to induce a state similar to post-exercise contracture. Control samples were obtained from the vastus lateralis and the rectus abdominis of two subjects with normal musculature. The extensor digitorum longus of the mouse, stimulated electrically in a similar manner to the biopsied muscle, was also used as a control. Single fibres were dissected away from the bundles under paraffin oil and the sarcolemma stripped away over part of their length. Calcium chloride (1 mM) and caffeine (5 mM) were focally applied to the stripped regions through glass micropipettes measuring approximately 5 μ in diameter. The fibres were observed under the microscope and their responses recorded cinematographically. All experiments were carried out at 20 ± 2°C.

Local contractures were obtained in both control and phosphorylase deficient fibres when challenged with either calcium or caffeine. Approximately four to 30 sarcomeres might be involved and these usually shortened to about two-thirds of their original length without any apparent differences in the degree of shortening as between control and affected fibres. In the controls (Figs. 1 and 4) contractures lasted approximately 1 to 3 sec and were completely reversible. The response to repeated focal applications of either calcium or caffeine to the control fibres showed no difference from single applications to such fibres. In the affected muscle fibres, however, repeated applications of either calcium or caffeine led to persistent residual contractures which lasted for up to 1 min. Such residual contractures occurred most readily in the stimulated fibres (Figs. 3 and 6) after two or more focal applications. The unstimulated affected fibres (Figs. 2 and 5) showed residual contractures only after five or more focal applications. The control mouse fibres that had been stimulated before removal did not differ in their behaviour from the unstimulated normal human muscle and residual contractures were never observed.

Some of the fibres from the electrically stimulated affected muscle had remained in a state of contraction. A few of these were locally superfused with a relaxing medium containing 5 mM ethyleneglycol bis (aminoethyl ether) tetra-acetic acid (EGTA) 5 mM ATP, 1 mM MgCl₂, 10 mM histidine (HCl), and 110 mM KCl. The fibres could be shown to relax by measuring their sarcomere spacings before and after the application of the relaxing medium (Fig. 7).

**PATHOLOGY**

**LIGHT MICROSCOPY** The complete absence of phosphorylase activity in the muscle was confirmed in sections stained histochemically by the method of Takeuchi (Pearse, 1960), whereas normal muscle stained in the usual manner.

Biopsy material fixed in formal saline or glutaraldehyde was available from the triceps and the vastus medialis, and from the sartorius both before and after electrical stimulation. The characteristic subsarcolemmal blebs containing glycogen (Schmid and Mahler, 1959; Pearson et al., 1961), were present in all sections, but there was a notable
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FIG. 1. Local application of calcium to a vastus lateralis normal fibre. (The bar—on this and the following plates indicates 20 μ. The figure in parentheses indicates average sarcomere length in microns.)
(a) During 7th application
(b) 1-5 sec after application
(c) 3-5 sec after application

(a) During 7th application (2.7)
(b) 1.5 sec after application (1.2)
(c) 3.5 sec after application (2.5)

FIG. 2. Local application of calcium to a phosphorylase deficient unstimulated sartorius fibre.
(a) before application
(b) during application
(c) 0.25 sec after application
(d) 1.0 sec after application

(a) before application (3.5)
(b) during application (2.0)
(c) 0.25 sec after application (2.0)
(d) 1.0 sec after application (3.1)
FIG. 3. Local application of calcium to a phosphorylase-deficient stimulated sartorius fibre.

(a) before application (3-1) (b) 0-5 sec after 4th application (2-2) (c) 1-0 sec after 4th application (1-9) 
(d) 5 sec after 4th application (1-9)

difference between the very frequent occurrence of these blebs in the leg muscles and their very rare occurrence in the triceps. In addition there appeared to be a slight generalized increase in glycogen, possibly more definite in the leg muscles. Single fibres showing floccular degeneration and invasion by phagocytes were seen in both the resting and the stimulated sartorius. There were a few chains of subsarcolemmal nuclei, more noticeable in the triceps, and in this muscle, though not in the others, there was a definite increase in the number of intracellular nuclei above normal. Fibres in the triceps appeared to be generally smaller than those in the leg but no abnormally small or large fibres were seen in any of the resting muscles. Cross striation was well preserved.

Additional changes were present in the sartorius that had been stimulated before biopsy. On longitudinal section a number of fibres when stained for glycogen were noticeably different in having lost their cross striation and also, in places, their longitudinal striation, and in being largely devoid of glycogen. Occasional segments appeared to be in contracture, as judged by the gross narrowing of the cross striations which were faintly outlined by the remnants of the glycogen. In one fibre the process could be followed from normality through a short portion in which the cross striations appeared rather widened to a length in which both cross and longitudinal striations had largely disappeared, though they contained areas with faint and very narrow cross striation. A much longer length then ensued, with uniformly narrowed cross striations but containing a few short segments having a ground-glass appearance due to loss of all striations. At two points there were narrow contraction bands. The direction of the cross striation of one of the fibres immediately adjoining the abnormal fibre was at an acute angle, and in a number of the other fibres the cross striations were arched rather than transverse.

The changes were considerably less noticeable in transverse section, though a few abnormal fibres were readily apparent, since they were swollen, rounded, ground-glass in appearance, and devoid of glycogen.

Similar changes were present in sections from the other biopsy specimens but were seen only in those fibres retaining a severed end that had been cut in excising the specimen and were present only for up
FIG. 4. Local application of caffeine to a vastus lateralis normal fibre.
(a) 0·25 sec after 6th application (1·2)
(b) 1·5 sec after 6th application (2·1)
(c) 3·5 sec after 6th application (2·5)

FIG. 5. Local application of caffeine to a phosphorylase deficient unstimulated sartorius fibre.
(a) before application (2·0)
(b) 0·25 sec after application (1·5)
(c) 0·5 sec after application (1·2)
(d) 1·5 sec after application (1·7)

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...to about 1 mm away from the cut end. They were clearly changes that had not been present during life. The same changes were also present in biopsied muscle from other disorders but they did not extend as far from the cut end.

It is evident, therefore, that these changes observed in the cut ends of these fibres are artefacts resulting from damage, but it is a distinct possibility that the phosphorylase deficiency predisposes to the development of these artefacts. What is less certain
is the extent to which the changes observed in some of the fibres in the stimulated muscle represent alterations that took place during life or are artefacts occurring in muscle that may have been partly damaged in vivo not only by the contracture itself, but by other effects of the disordered metabolism.

**ELECTRON MICROSCOPY** Subsarcolemmal and interfibrillar sarcoplasmic glycogen was markedly increased in the sartorius compared with normal human muscle (Price, 1963), but this increase was less in the triceps. Some glycogen granules were also observed between the actin filaments of the I-bands (Figs. 8 and 9) and a few were present in A-bands of the myosin filaments (Fig. 8).

Several muscle fibres in the stimulated sartorius were in contracture, showing a reduction in sarcomere length with disappearance of the I-bands, similar in appearance to those described by Schotland et al. (1965). The other fibrils in this muscle appeared normal, whereas in the clinically rather wasted triceps many of the fibres were reduced in cross-sectional area (Figs. 8 and 9).

The most striking ultrastructural change in the muscle was the accumulation of glycogen granules within the mitochondria of the triceps (Fig. 9) and of the sartorius both before and after stimulation (Figs. 10 and 11). Longitudinal section showed that glycogen accumulation and disruption of the cristae may be restricted to one region of the mitochondrion, while the rest of the organelle retains its normal structure (Fig. 9). Some of the glycogen granules were enclosed in single-membrane bound vesicles or appeared to be invaginating the outer mitochondrial membrane (Fig. 9). However, the majority of the granules were lying free within such mitochondria, and at times formed a continuous mass with the sarcoplasmic glycogen through a gap in the outer mitochondrial membrane (Fig. 9). Subsarcolemmal mitochondria were also involved; Fig. 11 shows a group of mitochondria where some have a normal cristal pattern, although others are partially disrupted, and may be recognizable as mitochondria only by the double outer membrane, a few remaining cristae, and the presence of normal dense granules (André, 1962). Vesicular outlines

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**FIG. 6.** Local application of caffeine to a phosphorylase-deficient stimulated sartorius fibre.  
(a) Before any treatment (2-6)  
(b) 60 sec after 4th application and just before 5th application (1-5)  
(c) 0-25 sec after 5th application (1-3)  
(d) 4 sec after 5th application (1-3)
Contracture of phosphorylase deficient muscle

FIG. 7  Local application of relaxing-medium to a contractured (stimulated) phosphorylase-deficient sartorius fibre.
(a) before application  (1-7)  (b) 0-5 sec after application  (1-8)  (c) 4 sec after application  (2-2)
(d) 10 sec after application  (2-0)

enclosing groups of glycogen granules were observed in all the specimens of muscle and may represent the remains of mitochondria.

Many of the mitochondria in the region of the stimulated sartorius in contracture were swollen; the cristae were disrupted but they contained no glycogen. However, because the same changes were present in some of the mitochondria in the resting sartorius and as similar appearances may occur as a result of preparative artefact, it is clear that these changes are difficult to evaluate.

The sarcoplasmic reticulum appeared normal in almost all cases (Fig. 9); occasionally, parts of the longitudinal anastomosing system were dilated, but this bore no relationship to the fibres in contracture. No increase in lysosomes was observed in any of the specimens.

DISCUSSION

The early myopathy noted in the present case is the only rather unusual feature in what is otherwise a very characteristic picture. It is possible, though, that it may be unusual only in the age at which it developed, for Schmid and Hammaker (1961) have suggested the natural history of the condition is for the myopathy to develop in later life. This is a difficult proposition to prove or disprove since only 33 cases have been recorded, about two-thirds of them under 40 years of age. In its favour is the fact that some degree of myopathy could not be excluded in about half the cases over 40 years and in less than a fifth of those under this age; but there are a number of factors that might distort these figures. The myopathy occurring in the two brothers described by Schmid and Hammaker (1961) seems to have been unusually severe, and the overall impression gained from the cases so far recorded is that the myopathy may appear at any age, that it is only very slowly progressive, and that, with avoidance of unduly vigorous exercise, the process might be halted. It would seem to be advisable to monitor the serum CPK levels, not only to assess whether a myopathy is likely to develop, but also to aid in the determination of the range of permitted activity. It is likely, however, that in this condition some
elevation of CPK above normal is nevertheless compatible with continued freedom from overt myopathy. This would seem to be the case in G.W. (McArdle, 1951) who, when recently examined at the age of 50 years, showed no signs whatever of myopathy but had serum CPK levels of 114 and 127 i.u. on separate occasions.

The myopathy appears to have a special predilection for the proximal muscles of the upper limb. Nixon, Hobbs, and Greenblatt (1966) remarked that the powerful lower limbs of their patient were disproportionately larger than the slim and relatively weak arms. The same disparity is a feature of the present case.

Why some individuals, and only some of the muscles in an affected individual, develop myopathy and others do not is not clear. It has been tentatively suggested (Schotland et al., 1965) that the increased glycogen in the muscle infiltrating the I-bands might contribute to the development of a myopathy by exerting a purely mechanical disruptive effect on the slender actin fibres. This seems unlikely, since from the literature there would seem to be little or no correlation between the level of glycogen in the muscle and any unusual weakness. The glycogen content of the vastus medialis of the present case was very moderately increased. Unfortunately that of the triceps was not estimated, but from the appearance of the sections stained for glycogen and from the electron microscopic studies, it can be confidently presumed that it was certainly not greater than that of the vastus and more probably slightly less. However, it could be maintained that it was not so much the absolute amount of glycogen but the number of granules infiltrating and displacing the I-bands that was important. The electron microscopic appearances would argue against this, for there was little difference between the degree of

FIG. 8. Electron micrograph of triceps muscle L.S. showing narrowed I-bands with glycogen (g) between the actin filaments. Sarcoplasmic reticulum normal. Triads (T) longitudinal anastomosing system (L). × 87,000.
I-band infiltration and displacement in the powerful leg muscles and the mildly myopathic triceps muscles.

What may well be of greater significance is the finding of the accumulation of glycogen within the mitochondria with dilatation and disruption of the normal cristal pattern. Any attempt to reconstruct the process by which this was brought about must be highly speculative. There would seem to be at least three possibilities. A similar phenomenon has been observed in the mitochondria of the digestive cells of Hydra. They usually contain no glycogen when the organism is fasting, but on feeding there is a rapid rise in the glycogen content of the cytoplasm, together with an accumulation of glycogen granules in the mitochondria (Lentz, 1966). Very similar changes are also seen within the mitochondria of the B-neurones of the lumbar spinal ganglia of the winter frog, a finding which Berthold (1966) considers may be concerned with storage. None the less, it seems highly improbable that the raised glycogen content of the muscle fibres induced its accumulation within the mitochondria. A more likely possibility is that the changes are degenerative, since Lampert (1967) describes similar glycogen accumulations within mitochondria in dystrophic axons in the spinal cord and medulla of rats with chronic vitamin E deficiency. The third possibility is that the abnormal metabolic or pH changes occurring during excessive exercise lead to rapid and gross swelling of some mitochondria or of localized portions of mitochondria. Further physico-chemical and/or mechanical forces might cause some of the mitochondria to burst, the glycogen containing...
sarcoplasm being then forced or just flowing into the mitochondria. Others, in collapsing, might become invaginated. It seems reasonable to suppose that the ease with which the mitochondria are damaged or degenerate and are replaced or repaired might well influence the development of a permanent myopathy. Schotland et al. (1965) had earlier noted the same, though less severe, changes 30 min after voluntary exercise sufficient to induce contracture. These changes had not been noted before or 3 min after exercise. Though the myofibrils were much distorted by glycogen accumulation, there was no clinical weakness or other evidence of myopathy.

**BIOCHEMICAL STUDIES** It is possible that it would be better to concentrate in future on mitochondrial function especially after exercise, rather than on glycogen synthesis and degradation. The enzymes investigated, apart of course from phosphorylase, have been found to have normal activity, though that of the debranching enzyme amylo-1,6-glucosidase might have been somewhat increased. This could scarcely have had any significant glycogenolytic effect. It seems very probable that the relatively small rise in muscle glycogen noted in many cases may be due, as has already been suggested (Hers, 1964; Larner, 1964), to the activity of the acid maltase (α-1,4-glucosidase). The activity of this lysosomal enzyme in muscle from one of the two cases described by Rowland et al. (1966) was somewhat higher than that of two normal controls, but was normal in our patient. The activity of this enzyme as judged by biochemical assay would appear to be small in normal muscle, but its importance in controlling the level of glycogen in vivo is shown by the high levels of glycogen (about 10% or more) found in Pompe's (Type II) disease in which α-1,4-glucosidase is deficient.

**PHYSIOLOGICAL STUDIES** An intriguing feature of the condition is the stiffness and shortening of the muscle caused by vigorous and even by moderate exertion. It has been shown electromyographically that this shortening of muscle is unaccompanied by any electrical activity and is therefore in the nature of a contracture. That is, it is a shortening of the muscle fibres due to changes, within the fibres themselves, which are initially independent, or become independent of the electrical propagation of impulses along the muscle membrane. Such a view...
is reinforced by the findings with fibres stripped of their sarcolemma, since the effects observed, the counterpart of those seen in life, could not possibly be due to a membrane abnormality.

It is therefore a disturbance of 'excitation-contraction coupling' a phase of muscle contraction which has been considerably clarified in the last decade (for a recent authoritative review see Huxley, 1968). The most widely held view of the excitation contraction process is that the spread of the impulse through the muscle fibre is by electrotonic conduction along the central tubular component of the triads—specialized portions of the sarcoplasmic reticulum (S.R.) running transversely through the fibres near the A–I junctions. This causes a release of Ca\(^{2+}\) stored in the S.R., particularly the terminal vesicles and lateral sacs of the triads. The Ca\(^{2+}\) released into the sarcoplasm triggers off contraction of the myofibrils by causing sliding of the interdigitating actin and myosin filaments on each other, though how this is brought about is still a subject for considerable speculation (for example, Davies, 1963). The process will continue so long as there are sufficient free calcium ions available and adequate regeneration of ATP. However, with repolarization of the membrane, release of Ca\(^{2+}\) from the S.R. ceases and the relaxing factor system ('the Ca\(^{2+}\) pump') very rapidly reaccumulates the Ca\(^{2+}\) from the sarcoplasm. From the work of Ebashi (1961) and Hasselbach and Makinose (1961), it is thought that this Ca\(^{2+}\) reaccumulation is linked to a highly active ATPase present in the S.R., and that it is dependent on a continuous supply of ATP which would seem to be present in several-fold higher concentration in the S.R. than in the surrounding fluid (Ebashi and Lipmann, 1962).

It would appear, therefore, in the light of these views, that our results might be tentatively interpreted as suggesting that the contraction mechanism is reasonably intact with all that this implies in terms of local ATP concentration, Ca\(^{2+}\) linking, myosin ATPase activity, etc. This would seem to follow from the observation that the addition of Ca\(^{2+}\) (or caffeine) induces shortening in those fibres that are already in a state of partial contracture or in residual contracture from previous applications. Furthermore, the Ca\(^{2+}\) release mechanism would appear to be relatively intact, since shortening uniformly followed the application of caffeine, which is supposed to act on the S.R. by releasing Ca\(^{2+}\) and inhibiting its subsequent reaccumulation (Herz and Weber, 1965). On the other hand, the residual contractures following Ca\(^{2+}\) and caffeine, especially in the previously stimulated fibres, and the relaxation following local superfusion with a medium known to relax Ca\(^{2+}\) induced contractions in muscle fibres, are all explicable if the Ca\(^{2+}\) pump mechanism were affected. The relaxing medium contained both EGTA and ATP, either of which might have been responsible for the relaxation; both would remove Ca\(^{2+}\) ions by chelation, and, in addition, the ATP would provide energy for the Ca\(^{2+}\) pump were the ATP, in fact, to be deficient. There is some evidence against the latter possibility, since Rowland et al. (1965) have found not only normal but unchanged levels of ATP in two patients who had exercised their muscles sufficiently to induce contracture before biopsy. However, the possibility exists that there is a local deficiency in the relatively high concentration of ATP in the S.R. This would presumably stem largely from the enzyme abnormality, but the mitochondrial changes might well contribute. Alternatively, the partial failure to reaccumulate Ca\(^{2+}\) could be due to structural or to permeability changes reducing the amount of Ca\(^{2+}\) stored in the S.R. Such changes would, of course, fit in with the dilated lateral vesicles observed by Schotland et al. (1965) in contractured fibres. However, these changes were not observed in the present material.

If a partial failure to reaccumulate Ca\(^{2+}\) is accepted as the cause of the stiffness, it could be argued that the weakness might also be due to the same cause, since less Ca\(^{2+}\) might be liberated by each nerve impulse. But it might then be expected that stiffness and weakness should occur together, whereas the history and the results of the double ischaemic work experiment suggest that they do not necessarily do so. This occasional divorce of weakness and stiffness might be explained by postulating the respiration-linked accumulation by mitochondria of such calcium ions (Lehniger, 1966) as were not taken up by the S.R., but it is clear that such speculations merely emphasize the need for more detailed knowledge. Further observations using the Natori technique might aid in resolving these problems.

**SUMMARY**

A 21-year-old man is described with the typical signs, symptoms, and findings of a deficiency of phosphorylase in his muscles. There was evidence also of early myopathic change in the muscles of his upper limbs. Muscle was biopsied before and in the sartorius after electrical stimulation sufficient to induce a contracture.

Single muscle fibres stripped of their sarcolemma at first responded normally with brief, reversible contractions to both Ca\(^{2+}\) and caffeine, but after five or more applications developed residual contractures. The fibres that had been electrically stimulated were extremely prone to develop residual
contractures, even after the first application. Those that were still in contracture following stimulation were relaxed by a Ca\textsuperscript{2+} chelating solution also containing ATP. The nature of the defect is discussed; the absence of a membrane in these preparations precludes the possibility that the effects seen were due to a membrane abnormality. It is suggested that Ca\textsuperscript{2+} reaccumulation by the sarcoplasmic reticulum becomes defective during vigorous exercise.

Light microscopy showed the usual glycogen containing subsarcolemmal blebs, early myopathic changes in the triceps seemingly unrelated to glycogen accumulation, and acute changes in some of the fibres that had been electrically stimulated. Electron microscopy confirmed most of the appearances noted in contractured fibres by Schotland et al. (1965), but the mitochondrial changes were more pronounced, especially the very considerable amount of glycogen within many of the mitochondria.

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