A mitochondrial myopathy characterised by a deficiency in reducible cytochrome b. By JA Morgan-Hughes, P Darveniza, SN Kahn, DN Langdon, RM Sherratt, JM Land and JB Clark (From the Institute of Neurology, Queen Square, London WC1 and the Biochemistry Department, St Bartholomew’s Hospital Medical College, London EC1) 


Hitherto, morphological changes seen in mitochondria obtained from samples of skeletal muscle in a variety of neuromuscular disorders have not been correlated with functional abnormalities and so have not been considered significant for the pathogenesis of these conditions. But more recently, investigators from Cornell University have linked these same ultrastructural features to a primary biochemical abnormality of mitochondrial function. The evidence hints at a disturbance of cellular respiration in the presence of various substrates. In Luft’s disease, hypermetabolism (not of thyroid origin) is observed but, for the rest, insights into the disease mechanisms are lacking. Now that situation changes. ‘The present paper reports the findings in a man whose weakness and striking muscular fatigability appeared to be due to a primary disturbance of mitochondrial function. Biochemical studies of isolated mitochondrial fractions have shown a specific deficiency of reducible cytochrome b’.

N.T., now aged 38 years, has had weakness and muscle fatigue since early childhood and has not been able to run or play games from his mid-teens. Nor was he able to continue his apprenticeship as a tailor through inability to work the foot-operated treadle of a sewing machine. Later, some bulbar symptoms emerged. Cold and alcohol seem to make him worse. On examination, he is short in stature with normal appearing if somewhat wasted muscles but showing weakness of the face (sparing the eyelids and eye movements), neck, and shoulder and pelvic girdles. The tendon reflexes are absent in the legs. ‘The most striking abnormality was a marked and progressive increase in muscle weakness during sustained activity…or repetitive manoeuvres…after a few minutes of complete rest, muscle strength returned to its pre-exercise level’. Screening investigations show only an excess of urinary lactate and pyruvate. Routine electromyography is equally unremarkable although his ability to exert maximal voluntary force at the elbow is reduced to 5.5 kg; and motor and sensory nerve conduction studies are also normal. Against this background, neurophysiological tests are carried out of electrical and mechanical responses to repetitive stimulation of the abductor digiti minimus and anterior tibial group, each constrained in a suitable jig for studying isometric responses to varying degrees of resistance, at rates that do not lead to fusion of the twitch responses, and before and after sustained voluntary muscle activity. Control observations on oxygen consumption and electrophysiology are made in ‘a healthy male subject’ (P.D.).

We might now ask how fair was this physiological comparison? Dr Paul Darveniza had arrived from Australia to work with John Morgan-Hughes bringing a record of sporting achievement that included international caps for Rugby Union although his career was curtailed by the courageous boycott taken by Paul and six other Wallabies in protest at handling of the 1971 tour from South Africa to Australia during the height of the apartheid influence on sport.

Next, John Morgan-Hughes and colleagues proceed to muscle biopsy from both the arm (for histochemistry and electron microscopy) and the leg (for in vitro mitochondrial studies and electron microscopy). Mitochondria are isolated for oxygen uptake studies and estimations made of pyruvate dehydrogenase and citrate synthase activity. The cytochromes are studied from low temperature spectra. On a separate occasion, N.T. undergoes exercise testing on a bicycle ergometer without added load for 5–15 min at a rate of around 10 km/h during which venous blood is sampled at regular intervals over 90 min and measurements made of serum lactate, pyruvate and ketones; and during a second test, N.T. is assessed for pulmonary respiratory function with measurement of exhaled oxygen and carbon dioxide. So what has been learnt?

First, over 75% of the type 1 and 2A muscle fibres are ‘ragged-red’ giving an intense reaction for succinic dehydrogenase, nicotinamide adenine dinucleotide (NADH)-tetrazolium reductase and lactate dehydrogenase, and with increased amounts of neutral lipid and periodic acid–Schiff (PAS)-positive material (Fig. 1). Perhaps, the affected fibres are slightly smaller than those that are histologically normal. Under electron microscopy both the arm and the leg muscles show separation of the myofibrils by tightly packed glycogen granules. But the striking feature is packing of the many mitochondria with an unusual matrix material and occasional dense crystalline inclusions orientated in the long axis of the organelle and with structures that produce a latticed arrangement of broad bars and cross striations (Fig. 2). Mitochondrial respiration rates for N.T. are markedly reduced in the presence of...
various substrates. Careful comparisons of the handling of pyruvate, succinate, glutamate and α-glycerophosphate, and measurements of mitochondrial pyruvate dehydrogenase and citrate synthase suggest that the citric acid cycle and the mitochondrial membrane system are intact. But crucially, although a normal complement of cytochromes is present, there is a marked reduction in the level of cytochrome b (<0.18 nmol/mg) representing <25% of that expected for other cytochromes (>0.4 nmol/mg; Fig. 3).

N.T. has a reduced cardiovascular response to exercise and is so weak that he has to be helped off the bicycle ergometer. Blood glucose and serum potassium rise transiently; but there are more prolonged increases in plasma lactate, pyruvate, acetocetate and 3-hydroxybutyrate with a corresponding fall in serum-free fatty acids (Fig. 4). The electrical correlates, both in the abductor digiti minimus and the anterior compartment of the leg, of his exercise-induced clinical exhaustion and these metabolic responses are a reduction, by 71%, of twitch tension that improves with rest—differing from the ‘normal’ subject both in their absolute reductions and variations following exercise.

Fig. 1 (A) Left biceps muscle stained with the modified Gomori trichrome method. Most fibres show abnormal subsarcolemmal deposits and possess a coarse punctate reticular network. (B) Increased subsarcolemmal activity of succinic dehydrogenase.

Fig. 2 (A) Transverse section through parts of three fibres from left biceps muscle (A, B and C). Fibre A is relatively normal in appearance; B shows an excess of peripherally located glycogen (g) situated between abnormally slender myofibrils; C contains a large subsarcolemmal accumulation of mitochondria (m) of widely varying size. Many of these have irregularly arranged cristae and a few contain dense rectangular inclusions. (B) Transverse section including two intramitochondrial crystals. (C) Non-scale diagrammatic representation of the structure and the location of an intramitochondrial crystal.
Whilst the concept of disordered mitochondrial function and the appearance of bizarre-looking mitochondria are well recognized, limited progress has been made in dissecting the biochemical defect present in any previous cases. 'The clinical, morphological and biochemical findings in the present case are all compatible with a primary disturbance of energy metabolism.' The magnitude of dissociation between electrical and mechanical responses during voluntary effort or repetitive nerve stimulation indicates that the fatigability results from failure of muscle contraction, unaltered nerve conduction or defects of the neuromuscular apparatus. The biochemical changes relating to exercise indicate a defect at the level of the mitochondrion and suggest a partially compensated lactic acidosis—

that, in passing, may indicate failure at a hepatic level to stimulate gluconeogenesis, perhaps explaining the additional effect of alcohol reported by NT—but without markedly impaired fatty acid oxidation. The in vitro mitochondrial studies reveal impaired respiration, unfluenced by artificial uncoupling indicating a defect of the electron transport chain rather than the phosphorylation system per se. Matters are clinched by the low level of reducible cytochrome b, relative to other cytochromes. Until now a deficiency of cytochrome b has only been reported in one pedigree manifesting as dementia, ataxia, proximal muscle weakness and reduced proprioception, and of cytochrome aa3 in a single example of Menkes disease. Can everything be understood in terms of perturbation affecting one biochemical pathway? A significant limitation of adenosine triphosphate (ATP) synthesis would be compensated by stimulation of muscle glycolysis but this will require an alternative acceptor having reducing properties equivalent to oxygen which, given the high lactate at rest and during exercise, identify this as pyruvate: 'the potential for exercise would be limited, therefore, by the ability of the plasma to buffer further increases in acid production'.

Thirty-one years on, John Morgan-Hughes recalls that work began early on the occasions when biopsies were taken. Their colleague Norman Grant removed a fair-sized chunk of N.T.’s arm and leg and John Land jumped on his bicycle (not N.T.’s ergometer) to race these down to St Bartholomew’s Hospital where John Clark (later appointed as Professor of Neurochemistry at Queen Square from 1990 to 2006, and Deputy Editor of Brain during the tenure of Ian McDonald) promptly got to work. As for N.T., he was treated gratis as a private patient visiting from northern Greece, remaining in London for over a year to participate in these investigations carried out using ad hoc equipment assembled in the day-room of Bentinck Ward at the
National Hospital, (courtesy of the ever obliging ward sister), including (Sir) Roger Bannister’s Douglas bag for measuring respiratory gases. Over this period, N.T. lived in a hostel run by the Greek Orthodox Church. When last in contact a few years ago, he was well. Although N.T. was easily exhausted by physical exercise, and had to be helped from his bike by the courteous John Morgan-Hughes, today’s mitochondriologists and neuro-myologists are more brutal. In being cruel to be kind, Marie-Louise Sveen and Julie Murphy, and their colleagues show (pages 2824 and 2832; and see page 2809) that endurance training may actually increase performance and muscle repair in Becker muscular dystrophy and the mitochondrial myopathies.

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