Modern Methods of Diagnosis of Muscle Diseases

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Muscle diseases are generally considered to be rare and therefore the province of the specialist. Yet there is no single specialty in medicine which sees all muscle diseases. At present patients with diseases or complaints referable to skeletal muscle find their way into the clinics not only of general physicians and neurologists but also those of rheumatologists, paediatricians, psychiatrists and orthopaedic surgeons. This is no surprise because skeletal muscle represents about 40 per cent (less in the diseases of muscle which result in wasting) of the body cell mass. Muscle does therefore reflect disorders in a variety of other body systems. The more a search is made for manifestations of disordered structure or function of muscle the more are changes found in diseases which are not at first sight classified as muscle diseases. Thus there are well-recognised changes of muscle structure or function in endocrine disorders whether or not there is weakness[1]. Patients with psychogenic weakness or fatigue of the kind which has variously been termed 'effort syndrome', neurasthenia, Da Costa's syndrome or vasoregulatory asthenia[2] may also have altered muscle mitochondrial function[3] which can result in excessive intracellular acidosis[4].

Skeletal muscle is not only the biological machine on which much of life and movement depends but it is also the tissue capable of the greatest range of alteration in its overall metabolic rate and most obviously related to the generation (and absorption) of mechanical forces. Not surprisingly, the factors that precipitate or aggravate muscle symptoms are those which pertain to energy exchanges or mechanical force. The 'machine' cannot be considered in isolation from its sophisticated control mechanisms, be they neural or metabolic. Because muscle is also the largest pool of protein in the body, those factors such as insulin, the sex hormones, prostaglandins or amino acids which influence protein metabolism in general also have an influence on size, structure or function of muscle.

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As yet the diseases of muscle in general and the inherited, progressive ones in particular (the dystrophies) tend to be named in 'syndrome' or descriptive terms, for example 'facioscapulohumeral muscular dystrophy' (FSH) with little knowledge of the underlying mechanism or explanation of the distribution of the pathological process except that the inheritance is clear (dominant in the case of FSH, see Fig. 1) and important for genetic counselling. New genetic studies will, it is hoped, help to explain why there is a high mutation rate in some muscular dystrophies (i.e. why so many patients have no family history) and confirm whether patients with the same clinical appearances have indeed got the same genetically determined disease. The inverse of this is whether there is a significant environmental influence on the course and distribution of the pathological processes, as suggested in the 'mechanical' hypothesis, to explain the largely proximal distribution of weakness in most myopathies[5]. Clearly the identification of the genetic defect in the dystrophies will have its most immediate and useful role in carrier detection and antenatal diagnosis[6].

With this background it is our purpose to present new developments in several fields that help in the diagnosis of muscle diseases. Some of these have been reviewed before[7] but this is a new appraisal of practical and cost-effective techniques we use in a clinical practice involving the diagnosis and management of paediatric and adult patients with muscle diseases.

Needle Biopsy

This technique is simple, harmless, rapid, and repeatable. It is, in our opinion, now the ethical alternative to open biopsy for the diagnosis of muscle diseases. Coupled with histochemistry and electron microscopy it is a powerful and cost-effective method of examining muscle for morphological and biochemical abnormalities with which there has now been years of experience[8]. The residual indications for open muscle biopsy are (a) motor point
biopsy for end-plate studies in myasthenia gravis, (b) the search for affected arteries in polyarteritis nodosa, (c) to obtain large samples of muscle for pharmacological investigation in suspected malignant hyperthermia or to obtain samples for isolation of muscle mitochondria[9]. Even here, recent developments in the needle biopsy technique make it possible to obtain adequate samples for such in vitro studies (Professor A. Stadthouder, Nijmegen, personal communication[10]). The most common criticisms levelled against needle biopsy are of poor orientation of cross-sections and over-contraction of sarcomere structure, and of not being sufficiently representative in heterogeneous (especially focal inflammatory) pathological conditions of muscle. These criticisms can now be countered in practice by orientating the specimen under a dissecting microscope before freezing and allowing the specimen for electron microscopy to ‘relax’ for some minutes before fixation. The problem of being representative applies equally to an open biopsy which is small in size in comparison with that of the muscle as a whole and which is usually more superficially located than a needle biopsy. This criticism can be countered by taking more than one needle biopsy through the same 4mm skin incision from widely separated superficial and deep sites in the muscle in those patients in whom a focal pathology is suspected. If this relativelyatraumatic approach is unsuccessful in yielding the information sought, there are still left the options of repeating needle biopsies at a future date from another muscle or reverting to open biopsy from the same or some other muscle.

In general the needle biopsy technique is sufficient to provide material for diagnosis and research and it is so valuable a diagnostic tool that it could (should) be carried out on all patients who are referred for diagnostic electrophysiology for suspected neuromuscular disease.

Needle biopsy of muscle and histological techniques are aimed at defining the state of the contractile machine. Conventional histological procedures demonstrate that muscle cells respond to damage in only a limited number of ways: atrophy or damage, adaptation to chronic mechanical stress by hypertrophy or splitting, and by-invasion (by inflammatory cells) or replacement with fat and fibrous tissues. Further information on the function of individual muscle fibres is obtained using frozen tissues and a variety of staining procedures in order to identify the activities of individual enzymes. In normal human muscle three different fibre types can be identified on the basis of their staining in the myosin ATPase reaction after pre-incubation at different pHs. Fibres in a given motor unit are all of one type and normal muscle consists of a mosaic of the different fibre types. In conditions resulting in denervation and reinnervation of muscle the mosaic pattern is lost and groups of fibres of one type are found.

Distinction between fibre types is also important in the recognition of disorders that predominantly affect a particular fibre type, e.g. congenital fibre type disproportion, where the diagnosis is made solely on the presence of small type 1 fibres, and the large range of disorders showing selective atrophy of type 2 fibres, e.g. steroid, hypothyroid and alcoholic myopathies. Other enzyme stains which are part of the routine armament of the muscle pathologist include stains for oxidative enzymes which are useful for displaying the disordered internal architecture of the fibres seen in many diseases of muscle. An example is shown in Fig. 2 where pale areas in many fibres enabled a diagnosis of ‘central core disease’ to be made. Of recent interest is the identification of a variety of metabolic myopathies in which abnormal enzyme activities can be identified histochemically or biochemically. The accumulation of substrates may give diagnostic clues, e.g. in type 2 glycogenosis (lysosomal acid maltase deficiency) there is increased muscle glycogen and in carnitine palmitoyltransferase deficiency there is increased lipid. Histochemical methods can also be used to assess the activities of specific enzymes. The best known example is that of McArdle’s disease (type V glycogenosis) where the absence of myophosphorylase activity can be readily identified histochemically. Phosphofructokinase deficiency, which presents clinically in a similar way to McArdle’s disease, can also be shown in biopsy specimens. Mitochondrial myopathies are conditions in which increased numbers of morphologically abnormal mitochondria are found in muscle fibres. This is best appreciated using electron microscopy but histologically a
The characteristic appearance of 'ragged red' fibres is seen on trichrome staining. Many different clinicopathological entities have been described which have this appearance on biopsy and, although they are presumed to be associated with disordered oxidative metabolism, in only a few cases have specific enzyme defects been identified, e.g. cytochrome C oxidase deficiency[11]. Myoadenylate deaminase (MAD) is another muscle enzyme whose deficiency may be of clinical significance. MAD deficiency was initially identified by routine screening of muscle biopsies[12] by workers who have suggested that deficiency may occur in up to 6 per cent of unselected biopsies. This has been disputed and experience in the UK[8,13] shows it to be far less common (<0.1 per cent), and indeed low levels can be found in other disorders, including Duchenne dystrophy. Clinically MAD-deficient patients suffer from mild exertional cramps but it is not clear to what extent these symptoms are related to the enzyme deficiency. Obviously there is great scope in the future for unravelling these metabolic myopathies and it may well prove practical to demonstrate them histologically as well as biochemically.

The recent revolution in general pathology following the introduction of immunohistological techniques and monoclonal antibodies has so far found limited application to muscle pathology. Its most significant application has been in elucidating the role of immune complexes and leucocytes in the polymyositis/dermatomyositis complex—immunoglobulins, complement and the various subclasses of T lymphocytes can now all be readily identified in cryostat sections[14,15]. Immunological methods for other components of muscle fibres, e.g. myoglobin, actin, myosin, and intermediate filaments are of limited diagnostic use except for the identification of the muscle origin of the various types of rhabdomyosarcoma. The main intermediate filament of muscle, desmin, is involved in forming the inclusion bodies of nemaline myopathy and is also increased in amount in regenerating cells[16].

Electron microscopy (EM) has a limited role in the diagnosis of muscle disorders. The ultrastructural changes seen tend to be non-specific and common to many human muscle disorders. The main use of EM is to confirm light microscopic findings of, for example, inclusion bodies or abnormal mitochondria.

**Measurement of Muscle Function**

The determination of the force of maximum voluntary contractions or those elicited by electrical stimulation at different stimulation frequencies is now seen as valuable in the assessment of possible drug effects on muscle or to follow the time course of disease. Measurements with a hand-held 'myometer' can be a useful adjunct to a physiotherapist's charting of muscle strength[17,18]. Serial force measurements can be a useful guide to muscle breakdown and repair in conditions such as polymyositis[19]. Electrical stimulation of muscle by an accessible motor nerve or percutaneously via large pad electrodes that stimulate the intramuscular nerves allows precise determination of the forces generated at different frequencies and the relaxation rate[20]. The maximum relaxation rate, determined as the first differential of the force signal, is a particularly useful measurement because it correlates closely with metabolism (determined as the heat production in the adductor pollicis)[21] both with ischaemia at rest and with fatiguing muscular activity. It is of great interest that these tests of muscle function vary with age, sex and nutritional status of the muscle[22,23] possibly due to effects of nutrition on the regulation of intracellular calcium concentration. Better known are the effects of endocrine disorders on muscle strength and relaxation rate[24]. Ischaemic exercise of forearm muscles followed by venous blood sampling for lactate and ammonia can be a valuable screening test for distinguishing a glycolytic defect (producing no lactate) from myoadenylate deaminase deficiency (producing no ammonia) with exercise[25].

**Electrophysiology**

Conventional electrophysiology contributes evidence based largely on recordings of action potentials that can help to establish whether the muscle wasting or weakness is primarily 'neuropathic' or 'myopathic'. Repetitive nerve stimulation is also a valuable test in the diagnosis of myasthenia gravis. New techniques involving computer analysis of the electrical recordings are now providing the opportunity to analyse the function of individual motor units.
Electromyographic techniques play an important part in the diagnosis of neuromuscular diseases and an increasing role in the understanding of the physiology of normal and abnormal muscle. With the introduction of special types of recording electrodes many new analytical techniques have been developed. The core of a single-fibre electrode (Fig. 3a) is 25 μm in diameter, comparable to the diameter of a normal muscle fibre, and the electrode records selectively a very small number of fibres. This electrode is widely used to study 'jitter' of pairs of potentials (Fig. 4), a sensitive index of the stability of neuromuscular transmission[26]. Neuromuscular transmission is disturbed in regenerating potentials[27] as well as in myasthenia gravis. A finding of abnormally low jitter occurs when muscle fibres are split[28]. By counting the frequency with which linked pairs of muscle fibres are recorded on random insertion into the muscle, the fibre density is measured, an index of the fine morphology of the muscle, increased in dystrophies as well as in denervation[29]. The macro-electrode (Fig. 3b) is an adaptation of the original single-fibre electrode in which the proximal part of the shaft is insulated and the distal part left exposed. A single fibre potential recorded from the core is used to trigger an averager and the macro-potential is then averaged between the exposed tip of the shaft and a remote surface electrode. The macro-potential is an indicator of the cross-sectional area of the motor unit[30].

The concentric needle electrode (Fig. 3c) has a core of 150 μm diameter and records motor unit potentials (MUP) from up to 12 of the closest fibres of an active motor unit[31]. This electrode remains the standard tool for diagnostic electromyography. Microprocessors are starting to make automatic measurement of MUP a reality in the clinical service department, bringing increased objectivity to the assessment of MUP[32]. In addition, computer modelling of MUP has given new understanding of the origin of the MUP and the conduction of the potentials through the complex ionic environment of the muscle[33,34]. The interpretation of MUP is expanding from the empirical associations of parameters with primary muscle disease or chronic denervation, established by the pioneers of the subject[35,36], to include the interpretation of those parameters in terms of the geometry of the electrical generators.

The interpretation of interference patterns, the complex activity recorded in a muscle at stronger contraction effort when multiple MUPs are superimposed, requires automated methods of signal analysis in time or frequency domains[37,38]. Methods of analysis which were developed with specifically designed hardware are now being implemented in general purpose microprocessors, making them much more readily available for diagnostic use. Analytical methods which deconvolute the interference pattern into its constituent potentials give additional insight into the functioning of muscle but require very considerable computing power and are confined to a few research laboratories[39].

**Magnetic Resonance Spectroscopy in the Study of Myopathy**

This is a new technique which offers much in the understanding of muscle energy metabolism and chemical content. A typical 31P MR spectrum of normal muscle (Fig. 5) shows the key energy metabolites of phosphocreatine (PCr), adenosine triphosphate (ATP) and inorganic phosphate (Pi). It is now possible to study non-invasively this high-energy phosphate metabolism along with the intracellular pH of muscle. This has come about with the advent of wide-bore magnets and the application of high resolution magnetic resonance spectroscopy (MRS) using surface coils. In principle MRS is a method of obtaining information about the behaviour and content of nuclei in differing chemical environments through interrogation with weak radio-frequency pulses.

Pioneering studies of topical 31P MRS in human muscle metabolism have come from Oxford[40-42], Philadelphia[43,44], and London[45,46]. A recent review[47] discusses this new technology. The first clinical application of 31P MRS[48] was in a patient with McArdle’s syndrome (myophosphorylase deficiency) in which lactic acid accumulation during ischaemic exercise is prevented by the failure of glycogenolysis. It was possible to show non-invasively the lack of an acid shift in the intracellular pH which normally occurs with lactic acid accumulation during ischaemic exercise. Phosphofructokinase (PFK) deficiency is a more rare glycolytic defect which presents with a similar clinical picture as
myophosphorylase deficiency. The MRS findings are strikingly characteristic, with a steady accumulation of a $^3$P sugar phosphate resonance during exercise, consistent with the level of the block in the glycolytic pathway. This MRS finding initially reported[45] and confirmed[44] in 1982 is illustrated in yet another patient (Fig. 6). The ability to repeat the measurements non-invasively enables changes with exercise to be followed (Fig. 7). By studying the kinetics of the metabolite changes it is possible to differentiate PFK deficiency from phosphoglyceratekinase deficiency[49].

Patients with mitochondrial myopathies have been studied[41,47,50]. At rest various abnormalities could be demonstrated of reduced PCr, high Pi, high calculated ADP, and in some abnormal intracellular pH. The kinetics of PCr metabolism, best seen in two patients with NADH-CoQ reductase deficiency[40] and in a defect in pyruvate oxidation[51], show excessive PCr utilisation with exercise and slowed oxidative recovery. Excessive intracellular acidosis on exercise has been demonstrated by MRS in a patient with a post-viral fatigue syndrome[4].

The question whether a reduced energy state exists in
dystrophic muscle has been studied by MRS, at rest and with exercise in boys with Duchenne muscular dystrophy[32]. The considerable problems of tissue standardisation, which complicate conventional analysis due to fat and fibrous tissue derangement of the muscle, have been addressed by a combination of $^1$H, $^{31}$P MRS and morphometry of needle biopsies. The ATP content was not reduced in the dystrophic muscle, and was not altered in a double-blind controlled trial of allopurinol. A lower PCr and higher Pi content was found indicative of a reduced phosphorylation potential, yet in a disease characterised by a massive leak of the enzyme creatine kinase no defect of intracellular activity of this enzyme could be demonstrated, with a normal oxidative PCr recovery.

Proton $^1$H MRS offers exciting possibilities now that techniques to suppress the dominant water resonance have been developed[53]. The combination with $^{31}$P MRS[54] allows lactate measurement to be added to the phosphate metabolite information. The study of urinary organic acids with $^1$H and the measurement of carnitine and acyl-carnitines following carnitine infusion[55] open up the possibility of studying the disorders of fat metabolism in muscle, which so far by $^{31}$P MRS have proved difficult[56]. The $^{13}$C techniques[57], along with $^{13}$C labelled glucose infusions[58], open up a large field of study, particularly that of glycolytic metabolism. As whole-body spectrometers (as in Oxford and shortly in Liverpool) become available, the study of a myopathy in relationship to other organ/system involvement will be a practical but expensive research and possibly diagnostic opportunity.

Imaging of Skeletal Muscle

It is obvious that a wasted muscle is weak but it is not so clear whether the muscle, whatever its size, can develop the force expected of it. What then is the force expected? It is proportional to the total cross-sectional area of the actomyosin interactions, which is only approximately proportional to the cross-section of the muscle as a whole. Since it is not possible to determine this from surface measurements (despite attempts to allow for the thickness of subcutaneous fat) there is interest in the use of modern imaging techniques, ultrasound (US)[59-61], X-ray computerised tomography (CT)[62] and magnetic resonance imaging (MRI)[63]. Each has its advantages and limitations but all can not only give an accurate measurement of the cross-section of the muscle but also an indication of the composition of the muscle (i.e. whether it has been replaced with fat or fibrous tissue as typically occurs in muscular dystrophy[62,64,65]). Radio-nucleide (e.g. 99m pyrophosphate) uptake in skeletal muscle is also a means of imaging muscle damage as in inflammatory myopathy[65] and following mechanical damage to muscle[66].

Conclusions

Several techniques are now becoming available to visualise the structure, electrical properties, contractile capabilities and metabolism in human muscle. As diagnostic tools not one of them is uniquely sufficient but in combination they promise rational explanations for the disorders of muscle structure or function that are currently known in descriptive terms as syndromes. However, these new opportunities for studying muscle also yield more descriptive pathological and biochemical diagnoses (e.g. ‘central core disease’ or ‘mitochondrial myopathy’) which require further analysis.

Accurate (or in practice ‘as accurate as possible’) diagnosis is necessary in this class of disease in which there are important differences in prognosis and inheritance. The new genetics, with the possibility of soon identifying the abnormal gene in the muscular dystrophies, offer an important theoretical and practical tool to determine the precise genetic diagnosis both antenatally and for a patient as well as for genetic counselling. It should also help to resolve the question as to whether patients with apparently similar clinical appearances have indeed the same muscle disease. Variation in severity could be due to environmental (diet or exercise) influences which might then be explored with possible therapeutic benefit. The field of interest in clinical muscle science is advancing rapidly, with several developments in basic science and technology, and it is therefore with guarded optimism that we and others look to a more promising therapeutic future for this class of diseases for which there is currently little or no effective treatment.

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