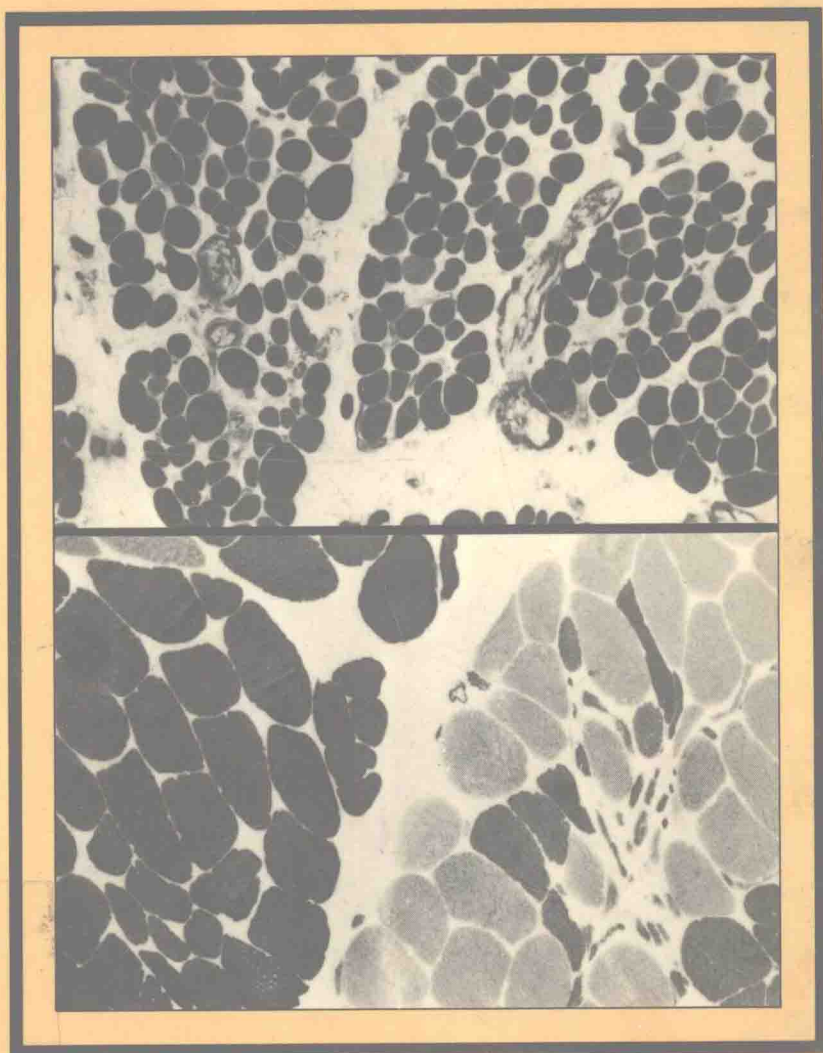# Biopsy Pathology of Muscle

M. Swash and M. S. Schwartz

Biopsy Pathology Series

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Biopsy Pathology of Muscle

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Preface

During the last 20 years the development of enzyme histochemical techniques has contributed greatly to knowledge of muscle pathology. However, these and other new methods, such as electron microscopy and immunocytochemistry, have only relatively recently become generally available for routine use in histopathology. Muscle biopsy is a long-established technique in clinical practice, having been introduced by Duchenne in 1868 (*Arch. Gen. Med.*, **11**, 5–179). However, the needle method used by Duchenne was not generally adopted, although Shank and Hoagland described a similar technique in 1943 (*Science*, **98**, 592). During this time muscle biopsies required a surgical procedure and this was a considerable disincentive to their use. It was not until Bergstrom (1962; *Scand. J. Clin. Lab. Invest.*, **14**, Suppl. 68) and Edwards (1971; *Lancet*, **ii**, 593–6) developed a simple biopsy needle suitable for muscle work in connection with exercise physiology that the advantages of needle muscle biopsies came to be appreciated. Since then, muscle biopsies have become a relatively minor procedure. This has led to the increasing use of muscle biopsy in clinical practice, both for diagnosis and for assessing progress in repeated biopsies during the course of a disorder and its treatment. The full range of enzyme histochemical and ultrastructural histological techniques can be applied to these small biopsies and many of the older histological staining methods can also be used.

This book is intended to serve as a practical guide in muscle pathology, particularly for histopathologists, and for those in training. As enzyme histochemistry has become more widely available, formalin-fixed methods, with their inherent limitations, have become less frequently used in muscle biopsy work, and there is a thus a need for a short but complete account of muscle biopsy pathology. In this book emphasis is placed on those features which are of specific diagnostic value, or which reflect changes occurring during treatment, as in polymyositis, or at different stages of neuromuscular disorders. Electron microscopy is largely a research tool and ultrastructural features are discussed only

when they are relevant to the investigation of particular disorders. Similarly, although quantitative methods are applicable to muscle biopsy pathology, microcomputer-based measurements are not generally available. Nonetheless, simple and accurate measurement can be made without technical aids of this sort and these are described. We have tried to illustrate the major problems likely to be encountered in all but the most specialized referral centres, and have provided sufficient references to guide our readers in any further exploration of the literature they may wish to undertake. A description of the clinical and physiological features of neuromuscular disorders is available in our earlier book *Neuromuscular Diseases: A Practical Approach to Diagnosis and Management*.

No book can be written without help from colleagues. We thank particularly Dr David Pollock, who kindly reviewed our account of muscle tumours as revealed by muscle biopsy, and helped with the illustrations for this chapter. Mr Ivor Northey and Mr Tim Bushnell in the department of medical photography of the Institute of Pathology at The London Hospital Medical College kindly prepared the illustrations. The work which has led to the preparation of this book has been supported, at least in part, by The London Hospital Special Trustees, The Wellcome Trust, The Medical Research Council and The London Hospital Medical College, and we gratefully acknowledge this support. A number of the illustrations are taken from our previous publications in various journals and these are reproduced here, with permission, as follows: Fig. 2.7, *Neurology (Minneapolis)*; Figs 2.13, 4.12, 7.3, 7.5 and 8.4, *Journal of Neurology, Neurosurgery and Psychiatry*; Fig. 3.5a, b, *Journal of Anatomy*; Figs 4.12c, 6.17 and 8.4, *Brain*; Figs 4.3 and 8.14, *Muscle and Nerve*; Figs 4.6, 4.7, 5.7, 5.8, 5.13, 7.1, 7.8, 8.9 and 9.4 are reproduced from our book, *Neuromuscular Diseases: A Practical Approach to Diagnosis and Management*, Springer-Verlag, Berlin, Heidelberg, New York (1981) (316 pp.).

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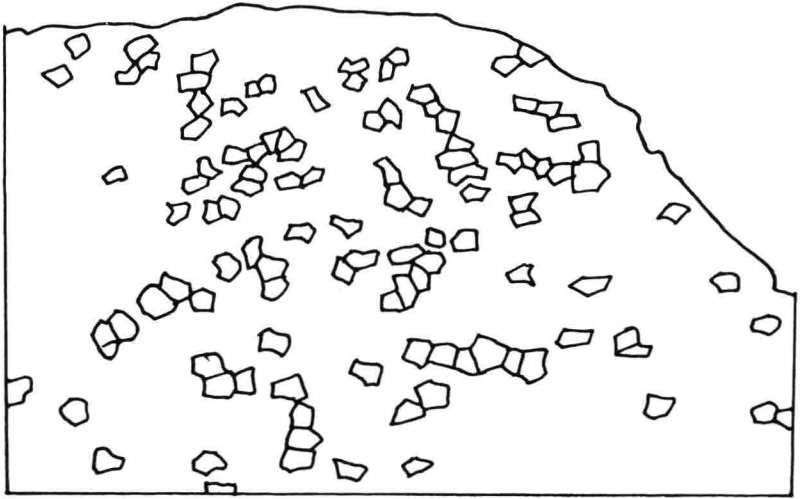
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Fig. 1.1 (a) The muscle fibres belonging to a single motor unit are distributed quasi-randomly through part of the muscle. This drawing of the cross-sectional plane of a cat soleus muscle is taken from the work of Edstrom and Kugelberg (1968). The motor unit was identified by glycogen depletion after supramaximal stimulation of a single efferent nerve fibre in the appropriate ventral root. (b) Drawing of the territories of three separate motor units in the cat soleus (after Edstrom and Kugelberg, 1968).

units (Edstrom and Kugelberg, 1968; Brandstater and Lambert, 1973). The individual motor units cannot be recognized in specimens of muscle without special physiological/pathological correlation techniques.

Muscle fascicles (Fig. 1.2) are separated from each other by connective tissue which itself contains neurovascular bundles. Muscle spindles are situated in close association with these neurovascular bundles. The fascicular pattern of individual muscles varies. The length of muscle fibres in different muscles varies greatly, from a few millimeters to as much as 40 cm. Within a fascicle the muscle fibres are arranged in parallel, but individual fascicles are not usually arranged in the plane of the long axis of a muscle, but in a bipennate distribution inserting into a centrally situated tendinous plane. This arrangement may or may not be symmetrical, and there is great variability between different muscles. Golgi tendon organs are found in the major tendinous insertions and origins of muscles. The nerve supplying a muscle usually enters it, with its accompanying blood vessels, near the mid-point of the muscle.

The pathological features of diseases of muscle are determined by the disease process itself, by the intrinsic properties of muscle and by the close structural/functional relationship of muscle and its nerve supply. Thus diseases of muscle are broadly classified into *myopathies*, in which the disorder primarily affects the muscle fibres, and *neurogenic disorders*, in which muscular abnormalities result from disturbance of the innervation. By historical convention certain inherited myopathies are classified as *muscular dystrophies*, a term which implies a progressive course with marked change in the normal structure of the muscles.

1.3 Classification of neuromuscular disorders

There are a large number of different disorders in which muscle may be affected but, in the majority of these, muscle biopsy is not a useful investigation since it does not provide specific diagnostic information, and a diagnosis can be obtained more easily by other methods. For example, peripheral neuropathies, in which marked abnormalities occur in affected muscle, are usually diagnosed by clinical and electrophysiological techniques, and occasionally by nerve biopsy. In addition, many of the disorders noted in comprehensive classifications of neuromuscular disorders are extremely rare. The classification given here (Table 1.1) includes those disorders likely to be encountered in the pathological laboratory. A more complete classification is available (Walton and Gardner-Medwin, 1981). The most common condition in which muscle biopsy is likely to be performed is polymyositis, but this is not the commonest neuromuscular disorder; for example diabetic polyneuropathy is far more frequent.
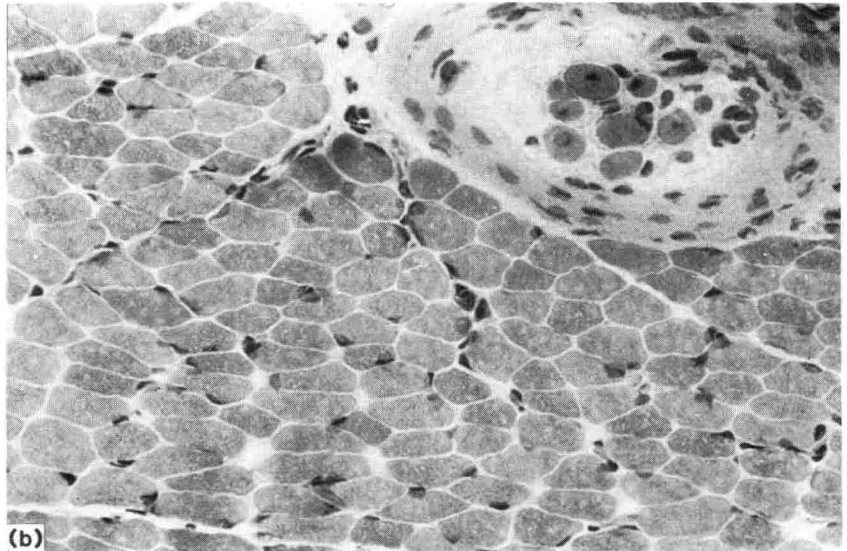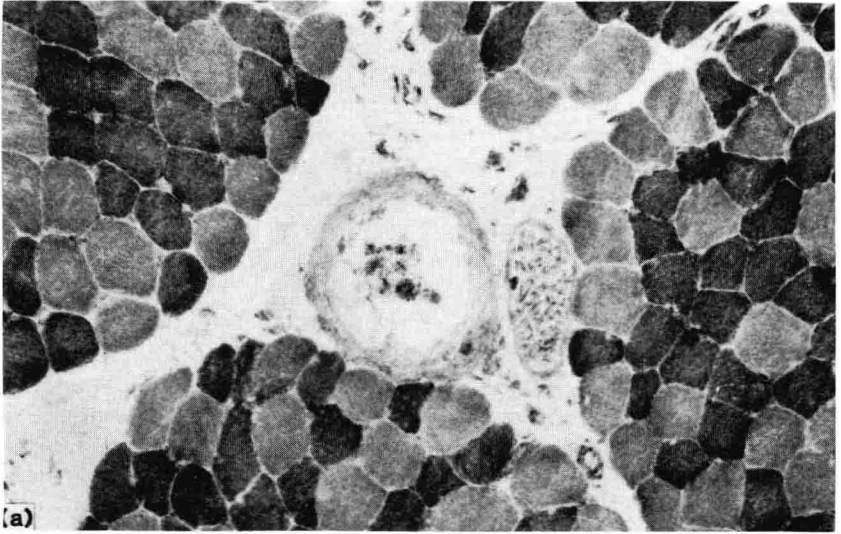
Fig. 1.2 (a) × 140; NADH. Normal muscle. A muscle spindle is seen in the interfascicular plane at the junction of several fascicles in the centre of the illustration, close to a small intramuscular nerve and a blood vessel – the neurovascular bundle. Two fibre types can be identified in the extrafusal muscle fibres; the intrafusal muscle fibres are very small and react intensely in this technique. (b) × 350; HE. Normal muscle spindle in a muscle biopsy from an infant. The intrafusal and extrafusal muscle fibres are approximately equal in size.

Table 1.1 A classification of neuromuscular disorders (modified from Swash and Schwartz, 1981)

MYOPATHIC DISORDERS

1. *Inflammatory myopathies*
   (a) *Idiopathic*
       Polymyositis
       Dermatomyositis
       Childhood dermatomyositis
       Polymyositis and dermatomyositis associated with carcinoma
       Polymyositis and dermatomyositis associated with collagen–vascular disease
       Granulomatous polymyositis (sarcoidosis)
       Eosinophilic polymyositis

   (b) *Infections*
       Viral
       Bacterial
       Infestations

2. *Drug-induced myopathies*

3. *Endocrine myopathies*
       Thyroid myopathies
       Osteomalacia and parathyroid disease
       Acromegalic myopathy
       Steroid myopathy

4. *Genetically determined myopathies*
   (a) *Muscular dystrophies*
       Duchenne muscular dystrophy
       Becker muscular dystrophy
       Limb-girdle muscular dystrophy
       Facio-scapulo-humeral muscular dystrophy
       Scapulo-peroneal syndrome
       Oculo-pharyngeal muscular dystrophy
       Ocular myopathy

   (b) *Myotonic syndromes*
       Myotonic dystrophy
       Myotonia congenita
       Other myotonic syndromes

   (c) *Metabolic myopathies*
       Glycogenoses, e.g. McArdle's disease
       Disorders of lipid metabolism, e.g. carnitine deficiency
       Mitochondrial myopathies
       Periodic paralyses
       Malignant hyperpyrexia
       Myoglobinurias

(d) *Benign myopathies of childhood*
    Central core and multicore disease
    Myotubular myopathy
    Nemaline myopathy
    Congenital fibre-type disproportion
    Congenital muscular dystrophy
    Other rare syndromes

NEUROGENIC DISORDERS

1. *Disorders of anterior horn cells*
    Spinal muscular atrophy (SMA)
      Type 1 Werdnig–Hoffmann disease
      Type 2 Intermediate SMA
      Type 3 Juvenile onset Kugelberg–Welander disease
      Type 4 Adult onset
    Motor neuron disease
    Poliomyelitis
    Other anterior horn cell disorders

2. *Disorders of motor nerve roots*
    Cervical and lumbar spondylosis with root compression
    Malignant infiltration of nerve roots
    Brachial neuritis (neuralgic amyotrophy)

3. *Peripheral neuropathies*
  (a) *Acquired polyneuropathies*
    (i) *metabolic*
      Diabetes mellitus
      Alcoholic neuropathy
      Renal and hepatic disease
      Vitamin deficiencies, e.g. B_{12} deficiency
    (ii) *Inflammatory polyradiculoneuropathy* (Guillain–Barré syndrome)
    (iii) *Drug-induced and toxic neuropathies*
      e.g. tri-ortho-cresyl phosphate poisoning, isoniazid neuropathy
    (iv) *Associated with malignant disease*
    (v) *Infections*, e.g. leprosy, diphtheria
    (vi) *Associated with collagen vascular disease*
      e.g. polyartertis nodosa and other vasculitides, rheumatoid arthritis

  (b) *Acquired mononeuropathies*
    Entrapment and compressive, e.g. carpal tunnel syndrome
    Traumatic
    Mononeuritis multiplex

  (c) *Genetically determined polyneuropathies*
    Charcot–Marie–Tooth syndrome
    Hereditary sensory neuropathies
    Amyloid neuropathy
    Porphyric neuropathy
    Metachromatic leukodystrophy
    Other rare syndromes, e.g. Refsum's disease

4. *Disorders of Neuromuscular transmission* (end plate disorders)
   Myasthenia gravis
   Myasthenic syndromes

1.4 Clinical features of neuromuscular disease

In the majority of patients in whom muscle biopsy is performed, the major problem is *weakness*. In most primary disorders of muscle, i.e. the myopathies, weakness is predominantly proximal, affecting the pelvic girdle muscles more severely than the shoulder girdle muscles. Proximal weakness is also a feature of spinal muscular atrophy, a disorder in which muscular weakness and atrophy develops as a result of progressive loss of anterior horn cells. In some peripheral neuropathies in which there is involvement of spinal roots as well of the peripheral nerves, e.g. Guillain-Barré polyradiculoneuropathy, proximal weakness may also occur but, in general, peripheral neuropathies are characterized by distal weakness, which is usually symmetrical, and distal sensory loss or paraesthesiae. In peripheral neuropathies the tendon reflexes are often absent, particularly the ankle and finger jerks and, if present, atrophy is also characteristically distal. In myopathies the tendon reflexes are usually present, although in Duchenne dystrophy, and in myotonic dystrophy, they are often reduced.

In most inherited myopathies and dystrophies, *proximal weakness* is symmetrical, but particular muscle groups are often selectively involved. For example, in Duchenne dystrophy, weakness of the hip flexors and extensors, quadriceps and tibialis anterior is often prominent. In limb–girdle muscular dystrophy the biceps and periscapular muscles are particularly weak and in facio-scapulo-humeral muscular dystrophy, facial, triceps, biceps and periscapular muscles are mainly affected. Other rare variants, such as scapulo-peroneal atrophy and quadriceps myopathy, are recognized. In myotonic dystrophy facial and distal limb weakness, also involving small hand muscles, is characteristic, and there is marked weakness and atrophy of neck flexor muscles.

Pseudohypertrophy, a clinical phenomenon in which weak muscles appear enlarged and unusually firm to palpation, is a particular feature of Duchenne dystrophy, in which it especially involves periscapular, deltoid and gastrocnemius muscles, but it also occurs in some patients with limb–girdle dystrophy and, rarely, in hypothyroid myopathy and certain other metabolic myopathies. Involvement of the bulbar muscula-ture is characteristic of myasthenia gravis and motor neuron disease. It also occurs in myotonic dystrophy and in the rare disorder, oculo-pharyn-geal dystrophy, but it is uncommon in other myopathies.

Muscular wasting is a feature of most progressive myopathies, and usually occurs in the distribution of the weakness. However, wasting is also a feature of neurogenic disorders. In the latter wasting may be accompanied by spontaneous fasciculation. This is an important and diagnostic feature of motor neuron disease, but it also occurs in some patients with spinal root lesions and, rarely, in thyrotoxicosis. In spinal muscular atrophies spontaneous fasciculation is uncommon, but fasciculation may occur after exercise and coarse tremulous contraction of individual muscle bundles can be recognized. In Werdnig–Hoffmann disease (infantile spinal muscular atrophy), however, fasciculation occurs at rest, especially in the tongue.

Myotonia consists of persistent contraction of a muscle or of part of a muscle after cessation of voluntary contraction. It can be recognized by its electromyographic features. It is usually a familial disturbance and may occur as an isolated clinical finding (e.g. myotonia congenita) or in association with other features of neuromuscular disease as in myotonic dystrophy or periodic paralysis.

Fatiguability is a common symptom noted not only by patients with neuromuscular diseases, but by patients with depression and as a reaction to undue psychological stress. It is thus difficult to define unless it is accompanied by definite evidence of a decrease in effort tolerance, or by the development of objective muscular weakness during exertion or movement. Fatiguability is particularly associated with myasthenia gravis. In this disorder weakness can usually be demonstrated by clinical tests of individual muscles during tonic or repeated movement. Commonly used tests include fist-clenching, abduction of the shoulder, and prolonged upward gaze leading to ptosis, but bulbar weakness is often evident during prolonged conversation or chewing. Fatiguability is also a feature of motor neuron disease, and of certain metabolic myopathies, particularly the mitochondrial myopathies. In polymyositis fatiguability is also a common complaint, especially in the early stages.

Muscular pain and stiffness occurs in inflammatory myopathies, in which it is particularly prominent in the mornings, or after rest, and may be particularly relieved by exercise. Similar symptoms occur in patients with inflammatory joint disease and it may be difficult for the clinician to be certain whether or not there is muscular involvement in such cases. Muscular pain is a particularly prominent feature of polymyalgia rheumatica, although weakness is usually only slight in this condition. Muscular pain also occurs in certain metabolic myopathies, especially myxoedema and McArdle's syndrome of myophosphorylase deficiency. In these patients muscular pain, stiffness or cramp usually develops during exercise, although it may be relieved with continued exercise. In neurogenic disorders, including peripheral neuropathies, muscular pain

is unusual. However, cramps at rest and with exercise are common in motor neuron disease, and severe muscular pain and tenderness may occur in Guillain-Barré syndrome and in alcoholic neuropathy.

Many neuromuscular diseases are accompanied by features of *involvement of other organs*. For example, in inflammatory myopathies there may be skin or joint involvement, in myotonic dystrophy cataract, diabetes mellitus and other features may coexist, in Duchenne dystrophy cardiac involvement is common, and in certain hereditary neuropathies pes cavus and other skeletal deformities are often found. These additional features may lead to recognition of the hereditary nature of the disorder. Careful enquiry about a possible family history is always important in the diagnosis of neuromuscular disorders.

The *age of onset* of symptoms is also important in diagnosis. In general, most hereditary conditions have specific patterns of presentation and progression and these can be readily recognized by experienced clinicians.

1.5 Methods of investigation

A wide variety of different tests can be used to assess patients with neuromuscular disorders but most of these are applicable only in rare instances. For example; the ischaemic lactate test is used in the diagnosis of McArdle's myophosphorylase deficiency. A low blood potassium level, often induced by exercise or by a glucose load, is important in the diagnosis of hypokalaemic periodic paralysis; various quantitative biochemical assays are used in the diagnosis of metabolic myopathies; thyroid function tests are useful in thyrotoxic and hypothyroid myopathies. Tests for myoglobinuria may be helpful on some occasions; and measurement of serum immunoglobulins can be used in the diagnosis of some inflammatory myopathies, paraprotein-associated neuropathies, and polyarteritis. However, the most useful laboratory test is the measurement of 'muscle enzyme' levels in the blood.

Although a number of different enzymes may be released from muscle in neuromuscular diseases, only aldolase, pyruvate kinase and creatine kinase (CK) levels are useful in diagnosis. In most laboratories CK levels are preferred, since they provide a more sensitive indication of active muscular disease. The CK level is raised when muscle breakdown occurs, or when the muscle fibre membranes are abnormal, as in Duchenne dystrophy, allowing the muscle CK_{MM} isoenzyme to leak from the muscle cells into the circulation. In most laboratories CK isoenzyme assays are not available and the total venous blood CK level is measured. It is sometimes important to recognize that CK levels are affected by exercise and by the phase of the menstrual cycle, particularly if CK levels

are being used in young women for genetic counselling when there is a risk of the carrier state for Duchenne dystrophy.

Since the CK level varies with the extent of muscle fibre damage, and with the muscle bulk, the level tends to be highest in the early and most active stages of a disease, and to be lowest at the end-stage, when there is marked muscle atrophy. The CK level is also relatively low during healing stages of inflammatory myopathies, and it may fall during steroid therapy, even if the disease remains active. In metabolic myopathies the CK level is usually normal since muscle destruction is not a feature of these disorders; if muscle fibre necrosis occurs, as in McArdle's disease, the CK level may be transiently raised. In neurogenic disorders the CK level is usually normal, but it may be slightly raised in chronic neurogenic disorders.

In the past, 24-hour urinary creatine/creatinine ratios were used for the diagnosis of progressive muscular disorders as an index of muscle bulk and muscle cell necrosis, but they are now little used. Recently, the 24-hour urinary 3-methylhistidine excretion has been used as a measure of muscle catabolism.

Electrocardiography is an important investigation since it provides evidence of an associated cardiomyopathy, e.g. in Duchenne dystrophy and Friedreich's ataxia.

Electromyography (EMG) is much used in the diagnosis of myopathic and neurogenic disorders. The technique consists both of needle electrode sampling of electrical activity in muscles at rest and during slight, graded muscular contraction, and of measurement of motor and sensory nerve conduction velocities. In the investigation of proximal weakness, the commonest presenting feature of patients submitted to muscle biopsy, needle EMG is particularly useful. Spontaneous electrical activity in resting muscle is uncommon except in neurogenic disorders, in which *fibrillations* representing spontaneous contractions of denervated muscle fibres, and *fasciculations*, representing spontaneous firing of parts or all of a motor unit, may occur. Fibrillations also occur rarely in myopathic disorders, in which segmental muscle necrosis may cause denervation of part of a muscle fibre by separating it from its nerve supply. Myotonic discharges can also be recognized during electrode insertion or movement.

During voluntary activation of a muscle the electrophysiological features of individual motor unit action potentials can be recognized, and the pattern and extent of their recruitment during increased activation can be studied. In neurogenic disorders individual potentials are larger and more complex than normal, owing to enlargement of the motor unit by reinnervation from axonal sprouts derived from surviving motor units. In myopathic disorders, however, individual potentials are smaller