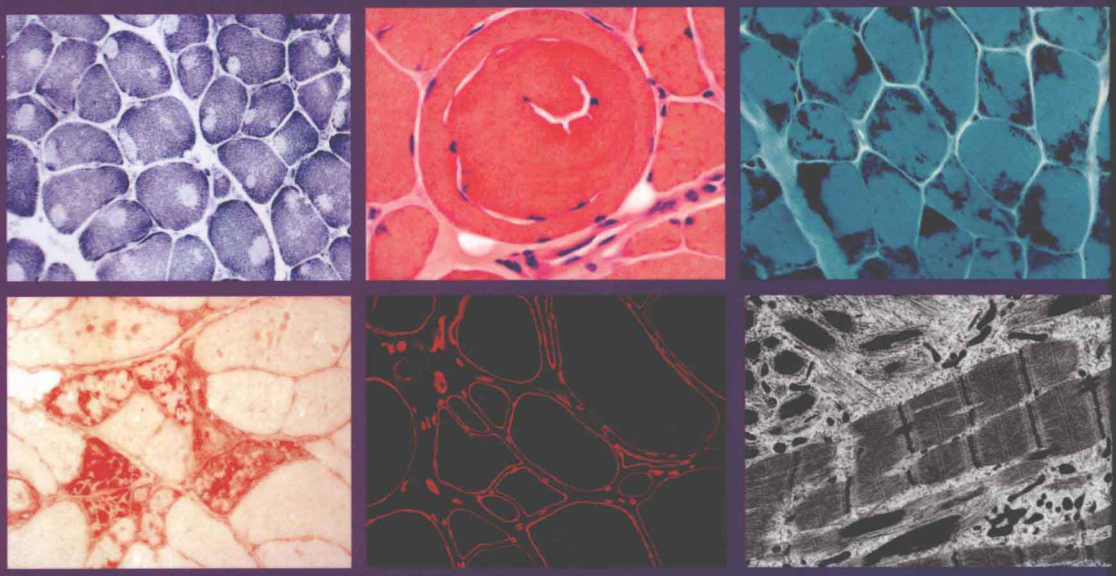
THIRD EDITION

Muscle Biopsy

A Practical Approach



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A Practical Approach

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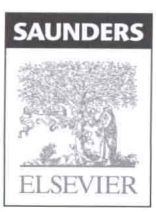

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MUSCLE BIOPSY

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Preface to the third edition

When Mike Brooke and I did the first edition of Muscle Biopsy in 1973, our main objective was to bring the application of the newly established enzyme histochemical techniques on rapidly frozen samples, as well as electron microscopy, to the routine study of muscle biopsies. This was rapidly achieved and within a few years most laboratories processing muscle biopsies were routinely identifying the basic fibre types and selective pathology in relation to them.

By the time of the second edition in 1985, there had been further major developments, particularly in relation to the introduction of immunohistochemistry and the use of specific antibodies. I was fortunate to have the contribution of a chapter dedicated to this early application of the technique to muscle pathology from Caroline Sewry and Robin Fitzsimmons.

Over the past two decades, there has been a tremendous advance in relation to the molecular genetic identification of many individual muscle disorders. Thus, for example, limb-girdle muscular dystrophy, which was initially looked upon as a single recessive disorder, now has 18 different genetic entities and, similarly, congenital muscular dystrophy has at least 10. This was also associated with a further quantum leap in immunohistochemistry and development of individual antibodies to specific proteins related to these genetic disorders.

This has now become a major player in the armamentarium of diagnosing neuromuscular disorders and I am extremely pleased that Caroline Sewry has agreed to come on board as a full co-author of this new edition, which has been totally revised and restructured. Caroline has been closely associated with the diagnostic and research activities of the Neuromuscular Unit at Hammersmith Hospital ever since its inception soon after my appointment to the Chair of Paediatrics in 1973.

We have retained a basic structure of the text, as in the earlier editions, with a fully comprehensive review of both normal and diseased muscle, using standard techniques of histology and electron microscopy and also the specific contributions of enzyme histochemistry, and protein specific immunohistochemistry.

The majority of illustrations are now in colour, compared to the black and white of the previous edition, and the immunohistochemistry is a major component of the book.

The molecular genetic advances have brought new clarity to the neuromuscular disorders but have also created complexity and some confusion. The same pathology may be related to different genetic disorders and, conversely, some genetic disorders may be associated with different clinical syndromes and different pathological features. This has raised controversy as to the appropriate nomenclature, with the geneticists and biochemists on one hand wanting to relate the diagnosis to the underlying abnormality, and the pathologists and clinicians on the other hand, wanting to retain some handle of diagnosis still based on the clinical presentation and pathological picture, which may be the initial diagnostic features following the patient's presentation.

The wider use of immunohistochemistry has thrown further light on the fibre types within muscle and the use of antibodies specific to different isoforms of fast and slow myosin, as well as neonatal and fetal isoforms, has opened the way for more specific designation of the fibre type profile within pathological muscle. This is also providing some insight into the pathogenesis of some of the disorders.

This is still a rapidly growing and expanding field and the coming years will undoubtedly see further major advances. Our current aim has been to provide an up-to-date and comprehensive overview of muscle pathology and to include clinical and molecular details that are relevant to the pathologist, in order to provide sufficient understanding and background into the various neuromuscular disorders.

Victor Dubowitz

Acknowledgements

The major component of the clinical material has come from our muscle clinic at Hammersmith and we are grateful to our current clinical colleagues, Francesco Muntoni and Adnan Manzur for the continuing flow.

Following a change of domicile in 1998, Caroline has divided her time between the Hammersmith unit and the Centre for Inherited Neuromuscular Disorders at Oswestry, where the diagnostic muscle biopsy service she established is an integral part of the clinical service and active research programme. We are grateful to her clinical colleague there, Ros Quinlivan, and to Glenn Morris, the Research Director, for further material. We are also grateful to the colleagues who have referred biopsy material that we have used for illustrations, including Natalie Costin-Kelly, Janice Holton, Jim Neal, and Waney Squier. The occasional illustrations we have obtained from outside colleagues are acknowledged in the captions to individual illustrations.

We are particularly appreciative of the contribution of our laboratory colleagues at Hammersmith over the years, including in earlier years Lesley Wilson, Carol Lovegrove, Rhoda McDouall, Christine Heinzmann, and currently Frederico Roncaroli, Sue Brown, Cecilia Jimenez-Mallebrera, and Lucy Feng, and also to Karen Davidson, for her help with the photographic work.

At the laboratory in Oswestry we are particulary grateful to Pat Evans, Nigel Harness and Martin Pritchard, and to Ellen Harrison for secretarial help.

Finally, a word of appreciation to Louise Cook and Glenys Norquay and their teams at Elsevier for the very friendly and productive working relationship we have had with them and also to our commissioning editor, Michael J Houston.

List of abbreviations

ABC	avidin–biotin complex
ADP	adenosine diphosphate
ALS	amyotrophic lateral sclerosis
AMP	adenosine-5-monophosphoric acid
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
AZT	azidothymidine
BAF	barrier-to-autointegration factor
BDMA	benzyl dimethylamine
BMD	Becker muscular dystrophy
BSA	bovine serum albumin
CD	cluster of differentiation
CK	creatine kinase
CMD	congenital muscular dystrophy
CoA	coenzyme A
CoQ	coenzyme Q
COX	cytochrome oxidase
CPT	carnitine palmitoyl transferase
CSF	cerebrospinal fluid
CT	computed tomography
DAB	3,3′-diaminobenzidine tetrahydrochloride
DAG	dystrophin-associated glycoprotein
DAPI	4′6-diamidino-2-phenylindole
DDSA	dodecenyl succinic anhydride
DM	myotonic dystrophy
DMD	Duchenne muscular dystrophy
DMP	dimethoxypropane
DMPK	myotonic dystrophy (DM) protein kinase
EACA	epsilon aminocaproic acid
ECG	electrocardiogram

EMG	electromyogram
ENMC	European Neuromuscular Centre
ESR	erythrocyte sedimentation rate
FCMD	Fukuyama CMD
FF	fast twitch, fatigue sensitive
FG	fast twitch, glycolytic
FITC	fluorescein isothiocyanate
FKRP	fukutin-related protein
FMN	flavin mononucleotide
FOG	fast twitch, oxidative glycolytic
FR	fast twitch, fatigue resistant
FSHD	facioscapulohumeral muscular dystrophy
GNE	UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase
H&E	haematoxylin and eosin
HIV	human immunodeficiency virus
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HMSN	hereditary motor and sensory neuropathy
IgG	immunoglobulin G
IGHMBP	immunoglobulin microbinding protein 2
KSS	Kearns–Sayre syndrome
LAMP	lysosomal associated membrane protein
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LEM	LAP2-Emerin-Man 1
LGMD	limb-girdle muscular dystrophy
LHON	Leber hereditary optic neuroretinopathy
MAC	membrane attack complex
MDC1A	congenital muscular dystrophy type 1A
MEB	muscle-eye-brain
MELAS	mitochondrial encephalopathy, lactic acidosis and stroke-like episodes
MERRF	myoclonic epilepsy with ragged-red fibres
MH	malignant hyperthermia
MHC	major histocompatibility complex
MHCf	myosin heavy chain fast
MHCn	myosin heavy chain neonatal
MHCs	myosin heavy chain slow
MILS	maternally inherited Leigh syndrome
MNGIE	myoneurogastrointestinal disorder and encephalopathy
MRF	myogenic regulator factors
MRI	magnetic resonance imaging
mtDNA	mitochondrial DNA
NAD	nicotinamide adenine dinucleotide
NADH-TR	reduced nicotinamide adenine dinucleotide-tetrazolium reductase
NAIP	neuronal apoptosis inhibitory protein

NARP	neuropathy, ataxia and retinitis pigmentosa
NBT	nitroblue tetrazolium
N-CAM	neural cell adhesion molecule
NFAT	nuclear factor of activated T cells
nNOS	neuronal nitric oxide synthase
OMIM	Online Mendelian Inheritance in Man database
OPMD	oculopharyngeal muscular dystrophy
ORO	oil red O
PABPN1	polyadenylate-binding protein nuclear 1
PAS	periodic acid-Schiff reaction
PCP	phencyclidine
PCR	polymerase chain reaction
PDHC	pyruvate dehydrogenase complex
PEO	progressive external ophthalmoplegia
PFK	phosphofructokinase
POLIP	polyneuropathy, ophthalmoplegia, leucoencephalopathy and intestinal pseudo-obstruction
PROMM	proximal myotonic myopathy
PTAH	phosphotungstic acid haematoxylin
rRNA	ribosomal RNA
RSMD	rigid spine muscular dystrophy
RYR1	ryanodine receptor 1
SCARMD	severe childhood autosomal recessive muscular dystrophy
SDH	succinic dehydrogenase
SEPN1	selenoprotein N1
SERCA	sarcoendoplasmic reticulum calcium ATPase
SMA	spinal muscular atrophy
SMARD	spinal muscular atrophy with respiratory distress
SMN	survival motor neurone
SO	slow twitch, oxidative
SR	sarcoplasmic reticulum
tRNA	transfer RNA
UCMD	Ullrich congenital muscular dystrophy
VCLAD	very long-chain acyl-CoA dehydrogenase
VVG	Verhoeff–van Gieson
WWB	Walker–Warburg syndrome
XMEA	X-linked myopathy with excess autophagic vacuoles
ZASP	Z line alternatively spliced PDZ protein

Contents

SECTION

The biopsy: normal and diseased muscle

CHAPTER 1

The procedure of muscle biopsy

Muscle biopsy is a relatively simple procedure; yet in the past it was frequently poorly done. The pathologist who receives a small fragment of an unnamed muscle, coiled into a disorientated ball after being dropped into formalin, is unlikely to get any meaningful information from it, no matter how careful the processing. With the upsurge of interest in neuromuscular disorders, clinicians and surgeons are now better informed on the handling of samples. The following are some guidelines worth following when planning a muscle biopsy.

Selection of the patient

A full clinical assessment of the patient is essential. Diagnosis should always be based on a detailed clinical and family history, and clinical examination, in conjunction with any special investigations such as serum enzymes, muscle imaging and electromyography, and the biopsy looked upon as an additional confirmatory test of an underlying muscle and/or neural disorder. In general, the main indication for muscle biopsy is some evidence of neuromuscular disease such as muscle weakness, muscle cramps or discomfort (especially on exercise) and muscle fatigue with activity. Pathological change may be found in some conditions in the absence of any apparent neuromuscular signs, for example collagen vascular diseases. On the other hand, the muscle biopsy may show no apparent morphological abnormalities in conditions such as myasthenia gravis or myotonia congenita in which the clinical diagnosis is more readily confirmed with electrodiagnostic methods.

With the spectacular advances in the identification of molecular defects, many clinicians question the need for a muscle biopsy if a defect in a gene can be identified. In some conditions such as spinal muscular atrophy, myotonic dystrophy and facioscapulohumeral dystrophy molecular analysis is so reliable that it can provide a direct confirmation of diagnosis without the need for a biopsy. Genotype and the results of DNA analysis, however, cannot always be related to phenotype and there are exceptions to every rule. This is well

demonstrated in Duchenne muscular dystrophy, in which the molecular defect may not always correlate with the protein expression seen in the muscle. More importantly, clinical severity cannot be judged by molecular analysis alone. We therefore feel that assessment of muscle pathology, with modern techniques, is an important component of patient assessment.

Selection of the muscle

This should be based on the distribution of the muscle weakness, as judged by detailed clinical assessment. In selecting the muscle for biopsy, it is important not to choose either a muscle which is so severely involved by the disease process that it will be largely replaced by fat or connective tissue and show little recognizable trace of the underlying disease process or, on the other hand, a muscle which is so little affected that it does not show sufficient change. Differential involvement of muscle occurs in several disorders and ultrasound imaging is a simple, quick technique for assessing this (Heckmatt et al 1982, Dubowitz 1995a) and can help in the selection of the biopsy site. Magnetic resonance imaging (MRI) of muscle gives superior quality, and patterns associated with individual diseases are now emerging (Mercuri et al 2005, Jungbluth et al 2004a,b, Pichiecchio et al 2004) but ultrasound is a rapid and practical method to apply before a biopsy and can be done in the outpatient clinic.

In general, where the distribution of the weakness is proximal, we select a moderately affected proximal muscle which is also reasonably accessible, such as the quadriceps (rectus femoris or vastus lateralis) in the leg or the biceps in the arm. In other circumstances, the deltoid or gastrocnemius are also suitable muscles for biopsy. Where weakness is mainly distal, a more distal limb muscle may be selected, but even in these circumstances biopsy of a proximal muscle may reveal the underlying pathological process adequately.

In a chronic disease such as muscular dystrophy, a muscle with only moderate weakness may be the ideal site for biopsy. In an acute disease, on the other hand, because the process has not had time to progress to extensive destruction, a more severely involved muscle may be chosen. In addition, the biopsy technique (see below) may influence the choice of muscle. For example with a needle technique the quadriceps is often considered relatively safe as the muscle is readily accessible and major nerves and blood vessels lie close to the femur and are unlikely to be damaged.

There are advantages in trying to limit the biopsies to certain muscles so as to be familiar with the normal pattern in that particular muscle. It is important to be aware of anatomical differences between muscles, and be familiar with possible age-related changes. Thus, the distribution of fibre types and fibre sizes is well recognized in the biceps and the quadriceps but the pattern may be unfamiliar in such muscles as the intercostals, the abdominal muscles or the hand or foot muscles. In certain circumstances, for example when studying motor endplates, the muscle selected will be determined by the particular line of investiga-