

MUSCLE BIOPSY IN NEUROMUSCULAR DISORDERS

**THESIS SUBMITTED FOR THE DEGREE OF
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INTRODUCTION

The human civilisation has ascended to its present scientific pinnacle with the help of brain as well as brawn. As a matter of fact, it was brawn rather than brain which rules the roost. The sculptures of Michaelangelo and others depicting fine muscular young men adorning the countless palaces of the middle ages strewn across the European continent clearly indicate the importance attached to muscle developments in those days.

Our own epics and puranas are replete with the epitomes of muscularity. Speaking on more scientific lines it is not surprising to see why so much importance is attached to muscle as very few vital functions in the body are carried out without the intervention of the muscle tissue, either directly or indirectly.

The word muscle is derived from the Latin word *Mus*(mouse) and refers to the resemblance of the muscle belly to a mouse. The muscular flesh constitutes an important body tissue. In the human there are no less than 434 muscles; these make up an estimated 25% of the total body weight at birth and 42-45% in adult man. The skeletal muscles are composed of a large number of muscle fibres. According to Eftman there are about 250 million individual striated muscle fibres in the body.

Diseases of muscle have for many years been the Cinderella of both the pathologists and the physicians. In the face of the relative neglect, it is not surprising that so little was added to the early descriptions after the turn of the century.

As early as 1861, Duchenne de Boulogne , whose name we associate with the childhood form of muscular dystrophy already stressed the need for muscle biopsy and devised the ingenious emporte-pièce histologique, a trocar with a sliding blade especially designed for percutaneous biopsy and the for runner of all subsequent biopsy needles. Open biopsy was not without hazard, and the wound from the first recorded muscle biopsy (Griesinger, 1865), taken from the deltoid under chloroform anaesthesia, by Prof. Billroth, was still suppurating five weeks later.

By 1891, Erb had defined the essential histological changes in deciding whether the muscle was normal or abnormal (often a difficult task in the phase of fixation or other artefacts). If the biopsy was abnormal, some opinion was then expressed as to whether the changes conform to a myopathic or a denervation (neurogenic) pattern. Routine histological stains on formalin fixed paraffin embedded material had very little more to offer beyond perhaps also identifying some specific changes such as vacuolation of the fibre or inflammatory response in the supporting tissue.

With the advent of enzyme histochemical methods, the perspective have changed. It is now recognized that human muscle is not a uniform tissue, but composed of different fibre "types", with specific biochemical and presumably physiological functions. The differentiation of embryonic muscle into these fibre types can be followed through foetal life. The selective influence of disease processes on one or other fibre types have been identified in muscle which would pass as histologically normal.

Histochemical techniques have also helped to identify certain structural and enzymatic changes within the individual fibre, which are not apparent on routine histological staining. In this way, new disease entities have been recognized. Histochemical methods are of value in the study of muscle biopsy because a) they may be used to demonstrate the specific fibre type on the basis of various enzyme reaction and thus the selective fibre type involvement in certain disease processes. b) They may show an absence of the particular enzyme e.g. phosphorylase in McArdle disease or an excess of a particular substrate as in glycogen storage disease. c) They may show various structural changes in the muscle which would not be apparent with routine histological stains , such as the enzyme deficient course in central core disease , moth eaten fibres and abnormalities in the distribution of mitochondria.

In addition, electron microscopy has also contributed to the advance in our knowledge of muscle disease by identifying certain ultrastructural changes in muscle not visible by histological or histochemical methods.

These advances have made muscle biopsy an important determinant in the diagnostic enigma of muscle in neuromuscular disorder.

REVIEW OF LITERATURE

As early as 1678, Stefano Lorenzini commented on the different types of muscles in animals and suggested that, on the basis of difference in colour, they were referred to as red and white muscle (Ciaccio, 1898). Ranvier (1873, 1874, 1880) suggested a physiological difference between the red and white fibres in the rabbit and he correlated these with slow and fast contraction. He also confirmed that red muscle had more extensive blood supply than the white.

Grützner (1884) drew a correlation between the thinner dark fibres of the gastrocnemius of the frog and the red fibres in the rabbit muscle. He concluded that the muscle of vertebrates, including man, was composed of two types of fibres, one thin and dark in colour, the other larger and lighter. He ascribed the dark colour to granules in the sarcoplasm. In 1857, Kolliker noted presence of interstitial granules in muscle fibres and suggested their connection with its metabolism. Retzius (1890) called these granules "sarcosomes".

Knoll (1891) stated that the difference in colour was due to more protoplasm between the myofibrils of the darker fibres than the light and he divided them into protoplasmareiche (rich in protoplasm) and protoplasmaarme (poor in protoplasm) fibres. He found that active muscles were composed mainly of dark fibres. In some animals muscle was either entirely red or white, while in others mixed. In frog muscle which is white he observed some dark fibres among the large pale ones. In higher animals least active muscles were white. He also found that the granules were of lipid nature.

Lee et al (1916) found chemical and physiological differences between these muscles. Bullard (1919) was able to show with Sudan III stain for lipids that muscles consisted of light dark and intermediate fibres with a variation in the proportion and size of these muscles. Denny Brown (1929) noted that the dark fibres of the cat muscle had a higher fat content than the light.

Benzley and Hoerr (1934) introduced the differential centrifugation for separating various structural components of liver cells which helped to localize certain enzymes to different fractions of the muscle fibres. This aided the study of mitochondrial density with respiratory activity in different types of muscles. Paul and Sperling (1952) demonstrated that white muscle had a low content of mitochondria with low respiratory activity while red muscle was rich in mitochondria and had a high respiratory activity.

Histochemistry: As early as 1935, Semenoff using methylene blue for succinate dehydrogenase noted a variation in enzyme activity of individual fibres. With more reliable methods, Seligman and Rutenburg (1951) , Padykula (1952) demonstrated the same in rat. Wachstein and Meisel (1955) also saw the same in rat and rabbit with a variability in their proportion. Buno and Germino (1958) , Nachmias and Padykula (1958) found a variability in proportion of different muscle fibres in different muscles and observed a stronger reaction in the fibres of smaller diameter and suggested that they contained more mitochondria per unit area. George and Searia (1958) were able to correlate high oxidative enzyme activity with red muscle and with high mitochondrial content.

Wachstein and Meisel (1955) first applied these methods to human muscles and found that the more reactive fibres tended to be smaller in diameter. Beckett and Bourne (1958) found a similar variation but were unable to demonstrate a correlation between cytochrome oxidase and succinate dehydrogenase.

Dubowitz and Pearse (1960a,b) showed a reciprocal relationship between phosphorylase activity and various oxidative enzymes in muscle fibres and subdivided them into two fibre types - type I having a high oxidative and low glycolytic activity and type II having a low oxidative and high glycolytic activity. Intermediate enzyme activity was also observed but not delineated as a separate fibre type.

Engel (1962) also favoured a two fibre type system but based it on the myofibrillar ATPase activity, type I having low and type II a high activity. In general type I and II fibres defined by ATPase corresponded to oxidative enzyme or phosphorylase but in some these relationships did not hold.

Brooke and Kaiser (1970) defined three fibre types in normal muscle on the basis of an augmented ATPase reaction with free incubation at pH (9.4, 4.6, 4.3) . In addition to the type I fibres (weak in ATPase at pH 9.4) there were two subtypes of the type II fibres (strong in ATPase at pH 9.4) which they call type II a (weak reaction at pH 4.6 and 4.3) and IIb (strong reaction still at pH 4.6 but weak at 4.3). A third subtype type IIc were seen which had residual positive reaction at pH 4.3 but negative at still lower pH.

Ogata (1958a) recognized three fibre types, a large white fibre with weak enzyme activity, a small red fibre with strong activity and a medium fibre intermediate in size and enzyme activity. Their proportion varied in different muscles. He noticed a variation in their cytochrome oxidase , diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphorase activity. Stein and Padykula (1962) recognized three fibres A,B and C in rat based on the size and distribution of mitochondria with succinate dehydrogenase reaction. Not satisfied with three fibre types, Romanul (1964) defined eight different fibre types based on enzyme reaction.

A number of attempts were made to correlate the physiological properties and the histochemical characteristics of fibres within the motor units (Henneman and Olson, 1965; Wuerker et al, 1965; McPhederan et al, 1965; Kugelberg and Edstrom ,1968;Burke et al, 1971). Muscle fibres of all motor units were found to be uniform in histochemical type. They tended to be confined to a limited area but randomly scattered though of an individual motor unit. Motor units composed of small, mitochondria rich fibres or of intermediate fibres,were as fast physiologically as those composed of mitochondria poor fibres.

Barnard et al (1971) attempted to correlate histochemical type of fibre with its physiological function. They accepted the classification of red, white and intermediate on the basis of NADH-TR reaction as of strong, weak and intermediate activity respectively. In the guineapig "fast twitch white" were histochemically strongly reactive for ATPase and thus "fast weakly reactive" with NADH-TR white. The other fibres were fast twitch red (strongly reactive for ATPase, strongly reactive for

NADH-TR) and slow twitch intermediate (weakly reactive for ATPase and intermediate with NADH-TR). Peter et al (1972) suggested appropriately slow oxidative (SO₂), fast glycolytic (FG) fast oxidative glycolytic (FOG).

In recent years, the assay of enzyme activities has shifted towards microbiological estimation in microdissected fibres (Spamer and Pette, 1977;1979; Lowrey et al, 1978; Hintz et al, 1980; Nemeth et al, 1981) or microphotometric measurement of histochemical enzyme reaction (Spurway, 1981; Nemeth and Pette,1981; Reichmann and Pette, 1982) .

Immunohistochemically, Pierobon-Bormioli et al (1981) demonstrated selective staining of fast white type IIb and fast red type IIa muscle fibres in rat. In addition to the three major fibre types, they also identified two groups of fibres with double reactivity, a) fibres reacting to anti slow twitch and anti fast red antibodies, corresponding in serial sections to type IIc fibres histochemically and b) fibres reactive with antifast red and antifast white antibodies.

With the advent of the knowledge of histochemistry and its use, now in the present years it is being utilized to broaden scientific knowledge regarding the various neuromuscular disorders and their histological and histochemical changes.

INDICATIONS FOR MUSCLE BIOPSY

The pathological changes in the muscle are seen in primary neuromuscular disorder characterised by muscular weakness and also in many systemic diseases such as rheumatoid arthritis, collagen vascular diseases, parkinsonism and endocrinopathies with no apparent symptoms.

Medical indications for a muscle biopsy are:

1. Progressive muscular atrophy and weakness; differential diagnosis between neurogenic and myogenic disease, distal atypical dystrophy, proximal forms of motor system disease.
2. Localised or diffuse inflammatory myopathy; identification of infectious agents or immunological disease
3. Obscure fever and multiple visceral and cutaneous lesions; search for diffuse vascular disease or connective tissue disease
4. Trauma to nerve or blood vessels of limb; assessment of state of muscle and nerve twigs to direct further treatment and determine prognosis.
5. Metabolic disease; assessment of histologic and biochemical changes such as in glycogen storage diseases
6. "Floppy baby" evaluation of specific congenital myopathies; infantile motor neuron disease.

AIMS AND OBJECTIVES

The aim of this dissertation was

1. To establish the diagnosis of neuromuscular diseases by a muscle biopsy.
2. Evaluation of histochemical techniques in aiding precise diagnosis of disease which cannot be detected by conventional and clinical methods.

MATERIAL AND METHODS

For the present study, patients of muscle disorders referred to a large teaching hospital in Bombay were selected. These included the outpatients and inpatients. Sixty Four cases were studied from 1989 to 1992. They were all subjected to detailed clinical evaluation. Other investigations carried out included, routine haematological & serum creatinine kinase. The electrophysiological investigations done were ECG, EEG, and EMG.

A muscle with moderate clinical or EMG involvement was chosen. Muscle biopsy was done on five patients with no neurological manifestations who underwent surgery for orthopaedic or general surgical diseases. These served as control.

The muscle excised was not the site of previously implanted EMG needles since the reaction elicited by this type of minimal trauma confused the histological picture. The pelvic girdle and thigh muscles are affected in the majority of muscular diseases. In most cases, therefore, the quadriceps was chosen for biopsy. The biopsy was done under local anaesthesia. General anaesthesia was used for children. The muscle was not infiltrated with local anaesthesia and handled with as little surgical trauma as possible. The operative field was maintained moist with saline so that the muscle was not damaged artifactually at the time of removal.

The minimum size of muscle removed was 2x1x1 c.m.

It was then divided into three bits as follows :

1. First bit was fixed in 3% glutaraldehyde for 5 hours and then transferred to cacodylate buffer solution and processed for electron microscopy.

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2. Second bit was fixed in formalin and then paraffin embedded for light microscopy.

3. Third bit was transferred to n-Hexane solution kept in a dry ice mixture to freeze the tissue to -70 C. The frozen tissues were transferred to a cryostat and sections of 4 μ thickness were cut and stained.

The stains used were :

1. Haematoxylin & Eosin (H & E)
2. Phosphotungstic acid haematoxylin (P T A H)
3. Para Amino Salicylic Acid (P A S) and fat stains when required.

Histochemical stains for enzymes :

1. A T Pase (pH 9.4)
2. NADH-TR
3. Phosphorylase.

The procedure for the stains were :

NADH-TR

1. Tissue was incubated for 30 minutes at 37 C in the following solution.

0.2 M - Tris Buffer (pH 7.4) - 10 ml.

NBT (2,2-di-p-Nitrophenyl-5-5-biphenyltetrazolium Chloride) - 10 mgm.

NADH (Reduced di-phosphopyridine nucleotide) - 8 mgm.

The pH was adjusted to 7.4

2. Dipped in acetone 30%, 60%, 90% sequentially.
3. Washed in water.
4. Mounted in glycerin jelly.

PHOSPHORYLASE

1. Tissue incubated at 37 C for sixty minutes in
0.2 M - Acetate buffer (pH 5.6) - 10 ml
Adenosine mono phosphate - 10 mgm
Glucose - 1 - Phosphate - 50 mgm
Soluble insulin (40 mu / ml) - 1 drop
Glycogen - 1 mgm
Distilled water - 625 ml
2. Rinsed in distilled water.
3. Placed in Lugol's Iodine for one minute.
4. Mounted in glycerin jelly.

ATPase (9.4)

1. Tissue incubated at room temperature for 15 minutes in
solution A i.e.
0.1 M Sodium Barbital - 2 ml
0.18 M calcium Chloride - 2 ml
Distilled water - 6 ml
Adjust pH to 9.4
2. Then incubated for 30 min. in solution B i.e.
0.1 M Sodium Barbital - 2 ml
0.18 M Calcium Chloride - 1 ml
Distilled water - 7 ml
Adenosine Tri Phosphate Di Sodium Salt - 25 mgm.
Pinch of 2,4 Dinitro Phenol
Adjust pH to 9.4
3. Washed in 3 changes of 1% Calcium Chloride for a total of 10
min.
4. Transferred to 2% Cobalt Chloride for 3 min.

5. Washed in water - 1 min.
6. Placed in 1% Yellow Amonium Sulphide for 20 to 30 sec.
7. Poured.
8. Mounted in glycerin jelly

In histological sections following changes were studied :

Architecture

Fibre Size

Atrophy

Hypertrophy

Fibre Shape

Round

Angulation

Nucleus

Internal

Central

Fibre Degeneration

Hyaline Change - Loss of Cross Striations

Vacuolar Change

Necrosis

Phagocytosis

Intracytoplasmic Inclusions

Fibre Regeneration

Inflammation

Connective Tissue Increase

Adipose Tissue Increase

Blood Vessel Abnormalities

Nerve Abnormalities

Frozen sections stained with histochemical stains

Fibre Size and Distribution

Alteration of Architecture Within Individual Fibres

Targets, Targetoids and Cores

Abnormal Mitochondria

Vacuoles

Enzyme Deficiency

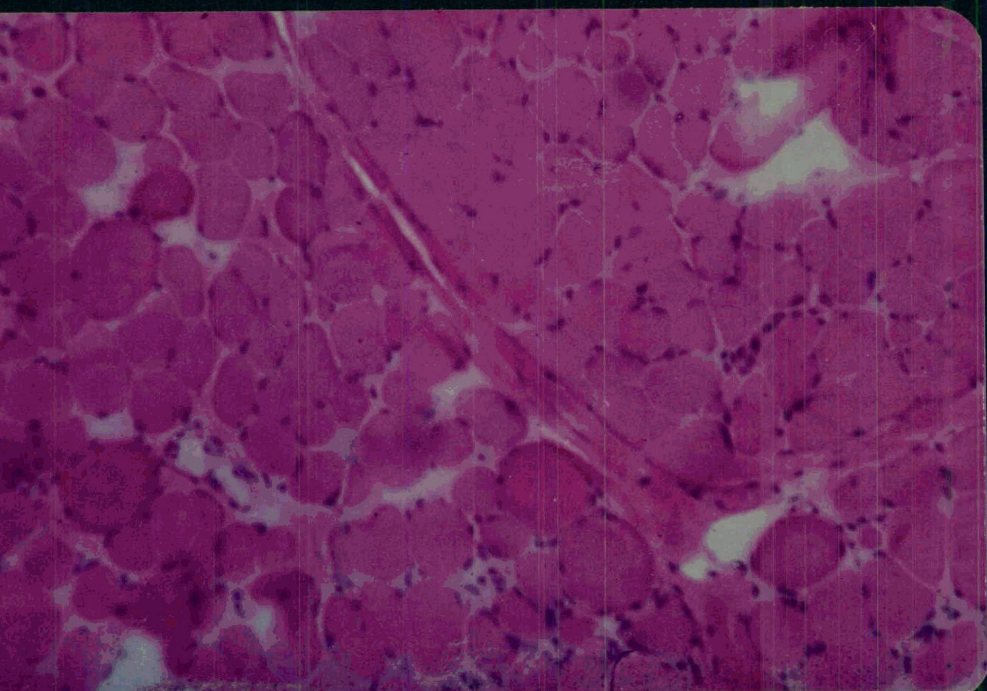


Fig.1: Photomicrograph of normal muscle showing fascicular architecture
Frozen H & E X 200.

FEATURES OF NORMAL MUSCLE

To understand the features of a muscle as seen in a biopsy it is imperative to understand the histochemical techniques, the normal structure and function of the muscle and its histochemistry.

These are as follows -

Anatomy

Individual skeletal muscle consists of a bundle of muscle "fibres" within a connective tissue frame work. These muscle fibres or myofibres are elongated, multinucleate syncytia, upto 10 cms. or more in length, with diameter in the range of 10-100 μ . Muscle fibre in general run from one end of a muscle, or muscle fasciculus, to the other, without interruption or overlap with another fibre.

The striated muscle fibres that constitute an individual muscle are bound together by collagenous connective tissue, divided into three components. The epimysium provides a tough elastic envelope of orientated collagen fibres; however, it delimits the muscle from adjacent structures, and its end merges with tendon, aponeurosis or periosteum. Deep extensions of this layer of organised collagen subdivide the enclosed muscle fibres into smaller fascicles, and these septa are referred to collectively as the perimysium. The individual muscle fibres are separated by an interlacing network of fine collagen fibrils, the endomysium.

The cross sectional shape of the adult muscle fibre (fig 1) is described as "polygonal" or "multifaceted", except those of the infant and the extrinsic eye muscles and some muscles of the face as they are round. In an ordinary histologic section, 6 to 10 microns thick, the adult cell is bounded by a plasma

membrane(sarcolemma), and a basement membrane. The nuclei are positioned beneath the sarcolemma and are spaced about 10-50 microns apart. Serial sections show that the nuclei actually spiral across the fibre. The nuclei are slender, oval, and oriented parallel to the length of the fibre. The nucleoplasm is fine and evenly distributed, and nucleoli are ordinarily not conspicuous. Internalized nuclei are rare, present in three percent of normal. Satellite nuclei lie between the basement and sarcolemmal membranes. They comprise about 4-10% of all nuclei seen in the cells and have an important function in regeneration. The familiar striations of the muscle fibres are imparted by repeating units of interlaced, longitudinally directed thin or light filaments, (actin) and thick or dark filaments(myosin). The thin filaments emerge from perpendicularly disposed Z lines (disks or bands). The myofibrils are cords of thick and thin filaments that run throughout the length of the fibre. In longitudinal sections, the contractile units between two Z bands, (sarcomeres) of adjoining myofibrils gives the characteristic striations. The number of myofibrils in each muscle fibres varies depending on the size of the fibre. At the tendinous insertions myofibrils tend to fuse with the connective tissue and nuclei are internalized and more numerous.

Muscle receives a rich blood supply from the branches of regional arteries. Within the muscle, the apparent vessels breakup to supply an extensive capillary network between the myofibres.

Nerves are seen in the perimysium and the terminal ramifications go into the fascicles in the endomysium in the region of the neuromuscular junction.

Histochemical reactions in human muscle.

Muscle fibre type	1	2A	2B	2C
Routine ATPase (pH 9.4)				
ATPase pre-incubated pH 4.6				
ATPase pre-incubated pH 4.3				
NADH-TR				
SDH				
Menadione-linked α -glycerophosphate				
PAS	 + 			
Phosphorylase	 + 			
 = 0  = 1+  = 2+  = 3+				

Fig. 2.

Muscle spindle:

This is a specialised structure of a striated fibres within a connective tissue capsule. They are located between the muscle fascicles in the perimysial connective tissue adjacent to nerve or vessels. It acts as a sensory organ and is associated with the coordination of muscle activity and maintenance of muscle tone. (Andrews, 1966; Granit, 1966). The muscle spindles seem to be selectively affected in certain pathological circumstances such as myotonic dystrophy (Daniel and Strich, 1964; Swash and Fox, 1975a,b).

Histochemistry of normal muscle (fig 2) :

The identification of functional and metabolic differences between individual muscles was followed by the recognition that the majority are composed of fibres which in turn can be demonstrated to possess a range of metabolic activities , and that the redness or whiteness of a muscle reflects the sum of the properties of its constituent fibres. Detection of the metabolic characteristics of the individual myofibrils depended upon the histochemical techniques and their application to frozen section, fresh or fixed muscle (Padykula, 1952; Padikula and Herman, 1955; Wachstien and Meisel, 1955) .

In man and certain experimental animals, two prototype fibres have been defined on the basis of certain physicochemical properties . In man, the two fibre types are more or less equally intermingled in every muscle; however, the proportion of one type over another may vary somewhat, depending on the function of the muscle. Type I fibres (37%) are the red muscles. They are capable of slow twitch and sustain or weight bearing action. Type II fibres (63%) are the white muscles and are capable of fast twitch and sudden action.

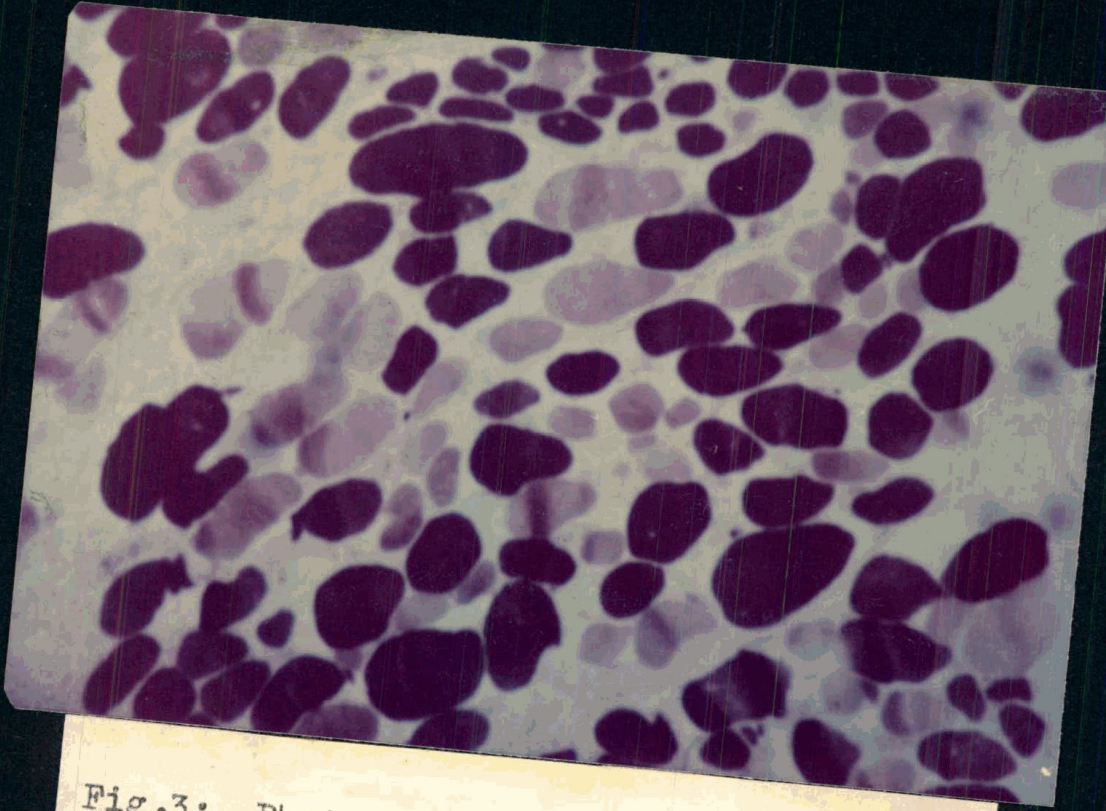


Fig.3: Photomicrograph of muscle stained for ATPase showing dark Type II and light Type I fibres X 200.

Histochemical reactions performed on fresh frozen sections demonstrate the two types. The fibres are randomly distributed and stained either dark, light or intermediate. This differential staining gives rise to the so called checkerboard pattern. Type I fibres are high in oxidative enzymes and low in myofibrillar adenosine triphosphatase (ATPase) activity. Type II fibres are low in oxidative enzyme and high in ATPase activity. Type II fibres can be further segregated into a, b, and c subtypes with the use of ATPase reaction incubated at pH 9.4 , 4.6, or 4.3 and to some extent with the oxidative enzyme reaction. Type IIc fibres are rare in human muscle and thought to represent an undifferentiated, or precursor fibre. In the experimental animal developmental maturation is associated with differentiation of type IIc fibres into type IIa and b. It is worthy of note that with ATPase reaction, the staining of the fibres can be reversed from light to dark by changing the pH of the incubating solution. Quantitative enzyme activities are different for ATPase and the oxidative enzymes in type I fibre and the subtypes of type II fibres.

Adenosine triphosphatase (ATPase) reactions (fig 3) :

In the routine reaction for ATPase both the preincubation and the incubation in the ATP mixture is carried out at the pH of 9.4. The reaction develops in the myofibrils; the intermyofibrillar network dissolves out of the tissue section at some stage of the reaction. The myofibrils can be seen separated by an unstained intermyofibrillar network. The stain develops in the region of A bands. Since this region is occupied by myosin, the reaction has been termed "myosin ATPase" reaction. In examining the muscle as a whole, there is a clear differentiation into two fibre types.

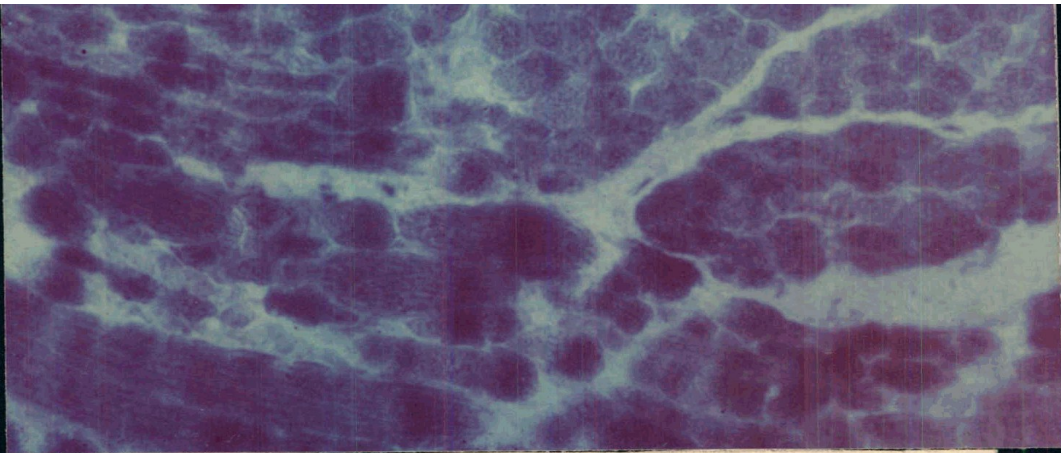


Fig.4: Photomicrograph of muscle stained for NADH-TR showing dark Type I and light Type II fibres X 200.

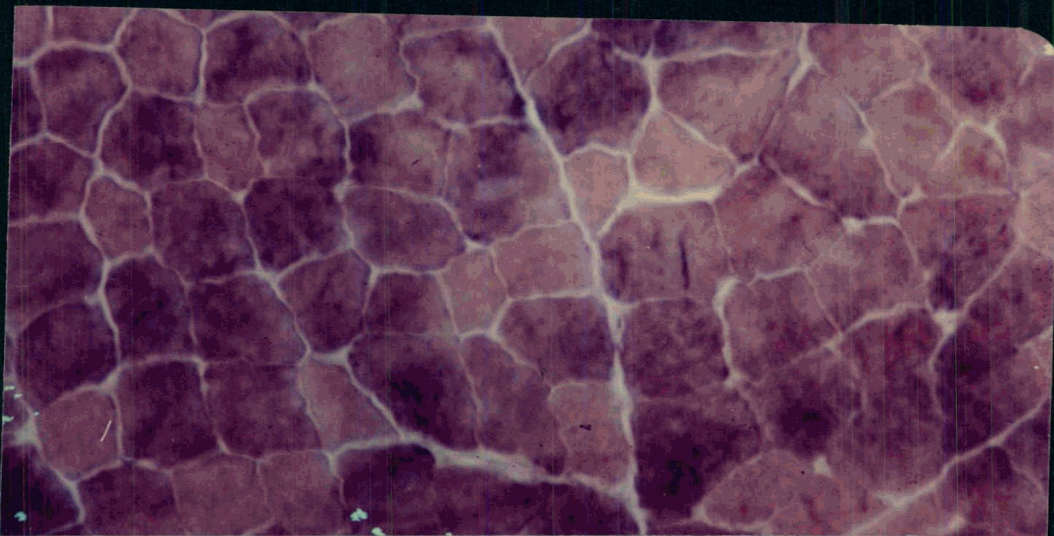


Fig.5: Photomicrograph of muscle stained for phosphorylase showing yellow Type I and dark Type II fibres X 200

The type I fibres are more lightly stained and the type II fibres are more heavily stained. Intermediate fibres are usually not seen with these reaction.

Following preincubation, at pH 4.3, the type I fibres stained darkly and the type II lightly. Occasionally type II may retain a moderate reactivity- these are the type IIc fibres which are rare in normal human muscles, but are present in developing muscles and may appear under pathological circumstances.

Following preincubation at pH 4.6 the type I fibres are strongly reactive at pH 4.3, but the Type II fibres vary. Some will be inhibited and stained lightly (IIa), others strongly (IIb).

Oxidative enzyme (NADH-tetrazolium reductase) (fig 4) :

With this reaction, two fibre types can be recognized. Type I fibres stain darkly and type II fibres lightly. The myofibrils are unstained, but the intermyofibrillar network, comprising the mitochondria and sarcoplasmic reticulum, is well demonstrated.

This network pattern is slightly different in the two fibre types. Division into fibre types is ,however, not absolute and some fibres show intermediate activity. The correlation of NADH-TR reaction with ATPase activity shows that in human muscle, type I fibres (weak in ATPase) react more intensely to NADH-TR, type IIb fibres show the least reaction with NADH-TR and the type IIa fibres have an intermediate activity.

Phosphorylase (fig 5) :

Phosphorylase is present in the aqueous sarcoplasm and thus this reaction shows the intermyofibrillar pattern in transverse sections. In longitudinal sections, the enzyme activity is concentrated at the level of I bands.

In the earlier methods of phosphorylase, (Takeuchi and Kuriaki, 1955), the result was a fairly clear cut division into a two fibre type pattern, one group of fibres (II) giving an intense blue black or dark purple colour, while the other group (type I) was practically negative, with a yellow stain from the iodine. With modifications and improvements in the technique (Eranko and Palkama, 1961), the colour product resembled that obtained in an equivalent biochemical reaction described by Swanson(1948). The result was the demonstration of intermediate fibres between the two extremes of reaction. The colour product is dependent on the chain length of the polysaccharide units, and there is a tendency for varying gradations of intermediate colours from red through purple through the blue black colour for the longest chains. It is thus not possible to define a uniform, single, intermediate fibre type and this has made the reaction more difficult for fibre typing. The intermediate fibres with this reaction occur among the type I fibres rather than the type II. The two extremes however are easy to recognize.

GENERAL REACTIONS OF MUSCLE

Introduction:

The muscle undergoes pathological reactions common to other tissues, but a) every fibre is a syncytium and b) the greatest part of the volume is occupied by contractile filaments. The first property means that an injury affects only a small part of the fibre, and a genetic lesion may manifest at different times in different parts of the fibre. The second is associated with the functional specialization of the skeletal muscle. The myofibrils contract and relax in response to calcium. Maintenance of low intracellular calcium is dependent on an intact plasma membrane and therefore an injury to the plasma membrane results in gross changes in the morphology of the fibres.

Problems regarding interpretations are a) distinguishing pathological changes from artefacts b) distinguishing changes in the muscle secondary to the initial lesions c) sampling of nonrepresentative areas d) problem regarding the specificity of the pathological changes which are found. Nevertheless, the pattern of abnormality in a muscle can be characteristic of a particular disease and thus a whole spectrum of changes are taken into account.

Basic reactions:

Atrophy:

It is a common response which involves a phase of negative growth and regression in volume. It is a common finding in a variety of chronic myopathies both inherited and acquired but the most striking degrees of atrophy results from denervation of the muscle. Various patterns of atrophy are seen. Atrophy on

randomly distributed single fibres is a very nonspecific finding. In chronic myopathies, atrophic fibres are randomly distributed and there is a nonselective involvement of both major fibre types. In some cases of dermatomyositis a perifascicular atrophy occurs which is of uncertain significance. In denervation, the atrophic process is again a nonselective type and a number of histological patterns are found. In a totally denervated muscle all fibres undergo reduction but in partial they are distributed between fibres of normal size. Denervated fibres are often angulated. In man different fibres atrophy to the same extent although there may be a preferential atrophy of type I.

Selective type I atrophy is uncommon and occurs in myotonic dystrophy, rheumatoid arthritis, centronuclear myopathy, nemaline myopathy and congenital fibre type disproportion. (Dubowitz and Brooke, 1973). Selective type II fibre atrophy is very common and is quite nonspecific being found in various conditions like myaesthesia gravis, polymyositis, polymyalgia rheumatica,, connective tissue diseases, corticosteroid myopathy and Cushings disease.

Histologically they are indistinguishable from normal fibres except in their size and shape. Ultrastructurally one see an irregular fibre outline thrown into folds especially at the "angles" of the fibres. Exterior to the folds, the redundant basal lamina also cast into complex folds. Histogencally some changes may be seen. In denervation, oxidative enzymes show an increased intensity of staining whereas glycogen and phosphorylase are lost at an early stage. (Karpati, 1979). In selective type II fibre atrophy no cytoarchitectural or histochemical profile remains normal.

The quantitative assessment of the extent of atrophy aids in the diagnosis of neuromuscular disorders (Dubowitz and Brooke, 1973).

Hypotrophy:

It is less common and involves an arrest or slowing of normal growth of the muscle fibre. The small type I fibres seen in nemaline myopathy, centronuclear myopathy and congenital fibre type disproportion fall into this category. It may be due to a defective neural influence during myogenesis. A similar feature may also be seen in neonatal myotonic dystrophy (Sarnat and Silbert, 1976 and Werdnig Hoffmann disease (Fidzianska, 1976).

Hypertrophy:

It occurs physiologically in response to increased workload and it may occur in certain disease states. The physiological hypertrophy may involve both fibre types but endurance training shows selective type I hypertrophy. The type II fibres are larger in men than in women probably due to a hormonal cause.

In pathological state, it is seen in myotonia congenita and slowly progressive forms of muscular dystrophy. (for e.g. Becker and limb girdle varieties). It is also seen in long standing hypothyroidism and acromegaly. Hypertrophy results principally from an increase in the number of myofibrils which may be due to a longitudinal splitting of myofibrils (Goldspink, 1972) or due to an incorporation of satellite cells into the muscle fibres syncytium (Reger and Craig, 1968).

True hypertrophy must be distinguished from pseudohypertrophy in which the volume of the muscle is increased due to fatty infiltration, inflammatory process such as cysticercosis or presence of a tumour (Adams et al, 1962). In Duchenne form of muscular dystrophy the pseudohypertrophy may be due to a

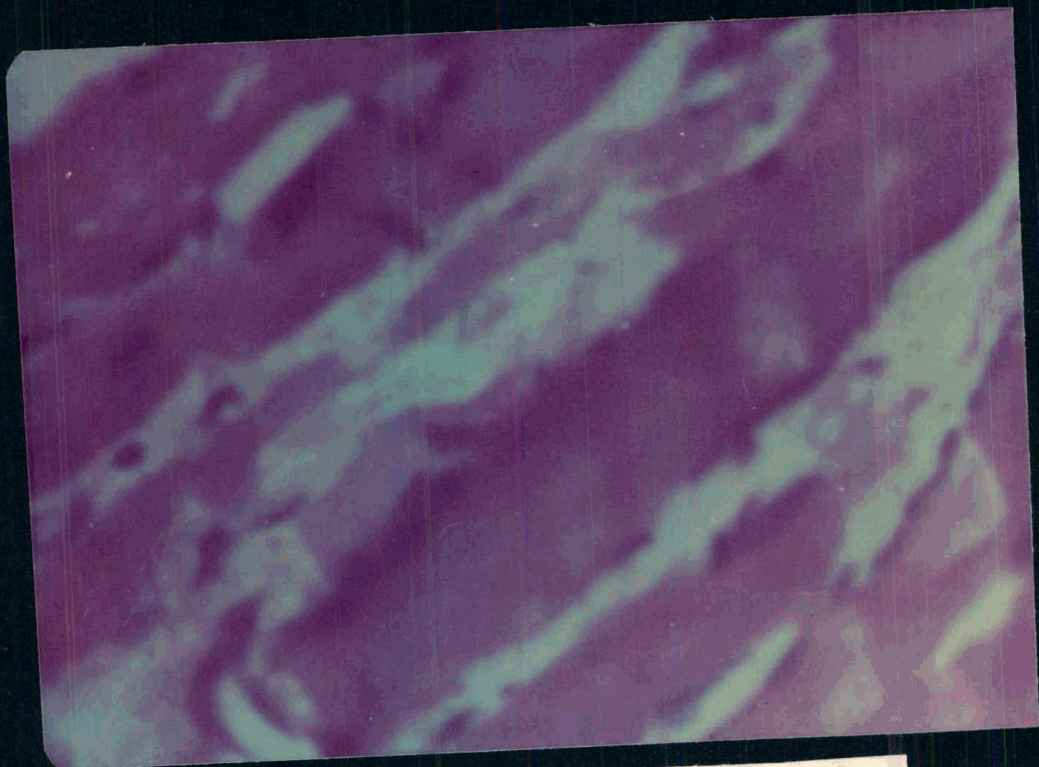


Fig.6: Hyaline degeneration
HE X 500

combination of true hypertrophy and an increase in the amount of interstitial adipose tissue. (Mastalgia et al, 1970).

Necrosis:

It usually occurs as a result of an acute injury. Often there is segmental necrosis that is it is confined to only a segment of variable length of the muscle fibres. It may be due to a focal injury to the plasma membrane of muscle fibre (Karpati and Carpenter, 1980). It leads to the surviving portions to synthesize new membranes to separate them from irreversible damaged segment. Thus necrosis and phagocytosis are confined to the fibre. A fundamental distinction must be made between the selective type of muscle fibre necrosis which occurs in muscular dystrophies and acquired metabolic nutritional and inflammatory myopathies and the nonselective damage which occurs after trauma, haemorrhage, ischaemia or certain forms of infection. In the latter a pan necrosis with a less likely regeneration of fibres is seen.

Histologically a classical Zenkers hyaline degeneration (fig 6) and waxy and granular degeneration are seen. The light microscopic changes form a continuum the final stage of which is myophagia. Hyalinization may be produced artifactually due to mishandling or improper fixation. The intermediate stages of breakdown of muscle fibres show a reduced intensity of staining with a loss of enzyme activity and glycogen. The contents of the fibres undergo progressive disorganization and fragmentation with the appearance of mononuclear phagocytic cells. Another type of change seen especially in metabolic myopathies is of a finely granular and vacuolated muscle fibres.

Patterns of necrosis seen are as such (Kakulas, 1975):

- a) Monophasic, monofocal
- b) Monophasic, polyfocal
- c) Polyphasic, polyfocal i.e. in chronic progressive human myopathies.

Regeneration:

It occurs at the sequel to necrosis and in a healthy muscle with an intact innervation and blood supply will lead to functional and structural reconstitution of the muscle. The crucial factor in determining the effectiveness is whether the scaffolding form by the basal lamina and the surrounding tissue remain intact and the pathological process subsides. The new fibres are found within the original sarcolemmal tubes (Isomorphic regeneration).

In contrast after trauma, haemorrhage, infection the regeneration is less orderly (anisomorphic regeneration) (Adams et al, 1962).

In this type of regeneration the new fibres are surrounded by proliferating fibroblasts which appear as muscle giant cells giving rise to a localised swelling or pseudotumour.

Muscle fibres regenerate from mononucleated myogenic stem cells (myoblasts) with continued incorporation of these cells formation takes place which ultimately join the intact muscle fibres. The end result is a production of a segment in the muscle fibre which is forked and appears to undergo longitudinal splitting.

The myoblasts are derived from undifferentiated subsarcolemmal satellite cells (Katz, 1961; Mauro, 1961). The satellite cells represent a population of resting cells which are attracted to the area of damage and can be stimulated to undergo myogenic differentiation. The primitive myogenic cell may arise within the degenerating fibre by the sequestration of myonuclei and the

surrounding cytoplasm by newly formed membranes.

Histologically by H and E staining they show a basophilia of the cytoplasm which reflects the high content of RNA and the appearance of nuclei which are large with dispersed chromatin and a prominent nucleolus. Myoblasts are thin , spindle shaped mononucleated cells seen on the inner aspect of the basement lamina. They are richly endowed with mitochondria and as a consequence the cytoplasm stains deeply in histochemical preparations for oxidative enzymes. They may show mitotic division. Regenerating fibres have a high content of oxidative enzyme such as succinic dehydrogenase and NADH-TR but do not show phosphorylase activity until late in maturation. Though they have a high level of myofibrillar ATPase activity, there is no differentiation into distinct fibre types until late stages of maturation. (Rezinik, 1972; Snow, 1973).

Autophagy:

It is a normal physiological activity of living cell and is a mechanism for the turnover of cytoplasmic constituents, for disposing of redundant components and using them as emergency sources of energy. It is stimulated when the tissue is exposed to starvation, dietary deficiency, or inhibition of protein synthesis. When the stress is prolonged, it leads to death and necrosis.

Autophagic vacuoles are seen in a wide variety of neuromuscular diseases where there is a myofibre breakdown i.e. muscular dystrophy, myotonic dystrophy, distal myopathy, polymyositis, idiopathic inflammatory myopathy, and many toxic and metabolic myopathies. In these the vacuoles are membrane bound and the contents show evidence of breakdown or condensation. In the distal myopathy, the vacuole contents are vesicular and granular.

They are usually found towards the periphery of the muscle fibres and from here they are expelled to the extracellular space. Stauber (1980) has postulated that autophagic vacuoles in muscle functions specifically in the degradation of nonmyofibrillar constituents.

Vacuolar degeneration:

It is one of the common histological changes seen. They are subdivided as

a) Dilatation of sarcotubular system - seen in familial forms of hypo and hyperkalaemic periodic paralysis. The vacuoles are empty or may contain PAS positive material.

b) Autophagic vacuolation - seen in necrobiotic myopathies, glycogen storage disease, drug induced myopathies. Such vacuoles should be distinguished from the rimmed vacuoles seen in oculopharyngeal dystrophy. (Dubowitz and Brooke, 1970).

c) Storage vacuoles - seen in lipid storage, glycogen storage diseases, and mitochondrial myopathy. In lipid storage, the type I fibres are most severely affected.

d) A distinctive variety have been described in SLE. (Pearson and Yamazaki, 1958).

NEUROMUSCULAR DISORDERS

Neuromuscular disorders, as one can see may result from abnormalities in the lower motor neurons, (including the motor nuclei of the cranial nerves, the anterior horn cells of the spinal cord, the spinal motor roots, the motor fibres of the peripheral nerves), the neuromuscular junction as well as the voluntary muscles themselves. (Walton, 1981). Diseases of muscles could be classified into two broad categories as "Neurogenic" and "Myopathic".

Neurogenic muscle disorders:

In these disorders, the pathological lesions primarily affect the spinal cord, spinal roots, or peripheral nerves. The changes in muscle are less pronounced or are due to secondary effects of denervation. These include: a) motor neuron disease b) Peripheral neuropathies and radiculopathies, c) spinal cord disorders.

a) Motor neuron disease can be classified as follows: (Campbell, and Liversedge, 1981).

1. Motor neurone disease (amyotrophic lateral sclerosis)

- a) Progressive muscular atrophy
- b) Progressive bulbar palsy
- c) Amyotrophic lateral sclerosis.

2. Spinal muscular atrophies:

- a) Proximal forms
- b) Distal forms
- c) Scapuloperoneal form
- d) Facioscapuloperoneal form
- e) Juvenile progressive bulbar palsy

3. Motor neurone disease associated with other diseases of the central nervous system:

- a) Mental disorder
- b) Extrapyrmidal disorder
- c) Spinal diseases including the hereditary spinocerebellar ataxia.

4. Miscellaneous diseases

- a) Infections
- b) Metabolic including hypoglycaemia
- c) Toxicity - heavy metals, organophosphorous compound
- d) Ischaemic myelopathy including radiation damage.
- e) Trauma including electrical injury
- f) Nonmetastatic carcinomatous neuromuscular disease.

B. Peripheral neuropathy.

Diseases arising out of disorder function and structure of peripheral nervous system which encompasses spinal nerves, plexuses, main nerve trunks and terminal ramifications. They could be further classified for diagnostic purposes into

- a) Acute severe polyneuropathies
- b) Mononeuritis or mononeuritis multiplex
- c) Subacute chronic distal symmetrical sensorimotor polyneuropathy.
- d) Recurrent and hypertrophic polyneuropathy

Type C neuropathies constitute the larger group and include chronic neuropathies such as peroneal muscular atrophy (Charcot-Marie-Tooth disease) which is hereditary in nature.

C. Spinal cord disorders:

- a) Spina bifida
- b) Syringomyelia
- c) Tumours

Disorders of neuromuscular transmission

- a) Myaesthesia gravis
- b) Myaesthetic syndrome

Myopathic disorders:

The term myopathy is defined as only disorder which can be attributed to pathological, biochemical, or electrical changes occurring in the muscle fibres or in the interstitial tissue of the muscle fibres or in the interstitial tissue of the voluntary musculature, and in which there is no evidence that such changes are in any way secondary to disorder function in the central and peripheral nerve tissue (Walton and Gardner, Medwin, 1981). Myopathies hence can be caused by a variety of factors like infection, inflammation, metabolic deficiencies, endocrine disorders, genetic aberration etc. Myopathies are characterised by muscle weakness. Muscular dystrophies constitute the largest proportion of these disorders. The term dystrophy was coined by Erb in 1891. They are defined as progressive, genetically determined primary degenerative myopathy (Walton, 1981).

Myopathies are classified as:

A) Destructive myopathies

1. Polymyositis and dermatomyositis

- a) Idiopathic
- b) Childhood
- c) Associated with collagen vascular disease or malignancy

2. Dystrophies

- a) X linked recessive muscular dystrophies

Severe(Duchenne)

Benign(Becker)

?Benign with early contractures(Dreifuss)

?Scapuloperoneal

- b) X linked dominant muscular dystrophies
 - ?Muscular dystrophy confined to females.
- c) Autosomal recessive muscular dystrophy
 - Scapulohumeral (limb girdle)
 - ?Quadriciceps myopathy
 - Autosomal recessive muscular dystrophy in childhood
 - Congenital muscular dystrophies
- d) Autosomal dominant muscular dystrophies.
 - Facioscapulohumeral
 - ?Scapuloperoneal
 - Proximal (late onset)
 - Distal (infantile onset)
 - Distal (adult onset)
 - Occular
 - Occulopharyngeal
 - Myotonic

3. Vascular disease

4. Trauma

5. Myopathies associated with viral infections.

Influenza, coxsackie

6. Toxic or drug induced myopathies

Alcohol, emetine, senna, penicillamine.

B) Myopathies with selective type II fibre atrophy

Polymyalgia rheumatica

Steroid myopathy

Endocrine myopathies

Some cases of polymyositis

C) Inflammatory myopathy

Sarcoid

Pyogenic infection

Parasitic infection .pa

D) Congenital myopathies

Congenital fibre type disproportion

Myotubular (centronuclear) myopathy

Central core disease

Minicore disease

Nemaline myopathy

Mitochondrial myopathy

E) Metabolic myopathies

Glycogenosis

Periodic paralysis

Malignant hyperpyrexia

TABLE I

AGE AND SEX DISTRIBUTION

TOTAL NUMBER OF CASES.:	64.
MALES	51.
FEMALES	13.
AGE - 0 - 10 YRS.	18.
11 - 30 YRS.	35.
MORE THAN 30 YRS.	11.

TABLE II

DISTRIBUTION OF CASES

BIOPSY DIAGNOSIS	NO.	BASED ON HISTOLOGY & HISTOCHEMISTRY	BASED ONLY ON HISTOLOGY
NORMAL MUSCLE	13	7	6
INFLAMMATORY MUSCLE DISEASE	13	6	7
MUSCLE DYSTROPHY	18	8	10
NEUROGENIC MUSCLE DISEASE	9	4	5
CONGENITAL MYOPATHY	1	1	-
ADVANCED MUSCLE DISEASE	10	7	3
TOTAL.	64	33	31

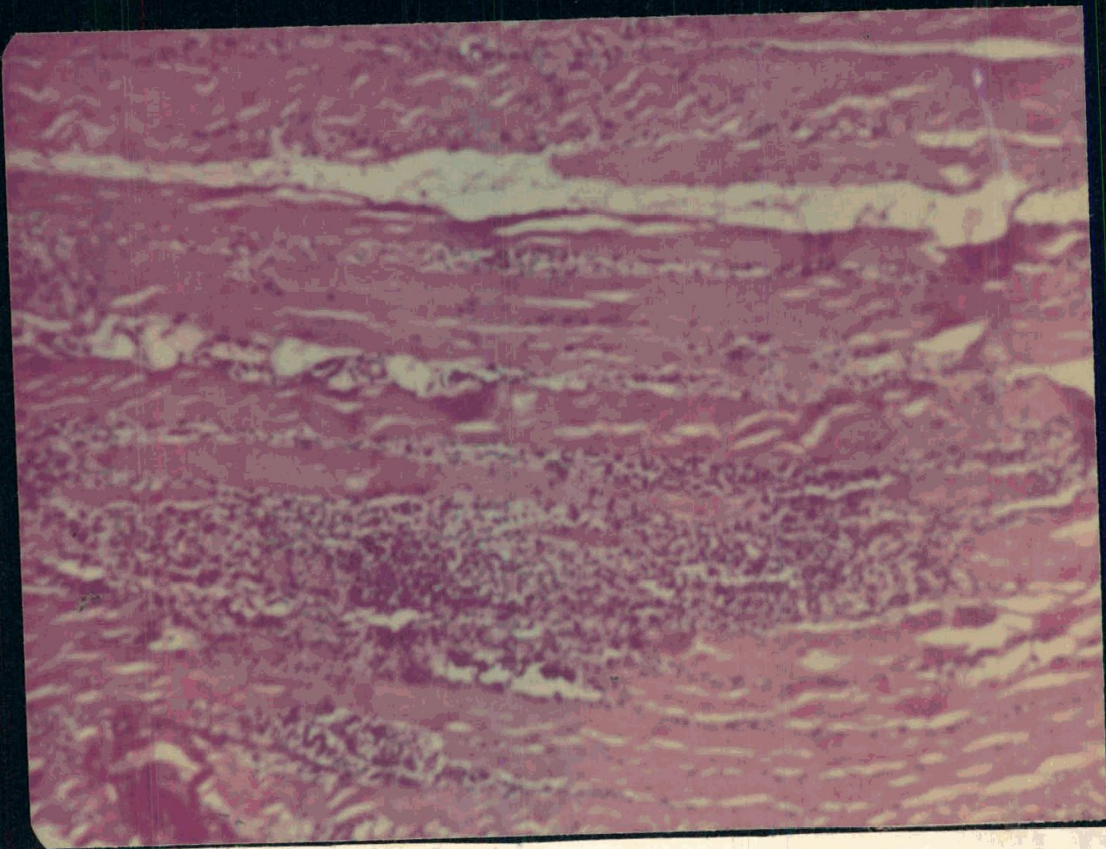


Fig.7: Photomicrograph showing dense inflammatory infiltrate in interstitium and edema of fibres. H & E X 80.

RESULTS AND DISCUSSION

The muscle biopsy done on each of the control patient a routine H&E staining and histochemistry by ATPase (pH 9.4), NADH-TR and Phosphorylase was performed on transverse sections.

These revealed tightly packed polygonal eosinophilic muscle fibres of uniform size with small dark peripherally placed nuclei, few showing a centrally placed nuclei. These fibres showed very little endomyseal connective tissue surrounding them. The histochemistry revealed a chequerboard pattern.

INFLAMMATORY MUSCLE DISEASE

13 cases studied were diagnosed as inflammatory myositis. On histology as in table III, their fascicular architecture was preserved and majority showed a preservation of fibre size and shape. There was degeneration seen in most of them and group atrophy or hypertrophy was not significant. Regeneration also was not seen and the fibres were mostly multifaceted. Macrophages, however were seen in most of them with all nuclei peripheral in each fibre. There was evidence of inflammatory infiltrate in the muscle fibre and in the interstitium (fig 7) with no significant fibre splitting or edema or an increase in connective and fibrofatty tissue infiltration. Their histochemistry showed a mosaic pattern. Keeping these features in mind they were diagnosed as cases of inflammatory myositis.

Of the 13 cases studied as shown in table with a median age of 25 year and mean of 27 year, 8 cases were clinically diagnosed as cases of polymyositis. 2 had associated skin involvement ie. dermatomyositis. 2 were diagnosed as cases of dystrophy of which 1 was thought to be Duchennes. 1 case with a high CPK level was

TABLE III

INFLAMMATORY MUSCLE DISEASE

n = 13

FASCICULAR	PRESERVED	12
ARCHITECTURE	DISTORTED	1
FIBRE SIZE & SHAPE	NORMAL	9
	VARIED	4
ATROPHY	PRESENT	3
	ABSENT	10
HYPERTROPHY	PRESENT	2
	ABSENT	11
DEGENERATION	PRESENT	8
	ABSENT	5
REGENERATION	PRESENT	-
	ABSENT	13
PHAGOCYTOSIS & NECROSIS	PRESENT	8
	ABSENT	5
ROUND OR ANG- ULATED FIBRES	PRESENT	2
	ABSENT	11
FIBRE SPLITT- ING EDEMA	PRESENT	3
	ABSENT	10
NUCLEI	INTERNAL	-
	PERIPHERAL	13
INFLAMMATION	PRESENT	13
	ABSENT	-
CONNECTIVE TISSUE	INCREASED	2
	N/DECREASED	11
FIBRO FATTY TISSUE	INCREASED	1
INFILTRATION	N/DECREASED	12

n = total number of cases

TABLE IV

TOTAL NUMBER OF CASES....13

MALE.....10 ADULT - 11

FEMALE.....03 CHILD - 02

CLINICAL DIAGNOSIS	NO.	HISTOLOGICAL DIAGNOSIS	HISTOCHEMISTRY NO. FEATURES	FINAL DIAGNOSIS
MYOSITIS	10	MYOSITIS	4 MOSAIC PATTERN WITH	INFLAMMATORY MYOSITIS
DYSTROPHY	3	MYOSITIS	2 ONE CASE EACH OF TYPE I FIBRE PREDOMINANCE	INFLAMMATORY MYOSITIS
TOTAL	13		6	

CORRELATION OF CLINICAL & BIOPSY DIAGNOSIS 10/13 CASES - 76%

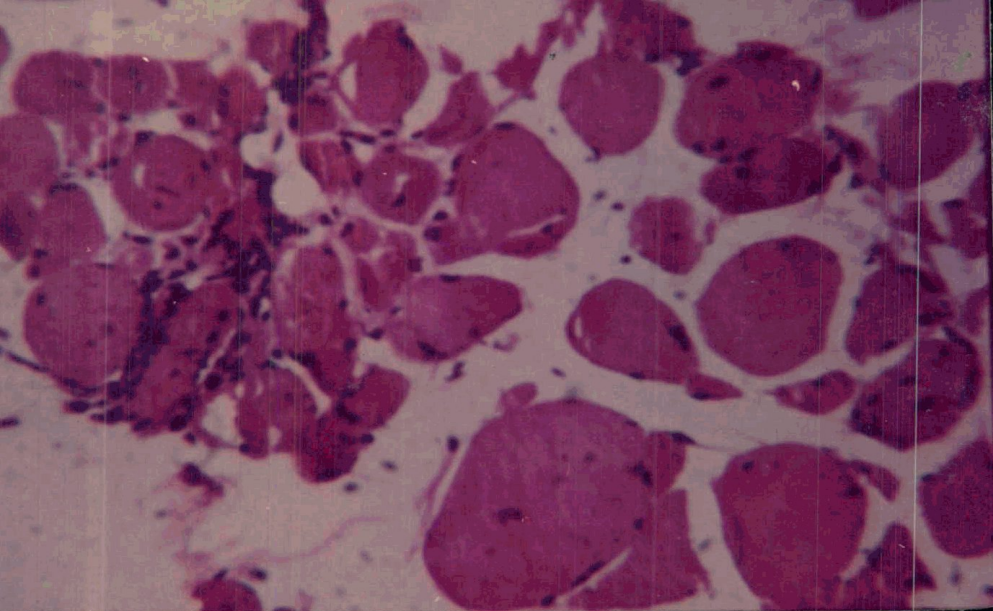


Fig.8: Focal myositis
HE X 200

9: Focal myositis
HE X 500

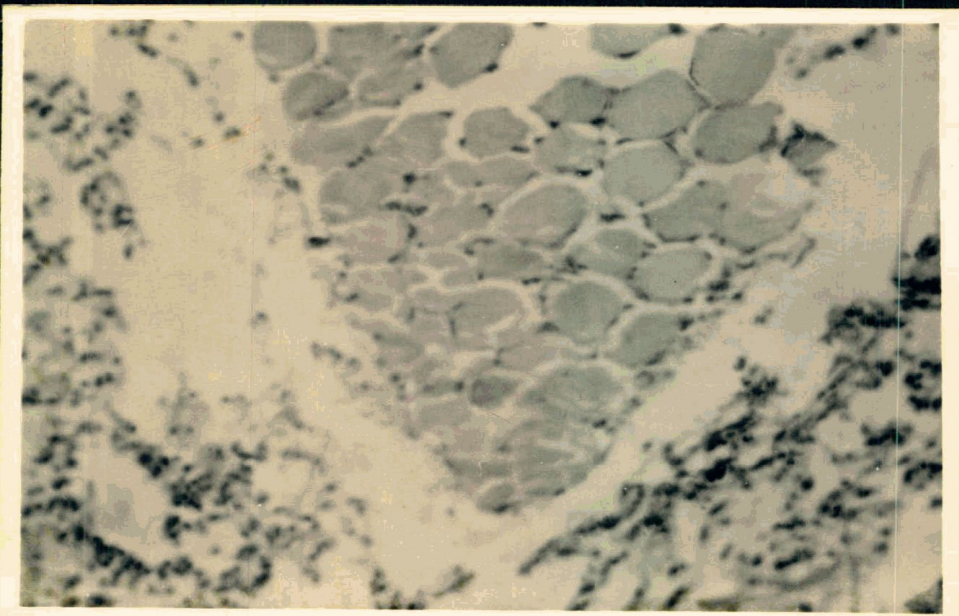
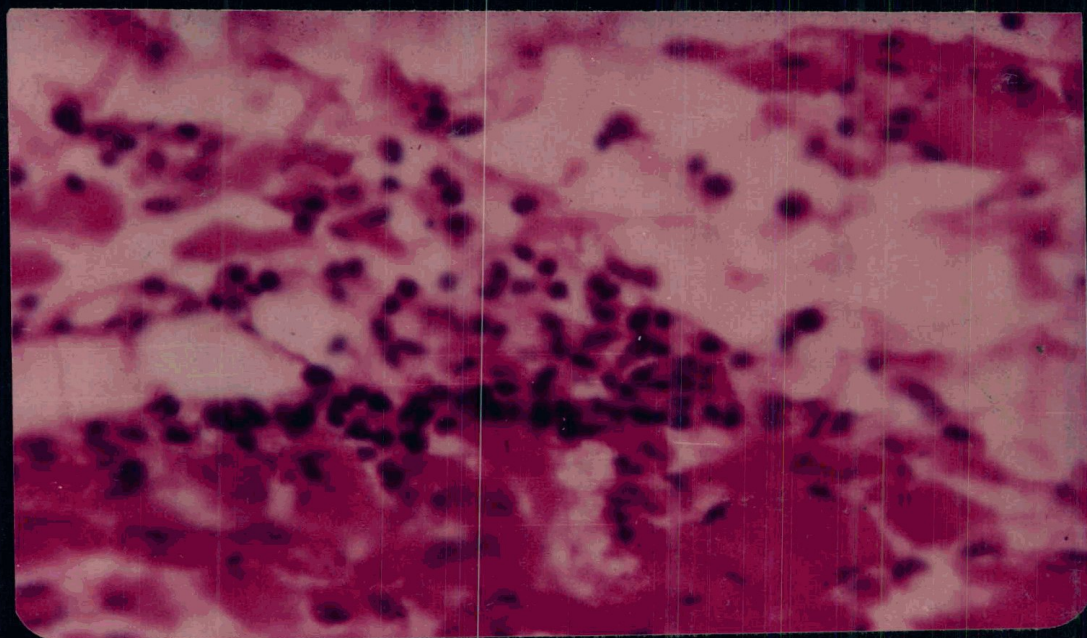


Fig.10: Interstitial
eosinophilic
myositis
HE X 200

thought to be either chronic polymyositis or Becker's disease.

2 cases of clinical myositis on histology showed a well preserved fascicular architecture with no other histopathological changes except in one which showed an acute interstitial eosinophilic infiltrate (fig 10) and another a focal mononuclear infiltrate (fig 8 & 9).

Histochemistry showed a chequerboard pattern and hence a diagnosis of interstitial eosinophilic myositis and focal inflammatory myositis was given respectively.

1 case of clinical Duchennes showed an inflammatory infiltrate with few degenerated fibres establishing a diagnosis of minimal inflammatory myositis. 7 cases of which 1 was thought to be dystrophy showed a preservation of fibre architecture and few showed a variation in the size and shape of fibres. Also noticed in them was necrotic eosinophilic fibres with a lack of nuclei, presence of macrophages, inflammatory cell infiltrate with evidence of hyaline degeneration. No perivascular infiltrate or atrophic fibres were seen. Histochemistry in some of them showed a chequerboard pattern suggesting subacute polymyositis. 3 cases of clinical polymyositis of which 1 was suspected of dystrophy on histology showed a varied fibre size with atrophic degenerated fibres. 2 of these showed interstitial inflammation with a few rounded fibres. A hypertrophy of few fibres was also noticed in them. Histochemically in 1 type II fibres were atrophic and type I predominant with no increase in connective tissue aiding a diagnosis of chronic polymyositis in remission. The other was diagnosed as to be consistent with chronic polymyositis in the absence of histochemistry. The third case showing a distorted fascicular architecture with no definite hypertrophy and

increased connective tissue. Histochemistry also showed type II fibre atrophy with type I fibre predominance. Though this patient was young (18 years) these findings were found to be consistent with polymyositis.

Weller in his studies for similar cases has noticed that the pathological picture in a biopsy depends on the stage and progression of the disease. The acute stage, shows muscle fibre atrophy which involves mainly the type 2 fibres. Most biopsies, however, are taken in the subacute phase, when muscle fibre death, phagocytosis and regeneration predominate or in the chronic phase when the muscle is replaced by fibrous tissue and fat.

An H & E stained section of a biopsy with subacute polymyositis shows varying degrees of muscle fibre necrosis and phagocytosis (Bohan & Peter, 1975B). Necrotic fibres are usually eosinophilic or unstained and lack the peripheral ring of nuclei. In addition to the presence of necrotic and regenerating fibres, there is variation in individual muscle fibre size but without distinct grouping. The atrophy is often more pronounced around the edges of fascicles (Perifascicular atrophy). Lymphocytic infiltration around blood vessels and within the endomysium is variable and seen in 50% of subacute cases.

Myosin ATPase stains show fibre type differentiation i.e. type 1 fibre predominance and type 2 fibre atrophy. 50% of patients pass into a chronic phase with slow but continuous destruction and regeneration of muscle fibres. Biopsies often show wide variation in fibre size and an increase in endomysial fibrous tissue. There is type 1 fibre predominance but type 1 fibre hypertrophy is rarely seen.

The different types of polymyositis vary in their age of onset and in their clinical features (Swash & Schwartz, 1981). 66% of male patients with polymyositis are over the age of 40 years.

Lymphocytes from patients with acute polymyositis have been shown to be cytotoxic to cultured striated muscle cells (Dawkins & Mastaglia, 1973).

The diagnosis of myositis is rarely made in the early stages. Reasons for this include : (a) General unfamiliarity with the condition, (b) Variability in the mode of onset, (c) Insidious onset of weakness in many cases simulating muscular dystrophy, (d) Initial symptom is non muscular in one third of cases.

About one half of all cases of polymyositis are linked either with one of the collagen or connective tissue disorders or, with a malignant tumor. The remainder develop as isolated primary myopathies or occur in conjunction with skin manifestations (dermatomyositis).

The typical rash of dermatomyositis occurs in 40% of the patients. In addition, another one quarter of the patients will show a variety of dermal changes. The Raynaud phenomenon also occurs with considerable frequency. In a large series it is found in about one third of all patients with polymyositis.

An unequivocal association has been established between malignancy and polymyositis in a sizeable proportion of cases. Nearly all such combined diseases have been noted in adults usually beyond 40 years. The overall incidence of tumor, when all cases of myositis are included, is nearly 20% (Dowling, 1955). When dermatomyositis develops in male over 40 there is more than 50% chance that he will demonstrate a tumor (Arundell, Wilkinson

& Haserick, 1960), and if the myositis appears after the age of 50 cancer will be found in 71% men and 24% women (Shy, 1962).

From the reports that have been submitted so far, we conclude (a) From among all cases of myositis, 15-20% harbour malignant neoplasm (b) With one or two rare exceptions, all these cases have occurred in adults over 40 (c) Almost without exceptions, the muscular disorder takes the form of florid dermatomyositis which is easily recognised. In a few persons the skin changes are only minor (d) In almost all cases the neoplasm is a carcinoma (e) Muscular weakness is characteristically proximal in distribution and dysphagia may be present (f) Dermatomyositis may be overt and the neoplasm asymptomatic or unrecognised upto death (g) Response to corticosteroid therapy is poor in contrast to that noted in polymyositis without neoplasia.

Thus we can say that inflammatory myopathy show a marked inflammatory response with a perifascicular atrophy. "Ghost fibres" and moth eaten whorled fibres may be seen. No hypertrophic fibres are seen.

TABLE V

MUSCLE DYSTROPHY

n = 18

FASCICULAR ARCHITECTURE	PRESERVED DISTORTED	- 18
FIBRE SIZE & SHAPE	NORMAL VARIED	- 18
ATROPHY	PRESENT ABSENT	15 3
HYPERTROPHY	PRESENT ABSENT	17 1
DEGENERATION	PRESENT ABSENT	16 2
REGENERATION	PRESENT ABSENT	- 18
PHAGOCYTOSIS & NECROSIS	PRESENT ABSENT	14 4
ROUND OR ANG- ULATED FIBRES	PRESENT ABSENT	13 5
FIBRE SPLITT- ING EDEMA	PRESENT ABSENT	5 13
NUCLEI	INTERNAL PERIPHERAL	12 6
INFLAMMATION	PRESENT ABSENT	1 17
CONNECTIVE TISSUE	INCREASED N/DECREASED	16 2
FIBRO FATTY TISSUE INFILTRATION	INCREASED N/DECREASED	8 10

TABLE VI

TOTAL NUMBER OF CASES....18

MALE.....18 ADULT...9

FEMALE.....-- CHILD...9

CLINICAL DIAGNOSIS	NO.	HISTOLOGICAL DIAGNOSIS	HISTOCHEMISTRY NO. FEATURES	FINAL DIAGNOSIS
DMD	10	DYSTROPHY	3 NORMAL CHEQUER BOARD	DYSTROPHY
FSH	1	CONSISTENT WITH FSH	- PATTERN WITH TYPE 1 FIBRE	DYSTROPHY
BECKER	2	DYSTROPHY	- PREDOMINANCE IN 1 CASE OF	DYSTROPHY
SMA	1	DYSTROPHY	1 DMD, DYSTROPHY AND MYOPATHY	DYSTROPHY
DYSTROPHY	1	DYSTROPHY	1 EACH.	DYSTROPHY
MYOPATHY	3	DYSTROPHY	3	DYSTROPHY

CORRELATION OF CLINICAL & BIOPSY DIAGNOSIS - 14/18 - 78%

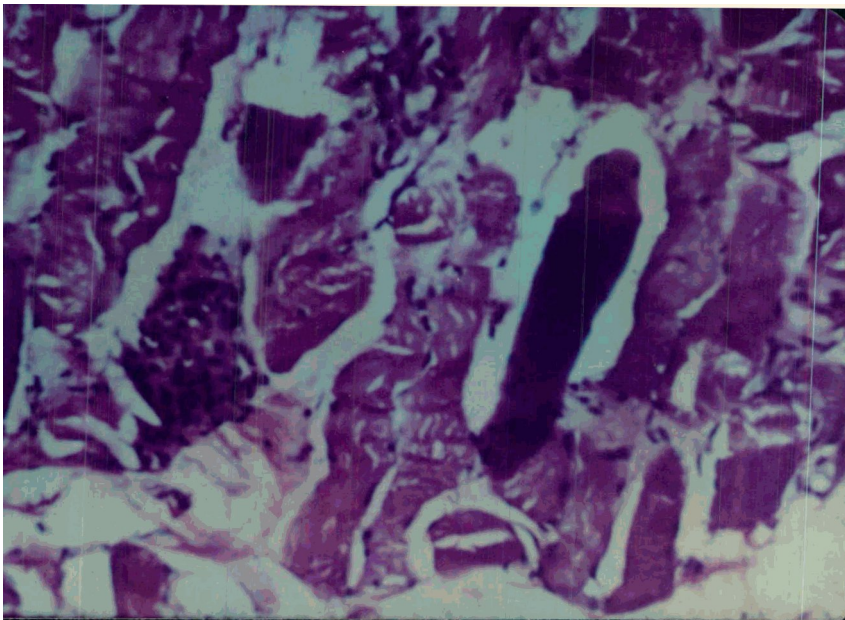


Fig.11: Photomicrograph showing dystrophic fibres with degeneration and regeneration. HE X 200

2: Photomicro-
graph of dystrophic
tissue showing large
fibres and
infiltration .
500.

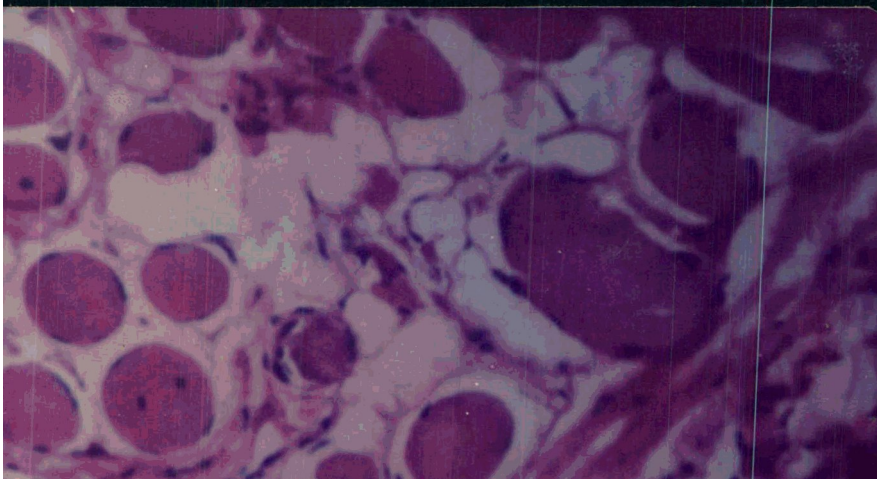
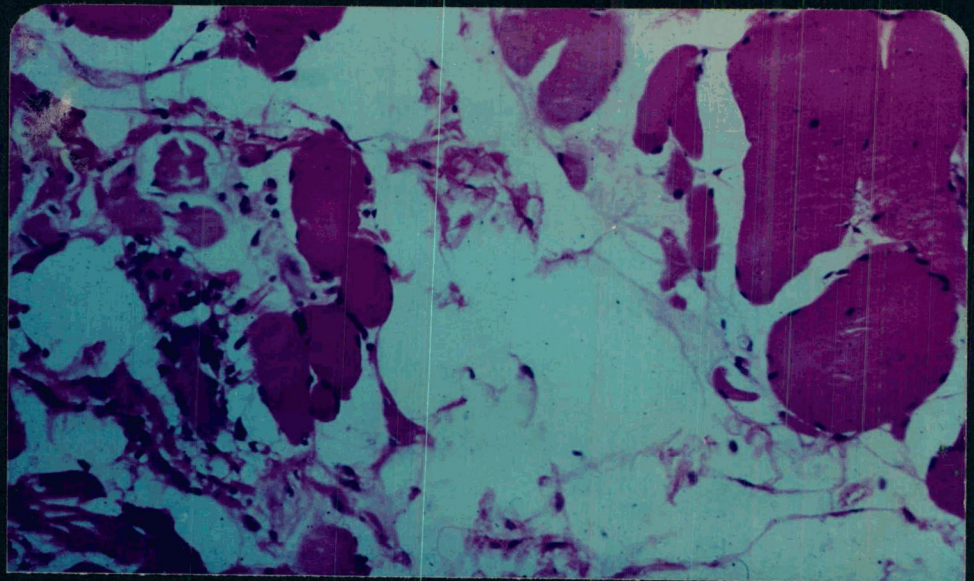


Fig.13: Dystrophy with internal nuclei and fibre splitting. HE X 500

Fig.14: Dystrophy
showing phagocyto-
sis. HE X 200

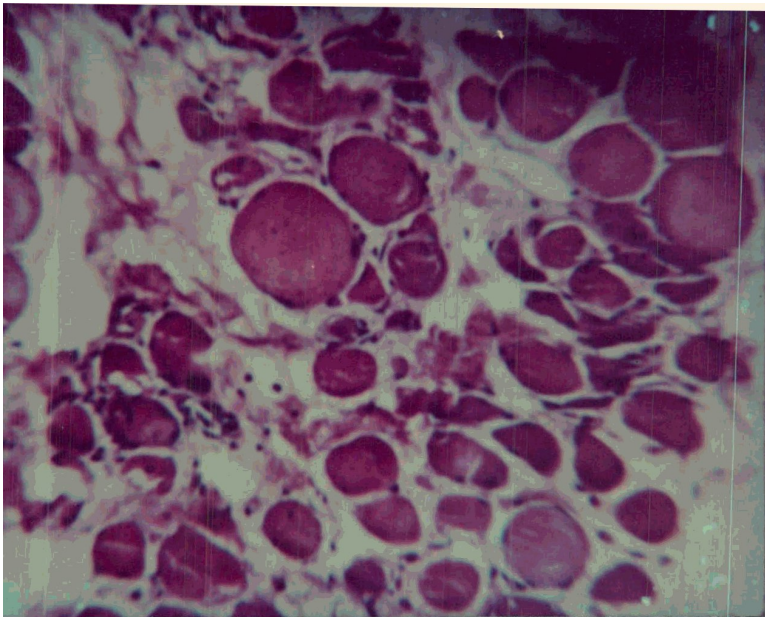


Fig.15: Muscle
dystrophy with
increased
connective
tissue
X 200

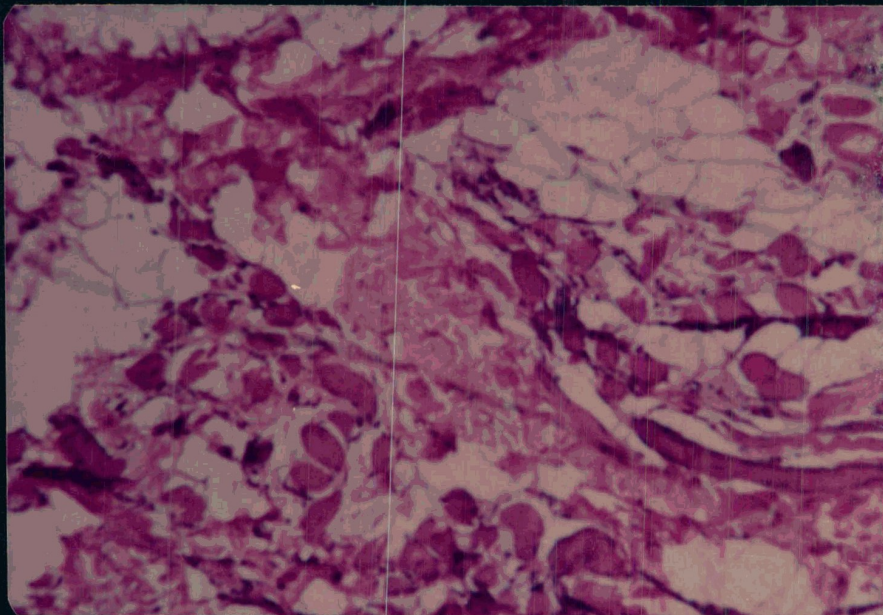
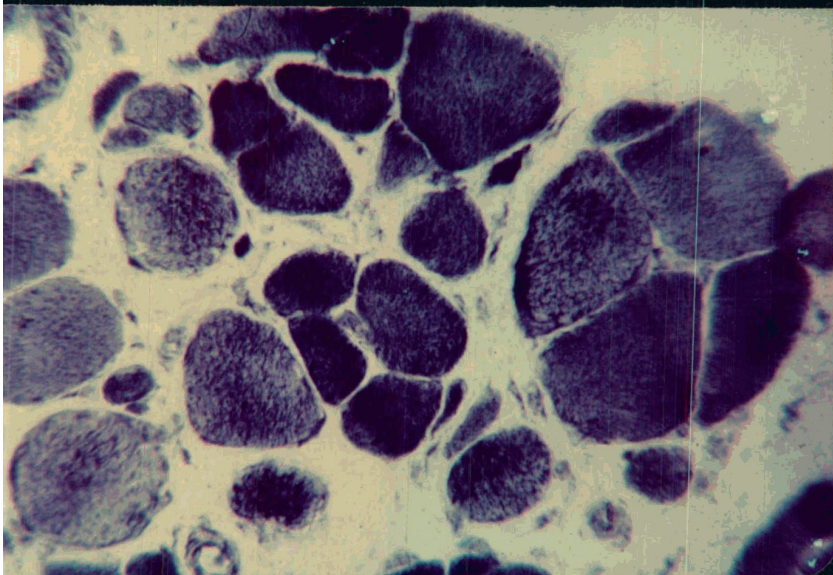


Fig.16: Photomicro-
graph of section
stained for NADH-TR
showing large round-
ed fibres predomina-
ntly Type I. X 500.



DYSTROPHY

17 cases were studied and diagnosed as dystrophy as shown in table V & VI.

On histology as in table V their fascicular architecture was lost and the size and shape of fibres was variable (fig 11 & 12). The fibres showed a significant atrophy, hypertrophy and degeneration of fibres. There was however no regeneration noticed. The fibres had a significant number of macrophages within them (fig 14) and the fibres were rounded or angulated. The nuclei in each were internalised (fig 13). There was no significant fibre splitting or inflammatory response with only a moderate increase in connective and fibrofatty tissue (fig 15). Histochemistry showed a mosaic pattern (fig 16) with a few showing type I fibre predominance. Keeping this in mind they were diagnosed as cases of dystrophy.

Based on their present age and mode of presentation, these cases of were diagnosed as 10 cases of Duchenne having a mean age of 9 years, 2 cases of Becker with a mean age of 20 years, 1 as dystrophy and 1 as fascioscapulohumeral dystrophy.

4 were diagnosed as cases of myopathy ie. dystrophy or neurogenic muscle disease. In all the ten cases of Duchenne, it was noticed on histology that there was a marked variation in fibre size and shape with a complete distortion of the muscle architecture. All the fibres showed hyaline degeneration with 70% showing roundness of fibres with phagocytic activity. There was hypertrophy of fibres noticed along with small atrophic fibres. Since all the cases were diseased since a long duration there was absence of basophillic or regenerated fibres with an increase in the

endomyseal and perimyseal connective tissue and fat and fibrous tissue infiltration. There was no evidence of inflammatory cell infiltration. There was an internalisation of nuclei observed with a 30-40% depending on the duration and age of presentation showing fibre splitting or edema. Histochemistry was not very helpful in these cases though it showed that type II fibres were atrophic with type I showing a tendency to be predominant. Thus with these microscopic findings due to long standing duration of disease a diagnosis of dystrophy was established without specifying the type.

1 case of suspected FSH disease however showed similar features on H&E with muscle fibre necrosis and inflammatory cell infiltrate. Though histochemistry was not done the findings were consistent with FSH. In 2 cases of suspected Becker's similar features on histology were seen. However, in the absence of histochemistry no comment on group atrophy or pyknotic nuclei could be made and hence a diagnosis of dystrophy was made. 4 cases of myopathy however were supplemented with histochemistry. These showed similar histological features as those of dystrophy, and histochemically type II fibre atrophy with 1 showing associated type I fibre predominance. One of these cases was clinically thought to be Becker's or SMA which was ruled out in the absence of fibre type grouping. Another case of dystrophy was found to be consistent histologically with the clinical diagnosis.

Weller states that there is a considerable range of muscular dystrophies with the rapidly progressive X-linked recessive Duchenne muscular dystrophy at one end of the spectrum and slowly progressive dystrophies in which there is little reduction of life expectancy at the other end. The diagnosis depends on a

combination of clinical and pathological features.

Duchenne muscular dystrophy (pseudohypertrophic muscular dystrophy) presents before the age of 5 years and muscle biopsies reflect the rapidly progressive and severely destructive nature of the disease. On histology, the amount of fibre destruction, regeneration and replacement of muscle by fibrous and adipose tissue varies according to the stage of the disease and the age of the patient. In H & E stained sections there is a wide variation in muscle fibre size with hypertrophic rounded fibres, small atrophic fibres, extensive phagocytosis and many small basophilic regenerating fibres. Except in the early stages there is also marked endomysial fibrosis. In older patients 5 years of age onwards, fibre splitting may be seen in the hypertrophic muscle fibres as in ours. In the late stages of the disease, the muscle is totally replaced by fibrous tissue and fat.

Our mean age of the patients was 9 years due to desire for treatment at a later age.

Facioscapulohumeral dystrophy - It shows a typical dystrophic pattern with muscle fibre necrosis, regeneration, muscle fibre hypertrophy and fibre splitting. In many cases there is prominent lymphocytic infiltration in the muscle which may suggest polymyositis. However, the muscle fibre hypertrophy and the clinical history helps to distinguish the 2.

Other dystrophies - Fibre splitting is seen in large fibres with splits extending to a varying extent across the fibre. Internal nuclei are increased in number and ring fibres may be present (Dubowitz & Brooke, 1973). In addition to the gross hypertrophy, there may be severe atrophy of other fibres and some may remain

only as small groups of nuclei with little associated cytoplasm. Muscle fibre phagocytosis and regeneration is variable.

Myosin ATPase reveals good fibre type differentiation i.e. type 1 fibre predominance and type 2B fibre deficiency (Dubowitz & Brooke, 1973).

The diagnosis on the type of dystrophy is made on the clinical distribution of the muscle involvement.

Thus we can summarise that the changes seen in different types of dystrophies are :

a) In cases of Duchenne muscular dystrophy, one sees many rounded fibres of variable size, including "opaque" fibres with much fibrosis, phagocytosis and little change in the myofibrillar net. Basophilic fibres are seen in early cases often in clusters.

b) Becker's dystrophy - here there is an increased variability in fibre size, with their splitting. A group atrophy and pyknotic nuclei (denervation) is observed. There is fibrosis with a lesser amount of necrosis and phagocytosis. Basophilic fibres are also seen.

c) Limb girdle dystrophy: This shows a good fibre type differentiation with a much fibre splitting. The type II b fibres are deficient. "Moth eaten" and "ring and coil" fibres are common. There is an internalization of nuclei with a lesser amount of fibrosis, phagocytosis and basophilia as compared with the above.

d) Facioscapulohumeral dystrophy: It shows large fibres often of type II variety with small angulated fibres. Few of the fibres are moth eaten and whorled. There is a lesser amount of fibrosis with often focal and inconspicuous changes. Inflammatory response

is common with an occasional extensive pathological change.

e) Congenital muscular dystrophy: In the early stages, one sees a mildly myopathic muscle with a variation in fibre size. Some degenerative changes with focal adipose tissue are seen.

In the later stages, irrespective of the age of patients, dystrophic changes are seen in the muscle fibres with a predominance of type I fibres. There is extensive adipose tissue replacement with connective tissue proliferation. Few moth eaten fibres are also seen.

f) Myotonic dystrophy: In this specifically one may see an atrophy of type I fibres with an extensive internal nuclei. Ring and moth eaten fibres with sarcoplasmic masses may also be observed.

g) Occulopharyngeal dystrophy: In this dystrophy, histologically one observes the presence of many rimmed vacuoles.

TABLE VII

NEUROGENIC MUSCLE DISEASE

n = 9

FASCICULAR	PRESERVED	8
ARCHITECTURE	DISTORTED	1
FIBRE SIZE & SHAPE	NORMAL	-
	VARIED	9
ATROPHY	PRESENT	9
	ABSENT	-
HYPERTROPHY	PRESENT	6
	ABSENT	3
DEGENERATION	PRESENT	3
	ABSENT	6
REGENERATION	PRESENT	-
	ABSENT	9
PHAGOCYTOSIS & NECROSIS	PRESENT	3
	ABSENT	6
ROUND OR ANG- ULATED FIBRES	PRESENT	6
	ABSENT	3
FIBRE SPLITT- ING EDEMA	PRESENT	-
	ABSENT	9
NUCLEI	INTERNAL	1
	PERIPHERAL	8
INFLAMMATION	PRESENT	-
	ABSENT	9
CONNECTIVE TISSUE	INCREASED	2
	N/DECREASED	7
FIBRO FATTY TISSUE	INCREASED	1
INFILTRATION	N/DECREASED	8

TABLE VIII

TOTAL NUMBER OF CASES...9

MALE.....6 ADULT...6

FEMALE.....3 CHILD...3

CLINICAL DIAGNOSIS	NO.	HISTOLOGICAL DIAGNOSIS	HISTOCHEMISTRY NO. FEATURES	FINAL DIAGNOSIS
DYSTROPHY (not specified)	4	4 DENERVATION ATROPHY	- -	3 DENERVATION ATROPHY 1 SMA
BECKER	1	NEUROGENIC ATROPHY	- -	NEUROGENIC ATROPHY
SMA	3	3 NEUROGENIC ATROPHY	AS IN TABLE X.	
WERDNIG HOFFMAN DISEASE	1	CONSISTENT WITH WERDNIG HOFFMAN		

CORRELATION OF CLINICAL & BIOPSY DIAGNOSIS 4/9 - 44%

TABLE IX

HISTOCHEMICAL FEATURES	CLINICAL DIAGNOSIS WITH AGE			
	WERDNIG HOFFMAN 7 MONTHS	SMA 25 YRS	SMA 14 YRS	SMA 4 YRS
ATROPHIC FIBRES	++	+	+	++
HYPERTROPHIC FIBRES	TYPE I	-	-	-
FIBRE GROUPING	++	-	-	+
FINAL DIAGNOSIS ON CORRELATION WITH HISTOLOGIC AND CLINICAL FEATURES	INFANTILE SMA	SUGGES- TIVE OF NEURO- GENIC ATROPHY	DENERVA- TION ATROPHY	SUGGES- TIVE OF SMA

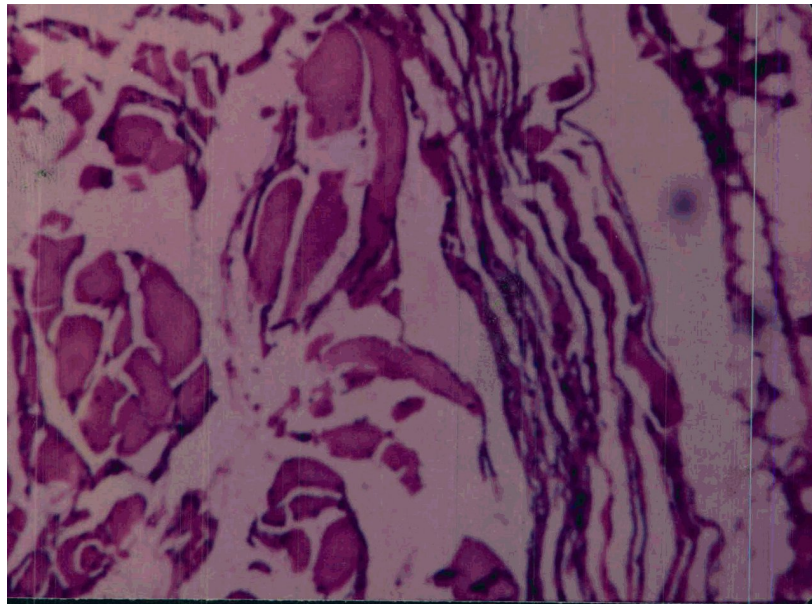


Fig.17: Photomicrograph showing thin atrophic fibres cut longitudinally
HE X 200.

Fig.18: Photomicrograph showing well preserved fascicles with small angulated fibres. HE X 200.

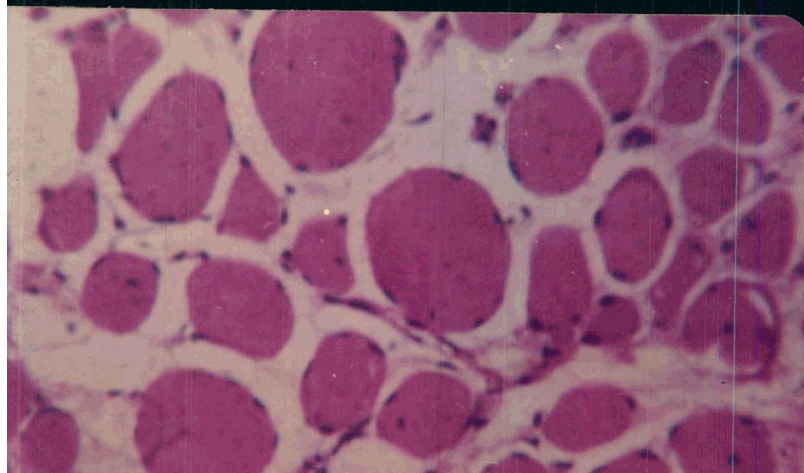
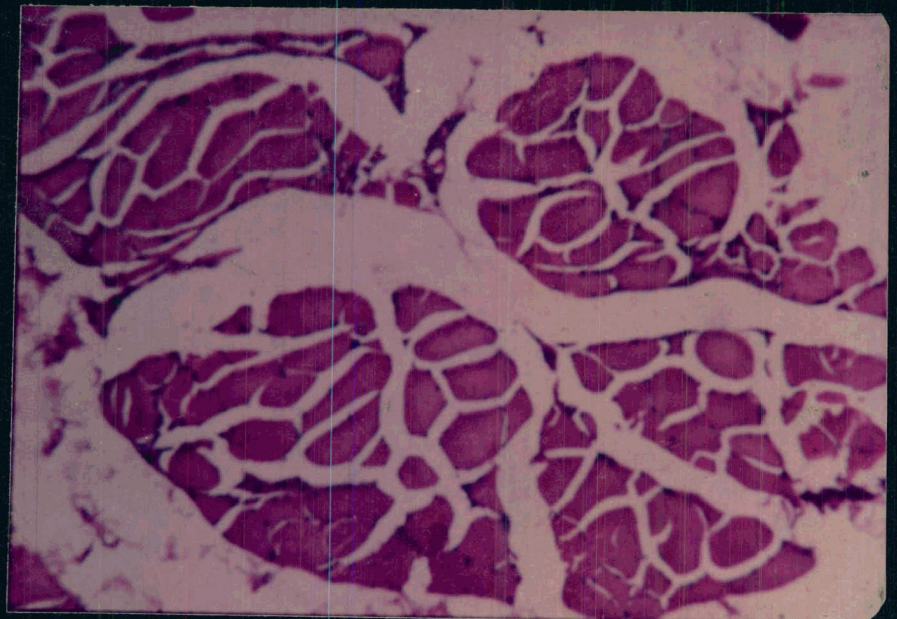


Fig. 19: Photomicrograph showing muscle cut transversely showing angulated fibres. HE X 500.

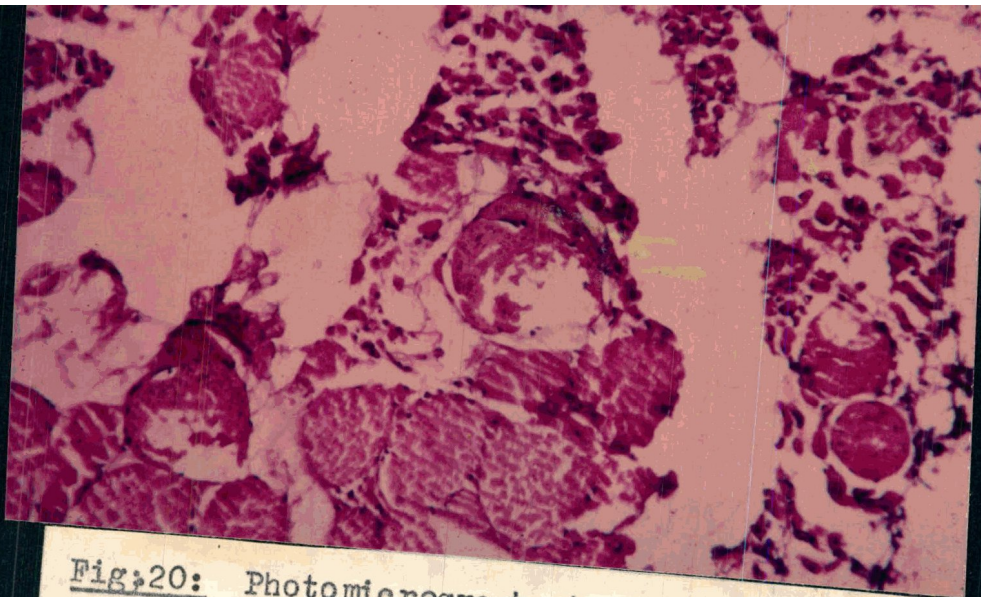


Fig.20: Photomicrograph showing atrophy of large group of fibres involving entire fascicle and hypertrophy of fibres. HE X 200.

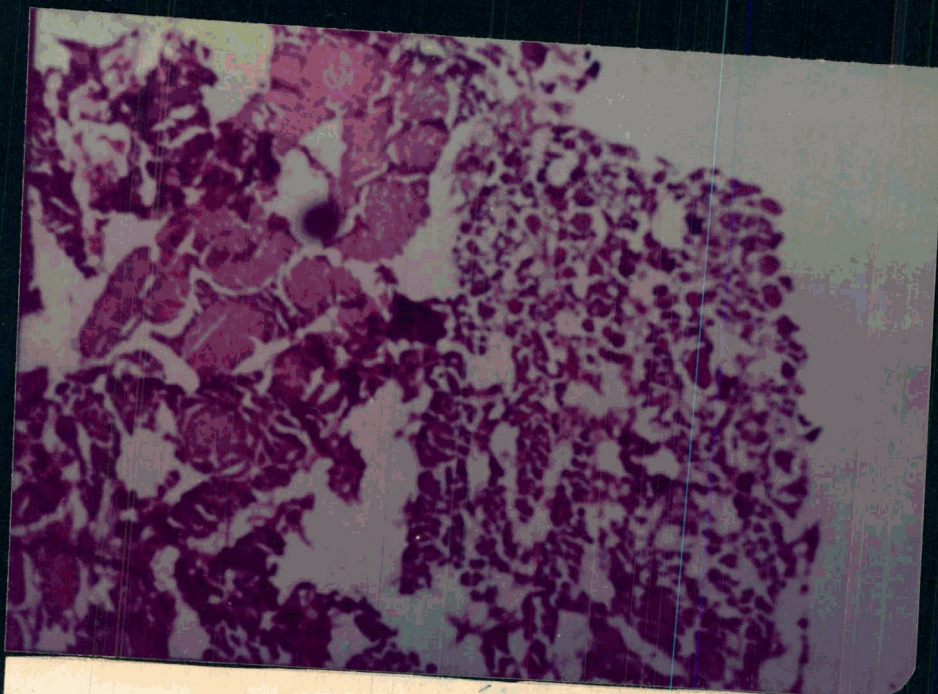


Fig.21: Photomicrograph showing atrophy of large group of fibres involving entire fascicle and hypertrophy of fibres. HE X 200.

NEUROGENIC MUSCLE DISEASE

9 cases were studied and diagnosed as neurogenic muscle disease as shown in table VII, VIII & IX.

On histology as in table VII, it was noticed that though the architecture was preserved, the fibre size and shape was varied. There was a significant atrophy (fig 20 & 21) and hypertrophy of fibres with no noticeable evidence of degeneration or regeneration. The fibres also appeared rounded or angulated. The nuclei however were peripheral. There was no significant phagocytosis, inflammation, fibre splitting or edema or increase in connective and fibrofatty tissue. Thus in all histology was suggestive of neurogenic atrophy (fig 17 & 18). However, no specific diagnosis could be made. On histochemistry as in table IX, when atrophy and grouping of fibres were noted then along with clinical correlation a specific diagnosis could be made.

Of these 5 cases were clinically suspected of dystrophy or primary muscle disease. 3 as cases of SMA and 1 as myopathy. Of the 3 cases of SMA 1 due to its age of presentation ie. 7 months was thought to be infantile SMA (Werdnig Hoffmann Disease). All these cases histologically showed a preserved fascicular architecture with a variation in the size and shape of fibres. All of them showed atrophic fibres with angulation and 70-80% showed hypertrophy. Some also showed degeneration and phagocytosis. Endomyseal connective tissue was increased depending on the duration of the disease. There was no evidence of regeneration, edema or fibre splitting. In 5 cases on which histochemistry was not done a diagnosis of neurogenic atrophy was given. No definite typing could be done. 1 of these showed small muscle group atrophy and considering the age a diagnosis of SMA

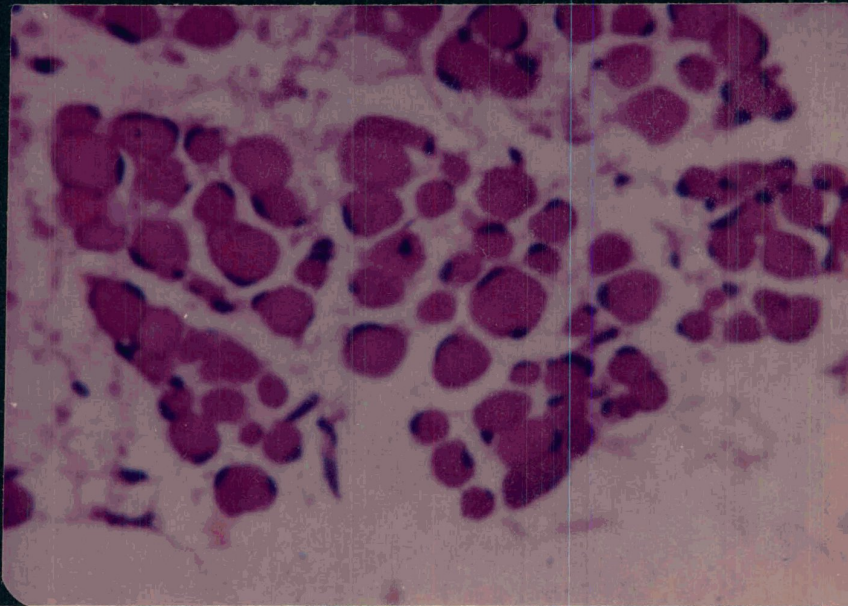


Fig.22: Werdnig Hoffmann disease showing two fibre size population. HE X 200.

was suggested. In 4 cases however histochemistry was done. 2 of these showed atrophy of type I and II fibres with a grouping of type I fibres. Hence a definite diagnosis of SMA was suggested. 1 case because of its clinical age of seven months was thought to be infantile SMA showed associated type I muscle fibre hypertrophy and hence a definitive diagnosis of infantile SMA was established. The other 2 cases showed both type of fibre to be atrophied ie. a mosaic pattern and hence a diagnosis of denervation atrophy was stated.

Weller states that there are several features in a biopsy which suggest the diagnosis of neurogenic muscle disease. Atrophic, angulated fibres are detected in H & E stained sections in small or large groups. Extremely atrophic denervated fibres remain only as small groups of darkly stained nuclei devoid of cytoplasm.

Most biopsies require enzyme histochemistry and fibre typing to confirm the diagnosis. Those stained with NADH-TR show dark blue staining of denervated atrophic fibres regardless of fibre types. ATPase stains show both atrophic type 1 and type 2 fibres which is a certain evidence of denervation.

Clinically neurogenic muscle disease is more severe in the distal parts of the limbs with wasting and weakness of the small muscles of the hands, lower legs and feet. Spinal muscular atrophy is an exception; the muscle involvement may be mainly in the shoulder and pelvic girdles and may be confused with dystrophy.

Spinal muscular atrophy - Infantile (SMA type 1 or Werdnig - Hoffmann disease) (fig 22) presents acutely early in life and is one of the causes of a hypotonic or floppy infant. This disease may present before birth or at delivery and give rise to severe

disability; 95% of patients are dead by the age of 18 months (Pearn & Wilson, 1973). A biopsy reveals large groups of atrophic rounded fibres. Hypertrophy of the remaining innervated fibres, especially type 1, is seen (Dubowitz & Brooke, 1973).

SMA type 2 also occurs in infancy and is indistinguishable from SMA type 1.

SMA type 3 is the juvenile form and presents at the age of 5 to 15 years. A biopsy shows the features of neurogenic muscle disease with groups of atrophic type 1 and type 2 fibres and fibre type grouping suggesting reinnervation. Myopathic changes with muscle fibre destruction, phagocytosis and regeneration may also be seen together with some fibrosis within the muscle (Dubowitz & Brooke, 1973).

SMA type 4, the adult form, presents in the fourth decade and may be confused clinically with a dystrophy. The biopsy shows group atrophy, fibre grouping and myopathic changes.

Thus we can summarise that

a) In spinal muscular atrophy i.e. of severe infantile type (Werdnig Hoffman disease), and of intermediate severity (late infantile/juvenile) we see a large group atrophy with rounded atrophic fibres of both types. The hypertrophied fibres seen are mostly type I. (ATPase). In cases of mild spinal muscular atrophy (Kugelberg-Wellander disease) we see hypertrophic fibres often of type II with type grouping and structural changes with internal nuclei in large fibres.

b) In cases of motor neuron disease, we observe a type II fibre hypertrophy with a type II fibre predominance. A type II fibre atrophy is seen if the upper motor neurone involvement is severe.

c) In cases of chronic neuropathy, one sees a type I fibre hypertrophy with a type I fibre predominance and "myopathic" changes.

d) In cases of simple neuropathy, no specific changes are seen other than general denervation changes.

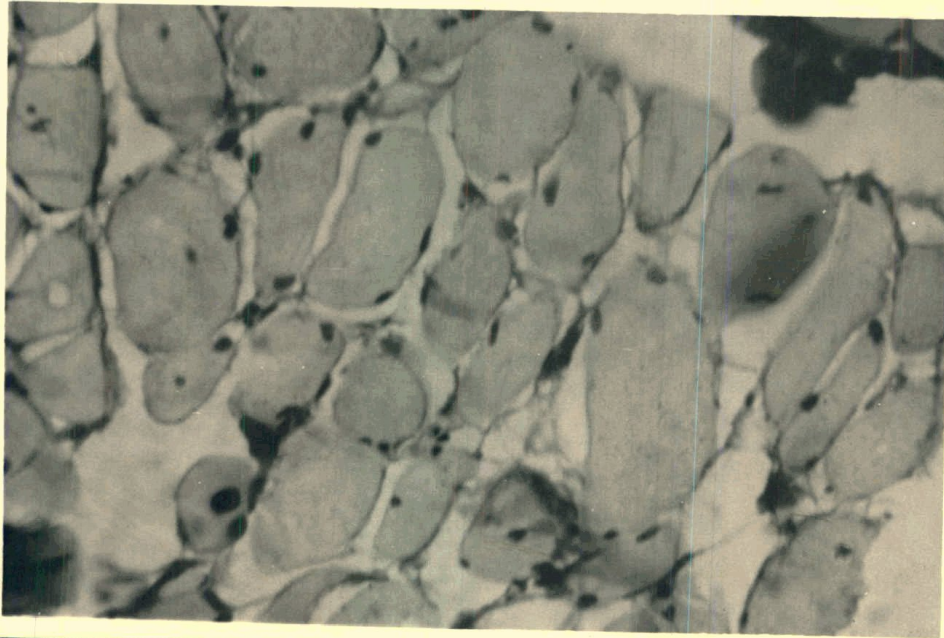


Fig.23: Centronuclear myopathy. HE X 200

CONGENITAL MYOPATHY

Table II shows a case of a 14 years adolescent clinically suspected of congenital dystrophy. The histology showed a variation in muscle fibre size with a large number of small round atrophic fibres. More than 75% of these showed a prominent central nuclei with a perinuclear halo. The histochemistry showed a mosaic pattern establishing a diagnosis of centronuclear (myotubular) myopathy (FIG 23).

Weller states that :

Myotubular (centronuclear) myopathy has a distinctive histological picture with wide variation in muscle fibre size and large numbers of small fibres with prominent central nuclei. Clinically, they form a heterogeneous group; some patients die in infancy, often soon after birth, whereas others survive to adult life (Van Wijngaarden et al, 1969; Hawkes & Absolom, 1975). Thus summarising other features of biopsies of muscle of other disorders we see that :

Along with other secondary changes, seen in the muscle, the primary differentiating features seen in other disorders are as follows:

- a) Myaesthesia gravis: Here we see lymphorrhages with focal type II fibres atrophy.
- b) Periodic paralysis: One sees tubular aggregates with vacuole formation.
- c) Occulocraniosomatic neuromuscular disease: This biopsy is not a very abnormal biopsy other than the observance of "ragged -red" or granular fibres.

d) Central core disease, nemaline myopathies, reducing body myopathy.

The distinctive pathological change seen in all these three are implied in their names. However, they may also show a selective type I fibre involvement with a type I atrophy and predominance.

e) Myotubular myopathy: This is diagnosed only if one sees a prominent single central nuclei (may also affect 10-20% of fibres in transverse section owing to spacing of nuclei). They show a dark central aggregation of stain with NADH-TR and a central "hole" with ATPase.

f) Congenital fibre type disproportion: A childhood biopsy in which type I fibres are smaller than the type II fibres (which may be hypertrophic).

g) McArdles (phosphorylase deficiency) disease: This disease shows a negative histochemical reaction for phosphorylase. Clinically it may be seen in childhood and adolescence, early adult life, fourth or fifth decade.

h) Myotonia congenita: It shows a complete absence of type IIb fibres.

i) Nemaline myopathy : This is another non progressive congenital myopathy. The characteristic rod bodies or nemaline rods were first recognised by Conen, Murphy & Donohue (1963) and Shy, Engel, Somers & Wanko (1963). The nemaline rods are devoid of enzyme activity and can be demonstrated by the Gomori trichrome or PTAH stains.

TABLE X

ADVANCED MUSCLE DISEASE

n = 10

FASCICULAR ARCHITECTURE	PRESERVED DISTORTED	- 10
FIBRE SIZE & SHAPE	NORMAL VARIED	2 8
ATROPHY	PRESENT ABSENT	6 4
HYPERTROPHY	PRESENT ABSENT	5 5
DEGENERATION	PRESENT ABSENT	8 2
REGENERATION	PRESENT ABSENT	1 9
PHAGOCYTOSIS & NECROSIS	PRESENT ABSENT	5 5
ROUND OR ANG- ULATED FIBRES	PRESENT ABSENT	3 7
FIBRE SPLIT- TING EDEMA	PRESENT ABSENT	- 10
NUCLEI	INTERNAL PERIPHERAL	2 8
INFLAMMATION	PRESENT ABSENT	1 9
CONNECTIVE TISSUE	INCREASED N/DECREASED	10 -
FIBRO FATTY TISSUE INFILTRATION	INCREASED N/DECREASED	10 -

TABLE XI

TOTAL NUMBER OF CASES....10

MALE.....9

ADULT....10

FEMALE.....1

CHILD.....-

CLINICAL DIAGNOSIS	NO.	HISTOLOGICAL DIAGNOSIS	HISTOCHEMISTRY NO.	FEATURES	FINAL DIAGNOSIS
DYSTROPHY	3	ADVANCED MUSCLE DISEASE	2	MOSAIC PATTERN	ADVANCED MUSCLE DISEASE
DMD	2	ADVANCED MUSCLE DISEASE	1	MOSAIC PATTERN	ADVANCED MUSCLE DISEASE
MYOPATHY	6	ADVANCED MUSCLE DISEASE WITH 1 CASE OF CHRONIC POLY- MYOSITIS.	4	MOSAIC PATTERN	ADVANCED MUSCLE DISEASE

ADVANCED MUSCLE DISEASE

10 cases were studied and diagnosed as advanced muscle disease as shown in table X & XI.

On histology as in table X all these cases showed a marked increase in connective and fibrofatty tissue with very few muscle fibres. Those which were seen had a distorted architecture, varied size and shape of fibres, atrophy and hypertrophy and evidence of degeneration. No significant regeneration, phagocytosis, inflammation or fibre edema was seen. Their nuclei were peripheral and angulation of fibres was not seen. Their histochemistry was inconclusive and hence a diagnosis of advanced stage of muscle disease was given.

These cases were of different age groups with long standing disease. They were clinically diagnosed as cases of dystrophy or myopathy. Their histology was not very informative. Histochemistry was done on five of these cases.

On histology 2 cases showed occasional degenerated muscle fibres and a marked increase in the connective tissue with fat and fibrous tissue infiltration. Histochemistry was not done on them and were diagnosed as muscle in advanced stage of disease. In 7 of these cases, whilst showing an increase in fibrofatty tissue and the connective tissue a few muscle fibres were also seen. these fibres showed a loss of architecture with a variation in their size and shape. Some were rounded and atrophic and others showed hypertrophic features with evidence of degeneration and phagocytosis. Their histochemistry showed a mosaic pattern and based on these features they were diagnosed as cases of advanced chronic dystrophy. Another case with similar features also showed

an inflammatory mononuclear cell infiltrate and it was diagnosed as a case of advanced chronic polymyositis.

TABLE XII

MYOPATHIES WITHOUT ESTABLISHED MICROSCOPIC CHANGE

TOTAL NUMBER OF CASES...13

MALE.....08

FEMALE.....05

CLINICAL DIAGNOSIS	NO.	HISTOLOGICAL DIAGNOSIS	HISTOCHEMISTRY NO FEATURES	FINAL DIAGNOSIS
MYOSITIS	2	NORMAL MUSCLE	- -	NO PATHOLOGY
MYOPATHY	11	NORMAL MUSCLE	7 NORMAL CHEQUER BOARD PATTERN.	SEEN
TOTAL	13		7	

CORRELATION OF CLINICAL & BIOPSY DIAGNOSIS 0/13 CASES - 0%

MYOPATHIES WITHOUT ESTABLISHED MICROSCOPIC CHANGE

There are many clinically recognisable diseases of muscle, with a severe and often fluctuant, functional disorder sometimes sufficient to terminate life, in which the pathologists can identify no characteristic or specific change in the muscle fibre. These are stated as below :

1. Thyroid disease
 - a. Hypothyroidism
 - b. Hyperthyroidism
2. Primary renal acidosis & Milkman's disease
3. Addison's disease with contractures
4. Acromegaly and hypopituitarism
5. Hyper and Hypo parathyroidism
6. Tetanus, botulism & black-widow spider intoxications
7. Adynamia episodica hereditaria and paramyotonia
8. Myasthenia gravis
9. Stiff man's syndrome

Some of these disease such as tetanus, acute botulism and stiffman's syndrome have revealed nothing after a careful study under the light microscope : and if the pathologists were uninformed of the clinical picture, the muscle tissue was stated as normal. In others, such as myasthenia gravis doubts still exists regarding the myopathology. Where as in many fatal cases of these disease the only visible change is a mild often insignificant disuse atrophy, in others isolated necrosis of single muscle fibre and a few aggregates of lymphocytes and monocytes have been found. When unusually prominent, the question of polymyositis or Zenkers degeneration secondary to a terminal infection or a myopathy associated with neoplasia always arises.

In a few cases however, if one sees group atrophy, it is indicative of denervation. In this type one is justified in assuming that the abnormality is at the neuromuscular junction which is not revealed in routine biopsies. Only the use of special techniques such as methylene blue staining method of Coers & Wollf or electron microscopy permit their study. Finally some diseases such as myopathies of hyper and hypo thyroidism, Addisons disease, renal acidosis and Milkman's disease, of adynamia episodica hereditaria and paramyotonia and the generalised endogenous myospasms, the pathological study is too limited to determine the status of fibre. By contrast, the mild early lesions as seen in a biopsy may not be considered abnormal.

A biopsy may look normal due to the possibility of sampling error, i.e. failing to expose the basic disease because of excising an unaffected part of the muscle chosen for biopsy, and even autopsy examination rarely surveys more than a few small parts of several parts of the muscle. Thus the biopsy may prove to be negative or fail to demonstrate the most significant attributes of the disease.

In the assesment of diseased muscle, the selection is even more critical. The muscle of choice is one which is clinically affected, but not to such an advanced degree that all muscle tissue has degerated beyond recognition. Where a disease processed is fairly focal care should be taken to select a muscle that is affected by the disease.

The 13 cases shown in table XII, 2 were clinically suspected of congenital mitochondrial myopathy, 1 as myositis and the remaining 10 as cases of myopathy (ie. either primary muscle disease or neurogenic atrophy).

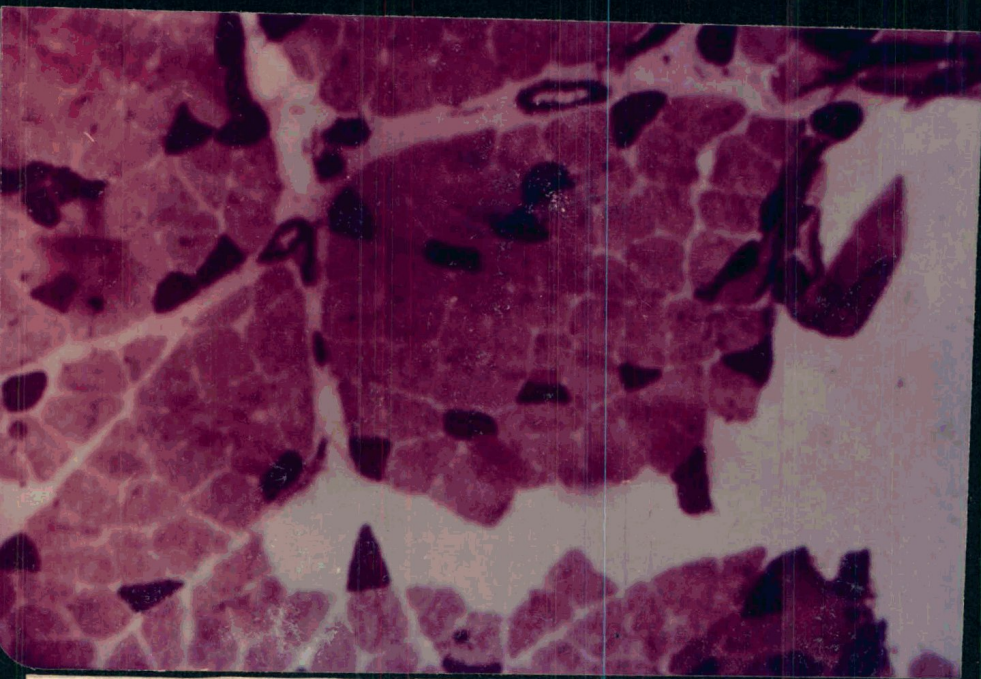


Fig.24: Section stained with ATPase showing fibre grouping. X 200.

Histological examination of these cases showed a normal fascicular architecture with a normal size and shape of fibres. There was no evidence of atrophy, hypertrophy, degeneration or regeneration of muscle fibres. The nuclei were small, dark and peripheral with no evidence of rounded or angulated fibres or splitting of fibres. No phagocytosis or an inflammatory cell infiltrate was noticed. There was minimal endomyseal and perimyseal connective tissue.

Histochemical study of seven of these cases with ATPase, NADH-TR and Phosphorylase revealed a normal chequerboard pattern ie. with ATPase type I were lightly stained and type II were intermediate or darkly stained. With NADH-TR type I were darkly blue stained and type II were intermediate or lightly stained. With phosphorylase type I were light and type II were intermediate or darkly stained. There was no predominance noticed of any particular type or any fibre grouping seen. Artifactual degeneration, roundness of fibres and fibre grouping (fig 24) was noticed in one case. They were hence reported as biopsies of normal muscle ruling out a possibility of a neuromuscular disorder.

CONCLUSION

We can thus see in our study of 64 cases that 13 were diagnosed as cases of Inflammatory muscle disease, 18 as muscle dystrophy, 9 as Neurogenic muscle disease, 1 as congenital myopathy, 10 as cases of advanced muscle disease and 13 as myopathies with no established microscopic change by utilising histology and histochemistry.

76% of cases of myositis, 78% of myopathies, 44% of neurogenic and 0% of cases without any diagnostic pathology were diagnosed the same clinically proving the efficacy and essentiality of a muscle biopsy. Of the neurogenic atrophy cases as we saw histochemistry was an essential tool to type the exact diagnosis proving histochemistry to be very essential. Also the clinical muscle weakness cases with no microscopic change advaents the need for newer modes to be utilised on a muscle biopsy to be able to determine the causative factor.

When one is asked to evaluate a muscle biopsy, the interpretation is based on experience and recognition of similarities between the biopsy and muscle from known diseases. In some instances, such as an advanced dystrophy or a spinal muscular atrophy, the changes may be striking and unequivocal. In others, the changes may be more subtle and a systematic approach is required in the evaluation and interpretation.

Once the type of pathology is recognised it is important to relate it to the clinical picture in order to make a rational overall diagnosis. Thus an overtly dystrophic picture in a 6 month old infant with hypotonia from birth may confirm the diagnosis of congenital muscular dystrophy, whereas a similar picture in a 14 year old boy ambulant until the age of 10 is

characteristic of Duchenne type dystrophy. Similarly, in relation to spinal muscular atrophy, an identical histological picture is seen in the muscle of a severely affected infant with Werdnig Hoffmann disease as in a milder case with a somewhat later onset and better motor function and prognosis. In childhood dermatomyositis with profound clinical weakness one may see relatively minor pathological change in the form of perifascicular atrophy with no overt degeneration or inflammatory response or, in a case of less marked weakness, one may see a striking and diffuse myopathy with extensive degeneration and inflammatory response. Similarly, in some of the congenital myopathies like nemaline myopathy, a mildly affected child may show very extensive involvement of almost all the muscle fibres, whereas a profoundly weak case may have a small proportion of fibres with characteristic rods.

Thus using the above features of a muscle biopsy cut in transverse sections and stained with routine H & E staining and by the use of histochemical techniques one can diagnose a neuromuscular disorder.

Hence we can conclude by saying that a muscle biopsy is an essential tool in solving the diagnostic dilemma of neuromuscular disorders.

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