CHAPTER VII
CONGENITAL MYOPATHIES
CONGENITAL MYOPATHIES

REVIEW OF LITERATURE

Congenital myopathy is the term used to refer to a group of relatively non-progressive, congenital and infantile muscular disorders. The recognition of this new entity was possible by the application of enzyme histochemical and electron microscopic techniques, where specific structural abnormalities were seen within the muscle fibres.

These disorders share several features in common including an onset in early life and hypotonia. Many of these patients present as "floppy babies" and generally have a non-progressive or slowly progressive clinical course. The mode of inheritance is variable. They are distinguished from one another by their unique pathologic features and the disease is named after the particular alteration seen within the muscle fibres.

Several congenital myopathies have been recognised. The major ones being central core disease, nemaline (rod body) myopathy, centro nuclear myopathy, congenital fibre type disproportion. The mitochondrial myopathies of early onset is considered as one of the congenital myopathies.

1. CENTRAL CORE DISEASE

Central core disease (CCD) was the first of the congenital myopathies to be described (Shy and Magee, 1956).
They referred to this disorder as "Congenital non-progressive myopathy". Greenfield et al., (1958) used the term "Central core disease" to describe the same.

The disease is clinically characterised by infantile hypotonia and mild non-progressive weakness involving the proximal muscles. Although the disorder is inherited primarily as an autosomal dominant trait, sex-linked recessive mode of inheritance has also been reported (Fitzsimons and Tyer, 1980). In the recent years it has been found that the gene responsible for the disease is located on chromosome 19q (Kaush et al., 1990).

Histopathological investigations on the muscle biopsy revealed rounded, centrally placed cores within the most muscle fibres (Shy and Magee, 1956). With histochemical staining, Dubowitz and Pearse (1960), showed absence of phosphorylase and oxidative enzymes in these regions. The core lesions were best seen in NADH-TR preparations, sometimes sharply demarcated by a rim of increased substrate activity. The other characteristic feature is predominance of type-I fibres. The cores were mostly detected in type-I fibres and rarely in type-II fibres (Fukunaga et al., 1980). According to Dubowitz and Roy (1970), the selective involvement of type-I fibres possibly suggests a neural influence in the pathogenesis of this disorder.
Two types of cores were identified based on the ATPase activity in the core region (Gonatas, et al., 1965). Cores with reduced enzyme activity (weaker than usual ATPase activity) were called unstructured cores and those with enhanced activity (stronger than usual ATPase activity), structured cores (Neville and Brooke, 1973). The term reversed cores was used by Radu et al., (1977) for increased NADH-TR activity in the core region. The presence of unstructured cores and structured cores within the same muscle fibre was thought to be different stages of the same basic process (Goebel, 1986).

Electron microscopic studies (Gonatas et al., 1965; Dubowitz and Roy, 1970; Cohen et al., 1978) showed unstructured cores to be devoid of mitochondria. The cores also showed complete derangement of the Z-band. The regions other than cores showed well maintained banding pattern and mitochondria. The structured cores on the other hand showed preserved banding pattern and mitochondria were diminished or totally absent. According to Dudley (1989) the zig-zag distortion or offsetting of the sarcomere and close packing of the myofibrils squeeze the mitochondria and other organell to the periphery of the muscle fibre resulting in the formation of cores. By the use of modified Golgi technique to study the ultrastructural features of myofibres, Hayashi et al., (1989) found that the sarcoplasmic reticulum and the
T-tubules were distorted from their usual parallel and perpendicular orientation resulting in 'V' shaped triads.

Cores have been produced experimentally by tenotomy and by triethyltin sulfate and emetine intoxication (Bender, 1979). Formation of cores by tenotomy seems to depend more on innervation of the myofibres than on fibre types, as cores were not found when neurotomy (Otte et al., 1980) or spinal cord lesions (De Reuck et al., 1982) were performed simultaneously with tenotomy. Tenotomy induced cores were of unstructured type, were not permanent and a complete recovery was achieved.

A variant of CCD known as multicore disease was described by Engel and Gomez (1966). The term "multifocal degeneration of muscle fibres" was used by the authors and was considered as a separate congenital myopathy. In a subsequent report Engel et al., (1971) used the term "multicore disease". "Minicores" with regard to the size of the lesion has also been used to describe the same (Currier et al., 1974; Ricoy et al., 1980). The term multicore and minicore are now used interchangeably.

The clinical features of multicore disease conform to the pattern of congenital myopathies. With enzyme histochemistry, the muscle biopsies were marked by multifocal small areas devoid of oxidative enzyme reactions with predominance of type-I fibres. The multicores were mostly
seen in type-I fibres and only occasionally in type-II fibres. The cores were structured, unstructured or showed either of the lesions (Fitzsimons and Tyer, 1980). The presence of type-I fibre hypotrophy and type-II fibre hypertrophy has been reported (Ricoy et al., 1980). Ultrastructural features resembled those seen in CCD. Other features observed were presence of enlarged mitochondria with electron dense inclusions and myeloid bodies (Fitzsimons and Tyer, 1980). They suggested that mitochondrial dysfunction was responsible for core formation. The increased number of central cores with advancing age of the patient probably due to failure of recovery has been suggested (Goebel, 1986).

The coexistence of other features like nemaline rods and central nuclei along with multicores and central cores has been reported (Afifi et al., 1965; Bethlem et al., 1978; Lee and Yip, 1981, Vallat et al., 1982). Evolution of multicore pathology into central cores and rod lesions and the possibility that a common mechanism was responsible for these lesions was suggested by the latter authors.

The similarities of the unstructured cores to the target fibres seen in denervation led Close (1965) to propose a possible defect in innervation. Engel (1967) has suggested an abnormality in innervation to foetal muscle during development to explain this phenomenon.
MATERIAL

2 cases of central core disease and one case of multicore disease were included in the study. The patients had infantile hypotonia and weakness. The clinical features are given in Table-6.

TABLE - 6

Clinical data - Central core disease/multicore disease

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at biopsy/sex</th>
<th>Hypotonia</th>
<th>Delay motor milestones</th>
<th>Loss of acquired milestones</th>
<th>Dysmorphic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11/M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>13/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>26/F</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Quadriceps muscle was biopsied in all. The tissues were processed for morphological studies as stated earlier.

OBSERVATIONS

HISTOLOGY: In the 2 cases (cases 1 and 2) diagnosed as central core disease (CCD), the muscle sections showed normal fascicular architecture in one of the cases (Fig.98), while in the other, it was mildly distorted due to increase in perimysial and endomysial connective tissue. In both the cases majority of the fibres were rounded and showed mild variation in fibre diameter. A few fibres also showed central nuclei. In one of the biopsies a moderate number of fibres of
smaller diameter were observed. A few opaque fibres and basophilic fibres were also noticed.

**ENZYME HISTOCHEMISTRY**: Oxidative enzyme reactions for NADH-TR and SDH showed predominance of the type-I fibres. Majority of the type-I fibres and a few type-II fibres showed central large areas devoid of enzyme activity. (Fig 100). These regions were also negative to PAS reaction (Fig 99). Such areas represent the cores. A few fibres, other than the ones with cores, showed multiple small areas devoid of enzyme activity - the multicores. These cores were of the structured type, as normal activity was seen on ATPase reaction (Fig 101). However, an occasional fibre showed absence of reaction - the unstructured cores.

**ELECTRON MICROSCOPY**: The muscle tissue in both the cases showed normal sarcolemma. In the transverse section of the myofibres, distinct central regions with distortion of myofilaments and paucity of mitochondria was noticed. These areas corresponded to the cores (Fig 102 & 103). In the longitudinal sections of such areas, disarray of filaments and smearing of the Z-band was observed (Fig 104). The adjoining regions showed fairly well preserved architecture and the mitochondria were normal in distribution. A few normal fibres were also seen amidst such pathological ones. Small fibres with central large nucleus and few myofilaments were noticed (Figs 105 & 106). These corresponds to the
small fibres seen at light microscopy. Fibres similar to ring binden fibres (Fig.107) were seen in a few number.

MULTICORE DISEASE

HISTOLOGY: Muscle biopsy from one case (case 3) diagnosed as multicore disease showed mild distortion of the fascicular architecture due to increase in fibrofatty tissue in the perimysial and endomysial regions. Moderate variation in fibre diameter with very small atrophic fibres inbetween was seen. The nuclei in a moderate number of fibres were internally placed, as compared to only a few internal nuclei seen in CCD.

ENZYME HISTOCHEMISTRY: In the muscle sections, predominance of type-I fibres was noticed in the enzyme preparations. Majority of these fibres showed multiple regions devoid of oxidative enzyme activity. A few type-II fibres also showed such core regions as illustrated (Fig 108). These were termed the multiple cores or minicores. The cores were of structured type as seen in ATPase reaction.

ELECTRON MICROSCOPY The muscle specimen showed majority of the fibres with normal pattern except for a few fibres which showed mild disarray of filaments and streaming of Z-band at few foci.

DISCUSSION

Central Core disease was observed in both sexes and the patients presented mainly with hypotonia.
Predominance of type-I fibres comprising 80-90% of the total number, a feature seen in both the cases suggest that the pathogenesis is due to lack of differentiation into type-II fibres resulting in predominance of type-I fibres. In addition, the presence of small fibres resembling foetal type fibres suggests immaturity as a factor in this condition.

The presence of cores devoid of oxidative enzyme reaction associated with myofibrillar disarray and Z-band streaming at electron microscopy constitute the diagnostic criteria in central core disease. The preservation of ATPase reaction and alteration of sarcomeric pattern to a moderate degree suggests these cores to be of structured type similar to that described in literature (Neville and Brooke, 1973). At ultrastructural level most of the fibres showed well preserved myofibrillar structure and organelles except at the core region. This possibly explains the preservation of the motor power in both the patients. None of the fibres showed redundant basal lamina to suggest denervation. One can attribute the absence of mitochondria and disorganisation of myofibrillar structure in the core region to be a result of defect in development in the foetal life. In the foetuses examined in the present study, mitochondria were seen by the 9th week of gestation and well developed sarcomeric pattern by the 20th week, cores were not observed in any of the stages of foetal development.
The presence of fibres resembling the ring binden' fibres suggest the atypical contraction causing peripheral placement of mitochondria and other organelles, probably resulting in the subsequent formation of cores as suggested by Dudley, (1989).

It has been shown experimentally that lesions similar to central core disease can be produced by tenotomy and certain chemicals like triethyltin sulfate. These resemble the target fibres seen in the human muscle denervation (Resnick and Engel, 1967). Based on these findings it has been suggested that neural influence to be the pathogenetic mechanism in the production of core lesions, even though the exact mechanism of neural influence bringing about this is not clear. However, in experimental animals, a stage of recovery is marked by the regeneration and reorganisation of myofilaments back to its normalcy, while in CCD normal myofilamentous pattern do not replace the cores. This is supported by the finding that the number of cores increase with advancing age (Goebel, 1986). In our study, in the cases of chronic denervation we have not been able to demonstrate such cores in the biopsies either in enzyme histochemical preparations or at ultrastructural level. Target fibres were observed in large numbers in a case of spinal muscular atrophy. These
fibres showed three distinct zones of enzyme activity comprising central zone of increased activity, intermediate zone of paler reaction and an outer zone of normal reaction which is the classical picture seen in target fibres (Fig 109). These target fibres however did not resemble the cores, since, in the latter, such distinct zones were not observed. Hence it is difficult to justify the neural hypothesis as the mechanism of formation of cores in CCD based on the morphological observations in our study.

In the multicore disease the main features observed were the type-I fibre predominance and multiple foci of lack of oxidative enzyme activity at histochemical level, and mild myofilamentous disarray in several foci at electron microscopy. These features also suggest arrest during differentiation of fibres into histochemical fibre types. The alterations like filamentous disarray are similar to those seen in CCD but of milder degree. Thus multicore disease may be a variant of CCD with similar pathogenetic mechanism.

Thus, it may be suggested that lack of differentiation due to arrest in development at certain stage is an important factor in the pathogenesis of central core disease and multicore disease. However, the mechanism's responsible for structural abnormality remains to be understood.
Legends to Photomicrographs – Central core disease

Serial transverse sections of quadriceps muscle biopsy from a female child aged 13 years:

Figure.98: Section showing several fascicles. The myofibres are polygonal with a few angulated fibres (\(\wedge\)) in between. The nucleus in majority of the fibres are peripherally placed. An occasional fibre shows central nucleus. 

H and E X 300.

Figure.99: Section shows several fibres to be devoid of PAS positive material. 

PAS X 300.
Legends

Figure 100: Section stained for oxidative enzyme reaction shows majority of the myofibres to be devoid of enzyme activity in the centre. These areas represent the cores (C). NADH-TR X300.

Figure 101: Section stained for myosin ATPase reaction shows predominance of type-I fibres. The preservation of reaction in each of the fibre suggest the cores to be of structured type. ATPase(pH 9.5) X 300.
Legends to Electron micrographs - Central core disease

**Figure.102**: Transverse section of muscle showing an entire fibre and portions of several fibres. The fibre in the centre shows well defined core (C). The nucleus (N) is seen in the subsarcolemmal region. X 2,240.

**Figure.103**: Higher magnification of a portion of above with core. The core region shows mild distortion of filamentous pattern and absence of mitochondria. X 5,760.

**Figure.104**: A portion of myofibre in longitudinal section showing cores (C). X 6,840.
Legends

Figure.105: Electron micrograph from the muscle biopsy of the male child aged 12 years (case 1) showing small fibres containing vesicular nucleus (N) and a few myofilaments (M) dispersed in the subsarcolemmal region. X 7,250.

Figure.106: Another small fibre showing two nuclei (N) and a few myofilaments (M) at the periphery. X 4,750.

Figure.107: Electron micrograph illustrating the possible formation of core. Note the absence of mitochondria in the central region of the myofibre and their accumulation at the peripheral region. X 2,660.
Legends

Figure.108: Transverse section of muscle from a female aged 26 years (case 3) showing multiple regions devoid of enzyme activity in the fibre.

SDH X 300.

Figure.109: Transverse section of muscle from an adult male aged 38 years showing target fibres.

NADH-TR X 300.
I. CENTRO NUCLEAR MYOPATHY

As the name indicates the "Centro nuclear myopathy" (CNM) is characterised by the presence of greater than normal number of fibres with central nuclei. The first description was given by Spire et al., (1966), who found that 45-50% of the fibres had central regions occupied by nuclei or by material that had high levels of oxidative enzymes. The authors postulated that the fibres with central nuclei represented a persistence of foetal myotubes and hence called the disease as "myotubular myopathy". In the later report, either et al., (1967) used the term "centronuclear myopathy" to describe the same.

In contrast to other myopathies, patients with CNM show prominent involvement of extra ocular and facial muscles in addition to infantile hypotonia and slowly progressive weakness in limb muscles. Most cases have their onset in infancy; however onsets during childhood and adult life has also been reported (van Wijngaarden, et al., 1969). The inheritance pattern is heterogenous and falls into 3 groups namely, autosomal recessive, autosomal dominant and sex-linked types. A non-hereditary late onset group has also been described (Engel, 1986). In the recent studies, De Angelis et al., (1991), have shown only two categories viz an autosomal dominant and an X-linked recessive types. By using linkage studies it has been shown that the defective gene is mapped to chromosome Xq28 (Lehesjoki et al., 1990).
In the histological studies of muscle specimens it was shown that the predominant feature was centrally placed nuclei in majority of myofibres. These central nuclei were often surrounded by a vacuolated area that was devoid of ATPase activity as seen in the enzyme preparations. The central nuclei were found in both the major fibre types. Histochemical studies have shown type-I fibre predominance, type-I fibre atrophy, type-I fibre hypotrophy and fibre size disproportion (Engel et al., 1968; Bethlem et al., 1969; Badruska et al., 1969; Campbell et al., 1969; Serratrice et al., 1978). Numerous fat cells within the fascicles and presence of multicores within the fibres were also observed (Lee and Yip, 1981; Fitzsimons and McLeod, 1982; Goebel, 1986).

Different views have been expressed by authors with regard to the presence of central nuclei. While, Kinoshita and Cadman (1968), suggested that the presence of central nuclei is likely to be due to arrest of regeneration or development at the myotube stage, Engel et al., (1968) suggested that the persistence of fibres resembling myotubes could be due to 1) functional deficiency of maturational "factor" from the motor nerve or 2) inability of the muscle fibres to utilize the maturational factor. Arrest in maturation which might have occurred beyond the 20th week of foetal gestation due to lack of trophic factor from the motor
nerve was proposed by Karpati et al., (1970) and Reske-Neilsen et al., (1987).

Serratrice et al., (1978), attributed the presence of central nuclei to the malfunctioning of central nervous factor controlling nuclear migration. However, Hulsmann et al., (1981), explained a postnatal secondary migration to the central region. The concept of dysmaturation myopathy was introduced by Peyronnard et al., (1982), as the muscle fibres with central nuclei resembled the foetal myotubes. Recent studies using antibodies to foetal myosin heavy chain (Sawchak, 1988) failed to demonstrate the presence of prenatal myosin, but the presence of foetal vimentin and desmin was reported by Sarnat et al., (1990).

Examination of the central and peripheral nervous system in the autosomal recessive group disclosed no abnormality (Campbell et al., 1969). Sugie et al., (1982) reported segmental demyelination in sural nerve from an adult patient with CNM.

MATERIAL

The material consisted of 6 cases of centronuclear myopathy. The age of the patients at biopsy was 2-40 years. The onset of the symptoms was at birth in all, except in one, at 2 years. A brief clinical data is given in the Table-7.
### TABLE - 7

**Clinical data - Centronuclear myopathy**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at biopsy/sex</th>
<th>Onset of symptom</th>
<th>Ptosis</th>
<th>Facial muscle weakness</th>
<th>Somatic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>40yrs/F</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>High-arched palate</td>
</tr>
<tr>
<td>2.</td>
<td>12yrs/F</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>Long face</td>
</tr>
<tr>
<td>3.</td>
<td>40yrs/F</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>2yrs/M</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>26yrs/M</td>
<td>2 yrs</td>
<td>-</td>
<td>+</td>
<td>Long face</td>
</tr>
<tr>
<td>6.</td>
<td>12yrs/F</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td>Dysmorphic facies</td>
</tr>
</tbody>
</table>

Quadriceps muscle was biopsied in each of the patient.

**OBSERVATIONS**

**HISTOLOGY** : The skeletal muscle in cross section showed mild distortion of fascicular architecture in 4 cases and marked distortion in 2 cases, due to, infiltration of fibrofatty tissue. In 3 cases (cases 1, 3, 5) the fibres were polygonal in shape with mild to moderate variation in their diameters, while in the remaining 3 cases, very large fibres (hypertrophic fibres) surrounded by fibres of smaller diameter was noticed (Fig 110). The striking feature seen in all the cases was the presence of sarcolemmal nuclei in the centre instead of at the periphery (Fig 112). These fibres in
longitudinal sections showed a row of nuclei in the centre (Fig 113). The number of fibres with central nuclei varied in each case (20-90%).

**ENZYME HISTOCHEMISTRY**: The cryostat sections stained for oxidative enzyme (NADH-TR & SDH) showed the fibres to contain a central halo and/or excessive reaction product. A radiating pattern of the reaction, giving "spokes" like appearance was seen in some of the fibres. These features are illustrated in (Fig 114). In cases with larger number of fibres with central nuclei such changes were predominantly seen. Predominance of type-I fibres was seen in 5 cases. Although, the central nuclei were mostly seen in type-I fibres, a few type-II fibres also revealed central nuclei.

In addition to the presence of central nuclei, one of the cases (case 5) also demonstrated multicores in a few fibres. In three of the biopsies (case Nos. 2, 3, 5) subsarcolemmal accumulation of the reaction product similar to that seen in mitochondrial myopathy was observed. However, sections stained with MGT failed to reveal ragged red fibres. In ATPase reaction predominance of type-I fibres containing central halo similar to that seen in oxidative enzymes reaction was noticed. The hypertrophic fibres were type-I and the small fibres were type-II in one case. In the other 2 cases the hypertrophic fibres were type-II and the small fibres were type-I (Fig 111).
ELECTRON MICROSCOPY: Fine structural observations showed myofibres of varying diameter. The "spokes" like appearance seen in the fibres at light microscopy corresponded to the widened intermyofibrillar spaces. The mitochondria were present within these spaces in a radiating pattern (Fig. 115). The central regions were occupied by the nuclei, and the number of fibres with central nuclei varied in each case. Absence of myofilaments around the nuclei corresponds to the halo seen at light microscopy. Accumulation of normal looking mitochondria was seen in the centre of some of the fibres. (Fig 116). Lipofuscin material (Fig 117) was observed in a few fibres. They were present both in the centre, especially at nuclear poles and in the subsarcolemmal region. In one case polyribosomes were seen in majority of the fibres. Accumulation of normal looking mitochondria at the subsarcolemmal region in a few fibres corresponding to the light microscopic finding of accumulation of oxidative enzyme reaction was seen in 3 cases. In all the cases structural alterations in the form of mild disarray of the myofilaments and streaming of the Z-band was observed in a few fibres. The remaining fibres appeared normal without any myofibrillar disarray.
Light microscopic observations revealed myofibres both small and large with centrally placed nuclei in all the six cases. However, the number of fibres with central nuclei varied in each case. Predominance of type-I fibres was seen in 5 cases. The presence of central nuclei suggests an arrest during development preventing migration towards the subsarcolemmal region. In the present study it was observed that in the foetal muscle of 14 weeks gestation some of the fibres contained nucleus in the subsarcolemmal region while, majority of the fibres with subsarcolemmal nucleus was seen by the 24th week gestation. Thus the arrest in development must have occurred early in the foetal life. In the present study the type-I and type-II fibre differentiation was initiated by the 22nd week gestation and distinct mosaic pattern was observed by the 28th week of foetal life. Thus the predominance of type-I fibres could be due to lack of differentiation of type-II fibres which might have occurred at the 22nd week of gestation. Both these developmental arrests could be due to neural influence at the early stages of foetal life before nuclear migration and fibre type distinction are known to occur as suggested by Engel et al., (1968). The same mechanism could bring about marked variation in fibre diameter in all the cases.
There was no evidence of neurogenic atrophy. Presence of myofibrillar disarray, Z-band streaming and lipofuscin material suggests simultaneous breakdown of myofilaments and degeneration of the fibre causing damage to the myofibre. In addition, the distribution of mitochondria in a radiating pattern may probably be due to persistence of nuclei in the centre. In no stage during the normal foetal development have we observed similar distribution of mitochondria.

Association of multicores in one of the case of CNM suggests a common factor influencing the migration of nuclei and structural organisation of myofibre. Similar suggestions have been made by Vallat et al., (1982).

The relative absence of type-II fibres and the presence of central nuclei may be responsible for the weakness. The functional activity of the patient was hampered to a variable degree as seen by the clinical presentation. This also correlated with the morphological findings. The loss of fibres and increase in fat replacing the fibres observed in patients who were biopsied at 40 years explains the severity and chronicity of illness.
Legends to Photomicrographs - Centro nuclear myopathy

**Figure 110**: Quadriceps muscle biopsy from a female child aged 12 years (case 6). Transverse section showing two fascicles with small and large fibres polygonal in shape. The fibres show centrally placed nuclei in many fibres. 

*H and E X 300.*

**Figure 111**: Section from biopsy same as above showing condensation of reaction product in many fibres. The type-I fibres (dark) are predominantly seen. A single large type-II fibre is also seen. 

*NADH-TR X 300.*
Legends

Figure.112: Quadriceps muscle from a female aged 40 years (case 1): Transverse section showing well preserved myofibrillar pattern central nucleus is seen in many of the fibres. MAT X 300.

Figure.113: Longitudinal section from biopsy same as above showing central row of nuclei in many fibres. H and E X 300.

Figure.114: Section stained for oxidative enzyme shows fibres of varying diameter. The fibres have central halo (site of nucleus). The reaction products are concentrated around the central halo and shows radiating pattern (R) in some of the fibres. NADH-TR X 300.
Legends to Electron micrographs-Centronuclear myopathy

Figure 115: Transverse section of muscle showing central nucleus (N), surrounding the nucleus are several mitochondria (mt). The myofibrils are arranged in a radiating pattern around the nucleus. Mitochondria are also seen in the intermyofibrillar region. X 10,000.

Figure 116: A portion of muscle fibre with several mitochondria (mt) in the centre. X 10,000.

Figure 117: Muscle biopsy same as above, a portion of muscle fibre in another field showing lipofuscin material (LF) in the centre of the fibre. X 10,000.
3. NEMALINE (ROD BODY) MYOPATHY

Shy et al., (1963) described a new congenital non-progressive myopathy associated with rod-like structures in the muscle fibres. The term nemaline (from Greek-nema-thread like) was used to describe such structures and the condition was called "Nemaline myopathy". It presents as a mildly progressive or non-progressive myopathy with proximal or generalised weakness, often associated with high-arched palate and other dysmorphic skeletal stigmata. Although in most instances the clinical course is benign an occasional case had rapidly progressive fatal course (Mc Menamin, 1984). Late onset forms, often marked by severe respiratory impairment have also been described (Engel, 1966).

The pathological hallmark of this disorder is the presence of small pockets of bacillus-like structures called nemaline or rod body beneath the sarcolemma (Price et al., 1965). Engel and Cunningham (1963) demonstrated the nemaline rods to stain red by the modified Gomori's trichrome technique. These rods in histochemical reaction to ATPase appeared as transparent areas confined to type-I fibres. Predominance of type-I fibres was also noticed by Engel, (1977). This led him to suggest a neurogenic etiology.

Electron microscopic studies on the muscle biopsies from nemaline myopathy was marked by an abundance of intermyofibrillar inclusions, that often clustered in the
subsarcolemmal area (Gonatas, 1966). The rod bodies were made up of regular lattice of the Z-disc, and were thought to resemble the Z-disc material (Price et al., 1965). By biochemical studies it was found that the composition of the rods was similar to the Z-disc material (Stromer et al., 1976).

The presence of increased number of satellite cells and myotube like cells were thought to be morphological signs of immaturity in this disorder (Tsujihata et al., 1983; Nonaka et al., 1989). Robertson et al., (1978) evaluated the number and the distribution of motor neuron cell bodies and axons of myelinated fibre in post mortem material from a 9-month-old infant with nemaline myopathy. A 2-month-old infant with Werdnig-Hoffmann disease and three neurologically normal infants between and 9 and 10 months of age served as controls. In the infant with nemaline myopathy motor neurons were not lost but showed reduction in the diameter of cell bodies. The histogram of myelinated fibre corroborated the alteration in anterior horn cells. According to the authors whether the reduction in diameter is primary or secondary developmental change was unclear.

Note: Case of Nemaline myopathy was not encountered in our study.
4. CONGENITAL FIBRE TYPE DISPROPORTION

The congenital fibre type disproportion (CFTD) encompasses the following types of disorder: i) congenital myopathy with type-I fibre hypoplasia ii) myopathy with type-I fibre predominance iii) myopathy with type-II fibre hypoplasia. (Brooke, 1973) The abnormalities seen in these disorders are in relation to the size and number of the major histochemical fibre types. Agrov et al., (1984) suggested that the abnormalities in the fibre type sizes and number were due to functional abnormalities of motor neurons.

Clinically, patients with these disorders present as floppy babies and have skeletal abnormalities. In general, the child improves with age. The mode of inheritance is unclear.

The criteria laid down by Brooke (1973), for the diagnosis of fibre hypoplasia is that the fibres should vary in their cross sectional diameter by at least 12% and predominance of fibre type by more than 55%. Subsequently, Brooke (1990), revised his definition by giving a difference of at least 45% for fibre size and 65% for fibre predominance.

Although no ultrastructural abnormalities were observed (Gardner-Medwin, 1988); a mild alteration in the organisation of myofibrils (Fardeau, 1982) and the presence of central nuclei (Spiro et al., 1977) were reported. However, no significance could be attached to these findings.
a) MYOPATHY WITH TYPE II FIBRE HYPOPLASIA

MATERIAL

The material consisted of two cases of type-II fibre hypoplasia. These patients came with hypotonia from birth and proximal muscle weakness. Clinical features suggestive of cerebral involvement was absent in both the cases. Biceps muscle was biopsied in both the patients.

OBSERVATIONS

HISTOLOGY: Examination of muscle under light microscope showed normal fascicular architecture in which myofibres were polygonal in shape. In all the fascicles a uniform bimodal distribution of the fibres was seen (Fig 118). By morphometry, it was found that the diameter of the small fibres differed by more than 12% when compared with normal age matched controls. The nuclei were peripherally placed in all the myofibres.

ENZYME HISTOCHEMISTRY: Sections stained for oxidative enzymes and ATPase reactions showed normal mosaic pattern. However the smaller fibres were of type-II variety, while the larger ones were type-I (Fig 119).

ELECTRON MICROSCOPY: The muscle sections when seen under the electron microscope showed normal morphological features except for the size of the fibres. No evidence of neurogenic atrophy such as presence of redundant basal lamina in the hypotrophied fibres could be observed.
b) MYOPATHY WITH TYPE I FIBRE PREDOMINANCE

MATERIAL

4 cases of type-I fibre predominance were included in the study. The patients presented with hypotonia and delayed acquisition of motor milestones. A brief clinical summary is given in Table 8.

TABLE - 8

Clinical data - Myopathy with type-I fibre predominance

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at biopsy/sex</th>
<th>Onset</th>
<th>Delay motor milestones</th>
<th>Hypotonia</th>
<th>Proximal muscle weakness</th>
<th>Congenital markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 yrs/M</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Dysmorphic facies</td>
</tr>
<tr>
<td>2</td>
<td>8 yrs/M</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>low set ears, high-arched palate</td>
</tr>
<tr>
<td>3</td>
<td>13 yrs/M</td>
<td>6 yrs</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Ptosis</td>
</tr>
<tr>
<td>4</td>
<td>15 m/M</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Quadriceps muscle was biopsied in all.

OBSERVATIONS

HISTOLOGY: The HE stained sections showed normal fascicular architecture with polygonal fibres in opposition to each other. The nuclei were peripherally placed (Fig 120).
ENZYME HISTOCHEMISTRY: Sections stained for histochemical reactions revealed 80-90% of the fibres to be of type-I variety, with very few type-II fibres scattered in between in all the 4 cases (Fig 121).

ELECTRON MICROSCOPY: The fibres showed normal morphological features in all the cases.

DISCUSSION

Classically described congenital fibre type disproportion (CFTD) shows type-I fibre hypoplasia and predominance, while type-II fibres are large. In our material, this type of CFTD was not encountered. In two of the biopsies CFTD was evidenced by the presence of small type-II fibres with normal appearing type-I fibres, the difference in diameter being more than 12%. This condition has been included under CFTD by Dudley (1989) and Goebel (1992). Histochemical and ultrastructural study did not reveal any pathological change in both the types of fibres. Thus it appears that development of muscle fibre in its size subsequent to differentiation could be defective.

In type-I fibre predominance, type-I fibres practically replaced the whole bulk, while about 10% of the fibres only were of type-II nature it is quite obvious that there has been defect in maturation and differentiation embryonic life so as to bring about type-I fibre predominance. This is likely to occur during early week of development that is before the 22nd wk of gestation.
Legends to Photomicrographs—Congenital fibre type disproportion

**Figure 118**: Transverse section of muscle (biceps) from a female child aged 4 yrs 4 months showing polygonal shaped fibres with a few small fibres in between. H and E (cryo) X 480.

**Figure 119**: Section stained for myosin ATPase reaction showing type II fibres small.

ATPase (pH9.5) X 480.
Legends

Figure 120: Transverse section, of muscle from a male child aged 8 years showing normal polygonal shaped fibres with subsarcolemmal nuclei.

H and E (cryo) X 300.

Figure 121: Section stained for myosin ATPase showing predominance of type I fibres.

ATPase (pH 9.5) X 300.
5. BENIGN CONGENITAL HYPOTONIA

A total number of 7 cases, were grouped under benign congenital hypotonia. These patients presented with hypotonia and delayed acquisition of motor milestones. Their muscle biopsy however showed normal features at histology and histochemistry without any suggestion of structural abnormalities in the fibres. A detailed electron microscopic study also did not reveal any structural disorganisation of myofilaments, mitochondria and other components. Atypical inclusions, such as Zebra bodies and others, were not detected. Hence these cases were grouped under benign congenital hypotonia. The hypotonia seen in these patients cannot be explained. Only follow-up studies in these patients may clarify the cause of hypotonia.