CHAPTER VIII
METABOLIC MYOPATHIES
A classification based on biochemical defects was proposed as follows: defect of mitochondrial substrate transport; Substrate utilization; defect of respiratory chain and energy conservation and transduction. Based on clinical features the mitochondrial myopathies were divided into three distinct groups:

1. Kearn's-Sayre syndrome (KSS)
2. The syndrome of myoclonus epilepsy with ragged red fibres (MERRF)

Engel and Cunningham (1963), used modified Gomori's trichrome technique which enabled them to recognise fibres with pathologic accumulation of mitochondria—the ragged red fibres (RRF). Enzyme histochemical stains for oxidative enzymes such as SDH and NADH-TR, showed ragged red fibres with abnormally intense reaction. There was often type-I fibre predominance (DiMauro et al., 1976). Swash and Schwartz (1978), found that RRF were virtually restricted to type-I fibres, which were smaller than the unaffected type-I fibres. An increased number of type-IIIC fibres and type-IIB fibre deficiency and selective atrophy of type-I and type-II fibres were reported by Yamamota and Nonaka (1988). Although, Mitumoto et al., (1981); Johnson et al., (1983), demonstrated that RRF lack cytochrome c oxidase (COX) activity either completely or segmentally, its absence also
in non-ragged red fibres was noticed by Yamamota and Nonaka (1988). The segmental COX deficiency within a single muscle fibre was thought to be due to the disturbances of nuclear or mitochondrial function.

Ragged red fibres were not seen in some primary mitochondrial diseases especially those due to defect outside the respiratory chain such as carnitine palmityl transferase (CPT), pyruvate dehydrogenase complex (PDHC), fumarase deficiencies and COX deficiency (DiMauro, et al., 1992).

Electron microscopic investigations showed marked accumulation of mitochondria under the sarcolemma and to a lesser extent between the myofibrils. The mitochondria varied in size from normal (pleoconial) to giant (megaconial); some of them spanned several sarcomeres (Shy et al., 1966). The mitochondria often showed disorientation and increased number of cristae. They also appeared as concentric whorls or forming honey comb like structures. Paucity of cristae giving the mitochondria a vacuolated or empty appearance has also been reported (Shy and Gonatas, 1964). The mitochondria often contained crystalline inclusions (Hammerson et al., 1980). These inclusions were of two types - type 1 and type 2. The type 1 inclusions called the "parking lot type" was seen in the intracrystal space and in the type 2, they were preferentially located in the intermembrane space. In addition to abnormal mitochondria excessive accumulation of
glycogen particles and triglyceride droplets were also seen (DiMauro et al., 1985). Such changes in the mitochondria have been produced experimentally by respiratory toxins, some uncoupling agents of oxidative enzymes, such as 2,4, dinitrophenol (Melmed et al., 1975) and by ischemia (Heffner et al., 1978).

Shah et al., (1982), suggested that the various types of abnormal mitochondria encountered in the muscle of mitochondrial myopathies probably represent stages in the morphologic evolution of the lesion.

In an autopsy study of KSS (Oldfors et al., 1990), the neuropathological changes seen were neuronal degeneration and gliosis of basal ganglia and lenticular nuclei. Wide spread spongy degeneration in the white matter of cerebrum, cerebellum and brainstem was noticed. The possible cause for these changes was ascribed to a deficiency of respiratory chain enzymes resulting from deletions in mitochondrial DNA (mt DNA) (Oldfors et al., 1990). Mitochondrial changes were also seen in the schwann cells of sural nerve, smooth muscle and endothelial cells.

Holt et al., (1988), established an association between human disease and mitochondrial genome in patients with progressive external ophthalmoplegia (PEO). He found 2 populations of mt DNA in the muscle, one of which was deleted
upto 7kb. Similarly, deletions in patients with KSS was reported by Zeviani et al., (1989). Point mutations of mt DNA in Lebers hereditary optic neuropathy (LHON) was described (Wallace et al., 1988). Point mutations in MERRF and in patients with MELAS was also reported (Rosing et al., 1985; Goto et al., 1990). Multiple deletions (mt DNA depletion) was described in patients with autosomal dominantly inherited PEO (Zeviani, 1989).

The mt DNA related syndromes were subdivided into two groups (Zeviani and Antozzi, 1992)

1. Mitochondrial encephalomyopathies characterised by the presence of RRF as the morphological hall mark, which includes MERRF, MELAS, chronic progressive external ophthalmoplegia (CPEO), and a new entity called the "Maternally inherited myopathy and cardiomyopathy".

2. "Pure" encephalomyopathies with no gross morphological abnormalities in the muscle. This includes LHON and ataxia retinitis pigmentosa-dementia complex.

MATERIAL

6 cases of mitochondrial myopathy not associated with any of the morphologically identified storage disorder were encountered in our study. Clinically, the patients had hypotonia, with onset of symptoms at birth in all except in one. The brief clinical data is given in Table-9 below:
TABLE 9

Clinical data - Mitochondrial myopathy

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age at Biopsy/sex</th>
<th>Onset of symptoms</th>
<th>Hypotonia &amp; delay motor milestones</th>
<th>Ptosis</th>
<th>Ophthalmoplegia</th>
<th>Seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>12 yrs/M Birth</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>12 yrs/M Birth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>8 yrs/F Birth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>28 yrs/F Birth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>2 yrs/M Birth</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>13 yrs/M 1 1/2 yrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quadriceps muscle was biopsied in all. The tissues were subjected to morphological investigations.

OBSERVATIONS

HISTOLOGY: In the H and E stained muscle sections it was seen that the fascicular architecture was well maintained in all. Mild increase in endomysial and perimysial connective tissue was seen in 2 cases (cases 3 and 4). The fibres were mostly polygonal in shape, except in one case, in which the fibres were all rounded. 2 cases (cases 1, 6) showed normal histology except for mild variation in fibre diameter (Fig 122) while, the other 4 cases showed the following changes: there was mild variation in the fibre diameter, a few fibres...
in each fascicle were opaque and some had cracked appearance (Fig. 126). The nuclei were peripherally placed with an occasional fibre having central nuclei. Moderate number of fibres with vacuoles, containing amorphous and bluish material was observed in one of the 4 cases. A few fibres showing degenerative changes, necrosis and myophagocytosis were seen in 2 cases (cases 3 and 4). In the 4 cases mentioned above MGT stain revealed a large number of ragged red fibres (Fig. 128). No ragged red fibres were seen in cases 1 and 6 (Fig. 123). Oil red O stain failed to demonstrate the presence of neutral fats. PAS showed intensely positive reaction in one of the cases (case 2).

**ENZYME HISTOCHEMISTRY:** Histochemical reactions for myosin ATPase showed normal mosaic pattern in 2 cases (cases 1 and 6), which on histology were normal. But when stained for oxidative enzymes viz, NADH-TR and SDH, condensation of dark reaction product was seen at a few points on the margins of many of the type-I fibres (Fig. 124). In the other 4 cases mentioned above there was predominance of type-I fibres as seen on ATPase reactions. Many of these type-I fibres showed clumping of oxidative enzyme reaction products both at the subsarcolemmal region and in the centre of the fibres (Fig. 127). In some, the entire fibre showed dark reaction product. Cytochrome C oxidase reaction done only in two cases showed loss of enzyme activity in RRF.
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ELECTRON MICROSCOPY: The muscle tissues of the 2 cases (cases 1, 6) which did not reveal RRF but only showed thin margins of the reaction product at histochemical level showed a large number of normal looking mitochondria in the subsarcolemmal region (Fig 125). A few abnormal mitochondria were seen in an occasional fibre. No crystalline inclusions were seen. The sarcolemma and the filamentous pattern appeared normal.

The muscle biopsy in the other 4 cases which showed RRF under the light microscope, revealed varying degree of myofilamentous disorganisation and loss in the affected fibres by electron microscopy. The sarcolemma was normal. The striking feature seen was the increased number of abnormal mitochondria in the subsarcolemmal and inter myofibrillar regions. The mitochondria varied in number, size and contained cristae arranged in irregular form as illustrated in Fig 129. Some of the mitochondria appeared lobulated, while others had clear matrix devoid of cristae forming a ring. In some, electron dense material was also seen. Paracrystalline inclusions of type 1 variety were seen in all the 4 cases. These inclusions had 4 to 8 electron dense membranous layers oriented parallel to one another and seen in the intracristal region (Figs 130 & 131). Accumulation of excess of glycogen was seen around and within a few mitochondria, especially the ones with concentric whorls (Inset Fig 129). A large number of giant mitochondria with abnormal pattern of cristae were seen in 2 of the cases (Fig
In one of the cases, (case 2) excess of glycogen was seen in the sarcoplasm in many fibres. One of the case (case 4) which showed marked vacuolation at histology contained vacuoles with myelin figures (Fig 133). Also seen within the vacuoles were a few mitochondria with inclusions.

In all the 6 cases a moderate number of fibres with normal filamentous pattern and mitochondria having normal morphology were seen.

DISCUSSIONS

Based on the clinical data, the light microscopic finding of SDH and NADH-TR reaction, and the presence of ragged red fibres, the diagnosis of mitochondrial myopathy was offered. The diagnosis was further confirmed by the presence of abnormal mitochondria at ultra structural level.

Though, the morphological hallmark in the diagnosis of mitochondrial myopathies is the presence of ragged red fibres, two out of 6 cases in the present study did not reveal RRF but showed condensation of the reaction product in the SDH and NADH-TR preparations and accumulation of normal looking mitochondria. These two cases probably fall into the category of primary mitochondrial diseases caused due to defect outside the respiratory chain as suggested by Di Mauro, (1992). Alternatively MGT stain might be inadequate to pick up the normal appearing mitochondria encountered in these two cases.
In the remaining 4 cases, the percentage of abnormal myofibres showing increased SDH and NADH-TR activity in the subsarcolemmal and intermyofibrillar regions varied in each case. In all the four cases the affected fibres appeared ragged red in the MGT preparation. These findings appeared to correspond to the aggregation of structurally abnormal mitochondria seen under electron microscope.

The various forms of abnormal mitochondria seen in the fibre probably represents stages in the morphogenic evolution of the lesion as suggested by Shah et al., (1982). The presence of paracrystalline inclusions of the type 1 variety in all the four cases suggests that type 1 crystals are the commonest. According to Farrants et al., (1988), two crystal types are seen and that they occur in different muscle fibre types. The type 1 crystals seen only in type-I fibres and the type 2 crystals are seen in type-II fibres. In our observation it is likely that the type-I fibre predominance seen in all the cases could be the reason for the presence of type 1 crystals. However, the significance of paracrystalline inclusions and its relation to disease in the muscle fibre, is not clear.

In two of the cases, degeneration, myophagocytosis and mild fallout suggestive of frank myopathic change was seen at light microscopy. It is likely that muscle weakness which also varied in its degree from each patient is explained on
the basis of pathological changes like myofibril loss and disorganisation. The myofibrillar changes appear to be mostly associated with presence of abnormal mitochondria in the fibres.

The increase in number and size, shape and presence of inclusion bodies suggests an insult to mitochondria in response to energy deficit (Capaldi, 1988). The presence of excess of glycogen in and around mitochondria points to a metabolic defect responsible for such accumulation (DiMauro et al., 1988). The overall findings suggests metabolic abnormality in our patients. However, biochemical studies were not possible to further categorise our cases under biochemically defined mitochondrial myopathies.
Legends of Muscle biopsies - Mitochondrial myopathy

Serial transverse section of muscle biopsy from a child aged 13 years (case 6):

**Figure 1.22**: Section showing normal features except for mild variation in fibre diameter. H and E X 300.

**Figure 1.23**: Section of muscle, to show absence of ragged red fibres. MGT X 300.
Legends

Figure.124: Section stained for oxidative enzyme reaction. Many of the fibres show thin rim of accentuated reaction product at the periphery. SDH X 300.

Figure.125: Electron micrograph from the biopsy same as above illustrating a portion of two myofibres with accumulation of normal mitochondria (mt) in the subsarcolemmal region. X 7,200.
Legends

Quadriceps muscle from a male child aged 12 years (case 2)

Figure.126: Transverse section of muscle showing myofibres with mild variation in fibre diameter. Note a few fibres appear cracked.

H and E (cryo) X 300.

Figure.127: Biopsy from child same as above stained for oxidative enzyme reaction shows subsarcolemmal accumulation of reaction product in a few fibres. Some of the fibres appear normal.

SDH X 300.

Figure.128: Transverse section of muscle reveals several ragged red fibres.

MGT X 300.
**Legends**

**Figure.129**: Electron micrograph from muscle biopsy of a male child aged 12 years. Longitudinal section of a portion of muscle fibre showing loss of myofilaments in the subsarcolemmal region and accumulation of abnormal mitochondria. The mitochondria show abnormal cristae (a), concentric lamella (b), paracrystalline inclusions (c) and vacuolated (d) forms. X 7,200. Inset - higher magnification of mitochondrion with concentric lamella. Note the presence of glycogen granules (G) in the centre and around the abnormal mitochondria. X 14,400.

**Figure.130**: Transverse section of a portion of the myofibre showing a single mitochondria containing type 1 paracrystalline inclusions - "parking lot type". X 72,500.

**Figure.131**: Mitochondria with paracrystalline inclusions from another biopsy. X 28,000.
Legends

**Figure 132**: Giant mitochondria (mt) with abnormal cristae pattern. X 23,000.

**Figure 133**: Transverse section from muscle biopsy of a female adult aged 28 years (case 4) showing vacuoles (V) containing amorphous material and myelin figures (mf). X 17,400.
2. CARNITINE DEFICIENCY

REVIEW OF LITERATURE

Myopathies associated with the deficiency involving the transport of long chain fatty acyl units across the mitochondrial inner membrane are included under lipid storage myopathies. One such defect in the mitochondrial substrate transport is due to deficiency of carnitine. Carnitine (beta-hydroxy-gamma-N-trimethylaminobutyrate) plays an important role not only in the transport of long chain fatty acyl units into mitochondria but also in the modulation of intramitochondrial CoA/acyl CoA ratio (Fritz, 1963). Most carnitine deficiency syndromes are secondary to an inborn error of metabolism and the commonest cause of lipid storage myopathy. The first case of carnitine deficiency with lipid storage in the muscle was described by Engel and Angeline (1973).

Two types of carnitine deficiency were recognised (Karpati et al., 1975), the muscle carnitine deficiency and the systemic carnitine deficiency. In both these groups, the muscle syndrome is characterised by intermittent or progressive weakness, hypoglycemia and encephalomyopathy.

The striking pathological change seen was the presence of vacuoles within skeletal muscle particularly type-I fibres, seen in both systemic and carnitine deficiencies. The vacuoles contained neutral fats and RRF were demonstrated by
MGT stain. Type grouping and groups of small angulated muscle fibres were seen suggesting the possibility of denervation (Boudin, 1976, Isaacs, 1976). Deficiency of cytochrome-c oxidase in a fatal case of lipid storage myopathy was reported (Muller-Hocker et al., 1983). Electron microscopic investigations showed that the lipid vacuoles were not membrane bound and lay freely in the sarcoplasm resulting in disarray of filaments. The mitochondria showed paracrystalline inclusions in addition to the presence of abnormal pattern of cristae. Lysosomal bodies and lipofuscin aggregates were frequently seen.

The treatment of these patients with oral L.Carnitine proved beneficial (Engel and Siekert, 1973). However lack of response was noticed in patients who demonstrated features of both systemic and carnitine deficiency (Carroll et al., 1981).

MATERIAL

Muscle-quadriceps, biopsied from two infants aged 8 months and 18 months who had generalised hypotonia was submitted for routine analysis. A diagnosis of Carnitine deficiency based on clinical data and morphological findings of muscle biopsy described in the following paragraphs was offered. However, it was not possible to confirm carnitine deficiency by biochemical investigations.
OBSERVATIONS

HISTOLOGY: The muscle sections showed striking vacuolation (Fig 134). The fibres were rounded and varied in their cross-sectional diameter. The vacuoles were of varying sizes and replaced 10 to 90% of the normal constituents of the fibre. There was significant increase in endo and perimysial connective tissue distorting the fascicular architecture of the muscle. Ragged red fibres were seen in large numbers (Fig 136). Oil red O stain demonstrated accumulation of large and small droplets of neutral fats within the muscle fibres (Fig 137).

ENZYME HISTOCHEMISTRY: Muscle sections stained for oxidative enzymes showed marked condensation of the reaction product in many of the fibres (Fig 135). The distinction between different fibre types was not clear.

ELECTRON MICROSCOPY: In the electron microscopic investigations, majority of the fibres contained fat vacuoles of varying sizes. The fibres were round in contour. The sarcolemma was normal. The nuclei were subsarcolemmal in position and had dense chromatin. A few myofibrils that were present showed marked disarray of filamentous pattern, streaming and clumping of Z-band in majority of the fibres. The significant finding was the presence of abnormal mitochondria of varying sizes with marked changes in the configuration of the cristae. A few cytoplasmic bodies consisting of a dense core with radially arranged filaments...
of uniform size were seen in a few fibres (Figs 138 & 139). Microladders or leptomeres consisting of alternate light and dark bands were also seen.

DISCUSSIONS

Two cases were diagnosed as carnitine deficiency based on clinical and morphological findings. The muscle biopsy showed presence of excessive fat and abnormal mitochondria. The vacuolation seen in the muscle was due to excessive storage of undegraded material. This also caused replacement and compression of normal cellular organelles. Injury to the fibre also depended on the physical volume of the stored material, as evidenced by electron microscopy. The presence of abnormal mitochondria in majority of the fibres noticed at electron microscope, corresponds to a large number of RRF seen on MGT preparations. This points to a metabolic error which mainly involves the mitochondrial energy system (Karpati et al., 1975).

Carnitine acts as a carrier of long chain fatty acids into the mitochondria for β-oxidation (Fritz, 1963), the deficiency of which may probably be the cause of fat accumulation. However, it was not possible to confirm carnitine deficiency by biochemical estimation in our study.

The diagnosis of carnitine deficiency among vacuolar myopathies is important as carnitine can be supplemented in the diet or by oral therapy, which has proved effective as a mode of treatment (Engel and Siekert, 1973).
Legends to Photomicrographs - Carnitine deficiency disease

Serial transverse section of quadriceps muscle biopsy from a female child aged 18 months.

Figure 134: Section of muscle showing marked vacuolation. H and E (cryo) X 300.

Figure 135: Section shows condensation of oxidative enzyme reaction in majority of the fibres. SDH X 300.
Legends

Figure 136: Section reveals ragged red fibres in large numbers. MGT X 300.

Figure 137: Section stained for neutral fats shows intense reaction in many of the fibres. Oil red O X 300.
Legends to Electron micrographs—Carnitine deficiency disease

Figure 138: Biopsy from the same child showing portions of two muscle fibres. Striking accumulation of abnormal mitochondria (mt) in one of the fibre around empty vacuoles (V) probably representing fat droplets with severe filament loss. Cytoplasmic body (CB) is seen. Adjoining fibre shows same pathological features to a lesser degree. X 4,750.

Figure 139: Higher magnification of a portion of myofibre with a few mitochondria (mt) containing abnormal cristae pattern. Fat vacuoles (V) are also seen. X 25,000.
3. MITOCHONDRIA-LIPID-GLYCOGEN DISEASE

REVIEW OF LITERATURE

Jerusalem et al., (1973) noticed the presence of fat and PAS positive vacuoles within the same muscle fibre. As abnormal mitochondria were also noticed in the muscle, the term "mitochondria-lipid-glycogen disease" (MLG) was used. These infants had hypotonia, deficiency of carnitine and hyperlactic acidemia (Di Danato et al, 1978). The muscle biopsy showed vacuoles mainly in type-I fibres; although, a few type-II fibres were also involved. Strong reaction forming crescents or caps around the margins of the muscle fibres were seen in oxidative enzyme reactions. Abnormal accumulation of lipids, glycogen and mitochondria were seen by electron microscopy (Sarnath et al., 1982).

Although the etiology of the disease is not clear, presence of excess glycogen, mitochondria, and very few synaptic vesicles at the neuromuscular junctions led Dudley et al., (1981), to suggest an associated neural abnormality in MLG disease.

MATERIAL

Quadriceps muscle, from an infant aged 14 months, who came with hypotonia, delayed motor milestones and dysmorphic features was biopsied. The muscle tissue was processed for usual investigations.
A diagnosis of mitochondria-Lipid-glycogen disorder was offered.

OBSERVATIONS

HISTOLOGY: The histology of muscle showed well preserved architecture. The fibres were round with mild variation in fibre diameter. The nuclei were vesicular with prominent nucleoli. They were peripherally placed in most fibres with an occasional fibre showing central nuclei. Vacuoles of varying sizes were seen in almost all fibres giving a sieve like appearance (Fig 140). The MAT stain showed mild increase in endomysial and perimysial connective tissue. The vacuoles in the majority of the fibres contained PAS positive material (Fig 144), which was resistant to diastase digestion. Oil red O stain demonstrated presence of the neutral fat (Fig 145) in most fibres. Ragged red fibres (Fig 143) were seen in moderate numbers.

ENZYME HISTOCHEMISTRY The muscle sections stained for oxidative enzymes viz. NADH-TR and SDH showed condensation of the reaction product in many fibres, as illustrated (Fig 142). The vacuoles were seen in both type-I and type-II fibres in ATPase preparations (Fig 141).

ELECTRON MICROSCOPY: At electron microscope, the muscle fibres showed marked vacuolation. The membranes were normal. The nuclei were vesicular with prominent nucleoli and were seen in the subsarcolemmal region. Fat vacuoles and vacuoles
containing glycogen rosettes were seen within the same fibre. The mitochondria varied in size and configuration of the cristae (Fig 146). A few myofibrils that remained showed disorganisation of myofilamentous pattern.

DISCUSSIONS

The vacuolation seen in this case is due to the presence of undegraded material namely fat and glycogen due to metabolic blockade. The abnormal mitochondria seen is probably a reaction to biochemical disorder as suggested by Sarnath et al., (1982).
Legends to Photomicrographs—Mitochondria—Lipid—Glycogen disease.
Serial transverse sections of quadriceps muscle from a female child aged 14 months:

Figure.140: Section showing rounded fibres with variable diameter and peripherally placed nuclei. Many of the fibres show multiple small vacuoles giving the fibre a sieve like appearance.

H and E (cryo) X300.

Figure.141: Section stained for myosin ATPase reaction reveals type I and type II fibres. Both fibre types showing multiple small vacuoles.

ATPase (pH9.5)X 300.
Legends

Figure.142: Section stained for oxidative enzyme shows condensation of reaction product in many fibres.

SDH X 300.

Figure.143: Section reveals ragged red fibres in large number.

MGT X 300.
Legends

**Figure.144**: Section showing PAS positive material concentrated in some of the fibres (▲). PAS X 300.

**Figure.145**: Section shows some of the fibres to be positive to neutral fat (▲). Oil red O X 300.

**Figure.146**: Electron micrograph from the case same as above showing a portion of myofibre in longitudinal section, lipid vacuoles (V), glycogen particles (G), mitochondria (mt) with abnormal cristae pattern and a few myofilaments (M). X 20,000.
4. **ACID MALTASE DEFICIENCY DISEASE (POMPE'S DISEASE)**

**REVIEW OF LITERATURE**

Acid maltase is a lysosomal enzyme, which releases glucose from maltose, oligosaccharides and glycogen. Deficiency of this enzyme leads to storage disorder. Three variants of acid maltase deficiency (AMD) are described: the infantile, childhood and adult forms. The infantile form of AMD was first described by Pompe in 1932 and was later named after him. Illingsworth and Cori (1952) designated Pompe's disease as type 2 glycogenoses. Hers (1963), described the absence of 1,4-glucosidase (acid maltase) in the muscle tissue of infants with type-2 glycogenoses. These infants failed to hydrolyze maltase at acid pH. The infants usually have diffuse hypotonia and weakness. Macroglossia is common. Massive cardiomegaly and hepatomegaly are noticed and death occurs before age of 2 years.

The muscle pathology shows a vacuolar myopathy. The vacuoles contained PAS positive diastase digestable material and stain intensely for acid phosphatase. Electron microscopy confirms the presence of excess glycogen within lysosomal vacuoles and also found free in the sarcoplasm (Bordiuk et al., 1970). Autopsy study by Hudgson and Fulthrope (1975), showed excess glycogen especially in the heart and in the schwann cells of peripheral nerves.
Two Infants aged 4 months and 10 months diagnosed as acid maltase deficiency were included in the study. Both had hypotonia and cardiomegaly. One of them also had hepatomegaly and macroglossia. The skeletal muscle biopsies were taken for routine investigations.

**OBSERVATIONS**

**HISTOLOGY:** The muscle biopsies revealed pronounced vacuolation in majority of the fibres. The myofibres were rounded and showed variation in their diameter. H and E stained sections showed vacuoles to contain bluish material (Fig 147). The vacuoles contained PAS positive material which was totally digested by diastase. The sections stained for acid phosphatase showed positive reaction within the vacuoles. Lipid storage myopathy was ruled out, as the vacuoles did not contain neutral fats when stained with Oil red O.

**ENZYME HISTOCHEMISTRY:** Enzyme reactions showed clumping of reaction product to one side to all enzyme stains. Distinction into fibre types was not evident due to marked distortion of the myofibre by vacuolation.

**ELECTRON MICROSCOPY:** The muscle fibres showed marked vacuolation, the sarcolemma was normal and the nuclei contained dense chromatin. Lakes of freely dispersed glycogen not limited by membranes was seen in one case while, spaces
limited by continuous or discontinuous membranes with excess glycogen was seen in the other case (Fig 148, 149). Glycogen granules were in the rosette form and was also found outside the vacuoles. A fewer number of myofibrils present in the myofibres were pushed to one side while the vacuoles occupied a larger area. An occasional mitochondria in a few fibres contained glycogen granules, but their morphology was otherwise normal. Dense bodies and myelin figures were seen in some fibres.

DISCUSSION

Two cases, were diagnosed as acid maltase deficiency. This forms an important type of vacuolar myopathy where accumulation of glycogen was demonstrated by histochemical and electron microscopic studies. The bluish material seen within the vacuoles is probably the mucopolysaccharide as suggested by Bordiuk et al.,(1970). The lysosomal enzyme activity was indicated by the presence of large autophagic vacuoles. The presence of vacuoles and glycogen is probably due to entry of lysosomal enzymes into the myofibre damaging the normal organelles and inactivating the glycolytic enzymes as suggested by Bardiuk et al.,(1970).
Legends - Acid maltase deficiency

Quadriceps muscle from a female child aged 10 months:

Figure 147: Transverse section shows myofibres with marked vacuolation. Some of the vacuoles contain bluish stained material. H and E (cryo) X 480.

Figure 148: Electron micrograph from the biopsy same as above: portion of two fibres showing replacement of filaments by vacuoles with granular material glycogen (G). X 4,200.

Figure 149: Higher magnification of a portion of myofibre showing glycogen particles (G) in the rosette form. X 11,400.