CHAPTER V
INFANTILE SPINAL MUSCULAR ATROPHIES
INFANTILE SPINAL MUSCULAR ATROPHIES

REVIEW OF LITERATURE

Infantile spinal muscular atrophies (ISMA) constitutes one of the major disease entities presenting as floppy infant syndrome. In general, they are progressive diseases and the earlier they present with clinical symptoms, the swifter is the downhill course. The ISMA are genetically determined with autosomal recessive pattern of inheritance. In recent studies, Melki et al., (1990) have demonstrated that the disease gene is located on the chromosome between 5ql2-ql4.

Werdnig in 1891 was the first to describe the disease in two half brothers, who since the age of 10 months, had progressive symmetrical weakness of distal, proximal and axial musculature. The patients had never been able to sit without support and the tendon reflexes were absent. In an autopsy study of one of the patients mentioned above, he found atrophy of motor neurons in the spinal cord and in the Vth and VIIth cranial nerve nuclei. These observations prompted the author to propose a neural origin of the disease. Hoffmann in 1893 reported a family of four children, all of whom suffered from a similar muscle disorder with weakness greater in the upper limbs than the lower. Light microscopic observations demonstrated decrease in the number of anterior horn cells in the spinal cord and atrophy of muscle. He referred to this disease as hereditary progressive spinal muscular atrophy.
Since the original descriptions of Werdnig and Hoffmann much has been added to our knowledge regarding the clinical and histopathological features of this disease, often under different titles (See Brandt, 1950).

Walton (1956) in a review of 115 patients who had been previously diagnosed as Amyotonia Congenita found heterogeneous nature of this syndrome. The most common disorders according to him were infantile spinal muscular atrophy, congenital muscular dystrophy and benign congenital hypotonia. Subsequent reports described infants with variable clinical course some of whom died before the age of 3 years, while others lived up to 10 years or longer. Attempts were therefore made by various authors to classify ISMA based on onset of symptoms and the clinical course (Byers and Banker, 1961; Dubowitz, 1964; Pearn, 1978; Gomez, 1986 and Hausmanowa-Petrusewicz and Karwanska, 1986).

The classification proposed by Hausmanowa-Petrusewicz and Karwanska, (1986), based on onset of the disease has been adopted in our study. Thus ISMA was classified as:

SMA-1- Acute infantile Spinal muscular atrophy or Werdnig-Hoffmann disease (WHD) onset at birth, survival 2-4 years.

SMA-2- Intermediate form onset at 3 months, long survival

In the study of muscle biopsies, Dubowitz, (1964) noticed features of denervation with uniform group atrophy in a large number of fascicles. Amidst these atrophic fibres,
the normal sized or enlarged fibres were seen either singly or in clusters. By the application of enzyme histochemical techniques it was found that the atrophic fibres to be of type-I and type-II, while the larger fibres conformed to one type only. Dubowitz, (1964) suggested that the presence of one fibre type was due to reinnervation rather than unaffected normal fibres. Roy et al., (1971) noticed selective atrophy of type-I fibres and hypertrophy of type-II fibres. According to Kingma et al., (1991), selective type-II fibre hypertrophy indicated a relative sparing of the motor units with type-II fibres. An increase in number of type-II C fibres was reported by Nonaka and Chou, (1978).

Detailed fine structural studies (Hughes and Brownell, 1969; Roy et al., 1971) showed marked degree of denervation and disappearance of myofilaments. Dilatation and proliferation of the sarcotubular system, swollen and degenerated mitochondria were observed. Proliferation of basal lamina was also noticed. Numerous lysosomal bodies seen was considered to be due to degeneration. The presence of tubular structures observed by them were thought to represent abnormal mitochondria (Roy et al., 1971). According to these authors, the overall findings suggested a neurogenic component affecting the muscle to be the basic pathology in ISMA.

Fidzianska (1976), in a comparative study between the small muscle fibres of Werdnig-Hoffmann disease (WHD) and
Amyotrophic lateral sclerosis (ALS), observed the presence of myotubes only in Werdnig-Hoffmann disease (WHD). These myotubes were similar to those seen in the foetal stages of development. This prompted her to suggest a maturational defect in WHD. Hausmanowa-Petrusewicz and Fidzianska (1974) suggested maturation arrest at 20 wks gestation was due to defective neural innervation. Saito (1985), noticed a significant increase in number of satellite cells. This led the author to propose that the fibre immaturity is possibly due to a failure of satellite cells to fuse with the growing muscle fibres. Thus, it seems that both the defective neural mechanism and maturational arrest play a role in the disease process.

The presence of apoptotic bodies which are membrane bound bodies of muscle cell fragments were observed in the muscle of ISMA (Fidzianska et al., 1990). The term apoptosis was first used by Kerr et al., (1972) for focal elimination of cells during embryogenesis. Fidzianska et al., (1990) consider that the loss of motor neurons in the spinal cord of ISMA patients is secondary to the elimination of the peripheral targets by apoptosis, rather than a primary pathologic change. Cytoplasmic bodies in the muscle biopsies of WHD was reported by Buchino et al., (1990). However, no diagnostic significance could be attached to the presence of such bodies, as follow-up studies failed to reveal similar changes in the muscle biopsies.
To resolve whether maturation arrest plays a role in the disease process, immunohistochemical methods were employed (Schiaffino et al., 1986; Biral et al., 1989; Sawchak et al., 1990). The authors used antibodies specific for foetal myosin heavy chain (a protein present in the foetal muscle fibres) and found its presence only in a few small fibres, while the remaining small and large fibres showed a variable reaction. Thus the postulates laid down by earlier authors with regard to maturation arrest still remains unclear.

The pathological changes in spinal cord, brainstem, cerebellum, thalamus, cranial nuclei and cranial nerves in autopsy studies of ISMA patients have been reported (Norman and Kay, 1965; Chou and Nonaka, 1978; Shishikura et al., 1983 Towfighi et al., 1985; Lee et al., 1989). In the spinal cord, the changes seen were wide spread astrocytosis and multiple foci of necrosis, varying degree of neuronal enlargement (ballooned neurons), and neuronophagia, in the anterior horn and Clarke's column. Most of the cranial nerves showed marked axonal degeneration, while the optic nerve was normal. The ultrastructural examination mainly of the spinal cord revealed degenerative changes in the ballooned neurons such as diffuse increase in neurofilaments, accumulation of mitochondria and vesicular and membraneous profiles in the centre of the neuron. Thalamus and brainstem showed degenerative changes. The cerebellar cortex showed loss of Purkinje cells. Shishikura et al., (1983) suggested that WHD
is consistent with multisystem disease involving both the anterior and posterior root systems based on the observation that degeneration of lower motor neurons and glial bundles in the anterior roots and also degeneration of sensory neurons and thalamus.

Immunohistochemical studies using polyclonal and monoclonal antibodies against phosphorylated neurofilament (pNF), neurofilaments (NF), glial fibrillary acidic protein (GFAP), and ubiquitin (UBQ) have been reported (Lippa and Smith, 1988; Lee et al., 1989; Murayama et al., 1991; Kato and Hirano, 1990). Lee et al., (1989) found a variable degree of immunostaining with UBQ and pNF in the ballooned neurons. On the other hand Kato and Hirano (1990) found that the reaction to pNF was seen at the periphery as a ring and only occasionally at the centre. The antibody reaction to UBQ was seen as small granules aggregated at the centre. It has been suggested by Murayama et al., (1991) that increased pNF in the neurons reflect an alteration in the intrinsic metabolism, eventually leading to neuronal death.

MATERIAL

22 cases of SMA-1 and 13 cases of SMA-2 were included in the study. A brief summary of salient clinical features is given in Table 3 and Table 4. Spinal cord from an autopsy case of WHD was available for histological and immunohistochemical study.
### Clinical data - SMA-1

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age at biopsy/sex</th>
<th>Onset of symptoms</th>
<th>Foetal movements</th>
<th>Delay in motor milestones</th>
<th>Floppiness</th>
<th>Tongue fasciculations</th>
<th>Areflexia</th>
<th>EMG</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>18 m/M</td>
<td>Birth</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+ Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>2 1/2 yrs/F</td>
<td>Birth</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+ Neurogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>9 m/F</td>
<td>Birth</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ Neurogenic</td>
<td>Expired</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>3 m/F</td>
<td>Birth</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>- Neurogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>11 m/F</td>
<td>Birth</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>- Normal</td>
<td>Sits with support</td>
<td>Expired</td>
</tr>
<tr>
<td>6.</td>
<td>3 m/M</td>
<td>Birth</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+ Neurogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>6 m/F</td>
<td>Birth</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>4 yrs/F</td>
<td>Birth</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>6 m/F</td>
<td>Birth</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Neurogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>11 m/M</td>
<td>Birth</td>
<td>Normal</td>
<td>Not attained any milestones</td>
<td>+</td>
<td>+</td>
<td>? Sensory neuropathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>7 m/M</td>
<td>Birth</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>6 m/F</td>
<td>Birth</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>- Neurogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>20 days/M</td>
<td>Birth</td>
<td>Reduced</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>8 m/M</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+ Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>2 1/2 yrs/M</td>
<td>Birth</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+ Neurogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>3 yrs/M</td>
<td>Birth</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>Gradual increase in motor function</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>2 yrs/M</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>- Neurogenic</td>
<td>Status quo</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>11 m/M</td>
<td>Birth</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Neurogenic</td>
<td></td>
<td>Expired</td>
</tr>
<tr>
<td>19.</td>
<td>1 yr/M</td>
<td>Birth</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>7 m/M</td>
<td>2 mths</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>2 1/2 yrs/M</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Neurogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>8/12 M</td>
<td>Birth</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Neurogenic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Quadriceps muscle was biopsied in all.
* Autopsy done; + - present; - absent; ? not known; EMG - Electromyography; m - month; M - Male; F - Female.
<table>
<thead>
<tr>
<th>Cases</th>
<th>Age at biopsy/sex</th>
<th>Onset of symptoms</th>
<th>Foetal movements</th>
<th>Delay in motor milestones</th>
<th>Hypotonia</th>
<th>Congenital markers</th>
<th>Areflexia</th>
<th>EMG</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7 yrs/M</td>
<td>4 yrs</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>Over riding of toes</td>
<td>-</td>
<td>Neurogenic</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>5 yrs/F</td>
<td>1 yr</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Neurogenic</td>
<td>Improving</td>
</tr>
<tr>
<td>3.</td>
<td>4 yrs/F</td>
<td>Birth</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>Scoliosis</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>5 yrs/F</td>
<td>1 1/2 yrs</td>
<td>Reduced</td>
<td>+</td>
<td>+</td>
<td>Scoliosis</td>
<td>+</td>
<td>-</td>
<td>able to walk</td>
</tr>
<tr>
<td>5.</td>
<td>2 4/12 yrs/F</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Improving</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>8 yrs/F</td>
<td>Birth</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Scoliosis</td>
<td>+</td>
<td>Neurogenic</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>2 yrs/F</td>
<td>8 mths</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>Scoliosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>8 yrs/F</td>
<td>6 mths.</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>3 1/2 yrs/M</td>
<td>10 mths</td>
<td>10 mths</td>
<td>10 mths</td>
<td>10 mths</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>4 yrs/F</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>Neurogenic</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>3 3/12 yrs/M</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Neurogenic</td>
<td>Able to walk</td>
</tr>
<tr>
<td>12.</td>
<td>3 yrs/M</td>
<td>9 mths</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Neurogenic</td>
<td>-</td>
</tr>
</tbody>
</table>

Quadriceps muscles was biopsied in all
OBSERVATIONS

SPINAL MUSCULAR ATROPHY - 1 (SMA-1)

HISTOLOGY: The muscle biopsies from 19 of the 22 cases of SMA-1 showed fascicles composed of clusters of small rounded fibres. A few groups of large hypertrophic fibres were seen adjoining such fascicles as illustrated in Fig 49. In some fascicles admixture of both small and large fibres were seen (Fig 50). In the majority of the small fibres and a few hypertrophic fibres the sarcolemmal nuclei were vesicular with prominent nucleoli. They were seen beneath the sarcolemma in all except in a few small fibres where they were centrally placed. Clumping of nuclei was also seen at a few foci. In some biopsies (cases 1, 8) the muscle sections showed mild increase in endomysial and perimysial connective tissue (Fig 51).

In the other 3 cases (case no.11, 17 and 22) majority of the fibres were round in shape. Triangular small atrophic fibres were seen in between them (Fig 56). The nuclei were peripherally placed in most and centrally in a few fibres. There was no increase in either endomysial or perimysial connective tissue.

ENZYME HISTOCHEMISTRY: In the ATPase reaction at pH 9.5 and 4.6 type-I and type-II fibres could be distinguished. The small fibres were type-I and type-II in some fascicles while
indistinct staining was observed in other fibres in all the cases. The hypertrophic fibres were type-I in 13 cases (Fig 52). type-II in one case (case 4) and type-I and type-II in the remaining (Fig 53). Grouping of hypertrophic fibres was also seen (Fig 54). In 5 cases (case Nos.1, 10, 12, 13, 14) many of the small rounded fibres showed condensation of NADH-TR and SDH reaction products, similar to that seen in the foetal fibres (Fig 55).

The histochemical staining of muscle biopsies in the remaining 3 cases showed round shaped fibres to be of type I and type II. Grouping of type II fibres was seen at a few foci. The atrophic fibres in between the round shaped fibres showed dark reaction (Fig 57).

**ELECTRON MICROSCOPY**: In the muscle biopsies of the 19 cases, small fibres round in contour were predominantly seen. The larger fibres were also round but fewer in number. The basal lamina in the small and large fibres appeared normal in 5 of the 19 cases. In the remaining biopsies, majority of the small fibres showed redundant basal lamina (Fig 58). In two of these cases, there was also thickening of the basal lamina and mild increase in the endomysial collagen. The hypertrophic fibres also showed redundant basal lamina (Fig 59). Moderate to marked degree of disarray of filamentous pattern and streaming and clumping of the Z-band (Fig 60) both in the smaller and larger fibre populations was seen.
This led to the displacement of the T-tubular system and mitochondria. Several fibres from muscle specimen in all the cases showed formation of pentads and heptads. These consisted of complex multiple association of alternate lateral sacs and the tubular part of the T-system as illustrated (Fig 61).

The other changes seen were dilated sarcoplasmic reticulum, accumulation of normal mitochondria in the subsarcolemmal and intermyofibrillar region in an occasional large fibre in three of the cases. Presence of large vacuoles within a few small fibres containing amorphous material (Fig 62) was seen. The nuclei in some of these fibres showed condensation of chromatin and convoluted nuclear outline (Fig 63). A few hypertrophic fibres showed peripheral vacuolation (Fig 64).

In addition to the morphological changes mentioned above a few homogenised fibres and sarcolemmal tubes were noticed. The homogenised fibres showed dense filamentous mass. The nuclei in some of these fibres were convoluted with dense chromatin masses (Fig 65). These fibres were fewer in number seen mostly amongst the smaller fibre groups while an occasional larger fibre showed such changes.

Sarcolemmal tubes completely devoid of myofilaments but having a few mitochondria and nucleus (Fig.66) were seen infrequently. Moderate increase in number of satellite cells
was seen in 2 of the cases (case 2, 10), while in the others the satellite cells, though present, were not numerous. Myotubes were not encountered in any of the cases. A few normal fibres were observed in all the cases.

The other noticeable findings were the presence of concentric laminated structures and cytoplasmic bodies. The concentric laminated structures were seen in a few fibres in 3 cases (5, 9, 12). Several of these were present beneath the sarcolemma containing granular material in the interior. Some of these laminated bodies showed structures resembling cristae within them (Fig.67). The cytoplasmic bodies were seen in a few fibres in 2 cases (5, 10). They were of variable size, round or oval in profile present amidst the myofibrils. These bodies consisted of a dense core and an outer layer of radially arranged filaments (Fig.68). The radiating filaments were of uniform diameter probably representing thin filaments.

The rounded fibres seen in three of the biopsies (cases 11, 17 and 22) revealed normal morphology at electron microscopy. However a few small atrophic fibres which were present amidst normal looking ones showed redundant basal lamina and disarray of filamentous pattern.

**SPINAL CORD:** Different segments of the spinal cord specimen from a case of SMA-1 showed loss of anterior horn cells and chromatolysis of neurons. There was histological evidence of
the presence of ballooned neurons and neuronophagia of degenerating neurons in the anterior horn. Astroglial proliferation was a prominent feature (Fig.69 & 70). Immunohistochemical stains for phosphorylated neurofilaments (SMI-31) showed positive staining in the perikaryon of ballooned neurons and degenerating axons. In a few ballooned neurons the staining was restricted to the peripheral region leaving a small unstained central zone (Fig.71) while in some, the entire neuron showed positive staining (Fig.72). The neurons in the dorsal root ganglia were also ballooned and showed positive reaction to phosphorylated antibody.

Spinal cord specimen from three foetuses of gestational ages 14, 15 and 19 weeks were available for the study, as controls. Different spinal segments, from cervical, thoracic, lumbar and sacral regions were stained for histological and immunohistochemical studies. Histologically the spinal neurons were seen distributed in medial, intermediate and lateral groups in the anterior horn. None of the neurons showed ballooning. Immunostaining with monoclonal antibody to phosphorylated component of neurofilament revealed majority of the neurons to be unlabelled except for a few which showed mild diffuse staining. The axons on the other hand were immunolabelled. This, therefore suggests that in WHD, some of the ballooned neurons which were strongly immunolabelled in the anterior horn represent degenerated neurons due to disease process.
SPINAL MUSCULAR ATROPHY - 2 (SMA-2)

In SMA-2, the histomorphology of muscle biopsies showed many similarities with those of SMA-1. Among the distinguishing features on histology in SMA-2 were the presence of polygonal small fibres (Fig. 73), moderate number of hypertrophic fibres with internal nuclei and fibre splitting. An occasional fibre showed myophagocytosis (Fig. 74) in 2 of the biopsies. Presence of normal polygonal fibres with an occasional atrophic fibre interspersed in between normal ones was seen in 2 cases (case 9, 10). The features were similar to that seen in the 3 cases of SMA-1 mentioned earlier.

ENZYME HISTOCHEMISTRY: The ATPase reaction showed hypertrophic fibres to be of type-I nature in 8 cases, (Fig. 76), type-I and II in one case (case 4) (Fig. 77), and type-II in the other (case 11). A few hypertrophic fibres showed moth-eaten appearance (Fig. 75). 2 cases which showed normal polygonal fibres on H & E, revealed grouping of type-II fibres in one and grouping of both type-I and type-II fibres in the other. The atrophic fibres appeared dark in all enzyme reactions.

ELECTRON MICROSCOPY: Fine structural observations of muscle biopsies of SMA-2 showed the presence of redundant basal lamina membrane in the small and large fibres. The other finding was the presence of oval shaped structures near the
nucleus comprising of very fine filaments but not bound by any membrane. These inclusions were seen in a few fibres in 2 cases (case 2, 4) (Fig.78).

DISCUSSIONS

The essential features seen in ISMA were the presence of small atrophic fibres with a few hypertrophic fibres amidst them. The small fibres could be histochemically distinguished into type-I and type-II fibres which suggests that muscle fibres were well differentiated during foetal development. However, at ultrastructural level these fibres showed redundant basal lamina which is suggestive of a process of denervation occurring in the muscle fibres. Hence it is felt that the presence of small fibres resembling foetal type fibres (as seen in NAHD-TR preparation) is due to denervation rather than persistence of foetal myotubes as proposed by Fidzianska (1976). In the present study of foetal development it was noticed that the histochemical differentiation occurred at 22nd week gestation therefore it can be suggested that the denervation process in SMA-1 must have started after 22nd week of gestation as the small fibres in ISMA showed distinct histochemical types. Thus our observations suggest denervation atrophy occurring as early as 22nd week gestation to be the basic pathogenetic mechanism in SMA-1.
Grouping of hypertrophic fibre (which were often type-I in histochemical characteristic) could be due to reinnervation following denervation similar to the explanation offered by Dubowitz (1964) and Roy et al., (1971). The redundant basal lamina seen in some of these hypertrophic fibres suggests a continuation of the process of denervation in the reinnervated hypertrophic fibres or its persistence even in the reinnervated fibres.

In the present study of 22 cases of SMA-1 (WHD), it was noticed that both the histochemical fibre types were involved in atrophic and hypertrophic process. Therefore it is unlikely that any one type of motor unit is relatively spared. In this respect we do not agree with the suggestions of Kingma et al., (1991) who found selective type-II muscle fibre hypertrophy in one case of ISMA and suggested relative sparing of motor unit with type-II fibres.

Ultrastructural presence of pentads, heptads and dilatation of sarcoplasmic reticulum appear to represent the initial phases of degeneration following disorganisation and disarray of myofilaments.

Homogenised fibres and sarcolemmal tubes noticed, represent late stage of muscle fibre degeneration and ultimate loss, such changes were seen in the infantile forms and not in the other forms (juvenile form -SMA-3, adult form
which are denervation atrophies of chronic type. It is likely that these changes occur only in the rapidly progressing diseases.

In the present study the rapid progression of the disease in SMA-1 was reflected by marked fallout of motor neurons and the presence of dystrophic neurons which demonstrated neurofilaments in the different segments of the spinal cord as early as 20-days postnatal life. This also suggests that the disease process starts in the foetal life.

The concentric laminated structures seen in this study are similar to the tubular structures described by Roy et al., (1971). Such structures were also observed by Shafiq et al., (1967) in muscle specimens from unaffected mother of a child with nemaline myopathy. These authors considered such structures probably to represent altered mitochondria as they were similar to abnormal mitochondria described by Luft et al., (1962) from a case of hypermetabolic state of non-thyroid origin. In our study, concentric laminated structures showing dense matrix with cristae similar to that seen in the adjoining mitochondria strongly suggest that the concentric laminated structures are morphologically altered mitochondria.

The cytoplasmic bodies comprising dense core with radiating thin filaments noticed were similar to that seen by Buchino et al., (1990). Such bodies were also observed by
Engel (1962), in association with experimental muscle denervation. The occurrence and significance of such bodies however was not clear. Mc Donald and Engel (1969) speculated that Z-disc streaks coalesced to form cytoplasmic bodies based on the similarity in density of Z-disc and cytoplasmic bodies. However, relationship of cytoplasmic bodies to Z-disc could not be demonstrated by immunohistochemical methods - (Buchino et al., 1990). We consider cytoplasmic bodies to represent an early stage of homogenisation of myofilaments.

The sequence of changes seen in the muscle specimens following denervation are alteration of myofilaments resulting in disarray and disorganisation, cytoplasmic body formation, homogenisation of the myofibre and disappearance of myofilaments leaving a sarcolemmal tube. Gutmann et al., (1976) suggested that a continuous supply of neurotrophic factors (by axonal transport) and impulse mediated activity are essential for maintaining normal structure and function. In denervation atrophies the above mentioned changes are probably due to lack of such neurotrophic factors.

In a comparison between SMA-1 and SMA-2, it was found that in SMA-2 most of the small fibres appeared polygonal as opposed to the rounded fibres seen in SMA-1. This suggests that denervation process in the muscle fibre starts early in postnatal life in SMA-2 as against in SMA-1. It is possible
that the denervation process in SMA-1 starts in the foetus when the fibres are still round and therefore could not enlarge in the postnatal life to become polygonal. The difference in the age of onset and presentation between SMA-1 and SMA-2 explains this observations satisfactorily. A moderate number of hypertrophic fibres with internal nuclei and fibre splitting were seen in SMA-2 but not in SMA-1. Similar changes are seen in other slowly progressive denervation atrophies such as SMA-3 (Juvenile form) and SMA-4 (adult form). In SMA-2 these findings were seen in patients who presented at 5 years and 8 years, which explains the relatively slow progression of disease in SMA-2 as compared to SMA-1.

Our observations suggest that SMA-1 is due to denervation of skeletal muscle occurring in the foetal stage possibly after differentiation of the myofibres into the two major histochemical types. This denervation is due to degeneration of the anterior horn cells. Our studies do not suggest arrest in maturation to be the basic pathogenetic mechanism in SMA-1. On the other hand the pathogenesis in SMA-2 appears to be due to a denervation process starting early in the postnatal life.
Legends to Photomicrographs - Spinal muscular atrophy - 1

**Figure 49**: Quadriceps muscle biopsy from a female child aged 6 months (case 12): Transverse section showing several fascicles. The fascicles on the right shows groups of large hypertrophic fibres. The rest show small atrophic fibres in groups with a few larger ones in between. Nuclei are peripherally situated in most fibres. Muscle spindle (S), blood vessels (BV) and nerve (N) are also seen. H and E (cryo)x300.

**Figure 50**: Transverse section of muscle biopsy from a male child aged 18 months (case 1) showing admixture of hypertrophic fibres and atrophic fibres in each fascicle. H and E (cryo)x300.

**Figure 51**: Transverse section of muscle biopsy from a child aged 4 years (case 8) shows mild increase in the perimysial connective tissue. The left half of the picture shows small rounded atrophic fibres with a few larger ones in between. The other half shows very few large hypertrophic fibres. MAT x 300.
Legends

Figure 52: Transverse section of muscle biopsy from a male child aged 11 months (case 10): Myosin ATPase reaction reveals type I fibre hypertrophy. Both type I and type II fibres are distinguished among atrophic fibres. ATPase (pH 9.5)x 300.

Figure 53: Transverse section of muscle biopsy from a male child aged 6 months (case 12) showing both type I and type II fibre hypertrophy. ATPase (pH 9.5)x 300.
Legends

**Figure.54**: Transverse section of muscle biopsy from case 15, hypertrophic type-I fibres shows grouping. ATPase (pH 9.5)x300.

**Figure.55**: Transverse section of muscle stained for oxidative enzyme reaction showing type-I fibre hypertrophy some of the atrophic fibres reveals foetal character (\^\_). NADH-TR x 480.
Legends

Figure 56: Trasverse section of muscle from a female child aged 7 months (case 11) shows mostly rounded fibres. An occasional small atrophic fibre is seen in between. H and E (cryo) x 300.

Figure 57: Muscle biopsy from the patient same as above : section stained for oxidative enzyme reaction shows atrophic fibres to be intensely stained as against the large fibres. NADH-TR x 300.
Legends to Electron micrographs - SMA-1

Figure.58: Transverse section of muscle biopsy (quad) from a female child aged 18 months (case 1) showing several small atrophic fibres. The myofilaments show disarray, nuclei contain condensed chromatin. The atrophic fibres show redundant basal lamina (\textsuperscript{\textbullet}). X 4,560.

Figure.59: Transverse section from the case same as above shows two hypertrophic fibres with redundant basal lamina (\textsuperscript{\textbullet}). The myofilaments (M) are fairly well preserved. X 12,760.

Figure.60: Longitudinal section of muscle biopsy from a male child aged 6 months (case 7) showing a portion of myofibre with marked disarray of filamentous pattern, streaming and clumping of Z-band (\textsuperscript{\textbullet}). X 13,400.
Legends

Figure.61: Electron micrograph showing complex multiple assembly of triads consisting of alternate lateral sacs (L) and the tubular part (T) of the triad. X 30,400.

Figure.62: Transverse section of muscle biopsy from a male child aged 3 months (case 6) showing vacuolations with electron dense material (A) A few myofilaments (M) and mitochondria (mt) are seen. X 6,670.

Figure.63: Biopsy from the child same as above in another fibre showing degenerative changes, the nucleus is pyknotic with convoluted nuclear membrane. A few myofilaments (M) and mitochondria (mt) are seen. X 8,640.
Legends

Figure 64: Transverse section of muscle from case 8 illustrating hypertrophic fibres with peripheral vacuolation, disarray of myofilaments and small clumps of Z-band material. X 14,740.

Figure 65: Transverse section of muscle from another case (case 2) showing a part of homogenised fibre with dense mass of myofilaments (M), dilatation of sarcoplasmic reticulum (SR) and sarcotubular system (ST) and loss of myofilaments in the subsarcolemmal region. X 13,340.

Figure 66: Section of muscle from the case same as above showing the sarcolemmal tube with nucleus (N) and a few mitochondria. Note the total loss of myofilaments. X 18,400.
Legends

**Figure.67**: Longitudinal section of muscle biopsy from a female child aged 11 months (case 5) illustrating concentric laminated structures (CL) in the subsarcolemmal region. X 22,000.

**Figure.68**: Transverse section from biopsy same as above showing oval electron dense cytoplasmic body (CB) with radiating filaments. Atrophic nature of the fibre is shown by the presence of redundant basal lamina (∩). X 7,560.
Legends

Figure 69: Spinal cord section through the cervical segment from an infant aged 20 days showing fall out of motor neurons and glial proliferation in the anterior horn region. 

H and E X 192.

Figure 70: Spinal cord section through the sacral segment from infant same as above showing a single ballooned neurons (BN), neuronophagia (\_\_) and glial proliferation. 

H and E X 300.
Legends

Figure 71: Spinal cord sections through cervical segment showing ballooned neuron immunostained with antibody to phosphorylated neurofilament restricted to the periphery of the neuron.

SMI-31 X 480.

Figure 72: Spinal cord section through cervical segment from an infant same as above showing ballooned neuron immunostained with antibody to phosphorylated neurofilament.

SMI-31 X 480.
Legends to Photomicrographs—Spinal muscular atrophy—2

Figure. 73: Transverse section of muscle biopsy from a male child aged 3 yrs 3 months (case 2) showing groups of atrophic fibres with peripherally placed 'nuclei. Large hypertrophic fibres of varying sizes are seen amidst atrophic fibres. H and E (cryo) x 300.

Figure. 74: Transverse section of muscle from another case (case 4) showing hypertrophic fibres. One of the fibre shows necrosis and extensive phagocytosis, while a few others show internal nuclei. Fibre splitting is also evident (\Uparrow). H and E (cryo) X300.
Legends

Figure.75: Transverse section of muscle showing moth eaten appearance of the hypertrophic fibre.

NADH-TR X 300.

Figure.76: Transverse section of muscle from a male child aged 3 years (case 12) stained for myosin ATPase showing grouping of type-I fibres. Type II-fibre atrophy is very prominent.

ATPase (pH 9.5) X300.

Figure.77: Transverse section of muscle biopsy from case 8 stained for myosin ATPase showing type-I and type-II fibre hypertrophy. ATPase (pH 9.5) X 300.
Legends

Figure.78 : Electron micrograph from muscle biopsy of female child aged 3 yrs 3 months. Transverse section of a portion of myofibre showing oval shaped filamentous body (FB) comprising very fine filaments, seen near the nucleus (N). X 5,800.