Neurogenic Changes in Myasthenia Gravis

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SUMMARY

Twelve consecutive cases of myasthenia gravis have been studied histologically and histochemically. In addition 8 of these were studied ultramicroscopically, and 7 had nerve terminal and end-plate studies. Eleven of the cases show evidence of one type or another which supports a primary neuropathic aetiology. These findings are in keeping with some recent publications which have been reviewed.

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Although myasthenia gravis has been known for centuries, the over-all presentation was clarified and established in 1900 by Campbell and Bramwell,[1] when they fully described the clinical picture and published a review of the cases reported up to that time. By 1901 Laqueur and Weigert[2] were aware of the close relationship between the thymic gland and the occurrence of myasthenia gravis. Buzzard[3] noted the presence of lymphocytic reaction in myasthenic muscle and coined the term 'lymphorrhage'. From this time surprisingly little progress was made in muscle histology over the next 50 years. By 1955 authorities[4] still accepted that the motor nerve terminals and the end-plates were normal, a misconception which, like so many others, is perpetuated by the lack of adequate techniques of the period. The error was corrected when Coërs and Woolf[5] described the elongated motor end-plates of a case of congenital myasthenia in a 6-year-old patient, and subsequently consistently showed that the motor end-plates were abnormal in myasthenia gravis.

In 1962 Steadl *et al.*[6] reported on 1 patient with myasthenia gravis who showed histological evidence of denervation. Since this time reports of neurogenic involvement of muscle in myasthenia gravis have been accumulating, and it is with this background that 12 consecutive cases of myasthenia gravis were subjected to histological and histochemical analysis. In many patients the muscle was also studied ultramicroscopically, and nerve terminal and end-plate studies were carried out.

The 12 patients (7 female), with ages ranging from 11 to 55 years, are tabulated in Table I. The duration of the myasthenia varied between 1 and 28 years. Thymectomy had been performed 14 years previously in case 4, 25 years previously in case 10, within 1 - 2 years in cases 6 and 7 respectively, and within a year in case 9. In this last case there was evidence of marked thymic hyperplasia and evidence of the patella-nail syndrome (Fig. 1). In no case was there evidence of thymic neoplasia.

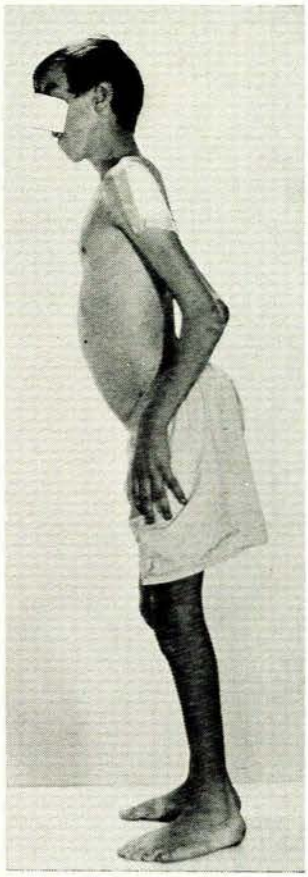

Fig. 1. Many of the muscular skeletal deformities associated with the patella-nail syndrome.

All cases were confirmed as myasthenia gravis by electrical stimulatory studies coupled with the edrophonium response. In all cases electromyography was considered abnormal in that there were abnormal numbers of low voltage motor units, and in the long-standing cases there was an increase in high voltage polyphasic activity indicating reinnervation with extension of motor unit territory. In no case was fibrillation activity recorded.

All patients were treated with neostigmine or pyridostigmine bromide, or both, in the maximum effective dosage up until the time of biopsy. After this the majority of patients were changed to a regimen of prednisolone and/or ACTH therapy.

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TABLE I. MUSCLE, NERVE TERMINAL AND END-PLATE FINDINGS

Case	Age (yrs)	Sex	Duration of illness at time of biopsy (yrs)	Muscle	Muscle fibre morphology	Infiltration	Evidence of atrophy	Fibre type abnormality	Motor-nerve terminals	End-plates	Electron microscopic features
1	30	Female	2	Biceps	Scattered small fibre atrophy	Nil	Scattered small angular fibres	Type 2 fibre atrophy	Multiple innervation of single fibres	Elongated dysplastic multiple. Some small and poorly developed	Loss of myofibrils. Areas of floccular change. Dilated sarcoplasm reticulum. Scattered Z-line material
2	26	Male	10	Deltoid	10 - 170 μm, marked variation in size. Several large round fibres. Increase in internal nuclei. Grouping of atrophic fibres	Lymphocytic about degenerating fibres. Increased fibrous tissue	Large groups of atrophic fibres and single fibre atrophy	Both fibre types involved	Not done	Not done	No EM
3	26	Female	5	Deltoid	Normal	Nil	Nil	Nil	Not done	Not done	No EM
4	38	Female	15	Deltoid	Grouped atrophy	Nil	Grouped atrophy	Both fibre types affected	No increase in branching seen	Most plates are small with few dense bodies	No EM
5	11	Male	½	Deltoid	Size 51 - 70 μm, scattered small fibres	Nil	Scattered small fibres	Type 2 fibre atrophy	Normal	17 - 34 μm, occasional elongated dysplastic plates	No EM
6	38	Male	2	Deltoid	Grouping of small fibres	Nil	Grouping of atrophic fibres	Both fibre types involved in the atrophic process	Excessive terminal branching TIR 1:30	Some hypoplastic plates, some large, others small and bulbous. Increased ultraterminal branching	Beading of plasma membrane. Increased glycogen in areas of regeneration. Loss of myofibrils
7	18	Female	4	Deltoid	Marked variation in size 12 - 90 μm, groups of small fibres	Nil	Groups of atrophic fibres	Both type 1 and 2 are involved in the atrophy	Multiple branching of terminal fibres TIR 1:27	Some large hypertrophic plates, others end in a bulb	Dilated sarcoplasm reticulum, loss of myofibrils, streaming of Z-lines, large areas of fibril destruction. Increased collagen formation with remnants of plasma membrane
8	55	Female	4	Deltoid	Normal	Nil	Nil	Nil	Not done	Not done	Loss of myofibrils, folding of plasma membranes
9	16	Male	1	Deltoid	Many scattered small fibres. 20 - 100 μm	Nil	Scattered small fibres	Type 2 fibre atrophy and fibre type grouping	Not done	Not done	Loss of myofibrils, streaming and loss of Z-lines
10	45	Female	28	Biceps	Variation in fibre size. Rows of central nuclei, groups of small round fibres	Lymphocytic about isolated atrophic cells	Groups of small cells with occasional atrophy of isolated cells	Both fibre types involved	Increased terminal branching TIR 1:35. Multiple innervation of single muscle fibres	Large dysplastic plates	Areas of loss of myofibrils. Increased glycogen and blurring of the Z-lines
11	39	Female	4	Deltoid	Marked variation in size 10 - 100 μm. Large round fibres with central nuclei. Hyaline change and fragmentation	Nil	Nil	The small fibres are of both fibre types and show excessive angulation	Not done	Not done	Large number of membranous bodies. Loss of myofibrils. Dilated sarcoplasma reticulum
12	45	Female	11	Deltoid	Size 40 - 55 μm. Normal	Nil	Nil	Nil	Increased terminal branching 1:35	Plate size 10 - 70 μm. Marked variation in form and size	Myofibril depletion. Excessive folding of plasma membrane. Streaming of Z-lines.

METHODS

Muscle tissue was removed from various sites under local anaesthesia without adrenaline. The muscle was removed by the usual technique, whereby the specimen is secured between two sutures, removed, and then maintained at resting length by tying the sutures over a wooden spatula. The specimen is then covered with gauze soaked in normal saline. At the same time further specimens were removed for electron microscopic study. These were held in clips to maintain the resting length, placed in gluteraldehyde and later embedded in aryldite or epon. Several muscle specimens were removed for motor end-plate and nerve terminal studies, and processed by employing a modified Ranvier method. The muscle for histochemistry was orientated on cork discs held in position by gum tragacanth. The specimens were frozen in isopentane, which had been previously cooled in liquid nitrogen. The sections were cut on a cryostat to a thickness of 10 μ and processed for NAD diaphorase, ATPase, myofibrillar and mitochondrial ATPase and phosphorylase. In addition, routine haematoxylin and eosin sections, a modified trichrome stain[7] and PAS stains were carried out.

RESULTS

Nine of the 12 cases studied show evidence of neurogenic atrophy when studied both histologically (Fig. 2) and histochemically. In 6 there was obvious grouping of fibre types of normal size (Figs 3 and 4) and groups of small muscle fibres which were seen histochemically or on histology, or both. One of these showed evidence of type 2 fibre atrophy (Fig. 5) (classification of Dubowitz and Pearce[8]). In 2 further cases there was evidence of type 2 fibre atrophy, and in 1 there were scattered atrophic and angular-looking cells (Fig. 6).

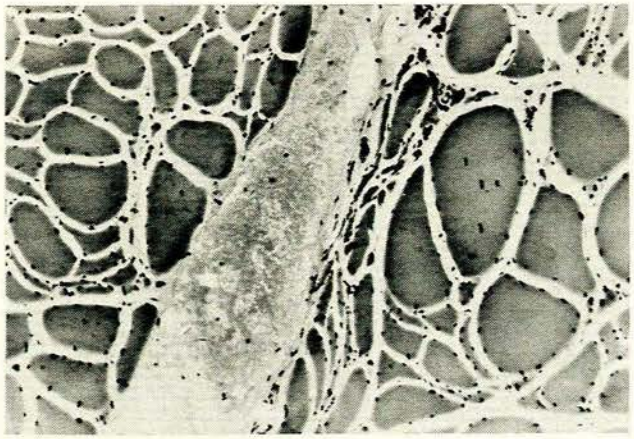

Fig. 2. Haematoxylin and eosin preparation showing grouped atrophy (× 360).

Nerve end-plate and motor nerve terminal studies were carried out in 7 of the 12 cases, and these revealed abnormalities in all the 7 cases, and provided evidence of

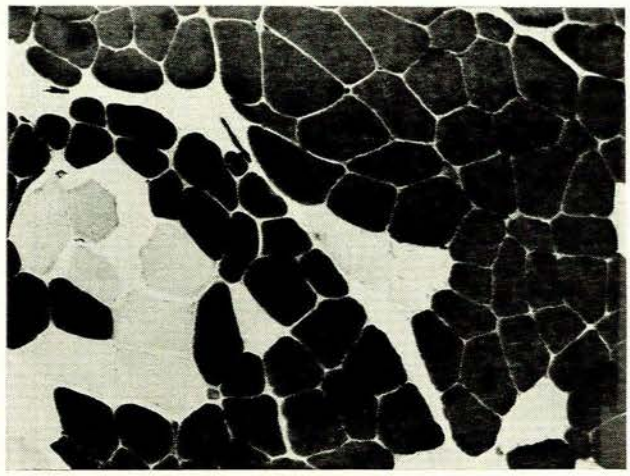

Fig. 3. ATPase preparation showing excessive grouping of type 2 fibres (× 80).

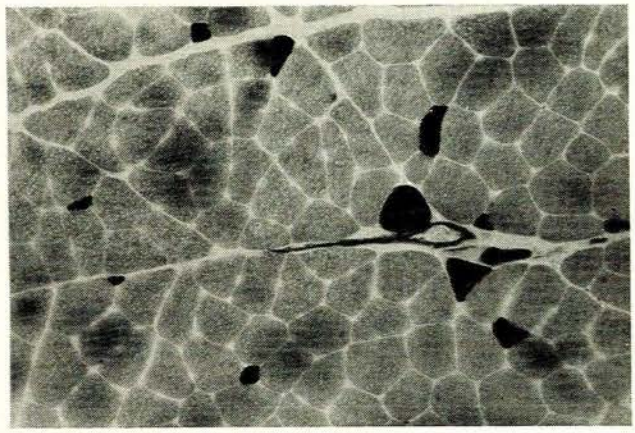

Fig. 4. ATPase preparation showing grouping of the type 1 fibres and angularity and smallness of the type 2 fibres (× 80).

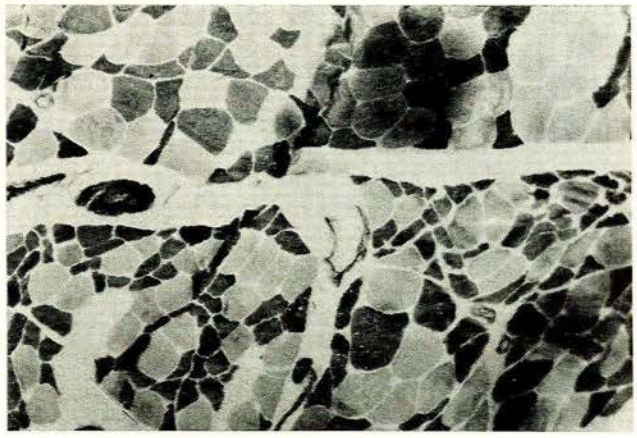

Fig. 5. ATPase preparation showing type 2 fibre atrophy (× 80).

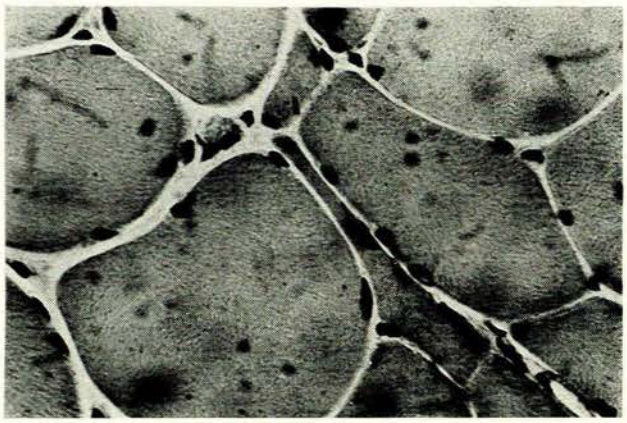

Fig. 6. ATPase preparation showing small atrophic fibres (× 100).

abnormality in 1 of the 3 cases considered to be normal on histology and histochemistry. The abnormalities consisted of excessive terminal branching with terminal innervation ratios (TIR) in excess of 1:25, which is taken as the upper limit of normal by Coërs *et al.*[9] The end-plates varied from large plates (Fig. 7), to long dysplastic-looking plates with immature branching, to very small plates containing few dense areas, to single bulbous endings on the muscle fibres. There was also evidence of increased ultraterminal branching extending from one end-plate to another, while in other areas multiple innervation of single muscle fibres was noted.

Fig. 7. A large lobulated end-plate (× 360).

Ultramicroscopic examination of the muscle was carried out in 8 of the 12 cases and was found to be abnormal in each case, the prominent abnormality consisting of blurring and streaming of the Z-lines (Fig. 8), loss of myofibrils, areas of complete disappearance of myofibrils with evidence of increased glycogen granules, areas which contained fragments of plasma membrane and large numbers of membranous bodies. In other areas

Fig. 8. Loss of fibrils and particularly the disappearance and blurring of the Z-lines (× 10 000).

there was evidence of swelling of the sarcoplasmic reticulum and increased deposition of collagen tissue (Figs 9-12). All these features are in keeping with denervation atrophy of the muscle.

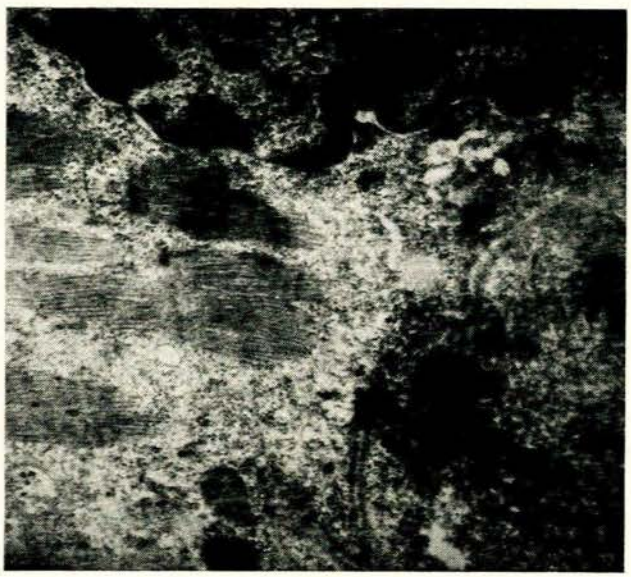

Fig. 9. Loss of myofibrils, glycogen deposition and scattered areas of plasma membrane (× 14 000).

stained for acetylcholinesterase showed poor concentrations of this substance. On an electrophysiological basis, Desmedt[14,15] suggested that the defect in myasthenia gravis occurred at a presynaptic level, and he was able to show that animals treated with hemicholinium developed a presynaptic defect similar to that of myasthenia gravis. Castello and Katz[16] calculated that acetylcholine is released in basic quanta (500 000 molecules), and Dahlbäck *et al.*[17] showed that there is a diminution in the rate of production of acetylcholine in myasthenia gravis. Elmqvist *et al.*[18] studied neuromuscular transmission in myasthenia gravis and showed that the end-plate potentials had a mean amplitude of 20% less than normal potentials, while the postsynaptic sensitivity to acetylcholine was found to be normal. They concluded that there is a deficiency in the amount of acetylcholine in the quanta released from the motor nerve terminals, thus indicating a primary pathology at the level of the motor nerve terminals. Defects in neuromuscular transmission, similar to those seen in myasthenia gravis, have also been reported in a number of varying conditions, including hyperthyroidism, lupus erythematosus, various malignancies, polymyositis, poliomyelitis, syringomyelia and amylotrophic lateral sclerosis.[6] Although the effect on the myoneural junction is a rare accompaniment of these disorders, it indicates that the end-plate region is vulnerable to a host of different pathologies.

As stated earlier, Steadl *et al.*[6] emphasised the presence of neuropathic lesions in myasthenia, and in 1963 Fenichel and Shy[19] studied muscle specimens from 37 patients with myasthenia gravis, and their observations were basically similar to those of Russel.[10] The abnormalities were classified into 3 categories — normal, small group lesions (collections of atrophic cells) and leucocytic infiltrations. Fifteen of their cases were normal, 11 showed small group lesions and 12 showed leucocytic infiltration. The white cells involved were dominantly lymphocytic and occurred largely around the small blood vessels, while degenerating fibres were frequently seen in the middle of or next to a lymphorrhage. The authors emphasised that the small group lesions should be considered as evidence of denervation, and further stressed that the group lesions indicated that either multiple terminal axones were involved or that the neurone itself was involved at a point proximal to the terminal sprouting.

In 1964 Brodie and Engel[20] reported a case of myasthenia gravis of long standing. This was the first published histochemical study, and this case provided evidence that advanced denervation may occur in the muscle of patients with long-standing myasthenia gravis. Fenichel[21] stressed the occurrence of both denervation and inflammatory myopathy, and supported the concept of auto-immunity as the basis for the disease as put forward by Simpson[22] in 1960.

Brownell *et al.*[23] presented 2 cases of myasthenia gravis, both showing severe neurogenic atrophy of the tongue. These studies revealed a remarkable degree of terminal proliferation of the axones supplying the tongue. Staining of the nerve bundles in the muscle revealed abnormal branching of the terminal axones with considerable elongation of the end-plates. Neurogenic changes were also found in muscles of the extremities, though involvement was slight. The brain and spinal cord were found to be normal, and sections of the median, femoral and popliteal nerves, sympathetic nerves and sympathetic ganglia were reported to be normal.

Oosterhuis and Bethlem[24] reported on the muscular pathology as found in 170 patients with myasthenia gravis. This series was analysed in various ways, but of the 10 biopsy specimens taken from atrophic muscles there was evidence of neurogenic changes in 8. One of the remaining 2 biopsy specimens showed lymphocytic infiltration only, and one showed type II fibre atrophy. No relationship with neurogenic change and age, sex, duration of disease, or with drug-resistant ophthalmoplegia could be established. In other biopsies they were able to show a correlation between the presence of thymoma and lymphocytic infiltrates, but no relationship could be established between thymoma and neurogenic changes. These authors also examined the spinal cord and peripheral nerves in 3 cases examined at autopsy, and no abnormalities were found. They concluded that neurogenic changes are found regularly in muscles of patients with myasthenia, and postulated that the denervation occurs at the myoneural junction as a result of a permanent absence of acetylcholine.

With the advent of the electron microscope, structural changes have frequently been demonstrated in the motor nerve endings, and Edwards[25] has shown consistent changes in the ultrastructure by way of an increased space between the pre- and postsynaptic membranes, and a general over-all reduction in the area of the postsynaptic membrane. Santa *et al.*[26] confirmed that the nerve terminal and postsynaptic areas were decreased, but found the mean synaptic vesicle diameter and vesicle count to be normal. Growing axon tips, immature-appearing end-plates and postsynaptic regions denuded of nerve terminals, were found. They suggest that the size of the acetylcholine quanta must be independent of the vesicle diameter, or the small miniature end-plate potentials in myasthenia gravis are not caused by the small size of quanta released. Whether the nerve terminal becomes abnormal because of an abnormal synapse or *vice versa* is still to be established.

By combining muscle histology with histochemistry, nerve terminal, end-plate and ultramicroscopy study, evidence of neuronal involvement was found to be present in 11 of our 12 cases. In the remaining case no electron microscopic or nerve terminal study was carried out. There were no specific features in the 12 biopsies which could be attributed to primary muscle fibre disease, and the existence of so-called 'myasthenic myositis' must be questioned. It is not good enough to regard the presence of lymphorrhages as an indication of myositis, since these collections of lymphocytes may occur in response to fibre death due to diverse causes. On the other hand, evidence is accumulating which points to a neurogenic basis for the muscle changes in myasthenia gravis. The isolated muscle nerve terminals and the motor end-plates have borne the brunt of the pathology. The grouped atrophy, on the other hand, seen histologically and especially with histochemistry, is highly suggestive of a lesion in

the more proximal site of the motor nerve, causing either deficient release of acetylcholine or faulty trophic function, or both.

The accepted immunological basis for the disease, the inadequate response to anticholinesterase therapy and the evidence of progressive denervation, prompted the use of steroids in most of these patients. The results of steroid and immunosuppressant therapy will be presented at a later stage.

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