Review article

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Neuromuscular disorders in childhood*

Old dogmas, new concepts

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In reviewing the advances in our understanding of neuromuscular disorders over the last 10 to 15 years, I should at the same time also like to pay tribute to some of the great clinicians of the last century for their foresight, and for laying the foundations of our present knowledge in this field.

Beginnings

I think it is logical for any discussion on neuromuscular disorders, particulary in childhood, to start with Duchenne, that rather eccentric physician from Boulogne who, though having no formal attachment or appointment to any of the Paris hospitals, spent the latter years of his life going around the various hospitals applying faradism to the muscles of patients with a wide range of chronic neurological disorders (Fig. 1). Not only was he the father of the application of electricity to medicine, both diagnostic and therapeutic, but he was also the inventor of a biopsy needle for muscle biopsy, the forerunner of all subsequent biopsy needles.

Muscular dystrophies. In the second edition of his famous book on the application of electricity to medicine, Duchenne (1861) described his first case of muscular dystrophy, 'paraplégie hypertrophique de l'enfance de cause cérébrale'. Because of the associated intellectual impairment of the child, he suggested that the condition might have a cerebral origin. However, in a series of further case reports in 1868 he drew attention to the striking prominence of the muscles in this condition and coined the term 'pseudohypertrophic'. This term was taken up by Gowers (1879) and remained firmly entrenched in the medical jargon until recently, but has now been dropped because we know that pseudohypertrophy may occur in many other forms of dystrophy and even in some of the neurogenic syndromes. We now prefer the eponymous title of Duchenne dystrophy for the childhood form of muscular dystrophy.

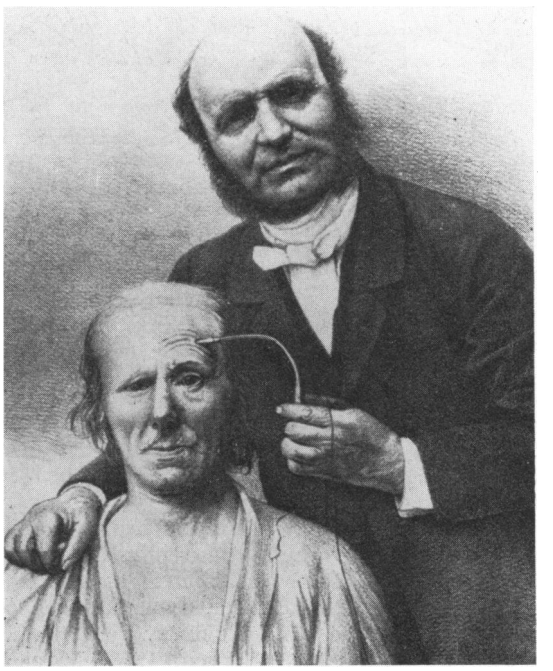

FIG. 1.—*Duchenne de Boulogne applying faradism to a patient.*

I suppose it is inevitable in any eponymous title for a disease that somebody is bound to have described it earlier. It is perhaps of particular interest that it was a London physician, Meryon, who some 10 years before Duchenne described in

*Based on the 1974 Philip Ellman lecture given before the Royal College of Physicians, London, 24 October 1974.

FIG. 2.—*The Queen of Punt (second from right) welcoming the Egyptians. From a relief on the Temple of Queen Hatshepsut (1500–1480* BC).

lucid detail a family in which 4 boys were affected with the condition (Meryon, 1852). He pointed out the striking pathological change within the muscle and in a necropsy on one of the cases also noted that the central nervous system appeared to be normal. He suggested that this was a primary disorder of muscle, possibly a fatty degeneration. One also gets a vivid impression of the clinical course of the disease from Meryon's writings. 'In May 1847, when nearly 9 years of age, he walked from Bruton Street to Westminster Bridge, but in November, 1848, he could neither walk nor stand, and in 1850 his arms were fast losing power.'

Following on the writings of Duchenne, various other forms of muscular dystrophy were soon described, based mainly on different distributions of the weakness. These included the facioscapulohumeral (Duchenne, 1872; Landouzy and Déjerine, 1884), the limb girdle (Leyden, 1876; Möbius, 1879), and the scapulohumeral (Erb, 1884) forms. It is unlikely, however, that muscular dystrophy suddenly appeared as a new disease in the middle of the last century, and it may well even date to antiquity (Pöch and Becker, 1955).

On the Temple in Egypt of the Queen Hatshepsut, who reigned between 1500 and 1480 BC, there is a relief showing the expedition to Punt which the Egyptians had undertaken during her reign. (Fig. 2). On this particular relief one sees the Queen of Punt (the lady to the right in the picture), together with various members of her family and her entourage, welcoming the visitors from Egypt. The striking appearance of this lady is immediately apparent and though the superficial appearances are of obesity rather than muscle pseudohypertrophy, she does also have a rather lordotic posture. It is perhaps of interest as well that the artist has chosen to depict nasolabial folds on her face, which are not present on any of the other individuals, suggesting perhaps that there was some abnormality of her facial appearance as well. The other thing of interest is her family. Her two sons behind her look quite normal in their posture, whereas the daughter also shows a somewhat lordotic posture. It thus appears that the Queen of Punt, back in the 15th century before Christ, may have been a case of muscular dystrophy, possibly of the facioscapulohumeral variety with a dominant inheritance.

Spinal muscular atrophies. In the 1890's Werdnig (1891, 1894) and Hoffmann (1893, 1897) gave a detailed description of another form of symmetrical muscle weakness in which the disorder lay in the anterior horn cells of the spinal cord. They described the severe form of spinal muscular atrophy occurring in infancy, which still carries their eponymous title 'Werdnig-Hoffmann' disease We now know that in addition to the most severe infantile form, with its onset in the first weeks of life or even *in utero*, and often leading to death within the first year, there are also milder forms of the condition in which the child has the ability to sit without support but is unable to take any weight on the legs; and a very mild form, the so-called Kugelberg-Welander syndrome, with ability to

walk, in which the muscle weakness is mild and confined to the proximal muscles of the lower limbs with little upper limb involvement. These children look very similar in presentation to muscular dystrophy. The diagnosis of this particular group of spinal muscular atrophies is important, particularly in the milder forms, because it carries overall a much better prognosis and tends to remain relatively static. Of interest in this disorder is the fact that whatever the age of onset it tends to come on abruptly in a child who may previously have shown no neuromuscular deficit at all, to reach its maximum involvement quickly, and then to remain relatively static over a long period. The inheritance of this form of disorder is usually autosomal recessive.

Application of new diagnostic techniques

Advances in techniques in relation to muscle biopsy have helped to open up the field of muscle pathology. Up to the 1950's routine histological methods involving formalin fixation and routine laboratory stains were able to distinguish the so-called 'myopathic' pattern of change in which there was variation in fibre size and associated degenerative changes within the muscle, details of which were already laid down by Erb (1884, 1891), and a neurogenic pattern in which one sees groups of uniformly atrophic fibres. This was as much as the routine methods were able to provide.

With the advent of enzyme histochemical techniques in the late 1950's and the application of these to the study of muscle, a new dimension was added to the interpretation of muscle pathology. In normal muscle it became obvious that in the human, as in animals, muscle is not a uniform tissue but is composed of a checkerboard of fibres with varying enzyme activity. With the enzyme reactions it became possible to define fibre types, which were arbitrarily called type 1 and type 2 on the basis of their histochemical enzyme activities (Dubowitz and Pearse, 1960a, b). It has subsequently been possible to draw correlations between these fibre types and the functional activity of the muscle, and also to make comparisons between corresponding animal and human muscle (Dubowitz and Brooke, 1973). Application of histochemical techniques and modern methods of rapidly freezing the muscle biopsy and cutting cryostat sections has not only eliminated a lot of the artefact of the older methods of fixation, but has also resulted in the recognition of a number of new disorders which are not recognizable at all by conventional stains. Electron microscopy has, in addition, helped to define some of the disorders associated with changes in the muscle at the subcellular level.

New myopathies. It is particularly the group of so-called congenital myopathies that has resulted from this advance in the application of modern techniques. These congenital myopathies tend to present in a nonspecific way, either as a floppy infant or at a later stage with muscle weakness, either localized and proximal, much like a muscular dystrophy or spinal atrophy, or more generalized. On the whole they are nonprogressive conditions. A number of entities have been defined, mainly on the basis of specific structural changes in the muscle, and have been given various romantic names like central core disease, nemaline myopathy (from the Greek *nema* meaning a thread), myotubular myopathy (because of the resemblance to myotubes of fetal muscle), mitochondrial myopathies, etc.

The pathology in a few of these can be illustrated to give an idea of the pattern of change in these conditions. The first example (Fig. 3) shows the characteristic pattern in central core disease in a 28-year-old woman with a mild, slowly progressive, proximal muscle weakness previously diagnosed as limb girdle muscular dystrophy. Routine stains revealed little change in the muscle, but histochemical methods showed two striking features. In the first place it was practically undifferentiated, the majority of fibres (99%) having a uniformly strong oxidative enzyme activity and low ATPase, thus conforming to type 1 fibres. Secondly, every type 1 fibre had a single, centrally-placed, central core in it.

Her 4-year-old son had minimal proximal weakness, almost subclinical in severity. His subsequent biopsy showed a normal distribution of fibre types, with the normal checkerboard pattern of light and dark staining fibres (Fig. 4), but a small proportion of the type 1 fibres did in fact show cores, which had a rather eccentric position, a sort of 'eccentric central core disease'. Since the initial description of central core disease additional variants have been noted, such as 'minicore disease', 'multicore disease', and at the electron microscopic level cores which have a normal structure with the appropriate A and I bands, the so-called 'structured cores', and those in which there is complete degeneration of the fibres in the core and loss of the band pattern, the so-called 'unstructured cores' (Brooke, 1973).

The next case, a 12-year-old boy, had a mild proximal muscle weakness, which was not a handicap to him until he tried an ascent of Snowdon and went into respiratory failure. On clinical

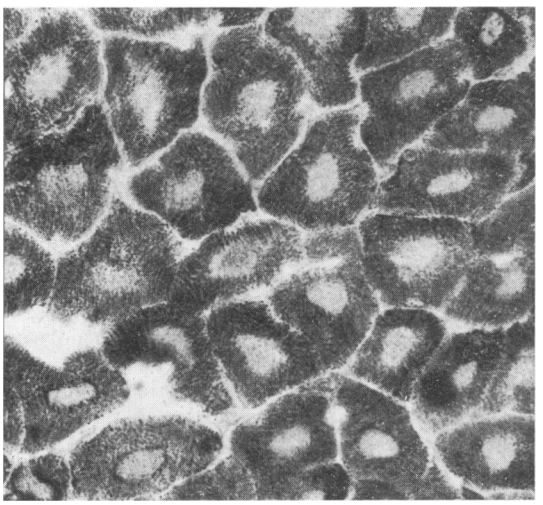

FIG. 3.—*Central core disease. Aged 28 years. Rectus femoris. Note uniformity of enzyme activity in fibres (type 1) and single central core in each fibre. (NADH-tetrazolium reductase. × 370).*

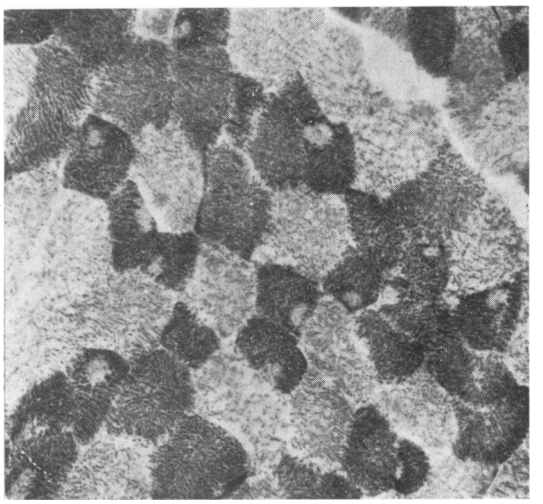

FIG. 4.—*Central core disease. Aged 4 years (son of patient in Fig. 3). Rectus femoris. Note checkerboard pattern of dark and light (type 1 and type 2) fibres, and eccentric cores in some of type 1 fibres. (NADH-tetrazolium reductase. × 370).*

grounds it was thought that the muscle problem might be a spinal muscular atrophy, and EMG also suggested a denervating condition. However, muscle biopsy showed unusual features. On routine haematoxylin and eosin stain there was no evidence of denervation or of a dystrophic process, but the striking feature was a marked variation in the size of muscle fibres. Some fibres were unusually large (more than 200 μm in diameter), while others were relatively small. With haematoxylin and eosin stain there was some suggestion of granularity in these fibres, and the trichrome stain revealed the characteristic red staining rods of rod body or nemaline myopathy, particularly in the small fibres (Fig. 5). Electron microscopy also showed these characteristic rods which have a dense appearance and are thought to be composed of a normal muscle protein and arise from the Z lines. We have subsequently considered that the respiratory problem may have been due to unusually severe diaphragmatic involvement, or central failure.

Another case that has recently presented us with a considerable diagnostic problem is that of a little girl who developed marked hypotonia and weakness of lower limbs, and subsequently of her trunk, from about 18 months of age, having developed normally to that time. Electromyography and nerve conduction velocities were completely normal and thus unhelpful in pinpointing any diagnosis. The muscle biopsy on routine staining showed a normal overall pattern, apart from the presence of some small degenerating fibres and a suggestion of granularity in some of the fibres. However, with the oxidative enzymes there was a striking picture of excessive activity and coarse granularity

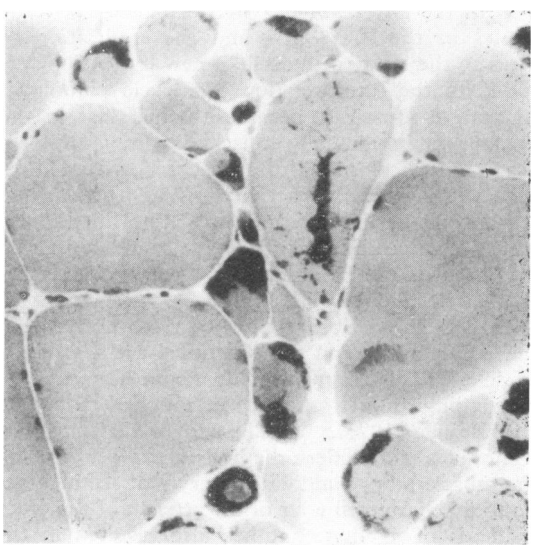

FIG. 5.—*Nemaline myopathy. Aged 12 years. Gastrocnemius. Note clusters of dark staining rods, particularly in the atrophic fibres. (Gomori trichrome. × 250.)*

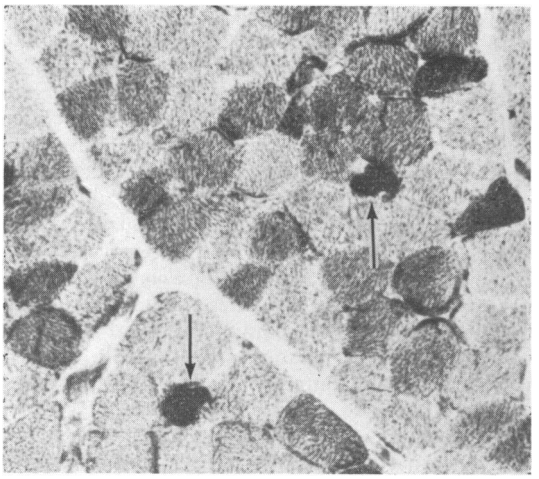

FIG. 6.—*Mitochondrial myopathy. Aged 2 years. Quadriceps. Note intensely reactive fibres (arrows). Remaining fibres appear normal. (NADH-tetrazolium reductase. × 250.)*

in some of the fibres (Fig. 6). This is characteristic of mitochondrial myopathies, subsequently confirmed on electron microscopy, showing the presence in isolated fibres of large numbers of mitochondria which were grossly enlarged in size and bizarre in appearance (Fig. 7). Mitochondrial myopathies are of particular interest since they tend to be associated with unusual syndromes of muscle weakness, such as oculopharyngeal. This is the first instance, I believe, of a child with a mitochondrial myopathy presenting in this particular way. This child subsequently had a steadily progressive course of weakness, with no apparent respiratory muscle involvement at all, until at Easter time last year she went into respiratory failure, which may have been associated with diaphragmatic weakness rather than intercostal.

It is tempting to think that in these mitochondrial myopathies there is a primary metabolic abnormality with secondary structural changes in the mitochondria and in this child we have been searching for a possible enzyme defect. Initially we had some possible clue to this from high lactate and pyruvate levels in her blood, but these subsequently settled to normal levels and may have been related to her state of inadequate nutrition before the observations.

Another congenital myopathy that I would particularly like to draw attention to is the so-called congenital fibre disproportion (Brooke, 1973; Dubowitz and Brooke, 1973). In this situation the muscle may look completely normal on routine

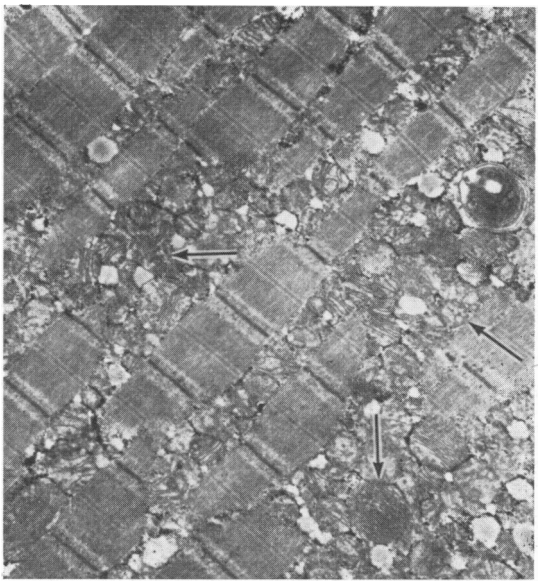

FIG. 7.—*Mitochondrial myopathy. Electron micrograph showing large clusters (arrows) of enlarged bizarre mitochondria between the myofibrils. × 7850.)*

histological stains, apart from some variation in the size of the fibres, but on histochemical preparations one sees a marked disproportion in size between type 1 and type 2 fibres (Fig. 8). The importance of recognizing this particular situation is that it may present exactly like a Werdnig-Hoffmann disease early on with severe weakness and

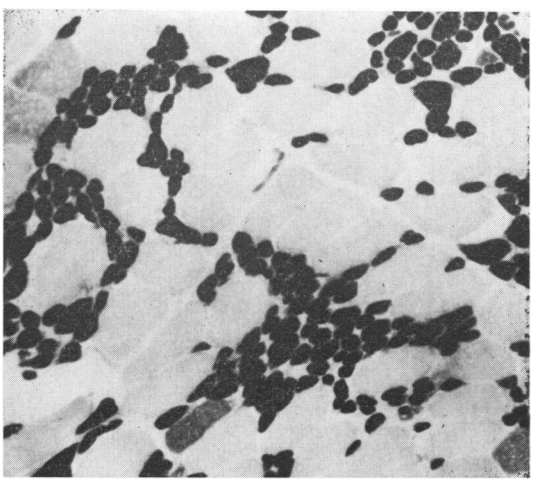

FIG. 8.—*Congenital fibre type disproportion. Note small dark-staining (type 1) fibres and larger weakly-staining (type 2) fibres. (ATPase (pH 4·6.) × 155.)*

hypotonia, but it does carry a good prognosis with a tendency to gradual improvement.

In summary, what has been gained from the modern techniques of investigation of neuromuscular problems is the recognition of a number of different disorders which can present in a clinical fashion identical to the dystrophies or the neurogenic syndromes, and which would not be diagnosed other than by histochemical and electron microscopic techniques. I think it is important for all children to have a muscle biopsy, even in the face of an apparently clear-cut clinical diagnosis of muscular dystrophy, or on the other hand in the face of a normal EMG and serum creatinine phosphokinase level. It is also essential that the biopsy, which can be done under local anaesthetic, be adequately investigated by modern techniques. It is probably unethical now to subject a child to a muscle biopsy and then to drop the specimen into a pot of formalin and essentially destroy it for any further meaningful investigation.

Duchenne muscular dystrophy—theories on aetiology. In spite of the many advances in relation to these new myopathies, many of the mysteries relative to Duchenne dystrophy still remain.

In recent years there has been extensive discussion on what is the most significant and the earliest lesion in relation to Duchenne muscular dystrophy. Some pathologists have had a particular fixation on the so-called hyaline fibres in Duchenne muscular dystrophy. These are fibres which are opaque and dark-staining on routine histological stains and they tend to be much more frequent in formalin fixed material than in frozen sections, though they are occasionally seen in frozen sections as illustrated in the section from a 6-year-old boy with Duchenne dystrophy (Fig. 9). With various stains these 'hyaline' fibres tend to show up more darkly and give the impression that they are not really hyaline in the ordinary pathological sense but of a more compact type fibre with a more intensive staining. A point of interest is that in cases of Duchenne dystrophy where one sees these fibres, they are not the only pathological change (Fig. 9), but there is already extensive change in other fibres, with variation in size, internal nuclei, proliferation of connective tissue, and proliferation of adipose tissue as well. Also of interest in these particular fibres is that they often have increased enzyme activity (Fig. 10).

Recently attempts have been made to try and identify pathological change in fetuses with potential Duchenne dystrophy. Toop and Emery (1974) have described such a fetus in a potential carrier of muscular dystrophy and thought that there were excessive numbers of large hyalinized fibres in the muscle of this particular fetus. It is still difficult at this stage in our knowledge, however, to try and define a pathological state of muscular dystrophy in a fetus purely on the basis of these so-called hyaline fibres, and extensive histochemical study of fetal muscle of equivalent age and also of potentially dystrophic muscle

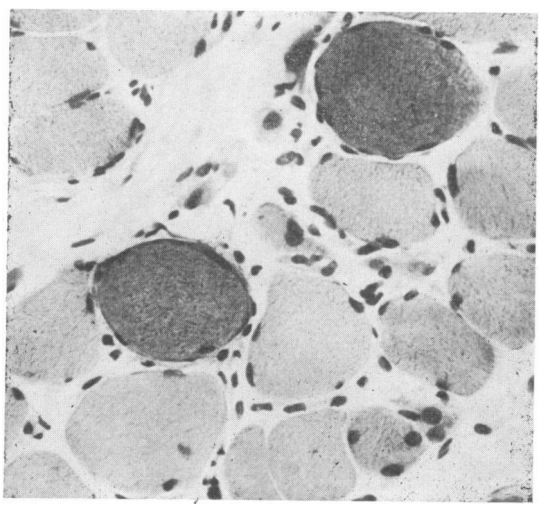

FIG. 9.—*Duchenne dystrophy. 'Opaque' or 'hyaline' fibres. (Haematoxylin and eosin. × 250.)*

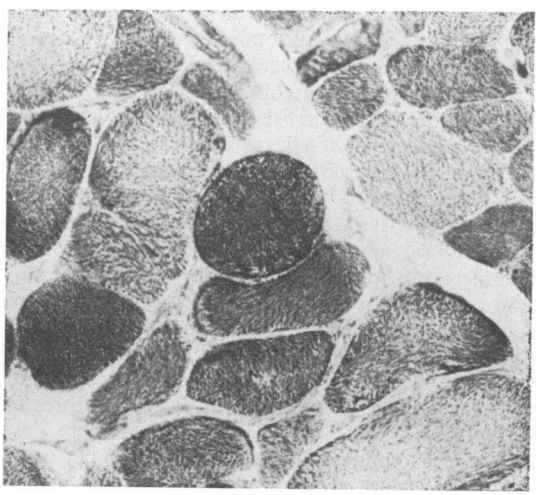

FIG. 10.—*Duchenne dystrophy. 'Opaque' fibres showing strong enzyme activity. (NADH-tetrazolium reductase. × 250.)*

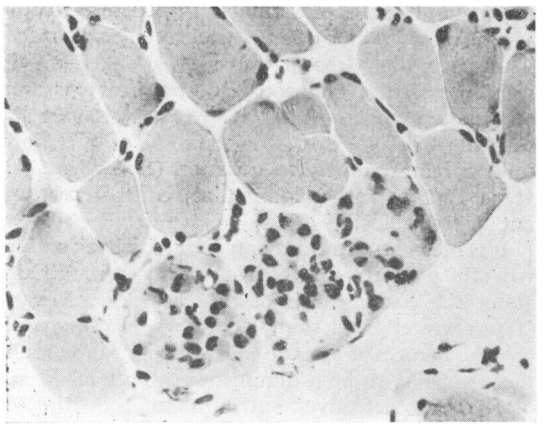

FIG. 11.—*Duchenne dystrophy. Necrotic fibres undergoing phagocytosis. (Haematoxylin and eosin. × 250.)*

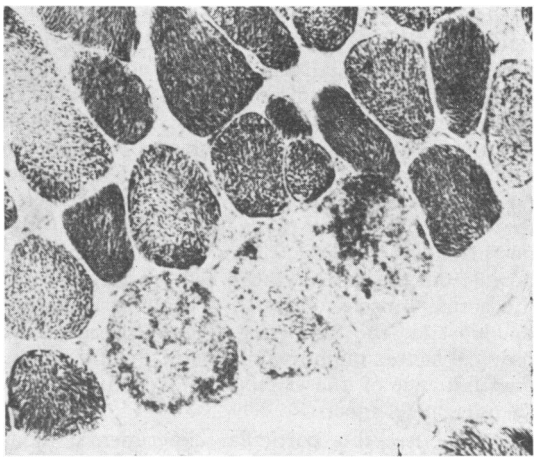

FIG. 12.—*Duchenne dystrophy. Serial section, showing loss of enzyme activity in necrotic fibres. (NADH-tetrazolium reductase. × 250.)*

may help to resolve whether this is in fact a variant of normal or not.

The other striking change seen in relation to dystrophy is the presence of necrotic fibres. These are different from the hyaline fibres in as much as they show a true liquefactive necrosis, and there is usually extensive phagocytosis of these particular fibres (Fig. 11). The enzymes in these fibres are usually diminished, that is the whole range of enzymes as well as the glycogen content (Fig. 12). These necrotic fibres have formed the basis of one of the theories of pathogenesis of muscular dystrophy, the vascular hypothesis. At the electron microscopy level one can pick up fibres which show degenerative change with complete loss of structure.

One other change that may be of significance in relation to pathogenesis of muscular dystrophy is the presence of regeneration within the muscle. We know that muscle has a tremendous potential for regeneration and in some of the acute syndromes of muscles, such as acute rhabdomyolysis and malignant hyperpyrexia, tremendous numbers of basophilic staining fibres are seen which show very high activity of RNA and are new myotubes forming and replacing the damaged muscle. It is possible that in muscular dystrophy these attempts at regeneration may be abortive and that the muscle may not be able to compensate for the degenerating process that is proceeding, whatever its cause may be. Regenerating fibres are seen particularly in early cases of Duchenne, but usually not later on, whereas in some of the milder forms of dystrophy, such as the Becker variety, more of these regenerating fibres may be seen.

Among the theories that have recently been put forward for the pathogenesis of muscular dystrophy is the so-called vascular hypothesis suggested by Engel and his colleagues (Hathaway, Engel and Zellweger, 1970; Mendell, Engel and Derrer, 1971). The basis for this theory is the presence of focal necrotic fibres, which they claim tend to occur in small clusters. They have been able to produce similar changes in experimental animals by drastic procedures such as ligation of the aorta in association with injection of 5-hydroxy-tryptamine and vasconstrictive substances, or by injection of fine pellets into the vessels, hence the vascular analogy. The other approach to a vascular abnormality was put forward a few years ago by Demos in Paris on the basis of abnormality of blood flow in the calf muscles of Duchenne dystrophy patients and also of carriers (Demos and Ecoiffier, 1957; Demos, Treumann and Schroeder, 1968). However, recent studies of blood flow in the muscle using isotopic studies have shown a normal pattern (Paulson, Engel and Gomez, 1974).

The other main hypothesis that has been put forward in recent years is the so-called neural hypothesis, which claims that muscular dystrophy may not in fact be a primary disorder of muscle but may be secondary to an abnormal neural influence. I have supported this view on the basis of some of the morphological changes observed in experimental situations and their possible relation to dystrophy (Dubowitz, 1967). I think part of the foundation of this neural hypothesis is the classical experiment of Buller, Eccles, and Eccles (1960) in which they showed that if you cross the nerves to fast and slow muscles in the cat, a slow

muscle such as the soleus, which normally has a slow twitch contraction, when innervated by the nerve from flexor hallucis, which is normally a fast contracting muscle, will become fast in its contractile properties. Conversely the flexor hallucis, normally a fast muscle, will become slow after reinnervation. In the control experiment of self-innervation no such change occurs. Buller *et al.* suggested that this might have a chemical basis and they postulated a chemical influence that might come down from the anterior horn cell via the axon into the actual muscle. Vrbová (1963) has suggested that possibly the frequency of impulses and other physical factors might be important in this controlling influence of the nerve, rather than simply a chemical mechanism.

If one uses this particular experimental model and studies the histochemical character of the muscles, one notes that the soleus, a slow muscle, has a uniform population of fibres with high oxidative enzyme content. On the other hand, the flexor hallucis which is the fast muscle, tends to have a large proportion of fibres low in oxidative content—so-called type 2 fibres—which would have a high ATPase content. If one then cross-innervates the muscle with the nerve to soleus, the flexor hallucis changes completely in its appearance and develops large areas in the muscle identical in appearance to what one normally sees in the soleus. So, under the influence of the soleus nerve one sees these large areas of muscle of predominantly type 1 fibres. This suggests that not only the contractile properties but also the histochemical and biochemical composition of the muscle is dependent on its innervation.

Also of interest in the cross-innervation experiment is that early in the state of regeneration or reinnervation of the muscle in the experimental animal a pattern is seen with variations in fibre size and histochemical pattern giving an impression somewhat similar to dystrophic muscle (Dubowitz, 1967). It is thus possible that what we look upon as dystrophic change in muscle could easily be an end result of some abnormal trophic influence in this way from the nervous system.

The neural hypothesis has been given a particular boost in recent years by the work of McComas and his colleagues, (McComas, Sica, and Campbell, 1971), who showed that by differential stimulation of the nerve to the extensor digitorum brevis one could show gradual increments in the response from the muscle, which he assumed reflected individual motor units being recruited. One could then calculate the number of units. In this way, McComas was able to show that there was fall-off in the number of motor units in muscular dystrophy as well as in various other neuromuscular disorders. This theory has recently been challenged on the grounds that the extensor digitorum brevis is not a reliable muscle for this type of analysis and also that the basis for the interpretation of the motor units might be open to question (Ballantyne and Hansen, 1974; Panayiotopoulos, Scarpalezos, and Papapetropoulos, 1974).

It is of interest in this context to look back and see what the physicians of the last century were thinking about the pathogenesis of the disorder. Meryon (1864) wrote, 'And such, I suspect, is the characteristic nature of the disease. From some cause there appears to be a diminished supply of arterial blood and defective nutrition in the diseased muscles, which are well nigh bloodless, just as is the case with the bones in rickets'. He thus appears to have been an early protagonist of the vascular theory.

Gowers (1879) reviewed the various possible pathogeneses of the condition, first of all invoking the sympathetic which was very much in vogue in those days, but subsequently turning to the possibility that it might be 'not an affection of the sympathetic but of "trophic nerves".' He even had the audacity to suggest that muscular dystrophy was possibly a primary disease of the muscular tissues, but was quick to defend the pathologists by adding that they were unlikely to accept such a suggestion since 'modern research has shown that almost every morbid state of the muscles, once thought to be primary is really due to disease of the nervous system'.

Some new approaches to old problems

Finally, I would like to discuss some of the ways in which the application of modern techniques and experimental models have perhaps helped to answer questions in relation to the pathogenesis of the dystrophic process. Animal models have, of course, always been of interest to the physician, and even Meryon (1852) made a bee-line for London Zoo. 'The ostrich is particularly obnoxius to this fatty degeneration of the voluntary muscles.' There are a number of animals which have been shown to have a genetically determined muscular dystrophy and these include the dystrophic mouse, the dystrophic hamster, and the dystrophic chick. Whether the disease is in fact the same as the human disease is still open to question, but they do at least provide some basis for studying some fundamental properties of behaviour of normal and diseased muscle. There are two particular experimental models that we

have been working on in the past few years; one is the behaviour of diseased muscle in tissue culture and the other the transplantation of muscle between normal and dystrophic animals.

Tissue culture. If human muscle is explanted it will proliferate and grow, much as fibroblasts do in culture. Initially there is a phase of mononucleate cells which undergo mitotic division and are indistinguishable at that stage from fibroblasts in their characteristics. However, they then start fusing, forming binucleate and multinucleate cells, and then elongate into myotubes, similar in appearance to those seen during normal fetal development. The nuclei in these myotubes initially are all centrally placed. One also sees the development of cross-striations in the muscle in isolation in culture, but these striations, though very similar in appearance to that of normal adult muscle, tend to be confined to one or other side of the myotubes rather than extending right across it. In all the various parameters we have been able to study, including growth, morphological characteristics, histochemical enzyme pattern, DNA and RNA metabolism, we have been unable to distinguish differences in behaviour of normal and diseased muscle in isolation (Bishop *et al.*, 1971; Gallup *et al.*, 1972a, b).

One additional technique developed in recent years by Peterson and Crain (1970) is that of combined culture of nerve with muscle. It is possible to make an explant of the embryonic spinal cord from the mouse and then to add a teased preparation of muscle fibres which can come from an adult animal. Under the influence of the spinal cord, these muscles then send out buds of new myoblasts which develop into myotubes in the same way as cultured muscle in isolation. Nerves grow out from the cord and make contact with the myotubes. One can trace neuromuscular contacts in this preparation, and also show these neuromuscular junctions with cholinesterase stains. Once these neuromuscular contacts are achieved, the striations within the muscle become much more mature than in isolated muscle and extend right across the myotubes and the nuclei migrate peripherally. In addition, synchronous contractions of these myotubes may be seen in culture. In a study of the normal and dystrophic mice by this technique, we obtained some interesting results (Gallup and Dubowitz, 1973). When combining the culture of embryonic spinal cord from a normal mouse with muscle from either a normal of a dystrophic animal, the result is a high proportion of functional neuromuscular contacts with frequent synchronous contraction of the myotubes and a high success rate of regenerating myotubes. On the other hand, when combining cord from the C57 variety of dystrophic mouse with either normal or dystrophic muscle, attempts at innervation are almost uniformly abortive, and in only a few cases were actual muscular contacts made and a few isolated asynchronous contractions observed. It thus appears that the spinal cord in the mouse has an important influence on the development of neuromuscular contact within the tissue culture and that dystrophic cord seems to have lost this ability to maintain functional innervation of the muscle. This suggests that in this particular variant of mouse dystrophy there is certainly a neural basis. Also, the tissue culture model transgresses any immunological problems in the culture situation and mouse spinal cord can thus be grown in association with human muscle explants (Witkowski and Dubowitz, 1975). With scanning electron microscopy the progression of these nerve outgrowths from the cord and the development of bipolar and multipolar neuronal cells (Fig. 13) can be vividly seen. We have been able to trace these nerves to their

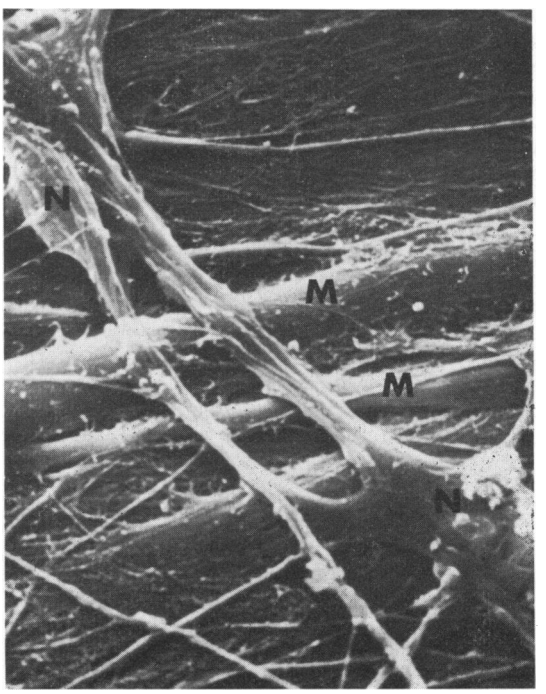

FIG. 13.—*Nerve outgrowths, with bipolar and multipolar neurons (N), from mouse spinal cord crossing over and making contact with myotubes (M) from human muscle explant. (Scanning electron microscopy. × 1885.)*

contacts with myotubes. We were hoping that by innervating human muscles from muscular dystrophy with mouse spinal cord we might be able to increase the maturation of the muscle beyond its situation in isolated culture, and perhaps bring out some of the abnormal features which have not been observed in any of the isolated muscle cultures. One particular combination which might give more information on this problem would be the culture of isolated human fetal cord together with either normal or dystrophic muscle from the human.

Muscle transplantation. The other model which has been of interest is the transplantation of muscle between the normal and dystrophic mouse. By the Studitsky technique (Studitsky, 1952; Carlson, 1972) a muscle removed from its bed and finely minced will regenerate into a functionally normal muscle again when replaced, as long as the nerve is left *in situ*. We have now completed an extensive series of studies on this particular model and followed the regenerative process from the first day to beyond 300 days (Neerunjun and Dubowitz, 1975a, b). We have noted in this experiment that if normal muscle is transplanted into a normal mouse, either the same mouse itself (autotransplant) or another normal mouse (homotransplant), after an intitial drop in weight of the transplanted muscle there is a regenerative process, and finally an innervation of muscle, and it then develops into a fully functional muscle again, achieving one-third to one-quarter of the bulk of the original muscle (Fig. 14). If dystrophic muscle from a dystrophic animal is transplanted into a normal animal (heterotransplant) the end result is a fairly reasonable muscle regenerate comparable to that in the normal, but slightly smaller. On the other hand, if either normal muscle or dystrophic muscle is transplanted to a dystrophic animal, the muscle gradually degenerates and disappears and there is no functional regeneration

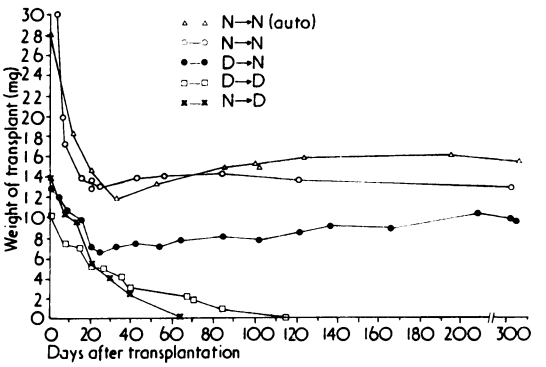

FIG. 14.—*The fate of normal (N) and dystrophic (D) transplants in normal and dystrophic hosts, respectively, is shown. ReJ 129 strain of mice.*

of this muscle at all in the long term (Neerunjun and Dubowtiz, 1975a, b). Early in transplantation, attempts at regeneration occur both in normal and dystrophic muscle in both normal and dystrophic hosts, but beyond 50 days there is a striking difference between the normal and dystrophic host (Table I). In first looking at autotransplants in the normal animal, there is a high degree of functional regeneration of the muscle between 50 and 100 days (8 out of 11), whereas in the dystrophic animal only 1 of 10 showed any residual muscle at that stage: from 101 to 200 days, 4 of 6 successes in the normals and none at all in the dystrophic autotransplanted situation. In the homotransplants a high success rate was noted at both times in the normal to normal, but in the dystrophic to dystrophic the success rate was low with no regenerates present at all beyond 50 days. Similarly, in the heterotransplant, transplanting dystrophic muscle from a dystrophic animal into a normal host gives a high incidence of positive successful regenerates, whereas in the normal muscle going into dystrophic host the majority of them completely degenerate with time and we have had no success beyond 100

TABLE I

Muscle transplantation long-term survival (129 ReJ mice)

Duration of transplant (d)	Type of transplant					
	Autotransplant		Homotransplant		Heterotransplant	
	N→N	D→D	N→N	D→D	D→N	N→D
51–100	8/11	1/10	4/4	0/4	8/11	2/7
101–200	4/6	0/4	5/6	0/4	5/7	0/4

N, normal; D, dystrophic.

days. The results suggest that it is the dystrophic host that is the key in this particular experiment, since both normal and dystrophic muscle regenerated in a similar way in the normal host, whereas in the dystrophic host neither the normal nor the dystrophic showed a long-term regenerative capacity. Furthermore beyond 200 days none of the dystrophic animals survived, so we were unable to assess the situation in them, but in the normal hosts beyond 200 days the position was exactly the same as in the earlier period. We have now followed some beyond 300 days and this has shown persistent regenerative capacity.

A further experiment in this context was to put a cap of silicone rubber onto the end of the nerve, thus leaving the nerve unable to reinnervate the muscle implant. With this procedure the position after 50 days and again after 100 days was exactly the same in the normal host as in the dystrophic (Table II). In other words, once the innervation

TABLE II

Muscle transplantation long-term survival (129 ReJ mice) + denervation

Duration of transplant (d)	Type of transplant			
	Homotransplant		Heterotransplant	
	N→N	D→D	D→N	N→D
51–100	0/7	0/4	1/9	0/6
101–200	0/4	0/3	0/5	0/3

was stopped in the regenerate, the normal animal was unable to sustain regeneration of the implanted muscle in the same way as the dystrophic animal was unable to do this even when the nerve was present.

Clinical applications? To return to Duchenne dystrophy, in spite of the advances in relation to some of the experimental situations, as far as Duchenne dystrophy goes we are still asking the question, 'Where is the lesion?' Could it be primarily in the muscles, could it be in the nervous system with an influence on the muscle, or are we dealing perhaps with a more general lesion affecting the nervous system as well as the neuromuscular, which would account also for some of the changes seen in Duchenne in relation to intellectual impairment, cardiac involvement, and other changes? We are also still asking the question, 'What is the lesion?' We do not yet know whether we are dealing with an enzyme defect which has not yet been pinpointed, or perhaps with some abnormality in a structural protein, or whether it might be a general defect in the cellular membrane. Recent work, particularly in relation to red cell membranes in cases of Duchenne, has shown certain abnormalities in the lipid composition (Kunze *et al.*, 1973) and also in the structural appearance of the membrane (Matheson and Howland, 1974),

We are in fact still groping in the dark, much as the newborn bird in the classic for 5-year-olds, *Are You My Mother?* (Eastman, 1962), setting out in search of a mother it had never seen. Hopefully some of the new pathways recently opened by application of new techniques may be pointing in the right direction.

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