THE MUSCLES IN CLUB FOOT—A HISTOLOGICAL, HISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDY

HYAM ISAACS, JOHN E. HANDELSMAN, MARGARET BADENHORST and AVONNE PICKERING, JOHANNESBURG, SOUTH AFRICA

From the Neuromuscular Research Laboratory, Department of Physiology and the Department of Orthopaedic Surgery, Medical School, University of the Witwatersrand, and the Transvaal Memorial Hospital for Children, Johannesburg

In talipes equino-varus the diminished bulk of the calf muscle suggests a neuromuscular defect. Accordingly, biopsies were taken from the postero-medial and peroneal muscle groups, and occasionally from abductor hallucis, in sixty patients mostly under the age of five years; 111 were studied histochemically and histologically, and a further fifty-three by electron-microscopy. Histochemical anomalies were revealed in ninety-two specimens; the muscle fibres in the other nineteen varied in size but were abnormal at the ultramicroscopic level, as were all specimens examined with the electron microscope. Evidence of neurogenic disease was seen in most instances and was more obvious in the older patients. The pattern of abnormality was similar in both muscle groups. It is thought that shortening of the postero-medial muscles may result from a small increase of fibrosis due to minor innervation changes occurring in intra-uterine life. There is evidence that immobilisation, stretching or relaxation of muscles does not account for the anomalies observed. This study of the extrinsic muscles in talipes equino-varus indicates a dominant neurogenic factor in its causation.

The causation of true congenital talipes equinovarus, in which the deformities cannot be passively corrected, is obscure. Primary bone dysplasia (Nichols 1897; Elmslie 1920), aberrations of tendon insertions (Flinchum 1953; Singer 1961) and abnormal intrauterine posture (Browne 1933) have all been accused but without any convincing evidence (Handelsman 1963). The bulk of the calf muscle, however, is consistently diminished in these patients (Irani and Sherman 1963), which suggests an underlying neuromuscular defect. Furthermore, the lower limbs of patients with severe club foot closely resemble those with arthrogryposis multiplex congenita (AMC). Rigid bilateral talipes equinovarus is a common deformity in this latter condition, and also in lumbo-sacral agenesis, where the calves have a similar appearance and the motor nerve supply is known to be anomalous or absent (Handelsman 1971, 1973). The occurrence of a deformity like club foot in other situations of neural imbalance, such as poliomyelitis and myelomeningocele, also suggests that a neuromuscular abnormality is an important factor in the causation of club foot.

Previous studies of muscle biopsies in cases of club foot using standard histological stains alone have been unrewarding. We therefore decided to extend the investigation by means of histochemical techniques and the electron microscope.

MATERIAL AND METHODS

Muscle was obtained from sixty patients undergoing operations to correct rigid talipes equinovarus. The sex distribution was equal and most of the patients were under five years of age. Histological and histochemical examination—Altogether 111 biopsies were examined. Sixty-three specimens were obtained from the shortened postero-medial group of muscles (Table 1). Most were from tibialis posterior and soleus but flexor hallucis longus, flexor digitorum longus and even abductor hallucis were sampled. The opposing peronei were examined on forty-eight occasions, no effort being made, however, to distinguish between longus and brevis.

At the time of operation the muscles were exposed above the malleolus and the biopsy specimen, about 5 millimetres in diameter and 10 to 15 millimetres in length, was secured at each end by two fine sutures. The muscle was maintained at resting length by fixing the sutures in slots at each end of a wooden spatula, then covered with a damp saline gauze.

Hyam Isaacs, M.D., F.R.C.P.(Ed.), Head, Neuromuscular Research Laboratory, Department of Physiology, Medical School, Johannesburg 2001, South Africa.
Professor John E. Handelsman, M.Ch.Orth., F.R.C.S., Department of Orthopedic Surgery, School of Medicine, Health Sciences Center, SUNY at Stony Brook, Stony Brook, N.Y. 11794, United States of America.
Margaret Badenhorst, Senior Technician, Department of Physiology, Medical School, Johannesburg 2001, South Africa.
Avonne Pickering, B.Sc., Technician

Requests for reprints should be addressed to Professor Handelsman.
After twenty minutes the muscle was orientated on pieces of cork and secured with gum tragacanth ready for both transverse and longitudinal sectioning. The tissue was then frozen in isopentane previously cooled to -160 degrees Celsius by liquid nitrogen. Sections were cut to a thickness of 10 micrometres on a cryostat at -25 degrees Celsius. These were stained with haematoxylin and eosin (H and E), a modified trichrome stain, and periodic acid Schiff (PAS) for glycogen. The histochemical study included NAD diaphorase, phosphorylase and ATPase at pH 9.4, 4.3 and 4.5 after treatment with EDTA.

**TABLE 1**

**Biopsies Obtained from Sixty Patients**

<table>
<thead>
<tr>
<th>For histology and histochemistry</th>
<th>For electron microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postero-medial muscles</td>
<td>64</td>
</tr>
<tr>
<td>Tibialis posticus</td>
<td>26</td>
</tr>
<tr>
<td>Soleus</td>
<td>15</td>
</tr>
<tr>
<td>Flexor digitorum longus</td>
<td>10</td>
</tr>
<tr>
<td>Flexor hallucis longus</td>
<td>8</td>
</tr>
<tr>
<td>Abductor hallucis</td>
<td>5</td>
</tr>
<tr>
<td>Peroneal muscles</td>
<td>47</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>111</strong></td>
</tr>
<tr>
<td></td>
<td><strong>53</strong></td>
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</tbody>
</table>

Fibre types were identified according to the classification of Dubowitz and Pearse (1960). Routine fibre type counts were made according to the method of Brooke and Kaiser (1970). Fibre smallness and hypertrophy were calculated according to the method of Brooke and Engel (1969). Grouping of fibres was considered to be present when twelve fibres of a specific type could be counted in a linear direction. Fibre size was measured on transverse sections stained with H and E. Occasional exceptionally large or small fibres were omitted, so that estimations were made on the basis of the presence of at least ten such large or small fibres per highpower field.

**Electron microscopic examination**—Fifty-three biopsies, twenty-two postero-medial and thirty-one peroneal, were taken at the same time as those for histochemical and histological examination (Table 1). The muscle was allowed to relax for five minutes and then fixed at its resting length in 5 per cent glutaraldehyde at 4 degrees Celsius, washed in phosphate buffer, post-fixed in 1 per cent osmic acid, dehydrated in alcohol and embedded in Araldite-Epon. Thin sections were cut on a Reichert ultramicrotome and stained with uranyl acetate and lead citrate. These were viewed on a Siemens's Elmiskop ultramicroscope.

**INTERPRETATION**

Histochemical stains are specific for the enzyme systems that differentiate Type 1 slow fibres, which have a mainly oxidative function, from the Type 2 fast fibres, which rely predominantly on phosphorylative activity. Bullett, Eccles and Eccles (1960) and Dubowitz (1967) have established that these metabolic differences in muscle are largely dependent upon the characteristics of its nerve supply. Using the cat, they crossed over the nerves to soleus and flexor hallucis longus, and by so doing converted an essentially slow twitch muscle to a muscle with fast twitch characteristics, and *vice versa*.

Differentiation between the essential skeletal muscle types has given insight into the changes that follow denervation of a particular motor-unit. After denervation the fibres either atrophy and remain so, or are reinnervated by axones of adjacent healthy units which sprout and penetrate the non-functional fibres. These muscle fibres then develop the characteristics of the invading axone. This produces an increase in the muscle fibre population of one type in the reinnervated area, and with histochemical staining the appearance of grouping of one fibre type becomes apparent. This change disrupts the normal checker-board pattern of human striated muscle of the limbs, where Type 1 and Type 2 fibres are usually present in a ratio of 1:2 (Fig. 1).

Histochemical stains also reveal atrophy or smallness and angularity of fibres of a particular type. There is evidence that these changes are also a function of nerve trophic activities (Karpati and Engel 1967; Guth 1968), which determine both fibre type and fibre size.

**RESULTS**

The common abnormal histological and histochemical findings in the present study are set out in Table II. Collections of very small fibres of both fibre types, often associated with marked angularity, were found in twelve of the biopsies (Fig. 2). Areas of gross underdevelopment or smallness of fibres of a particular fibre type were seen in thirty-two samples (Fig. 3), of which twenty-seven were of the Type 1 variety. An increase in the absolute number of Type 1 fibres was found in fifty-six samples (Fig. 4) and an increase in the absolute number of Type 2 fibres occurred in two instances. The direction of individual muscle fibres was found to be severely disturbed in thirty-five samples (Fig. 5). Target and targetoid fibres were a prominent feature in eleven specimens (Fig. 6). Grouping of individual fibre types was seen in twenty-six specimens (Fig. 1), all of which were obtained from twenty-four patients and were present more commonly in the older children. Non-specific changes, namely an increase in the number

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**TABLE II**

**Results of the Histochemical Study**

<table>
<thead>
<tr>
<th>Main pathological changes</th>
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<tbody>
<tr>
<td>Grouping of individual fibre types</td>
<td>26</td>
</tr>
<tr>
<td>Smallness and angularity of both fibre types</td>
<td>12</td>
</tr>
<tr>
<td>Type 1 fibre hypertrophy</td>
<td>27</td>
</tr>
<tr>
<td>Type 2 fibre hypertrophy</td>
<td>5</td>
</tr>
<tr>
<td>Profound loss of fibre direction</td>
<td>35</td>
</tr>
<tr>
<td>Presence of target and targetoid fibres</td>
<td>11</td>
</tr>
<tr>
<td>An absolute increase in Type 1 fibre population</td>
<td>56</td>
</tr>
<tr>
<td>An absolute increase in Type 2 fibre population</td>
<td>2</td>
</tr>
<tr>
<td>Scattered degeneration of individual fibre types, fragmentation, hyaline changes and an increase in the number of central nuclei</td>
<td>17</td>
</tr>
</tbody>
</table>
Grouping and an excess of Type 1 fibres. An area of much smaller fibres but with a normal fibre type distribution is evident in the middle of the preparation. ATPase preparation at pH 4.5 with prior treatment with EDTA. (× 80.)

Marked variation in fibre size with areas of small angular fibres. An increase in the number of central nuclei is also demonstrated. Haematoxylin and eosin. (× 80.)

Increased number of Type 1 fibres. ATPase preparations at pH 4.3. (× 80.)

Marked variation in the direction of the different fibre types. ATPase preparation at pH 4.6. (× 80.)
of centrally situated nuclei, fragmented and hyaline fibres, were seen in seventeen samples.

Muscle spindles, which were encountered in many of the sections, appeared to be normal. Blood vessels in the muscle and surrounding tissue were also normal. Fibrosis was not a feature in the sections studied. The most consistent deviation, present in almost every sample, was a significant variation in fibre size, seen either as groups or as scattered single, large or small fibres (Fig. 7). In nineteen samples an abnormal variation in fibre size was the only pathological change.

The general pattern of abnormalities seen in the peronei was similar to that of the postero-medial group. Ten of the nineteen specimens in which no major anomaly was found came from the peronei.

Each of the fifty-three specimens of muscle examined with the electron microscope showed significant anomalies (Table III). All contrasted sharply with the regular appearance of normal muscle. The commonest deviations consisted of loss of myofilaments (Fig. 8), abnormalities of mitochondria (Fig. 9), severe loss of filament direction (Fig. 10), and almost total loss of structure (Fig. 11). Excessive folding of the basement membrane with rows of degenerating fibres, vacuolation, distended sarcoplasmic reticulum, large nuclei with well-defined nucleoli, clumping of nuclei and Z-line abnormalities, were frequently seen.

Excessive glycogen deposition was not a common feature and very few sections revealed any increase in collagen fibres. Filamentous bodies, tubular aggregates,
myelin bodies, dilated transverse tubules, circular laminated bodies and phagocytes were found at random in many of the sections.

**DISCUSSION**

To date there have been no comprehensive studies on muscle tissue from patients with club feet. On the other hand, there have been several investigations of arthrogryposis multiplex congenita (AMC). We believe that club foot is a restricted form of AMC, and that the published studies of this condition are therefore relevant. From the clinical standpoint, the lower limbs of patients with talipes may be indistinguishable from the lower limbs of those with AMC.

**Arthrogryposis**—Otto (1841) first described arthrogryposis. Inherited forms have been described by Bargeton et al. (1961), Wynne-Davies (1964), Peña et al. (1968) and Lebenthal et al. (1970). Specialised techniques, however, were not used to study the muscle, and thus it is impossible to exclude congenital myopathies, such as central core disease, as causative factors in their patients.

Scarzella (1933) was the first to describe a neuropathic basis in foetal muscular dystrophy. In general, the classical findings consist of muscle atrophy, which is particularly noticeable in the distal part of the lower limbs and is associated with weakness, hypotonia and often inflexibility.
Although the respiratory muscles and those controlled by the cranial nerves tend to be normal, Brandt (1947) and Drachmann and Banker (1961) found evidence of loss of anterior horn cells in the spinal cord and reported involvement in the fifth, sixth and seventh cranial nerves. They postulated that the lesion occurred in the first trimester of pregnancy.

Further evidence of neuropathic involvement in AMC has been determined by electromyography and documented by Swinyard and Magora (1962) and by Amick, Johnson and Smith (1967). Electromyographic studies by Bharucha, Pandya and Dastur (1972) and by Dastur, Razzak and Bharucha (1972) revealed denervation activity in eight of their thirteen patients. In a later histochemical analysis of ten of this group, smallness of fibre size was found, and in three of these sections evidence of fibre type grouping occurred. All these findings suggest faulty innervation. Smith, Bender and Stover (1963) found electromyographic evidence of denervation in seventeen patients with AMC and claimed that the lesion was in the anterior horn cells.

Patients with non-progressive peripheral neuropathy associated with AMC have been recorded by Yuill and Lynch (1974). A neurogenic origin was established in four of the five patients with AMC described by Segawa et al. (1971).

The electron microscopic study confirmed that excessive collagenous tissue is not a feature of the extrinsic musculature in club foot, which suggests that degenerating muscle is actively removed by phagocytosis and that whatever fibrosis is present is a secondary and not a primary lesion. The loss of muscle filaments, the mitochondrial changes and the frequent total loss of structure are difficult to attribute to a specific pathology at the ultramicroscopic level. Certain features, however, in particular the clumping of smaller excessively folded nuclei, the abnormally folded basal membranes and the Z-line changes, suggest the presence of denervation.

This study has confirmed that the extrinsic muscles in club foot are grossly abnormal. It is noteworthy that the anomalies occurred in both the shortened postero-medial and the lengthened peroneal muscle groups, indicating that virtually all of the musculature between the knee and ankle is affected to a greater or lesser extent. Our patients are treated from soon after birth by gentle manipulation and serial plaster casts, which stretch the postero-medial muscles but relax the peronei. Because both muscle groups are similarly abnormal, the changes seen are not caused by the physical trauma of stretching. It has not yet been determined whether the muscles of the thigh are affected, but evidence in a few patients suggests that they are, though to a much lesser extent.

Further evidence incriminating denervation or faulty innervation as a major cause of peripheral deformity is available from the study of well-established neuromuscular disorders of a slowly progressive nature. In cases of peroneal muscular dystrophy the development of pes cavus, and occasionally of pes equino-varus, is well known. Similar deformities have been observed in Friedrich's ataxia, the Roussy-Levy syndrome (Isaacs 1958) and hypertrophic polyneuropathy (Isaacs 1960). We have recently followed such changes in a large family suffering from central core disease (Isaacs, Heffron and Badenhorst 1975) and in another with malignant hyperpyrexia associated with central core disease (Isaacs and Barlow 1974). Dubowitz and Sharrard (1968) have described a case of congenital club foot with central core disease in which the rectus femoris was normal, suggesting that the cores occurred only in the area of faulty innervation. The relationship between central cores, nemaline myopathy and target fibres (seen in eleven of our biopsies) is very close, and both signify faulty innervation (Afifi, Smith and Zellweger 1965).

The present evidence relating to nerve-muscle interaction infers a trophic as well as an excitatory action to the nerve (Karpatic and Engel 1967; Guth 1968). This trophic activity, as previously stated, is responsible for fibre type and fibre size. In the absence of adequate trophic growth factor the involved fibres remain small, though the fibre type distribution remains normal as seen in the central area of Figure 1. The effect of trophic type factor is seen when grouping of muscle fibres of the same type occurs, as illustrated in Figure 7 and on the right side of Figure 1. This disturbance occurs after denervation and subsequent reinnervation by the opposite fibre type.

We believe that a third trophic factor exists which controls fibre alignment and direction and which prevents the haphazard arrangement seen in Figure 5 and Figure 10, and also in conditions of established neuropathic origin. In thirty-five specimens there was profound loss of muscle fibre direction. In some areas this was so disturbed that effective contraction of muscle would be impossible. Although we have postulated that this lack of organisation into an effective unit is due to disturbed neurogenic control, faulty muscle feedback to the nerve may also be a factor.

Under normal circumstances there is very little difference in the size of the various fibre types in children; the mean diameters do not vary by more than 12 per cent (Dubowitz and Brooke 1973). A considerable difference in the size of muscle fibres was noted in all our specimens. In some samples there were small fibres adjacent to collections of fibres of a much larger size, whilst in others, areas of small fibres contained a scattering of fibres of a larger diameter. The overall picture was always abnormal in this respect, but in nineteen of the biopsies this extreme variation in size was the only anomaly present. We have commented that fibrous tissue was not significantly increased unless the muscle was close to the tendinous attachment. It is appreciated that slight increases are very difficult to determine, and for that reason we are at present studying the collagen and non-collagenous content of muscles of the postero-medial and peroneal groups, comparing them with each other and with more proximal muscles. We believe that tibialis posticus and soleus
bear the brunt of the neurological disturbance and that even minor fibrous tissue replacement in these muscles may result in major contracture or unstretchability, particularly when present before birth.

In most samples there were many muscle fibres which were much larger than normal. This feature, when taken together with the normal ATPase activity (Kurakami 1966), implies that disuse atrophy was not a factor.

The anomalies listed in Table II are evidence of neurogenic disturbance. A further seventeen specimens showed features which in the past had been regarded as myopathic. These are a marked variation in fibre size, increased numbers of central nuclei, rounded fibres and fibre fragmentation. Such changes, however, have also been found in neuropahties, particularly in spinal atrophy and in instances where interference with the blood supply has occurred (Mendell, Engel and Derrr 1971). These features are thus non-specific and may have a neurogenic basis.

In this series we were often able to obtain a clearer picture of the pattern of pathologic change by examining the clinically less involved peronei. It is noteworthy that the neuropathic changes were more obvious in the older children. This suggests that many of the very young patients with small but otherwise normal muscle fibres will reveal further neuropathic anomalies at a later stage as reinnervation from remaining neurones progresses.

A study of the extrinsic muscles of the foot in young baboons, in which one leg was splinted in a position of calcaneo-valgus for periods of eight, seventeen and twenty-five weeks respectively, has just been completed (Scher, Handelsman and Isaacs 1977). No abnormalities were found in the postero-medial or peroneal muscles of either the splinted or the control legs, both histochemically and with the electron microscope. These observations, in animals with muscles closely resembling those of humans, suggest that pre-operative stretching and immobilisation are not the cause of the changes reported here.

At this stage the evidence is in favour of an abnormal innervation as the prime factor in the development of this peripheral deformity. It is reasonable to presume the presence of a minor degree of muscle imbalance which may well produce a disproportionate deformity, particularly if the imbalance develops at an early intra-uterine stage.

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