## Spindle Induction and Differentiation in Murine Dystrophy

The etiology of mouse hereditary dystrophy remains an enigma. Although the muscle wasting is commonly attributed to an intrinsic abnormality of the skeletal muscle cell[1], other constituents of muscle, e.g., connective tissue and vasculature, have been implicated[2,3], and a growing literature ascribes a pathogenic role to the innervation[4,5]. The muscle sensory aspects of the disorder have received little attention[6,7] despite the relatively early onset of abnormal hindleg reflexes and function. In the present study, populations of neuromuscular spindles, as well as morphologic and histochemical characteristics of the individual receptors, were evaluated in an effort to disclose peripheral sensory factors associated with the disorder.

*Materials and methods.* These studies were conducted on Bar Harbor dystrophic mice (129/B6F$_1$ dydy), their unaffected littermates, and normal mice (129/B6F$_1$ Dy-Dy). The dystrophic and control mice were allowed free access to water and Old Guilfords Feed, the latter supplemented with oats and cod liver oil to prolong survival time of the affected animals[8]. Counts of spindles and their

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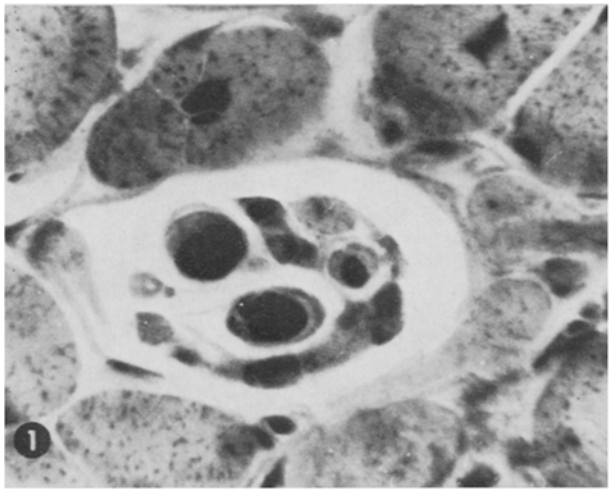


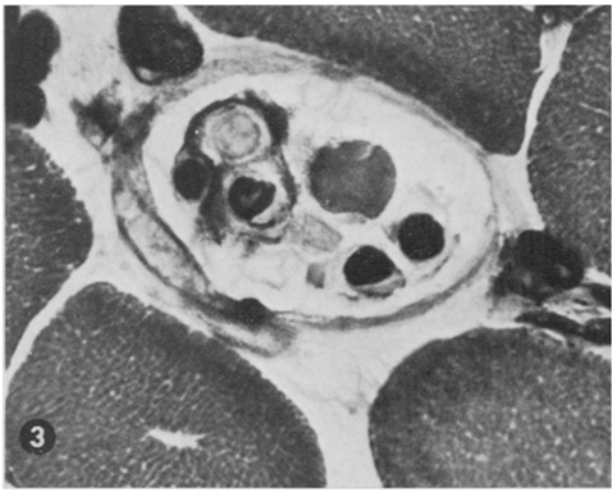


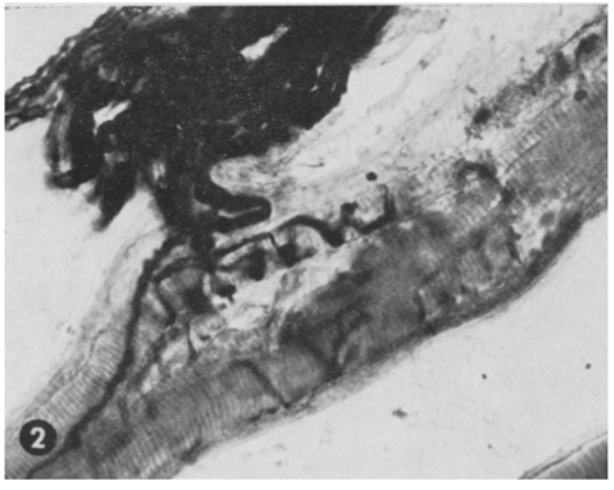


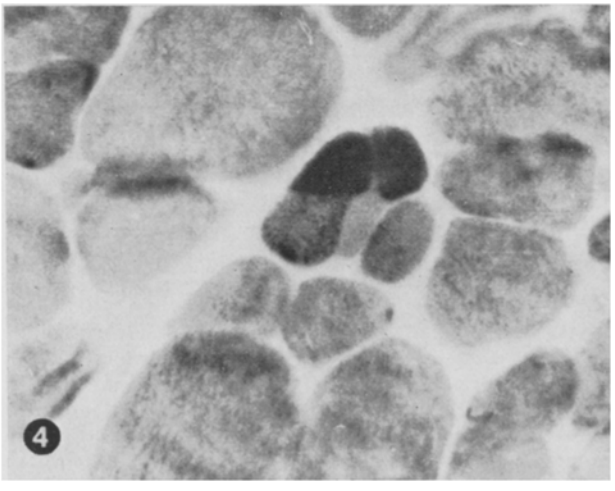


Fig. 1–4. Medial gastrocnemius muscles of dystrophic mice.

Fig. 1. A transverse section at the spindle equatorial region, revealing 2 large nuclear-bag and 2 small nuclear-chain intrafusal fibres within the axial tissue of the encapsulated receptor. (Trichrome stain of fresh frozen section.) ×900.

Fig. 2. A silver stained, teased preparation illustrating the intact spiral and branched innervation of the striated juxta-equatorial region of large and small intrafusal fibres. ×600.

Fig. 3. The myofibrillar ATPase reaction resulting in intense staining of the small intrafusal fibres, and light and moderate staining of the 2 large intrafusal fibres. ×900.

Fig. 4. Incubation for phosphorylase revealing 2 highly reactive and 3 less reactive intrafusal fibres in the polar region of a spindle. The surrounding extrafusal musculature is atypically unreactive as a result of the disorder. ×600.

included intrafusal fibres were made in paraffin embedded, serially sectioned (10 μm thickness), hematoxylin and eosin stained preparations of the left soleus and medial gastrocnemius muscles of 5 dystrophic and 4 control animals (all 4 months or older). The controls included 2 normal animals and 2 unaffected littermates of overtly dystrophic mice.

*Results.* The dystrophic muscles contained a virtually normal complement of spindles (Table). The latter were similar to those of control muscles with respect to receptor diamter, capsule thickness, and axial tissue content (Figure 1). The usual mean population of 4 intrafusal fibres per receptor was observed in spindles of 3 of the 5 dystrophic gastrocnemius and 4 of the 5 dystrophic soleus muscles. In those instances in which there were fewer intrafusal fibres per spindle (mean 3.5 to 3.8), the deficit was most evident in the subaponeurotic receptors. The latter normally exhibit greater variability in appearance and have a greater susceptibility to distortion by the histological procedure, particularly in the fibrotic dystrophic muscles. Although the mean diameters of nuclear-bag (10 μm) and nuclear-chain fibres (6.5 μm) of control and dystrophic muscles were similar, the range of the latter was slightly narrower.

The silver-impregnated teased[9] spindles of control and dystrophic muscles exhibited similar complex sensory and fusimotor innervations (Figure 2). Tendon organs were also comparably innervated in control and dystrophic muscles.

Histochemical reactions for succinic dehydrogenase[10], phosphorylase[11], and myofibrillar adenosine triphosphatase[12] revealed a characteristic pattern of 3 intrafusal fibre-types per spindle[13] in the overwhelming majority of receptors of both control and dystrophic mice (Figures 3 and 4). With rare exception, this pattern was retained in the spindles of young (2 week) and older (> 8 weeks) dystrophic animals.

*Discussion.* The presence of normal numbers of spindles in the muscles of chronically dystrophic mice, and the fact that most of the receptors are well differentiated, suggest that induction, differentiation, and maintenance of these receptors are relatively unaffected by the primary aspect(s) of the disorder.

*Résumé.* La présence d'un nombre normal de fuseaux neuromusculaires dans les muscles de souris distrophiques chroniques et le fait que la plupart des récepteurs sont bien différenciés suggèrent que l'induction, la différenciation et le maintien de ces récepteurs ne sont pratiquement pas atteints par le dérèglement.

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Spindles per muscle

| Soleus | | Medial gastrocnemius | |
|---|---|---|---|
| Control | Dystrophic | Control | Dystrophic |
| 11 | 12 | 12 | 10 |
| 10 | 10 | 12 | 9 |
| 12 | 11 | 10 | 12 |
| 10 | 11 | 12 | 10 |
| — | 11 | — | 13 |
| 10.8 | 11.0 mean | 11.5 | 10.8 |

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## Ultrastructural Evidence for the Localization of an Indolealkylamine in Supra-Ependymal Nerves from Combined Cytochemistry and Pharmacology[1]

When the cerebral ventricles of the rat are examined by electron microscopy varicoses nerve fibres can be observed just above the ependyma[2–7]. Recent fine structural investigations have characterized supra-ependymal nerves in certain brain regions as monoaminergic and in correlation an amine-specific formaldehyde-induced fluorescence could be demonstrated above the ependyma of these regions[8,9]. Moreover, the colour of the fluorescence and its reaction to drugs interfering with the synthesis, storage and/or metabolism of monoamines lead to the conclusion that the amine is an indolealkylamine, most probably 5-hydroxytryptamine (5-HT).

A more sensitive and specific cytochemical method, based on the chromaffin reaction, for the ultrastructural localization of biogenic monoamines has recently been developed in our laboratory[10] enabling the precise identification of endogenous amines in certain brain regions notably the above mentioned nerves on the ventricular surface[11]. In the present study the influence on amine localization of drugs affecting their synthesis or storage has been investigated by electron microscopy.

Male albino outbred rats of Wistar origin weighing 180–200 g were used for all experiments. Control animals and those given reserpine (Serpasil®, 10 mg/kg i.p. 18 h before sacrifice), α-methyl-*para*-tyrosine methylester

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