The Muscle Spindle in Mice with Hereditary Neuromuscular Diseases

Hereditary neurological and neuromuscular mutants of mice are promising materials for biological investigations because of their potential analogies to hereditary neuromuscular disorders of man. In our continuing studies of hereditary neuromuscular mutants we have become aware of an almost complete lack of information on the normal and abnormal occurrence, frequency, and types of muscle spindles in mice. Therefore, we have studied the behavior of the spindle in several hundred serial sections each from normal and mutant mice suffering from various muscular and neuromuscular diseases.

Materials and methods. To best evaluate muscle spindles in both normal and pathological material, sectioning was at a point between the equatorial region and the end of the lymph space or spindle pole. Most of the spindles studied were located in the musculi psoas, iliopsoas, semitendinosus and gastrocnemius. After fixation in 10% neutral buffered formalin and embedding in paraffin, transverse sections were cut at 8 μ and stained with hematoxylin and eosin. Periodic acid-Schiff-alcian blue, Luxol, Palmgren, and pyridine silver nitrate. In selected instances, phosphotungstic acid hematoxylin and van Gieson stains were used. We examined the musculature from shambling (gene symbol, shm)[1,2], ducky (du)[3], teetering (tn)[4], spastic (spa)[5], lethargic (lh)[6,7], disoriented (Do)[8], rabbit (rb)[8], and dystrophic (dy)[9–11] mice and compared it with that of the respective clinically normal homozygous or heterozygous littermates (+/+ or *m*/+). Each mutation (m) is being propagated within an inbred line or by repeated crosses to a standard inbred strain, so that 2 types of mice are available for comparison: m/m, the homozygous mutant, and m/+ or +/+, the homozygous or heterozygous normal controls.

Results and discussion. Atrophy of some muscle fibers and hypertrophy of others occur in shambling beginning at about 2 months of age. This denervation dystrophy, at least partially of 'motor' origin, is bilaterally symmetrical and becomes more severe the older the mice. Pathological changes include fiber vacuolization, central rowing of nuclei and fiber necrosis. We observed the largest diameters of intrafusal fibers in shambling and control mice, but no abnormalities were detected in mutant mice. Changes in the skeletal musculature of spastic mice are minor and consist of rare occurrences of nuclear rowing. No abnormalities were found in muscle spindles. In teetering, the muscle bundles of limbs and trunk are markedly smaller than in the controls. Muscle atrophy or underdevelopment is normally uniform affecting all fiber types; there is close packing of fibers within bundles. Occasionally there are fibers with centrally located nuclei and nuclear rowing; also there is atrophy of fiber bundles. Similarly, in ducky, the skeletal musculature of limbs and trunk are markedly but uniformly smaller than in the controls. In both mutant syndromes the muscle spindles were decreased in size in proportion to the extrafusal fibers. There are no qualitative differences between the mutants and controls.

The sciatic nerve may be smaller in the rabbit mutants compared with their controls, and there may be a selective type I fiber atrophy as determined enzyme-histochemically. In some muscle spindles, the intrafusal fibers are atrophied; in others there is discrete thickening of the capsule and widening of the lymph space. Similarly, in lethargic, there is an occasional spindle with a discretely thickened capsule.

The muscle spindles of dystrophic mice are entirely normal. Severe lesions occur, however, in the extrafusal fibers. Essentially, the histopathological findings consist in whole or part, of coagulation necrosis, regenerative activity, variation in fiber size, and internal rowing of

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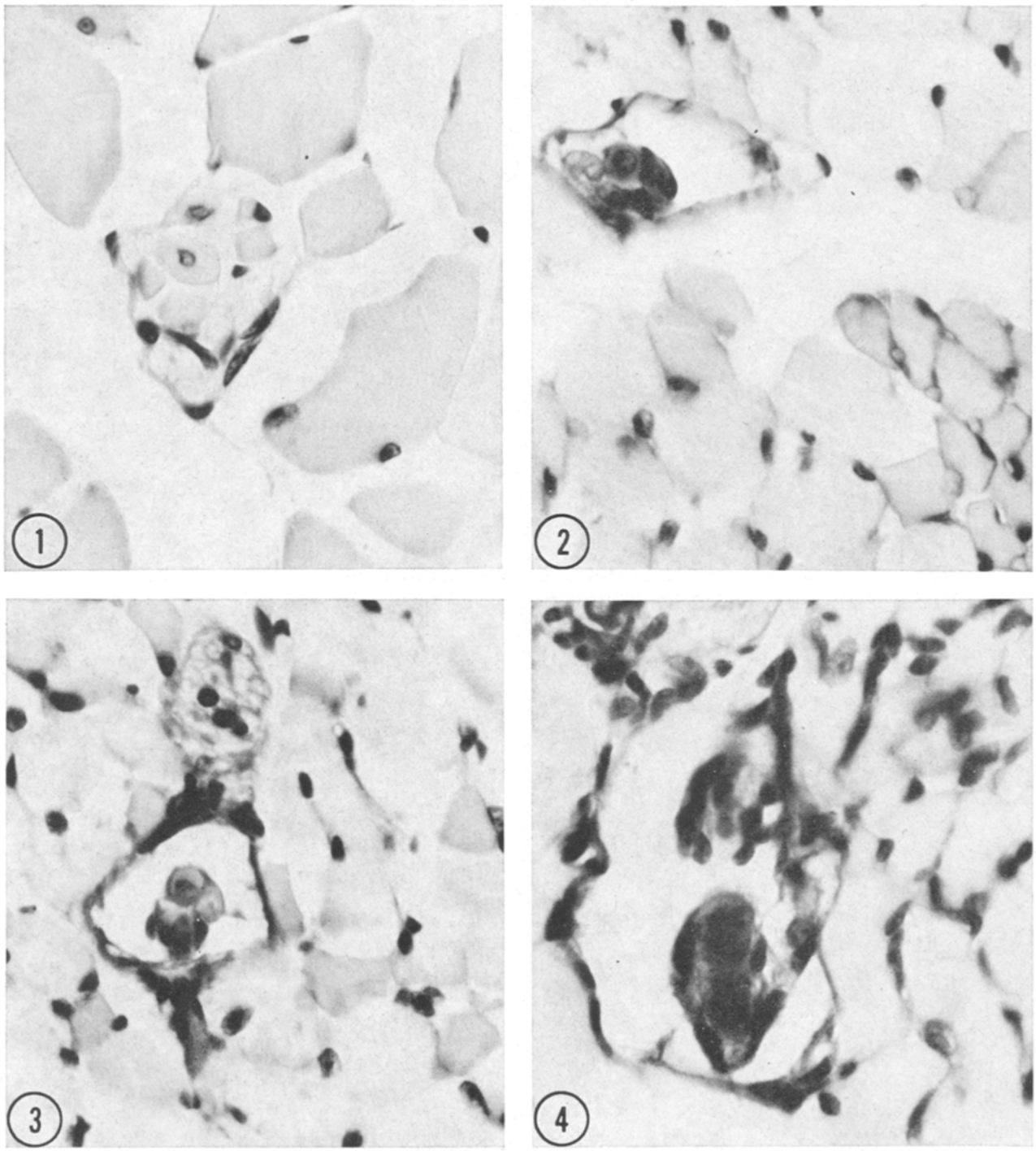
Findings in muscle spindles in normal and mutant mice

Genotype	Age (days)	Approximate disease state (days)	Muscle	Spindle diameter (μ)	Number intrafusal fibers	Number nuclear bag fibers	Diameter intrafusal fibers (μ)	Diameter nuclear bag fibers (μ)	Capsule
shm	60	30	psoas, iliopsoas	40–50	7		7–12		
+	60	–		40–50	7–8		8–13		
du	24	12	semitendinosus	40–50					
+	24	–		45–65					
tn	33	7	psoas, iliopsoas	25–40	3		6	7–11	
+	33	–		35–55	5		6–8	8–12	
spa	26	12	psoas, iliopsoas	30–40	5	3	5–6		
+	26	–		35–50	7	3	5–8		
lh	19	5	semitendinosus	50–70	3		8		discretely
+	19	–		50–70	3–5		6–9		thickened
Do	17	3	semitendinosus	30–50	5–6	3	4–5		
+	17	–		30–50	5–6	3	4–5		
rb	66	50	semitendinosus	55–75	3–5	3	4–6		thickened
+	66	–		60–80	4–6	3–5	4–8		
dy	14	preclinical	semitendinosus	25–45	5		3–5		
+	14			30–50	5–7		3–5		

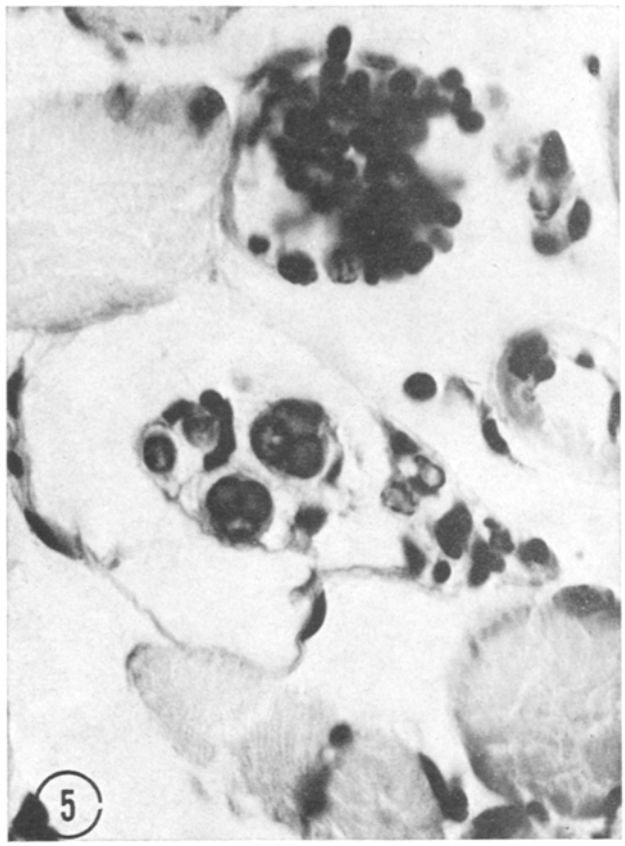
nuclei. These changes increase in severity with age. We have looked for abnormalities in muscle spindles from advanced cases of muscular dystrophy in mice over 2 months. It was not possible to count the number of intrafusal fibers, and the polar region of the spindles was not readily distinguishable from the surrounding fibrous tissue.

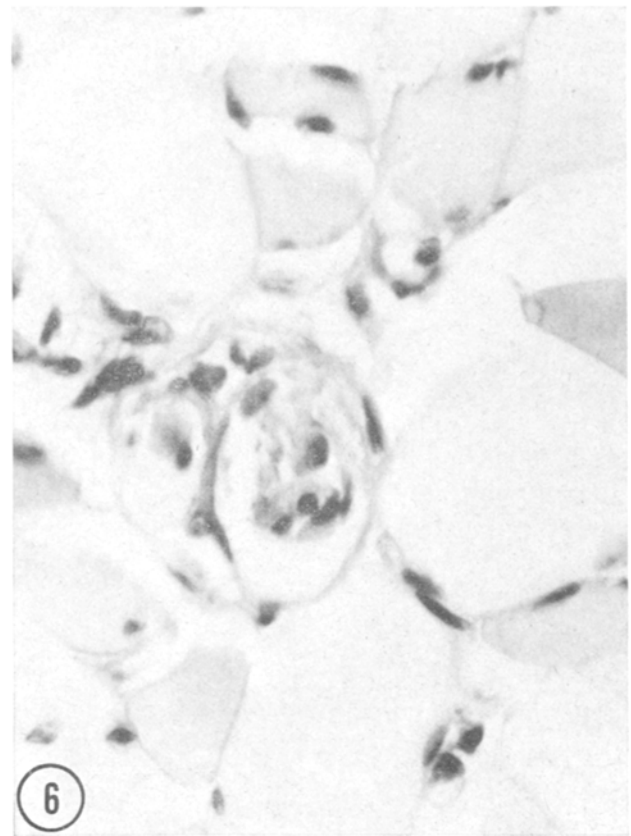
Certain of the morphological characteristics of normal and abnormal muscle spindles are depicted in the Figures 1 to 8. The Table presents data on spindles in mutants and their respective controls at different ages.

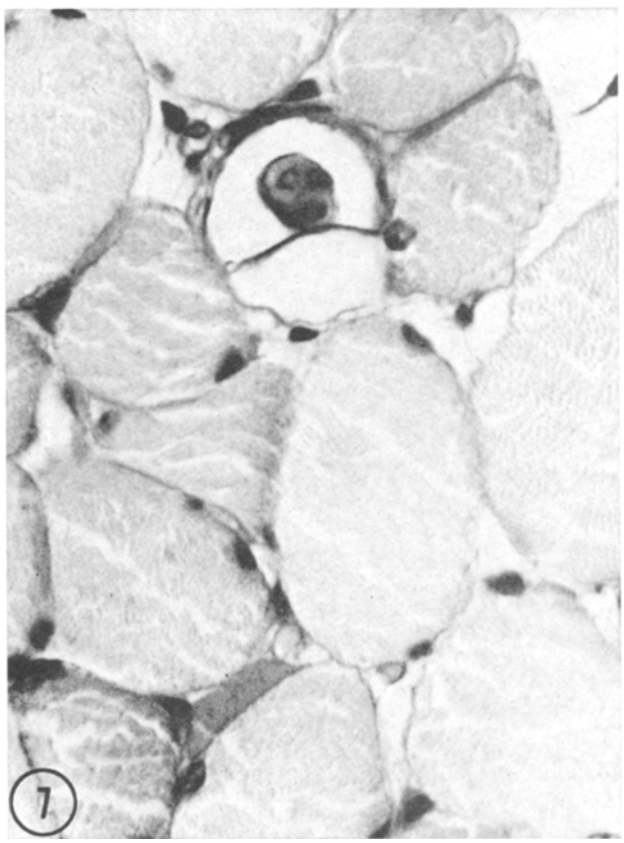
From our studies we cannot absolutely establish the extent to which the overall outer diameter of the spindles varies. It seems, however, that in mice the spindles are larger in the musculi gastrocnemii and semitendinosi than psoas and iliopsoas. This size difference is independent of the age of the mice. Generally, the nuclear chain fibers are smaller than the nuclear bag fibers. We have observed

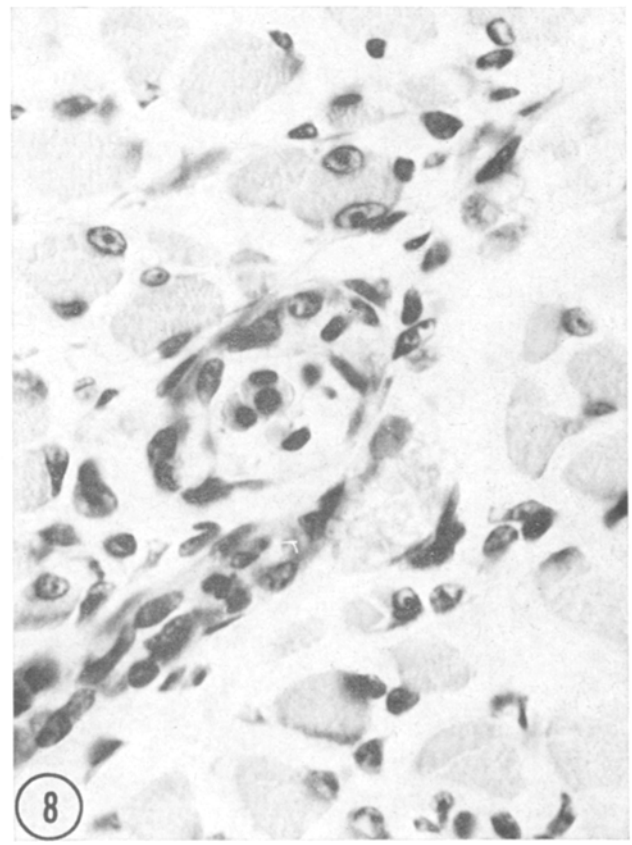


Figs. 1–4. Transverse sections through muscle spindles from shambling (1), teetering (2), spastic (3), and lethargic (4) stained by PAS-Alcian blue, × 900. M. semitendinosus (4) and psoas[1–3]. The constituent extrafusal fibers from the 2 muscles are not uniform in size and have different chemical-physiological characteristics. In teetering (2) selective atrophy of fibers in a muscle bundle may occur, but atrophy and underdevelopment is mostly uniform.









Figs. 5–8. Muscle spindles in M. semitendinosus of rabbit[5–7] and muscular dystrophy[8]. PAS-Alcian blue[7,8] and Luxol fast blue[5,6], × 900. Multiloculated muscle spindle with discretely thickened capsule and atrophy of intrafusal fibers[6]. Equatorial section showing 2 nuclear bag fibers[5].

only minor abnormalities in spindles from pathological materials. Apparently, they are 'disease-resistant', become involved late in disease so that they were not yet present in our material, or in fact never develop. Similar observations have been made in man and other animals[12]. For example, observations on experimental division of the sciatic nerve in dogs cats, monkeys lead to the conclusion that (1) intrafusal muscle fibers may survive independent of a nerve supply from both afferent and efferent fibers for at least 6 months and that (2) atrophic changes in extrafusal fibers are greater than in muscle spindles, although in a number of human muscular and neuromuscular diseases including denervation atrophies, progressive muscular dystrophy, polymyositis, etc., the degree and extent of spindle lesions is related to the stage of the disease[12]. Since the spindle is unaffected by nerve section, the muscle spindle is able to guide the regenerating nerve back to it[13–15].

Zusammenfassung. Der Aussendiameter der Muskelspindeln variiert in Mäusen von Muskel zu Muskel. Der Grössenunterschied ist altersunabhängig. In Mäusen mit vererblichen neuromuskulären Erkrankungen wurden nur geringe Abnormitäten der Spindeln gefunden. Anscheinend sind sie erkrankungsresistent oder entwickeln sich erst im späteren Erkrankungsprozess.

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[15] The principles of laboratory animal care as promulgated by the National Society for Medical Research are observed in this Laboratory.

Quinone-Tanning in the Reptilia and Aves

The presence of protein tanned by an orthoquinone has been established in the cuticle of arthropods[1], cysts of nematodes[2], shells of helminths[3], chetae of earthworm[4], byssus of the bivalve mollusc, *Mytilus edulis*[5] and central capsule of radiolarian, *Thalassicola* (Protozoa)[6]. Among the Chordata, the hardening of egg cases of the selachian, *Chiloscyllium griseum*[7] seems to involve a process very like sclerotization. During the course of histochemical studies on the eyes of the leaf-toed gecko, *Hemidactylus turicus turicus* (Linneaus) and the white-bellied petrel, *Fregetta grallaria*, it was found that the conical papilla of the reptile and pecten of the bird are phenolically tanned.

Material and methods. Required amount of conical papilla and pecten were dissected and fixed in 5% neutral formalin. Routine paraffin sections were made at 7 µ thick and stained with Mallory's triple stain. Histochemical tests employed include Millon's, potassium iodide, potassium bichromate, argentaffin, Nadi, Sudan Black-B and Liebermann-Burchardt tests. Unidimensional ascending chromatograph on Whatman No. 1 paper of acid hydrolysis of the materials was also used.

Results. The conical papilla and pecten are deep amber in colour. The papilla appears as a small cone measuring about 1–2 mm in length, while the pecten is larger than the cone formed of a series of parallel ridges. Transverse sections through the cone stained with Mallory's triple stain show that the cone is a solid structure with a circle contour. The outer portion of the core is amber-coloured and refractile to stains, while the inner central portion shows affinity to the red of acid fuchsin. The same picture is afforded by the ridges of the pecten.

Maceration of the frozen-sections of the cone and ridge of pecten with mineral acids show that the outer amber region is more resistant than the inner fuchsinophil zone. The Millon's, xanthoproteic, potassium iodide and potassium bichromate tests give positive results in the outer and inner regions indicating the existence of tyrosine, tryptophane and other phenolic compounds. Evidence that the amber-coloured region is tanned is given by the fact that, even after boiling, it induces a rapid oxidation of the Nadi reagent which has been used to indicate orthoquinones. The argentaffin reaction for polyphenols and polyamines is most marked in the outermost region of the amber zone. However, Sudan Black-B and Liebermann-Burchardt tests give negative reactions indicating the absence of simple as well as steroid lipids. Besides, upon detanning the cone and pecten by treating in diaphanol the amber regions become soft and white in colour. Histological inspection of such diaphanol-treated materials show that the region corresponding to amber and fuchsinophil regions are coloured red and blue respectively when stained with Mallory's stain. In view of these observations it may be assumed that the cone and ridge of the pecten are quinone tanned. This assumption is further supported by the chromatographic analysis for amino acids of them indicating the presence of aromatic amino acids like tyrosine and phenylalanine.

Discussion. The foregoing observations denote that the papillary cone and pecten are hardened by phenolic tanning, a process comparable to that found in the cuticle of insects and other arthropods. The outer mechanically resistant amber region is homologous to the amber-exocuticle, while the inner fuchsinophil portion to the mesocuticle of other insects. However, certain points of difference are significant: (a) unlike in the insect cuticle the substrate involved in tanning seems to be a protein rich in phenolic groups without a lipid component;

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