The development of muscle spindles in the embryonic and regenerative tail of *Hemidactylus bowringi* (Gray)

Hin-Ching Liu and R.B. Maneely

**Introduction**

Within relatively recent years a great deal of information has accumulated on the structure of the mammalian muscle spindle; but relatively little has been reported on this particular feature of neuro-muscular association in reptiles. Concerning non-mammalian spindles Cooper [1960] mentioned Kölliker [1862] as having studied the muscle spindle in the frog; and Cajal [1888, 1897], for a clear account of the spindle nerve-supply in the same animal. There are also later references concerning Kulitchsky's [1923] methylene blue dip method for demonstrating the innervation of python muscles, and more recently Cooper's [1960] review in which the author pointed out that in snakes and lizards two types of muscle spindle can be found: 1. a type having a short capsule and possessing a large (sensory) nerve terminating in a spiral surrounding a central modified (swollen) region of the myofibre which is rich in nuclei and poor in striations; and 2. a ‘long encapsulated’ type which retains its myofibrillar striations and has centrally and evenly disposed nuclei. In this latter type the neural end-elements are not spirally arranged, but spread out over the entire length of the myofibre. Apparently this type of ending was described by Cippolone [1897].

In addition to purely histological studies on mature striated muscle, there have been embryological observations including those of Felix [1888], Trichinese [1891], Sutton [1915], Langworth [1924], and Cujjuno [1927]; but these referred to mammalian muscle, and we have been unable to discover any direct references in this respect to the embryological development of the reptilian spindle.

Accordingly, in this paper we report our observations on the embryological development of the muscle spindle in the lizard *Hemidactylus bowringi* (Gray); compare and contrast this with the same structure in the regenerative tail; and relate both of these to the definitive spindle as seen in preparations of mature adult tail muscle.

**Materials and methods**

The material comprised three groups:

*Embryonic*: tail muscle of *Hemidactylus* embryos were collected in a series of 5 developmental stages (table I: 6, 7, 8, 9 and 10).

*Regenerative*: 6 stages of tail regeneration after autotomy, comprising specimens of 10, 12, 14, 19 and 27 mm length.

*Mature adult*: mature adult tails averaging 55 mm in length.
Methods

For general histology the material was fixed in Susa, embedded in paraffin wax (m.p. 48°C), sectioned, and stained with Masson's trichrome stain.

Sections were also treated by 1. the Bielschowsky-Gros method as reported by Gwyn and Heardman [1965]; 2. by Cole's modification of Ranvier's [1880] gold chloride technique, as described by us previously (Liu and Maneely [1967]); and 3. by the de Castro method [1925], as modified by Barker and Ip [1963], and further slightly modified by us to suit our material as follows: tail muscle was fixed for 4–6 days in freshly prepared chloral hydrate solution (chloral hydrate, 1.0 g; 95% alcohol, 45 ml; distilled water 50 ml; conc. nitric acid 1.0 ml). In these experiments we found that young embryos (e.g., those at 6, 7 and 8 mm) could satisfactorily be fixed by 4 days; while 6 days' fixation was preferable for older embryos. The adult and regenerative materials required 6 days' fixation.

After fixing, the tissue blocks were washed in running tap water (adult and regenerative material, 24 h; embryonic material, 6 h). The tissue blocks were then placed in a mixture of 95% alcohol, 25 ml, and ammonia, 1 drop, for 24–48 h at room temperature. The excess surface fluid was blotted, and the blocks were placed in a 1.5% solution of silver nitrate at 37°C for 5 days. This was followed by reduction for 3 days in a freshly prepared mixture of 2 g hydroquinone in 100 ml of 25% formic acid, followed by thorough rinsing in distilled water, and clearing in two changes of pure glycerine. Teased preparations were mounted in polyvinyl lactophenol.

The above method has the advantage that it can be used on small and large blocks of material, and, if successfully carried out, produces fairly uniform staining throughout the entire lengths of the muscle spindles.

Observations

A. Mature muscle

(Plate I, figs. 1–5; Plate III, figs. 17, 18)

In Hemidactylus bowringi only one type of muscle spindle was found to be present. This was a "long-encapsulated type", having the capsule composed of delicate collagenous connective tissue continuous with, or merging into, the intersegmental connective tissue at both ends of the muscle segments. In all cases a lymph space (particularly evident in transverse sections) could be demonstrated lying between the muscle fibre and its surrounding capsule. The single unbranched intrafusal muscle fibre itself was extremely thin, coextensive with the two intersegmental zones at each end, and had a slightly swollen mid-portion ("equatorial region"). The nuclei of this type of fibre were centrally placed, and distributed fairly evenly throughout the entire length of the fibre except in the swollen mid-portion where they were clumped close together. This type of intrafusal muscle fibre had the general appearance of a muscle at the
myotube stage of development; but differed from the myotube in that it showed both longitudinal and cross-striations.

Two types of nerve-supply could be differentiated in connection with the intrafusal muscle fibre:

a) a thin type running close to the lateral aspect of the myofibre at the swollen mid-portion, and penetrating the capsule to form delicate terminals (Plate I, figs.3 and 4). This type was quite uncomplicated by "spiral" formation in contradistinction to b) a type thicker than a), entering the capsule at its poles, where it divided into two principal branches, each of which gave rise to 1. a simple short secondary branch, and 2. terminal networks that clasped the fibre in light spiral formations (Plate I, figs.4 and 5).

Plate 1. – Fig. 1–6 (see legend page 66).
Table 1. Development of muscle spindles in five embryonic stages of *Hemidactylus bouringi* (Gray)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Dufaure and Hubert [1961]</th>
<th>Myogenesis</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>36</td>
<td>Premyoblasts</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>Myoblasts</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>Myotubes</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>Young myofibres</td>
<td>Thin myotube-like “intra-fusal” fibre identifiable among the myofibres. Neural elements from the intersegmental zones in the vicinity of the “equatorial” region of the fibres</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>Myofibres</td>
<td>Spindle-shaped intrafusal fibres with well-marked “equatorial” regions Capsule present Dual nerve-supply</td>
</tr>
</tbody>
</table>

N.B. The five stages are based on Dufaure and Hubert’s [1961] lizard embryo series (see Liu and Maneely [1967 a, b]).

*Plate I.* — Fig. 1–6 (see page 65).

*Note:* Figs.4–11 are of preparations impregnated with AgNO3 using Barker and Iv’s [1963] modification of the de Castro method.

*Plate I.* — Fig.1. Normal adult tail muscles: Illustrating the distribution of the neuromuscular spindle between the muscle fibres. The equatorial nerve before it enters the capsule divides into two branches (→). They enter the capsule (a) and spiral round the intrafusal muscle fibre (b). The extrafusal muscle fibres are seen at (c). Bielschowsky preparation. ×420.

*Fig. 2.* Adult tail muscle: The motor nerve fibre along the capsule has no branches; nor does it spiral on the surface of the intrafusal muscle fibre. Bielschowsky preparation. ×800.

*Fig. 3.* Adult tail muscle: The spiral sensory nerve (a) and the motor fibre (b) inside and outside the capsule respectively; intrafusal muscle fibre and the equatorial region (c) show the close arrangement of the nuclei and the sensory nerve spiral clasp ing it. Bielschowsky preparation. ×800.

*Fig. 4.* Adult tail muscle: The sensory nerve ending at the equatorial region divides into two primary branches (♂♀). From them secondary branches project and divide into many terminations inside the capsule. The blood vessels are just next to the spindle capsule (bv). AgNO3, ×800.

*Fig. 5.* Adult tail muscle: High power view showing the sensory terminal nerve endings forming networks (a); and the tips of the terminals in the form of small end-plates (→). AgNO3, ×1200.

*Fig. 6.* Embryo, stage 9: The earliest appearance of development of intrafusal muscle fibre (a) situated centrally; well marked striations; no nerve is present. Note the extrafusal muscle fibre at (b). AgNO3, ×600.
embryonic and regenerative tail of *Hemidactylus bowringi* (Gray)  

**B. Embryonic material**

In the following account the same criteria for the stages of myogenesis were used as in a previous contribution on motor end-plates (LIU and MANEELY [1967]). Regarding the development of muscle spindles, the muscular segments in stage 9 embryos were at the young myofibre stage; and among these a few extremely slender fibres could be found which were roughly one-eighth the diameter of the surrounding myofibres. These thin fibres retained more of the myotube characteristics than their neighbours, having centrally situated nuclei; but their striations were distinct (Plate 1, fig. 6), and they were invested in a delicate collagenous connective tissue sheath. It is difficult to avoid the view that these fibres represented the future intrafusal fibres of the adult muscle spindle. In the proximal portion of the tails of stage 9 embryos, it was possible to demonstrate the early stages of innervation of these delicate muscles. Branches from the intersegmental neural network were noted passing into the muscle segments, as in the development of motor end-plates, and some were traced to the primitive spindles, making contact with them at their mid-, “equatorial”, regions, where the centrally placed myonuclei were more vesicular in shape and packed more closely together.

**Table II. The development of muscle spindles at five stages of tail regeneration in *Hemidactylus bowringi* (Gray)**

<table>
<thead>
<tr>
<th>Tail regeneration</th>
<th>Myogenesis</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Myofibres*</td>
<td>Some delicate spindle-shaped cells present, having general appearance of myotubes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neural elements from intersegmental zone have not yet made contact</td>
</tr>
<tr>
<td>19</td>
<td>Myofibres</td>
<td>Thin, spindle-shaped muscle cells, with marked “equatorial” region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neural elements from intersegmental zone spreading along the fibre surface, and show branching in vicinity of “equatorial” region</td>
</tr>
<tr>
<td>22</td>
<td>Myofibres</td>
<td>Spindles longer than at 14 and 18 above. Capsule visible. Double nerve supply: a) thin, unbranched, running full length of spindles and b) thick, branching, and forming spiral round spindles</td>
</tr>
<tr>
<td>27</td>
<td>Myofibres**</td>
<td>The muscle spindles have reached their definitive form</td>
</tr>
</tbody>
</table>

* Upper third of tail; the lower two-thirds show less advanced stages of myogenesis.
** Upper two-thirds of tail.
In stage 10 embryos, the thin intrafusal muscle fibres were spindle-shaped and the “equatorial” regions showed a distinct enlargement containing closely-packed nuclei. The capsule, although thin and delicate, was more well defined and received a nerve at one pole. This nerve ran a short distance before dividing into primary and secondary branches, as described above (A a, b) and terminated as networks “clasping” the fibre surface. There was some evidence of the beginnings of spiral formation in this type of nerve.

As described in the adult muscle, each embryonic spindle was associated with a second type of nerve supply in the form of a single thin nerve running longitudinally along the fibre, and showing evidence of spiral formations.

In short, it would appear that at stage 10, the muscle spindle in these embryos was showing the characteristic nerve supply seen in the tail muscles of adult animals (Plate II, figs. 8–11; Plate III, fig. 16).

C. Regenerative material

The first signs of muscle spindle development were noticed at the 14 mm stage of regeneration. At this stage the extra-fusal muscle fibres were at the myofibre stage and showed well marked cross-striations and peripherally situated nuclei. However, it was possible at this stage to find thin spindle-shaped cells with centrally placed nuclei, having the general appearance of myotubes as described above.

By 19 mm regeneration these characteristics were much more marked and in the swollen mid-portion (“equatorial” region) of the cells, the nuclei were more vesicular and closely-packed than at 14 mm regeneration. Even at this stage in teased preparations these delicate fibres were not readily distinguishable among the extra-fusal myofibres, and on superficial examination could have been dismissed as “capillaries”, seemingly showing a central core of “red blood corpuscles”. However, in material stained by the gold chloride technique, nerves could be demonstrated in the vicinity of the “equatorial region”, and spreading along the fibre surface, with some indication of their dividing into branches which “clasped” the fibre surface (Plate II, fig. 12).

By 22 mm regeneration the spindle had become larger, and a capsule was more readily distinguished than at the earlier stages. Two nerve supplies, as reported in A and B above were distinguishable at
Plate II. — Fig. 7. Embryo, stage 9: The earliest sign of the “equatorial region” (↑↑). The nerve begins to approach, and spiral, the intrafusal muscle fibre. AgNO₃ × 600.

Fig. 8. Embryo, stage 10: The neuromuscular spindle. The sensory nerve enters the capsule and spirals at the equatorial region, and divides into two primary nerve branches (a) and (b). (C.f. fig. 10.) AgNO₃ × 480.

Fig. 9. Embryo, stage 10: A single long and thin neuromuscular spindle distributed between extrafusal muscle fibres. Many blood vessels (↑) supply the muscle fibres but no capillaries are present in the capsule of the neuromuscular spindle. ×480.

Fig. 10. Embryo, stage 10: Thin intrafusal muscle fibre, showing sensory nerves at the “equatorial region” and exhibiting secondary branches and terminal nets. Staining as in fig. 9. × 250.

Fig. 11. Embryo, stage 10: Intrafusal fibre is long and thin, and the “equatorial region” is slightly swollen. Motor and sensory nerves are present. ×600.

Fig. 12. 19 mm regeneration: A few developmental intrafusal muscle fibres are distributed in between the muscle fibres. Their nuclei are situated centrally and closely arranged. The fibre is also slightly swollen at this region. Gold chloride. ×480.
Plate III. – Fig. 13. 22 mm regeneration. More advanced form of the intrafusal muscle fibre showing the “equatorial region”. The sensory nerve enters the capsule, spirals and clasps the fibre (→). Spindle capsule shows clearly (a). Gold chloride. ×480.

Fig. 14. 27 mm regeneration: In the nearly mature neuromuscular spindle the nerves spiral in a more complex form, and the sensory nerve endings are within the capsule (→). Gold chloride. ×800.

Fig. 15. 27 mm regeneration: Transverse section of neuromuscular spindle. Intrafusal muscle fibre (→) which is covered by a capsule and nerve. Lymphatic space (a) in between the capsule and the fibre. AgNO₃ (Perez.) ×800.

Fig. 16. Embryo, stage 10: Transverse section showing myofibrils in the developing intrafusal muscle fibre. An extremely thin capsule can be observed at (→). Masson’s trichrome stain. ×800.

Fig. 17. Spindle capsule (→) in normal adult lizard tail muscle. Note the myofibrils within the intrafusal fibre. Masson’s trichrome stain. ×1500.

Fig. 18. Adult tail muscle: A thin muscle spindle is seen showing the “equatorial region” (a), and the capsular continuity with the connective tissue of an intersegmental zone (→). Masson’s trichrome stain. ×375.
this stage; the thin type unbranched, and running the length of the fibre; the thick type branching as described above, and forming a spiral round the thin spindle (Plate III; fig. 13).

By 27 mm regeneration, the muscle spindle had all the appearance of maturity and the capsule was thin, but distinct. In sections the intrafusal fibre had a small diameter and contained very few myofibrils within its sarcoplasm.

At this stage the dual nerve supply was readily demonstrable, the thin fibre terminating in numerous very thin branches inside the capsule, the thick fibres entering at the equatorial region dividing, and forming spirals, as in the adult spindle (Plate III; figs. 14, 15).

Discussion

Concerning the capsule surrounding the intrafusal fibre in the muscle spindles of this species, it seems clear that it covers the entire length of the intrafusal fibre, and is continuous with the connective tissue of the intersegmental zones at each end. It is difficult to avoid the view that during development, probably at the premyoblast stage, and before myoblastic differentiation occurs, some of the undifferentiated cells are destined to become fibroblasts and later develop as such in between the longitudinal rows of myotubes. As the spindles develop, these cells provide thin collagenous connective tissue covering, and the delicate collagen fibrils thus formed make contact (i.e. become continuous) with the collagenous elements in the intersegmental zones at the ends of the muscle segment. In this sense it is possible to agree with Sherrington [1894] that, essentially, the capsule is a sort of modified perimysium. Eichhorst's view [1888] that the capsule of the spindle is continuous with, and arises from the perineurium of the nerve supply, has not been accepted by most investigators, and is not borne out by these studies.

Although various authors have reported that the capsule differs in thickness in different species, e.g. 2–4 layers of connective tissue in amphibians, reptiles and birds (Huber and DeWitt [1897]), two layers in the boa constrictor (Hines [1932]) and 6–8 concentrically disposed cell layers in cat and monkey (Sherrington [1894]), there can be no doubt that in the adult Hemidactylus it is extremely thin, and consists of a single layer of interwoven delicate collagenous connective tissue fibres.
With regard to the nerve supply to muscle spindles, attempts have been made by various investigators in the past to differentiate its motor and sensory elements; but with conflicting results. The first attempt we have been able to trace was that of ONANOFF [1890] who cut the dorsal spinal root in dogs, and induced degeneration in only a few spindle fibres. However, SHERRINGTON [1894], using the same procedure on cats, demonstrated that the large myelinated fibres to the spindles remained intact. HINSEY [1927] removed the lumbar sympathetic trunks in the cat and later found that the primary or secondary endings of the sensory nerves were still intact, and HINES and TOWER [1920] confirmed the sensory nature of the spinal “equatorial” endings.

BOEKE [1927], using the cat, severed both the ventral and the dorsal roots, and reported finding degenerated motor endings in the muscle spindle. Using similar techniques, HINSEY [1927] found no nerves left at all, except for a few groups of non-myelinated fibres associated with the vascular supply to the spindle capsules; and this was later confirmed by HINES and TOWER [1928].

Electrophysiological methods, first developed by ADRIAN [1926] have brought a fuller understanding of the motor supply to the mammalian muscle spindle, but it has not been possible to trace any literature concerning this aspect in connection with the lizard.

The situation concerning the sensory and motor supplies of the muscle spindle after experimental ablation performed on the peripheral nervous system was summarised by HINES [1932] who opined that it is logical to interpret the equatorial ending of the spindle as sensory. In HINES’ experience a similar ending on the intrafusal fibre in the alligator’s m. flexor tibialis anterior and m. ambiens, degenerated when the dorsal root ganglia were removed. In like manner, the polar ending may be thought to be motor. Again, within these two muscles of the alligator, the nerve terminals degenerated when the ventral roots were severed.

Since our experiments did not include either ablation operations or electrophysiological techniques, it is not yet possible to state categorically which of the two types of nerve supplying the spindle in this species is sensory, and which motor. However, it is clear that a dual supply is present; and if the criteria set out above be correct, and accepted, one can assume that functionally the spiral “equatorial” ending probably is sensory and that the thick (more heavily myelinated) supply is motor.
Acknowledgment

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Summary

Muscle spindles in development and in regeneration of the tail of *Hemidactylus bowringi* (Gray) were studied, described, and compared and also related to the morphology of their nerve supply in the mature state. In embryos the first signs of spindle formation occurred at the late myotube or early myofibre stages. Dual nerve supply was acquired in the spindles at late embryonic stage. In regenerative tails, immature muscle spindles were identifiable at 14 mm regeneration; and they reached their definitive form by 27 mm regeneration.

Résumé

On a étudié les fuseaux neuromusculaires au cours du développement et de la régénération de la queue chez le lézard *Hemidactylus bowringi* (Gray). On en donne une description et on compare la morphologie des nerfs qui en partent à celle rencontrée chez l'adulte.

Les premiers indices du développement des fuseaux neuromusculaires chez l'embryon se rencontrent au moment où les myotubes sont entièrement formés ou au moment où apparaissent les premières fibres musculaires. L'inervation est démontrable au niveau des fuseaux dans les stades embryonnaires avancés. Dans la queue régénérée, on peut identifier des fuseaux en voie de formation lorsque le régénérateur mesure 14 mm; les fuseaux atteignent leur développement définitif lorsque la nouvelle queue atteint la longueur de 27 mm.

Zusammenfassung


References

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