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# The formation of muscles in regenerating limbs of the newt after denervation of the blastema

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SUMMARY

The purpose of this experiment was to examine the relationship, if any, between nerve fibers and the formation of muscle pattern in the regenerating amphibian limb. During embryogenesis, nerve fibers grow into the limb bud at the time when the common muscle blastemas subdivide into individual muscle primordia, whereas in regeneration nerve fibers are always present. In order to learn whether or not the muscle pattern could be laid down in the absence of nerves we amputated 58 limbs of newts (*Notophthalmus viridescens*) at the mid humeral level and allowed them to regenerate to the medium-bud or late-bud stage. The limbs were then denervated. The majority of limbs denervated at the medium-bud stage either regressed or failed to regenerate further. Regeneration after denervation failed in 9 of 25 limbs denervated at the late-bud stages.

In those limbs that continued to regenerate after denervation, the formation of individual muscle primordia did occur, following the same sequence with respect to the gross stage of regeneration as innervated regenerates. In comparing these results with our previous results on the development of muscular pattern in aneurogenic limbs of the axolotl, we conclude that in neither the embryonic nor the regenerating amphibian limb are nerve fibers directly involved in the subdivision of common muscle blastemas into the primordia of individual muscles.

## INTRODUCTION

It has been known for decades (Harrison, 1904, 1907; Hamburger, 1929, 1939; Yntema, 1959) that in the embryo gross morphogenesis of limbs can occur quite normally in the absence of innervation. These, and other, studies have also shown that differentiation of individual tissue types occurs in these limbs, but only recently have detailed analyses of the development of muscular pattern in aneurogenic limbs been undertaken (Čihák, Doskočil & Seichert, 1978; Shellswell, 1977). The morphogenesis of muscles in aneurogenic axolotl limbs has been recently studied (Grim & Carlson, 1978), but due to nutritional inadequacies the entire limbs were somewhat hypomorphic.

In regenerating limbs of urodeles, the blastema ultimately becomes independent of nerves and can undergo further development in their absence (Schotté,

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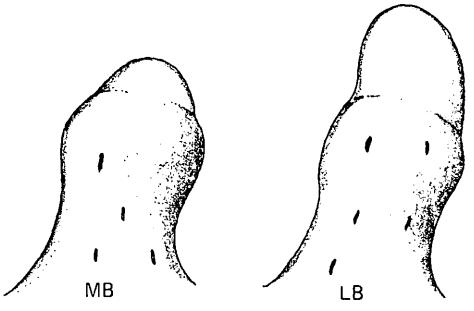



Fig. 1. Illustration of medium-bud (MB) and late-bud (LB) regenerates in forearms of the newt.

1926; Singer & Craven, 1948; Powell, 1969). Gross morphogenesis of these denervated regenerates may be normal, but typically growth of the regenerates is retarded. Because in regeneration nutrition is not a problem, we have used the regenerating limb as a model to study the establishment of muscle pattern in amphibians in the absence of nerves.

A major question regarding the development of muscular pattern within embryonic and regenerating limbs concerns the nature of the factors underlying the separation of common muscle blastemas into individual muscle primordia. In this communication we examine the relationship between nerve fibers and the morphogenesis of individual muscles within the regenerating limb. The specific question is whether or not the common muscle blastemas within a regenerating limb can separate into identifiable muscles in the absence of nerves.

## MATERIALS AND METHODS

This experiment was carried out on 48 adult newts, *Notophthalmus* (*Triturus*) *viridescens*, obtained from Bill Lee in Oak Ridge, Tennessee. The animals were maintained at 20–22 °C and fed three times per week with beef liver. At the time of both amputation and denervation the newts were anesthetized with MS-222 (Sandoz).

Both forelimbs were amputated through the mid-stylopodium and were allowed to regenerate. In most animals one limb was denervated, and the other was allowed to regenerate normally as a control. In some animals both limbs were denervated. Bilateral denervation did not produce any effects not seen in unilaterally denervated animals. Fifty-eight regenerates were denervated at stages from medium-bud (33 extremities, 21–29 days after the amputation) through late-bud (25 extremities, 24–34 days after amputation) (stages according to Iten & Bryant, 1973). Medium- and late-bud regenerates are illustrated in Fig. 1. The strategy was to denervate the regenerate soon after it became nerve independent so that the regenerate and the future muscle cells would be as immature as possible at the start of the test period. Denervation consisted

of severing spinal nerves 3, 4 and 5 at the mid-scapular level. Redenervations were done at 6- to 7-day intervals to maintain the blastemas in a denervated condition. Originally, the proximal ends of the nerves were tied off with 8-0 silk sutures (Ethicon) to prevent regeneration from the proximal segments of the nerves and thus eliminate the need for redenervation, but silver-stained sections showed that suturing of the nerve stumps did not always prevent the outgrowth of nerve fibers. Regenerates containing nerve fibers were not used for further analysis. The limbs were fixed at various periods (5–23 days) after denervation. In all cases the stage of the regenerate at both the time of denervation and the time of fixation was recorded.

Both denervated and innervated control regenerates were fixed in Bouin's fluid and decalcified in a solution of 5 % $HNO_3$ in 70 % ethanol. All limbs that did not regress after denervation, and six limbs which had regressed, were serially cross-sectioned at 10 $\mu$m and stained with Weigert's hematoxylin and eosin. Palmgren's silver stain (Palmgren, 1960) was used on cross-sections of the bases of the limbs to check on the completeness of denervation.

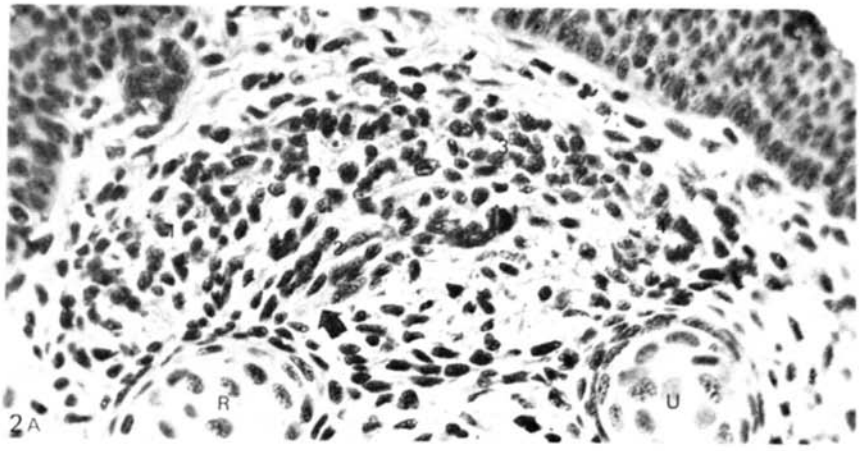
## RESULTS

### *Normal muscle morphogenesis in innervated regenerates*

This review of muscle morphogenesis in control regenerates is provided as a basis for comparison of the morphogenesis of muscles in denervated limbs. The anatomical arrangements of the muscles in the newt, as well as their morphogenesis, are in general similar to the muscles of the axolotl, which we have described previously (Grim & Carlson, 1974*a*). For the description of morphogenesis, we shall include only those muscles and the stages of their development that are relevant to the description of denervated limbs. As a point of departure, we are using the study of muscle morphogenesis of the forearm and hand in embryonic and regenerating limbs of the axolotl (Grim & Carlson, 1974*b*).

In the late-bud stage (stages of Iten & Bryant, 1973) the earliest stages of chondrogenesis of the humerus are present in the most proximal region of the regenerate. Distally, the level of differentiation is less, and the zeugopodial and autopodial regions of the regenerate consist of a homogeneous mass of mesenchymatous cells (Fig. 4). The differentiation of histologically identifiable muscle cells has not yet begun in regenerates of this stage.

Cartilaginous models of radius and ulna are established during the palette stage, and common flexor and extensor muscle blastemas appear as accumulations of mononuclear cells on either side of the skeletal elements. Late in the palette stage individual muscle primordia begin to form in the proximal half of the zeugopodium, but distally common pre-muscle masses still prevail. The individual muscle primordia that can be identified in late-palette stage regenerates are shown in Fig. 2.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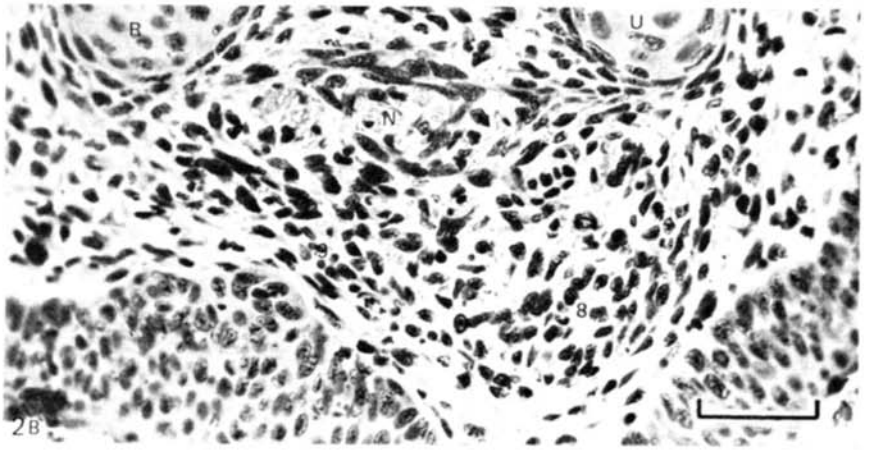
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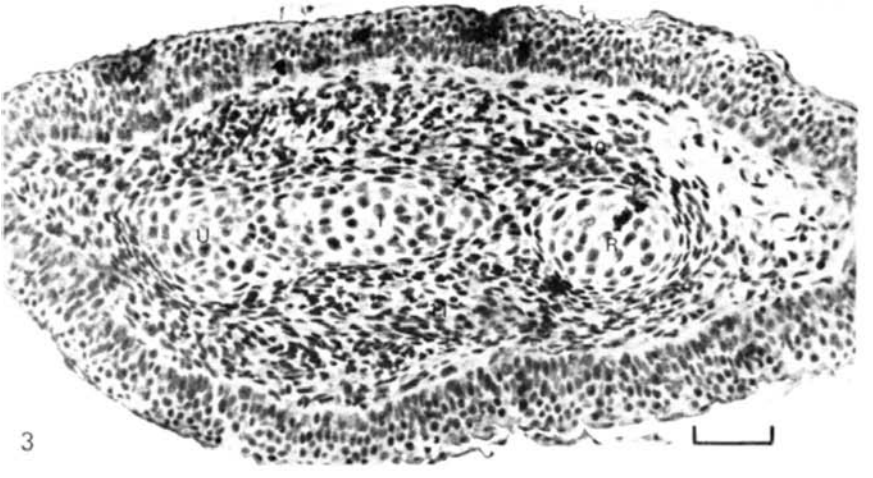
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The first signs that allow one to determine, in serial paraffin sections, that the morphogenesis of muscle primordia is beginning are differences in the density and in the orientation of cells of the muscle blastemas. In the newly forming muscle primordia, the elongated blastemal and muscle cells are oriented with their long axes in the direction of the muscle fibers of the definitive muscle (Cihák, 1972). These first signs of morphogenesis of individual muscles are similar to those occurring in the proximal half of the forearm in both the embryonic and regenerating limb of the axolotl, at the stage at which condensations of mesenchymal cells are formed in the sites of the anlagen of the first two metacarpals (Grim & Carlson, 1974*b*).

Individual muscle primordia can be identified throughout the length of the zeugopodium at the early-digits stage. Nevertheless, their identification, like that in the axolotl, is more difficult than the identification of muscle primordia in birds or mammals. In the latter, the muscle primordia are formed from a significantly greater number of cells, and more mesenchymal cells lie between them (Sullivan, 1962; Čihák, 1972). In the autopodium (Fig. 3) one can make out the primordia of the *m. abductor digiti I et extensor brevis digiti I*, but the remaining muscle primordia of the autopodium cannot be clearly distinguished.

## *Development of denervated regenerates*

### *Gross reactions of regenerating limbs to denervation*

A summary of the gross responses of medium-bud and late-bud regenerates to denervation is given in Table 1. Only 12 % of the regenerates denervated at the medium-bud stage continued to progress in their development, whereas 64 % of those denervated at the late-bud stage did so. In consonance with the report of Singer & Craven (1948), the denervated regenerates that continued to develop were noticeably smaller than normal regenerates of the same developmental stage.

The principal question asked in this study necessitates analysis of regenerates

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FIGURES 2 AND 3

Primordia of the extensor (A) and flexor (B) muscles seen in cross-section through the middle part of the regenerating innervated forearm in the newt at the late palette stage. The groups of cells which constitute the newly forming muscle primordia are designated by numbers: (1) *m. extensor carpi radialis*, (2) *m. extensor antebrachii radialis*, (3) *m. extensor digitorum communis*, (4) *m. extensor antebrachii et carpi ulnaris*, (5) *m. pronator quadratus*, (6) *m. ulnocarpalis*, (7) *m. flexor carpi ulnaris*, (8) *m. palmaris superficialis*, (9) *m. flexor antebrachii et carpi radialis*. The primordia indicated by numbers 7–9 are still poorly defined. R, radius, U, ulna, N, nerve fibers. Arrow – muscle cells with myofibrils. H and E. Bar = 100 $\mu$m.

Fig. 3. Newly forming muscle primordia seen in a cross-section of the proximal part of the autopodium of an innervated regenerate in the early-digits stage. (3) *m. extensor digitorum communis*, (10) *m. abductor digiti I et extensor brevis digiti I*, (11) common primordia of the palmar muscles. R, radius, U, ulna, I, os intermedium, H and E. Bar = 100 $\mu$m.

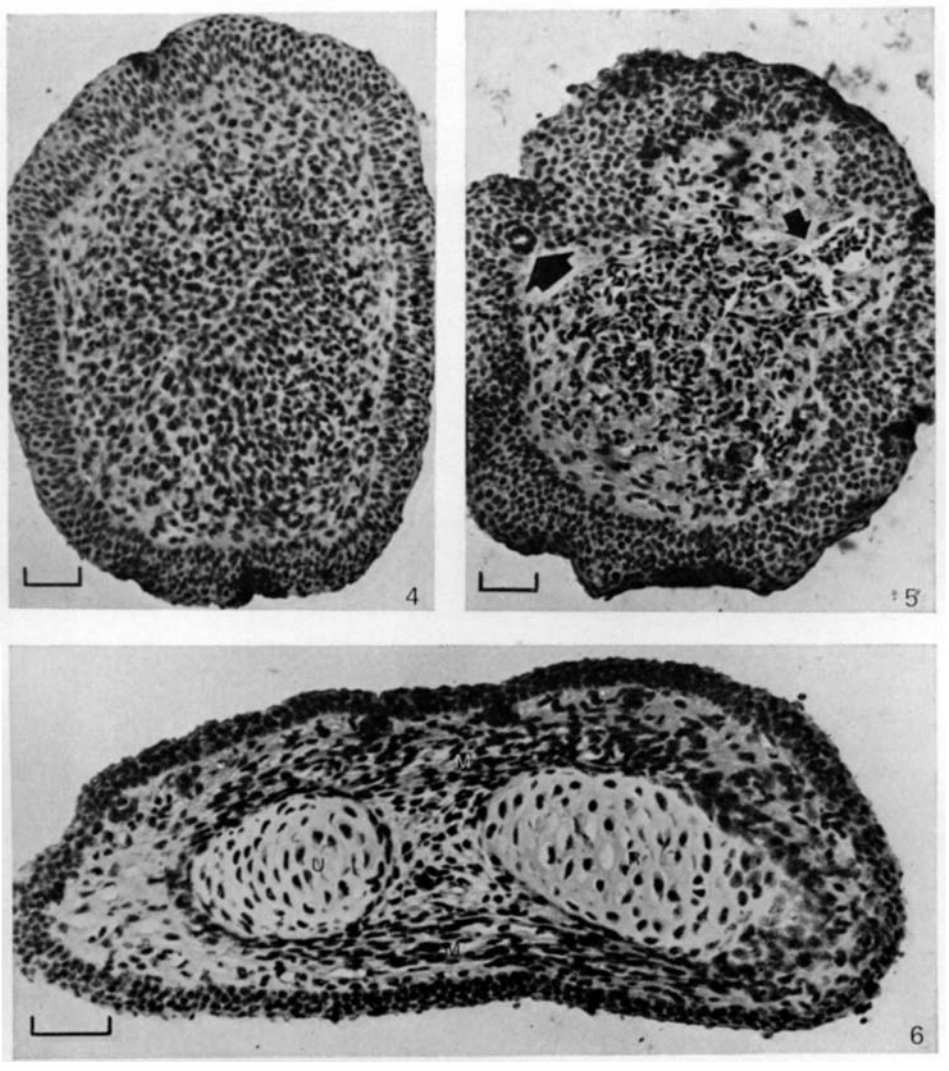
Table 1. *Summary of responses of regenerates to denervation at medium-bud and late-bud stages*

| Stage | Regression | No change | Progression | | |
|---|---|---|---|---|---|
| | | | Late bud | Palette | Early digits |
| Medium bud | 22 | 7 | 1 | 3 | 0 |
| Late bud | 6 | 3 | — | 7 | 9 |

that continue to develop after denervation. Therefore the histological appearance of the regenerates that regressed or remained static after denervation is only briefly summarized below. The regenerates that continued to develop fell into two categories with respect to muscle development. In some (Group 1) both the gross form of the regenerate and the skeletal pattern were normal. The pre-muscle masses, however, were very small in proportion to the size of the limbs. In others (Group 2) gross form of the regenerates and the morphological pattern and proportions of both the skeleton and musculature were normal. Of the four regenerates that continued to develop after denervation at the medium-bud stage, two fell into the Group 1 category and two could be assigned to Group 2. In contrast, only three of the regenerates that continued to develop after denervation in late-bud stage could be assigned to Group 1, and the remaining 13 regenerates fit into Group 2.

*Regenerates which failed to develop after denervation*

Twenty-nine limbs denervated at the medium-bud stage (particularly in early-medium-bud) and nine limbs denervated in the late-bud stage did not develop further (Table 1). The growth of the regenerates either remained at the stage at which the denervation was done, or sometimes the regenerates gradually decreased in size. In one case regressive changes progressed to the point where approximately 2 mm of the humerus protruded past the distal surface of the limb. Six of these limbs, fixed from 9–12 days after denervation, were examined histologically. Around the end of the humerus could be seen the formation of cartilage and isolated regenerating myotubes. Distal to the humerus we could not detect any signs of the formation of the zeugopodial skeleton or muscles. The regenerates were composed of irregularly distributed blastemal cells and had an appearance similar to that of comparable stages of regeneration in innervated limbs, but they contained fewer cells, more intercellular substance and dilated capillaries (Fig. 5). We were also able to recognize the presence of skin glands, which in innervated regenerates form only at the early-digits stage (Iten & Bryant, 1973).




FIGURES 4–6

Fig. 4. Cross-section through a regenerate in the late late-bud stage of an innervated limb of the newt. Accumulation of cells in the central and middle zones of the regenerate at a level distal to the end of the humerus. Prochondral and muscle blastemas have not yet formed. H and E. Bar = 100 $\mu$m.

Fig. 5. Cross-section through a regenerate on a denervated limb at level distal to the humerus. Nine days after denervation (at which time the regenerate was in the medium-bud stage) the regenerate remains at the same stage. There are no signs of morphogenesis: the density of cells is minimal; blood capillaries (small arrow) are dilated; and skin glands (large arrow) are forming. H and E. Bar = 100 $\mu$m.

Fig. 6. Cross-section (slightly oblique) through the forearm of a regenerating limb 12 days after denervation. Palette stage. Muscle blastemas (M) are rudimentary. R, radius, U, ulna. H and E. Bar = 100 $\mu$m.

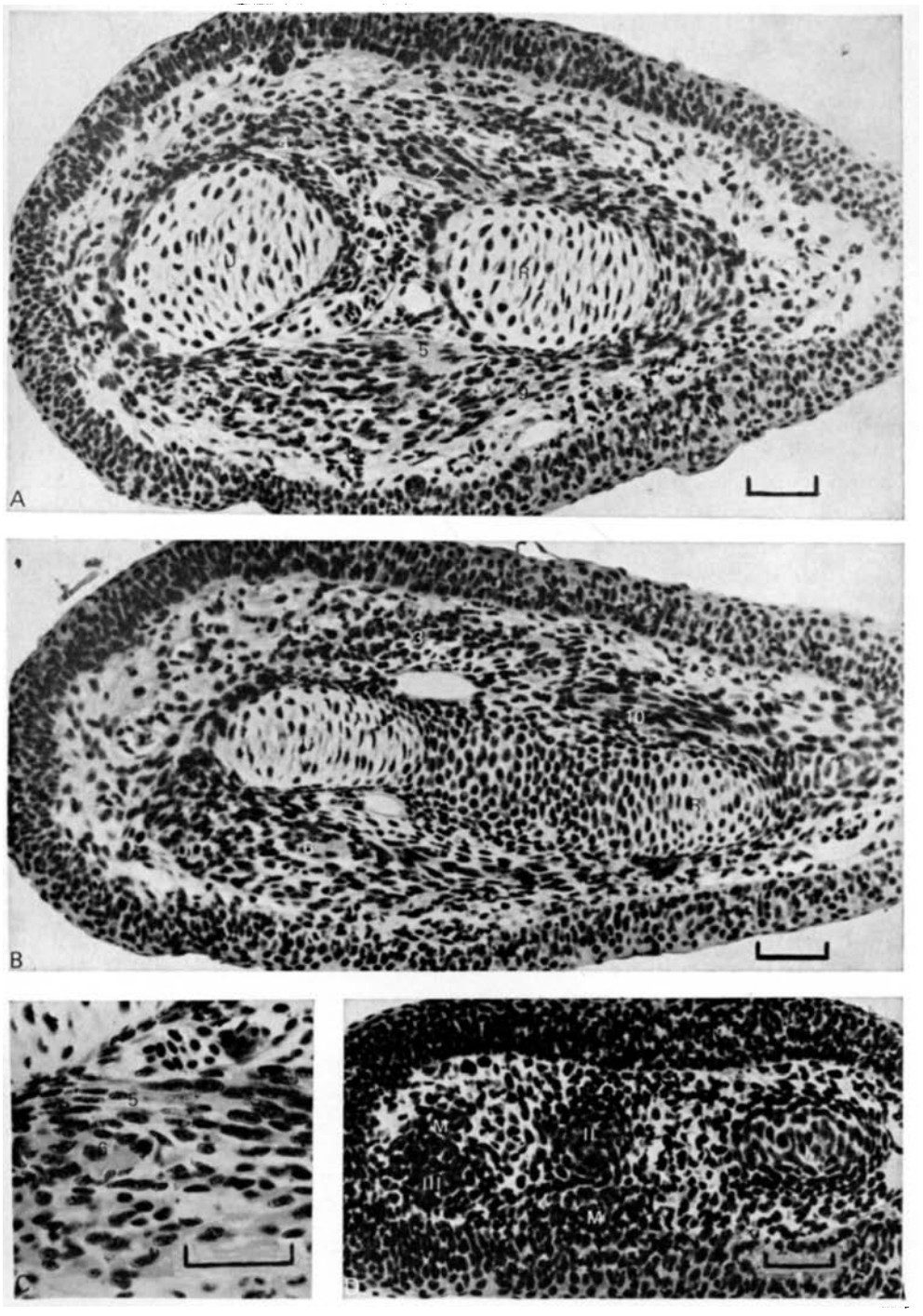



Fig. 7. Cross-sections (slightly oblique) through a denervated regenerate 12 days after denervation. Late-palette–early-digits stage. Mid-zeugopodium (7 A–C); proximal autopodium (7 B) and distal autopodium (7 D). The newly forming muscle primordia, which contain differentiated muscle cells, can be identified throughout the entire zeugopodium and in the proximal part of the autopodium. The muscle primordia are indicated by the same numbers that are listed in the legends of Figs. 1 and 2. The separation from one another of the muscle primordia indicated by numbers 7–9 is not yet complete. In the distal autopodium (7 D) muscle blastemas (M) have formed on the flexor and extensor side of the first three (I, II, III) metacarpals. H and E. Bar = 100 $\mu$m.

*Regenerates which continued to develop after denervation*

*Group 1.* The cross-sectional areas of the forearms and hands of these limbs were considerably less than those of innervated regenerates. The cartilaginous primordia of the skeleton of the zeugopodium and autopodium were formed at a level corresponding to that in normal regenerates of the same stage. The muscle blastemas, however, were hypomorphic. They were formed from a small number of cells, and in some cases only a thin layer of mesenchymal cells filled the narrow space between the cartilaginous primordia of the skeleton and the epidermis (Fig. 6). In the small space which these rudimentary muscle blastemas occupied, and because of the small number of cells that formed them, it was not possible to delineate the individual muscle primordia. This is evidently due both to the small size of the common muscle blastemas and to the beginning of atrophic changes in them, the atrophic changes being most prominent in regenerates taken late after denervation.

*Group 2.* In these regenerates common muscle blastemas formed, and from the blastemas arose the primordia of the individual muscles. The progression and degree of morphogenesis attained by these limbs were comparable with innervated limbs of a corresponding developmental stage, and the formation of muscle primordia was not delayed relative to the development of the skeletal elements. In both innervated and denervated regenerates muscle primordia began to form in the proximal part of the zeugopodium at the time when condensations of mesenchymal cells formed in the primodia of the first two metacarpals. Likewise, the differentiation of muscle cells began at the same time as the first stages of morphogenesis of the muscle primordia. The most advanced stage of morphogenesis of muscles which we encountered in limbs regenerating after denervation is illustrated in Fig. 7A–D. In the middle part of the zeugopodium it is possible to recognize the formation of the primordia of all four extensor muscles (Fig. 7A). In the flexor group of muscles, the primordia of two muscles in the deeper layer – the *m. pronator quadratus* and the *m. ulnocarpalis* – have formed (Fig. 7C). The separation of muscle primordia in the superficial flexor layer is not yet obvious. By their position and number, these muscles are comparable to those of a similar developmental stage in an innervated extremity. Likewise, the degree of individuation of these muscle primordia is not less than in innervated regenerates of the same stage.

In the proximal part of the autopodium is formed the primordium of the *m. adductor digiti I et extensor brevis I* (Fig. 7B). In the distal part of the autopodium are formed muscle blastemas of the short flexors and short extensors of the first three digits (Fig. 7D).

## DISCUSSION

From our experiments it is evident that there is no clear cut stage at which the regenerating limb of the newt becomes independent of nerves for its further morphogenesis. Within both the medium-bud and late-bud stages the response to denervation ranged from actual regression, to the retention of the developmental *status quo*, to the progression of differentiation and morphogenesis. In the 20 limbs that showed the progression of regeneration after denervation, it was possible to obtain evidence relating to the main question at hand – namely, whether or not the common muscle blastemas within a regenerating limb can separate into identifiable muscle primordia in the absence of nerves. The remainder of the cases, in which the overall regenerative process was inhibited or retarded, were not directly relevant to the question.

It is clear that normal muscle morphogenesis can occur in regenerates that have been denervated at the medium-bud or late-bud stage. This is contingent upon the continued development of the limb regenerate as a whole. In some regenerates, particularly those which were examined at long periods after denervation, the muscle blastemas were hypoplastic. It is not known whether this is due to primary hypoplasia of the myoblastic cells or to secondary atrophy.

In both embryonic and regenerating limbs of Urodeles, muscles are formed in the following sequence (Grim & Carlson, 1974*b*). Mesenchymal cells of the limb bud condense and accumulate as common flexor and extensor blastemas on opposite sides of the skeletal rudiments. The muscle blastemas then become subdivided into the primordia of individual muscles. In the embryonic limb, the subdivision of the muscle blastemas into muscle primordia occurs at about the same time when the first nerve fibers are penetrating into those areas of the limb. On the other hand, nerve fibers are present in the regenerating limb from the very beginning, because without an adequate nerve supply regeneration will not occur (Singer, 1952). Within a given segment of the limb, the muscle primordia take shape following a proximo-distal gradient rather than splitting at the same time throughout the length of the segment. At the time of splitting of the blastemas the elongated cells of the future muscle primordia are oriented in the direction of the definitive muscle in the adult. This orientation can be seen in the plane of the equatorial plate of the mitotic spindles. In the early muscle primordia some muscle cells show evidence of differentiation into myotubes, whereas the others remain undifferentiated at the light microscopic level.

Muscle morphogenesis in nerveless embryonic limbs and in nerveless regenerates is similar, in that primordia of individual muscles begin to form from the common blastemas in each case. Full morphogenesis of the separate muscle primordia did not occur in aneurogenic embryonic limbs, and not only were the early muscle primordia as well as the limbs themselves hypomorphic, but the course of differentiation and morphogenesis was also delayed (Grim & Carlson,

1978). This is likely due to overall nutritional deficiencies in aneurogenic axolotl embryos. In regenerating limbs which continue to develop after denervation, the level of muscle morphogenesis, as a whole, as well as the level of cytodifferentiation are higher than they are in embryonic aneurogenic limbs.

From statements made in the literature, one can infer that in both the aneurogenic, embryonic limb and the denervated, regenerating limb, muscle development proceeds quite normally (reviews by Zelená, 1959; Popiela, 1975). It has, in fact, been shown that the cytodifferentiation of muscle can take place to some degree, but except for some recent work (Shellswell, 1977; Čihák *et al.*, 1978; Grim & Carlson, 1978) the morphogenesis of individual muscles has not been analyzed. Both Shellswell (1977) and Čihák *et al.* (1978) used the embryonic chick wing as their experimental model, but they employed different methods and used them at different times. Shellswell (1977) chemically denervated the wing buds at stage 25/26 (Hamburger & Hamilton, 1951) by using the nicotinamide analogue, 3-acetylpyridine, and reported no disturbance in the development of muscular pattern.

In contrast, Čihák *et al.* (1978) produced aneurogenic extremities either by excision of the neural tube in the extent of the extremity in stages 13–15 HH, or by grafting of wing primordia (stages 11–20 HH) onto the chorioallantoic membrane. In the *in situ*-produced aneurogenic limbs the migration of pre-muscle cells into the limb bud from the somites was probably not disturbed. In the majority of explants pre-muscle cells have begun to migrate into the limb bud from the somites, but the normal immigration of cells has not been completed. Čihák *et al.* (1978) reported a range of abnormalities in the form and structure of extremities in *in situ* aneurogenesis and defects of subdivision of the muscle blastemas into muscle primordia in the explants. With such greatly differing methods, it is not easy to compare the results of Shellswell (1977) and Čihák *et al.* (1978). In the study of Čihák *et al.* (1978) the muscle morphogenesis in explants was influenced by the time of explantation of the limb bud (particularly with respect to the immigration of pre-muscle cells) or, possibly, by conditions surrounding the explant. Because of the relatively late period at which denervation was performed in Shellswell's study, it is possible that the basis for the muscular pattern had already been laid down in the wing bud at the time of chemical treatment. In the present experiments on the regenerating limb, it is possible as well, that by the time the regenerate became nerve-independent certain elements of muscular pattern had been imprinted upon the mesenchymal cells of the blastema.

On the basis of the experimental work performed to date, what can be said regarding the role of nerves in muscle morphogenesis? In neither embryonic nor regenerating limbs of Urodeles does the subdivision of the common muscle blastemas appear to require the direct mediation of nerves. Nevertheless, an indirect effect related to the action of nerves on cell proliferation may yet be possible. If cell proliferation is sufficiently depressed, possibly a minimum critical

mass of cells in the limb buds is not present and hypomorphic development could result. Experiments performed to date do not allow one to make any statements regarding the possible role of nerves in the later stages of muscle morphogenesis; or in its special developmental processes known in many vertebrates, such as the migration of the diaphragm and tongue muscles and the extension of the latissimus dorsi muscle; or in the secondary fusion of two muscle primordia into one interosseus muscle as described by Čihák (1977) in the human hand.

At this point it is not yet possible to attribute the morphogenesis of muscle pattern to any one specific factor. Experiments involving removal of either nerves (see above) or bones (Slačálková, 1974) from developing limbs have demonstrated that muscular pattern is relatively independent of these structures. It has proven to be very difficult to dissociate abnormalities in the musculature from abnormalities of the limb as a whole. Thus it appears likely that the pattern of the muscles within a limb is a component of the broad set of instructions that guides the development of the entire limb.

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## REFERENCES

ČIHÁK, R. (1972). Ontogenesis of the skeleton and intrinsic muscles of the human hand and foot. *Ergebn. Anat. Entw. Gesch.* **46**, 1–194.

ČIHÁK, R. (1977). Differentiation and rejoining of muscular layers in the embryonic human hand. *Birth Defects: Orig. Article Ser.* **13** (1), 97–110.

ČHIÁK, R., DOSKOČIL, M. & SEICHERT, V. (1978). Some morphogenetic aspects of the aneurogenic and explanted limb primordia. In *XIXth Morphol. Congr. Symp. 1978* (ed. E. Klika, pp. 119–125. Praha: Univ. Karlova.

GRIM, M. & CARLSON, B. M. (1974*a*). A comparison of morphogenesis of muscles of the forearm and hand during ontogenesis and regeneration in the axolotl (*Ambystoma mexicanum*). I. Anatomical description of muscles of the forearm and hand. *Z. Anat. Entw. Gesch.* **145**, 137–148.

GRIM, M. & CARLSON, B. M. (1974*b*). A comparison of morphogenesis of muscles of the forearm and hand during ontogenesis and regeneration in the axolotl (*Ambystoma mexicanum*). II. The development of muscular pattern in the embryonic and regenerating limb. *Z. Anat. EntwGesch.* **145**, 149–167.

GRIM, M. & CARLSON, B. M. (1978). Morphogenesis of muscle primordia in aneurogenic forelimbs in the axolotl (*Ambystoma mexicanum*). In *XIXth Morphol. Congr. Symp.* 1976 (ed. E. Klika), pp. 127–133. Praha: Univ. Karlova.

HAMBURGER, V. (1929). Die Entwicklung experimentell erzeugter nervenloser und schwach innervierter Extremitäten von Anuren. *Wilhelm Roux Arch. EntwMech. Org.* **114**, 272–363.

HAMBURGER, V. (1939). The development and innervation of transplanted limb primordia of chick embryos. *J. exp. Zool.* **80**, 347–390.

HAMBURGER, V. & HAMILTON, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49–92.

HARRISON, R. G. (1904). An experimental study of the relation of the nervous system to the developing musculature in the embryo of the frog. *Am. J. Anat.* **3**, 197–220.

HARRISON, R. G. (1907). Experiments in transplanting limbs and their bearing upon the problems of the development of nerves. *J. exp. Zool.* **4**, 239–281.

ITEN, L. E. & BRYANT, S. V. (1973). Forelimb regeneration from different levels of amputation in the newt, *Notophthalmus viridescens*: length, rate, and stages. *Wilhelm Roux Arch. EntwMech. Org.* **173**, 263–282.

PALMGREN, A. (1960). Specific silver staining of nerve fibers. I. Technique for vertebrates. *Acta zool. Stockh.* **41**, 239–265.

POPIELA, H. (1975). *Aneurogenic limb development and muscle degeneration.* Ph.D. dissertation, Michigan State University, Lansing, Michigan.

POWELL, J. (1969). Analysis of histogenesis and regenerative ability of denervated forelimb regenerates of *Triturus viridescens*. *J. exp. Zool.* **170**, 125–148.

SCHOTTÉ, O. (1926). Système nerveux et régénération chez le Triton. *Rev. Suisse Zool.* **33**, 1–211.

SHELLSWELL, G. B. (1977). The formation of discrete muscles from the chick wing dorsal and ventral muscle masses in the absence of nerves. *J. Embryol. exp. Morph.* **41**, 269–277.

SINGER, M. (1952). The influence of the nerve in regeneration of the amphibian extremity. *Q. Rev. Biol.* **27**, 169–200.

SINGER, M. & CRAVEN, L. (1948). The growth and morphogenesis of the regenerating forelimb of adult Triturus following denervation at various stages of development. *J. exp. Zool.* **108**, 279–308.

SLAČALKOVÁ, J. (1974). *Manus vara congenita* (Czech). Candidatus scientiarum dissertation, Charles University, Prague.

SULLIVAN, G. E. (1962). Anatomy and embryology of the wing musculature of the domestic fowl (*Gallus*). *Austr. J. Zool.* **10**, 458–518.

YNTEMA, C. L. (1959). Regeneration in sparsely innervated and aneurogenic forelimbs of *Ambystoma larvae*. *J. exp. Zool.* **140**, 101–124.

ZELENÁ, J. (1959). *Vliv inervace na vývoj svalu* (*The Influence of Innervation of the Development of Muscle*). Praha: Státní zdravotnické nakladatelství.

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