THE EFFECT OF COLCHICINE ON MYOGENESIS IN VIVO IN *RANA PIPIENS* AND *RHODNIUS PROLIXUS* (HEMIPTERA)

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ABSTRACT

The effect of colchicine on myogenesis in vivo has been studied in the regenerating tadpole tail of the frog, *Rana pipiens*, and in the abdominal molting muscles of a blood-sucking bug, *Rhodnius prolixus* Stål. Colchicine is shown to disrupt microtubules in the differentiating muscle cells of both these organisms. The disruption of microtubules is correlated with a loss of longitudinal anisometry in the myoblasts and myotubes of the regeneration blastema in the tadpole tail. Before colchicine treatment, the myotubes contain longitudinally oriented myofibrils. After colchicine treatment, rounded, multinucleate myosacs containing randomly oriented myofibrils are present. It is suggested that the primary function of microtubules in myogenesis in the *Rana pipiens* tadpole is the maintenance of cell shape. The abdominal molting muscles of *Rhodnius* undergo repeated phases of differentiation and dedifferentiation of the sarcoplasm. However, the longitudinal anisometry of the muscle fibers is maintained in all phases by the attachments of the ends of the fibers to the exoskeleton, and microtubule disruption does not alter cell shape. The orientation of the developing myofibrils is also unaltered, indicating that the microtubules do not directly align or support the myofibrils in this system.

INTRODUCTION

The differentiation of vertebrate striated muscle is characterized by the fusion of mononucleated myoblasts to form elongate, multinucleated myotubes, and by the ordering of the developing myofibrils in the longitudinal axes of the myotubes. Godman and Murray (1953) first reported that colchicine, when applied to cultures of differentiating rat skeletal muscle containing elongating myotubes, caused a reversible fragmentation of the myotubes into short, rounded segments. Recently, Bischoff and Holtzer (1968) studied similar myotube fragments, or myosacs, produced by colchicine in cultures of differentiating chick skeletal muscle, and showed that the developing myofibrils in the colchicine-induced myosacs lost their longitudinal orientation.

There is now evidence that colchicine specifically causes the disruption of microtubules in diverse types of cells. Robbins and Gonatas (1964) observed a loss of microtubules from the mitotic spindle in colchicine-treated, dividing HeLa cells. Tilney (1965) demonstrated that the reversible, colchicine-induced retraction of the axopodia of a Heliozoan, *Actinosphaerium nucleofilum*, was correlated with the disruption of microtubules in the axopodia. Malawista and Bensch (1967) have shown that microtubules are disrupted in human polymorphonuclear leukocytes cultured in the presence of colchicine.

Microtubules are present in large numbers in the longitudinal axes of differentiating muscle cells in vertebrates and invertebrates (Auber,

1962; Okazaki and Holtzer, 1965; Fischman, 1967). In this paper, the simultaneous disruption of microtubules and the alteration of cell shape and myofibril orientation in myotubes of regenerating striated muscle of *Rana pipiens* tadpoles treated with colchicine in vivo is reported. In addition, observations on the effect of colchicine in vivo on the differentiation of another type of striated muscle, the abdominal molting muscles of an insect, *Rhodnius prolixus* Stål, are presented.

The observations support the hypotheses that (*a*) microtubules are essential to the formation of the longitudinal axis of the myotube during myogenesis, and (*b*) microtubules are not specifically required for the assembly of individual myofibrils.

MATERIALS AND METHODS

Rana pipiens

Rana pipiens tadpoles were raised in the laboratory following techniques suggested by Rugh (1962). Adult male and female frogs were obtained from J. M. Hazen, Alburg, Vermont. Eggs were stripped from female frogs which had been injected with pituitary glands, and were artificially fertilized. The tadpoles were raised in springwater at 20°C and were fed boiled, fresh spinach. Tadpoles 15–20 mm long were used for the study of tail muscle regeneration. For each experiment, a group of tadpoles of approximately the same length was selected, and half the tail of each tadpole was amputated with a razor blade while the unanesthetized tadpole was trapped in a drop of springwater on a wax surface. The tadpoles were placed briefly in 200% Holtfreter's solution to stop bleeding and were then returned to springwater at 20°C. Feeding with fresh spinach was continued and the water changed daily. Under these conditions, there was no mortality from the operation, and tail regeneration began immediately. For colchicine experiments, tadpoles which had had their tails amputated on the same day were divided into two groups: colchicine was added in concentrations between 10^{-5} and 10^{-3} M for 12–24 hr to the springwater of one group, while the other group was kept as a control. Preparation of tissues for electron microscopy is described below.

Rhodnius prolixus

Rhodnius prolixus undergoes five nymphal molts before reaching the adult stage of development. Each molt is triggered by the ingestion of a blood meal. The abdominal intersegmental muscles, or molting muscles, of *Rhodnius* nymphs display a repeated differentiation and dedifferentiation correlated with the molting cycle of the nymph (Wigglesworth, 1956). Between molts, when the nymph is denied a blood meal, the molting muscle fibers are present, but the sarcoplasm is undifferentiated and lacks myofibrils. As soon as a blood meal is taken, the muscles begin to differentiate, and by the time of molting (about 15 days after feeding in fourth stage nymphs at 25°C) a full complement of striated myofibrils is present. The muscles are functional only during molting, and begin to dedifferentiate immediately after molting. They remain undifferentiated until the next blood meal is taken.

The *Rhodnius* nymphs were kept in a constant temperature room at 25°C. Groups of nymphs of the same stage were cultured in small glass jars with screen-wire tops, and the nymphs were given a blood meal by inverting the jars on the shaved belly of a rabbit.

For studying the effect of colchicine upon muscle differentiation, a group of starved fourth stage nymphs was fed to initiate myogenesis. Colchicine, dissolved in insect Ringer solution (Ephrussi and Beadle, 1936), was injected into some of the nymphs of this group before or after feeding. The remainder of the group served as untreated controls or were given an injection of the insect Ringer without colchicine. Glass microneedles attached to a motor-driven syringe filled with an inert damping oil were used for giving injections of 1-μl volume through the soft cuticle at a leg joint of a nymph anesthetized by water immersion.

Fixation Schedules

The first fixation of the *Rana pipiens* and *Rhodnius prolixus* tissues was at room temperature in 3–6% glutaraldehyde in 0.05–0.1 M cacodylate buffer at pH 7.2–7.3 with 0.025 M $CaCl_2$ added. The *Rana* tissue was fixed for 1 hr; the *Rhodnius* for ½ hr. The tissues were rinsed in 0.1 M cacodylate buffer at pH 7.2 with 10% sucrose for 60 min (*Rana*) and 15 min (*Rhodnius*). At this point, the *Rana* tissues were cooled to 4°C, and maintained at that temperature through the dehydration stages. The *Rhodnius* tissues were kept at room temperature throughout. Postfixation was carried out in 1% osmium tetroxide in 0.1 M Sorenson phosphate buffer at pH 7.2–7.3 for 60 min (*Rana*) and 15 min (*Rhodnius*). The tissues were dehydrated in ethanol and embedded in Epon 812. Thick sections were stained with toluidine blue and azure II, and thin sections (cut with a diamond knife on a Porter-Blum MT-2 microtome) were stained with 0.5% uranyl acetate in 50% ethanol for 30 min and with lead citrate for 5–10 min. The thin sections were examined in either an RCA EMU 3-f or a Siemens Elmiskop I electron microscope.

OBSERVATIONS

Rana pipiens

The regeneration of the tadpole tail muscle of *Rana pipiens* was first studied in detail by Speidel (1937). Hay (1962, 1963) has described the fine structure of myoblast differentiation in the regeneration blastemas of amputated limbs and tails of amphibians.

Regeneration of the tadpole tail occurs most rapidly in the region around the notochord, which begins to elongate within a day after amputation of the distal half of the tail. A regeneration blastema forms around the notochord, and by the 3rd day of regeneration numerous mononucleate, spindle-shaped myoblasts are present. These fuse with one another to form slender, multinucleate myotubes oriented in the longitudinal axis of the regenerating tail. On the 5th day of regeneration, the tail has elongated 1½–2 mm around the notochord (Fig. 1, inset). A longitudinal section of the regeneration blastema adjacent to the notochord (Fig. 1) reveals myotubes containing myofibrils visible in the light microscope, spindle-shaped myoblasts, and undifferentiated, fibroblast-like cells. Many of the mononucleated cells are undergoing mitosis.

Electron micrographs of the myotubes at this stage reveal developing myofibrils, free filaments, and microtubules, all longitudinally aligned in the peripheries of the myotubes (Fig. 3). Fig. 5 shows portions of a myotube and two myoblasts cut in cross-section. Slender myofibrils, together with free, thin filaments and microtubules are seen in the cortex of the myotube running perpendicular to the plane of section. While a few microtubules may be closely associated with the developing myofibrils in the myotube, the majority of the microtubules are distributed in the cortical sarcoplasm without apparent relation to the myofibrils. The adjacent myoblasts contain longitudinally aligned, cortical microtubules and free, thin filaments. Free, thick filaments and myofibrils are rarely observed in the myoblasts.

The effect of colchicine on the differentiating muscle cells of the regeneration blastema was studied on the 5th day of tail regeneration, when several myotubes have formed. Tadpoles placed in spring water containing 10^{-3} M colchicine for 24 hr are able to swim normally during and immediately after the treatment. However, they become immobile and die within 4 days after their return to plain springwater with fresh food. At the end of the 24-hr colchicine treatment, tail regeneration has stopped. Electron microscope examination shows that essentially all the microtubules have disappeared from the myoblasts and myotubes in the regeneration blastema.

The effects of a lower dose of colchicine, 5×10^{-4} M for 12 hr, can usually be reversed if the tadpoles are returned to springwater and fed. Tail regeneration resumes after a short lag period, and normal growth and development follow. A few tadpoles were allowed to develop to normal metamorphosis several weeks after this colchicine treatment.

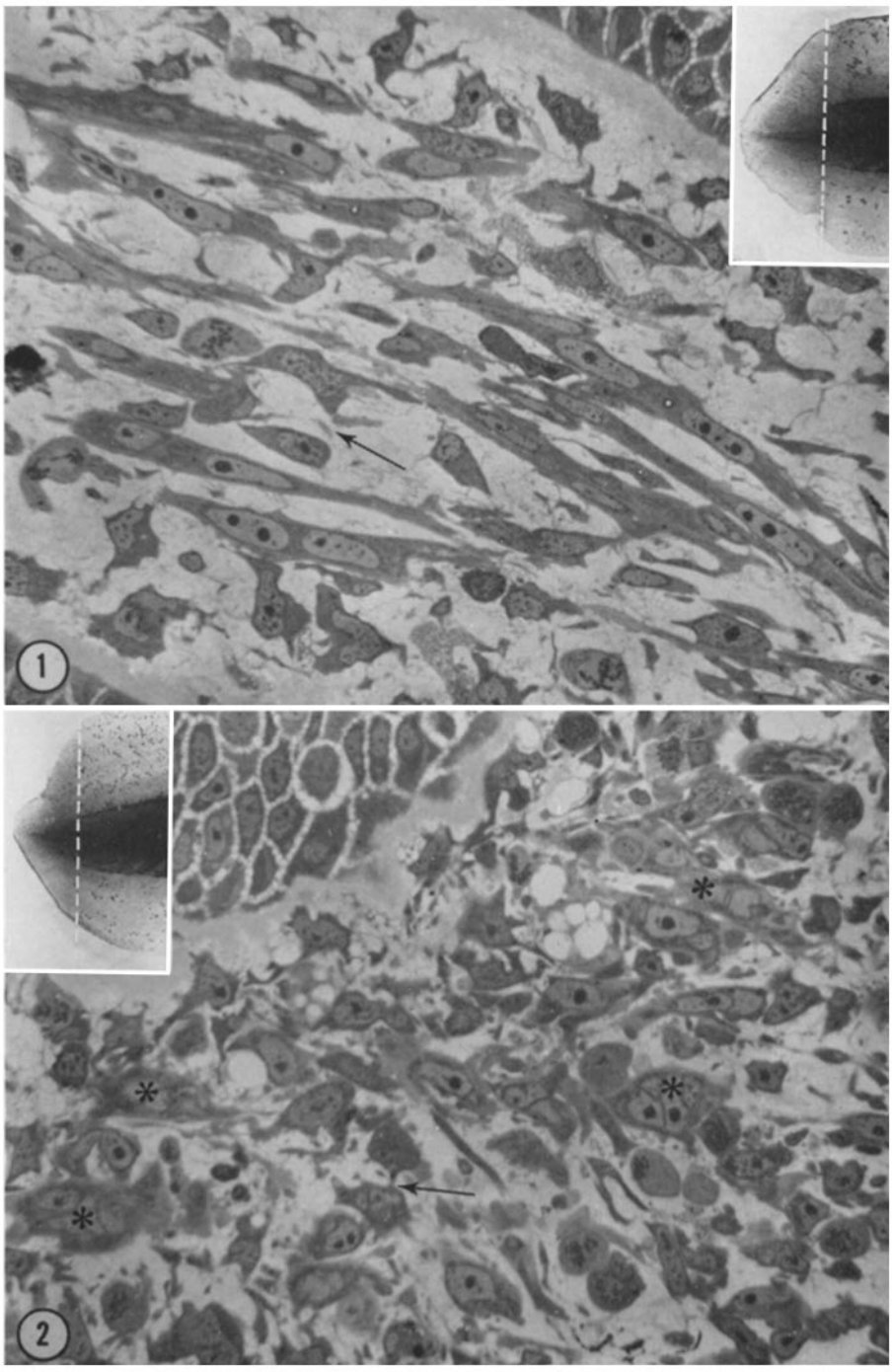
A comparison of the numbers of microtubules appearing in myosacs in tadpoles treated with the lower dose of colchicine, 5×10^{-4} M for 12 hr and in myotubes of control tadpoles was made in the following manner. Cross-sectional micrographs of myosacs and myotubes were printed at the same

FIGURE 1 Longitudinal section through the regeneration blastema around the notochord after 5 days of tail regeneration. Long, slender, multinucleate myotubes, spindle-shaped, mononucleate myoblasts, and undifferentiated fibroblast-like cells are present. The arrow indicates the longitudinal axis of the tail tip. × 485.

The inset shows the regenerating tail from which this section was taken. The intact myotomes are at the right. The area at the left of the dotted line is the regenerated portion of the tail. × 8.

FIGURE 2 Longitudinal section through the regeneration blastema around the notochord after 5 days of tail regeneration, including 12 hr of immersion in 5×10^{-4} M colchicine prior to fixation. The longitudinal anisometry of the cells in the regeneration blastema is greatly reduced, and several multinucleate myosacs (*) are present. The arrow indicates the longitudinal axis of the tail tip. × 485.

The inset shows the colchicine-treated, regenerating tail from which this section was taken, with the regenerated portion at the left of the dotted line. × 8.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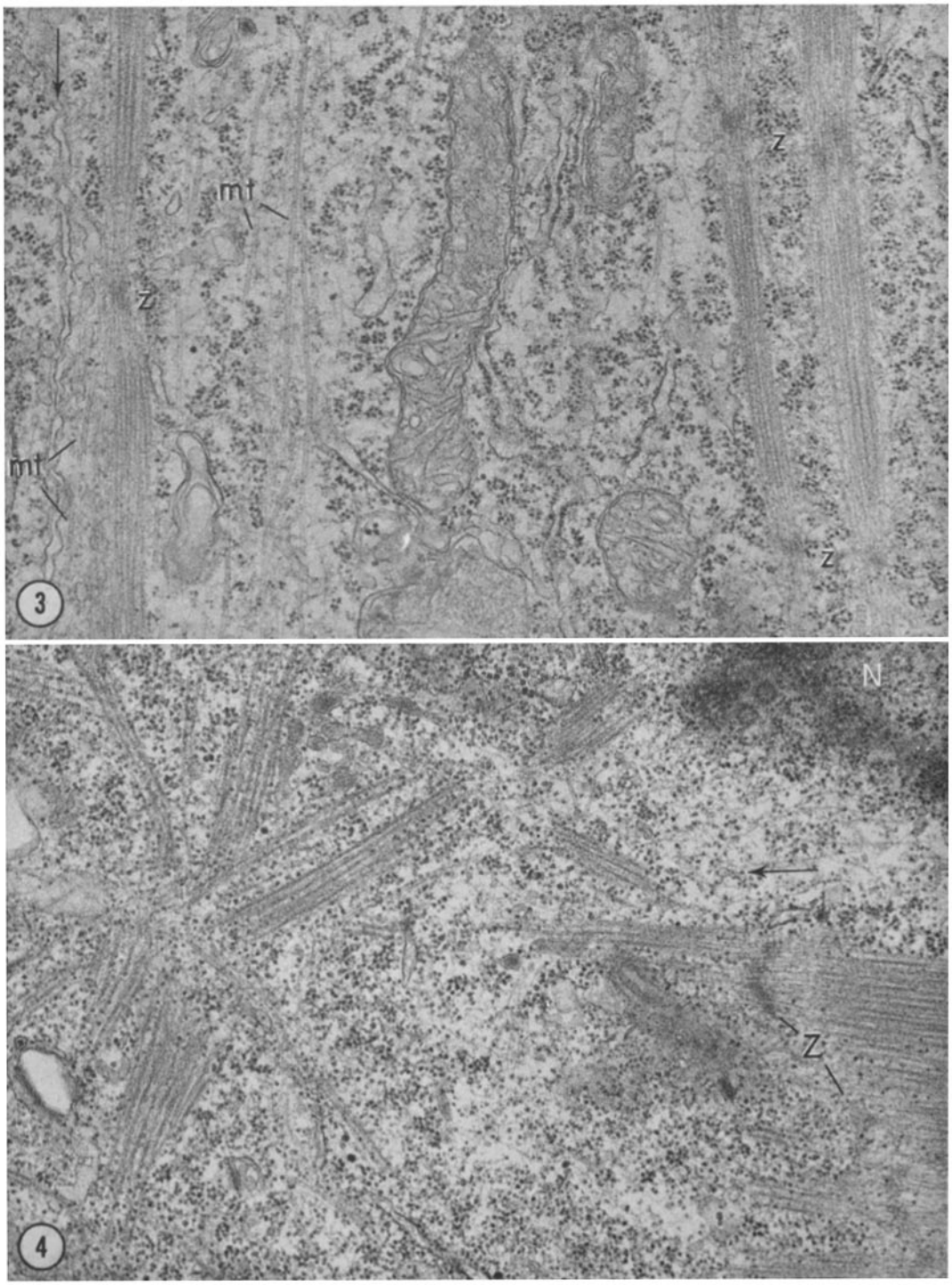


FIGURE 3 Longitudinal section through the peripheral region of a myotube in the specimen shown in Fig. 1, with the longitudinal axis oriented vertically. Three slender myofibrils, as well as several cortical microtubules (*mt*), are oriented in the longitudinal axis. The arrow indicates a region of close contact between a myoblast at the left and the myotube at the right. *Z*, Z bands. × 32,000.

FIGURE 4 Longitudinal section of a myosac in the colchicine-treated specimen shown in Fig. 2. Microtubules are absent. The arrow indicates the longitudinal axis of the tail tip. A large myofibril at the right retains its longitudinal orientation, while the smaller myofibrils at the left are randomly oriented. *N*, nucleus; *Z*, *Z* band. × 32,000.

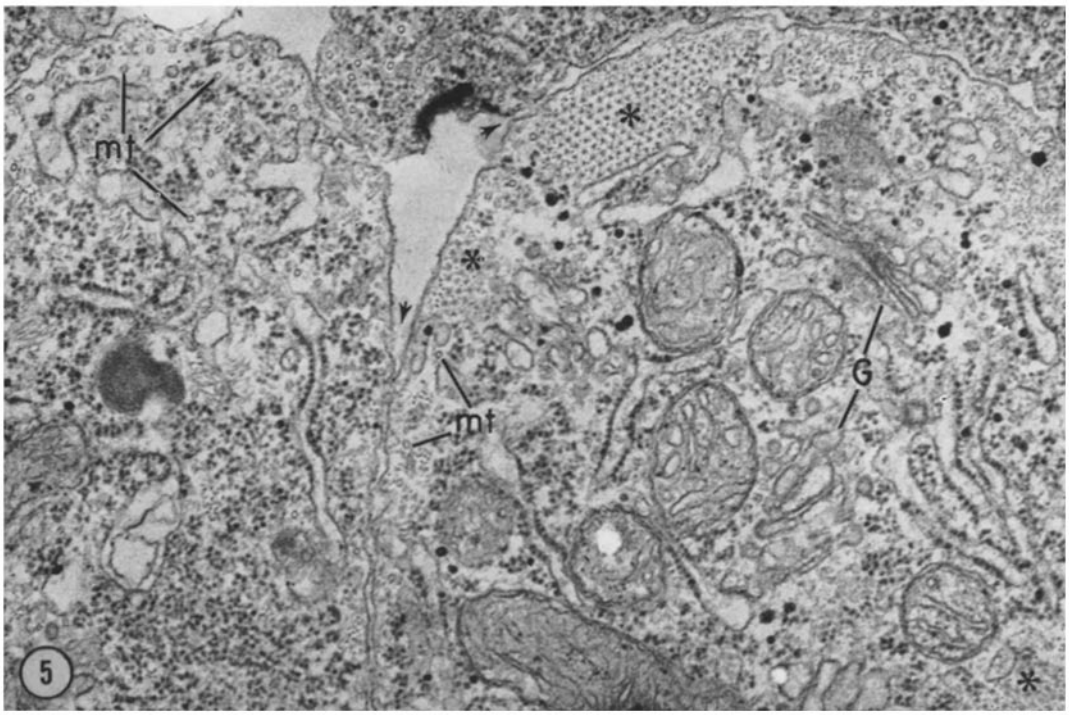


FIGURE 5 Cross-section of a myotube (right) and myoblasts (top and left) in a 5-day regeneration blastema in the tadpole tail. The arrowheads indicate close-contact regions between the myoblasts and myotube. Microtubules (*mt*) and thin myofilaments are present in the cortices of both cell types, while organized myofibrils (*) appear only in the cortex of the myotube. *G*, Golgi apparatus. × 40,000.

magnification on the same grade of photographic paper. The microtubules appearing in the sarcoplasmic areas of six colchicine-induced myosacs and six control myotubes were counted, and then these sarcoplasm areas were cut out and weighed so that the number of microtubules present in a cross-sectional area of sarcoplasm could be determined. A total of 287 microtubules was counted in the colchicine-treated and control specimens. The ratio of microtubules present in cross-sections of the former versus the latter was 1:16, representing a reduction of over 90% in microtubules of the colchicine-treated cells.

Although the higher dose of colchicine is more effective in disrupting microtubules, the morphological changes in the cells of the regeneration blastema are nearly the same in each case. There are an increase in the number of metaphase figures observed in the epithelium and regeneration blastema of the tail and a reduction in the length of the regenerated portion of the tail in comparison with control tadpoles (Figs. 1 and 2, insets). The most striking change in the colchicine-treated tadpoles is the loss of longitudinal anisometry of the myoblasts and myotubes in the regeneration blastema (Fig. 2). Multinucleate myosacs are present, in which the smaller myofibrils are randomly oriented (Figs. 4 and 6). The myofibrils at the ends of the remaining myotubes are disoriented, and there are frequent bulges along these myotubes which contain similarly disoriented myofibrils. The myoblasts have lost their spindle shape, and the myofilaments in them are randomly oriented.

These observations are similar to those of Godman and Murray (1953) and Bischoff and Holtzer (1968) on colchicine-treated myotubes in tissue culture, although those investigators were able to use much lower concentrations of colchicine in their in vitro systems. They observed that 10^{-6} M colchicine induced bulges and local swellings in myotubes after 2–5 hr and caused complete fragmentation of myotubes after 24 hr.

The sarcoplasm and organelles of the differentiating muscle cells in the regeneration blastema

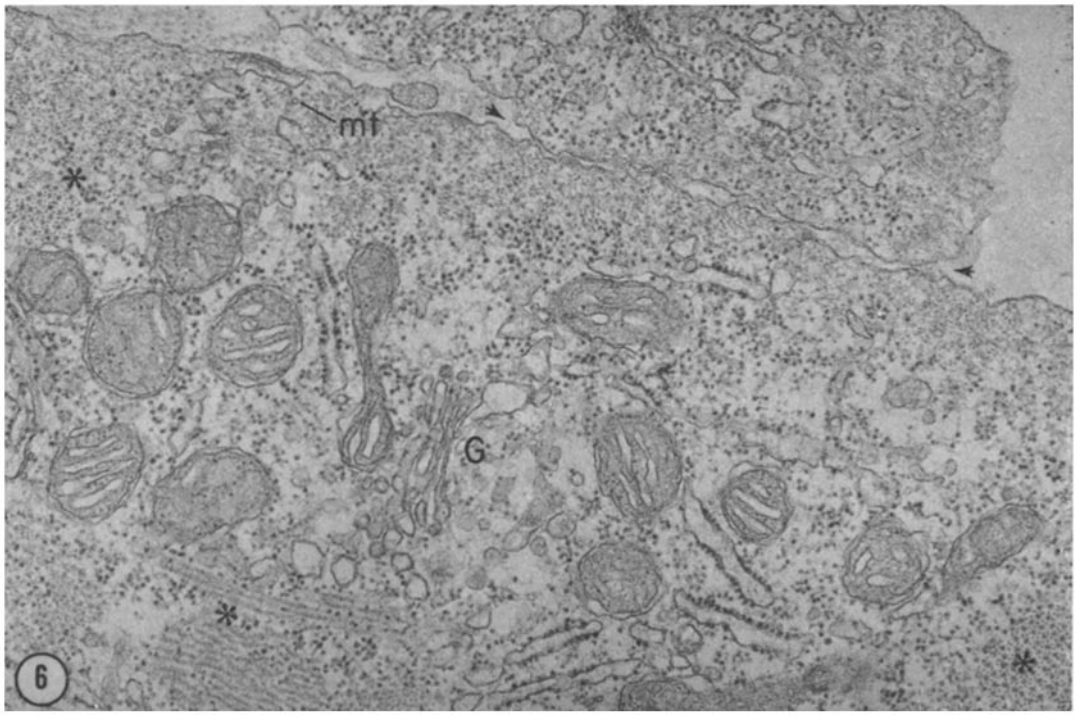


FIGURE 6 Cross-section through the periphery of a myosac in the colchicine-treated specimen shown in Fig. 1. One microtubule (*mt*) appears in the cortex of the myosac; only six microtubules appeared in the entire cross-section of this large myosac. The myofibrils (*) are oriented in both longitudinal and transverse axes. The arrow heads indicate a region of close contact with an adjacent myoblast (top). *G*, Golgi apparatus. × 36,000.

appear in good condition after treatment of the tadpole with 5×10^{-4} M colchicine for 12 hr. The microtubules and myofibrils appear to be the only organelles markedly affected by the colchicine treatment. The mature muscle fibers in the tail proximal to the regeneration zone appear normal, as would be expected from the swimming activity of the colchicine-treated tadpoles. Godman and Murray (1953) noted earlier that myotubes of older, more mature muscle cultures resisted fragmentation by colchicine.

Rhodnius prolixus

The developmental cycle of the abdominal molting muscles in *Rhodnius* was first studied with the light microscope by Wigglesworth (1956). Auber-Thomay (1967) and Warren and Porter (in press) have studied myofilament deposition in these muscles with the electron microscope.

The molting muscle fibers are longitudinally oriented on the ventral cuticle of the abdomen of the insect, and each fiber extends the length of a cuticular segment of the abdomen. The full length of a fiber is maintained throughout the developmental cycle by the attachments of the ends of the fiber to the epidermal cells lining the cuticular exoskeleton of the insect. The undifferentiated muscle fiber contains a longitudinal row of nuclei, but little sarcoplasm and no myofibrils. The fiber usually contains, however, a few small, longitudinally oriented, dense bundles of 50–70-A fine filaments of undefined nature. These filament bundles are usually found in the cortex of the fiber, adjacent to small plaques of amorphous dense material on the sarcolemma. The surface of the fiber displays prominent longitudinal outfoldings.

Muscle differentiation begins when the nymph takes a blood meal. A few myofilaments are present by 10 hr after feeding, and by 24 hr (Fig. 7) many

longitudinally-oriented groups of thick and thin myofilaments are present in the longitudinal outfoldings of cross-sectioned fibers. It should be noted that the new myofilaments are preferentially deposited near the complexes of 50–70-A fine filament bundles and sarcolemmal dense plaques. Such complexes are illustrated in Figs. 7 and 8.

After the 1st day of differentiation, the number of myofilaments increases rapidly, and striated myofibrils are present on the 5th day.

The effect of colchicine on differentiation of the molting muscles was studied in starved fourth stage nymphs which were fed and then immediately injected with 1 μl of insect Ringer's containing colchicine. In order to determine the dependence of toxicity of colchicine upon the amount injected, an initial experiment was done in which the concentration of colchicine in the injection was changed from 10^{-2} to 10^{-7} M in tenfold dilution steps. Three or four nymphs were injected at each dilution step. Although all nymphs appeared normal at 24 hr after the injection, those receiving 10^{-2} and 10^{-3} M injections were immobilized within 4 days and five of six died within 10 days. Those receiving 10^{-4} M injections appeared sluggish after 4 days, but three of four recovered and later molted normally. Injections of 10^{-5} M colchicine or lower caused no obvious ill effects.

The dye dilution technique of Yeager and Munson (1950) was used to estimate the blood volume of fourth stage *Rhodnius* nymphs. 1 μl of a solution of amaranth red dye in insect Ringer's was injected into three each of starved and freshly fed fourth stage nymphs. After allowing the dye to circulate in the blood for 15 min, a 1 μl sample of blood was withdrawn in a capillary tube and compared with a series of capillary tubes containing known dilutions of the dye solution used for injection. The approximate blood volume of both groups of nymphs was 15 μl as determined by this method. Thus, the immediate dilution in the blood of an injection of 1 μl of colchicine solution can be estimated, but the amount of colchicine remaining in the blood over a 24-hr period is unknown.

The effectiveness of colchicine in disrupting microtubules in the molting muscle fibers was determined in muscle fibers fixed 24 hr after the nymphs were fed and injected with colchicine. It was found that injections of 10^{-2} M colchicine were required to remove most of the microtubules from the muscle fibers over this 24-hr period. A comparison of the number of microtubules appearing in the muscle fibers of these colchicine-treated nymphs with the number of microtubules appearing in muscle fibers of control nymphs was made by using the same method described in the previous section of the paper on the *Rana pipiens* muscles. A total of 424 microtubules was counted in equal numbers of colchicine-treated and control muscle fibers. The ratio of microtubules appearing in the former versus the latter was 1:35, indicating a loss of about 97% of the microtubules from the colchicine-treated fibers.

The injection of 1 μl of 10^{-2} M colchicine, followed by the disruption of the microtubules, does not significantly affect the initial deposition of the myofilaments in the molting muscles during the first 24 hr of differentiation. The thick and thin myofilaments are present in their usual number and longitudinal orientation in muscle fibers fixed at the end of the 24-hr colchicine treatment (Fig. 8). The typical association of groups of myolfilaments with dense plaques on the sarcolemma is also observed. The only difference noted in the pattern of myofilament deposition after colchicine treatment is that the 50–70-A fine filament bundles are often slightly larger than usual, and the myofilaments are generally grouped in a more orderly pattern around them (Fig. 8).

The time course of microtubule disruption after injection of 10^{-2} M colchicine was investigated, and it was found that there was no significant reduction in number of microtubules in the muscle fibers 4 hr after feeding and injection. After 9 hr, the ratio of microtubules present in colchicine-treated specimens versus controls was 1:6, based on a total count of 196 microtubules. Thus, although there was a time lag before the microtubules disappeared, approximately 80% of the microtubules disappeared before the myofilaments began to form 10 hr after feeding. In one experiment, 1 μl of 10^{-2} M colchicine was injected 6 hr before the nymphs were fed. Feeding activity was unaffected by the colchicine injection, and myofilament disposition was unaltered 24 hr after feeding.

The toxic effect of an injection of 10^{-2} M colchicine was detected in the molting muscle fibers by 48 hr after the injection, when the myofilament number was lower than in controls, and when the sarcoplasm showed limited degenerative areas. Injection of 10^{-3} M colchicine produced fewer

toxic effects, but was also less effective in removing microtubules. The pattern of myofilament deposition at this dose was identical to that in controls. Among control of muscle fibers, there was no difference between those from untreated nymphs and those from nymphs which received injections of insect Ringer's without colchicine.

DISCUSSION

The observations presented here show that the specific effect of colchicine at the cellular level in myogenesis in two phylogenetically separate organisms is the disruption of cytoplasmic microtubules. This is in accord with the observations of the effect of colchicine on fine structure of other types of cells (cited in the introduction), and parallels the biochemical work of Borisy and Taylor (1967) and Shelanski and Taylor (1967), who demonstrated the binding of colchicine to protein extracts from a wide variety of cells known to contain large numbers of microtubules.

The loss of longitudinal anisometry of the myoblasts and myotubes in the colchicine-treated tadpole tail is attributed to the disruption of the longitudinally oriented microtubules in these cells. There is now a large body of evidence, recently summarized by Porter (1966), that microtubules are cytoskeletal elements, and that they are particularly prominent in cells which are undergoing changes in shape. Among many examples are the descriptions of oriented microtubules in elongating sperm cells (Burgos and Fawcett, 1955), chick lens cells (Byers and Porter, 1964), and primary mesenchyme cells in the sea urchin embryo (Tilney and Gibbons, 1966), in the microspikes of HeLa cells (Taylor, 1966), in the developing flagella of a water mold (Renaud and Swift, 1964), and in a protozoan during ameba-to-flagellate transformation (Outka and Kluss, 1967).

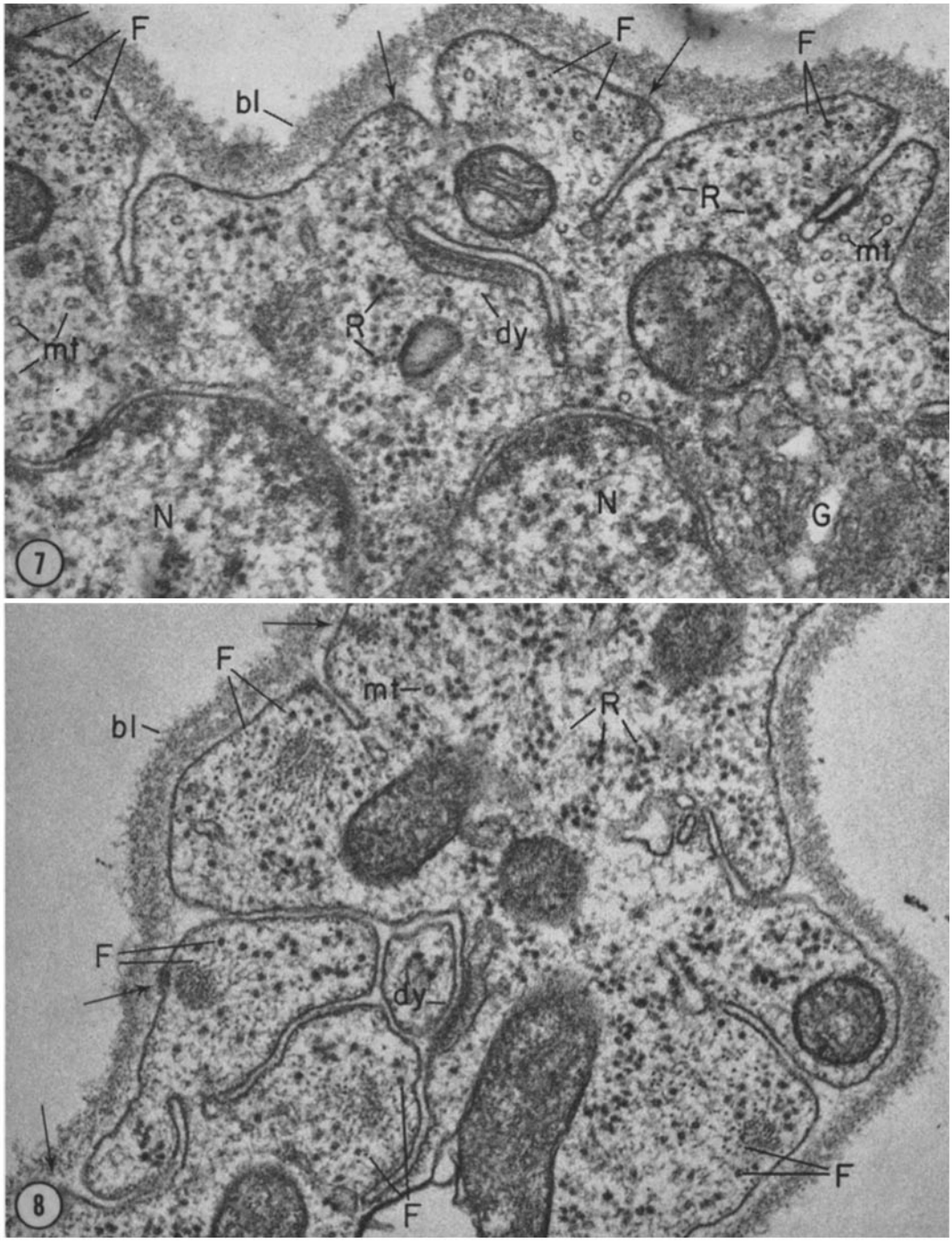
The effect of colchicine on differentiation of the molting muscles in *Rhodnius* provides evidence that microtubules do not directly interact with myofilaments during the initial stages of myofibrillar organization. In this highly specialized muscle system, the longitudinal anisometry of the muscle fibers is maintained throughout the developmental cycle by the myo-epidermal insertions of the fibers on the rigid abdominal cuticle. Hence, the effect of colchicine treatment can be studied without causing changes in the lengths of the muscle fibers. Since the general pattern of cortical myofilament deposition and orientation is relatively unchanged after colchicine treatment, it is concluded that these processes operate independently of the colchicine-sensitive system.

The specific association of newly deposited myofilaments with 50–70-A filament bundles and sarcolemmal dense plaques suggests that these latter structures are important in the organization of the developing myofibrils. In previous studies of the *Rhodnius* molting muscles (Auber-Thomay, 1967; Warren and Porter, in press), it has been shown that the 50–70-A filament bundles in the undifferentiated muscle fibers are derived from degenerating Z bands during the degradation of the myofibrils after the previous molt. Most of the filament bundles persist in the undifferentiated muscle fibers until the nymph takes another blood meal, and they are re-incorporated into new Z bands in the differentiating muscles. Since both the 50–70-A filament bundles and the Z bands in the differentiating and mature muscle fibers are associated with similar plaques of amorphous dense

FIGURE 7 Cross-section of a *Rhodnius* molting muscle fiber after 24 hr of differentiation. Thick and thin myofilaments (*F*) are present in the cortex adjacent to small bundles of 50-70-A filaments and amorphous dense plaques on the sarcolemma (arrows). Microtubules (*mt*) are distributed throughout the sarcoplasm. × 61,000.

On Figs. 7 and 8: *N*, nucleus; *G*, Golgi apparatus; *bl*, basement lamella; *dy*, dyad; *R*, ribosomes.

FIGURE 8 Cross-section of a molting muscle fiber after 24 hr of differentiation in a nymph which received an injection of 1 μl of 10^{-2} M colchicine immediately after feeding. Most of the microtubules have disappeared, although one (*mt*) may be seen in this field. The number and orientation of the thick and thin myofilaments (*F*) are undisturbed. The 50–70-A filament bundles associated with the myofilaments are larger than usual in this specimen; arrows indicate filament bundles contiguous with dense plaques on the sarcolemma. × 58,000.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material on the sarcolemma, it seems likely that the complexes of 50–70-A filament bundle and sarcolemmal dense plaque function as organizing centers for the initial myofilaments, and serve to anchor the developing myofibrils to the sarcolemma.

In the regenerating tadpole tail muscle of *Rana pipiens*, the microtubules are distributed throughout the cortices of the elongating muscle cells and are not preferentially associated with the myofilaments or myofibrils. Hence it appears that the microtubules do not directly support or align the myofilaments and myofibrils in this system, and the disorientation of these elements after colchicine treatment is interpreted at this time to be an indirect consequence of the changes induced in cell shape. However, further analysis of the factors which govern myofibril development in the cortex of the myotube may show that the microtubules, as well as supporting cell elongation, also have more specific functions in the organization of the cortical sarcoplasm.

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The material presented here will be included in a thesis which will be submitted by Mr. Warren in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Harvard University.

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REFERENCES

AUBER, J. 1962. Mode d'accroissemont des fibrilles au cours de la nymphose de *Calliphora erythrocephala*. *Compt. Rend. Acad. Sci. Paris.* **254**:4074.

AUBER-THOMAY, M. 1967. Modifications ultrastructurales au cours de la dégénérescence et de la croissance de fibres musculaires chez un insecte. *J. Microscop.* **6**:627.

BISCHOFF, R., and H. HOLTZER. 1968. The effect of mitotic inhibitors on myogenesis in vitro. *J. Cell Biol.* **36**:111.

BORISY, G. G., and E. W. TAYLOR. 1967. The mechanism of action of colchicine. *J. Cell Biol.* **34**:525.

BURGOS, M. H., and D. W. FAWCETT. 1955. Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids in the cat (*Felis domestica*). *J. Biophys. Biochem. Cytol.* **1**:287.

BYERS, B., and K. R. PORTER. 1964. Oriented microtubules in elongating cells of the developing lens rudiment after induction. *Proc. Natl. Acad. Sci.* **52**: 1091.

EPHRUSSI, B., and G. W. BEADLE. 1936. A technique of transplantation for *Drosophila*. *Am. Nat.* **70**:218.

FISCHMAN, D. A. 1967. An electron microscope study of myofibril formation in embryonic chick skeletal muscle. *J. Cell Biol.* **32**:557.

GODMAN, G. C., and M. R. MURRAY. 1953. Influence of colchicine on the form of skeletal muscle in tissue culture. *Proc. Soc. Exptl. Biol. Med.* **84**:668.

HAY, E. D. 1962. Cytological studies of dedifferentiation and differentiation in regenerating amphibian limbs. *In* Regeneration. D. Rudnick, editor. Ronald Press, New York.

HAY, E. D. 1963. The fine structure of differentiating muscle in the salamander tail. *Z. Zellforsch.* **59**:6.

MALAWISTA, S. E., and K. G. BENSCH. 1967. Human polymorphonuclear leukocytes: Demonstration of microtubules and the effect of colchicine. *Science.* **156**:521.

OKAZAKI, K., and H. HOLTZER. 1965. Aspects of myogenesis *in vitro*. *J. Cell Biol.* **27**:75A. (Abstr.)

OUTKA, D. E., and B. C. KLUSS. 1967. The ameba-to-flagellate transformation in *Tetramitus rostratus*. II. Microtubular morphogenesis. *J. Cell Biol.* **35**:323.

PORTER, K. R. 1966. Cytoplasmic microtubules and their function. *In* Principles of Biomolecular Organization. G. E. W. Wolstenholme and M. O'Connor, editors. Churchill, London. 308–356.

RENAUD, F. L., and H. SWIFT. 1964. The development of basal bodies and flagella in *Allomyces arbusculus*. *J. Cell Biol.* **23**:339.

ROBBINS, E., and N. K. GONATAS. 1964. Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell. *J. Histochem. Cytochem.* **12**:704.

RUGH, R. 1962. Experimental Embryology. Burgess, Minneapolis.

SHELANSKI, M. L., and E. W. TAYLOR. 1967. Isolation of a protein subunit from microtubules. *J. Cell Biol.* **34**:549.

SPEIDEL, C. C. 1937. Studies of living muscles. I. Growth, injury, and repair of striated muscle, as revealed by prolonged observations of individual fibers in living frog tadpoles. *Am. J. Anat.* **62**:179.

TAYLOR, A. C. 1966. Microtubules in the microspikes and cortical cytoplasm of isolated cells. *J. Cell Biol.* **28**:155.

TILNEY, L. G. 1965. Microtubules in the asymmetric

arms of *Actinosphaerium* and their response to cold, colchicine, and hydrostatic pressure. *Anat. Record.* **151**:426 (Abstr.).

TILNEY, L. G., and J. R. GIBBINS. 1966. Microtubules and morphogenesis. The role of microtubules in the development of the primary mesenchyme in the sea urchin embryo. *Biol. Bull.* **131**:378 (Abstr.).

WIGGLESWORTH, V. B. 1956. Formation and involution of striated muscle fibers during the growth and moulting cycles of *Rhodnius prolixus*. *Quart. J. Microscop. Sci.* **97**:465.

YEAGER, J. F., and S. C. MUNSON. 1950. Blood volume of the roach, *Periplaneta americana*, determined by several methods. *Arthropoda.* **1**:255.