SYNTHETIC STRANDS OF CARDIAC MUSCLE

Formation and Ultrastructure

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ABSTRACT

Spontaneously active bundles of cardiac muscle (synthetic strands) were prepared from isolated cells of 11–13-day old embryonic chick hearts which were disaggregated with trypsin. Linear orientation of the cells was obtained by plating them on agar-coated culture dishes in which either grooves were cut in the agar film or a thin line of palladium was deposited over the agar. The influence of cell-to-cell and cell-to-substrate interactions was observed with time lapse cinematography and the formation of the synthetic strand was shown to involve both random and guided cell movements, enlargement of aggregates by accretion and coalescence, and the compact linear arrangement of cells along paths of preferential adhesion. Electron microscope investigations of these strands showed that a dispersed population of heart cells organized into an inner core of muscle cells and an outer sheath of fibroblast-like cells. The muscle cells contained well-developed, but widely spaced myofibrils, a developing sarcoplasmic reticulum associated in part with the myofibrils and in part with the sarcolemma, an abundance of nonmembrane bound ribosomes and glycogen, and a prominent Golgi complex. Numerous specialized contacts were observed between the muscle cells in the strand, e.g., fasciae adherentes, desmosomes, and nexuses. A distinct type of muscle cell characterized by its pale appearance was regularly observed in the strand and was noted to be similar to Purkinje cells described in the adult avian conduction system and in developing chick myocardium. The present findings were compared with other observations of the developing myocardium, *in situ*, and it was concluded that, by a number or criteria, the muscle cells of the strand were differentiating normally and suitably organized for electrophysiological studies.

INTRODUCTION

The complex geometry of naturally occurring cardiac muscle limits our ability to isolate the extent to which excitation, conduction, and contraction are specifically related to the membrane properties, intercellular connections, and contractile apparatus of individual cardiac muscle cells (1). Similar problems are unavoidably encountered when cells from embryonic and neonatal hearts are grown to confluency in tissue culture and so consist of a heterogeneous population of cells, randomly organized in mono- or multilayers (2–7). Consequently, an attempt was made to develop a suitable preparation of cardiac muscle for electrophysiological analysis by orienting the growth of heart cells in tissue culture into a linear array or "synthetic strand" (8). The methods used to promote the formation of the synthetic strand were based on the observations

that (*a*) dissociated cells from embryonic ventricles could reassemble and organize as spherical aggregates composed of a myocardial core and peripheral sheath of fibroblasts (9), and (*b*) isolated cells could be aligned within grooves (10, 11) and along lines of deposited palladium (12). Action potentials and conduction velocities comparable to those of intact cardiac muscle were recorded from synthetic strands which contained many aligned muscle cells (8).

The present study was initiated to elucidate details of the formation and ultrastructure of this new preparation of cardiac muscle. To date, attempts to characterize the morphologic details and extent of cellular development in cultures from trypsin-dissociated embryonic chick hearts (13–17) have neither been systematic nor as extensive as those from neonatal rat hearts (18–23). Therefore, special consideration is given to the spatial orientation of cells and the degree of differentiation of the myofibrils, the sarcoplasmic reticulum, and junctional specializations. The relationships of the different cell types present in the synthetic strand are also investigated and thus contribute to the limited number of electron microscope studies concerned with "sorting-out" of cardiac cells (14, 16).

MATERIALS AND METHODS

Growth Orientation

The methods for growing isolated heart cells in linear arrays have been described in detail elsewhere (8). In brief, the growth orientation procedure was as follows. Hearts were dissected aseptically from chick embryos incubated 11–13 days, minced in warm saline G, and disaggregated in 0.1% trypsin in saline G at 37°C for 10 min. The trypsinization was stopped by adding cold culture medium and the cell suspension was then filtered, centrifuged at 260 *g* for 5 min, and the pellet was resuspended in fresh culture medium lacking embryo extract. Cells were plated at known densities (10^5–10^6 cells per 60 mm culture dish) on differentially treated surfaces. The cultures were incubated at 37°C in a humidified chamber containing 5% CO_2/95% air. The final culture media contained Eagle's Minimal Essential Medium (MEM) (Grand Island Biological Co., Grand Island, N.Y.) or Medium 199 supplemented with 10% fetal calf serum, 2% chick embryo extract, and a 1% mixture of penicillin G (100 units/ml) and streptomycin sulfate (50 μg/ml). Some of the cultures were grown in Medium 199 which had been conditioned by dissociated chick heart cells (8). Culture dishes were coated with rat tail collagen which was subsequently dried, overlaid with 2% washed agar (purified, Difco Laboratories, Inc., Detroit, Mich.), and then dried to a film. Differential surfaces were obtained either by pulling a scalpel blade through the agar film to form channels approximately 25 μ wide or by placing a template over the agar base and depositing a thin film of palladium by vacuum evaporation, thereby forming lines about 25 μ wide on the agar surface. The prepared culture dishes were sterilized by UV light and stored at room temperature. Since both methods produced preparations with similar configurations, the results are concerned chiefly with those strands formed in the channels.

Time Lapse Cinematography

The development of the preparation was observed with time lapse and normal speed photography. For these studies, the surface of the culture dish was placed on a temperature-controlled (37°C) stage of an inverted microscope (Model MS, Nikon) and viewed with a phase objective (16 ×, NA 0.40, Carl Zeiss Inc., New York) and Köhler illumination. A circular, lucite incubator was placed around the condenser and positioned over the culture dish on the microscope stage. The pH of the medium was kept constant by continually streaming a prewarmed mixture of 95% air and 5% CO_2 into the incubator. The time lapse photographic equipment consisted of a cinemicrography system (Model 503, Sage Laboratories, Inc., East Natick, Mass.) and exposure meter (Model 293, Sage Laboratories, Inc.). Time lapse sequences were taken at one frame/min using 16 mm Plus X negative and Plus X reversal film (Eastman Kodak Co., Rochester, N.Y.), exclusively. Periodic filming at normal speed enabled us to document the synchronization of spontaneously beating areas along the strand.

Electron Microscopy

The fixation and embedding procedures were those previously reported by Coon and Manasek (24). Cultures were washed twice in cold Hanks' salt solution and then fixed for 15 min with cold 2.5% glutaraldehyde in 0.025 M sodium cacodylate buffer (pH 7.6). After three, 5-min rinses in cold Hanks' solution, the cultures were postfixed for 1 hr with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.6) at 0°C, dehydrated in graded concentrations of ethanol, and embedded *in situ* in Epon 812. Some cultures were treated with 2% uranyl acetate in maleate buffer for 60 min after osmification. The cultures were infiltrated with Epon for 24 hr at room temperature. After polymerization at 60°C, we found that the preparation, embedded in the Epon sheet, could be snapped free from the culture dish after a short period of cooling in liquid nitrogen.

The strands were then examined with bright-field microscopy and regions of special interest marked, cut out, and reembedded for longitudinal or transverse sectioning. Silver to gold sections were cut with a diamond knife on a Cambridge-Huxley ultramicrotome (Cambridge Instrument Co. Inc., Ossining, N.Y.), mounted on naked 300-mesh copper grids or colloidin-coated 200-mesh grids, and double-stained with saturated uranyl acetate and lead citrate (25). After carbon stabilization, the sections were examined with an AEI-EM6B operated at accelerating voltages of 50 kv or 60 kv.

RESULTS

Formation of the Strand

The formation of the strand was studied with time lapse cinematography to observe the cell-to-cell and cell-to-substrate interactions responsible for the organization and configuration of the preparation. Freshly dissociated cells, either singly or as very small aggregates, settled randomly to the bottom of the dish and preferentially attached within the channels of exposed collagen (Fig. 1) or along lines of deposited palladium. Since agar acts as a poor adhesive substrate (26), the cells which settled initially on the agar formed spherical aggregates with nearby cells. These aggregates, often contracting spontaneously, either detached from the substrate and became suspended in the culture medium or remained stationary. However, when isolated cells and small aggregates settled on the agar close to the orienting surfaces they appeared to move in the direction of the regions of preferential adhesion. Aggregates tended to spread, enlarge by accretion, and coalesce along the orienting substrata (Fig. 1). Specific cell movements were difficult to monitor when cuts in the agar interfered with the phase optics (Fig. 1). However, since this was not a problem with preparations grown on palladium, it was possible to observe the envelopment of nonmotile, contracting muscle cells by fibroblast-like cells which moved either separately or as the leading edge of an aggregate. Such cells within the peripheral layer also appeared to "pull" adjacent aggregates into juxtaposition. In general, the strand attained its final shape within 3–4 days.

Although conditioned medium promoted aggregation and adhesion of cells, a finding similar to those reported by Moscona (27) and Roth (28), the cell growth rate was retarded; doubling time in the log phase was 23 hr in fresh medium as compared with 72 hr in conditioned medium (unpublished observations). In general, the fibroblast-like cells divided much more frequently than the muscle cells. Spontaneous activity was markedly enhanced when the strands were grown in conditioned medium (8).

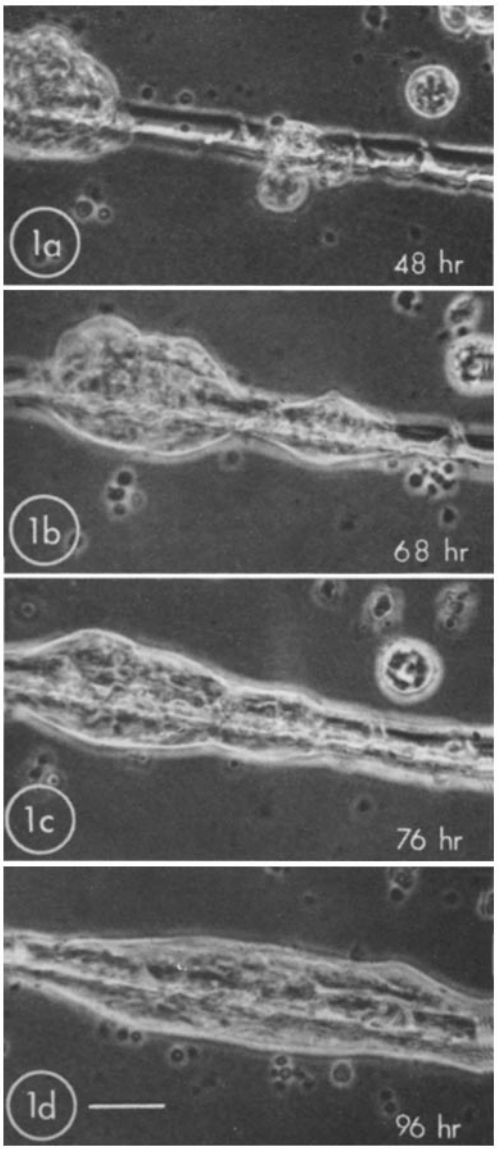


FIGURE 1 Development of actively contracting synthetic strands followed with time lapse cinematography and illustrating the attachment and coalescence of spontaneously beating aggregates along the channel cut in agar. Times shown represent hours after plating of cells. Scale, 25 μ. $\times$ 400.

Evidence that sorting out of homogeneous cell types occurred during the formation of the strand is presented in the section below (see *General Morphology of the Strand*). Cell-specific markers would be required to accurately follow sorting out with time lapse cinematography. For example, Trinkhaus and Lentz (29) were able to follow photographically, at closely timed intervals, the sorting out of mixed aggregates of embryonic chick retinal and heart cells only because the former contained visible melanin granules.

General Morphology of the Strand

In this section we describe the gross appearance of 6–10-day old preparations formed in channels of exposed collagen. The preparations varied in length up to 30 mm and were irregular in width due to the presence of large bulbous segments up to 400 μ in diameter which tapered down to 25 μ between segments (Fig. 2). The cylindrical shape of the preparations is illustrated in Fig. 3. The linear organization of muscle cells within the strand was clearly demonstrated by both polarization and light microscopy; the longitudinal alignment of the myofibrils was evident throughout the length of the strands.

It is apparent in Fig. 3 that the heart cells have sorted out within the strand forming an inner core consisting primarily of muscle cells and an outer sheath of loosely associated nonmuscle cells. Cells with long cytoplasmic processes, shown at the top of the strand in Fig. 3, are reminiscent of the epicardial (30) or endocardial (31) cells of intact embryonic hearts. The two cell populations were separated by a space of 0.5–8.0 μ. The outer sheath of cells varied in thickness from one to several layers and was approximately 8–10 μ in width. The muscle cell content along a strand could vary from regions which contained a thick multicellular core to regions with but a few muscle cells. In the bulbous portion of the strands muscle cells often formed more than one discrete group of closely associated cells internal to the outer sheath. In addition, single cells which had neither become closely associated with the outer sheath nor with the central core were more numerous in the wide intercellular spaces in such regions.

Ultrastructure of the Strand

In this section we categorize the cells within the strand on the basis of ultrastructural criteria and describe in detail the muscle cells because their structural as well as functional properties are of prime concern in physiological studies.

Nonmuscle Cells

The peripheral sheath of the strand contained flattened cells with the extensive lamellae of rough-surfaced endoplasmic reticulum and the long cytoplasmic processes characteristic of fibroblast-like cells (Fig. 3). The cells were generally separated by relatively wide spaces (Fig. 3), and junctional specializations such as those occurring between muscle cells were rarely observed.

Another group of cells was poorly differentiated and could not be definitively classified since they lacked either the well-formed myofibrils or the extensive, lamellar rough-surfaced endoplasmic reticula which characterize muscle cells and fibroblast-like cells, respectively. They often contained large amounts of nonmembrane bound ribosomes, numerous cytoplasmic filaments, and elements of both smooth- and rough-surfaced endoplasmic reticula. Such cells were observed both in the central core and peripheral sheath of the strand.

Muscle Cells

Definitive cardiac muscle cells were distinguished from nonmuscle cells by the presence of myofibrils. They were irregularly shaped and variable in diameter (3–10 μ). On the basis of criteria established by Manasek (32) two distinct types of muscle cells were identified (Fig. 4): one with a cytoplasm dense in appearance was classified as "working muscle" and the other with a pale cytoplasm due to the relative lack of granulations (ribosomes and glycogen) was classified tentatively as conduction tissue or "Purkinje cells". The pale cell types were present in most of the sections examined.

FILAMENTS: Myofibril content in the cells of the strand varied considerably (Figs. 4–7). Although many cells contained numerous well-developed myofibrils, few demonstrated the content typically seen in the embryonic chick heart at comparable developmental stages *in situ* (stage 39 [day 13] to stage 41 [day 15]; 33).

In most cells, the myofibrils appeared to be dispersed throughout the cytoplasm and were aligned with the long axis of the strand. Sarcomere length varied; in general H bands were absent and I bands rarely present. These findings might be attributed to a nonuniform contraction of the

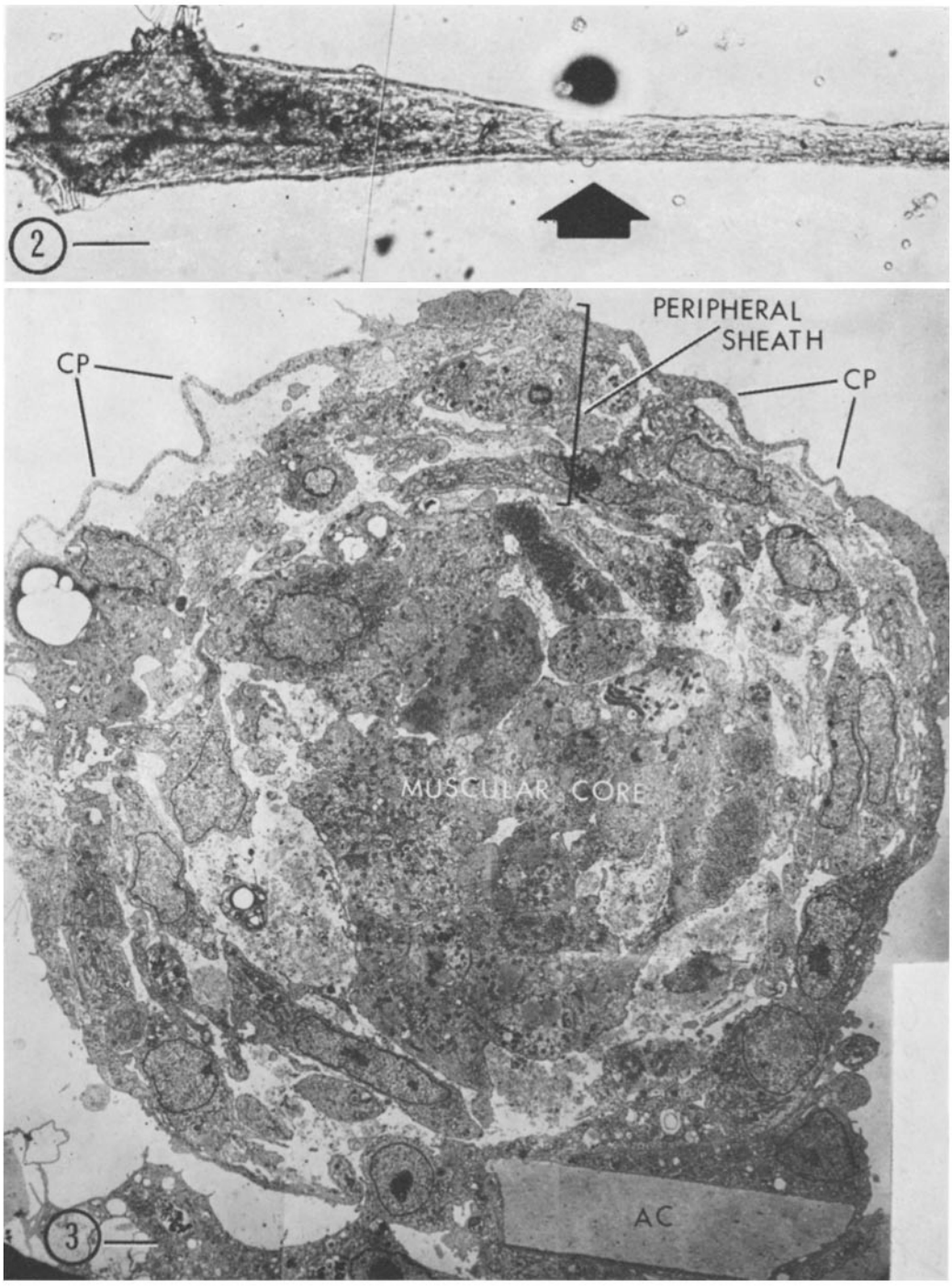


FIGURE 2 Phase contrast photomicrograph of a beating strand formed along a channel cut in agar. The arrow marks the region from which the section shown in Fig. 3 was taken. Scale, 100 μ. $\times$ 110.

FIGURE 3 Low magnification composite of a transverse section of a strand formed along a channel cut in agar showing the segregation of cells within the strand. Note that the central muscular core is separated by a space from a peripheral sheath of fibroblast-like cells. The long cytoplasmic processes (*CP*) of some of these cells are apparent at the upper edge of the strand. The agar chip (*AC*) within the channel is surrounded by cells at the bottom of the micrograph. The strand from which this section was taken is shown in Fig. 2. Scale, 3.0 μ. $\times$ 2440.

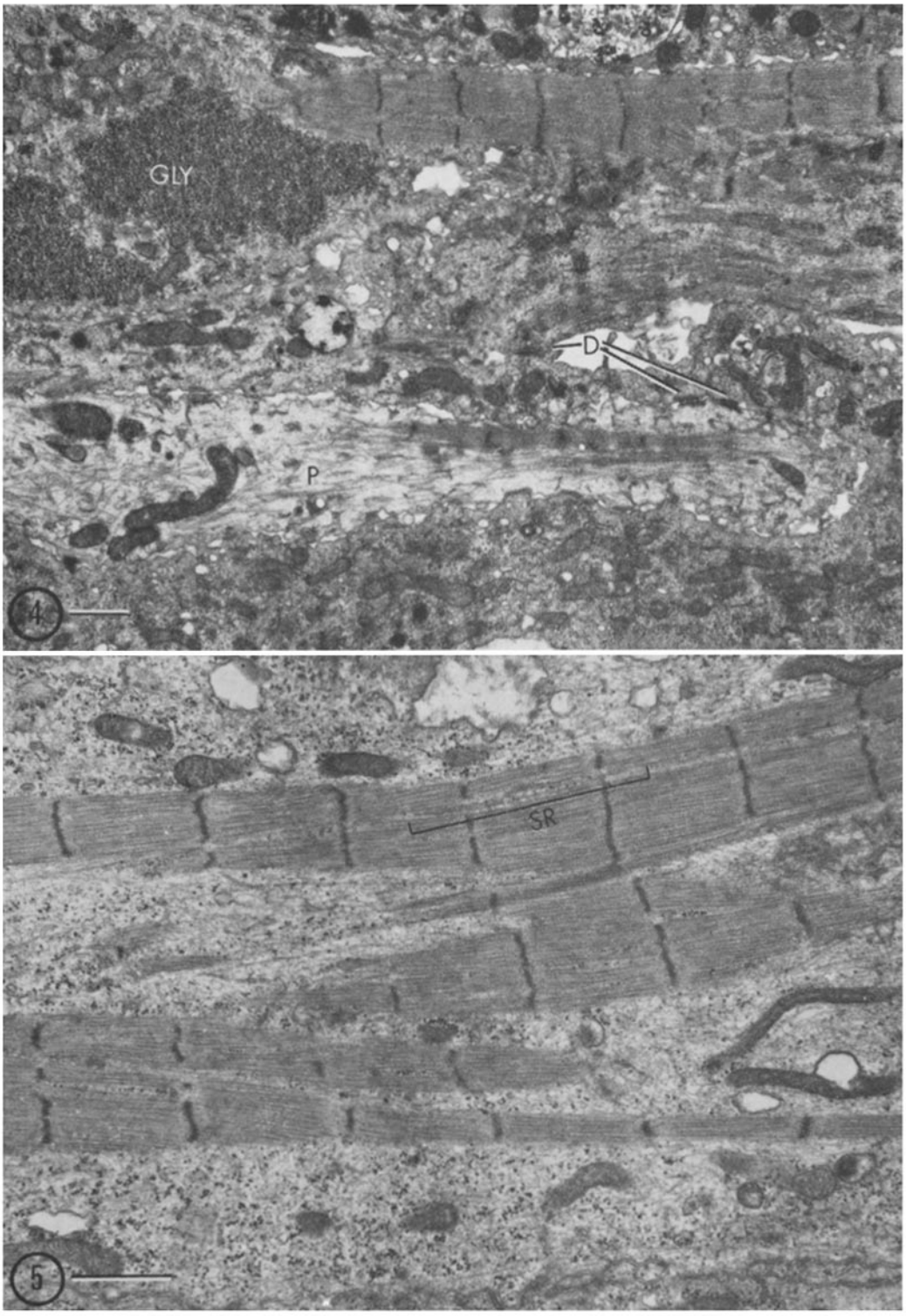


FIGURE 4 Low magnification micrograph of several longitudinally-sectioned muscle cells. A pale cell (*P*) lacking ribosomes and glycogen can be compared with the adjacent cells which contain a dense appearing cytoplasm. Desmosomes (*D*), glycogen (*GLY*). Scale, 1.0 μ. $\times$ 9000.

FIGURE 5 Typical appearance of a muscle cell in longitudinal section. Nonmembrane bound ribosomes and cytoplasmic filaments are apparent in the cytoplasm and an elongate element of the sarcoplasmic reticulum can be seen in the upper myofibril. Scale, 1.0 μ. $\times$ 15,000.

myofibrils during fixation (34). The characteristic arrangement of thick and thin filaments was evident in transverse sections of well-developed myofibrils (Figs. 17, 18). Both straight and irregular, jagged Z lines were observed (Figs. 9, 11).

A population of wavy, randomly oriented cytoplasmic filaments which measured 80–100 A in diameter was very apparent in areas with few other subcellular organelles (Fig. 8). These resembled the class of "intermediate" filaments reported by Ishikawa et al. (35) and Rash et al. (36).

SARCOPLASMIC RETICULUM (SR): Smooth-surfaced, tubular profiles (200–800 A in diameter) were commonly observed throughout the cytoplasm (Fig. 6) and resembled elements of the SR of adult chicken cardiac muscle (37). The myofibrils were shown to be discontinuously invested with SR tubules (Fig. 6). Although moderately developed as a tubular system, the SR appeared prominently at the level of I bands and Z lines (Fig. 13). In longitudinal sections, the SR appeared as vesicular profiles associated with the Z lines (Fig. 11) as well as elongate tubules in parallel with the myofibrils (Figs. 5, 9). The characteristic honeycomb structure of well-developed SR as seen in surface view was noted in a few sections (Fig. 10).

Elements of the SR were regularly observed in close apposition to the sarcolemma (peripheral couplings, Figs. 14, 15). Most of these couplings appeared as flattened saccules which were separated by a 120–180 A interspace and extended for 0.1–1.0 μ. A few contained a dense substratum and exhibited periodic densities between the apposed SR and surface membrane (Fig. 15). These structures appeared indistinguishable from the peripheral couplings of adult avian myocardial cells (37, 38).

A profusion of smooth-surfaced membranes was noted in some muscle cells (Fig. 12). This finding may be related to culture conditions since Manasek (39) has shown that little smooth-surfaced endoplasmic reticulum was present in the cytoplasm of chick heart cells *in situ* and that which was present was associated with the myofibrils. The proliferation of SR shown in the cytoplasm of denervated skeletal muscle (40) resembles the tubular profiles seen in Fig. 12.

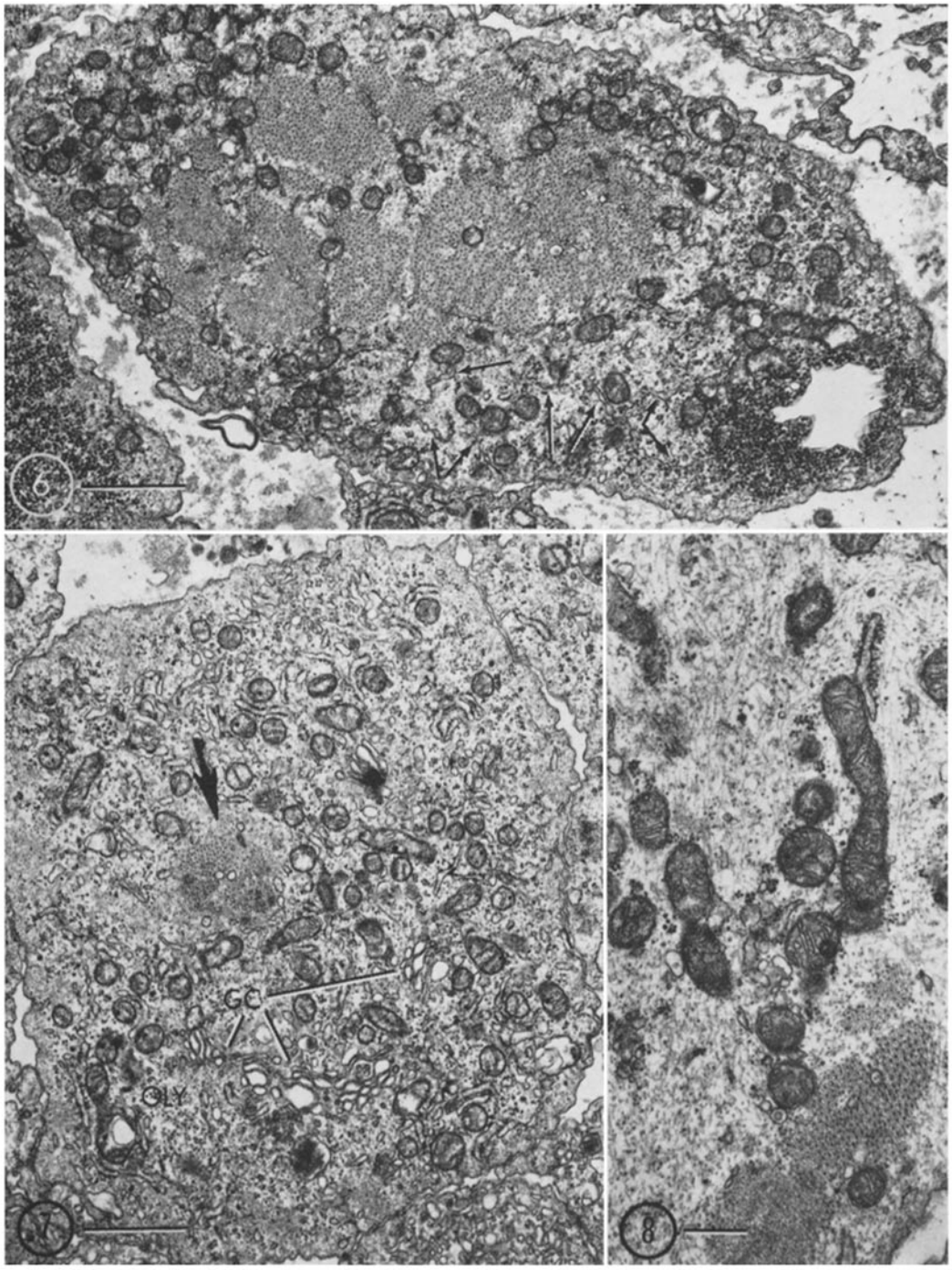
CYTOPLASMIC ORGANELLES AND INCLUSIONS: The most prominent cytoplasmic features of the muscle cells were an extensive Golgi complex (Fig. 7) and an abundance of glycogen (Figs. 4, 14) and nonmembrane bound ribosomes (Figs. 4, 5, 7). Manasek reported similar findings for embryonic chick hearts, *in situ* (31, 39). Numerous pinocytotic vesicles were noted particularly in grazing sections of the cell surface (Fig. 16).

CELL CONTACTS: Appositional regions of muscle cells situated in the core of the strand were categorized as follows: (*a*) relatively wide gaps (300–1000 A) (Fig. 3), (*b*) close but unspecialized appositions (250–300 A) (Fig. 14), (*c*) specialized junctional contacts including fasciae adherentes (Figs. 14, 17–19), desmosomes (Figs. 4, 18, 19), and nexuses (i.e. gap junctions as distinguished from tight junctions) (Figs. 14, 18–21). No difference in the distribution of the specialized junctions was observed between the two types of muscle cells in the strand; in contrast to the adult chicken heart (37), nexuses between working muscle cells were frequently seen. The predominant type of cell apposition was close but unspecialized (Fig. 14).

Fasciae adherentes, the most frequently occurring specialized junctions, were generally distributed about the cell perimeter. They were characterized by accumulations of electron-opaque material on the inner surface of the apposed membranes which were separated by a gap (200–300 A wide) filled with an undefined material (Fig. 17). The fasciae adherentes shown in Fig. 11 resembles an early stage in the development of intercalated discs (31, 41–43). Fig. 19 illustrates complex interdigitations of adjacent membranes of a developed intercalated disc exhibiting all three forms of specialized junctions. This structure is comparable to intercalated discs seen in the adult avian (38) and mammalian heart (43).

Desmosomes were numerous and regularly observed. In some cells, they were particularly well structured (Fig. 18); the dense condensation on the cytoplasmic side of the apposed membranes and the dense intercellular substratum were clearly evident.

Nexuses were characterized by a pentalaminar structure with a minimum over-all thickness of less than 190 A (Fig. 20). En bloc staining with uranyl acetate revealed that the apposed nexal membranes were separated by a gap of about 20 A in width (Fig. 21). The nexuses varied in length up to distances of 0.7 μ.



FIGURES 6–7 Transverse sections of two muscle cells illustrating the variability of myofibril content among cells in the strands. Smooth-surfaced tubular elements are seen in the cytoplasm (arrows) as well as in association with myofibrils in Fig. 6. The cell in Fig. 7 contains only one myofibril (arrow) but numerous ribosomes, mitochondria, a Golgi complex (*GC*), and some glycogen (*GLY*). Fig. 6, scale, 1.0 μ. $\times$ 15,000; Fig. 7, scale, 1.0 μ. $\times$ 15,000.

FIGURE 8 Transverse section of a muscle cell in which wavy, somewhat randomly oriented cytoplasmic filaments are easily distinguished. Scale, 0.4 μ. $\times$ 22,500.

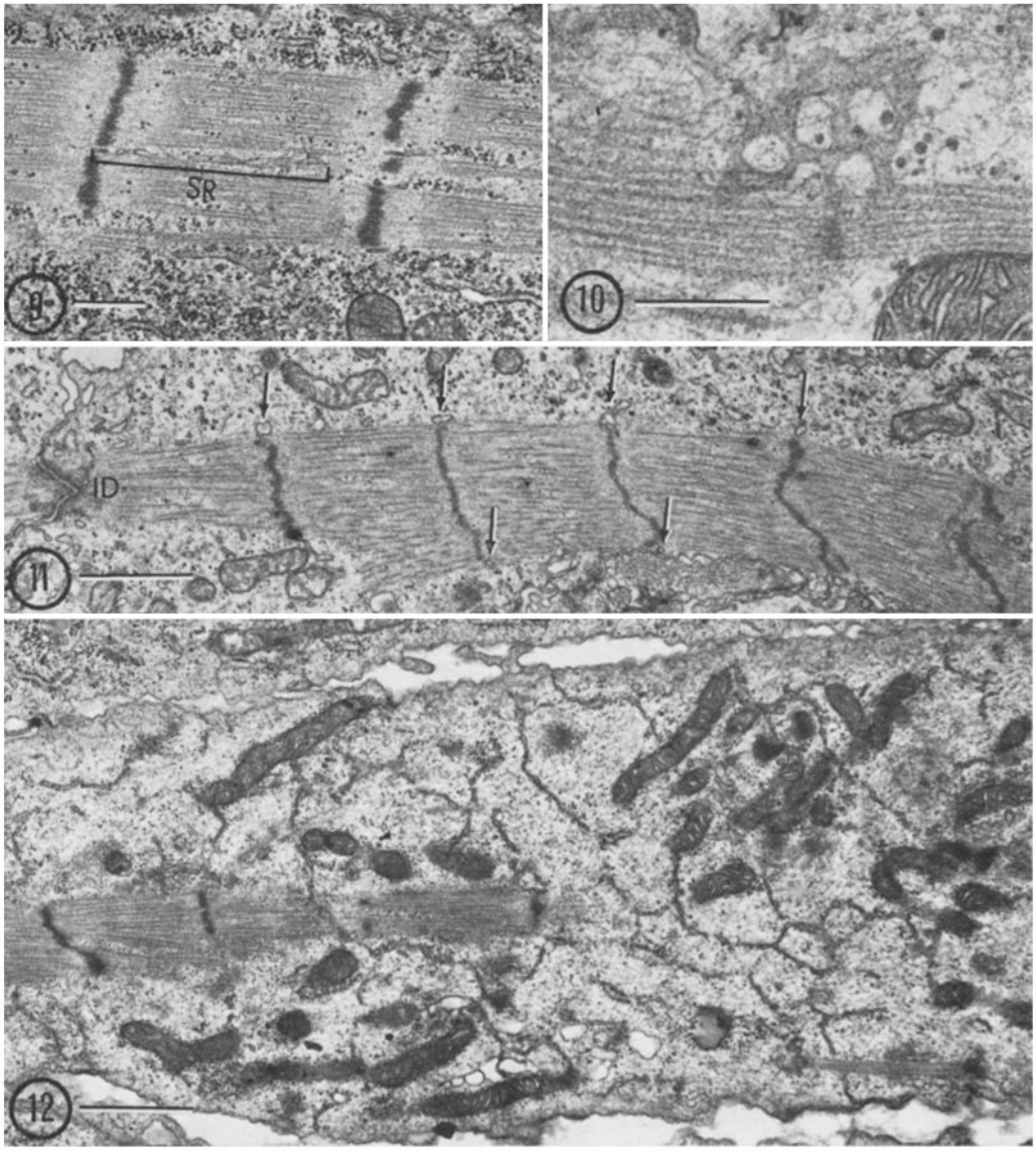


FIGURE 9 Longitudinal section of a muscle cell showing elements of the sarcoplasmic reticulum (*SR*) in parallel with a myofibril. Scale, 0.5 μ. $\times$ 20,000.

FIGURE 10 Longitudinal section of a muscle cell showing the characteristic honeycomb appearance of the sarcoplasmic reticulum in surface view. Scale, 0.4 μ. $\times$ 45,000.

FIGURE 11 Longitudinal section of a myofibril showing small vesicular elements of the sarcoplasmic reticulum situated at the Z lines (arrows). Note the jagged appearance of the Z lines and the developing intercalated disc (*ID*). Scale, 1.0 μ. $\times$ 15,000.

FIGURE 12 Longitudinal section of a muscle cell containing numerous smooth-surfaced tubular profiles. Scale, 1.0 μ. $\times$ 15,000.

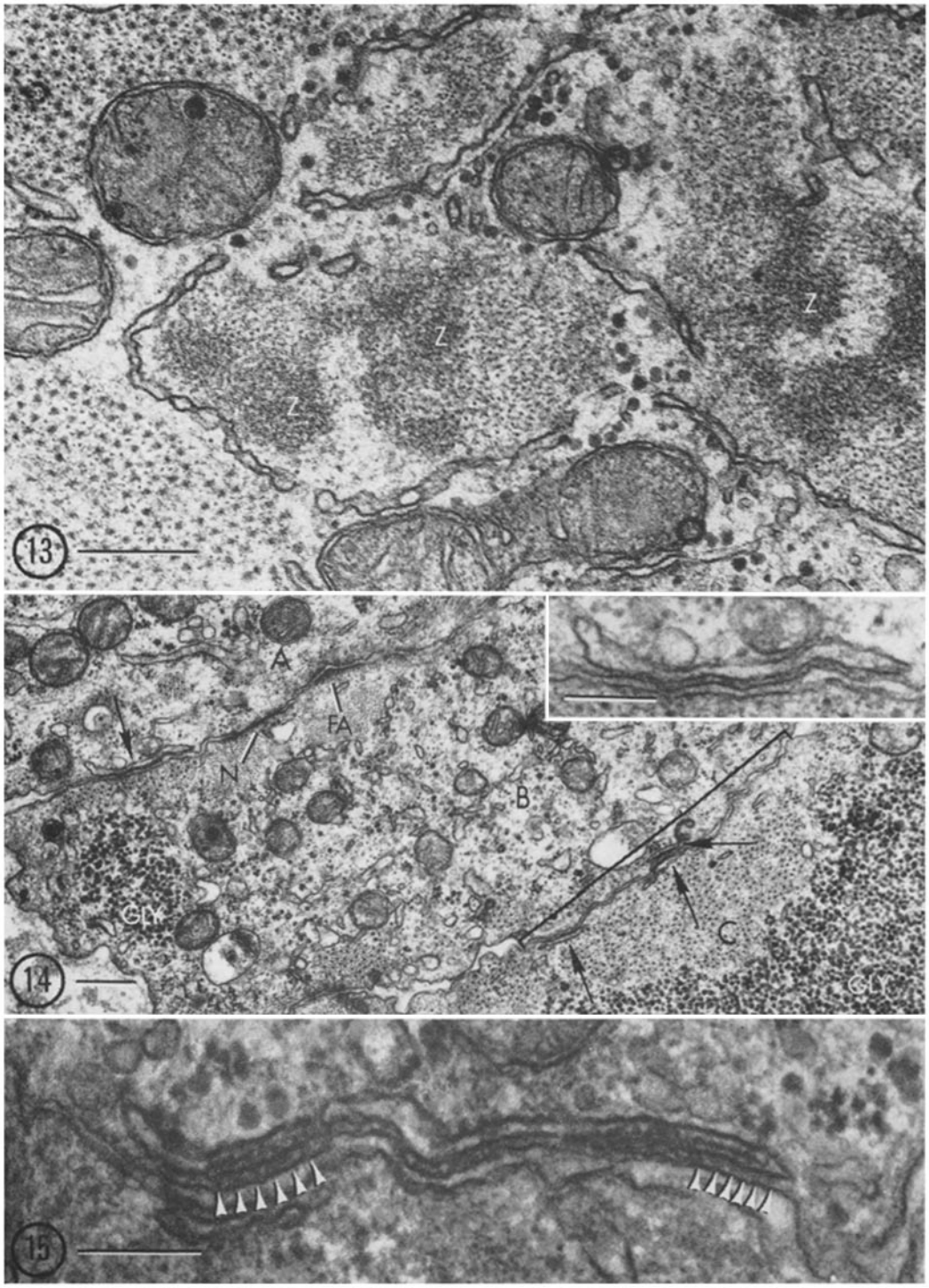


FIGURE 13 Transverse section of a muscle cell illustrating the development of the sarcoplasmic reticulum at the Z lines (*Z*) and I bands of myofibrils. Scale, 0.3 μ. $\times$ 60,000.

FIGURE 14 Transverse section of muscle cells showing peripheral couplings (arrows). A high magnification view of the peripheral coupling in cell A is shown in the inset. Note the presence of a fascia adherens (*FA*) and nexus (*N*) between cells A and B. Most of the region of contact between cells B and C is typical of a close but unspecialized apposition (bracket). Glycogen (*GLY*). Scale, 0.4 μ. $\times$ 22,500; Inset, scale, 0.2 μ. $\times$ 69,000.

FIGURE 15 A peripheral coupling which exhibits a dense substratum and periodic densities (arrows). Scale, 0.2 μ. $\times$ 93,000.

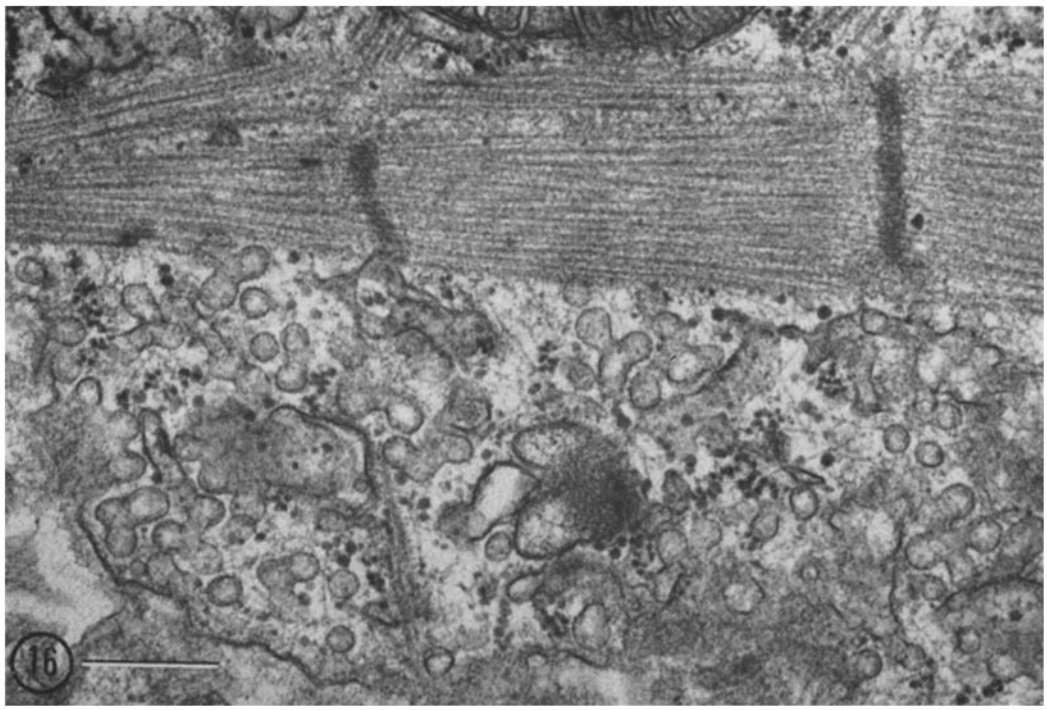


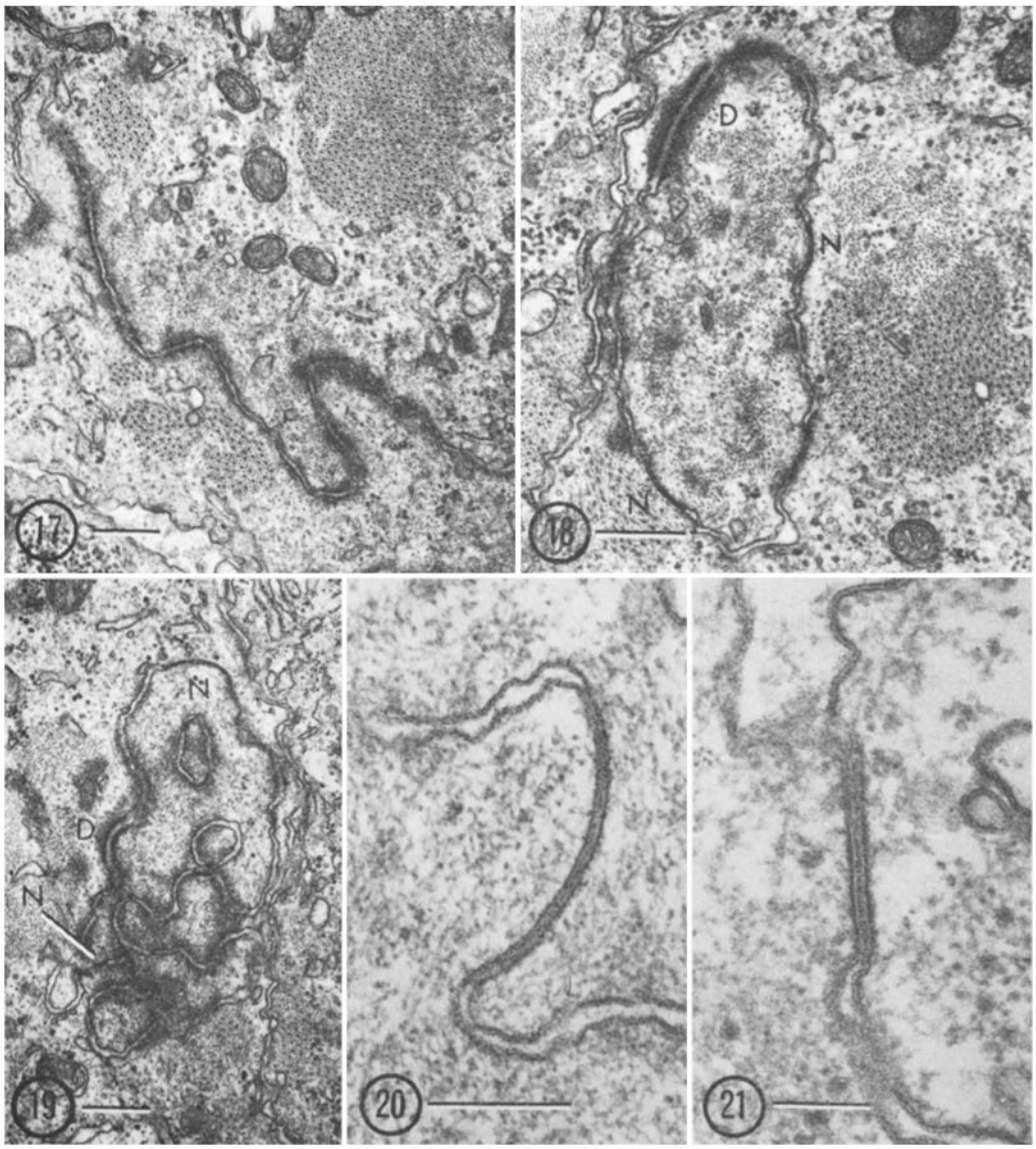
FIGURE 16 Longitudinal section of a muscle cell showing numerous single or fused pinocytotic vesicles. Scale, 0.4 μ. $\times$ 45,000.

DISCUSSION

Formation of the Strand

The formation and organization of the strand depends upon the ability of a heterogeneous population of cells from embryonic hearts (i.e. muscle and nonmuscle) to reassemble in a manner which simulates the original histological pattern of the intact tissue. To date, studies concerned with cell reaggregation, segregation, and selective adhesion (sorting out) in mixed cell suspensions have established that heart cells are capable of sorting out from other tissue types (9) and according to species (44). Whether a population of cells derived solely from embryonic hearts is capable of sorting out according to cell type has not been extensively studied. Steinberg (9) showed that suspensions of dissociated heart ventricle produced spherical aggregates in which a sheath of fibroblasts enveloped the muscle cells. Using different techniques, we found that the entire length of linear strands was composed of an inner core of muscle cells surrounded by an outer sheath of fibroblast-like cells. There was no clear segregation of muscle and Purkinje-like cells within the myocardial core; the latter were in close appositional contact with the muscle cells.

Although several hypotheses have been advanced to account for cellular segregation, evidence concerning the actual mechanism by which cells sort out within an aggregate is presently unresolved. The hypotheses of Steinberg (9) and Moscona (27) are similar in assuming that cell segregation results from random migration and differences in adhesive properties of cell types within an aggregate. Alternatively, Curtis (45) offers the hypothesis that sorting out and positioning of cell types is achieved according to the cell's ability to recover adhesiveness. In this regard, cells positioned peripherally would reflect a delayed aggregation around an internal core of rapidly aggregating cells. Although the results obtained in the present study do not resolve the dilemma concerning the mechanism of cell segregation, the relative absence of specialized junctional contacts between the outer fibroblast-like cells in contrast to their abundance between the inner muscle cells seems to support Steinberg's hypothesis of differen-



FIGURES 17–21 Transverse sections illustrating specialized junctional contacts between muscle cells. Fig. 17 shows an extensive fascia adherens which is part of a simple intercalated disc. The structure in Fig. 19 demonstrates the complex interdigitation of a more developed intercalated disc. Most of the complex is composed of fasciae adherentes but nexuses (*N*) and a desmosome (*D*) are also evident. Fig. 20 shows the pentalaminar appearance of a nexus fixed in glutaraldehyde-osmic acid. The nexus in Fig. 21 was stained en bloc with uranyl acetate and shows a gap of ~20 A separating the apposed cell membranes. Fig. 17, scale, 0.4 μ. × 22,500; Fig. 18, scale, 0.4 μ. × 30,000; Fig. 19, scale, 0.4 μ.× 22,500; Fig. 20, scale, 0.2 μ. × 95,000; Fig. 21, scale, 0.1 μ. × 129,000.

tial adhesion which states that the strength of cell adhesions differs for different cells (9). Our findings do not agree with those of Armstrong (16), who reported no differences in the contact junctions between like cells and unlike cells.

The importance of the substratum in providing orientation for cells was considered by Weiss (10), who described the phenomenon "contact guidance" as a behavioral reaction of cells to macromolecular exudates which oriented the movements

of cells within a groove. More recently, Carter (12) related cell movement and orientation to relative strengths of substrate adhesions, a concept he called, "haptotaxis". The physicochemical basis of cell orientation, whether it involves contact guidance or haptotaxis, is presently highly speculative and clearly beyond the scope of this study (see Trinkhaus (46) for discussion of this problem). We were surprised to discover that heart cells readily adhered to a surface of oriented cellulose acetate (unpublished observations) prepared according to the method of Carter (12). In support of our findings, Taylor (47) reported that cultured cells from human conjunctiva were capable of rapidly attaching to a substrate of cellulose acetate.

Ultrastructure of the Strand

By a number of ultrastructural criteria we have shown that the muscle cells are differentiating normally and resemble embryonic chick heart cells *in situ* (31, 39). Myofibrils are similar in arrangement and orientation to those in muscle cells of intact embryonic chick hearts but are more widely spaced. The apparent reduction in myofibril content in some cells could be due, in part, to the incomplete reassembly of the myofilaments after their disruption during disaggregation or to a lag in synthesis in vitro. Alternatively, culture conditions may favor the attachment and growth of myoblasts and immature myocytes whose developmental capabilities are decelerated because of a lack of essential nutritional factors needed to fully support the synthesis and oranization of muscle proteins.

Junctional specializations, e.g., highly structured desmosomes, intercalated discs, and nexuses are an important developmental feature of the strand. Their presence indicates *de novo* formation, in vitro, since the junctional contacts between the cells of the intact embryonic heart were undoubtedly disrupted by the dissociative procedures. The development of nexuses between muscle cells is particularly significant in light of earlier reports that the embryonic chick heart is nearly devoid of such structures, *in situ* (37, 38). Recently, however, Spira (48) described 30–50 A gap junctions in all but the deeper layers of embryonic chick myocardial cells from stage 7 (24 hr) to stage 30 (7 days). Nexuses were also shown in tissue culture between mammalian cardiac myoblasts (5, 49), fibroblasts (5), and smooth muscle cells (50, 51). DeHaan and Hirakow (17) reported that small punctate regions of close membrane apposition formed between isolated heart cells within minutes after they established contact.

The presence of a developing SR is another indication that these cells are undergoing differentiation. Information concerning SR formation in the embryonic myocardium is incomplete (20, 52). By contrast, it has been established that the proliferation of SR in immature skeletal muscle follows myofibril formation, both in vivo and in vitro (53–56). Most investigators postulate that SR is derived from rough-surfaced endoplasmic reticula throughout the cytoplasm and then becomes organized around the myofibrils, initially in the region of the I bands and Z lines (53, 55). Sarcoplasmic reticulum development in the strand appears to conform to this general scheme; however, details of initial SR formation were not investigated.

The SR is also observed in association with the sarcolemma in the form of peripheral couplings. Kelly (56) suggested that in developing skeletal muscle, simple subsurface cisterns of SR are precursors of the more structured junctional SR of the peripheral coupling. These structures have been implicated in excitation-contraction coupling and are of particular importance for chick as well as other avian cardiac cells, where they represent the only form of coupling between the sarcolemma and the SR (37, 38).

Pinocytotic activity is clearly more evident in muscle cells of the strand than is generally observed in the intact embryonic chick heart (31, 39). Some investigators have suggested a relation between the development of the transverse tubular (T) system and pinocytosis. For example, it has been shown that many pinocytotic vesicles were present in cardiac muscle when the SR was poorly developed and the T system was absent (20, 57). Ishikawa (54) has speculated that, in cultured skeletal muscle, the T system develops from repeated fusions of pinocytotic vesicles. As in embryonic (31) and adult chicken (37) cardiac muscle, no T system was present in cardiac muscle cells of the synthetic strand. Although simple pinocytotic fusions were evident, the extensive, beaded tubular profiles said to represent developing T system tubules (53, 54, 56) were never observed.

CONCLUSION

The synthetic strands represent reconstructed fibers of cardiac muscle with a linear and relatively simple geometry. The organizational de-

velopment of the strands minimizes the complications introduced into electrophysiological studies by nerves, blood vessels, thick collagenous matrix, and the tightly organized enveloping endocardial sheath of its naturally occurring counterpart (37). Furthermore, preparations containing areas with fewer muscle cells have provided a useful model system for investigating the factors contributing to slow conduction as it is known to occur in the intact heart (58). Finally, the synthetic strand appears to behave electrophysiologically, as an ideal one-dimensional cable (Sawanobori, Lieberman, and Johnson, unpublished observations) with a naturally occurring end.

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