THE ULTRASTRUCTURE OF CARDIAC DESMOSOMES IN THE TOAD AND THEIR RELATIONSHIP TO THE INTERCALATED DISC

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ABSTRACT

The fine structure of desmosomes and intercalated discs in the toad heart is discussed. A definite relationship between the dense components of these structures and the dense region of the Z band is demonstrated. The dense region of the Z band characteristically widens at its approach to the plasma membrane, and often terminates beneath it in a distinct discoidal plaque. Cardiac desmosomes appear to be structures which result from the intimate apposition of plaques of Z band material. These desmosomes retain the Z band function as sites of attachment for myofilaments. The suggestion is made that rotation of a desmosome through 90° and splitting of filaments from the adjacent sarcomere could result in the formation of a simple step-like intercalated disc. Intermediate stages in this process are illustrated. Complex discs present in the toad probably represent the alignment of groups of simple discs produced by contractile forces. Possible physiologic functions of the disc and desmosome are discussed. Other morphologic features of toad cardiac cells include a distinct amorphous outer coat to the sarcolemma, a prominent N band, and a granular sarcoplasm with poorly developed reticulum.

INTRODUCTION

Desmosomes have been observed in various epithelia with both light and electron microscopes (4, 8, 12, 20). Traditionally, they were considered to be bridges of protoplasm connecting adjacent cells, and the short filaments or tonofibrillae seen in these regions were believed to course directly from cell to cell (8). Agreement by light microscopists on the exact morphology of desmosomes was never really achieved and an historical sketch of their controversies has been presented by Odland (12). Using the electron microscope, Porter (14) showed that protoplasmic continuity did not exist in the desmosomal regions but rather that two opposing plasma membranes were each modified on the cytoplasmic side by accumulations of dense material. Fine filaments frequently attach to this material and may fan out from it toward the cell interior. Fawcett (4) discusses these electron microscopic observations in detail. The composition of the desmosomal interspace is still subject to question. Though the adjacent cellular membranes seem to remain firmly attached at the desmosome, a precise mechanism for cohesion has been demonstrated only in the case of *Hydra*.

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Wood (21) reviews the literature bearing upon this problem.

In a recent study, Fawcett and Selby (5) reported upon the fine structure of desmosomes in the turtle myocardium. Transitional forms between desmosomes and simple intercalated discs were demonstrated. With this evidence it was concluded that the intercalated disc and cardiac desmosome are basically similar modifications of the cell surface. Earlier work by Muir (11) on the development of intercalated discs in rabbits from embryos to adults had shown that dense bipartite structures, in all respects resembling desmosomes, underwent a progressive maturation to intercalated discs of increasing complexity. In the mouse heart, structures resembling desmosomes were occasionally found by Sjöstrand, Andersson-Cedergren, and Dewey (19) in the disc regions and were designated "S-regions" of the intercalated disc.

Previous electron microscopic studies of the frog myocardium have indicated that the disc is less pronounced than in the heart of the guinea pig or mouse (18) and less tortuous than in that of the guinea pig (13). These observations probably account for the omission of reference to the intercalated disc in early light microscopic studies of the amphibian heart (17). Jordan and Steele (7), however, observed that the discs of Amphibia were generally very narrow, though occasionally the width of an entire fiber.

In the present study of the toad heart, in addition to the classical intercalated disc, varieties of desmosomal structures are illustrated and described in detail. The mutual relationships of these surface modifications to the Z band and the myofibrillar pattern are discussed. Because of these relationships and other observations, a sequence of events in the evolution of desmosomes and discs from initially simple modifications of the Z band is postulated.

MATERIAL AND METHODS

Small blocks of fresh cardiac tissue from the common toad (*Bufo*) were fixed in 2 per cent buffered osmium tetroxide solution and rapidly dehydrated through a series of graded ethanols. The tissues were embedded in *n*-butyl methacrylate containing 10 per cent methyl methacrylate as hardener and 1.5 per cent lucidol as initiator. Standard technics were employed (9). Sections were cut with diamond knives on Porter-Blum microtomes, supported on copper grids by formvar films lightly stabilized with carbon, and examined in a Siemens Elmiskop I at original magnifications of 1000 to 20,000.

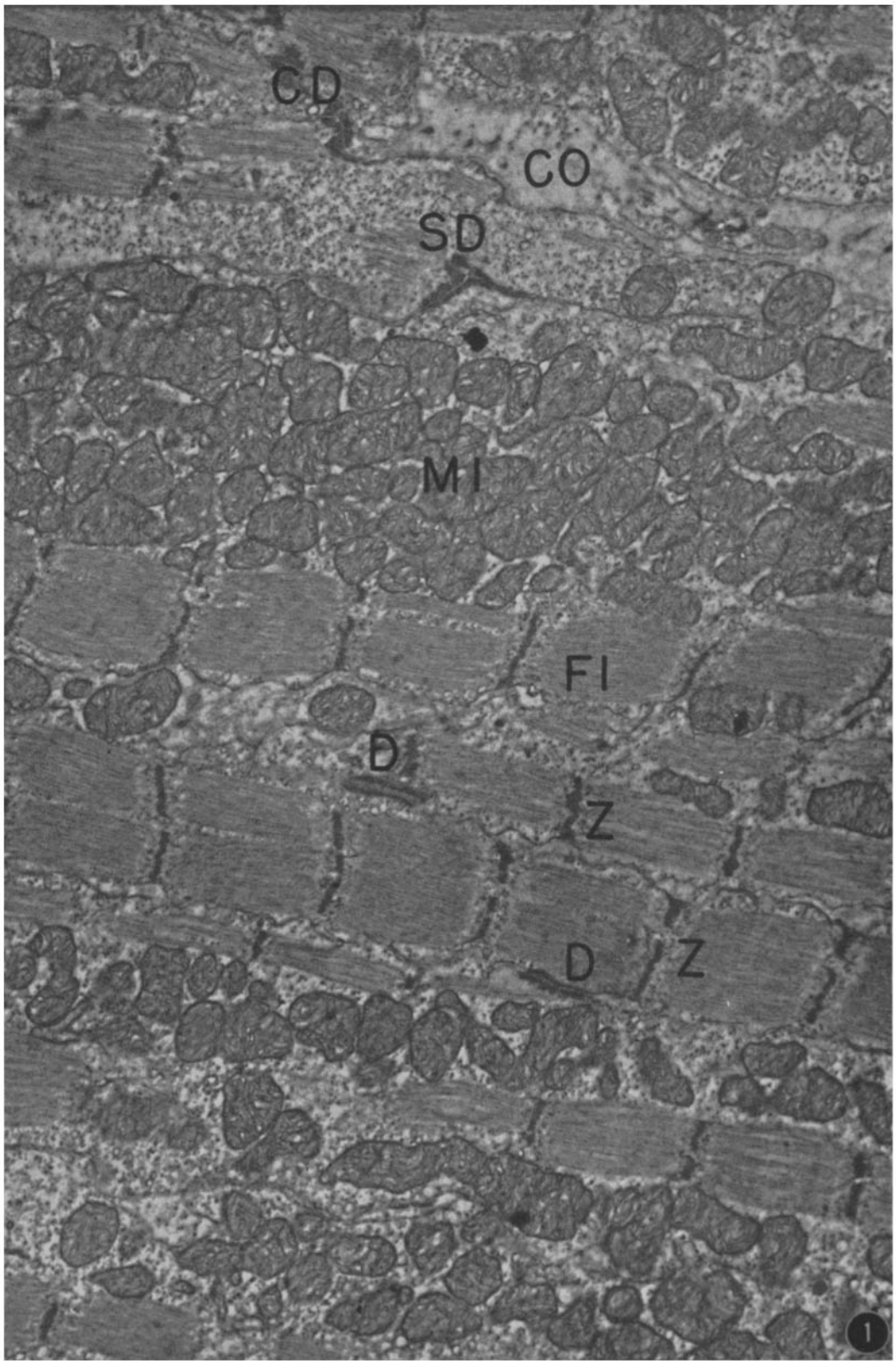
RESULTS

Cardiac cells of the toad, whether auricular or ventricular, are generally long and narrow. They are delimited from the vascular compartment by a thin layer of endothelium which overlies an intervening meshwork of collagen fibers and neural elements. Basement membrane and intercellular collagen fibrils in small amounts may occur between toad cardiac cells to an extent greater than that usually observed in higher vertebrates (10). Naked axons, singly or in groups, commonly come to be in close proximity to the cardiac cells and are separated from them only by a thin layer of ground substance. While no specialized neuromuscular endings were seen, many of the terminal axons contain clusters of synaptic vesicles (Fig. 4). In section, the tapered ends of the cardiac cells frequently enclose only one myofibril between the sarcolemmas (Fig. 1). Cell borders tend to interdigitate quite irregularly and the protuberances formed may contain one or more well delimited sarcomeres (Fig. 5).

In many respects the fine structure of the toad heart resembles that of the turtle (5). As in the turtle, the interfibrillar sarcoplasm is relatively more abundant than in the mammalian heart and is sprinkled with granular material arranged

FIGURE 1

View of several cells in the toad ventricle. The cells are typically narrow, elongate, and each contains few myofibrils (*FI*). Abundant mitochondria (*MI*) are distributed in the sarcoplasm between the fibrils and beneath the sarcolemmas. Collagen (*CO*) is present in the intercellular spaces. The dense component of the Z bands (*Z*) is irregular in thickness and expansions of this material subjacent to the plasmalemma are present. Several types of specializations of the cell surface are illustrated: (1) complex disc (*CD*), (2) simple disc (*SD*), (3) desmosomes (*D*). Details of these features are illustrated in succeeding plates. × 17,500.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in small clusters or rosettes (Figs. 4 and 8). The endoplasmic reticulum (sarcoplasmic reticulum) is generally not well developed and apparently lacks the elaborate organization characteristic of mammalian cardiac muscle (15). A distinct M line was not observed but the other cross-striations are well formed. A prominent N line is present and appears as a row of bead-like thickenings along the myofilaments on either side of, and parallel to, the Z band (Figs. 4, 8, 9). The N line thickenings sometimes seem to be connected directly with extending strands of the dense portion of the Z band (Figs. 8, 9).

The Z band comprises filaments, tiny vesicles, and an abundant dense material which often masks the other components. The Z band may be connected with the plasma membrane by a series of vesicular elements of the endoplasmic reticulum or by the dense Z band material. If connection is by vesicular elements, the plasma membrane is invaginated at Z band level and continuous with sarcoplasmic vesicles, which in turn are continuous with the Z band reticulum (Figs. 1, 2, 8). Neither triads of the sarcoplasmic reticulum (10) nor aggregates of vesicles of the reticulum near the level of the A-I junction were observed.

The dense, sarcoplasmic portion of the Z band varies considerably in thickness regardless of the state of contraction of the fibril or the angle of sectioning. This is particularly true of Z bands located in peripheral myofibrils. Here the dense sarcoplasmic component of the Z band is frequently noted to expand as it approaches its plane of intersection with the plasma membrane (Fig. 3) and may terminate in a distinct plaque subjacent to the membrane (Figs. 5, 9). The expanded, peripheral portions of Z bands apparently retain connections with myofilaments. They closely resemble portions of desmosomes but are unpaired structures, whereas desmosomes are generally considered to be bipartite. However, when the sarcolemmas of two adjacent cells are closely apposed with an interspace of about 25 to 50 mμ, two plaques of expanded Z band material may be coupled to form an integral structure (Figs. 6 and 10). This includes the intercellular space and resembles a classical type of desmosome.

The relationship of the dense sarcoplasmic components of the desmosome to the Z band proper is variable, as illustrated in Figs. 6 and 7. The Z bands are confluent with the midportion of the desmosomal densities in the former, and with the diagonally opposed margins of the densities in the latter. A 60° rotation of the desmosome in the plane of the picture (Fig. 7) into a line parallel with the pattern of muscular cross-striations would result in a simple step-like disc, provided that filaments remain attached. In many instances, one of the dense regions of the desmosome lacks any visible direct connection to the Z band or other intracellular component (Figs. 8 and 11). Most frequently the unconnected half of such a desmosome lies at the midportion of a sarcomere about equidistant from the I bands (Fig. 11, D_2, D_3). The origin of desmosomal components unconnected with a Z band is not clear. The dense material

FIGURE 2

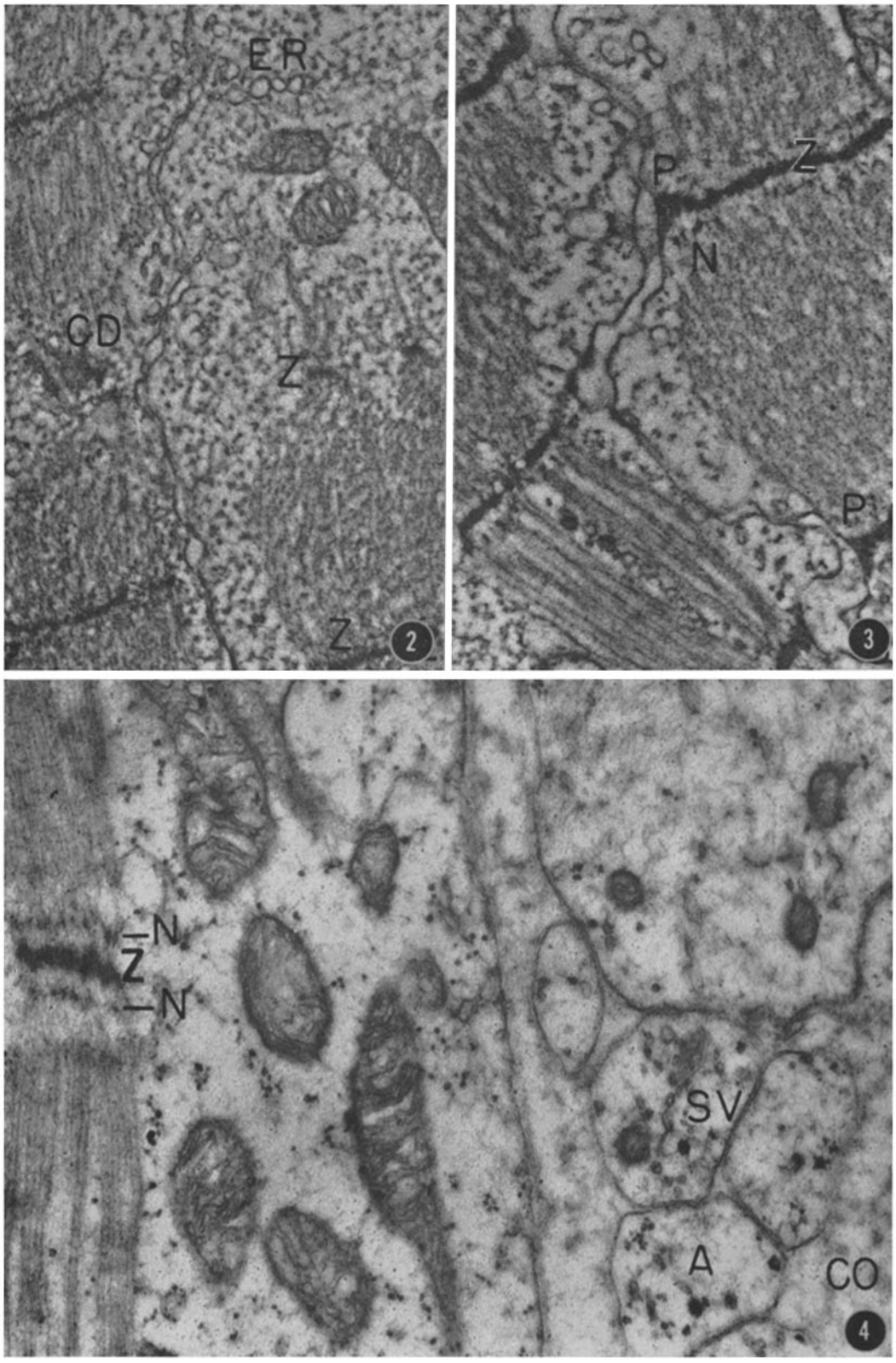
Section secant to the longitudinal plane of the myofibrillar axis, revealing the presence of endoplasmic reticulum (*ER*) at the projected level of a Z line, with multiple connections to the plasma membrane. A portion of a complex disc (*CD*) is present at the left. × 30,000.

FIGURE 3

The peripheral attachment of each of five Z bands to the plasma membranes of their respective cells is illustrated. The expansion of the Z band material as it approaches the plasma membrane is best seen at the regions labeled *P*. Notice the continuity of the N band (*N*) on either side of these terminal expansions. × 35,000.

FIGURE 4

Region of myoneural contact showing several small naked axons (*A*) filled with clusters of synaptic vesicles (*SV*). The axons are separated from the cardiac cell membrane by an amorphous material similar to basement membrane. Collagen fibrils (*CO*) are present to the lower right. These lie between the neural elements and endothelial cells (not illustrated). Granular material in the sarcoplasm of the cardiac cell is seen, but reticular elements are not conspicuous. Distinct N lines flank the Z band. × 45,000.


ER
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might be considered either to have arisen *de novo* from the sarcoplasm or to have become independent after migration from the Z region. One might speculate that bioelectric or chemotactic forces originating in a Z band and its expanded peripheral portion may be active in directing the aggregation of the dense material beneath an adherent, neighboring plasmalemma. A detailed discussion of such intercellular forces has been given by Weiss (20). Sections of desmosomes in single micrographs may appear long and thin (Fig. 11, D_2) or quite short (Fig. 11, D_3). After numerous observations there is the impression that these represent sections of discoidal structures of graded circumference and probably reflect different degrees of development.

Intercalary discs in the toad myocardium resemble those previously described in various other species, including Reptilia and Mammalia (3, 5, 9, 11, 13, 18, 19). A typical intercalated disc might best be described as a junctional specialization of cardiac cells, comprising two or more closely apposed and modified sarcolemmas with a modified interstitial material at the interface. In contrast to desmosomes, discs generally occur in planes parallel to those of the muscular cross-striations and intersect the myofibrillar axes at levels in which a Z band would otherwise be expected. In a detailed comparative study of disc ultrastructure, Sjöstrand, Andersson-Cedergren, and Dewey (19) reported that the disc interspace is filled by a material more opaque than the surrounding methacrylate. These authors further noted a definite orientation of this interstitial material in the form of fine cross-filaments at certain regions (S regions) of the mouse disc. In the toad disc there is a suggestion of interstitial structure similar to that of the S regions (Figs. 5 and 14). Desmosomal interfaces are sometimes observed to contain dense amorphous material (Figs. 1 and 7) but no interstitial structure comparable to that of the septate desmosomes of *Hydra* (12) has been seen. Rigorous differentiation between discs and desmosomes in the toad is quite difficult. In fact, the problems encountered in such an attempt reveal the basic identity of these structures. Figs. 1, 6, and 11 illustrate structures which are clearly similar to desmosomes previously described in epithelium and in the turtle heart. These are oriented parallel to the long dimension of the myofibril and do not participate in the pattern of cross-striations. Figs. 11 and 12 illustrate desmosomes that are attached to filaments split away from the main portion of a sarcomere. Rotation of such desmosomes toward the Z band plane would result in structures of the simple disc type (Fig. 5). Of particular interest is the observation that only some of the simple discs are strictly parallel to the Z band pattern (Fig. 14). These resemble the step-like discs described in the adult turtle (5) and juvenile rabbit (11)

FIGURE 5

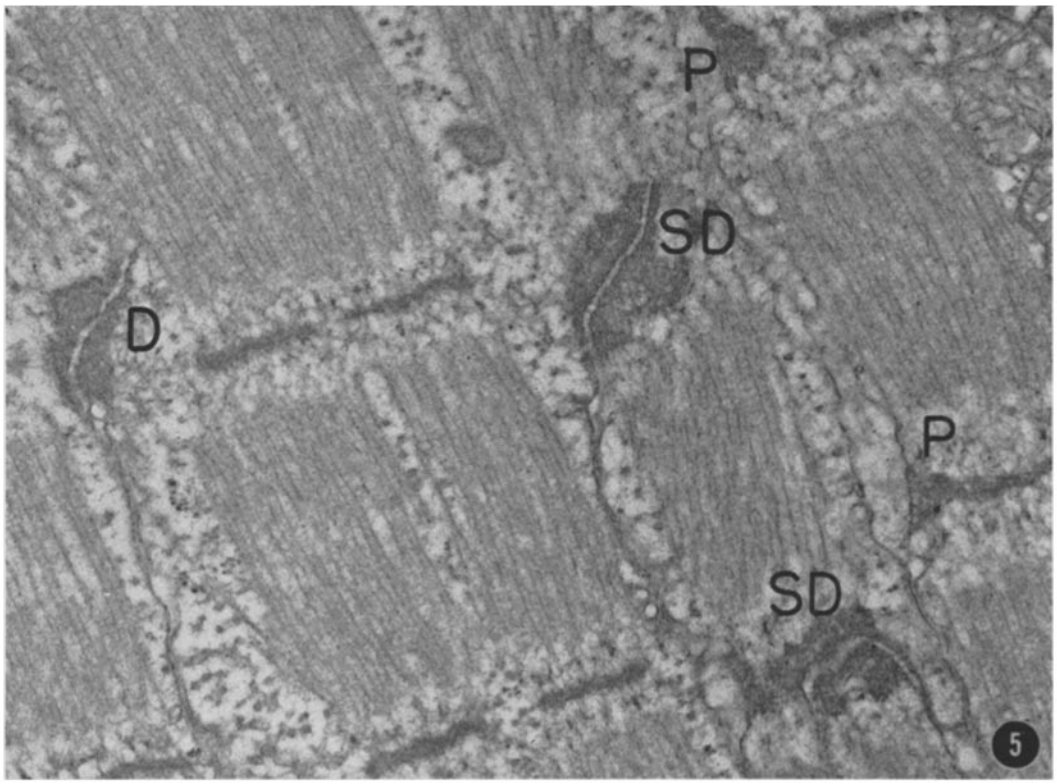
Section through a segment of a ventricular cell which interlocks with three adjacent cells and contains a single sarcomere. This sarcomere is attached to the plasma membrane at both ends in the regions of dense material that form simple discs (*SD*). At far left may be seen a desmosome (*D*). It is associated with a few diagonally oriented myofilaments. The similarities in composition and angle of orientation of this desmosome and the upper simple disc are apparent. The dense material of the lower simple disc is confluent with the Z band material. Plaques (*P*) of the Z band material are seen in the cell at the far right. × 40,000.

FIGURE 6

A classical desmosome (*D*) consisting of paired, modified sarcolemmas. The sarcoplasmic portions of the desmosome consist of dense regions confluent with the Z band density. Two juxtaposed Z band plaques would form a similar structure. A simple disc (*SD*) is illustrated at the lower right. × 50,000.

FIGURE 7

Another desmosome (*D*), illustrating a variation in the attachment of the dense Z band material to the sarcoplasmic component of the desmosome. The Z bands are attached at diagonally opposed ends of the desmosome. Note the greater interstitial density at the desmosomal interface. × 40,000.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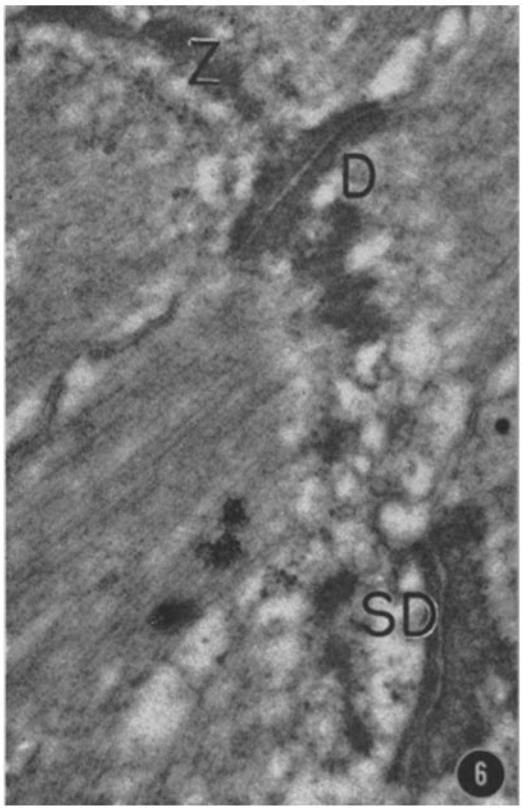
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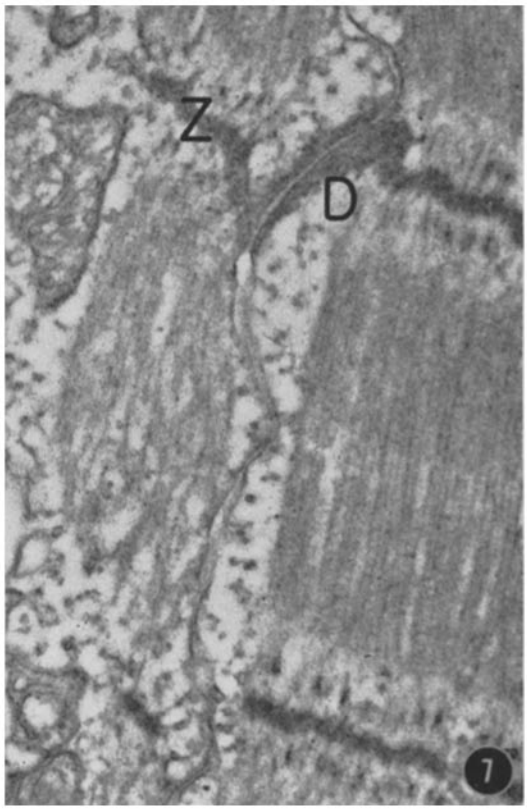
Z
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The component membranes of other discs, although occurring in the spatial interval of an expected Z band (Fig. 5), intersect the fibril in a direction not absolutely parallel to that of adjacent Z bands. They resemble the type of early disc described in the embryonic rabbit heart (11).

Simple forms of discs that cross only a single myofibril may occur singly or in groups of steplike offsets, which, in the light microscope, give a true staircase impression. These offsets of the sarcolemmas lie transversely to the long axis of the fibers at a Z line level for the width of one myofibril, and then return to follow the original longitudinal path. More complex discs similar to those observed in mammalian hearts also occur. They are formed either at a multicellular junction (Fig. 17) or at the junction of only two cells (Fig. 15). The number of myofibrils thus interrupted may vary from two to six or more. The complex disc assumes a more tortuous and corrugated appearance. Definite connections with the Z band are frequently retained by simple and complex discs (Figs. 5, 14, 16), supporting the notion of their earlier origin from the Z band.

DISCUSSION

Muir investigated the development of intercalary discs in the rabbit from embryonic to adult stages (11). He noted that the dense regions at the junction of embryonic cardiac cells, where the myofibrillae were interrupted, evolved in a stepwise fashion to form simple discs and eventually more complex, tortuous discs of the type usually seen in mammalian myocardia. Fawcett and Selby (5) described desmosomes in the turtle heart and commented upon the similarity of simple discs in the adult turtle and the young rabbit. Intermediate stages between cardiac desmosomes and intercalated discs in the turtle atrium were illustrated. Comparing the types of discs in the adult turtle with those in embryonic and young rabbits, these authors suggested that disc formation in the rabbit might be an example of ontogeny recapitulating phylogeny. They postulated that the mechanical forces produced by contraction in the turtle atrium were insufficient for the complete development into a complex disc.

The present investigation in an amphibian

FIGURE 8

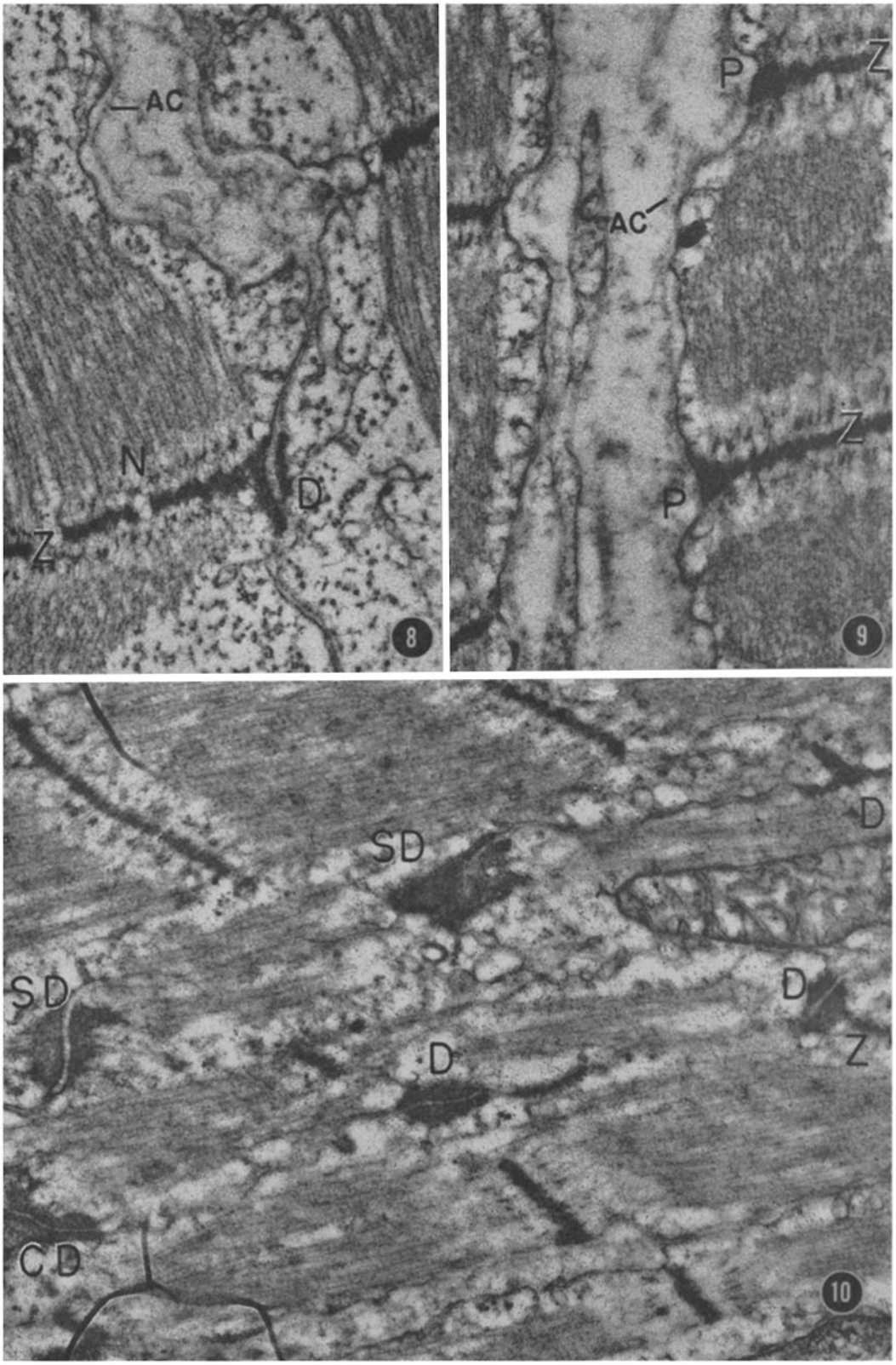
Section through a desmosome (*D*), that is connected to the Z band (*Z*) in one cell only. The dense material beneath the sarcolemma of the adjacent cell is not visibly connected to a Z band. A similar phenomenon may be observed occasionally in other illustrations (Figs. 1, 11). At the upper right may be seen an invagination of the plasma membrane linked to the Z line by means of a reticular vesicle. A coating of amorphous material (*AC*) that covers the cell membranes apparently became separated during fixation. × 40,000.

FIGURE 9

Portion of a myofibril showing the attachment of the two Z bands (*Z*) to the plasma membrane in distinct fan-like plaques of dense material. The N band consists of accumulations of dense material on the fine filaments of the I region on either side of and parallel to the Z band. These accumulations occasionally appear connected directly with the Z band material. The N bands also extend into the regions occupied by the plaques or desmosomes; illustrated both in this and previous figures (3, 8). The identity of plaque or desmosome density with the Z band density is thus emphasized. The amorphous outer coat (*AC*) of the sarcolemma is again demonstrated. × 40,000.

FIGURE 10

Section illustrating several discs and desmosomes in a single cell. The simple discs (*SD*) interrupt single sarcomeric patterns. The complex disc (*CD*) is not completely included, but interrupts several sarcomeres and is associated with a greater abundance of dense material. Three desmosomes are illustrated (*D*). At the right a desmosome is connected to a Z band on one aspect and to the terminal portion of a sarcomere on the other. × 40,000.


AC
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10

demonstrates the occurrence of new types of cardiac desmosomes and intermediate stages between desmosomes and discs. The evidence leads the authors to postulate that the first stage in desmosome or disc formation is a simple expansion of peripheral Z band material (Fig. 3). Further aggregation of this dense material subjacent to the plasmalemma would result in the formation of distinct discoidal plaques which may retain connections to myofilaments (Figs. 5 and 9). Close apposition of pairs of plaques in adjacent cells would form an integral structure, including a modified interspace and resembling epithelial desmosomes (Figs. 6, 7, 10). Transition of desmosomes to discs may be considered to involve essentially a rotation of the sarcolemmas in the immediate region of the desmosome. Filaments that remain attached to the desmosome may split off from the sarcomere proper and course at an angle from it to the desmosome (Figs. 11 to 13). The frequent association of fine filaments with epithelial desmosomes (4) leads one to believe that cardiac desmosomes also may serve as centers of orientation for the addition of new filaments; thus, rotating desmosomes might either retain connection to the myofilaments of their original Z band or acquire new filaments and thus add new sarcomeres to the muscle fiber.

When according to this hypothesis the desmosome is fully rotated into the Z band plane, it is a true simple disc (Figs. 5 and 14); that is, a disc which intersects a single myofibril. More complex discs (Figs. 15 to 17) would result either from the "staircasing" of many simple discs and eventual alignment due to contractile forces or from the close apposition of more than two cell surfaces. In the definitive disc stage, and even before, retention of connections between discs or desmosomes and the Z band is not always apparent. Such continuity, however, either through the reticulum (Fig. 12) or through the dense sarcoplasmic constituent of the Z band (Figs. 5, 10, 14, 16) may be observed in advantageously oriented sections.

It is tempting to speculate that the extensive variation of desmosomes or discs seen in the toad heart represents stages of transition which may occur also in mammalian disc development. The embryonic cardiac cell probably possesses a greater potential for desmosome formation. Those intercellular structures best adapted to function in the adult heart would remain as permanent features, whereas others would be represented only as stages in development or perhaps not at all, the pattern varying from one species to another. Evolution might thus be reflected at a cellular as well as at an organismal level.

Earlier workers have attributed various functions to discs. These include uninterrupted trans-

FIGURE 11

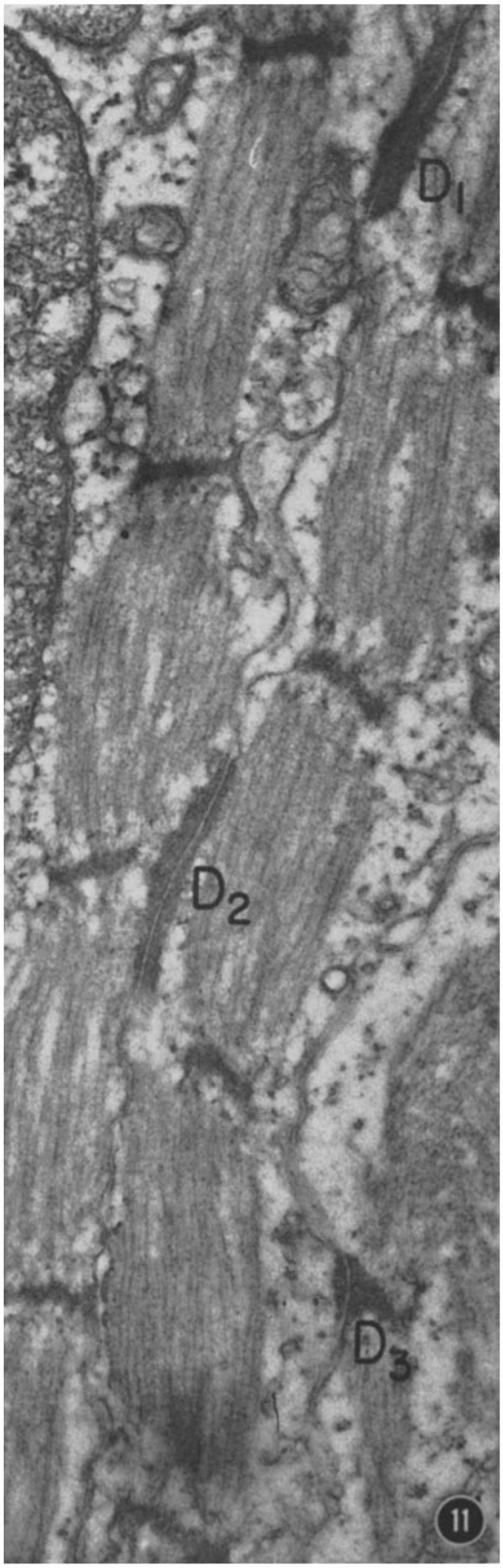
Three desmosomes are shown (D_1–D_3). In contrast to the desmosomes illustrated in Figs. 6 and 7, these are located at mid-sarcomeric level on one side, though opposite a Z band on the other. The uppermost desmosome (D_1) is associated with filaments. The lower desmosome (D_3) is free of any visible connection to the Z band or filaments on its left side and thus resembles the type of structure described in Fig. 8. A single myofibril lies between nucleus and sarcolemma in the cell at left. A long process of the centrally located cell is wedged between the left- and right-hand cells and contains but a single myofibril at this level. Narrow cardiac cells are quite characteristic in the toad. × 30,000.

FIGURE 12

Section through a desmosome (D) which bears a striking resemblance to a simple disc. The filaments attached to this desmosome are not part of a definitive myofibril, however. They are few in number and oriented at an angle to the longitudinally aligned definitive myofibril. Connection of the desmosome to the Z band by a strand of endoplasmic reticulum (ER) is indicated. A bulbous expansion (P) of the Z band subjacent to the plasmalemma resembles the plaques of Z band material previously illustrated (Fig. 3). × 25,000.

FIGURE 13

High magnification of a single desmosome (D) attached to myofilaments on both sides. No structure is apparent in the desmosomal interspace. × 50,000.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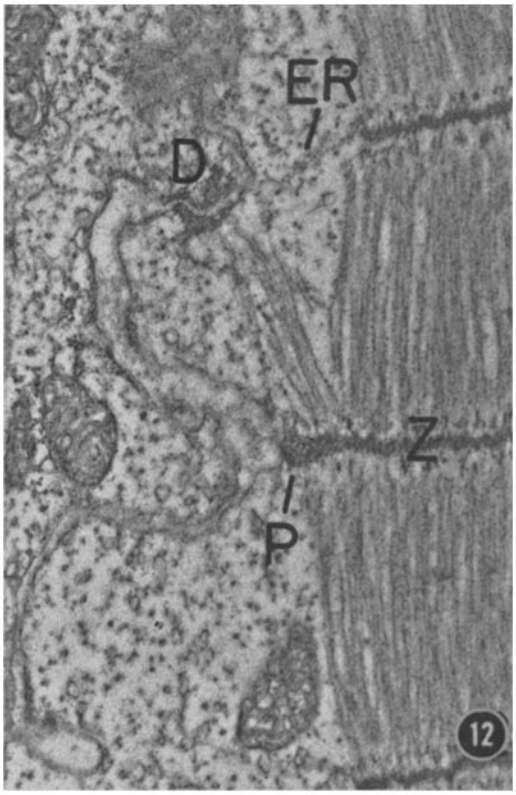
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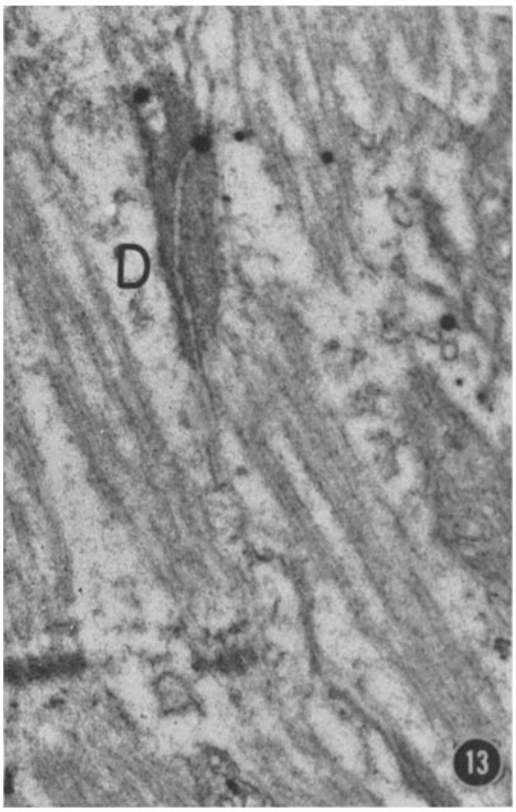
D
13

mission of mechanical forces between the fibrils of adjacent cells (5), intracellular cohesion (3), participation in fibrillar growth (6), and transmission of electrical impulses (2, 11). Many years ago Heidenhain (6) proposed a role for the disc in relation to the growth of new sarcomeres. The present investigation in the toad illustrating the splitting of filaments from established sarcomeres and stages of transition in the formation of discs, in conjunction with the work of Fawcett and Selby (5) and of Muir (11), provides a better basis for considering that the discs, or more accurately their precursors, are involved in the formation of new sarcomeres. Our knowledge concerning this question would be increased by further study of embryonic and hypertrophic myocardia.

Bourne's work (2) in localizing a differential enzymatic activity at the adult mammalian disc may be important evidence of the role of the disc either in myofilament orientation and fibrillar growth or in the transmission of impulses. Although sites of activity of dehydrogenase systems in cardiac muscle have been studied (1), localization of specific enzymes in the disc or desmosome by the electron microscope has not yet been accomplished. The intimate relationship of disc and desmosome to the Z band observed in the toad, coupled with a theory that the Z band reticulum acts as a pathway for conduction of impulses into the cell interior (16), makes it reasonable to assume that the intercalated disc also plays an important role in the processes of cardiac conduction and must be considered in any discussion of cardiac physiology.

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BIBLIOGRAPHY

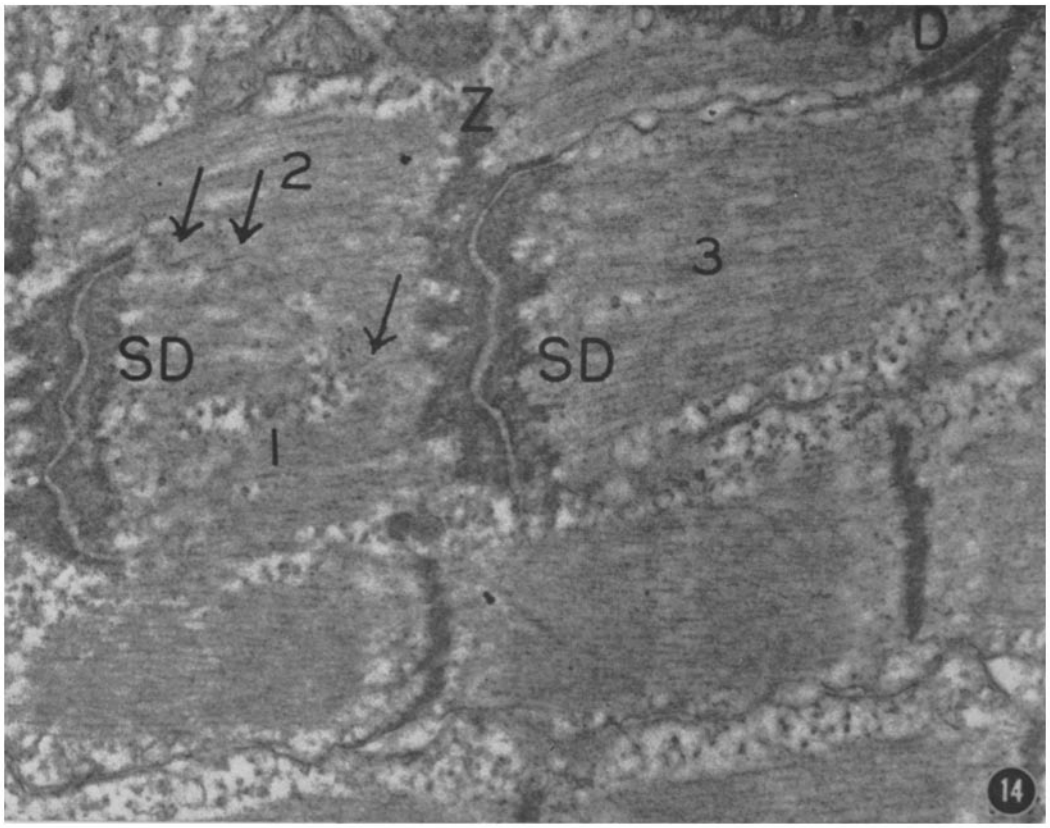
1. Barrnett, R. J., and Palade, G. E., Enzymatic activity in the M band, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 163.
2. Bourne, G. H., Enzymes of the intercalated disks of heart muscle fibres, *Nature*, 1953, **172**, 588.
3. van Breemen, V. L., Intercalated discs in heart muscle studied with the electron microscope, *Anat. Rec.*, 1953, **117**, 49.
4. Fawcett, D. W., Structural specializations of the cell surface, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 19.
5. Fawcett, D. W., and Selby, C. C., Observations on the fine structure of the turtle atrium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 63.
6. Heidenhain, M., Ueber die Structur des menschlichen Herzmuskels, *Anat. Anz.*, 1901, **20**, 33.
7. Jordan, H. E., and Steele, K. B., A comparative microscopic study of the intercalated discs of vertebrate heart muscle, *Am. J. Anat.*, 1912, **13**, 151.
8. Maximow, A., and Bloom, W., A Textbook of Histology, Philadelphia, W. B. Saunders Co., 6th edition, 1952, 22–35.
9. Moore, D. H., and Grimley, P. M., Problems in methacrylate embedding for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 255.

FIGURE 14

Section through parts of four cells, three of which (*1, 2, 3*) share two simple discs (*SD*). The border between cells 1 and 2 is marked by arrows. The disc at left delimits the broader portion of a wedge-shaped projection of cell 1, and a peripheral fibril of cell 2 extending to the left of the micrograph. The left hand border of the central disc is shared in the plane of section by the narrow portion of the wedge of cell 1 and the broad portion of the interlocking process of cell 2, the Z band of which is continuous with the disc. A single sarcomere of cell 3 is shown, delimited to the left by the central disc. A desmosome (*D*) is present at the upper right. × 40,000.

FIGURES 15, 16, 17

Sections through complex, corrugated discs (*CD*) which intersect several myofibrils in two (Fig. 15) or in three cells (Fig. 17). As observed in the simple discs above the dense disc material may be continuous with Z band material (*Z*, Fig. 16). Fig. 15, × 17,500; Fig. 16, × 25,000; Fig. 17, × 22,500.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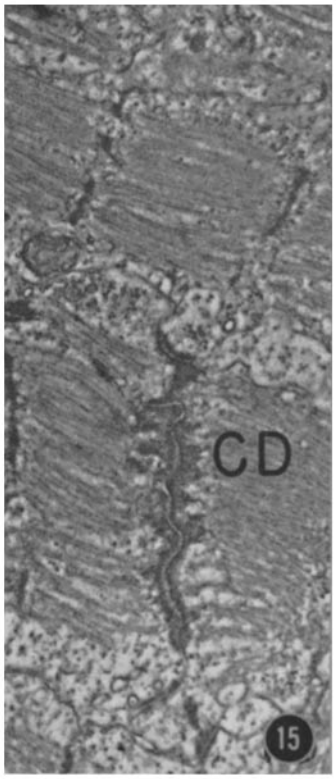
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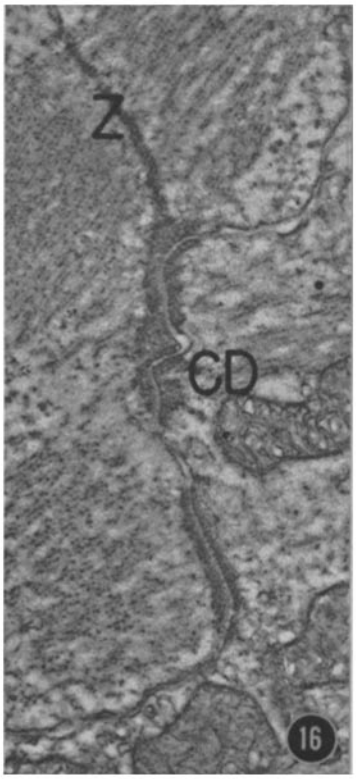
15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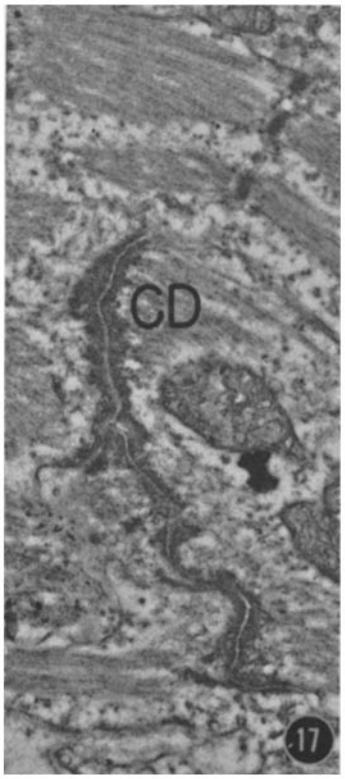
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10. MOORE, D. H., and RUSKA, H., Electron microscope study of mammalian cardiac muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 261.
11. MUIR, A. R., An electron microscope study of the embryology of the intercalated disc in the heart of the rabbit, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 193.
12. ODLAND, G., The fine structure of the interrelationship of cells in the human epidermis, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 529.
13. POCHE, R., and LINDNER, E., Untersuchungen zur Frage der Glanzstreifen der Herzmuskelgewebes beim Warmblütter und beim Kaltblütter, *Z. Zellforsch.*, 1955, **43**, 104.
14. PORTER, K. R., *Anat. Rec.*, 1954, **118**, 433; Proceedings 3rd International Conference on Electron Microscopy, London, 1954, London, Royal Microscopical Society, 1956, 539.
15. PORTER, K. R., and PALADE, G. E., Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.
16. RUSKA, H., EDWARDS, G. A., and CAESAR, R., A concept of intracellular transmission of excitation by means of the endoplasmic reticulum, *Experientia*, 1958, **14**, 117.
17. SCHWEIGGER-SEIDEL, F., DAS HERZ, *in* Handbuch der Lehre von den Geweben des Menschen und der Thiere, (S. Stricker, Editor), Leipzig, Wilhelm Engelmann, 1871, 177.
18. SJÖSTRAND, F. S., and ANDERSSON, E., Electron microscopy of the intercalated discs of cardiac mucle tissue, *Experientia*, 1954, **10**, 369.
19. SJÖSTRAND, F. S., ANDERSSON-CEDERGREN, E., and DEWEY, M. M., The ultrastructure of the intercalated discs of frog, mouse and guinea pig cardiac muscle, *J. Ultrastruct. Research*, 1958, **1**, 271.
20. WEISS, P., Cell contact, International Review of Cytology, (G. H. Bourne and J. F. Danielli, editors), New York, Academic Press, Inc., 1958, **7**, 391.
21. WOOD, R. L., Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 343.