

# TRANSITIONAL CARDIAC CELLS OF THE CONDUCTIVE SYSTEM OF THE DOG HEART

## Distinguishing Morphological and Electrophysiological Features

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### ABSTRACT

Cardiac cells with distinctive electrophysiological and morphological features were found at the junctional region between Purkinje and ventricular cells of the dog heart. The electrophysiological exploration of these "transitional" cells revealed action potentials markedly different in configuration from those generated by Purkinje or by ventricular cells. The impaled cardiac cells which generated transitional action potentials were identified in serial sections and studied with the light and the electron microscopes. The transitional cells were found to be characterized cytologically by: (a) their subendocardial location, (b) their small diameter, (c) the absence of T system and sarcoplasmic reticulum, and (d) the lack of intercalated discs under the light microscope and the sparsity of specialized intercellular junctions under the electron microscope. Purkinje, transitional, and ventricular cells were found to be joined by gap junctions permeable to lanthanum. A quantitative difference in the extent and distribution of specialized intercellular junctions may be one of the factors responsible for the slow velocity of conduction characteristic of the Purkinje-ventricular junctional region.

Electrophysiological studies on the specialized conductive system of the mammalian heart have demonstrated the presence of regions in which the impulses propagate under critical conditions, as evidenced by the conduction delays which occur under normal circumstances and the blockages of propagation induced by various experimental conditions (1-3). These regions are located at the junctions between different myocardial tissues, e.g., auricle and atrioventricular (A. V.) node; A. V. node and bundle of His; and at the union between Purkinje fibers and ventricular cells (P-V junction). Intracellular recordings from the junctional regions have shown cells which generate "transitional" action potentials distinctive from those of neighboring fibers. These cells are likely to be responsible for some physiological properties of the junctional areas (3, 4, 7).

Morphological examination of the conductive system of the heart has suggested that terminal Purkinje fibers are followed by a transitional cell type which makes contact with the adjacent ventricular fibers (35). The identification of transitional cells is not readily achieved by means of light microscopy alone, since the morphological differences reported with this technique are based on minor variations of shape and cell diameter. The present study was undertaken with the aim of identifying at the ultrastructural level the transitional cells located at the P-V junctional region. The fine structural features of those cardiac cells which generate a transitional type of action potential were found to be distinctive from those of adjacent Purkinje and ventricular cells, particularly in regard to the extent and distribution of specialized intercellular junctions.

## MATERIAL AND METHODS

Adult mongrel dogs were used. Under nembutal anesthesia, the heart was removed and immersed into a bath containing Tyrode's solution at 37°C. After the right ventricle was opened, a papillary muscle with an attached false tendon was excised and fixed with glass needles in a small chamber filled with oxygenated, flowing Tyrode's solution kept at 36–37°C. The electrical activity of the various types of cardiac cells was recorded through glass micropipettes (Ling-Gerard type) connected to a P6DC Grass<sup>1</sup> preamplifier and to a 565 Tektronix<sup>2</sup> oscilloscope. After a transitional cell was identified by its typical action potential (3), the micropipette was advanced downwards in order to prevent its detachment during the fixation procedure. The Tyrode's bathing solution was then replaced by cold fixative.

The following fixatives were used: (a) 2.5% glutaraldehyde in phosphate buffer; (b) 2.5% glutaraldehyde in cacodylate buffer; and (c) a glutaraldehyde-paraformaldehyde fixative (23). After fixation for 2–4 hr, the specimen was placed either in phosphate or in cacodylate buffer solutions. The region from which transitional potentials were recorded was trimmed off from the papillary muscle under a dissecting microscope. A piece of tissue of approximately 4 × 2 × 1 mm containing a segment of a false tendon and the adjacent portion of the papillary muscle was left. For facilitating the identification of the impaled transitional cell, one or several of the following procedures were used: (a) coordinates were drawn on a diagram of the endocardial surface of each trimmed block and used as a spatial reference to identify the location of the micropipette tip; (b) a small particle of graphite was placed next to the tip of the micropipette; (c) in some instances, the depression produced on the endocardium by the penetration of the micropipette was used as a reference. After the micropipette was withdrawn, the blocks were post-fixed either in 1% osmium tetroxide in phosphate buffer, in 1% osmium tetroxide with 1% lanthanum nitrate at pH 7.8 (30), or in 1% osmium tetroxide in cacodylate buffer containing 0.05% ruthenium red. Some blocks were treated in a 0.5% uranyl acetate solution (14) for 2 hr prior to dehydration. After dehydration in alcohol, the fragments were flat-embedded in Epon in small plastic dishes.

Following the identification of the site at which the microelectrode penetrated the endocardium, the blocks were trimmed under a dissecting microscope. The blocks were oriented first for obtaining sections perpendicular to the endocardial surface, and reoriented if needed, parallel to the long axis of the superficial myocardial cells. Serial sections 1–2 μ

thick were stained with toluidine blue; serial sectioning was performed until the tract left by the recording micropipette was detected. Subsequent thin sections containing the impaled cells and the adjacent myocardial fibers were observed under the electron microscope, after being stained with uranyl acetate and lead oxide. Sections of tissues treated with lanthanum hydroxide were studied without counterstain.

The fine structural features of Purkinje cells from the middle and terminal parts of the false tendon and those of ventricular cells from the papillary muscle were compared with the cytological characteristics of transitional cells. Observations were made with a Zeiss EM9a and a Siemens Elmiskop I electron microscopes.

## RESULTS

### *Electrophysiological Findings*

The false tendon and the papillary muscle from the dog heart were driven at a constant frequency (2/sec, 1 msec pulses, twice the threshold), placing the stimulating electrodes on the free end of the false tendon. The transmembrane potentials of Purkinje and ventricular fibers were recorded from the superficial cell layers through glass micropipettes (3M KCl, 20–50 megohms). Successive impalements of the false tendon were made, displacing one micropipette by 300–500-μ steps from the free end of the tendon up to its attachment in the papillary muscle. The shape, amplitude, and rate of rise of the action potential recorded from the cells distributed along the false tendon (*P* in Figs. 1 A, 1 B, and 2 A) were similar to those described for typical Purkinje fibers (12). The values for the resting membrane potential of Purkinje cells ranged from 70 to 90 mv (Fig. 1 B).

The subendocardial cells of the papillary muscle located within the region in which the false tendon merges into the myocardium were explored with a second recording micropipette (see diagram in Fig. 1 A). Within this narrow junctional region, the transmembrane potentials recorded from the most superficial layers of cells had the following distinctive characteristics: (a) a small resting membrane potential as compared with that of Purkinje or myocardial cells (*T<sub>r</sub>*, in Fig. 1 B); (b) the rate of rise of the initial phase of depolarization (*T<sub>r</sub>*, in Fig. 1 B) was slower than that of Purkinje or myocardial responses (*P* and *M* in Fig. 1 B); (c) a notch appeared either in the depolarization (*T<sub>r</sub>* in Fig. 1 A, 1 B) or in the repolarization phases (*T<sub>r</sub>* in Fig. 2 A, 2 B); and (d) the amplitude and dura-

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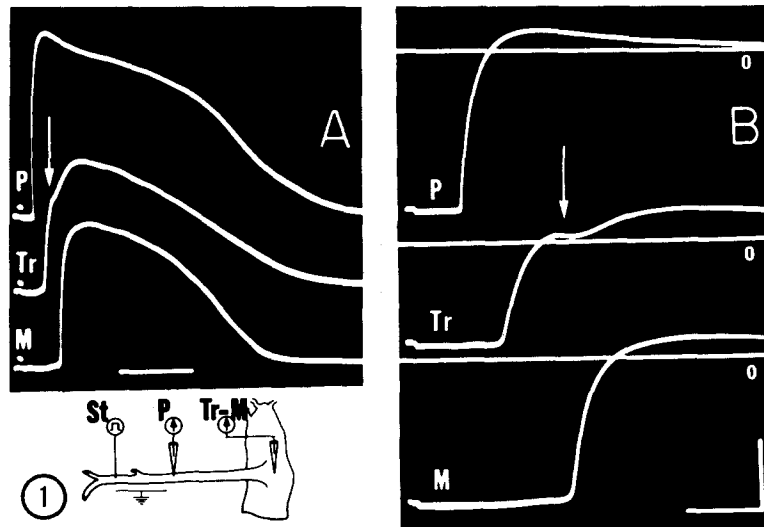


FIGURE 1 Distinctive characteristics of transmembrane potentials recorded from Purkinje (*P*), transitional (*Tr*), and myocardial (*M*) cells. The diagram in Fig. 1 A shows the arrangement of the stimulating (*St*) and recording micropipettes (*P*, *Tr-M*). Transitional potentials (*Tr*) are recorded only when the micropipette is within the junctional region; the notch of transitional potentials is indicated by the arrows. With a downward displacement, the same micropipette impales a myocardial cell, obtaining a typical myocardial action potential (*M*). In Fig. 1 B, the resting membrane potential of these cells and the upstrokes of *P*, *Tr*, and *M* responses are illustrated. Note that the lowest resting membrane potential and the slowest rate of rise of the action potential correspond to the transitional cells. Calibration in Fig. 1 A, 100 msec; in Fig. 1 B, 10 msec; 40 mv for Figs. 1 A and B.

tion of the response were small when compared with those of Purkinje cells (Fig. 2 A). These peculiar features of the responses generated by junctional cells led us previously to designate them as "transitional potentials" (2). The variable temporal location of the notch in these electrograms was due to the different activation time between Purkinje cells and the ventricular fibers adjacent to junctional cells. For example, when simultaneous recordings were taken from transitional and ventricular fibers, the notch of the transitional potential appeared concomitantly with the ventricular fiber upstroke (Fig. 2 B). Regardless of the temporal variation of the notch, the transitional potentials were markedly different in configuration from those originated by Purkinje or by ventricular cells. The more outstanding electrophysiological differences were the small resting membrane potential and the slow rate of rise of the transitional potentials.

The transitional potentials were usually recorded 20–50  $\mu$  in depth from the endocardial surface. When the same micropipette was advanced farther downward, a second transmem-

brane potential was recorded which had characteristics similar to those of the potential shown by the first transitional cell impaled, i.e., a slow rate of rise and the presence of a notch. In a few experiments, it was possible to record transitional potentials from three or four layers of cells, but usually these peculiar responses were observed in only the first two subendocardial layers. With a farther downward displacement of the same micropipette (see diagram in Fig. 1 A), a typical "working" myocardial action potential was obtained (*M* in Fig. 1 A). Exploration of deeper layers gave always action potentials with the characteristics of those of ventricular cells.

#### *Detection of Impaled Transitional Cells in Serial Sections*

Terminal Purkinje cells from the false tendon enter the subendocardial zone of the papillary muscle and spread over the superficial ventricular cells. This P-V junctional region from which transitional action potentials were recorded (diagrams, Fig. 2 A, 2 B) was studied in serial sections with the light microscope.

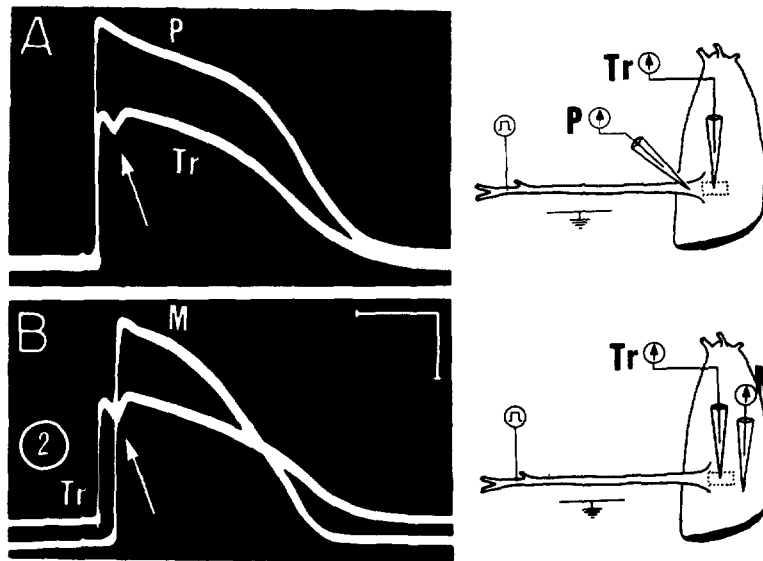


FIGURE 2 Temporal relationship between a transitional potential and the Purkinje and myocardial responses. The Purkinje (*P*), transitional (*Tr*), and myocardial cell (*M*) responses were recorded. The diagrams at the right show the distribution of the stimulating and recording electrodes. The region from which transitional potentials were obtained is enclosed by the dotted line. Note, in Fig. 2 A, that the upstroke of both the Purkinje (*P*) and transitional (*Tr*) responses occurred simultaneously. In Fig. 2 B, one of the recording micropipettes was used to impale a "working" myocardial fiber. Observe that the upstroke of the myocardial response (*M*) coincided with the appearance of the notch (arrow) in the transitional potential (*Tr*). Calibration: 100 msec, 25 mv.

Light microscopic identification of the cells which generated a transitional type of action potential was achieved in five different preparations. These cells were found to be located in a large subendocardial space containing abundant connective tissue elements, unmyelinated nerve fibers, and small blood vessels. The tract left by the recording micropipette was detected as a vertical discontinuity which passed through the endocardium, the subjacent connective tissue, and the first layers of cardiac cells (Fig. 3). Since transitional action potentials were always recorded from the first two or three layers of cells underlying the endocardium, the superficial cardiac cells which appeared transpierced by the microelectrode were considered to be those which generated the recorded transitional potentials.

Under the light microscope, transitional cells differed from Purkinje cells in being noticeably narrower (Figs. 4 and 5). In a series of measurements from various specimens, Purkinje cells from the false tendon of the dog were found to have a mean diameter of 25  $\mu$  (10–28) whereas the mean diameter of transitional cells was 8  $\mu$  (3–12). With

respect to the cell length, no differences were observed: Purkinje cells had a mean length of 165  $\mu$  (120–300) while transitional cells had a length of 154  $\mu$  (100–180). No clear-cut differences based on the intracellular distribution of myofibrils or the glycogen content could be detected between both types of cells. In contrast, the absence of cellular branching, the high content of glycogen, and particularly the lack of clearly defined intercalated discs in transitional cells (Figs. 3 and 5) facilitated their differentiation from ventricular cells.

#### *Electron Microscopic Findings*

The ultrastructural features of transitional cells were studied in sections consecutive to those light microscopy preparations in which the impaled cells were identified. Transpierced cells and those which join with them forming a slender subendocardial bundle were compared with terminal Purkinje fibers from the false tendon and with ventricular cells from the papillary muscle of the same preparation. Adequate fixation of the perfused specimen was obtained along the false tendon, in the



FIGURE 3 Photomicrograph of a portion of a P-V junctional region from which a transitional action potential was recorded. The tract of the recording micropipette disrupts the endocardium (*E*) and penetrates into the subjacent connective tissue. In this specimen, the micropipette was introduced farther down after the first subendocardial cell (*Tr*) gave a transitional type of action potential. As a consequence, three cardiac cells located at different depths appear transpierced by the microelectrode. There are some shrinkage and distortion of the impaled cells. In the lower part, a bundle of "working" ventricular cells (*V*) with abundant intercalated discs is seen. One- $\mu$ -thick section stained with toluidine blue.  $\times 500$ .

subendocardial region, and in the first 10 - 15 layers of myocardial cells of the papillary muscle. In these regions, no important differences were observed between the perfused specimens and the controls immersed in the fixative 2-3 min after the heart was removed from the animal.

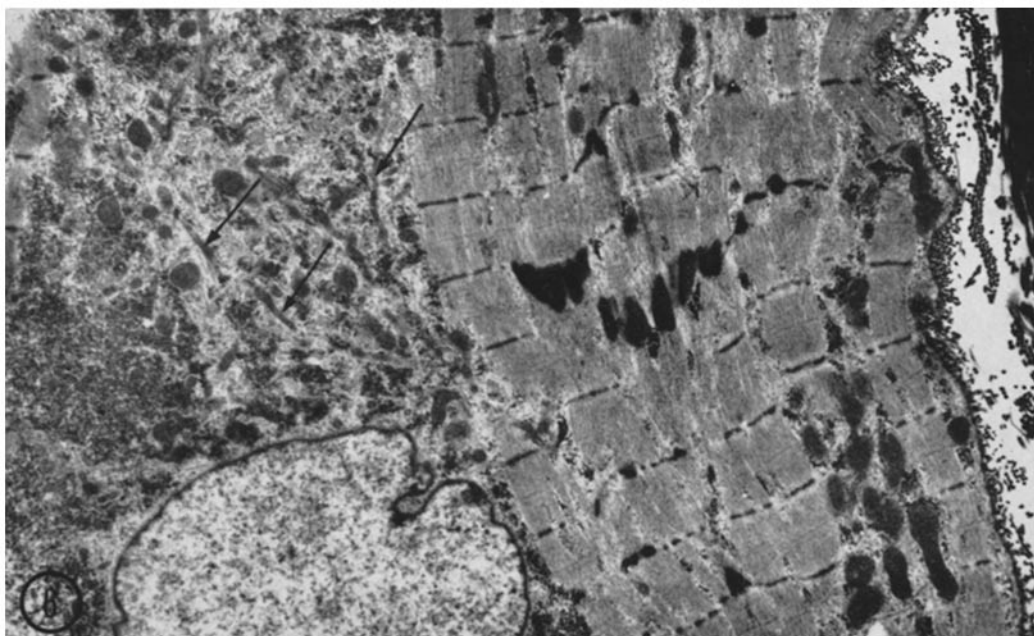
**TERMINAL PURKINJE CELLS:** The false tendon is formed by a bundle of Purkinje cells surrounded by a thick connective tissue sheath. Since the morphology of Purkinje fibers in the heart has been studied in a variety of mammals

(6, 24, 33, 34), only those fine structural features pertinent to differentiate Purkinje cells from transitional fibers will be mentioned. Most of the cytoplasm of Purkinje cells is filled with contractile material organized into ill defined myofibrils, separated by irregular clefts containing rows of mitochondria, glycogen granules, and tubular elements of the sarcoplasmic reticulum (Fig. 6). The basic arrangement of the myofilaments was similar to that reported for ventricular cardiac fibers (16). In longitudinal sections of Purkinje cells, cross-striations of the myofibrils appeared in



FIGURE 4 Purkinje cells from a portion of the false tendon near the P-V junctional region. Notice the considerable thickness of Purkinje cells as compared with that of transitional cells in Fig. 5. An intercalated disc (arrow) joins two Purkinje cells. Toluidine blue.  $\times 500$ .

FIGURE 5 P-V junctional region. Two transitional cells (*Tr*<sub>1</sub>, *Tr*<sub>2</sub>) are located immediately below the subendocardial connective tissue. Notice the small diameter of transitional cells as compared with that of Purkinje cells. No intercalated disc is seen at the junction between the cells (arrow). Toluidine blue.  $\times 500$ .



**FIGURE 6** Electron micrograph of a longitudinal section of a Purkinje cell from the false tendon. The large paraneuronal zone is occupied by abundant glycogen granules and by several filamentous mitochondria (arrows). The remainder of the cytoplasm is occupied by contractile material; broad irregular Z bands are seen in various sarcomeres. Abundant collagen fibers are seen in the extracellular space.  $\times 7,200$ .

lateral register over only a small portion of the cell width, owing to the frequent separation of sarcomeres by wide sarcoplasmic spaces containing mitochondria and glycogen granules (Fig. 6). The extent of the glycogen deposits varied considerably from cell to cell, but nevertheless these deposits constituted one of the distinctive characteristics of the Purkinje fibers.

In a large proportion of Purkinje cells, unusually broad Z bands were observed (Figs. 6 and 7). The dense material forming these broad Z bands was similar to that reported by other workers in normal (6, 15) and abnormal conditions (8, 18, 29). In this respect, it is interesting to emphasize that in our material these broad Z bands were seen exclusively in Purkinje cells; neither ventricular myocardial fibers nor transitional cells showed them. The broad Z bands showed cross-striation with an interperiod space of approximately 200 Å (Fig. 7). According to Fawcett (15), this periodicity, being similar to that of crystallized tropomyosin, suggests that the broad Z bands represent excessive production of a Z-band substance containing tropomyosin.

Peripheral elements of the sarcoplasmic retic-

ulum were prominent in Purkinje cells. They appeared as long tubules approximately 250 Å in diameter located either under the cell membrane (subsarcolemmal cisterna, Fig. 8) or between the most superficial sarcomeres. No evidence of continuity of these peripheral elements with the extracellular space was found either in specimens treated with lanthanum hydroxide or in those stained with ruthenium red (Fig. 8). In addition, we have confirmed with the use of lanthanum and ruthenium red the lack of transverse tubules in Purkinje fibers, as reported earlier by Sommer and Johnson (31, 32) and by Bencosme et al. (6).

Mitochondria were less abundant and more irregularly distributed in Purkinje cells than in ventricular fibers; whereas in ventricular cells mitochondria tend to parallel myofilaments and their length corresponds roughly to that of individual sarcomeres (16), mitochondria of Purkinje cells were disposed in irregular clumps in the paraneuronal region and in the intermyofibrillar spaces (Fig. 6). The pleomorphism of Purkinje mitochondria was more accentuated than that of mitochondria of ventricular fibers. Numerous slender mitochondria having a distinctive doughnut ap-

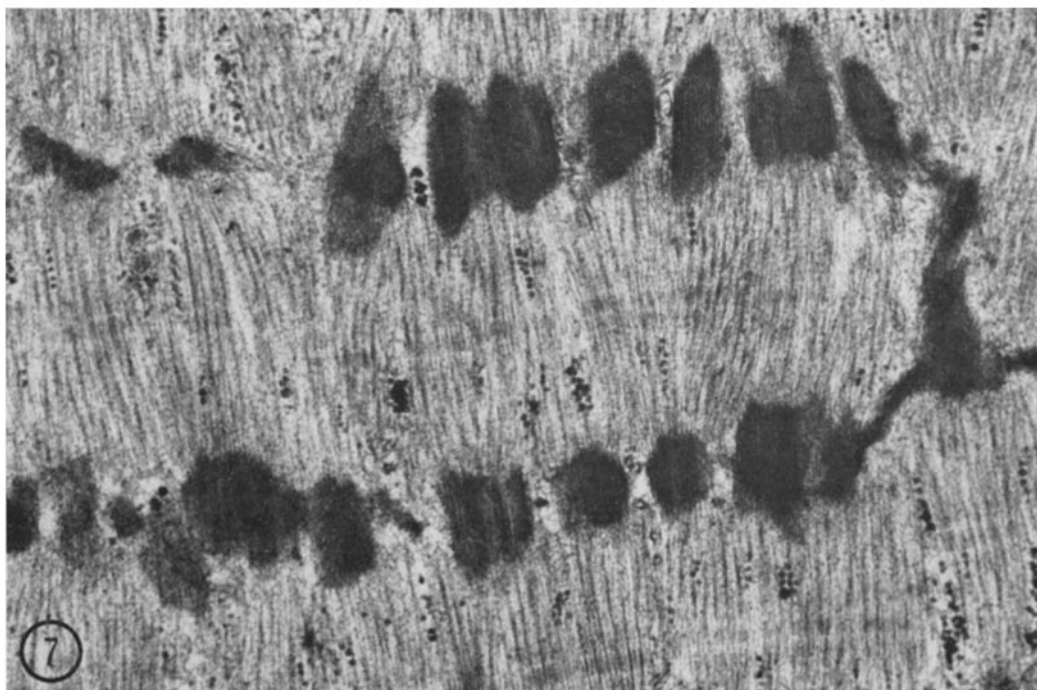


FIGURE 7 Higher magnification of a sarcomere of a Purkinje cell from the false tendon. The dense material of two Z bands is irregularly widened over the entire width of a sarcomere.  $\times 28,000$

pearance on transverse sections (Fig. 9) were seen in Purkinje cells, and they are so frequent as to give the impression of being a second population of mitochondria. Although doughnut-shaped mitochondria were also found in ventricular cells (16), their proportion was strikingly lower than in Purkinje fibers.

**TRANSITIONAL CELLS:** The identification of transitional cells under the electron microscope was facilitated by the small diameter of these cells, their subendocardial location, and, in some sections, by the presence of the hole produced by the recording micropipette (Fig. 10).

Transitional cells resemble Purkinje fibers in that most of their cytoplasm is filled with contractile material which does not form organized myofibrils. Additional resembling features between both types of cardiac cells were the presence of a sparse population of small and irregularly oriented mitochondria (Figs. 10 and 11) and the absence of transverse tubules, as evidenced in specimens treated with lanthanum hydroxide or with ruthenium red. The latter electron-opaque substances delineated clearly the T tubules of "working" myo-

cardial cells (25, 30). In contrast, transitional cells differ from terminal Purkinje fibers in that the former lack both the peripheral elements of the sarcoplasmic reticulum and the broad Z bands characteristic of Purkinje cells. By far, the most distinctive ultrastructural feature of transitional cells was the paucity of junctional end-to-end elements (Figs. 10-12 and 14), when compared with the complex succession of intercellular junctions which form the intercalated disc of Purkinje (Fig. 14) and ventricular cells (Fig. 16). Longitudinal (side-to-side) intercellular junctions frequently seen in other types of cardiac cells (16, 20) were usually lacking in transitional fibers, since the former appeared, in general, as single, isolated cell bundles.

#### *Intercellular Junctions*

**PURKINJE CELLS:** The plasma membranes of adjacent Purkinje cells form a succession of end-to-end specialized intercellular junctions appearing under the light microscope as a more or less straight intercalated disc (Fig. 4). When studied with the electron microscope, three types of surface

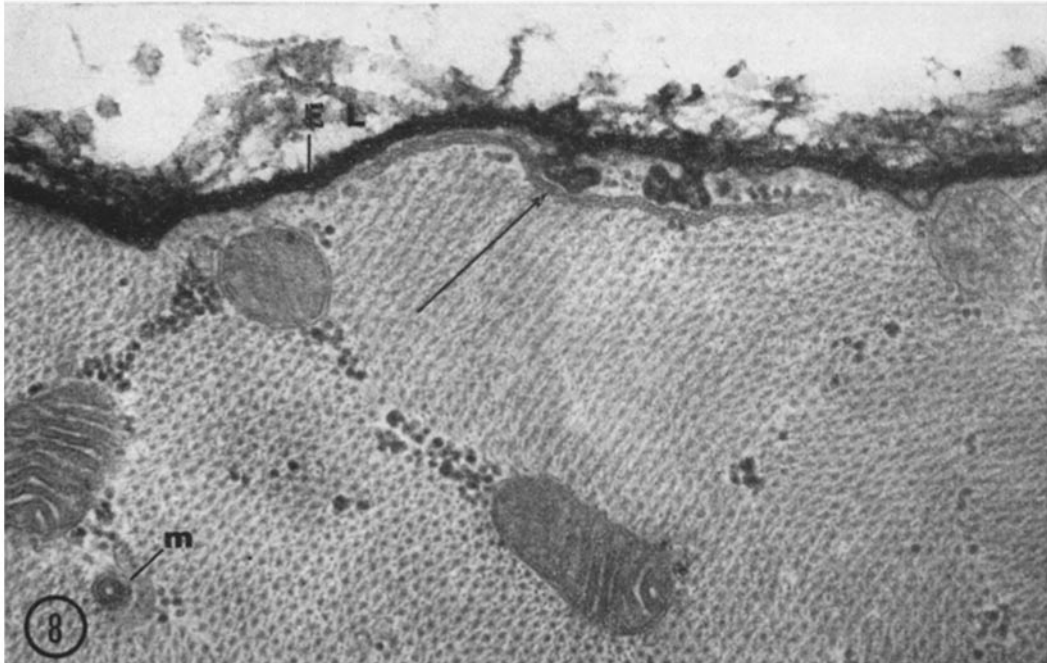


FIGURE 8 Peripheral region of a Purkinje cell from the false tendon. Ruthenium red has stained densely the external lamina (*EL*) (basement membrane), but has not penetrated into a subsarcolemmal cisterna of the sarcoplasmic reticulum (arrow). A doughnut-shaped mitochondrion (*m*) is seen in the lower left.  $\times 50,000$ .

junctions may be recognized in the intercalated disc: (a) gap junctions, previously designated as tight junctions (13, 18) or nexuses (5); (b) intermediate junctions, Z-band junctions (20), or fasciae adhaerentes (16); and (c) desmosomes or maculae adhaerentes (14). In tissues fixed with aldehydes and post-fixed with osmium tetroxide (Fig. 14), gap junctions appeared as focal regions in which adjacent cell membranes fused and obliterated the intercellular space. However, these junctions were freely permeable to both lanthanum hydroxide (Fig. 17) and ruthenium red (Fig. 13). Therefore, gap junctions in Purkinje cells were similar to those described first by Revel and Karnovsky (30) in the myocardium of the mouse and identified subsequently by others in a variety of tissues (9, 26). In specimens stained with uranyl acetate *en bloc*, the outer membrane leaflets of converging cell membranes do not touch but leave in between a narrow intercellular gap 20 Å in width (30). These junctions are not true tight junctions (zonulae occludentes) as previously supposed, but should be considered as specialized

regions of close membrane apposition. In addition, an hexagonal array of subunits with a center-to-center distance of 90 Å has been described in gap junctions of ventricular cells (30). A similar pattern was detected in the present study in gap junctions of Purkinje cells. The width of the intermediate line of the gap junctions was approximately 50 Å when lanthanum hydroxide or ruthenium red was used. In longitudinal sections of Purkinje cells from the false tendon, several gap junctions were seen to be distributed along the surface of end-to-end contacts. The remainder of the intercalated disc of Purkinje cells was formed by the successive disposition of desmosomes and intermediate junctions.

**TRANSITIONAL CELLS:** Only a few specialized intercellular contacts were seen at the junctional region of adjacent transitional cells. The scattered desmosomes and intermediate junctions of transitional cells (Figs. 10–12 and 15) did not form an intercalated disc recognizable with the light microscope (Fig. 5). Gap junctions were seldom observed in transitional cells (Fig. 15) and,



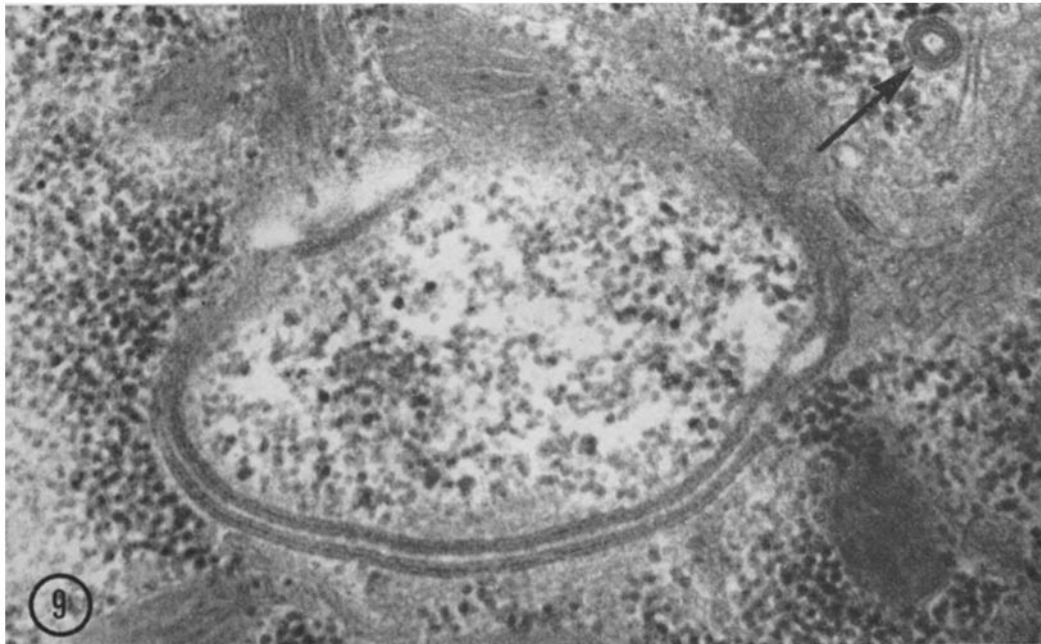


FIGURE 9 Long, filamentous mitochondria are particularly numerous in Purkinje cells. In transverse sections (arrow), they have a characteristic doughnut shape. In the lower part of the micrograph, a filamentous mitochondrion appears in a longitudinal view.  $\times 90,000$ .

when present, they were permeable to lanthanum (Fig. 18), as were gap junctions of terminal Purkinje cells (Fig. 17). In the remainder of the region of end-to-end apposition of adjacent cell membranes, where specialized intercellular junctions were lacking, the intercellular space appeared frequently dilated (Figs. 11, 12, and 15). It should be emphasized that this sparsity of end-to-end junctional elements was never seen in Purkinje fibers from the false tendon (Fig. 14) or in "working" myocardial cells (Fig. 16) from the papillary muscle.

**VENTRICULAR CELLS:** The ultrastructure of the intercalated disc of "working" myocardial cells from the papillary muscle of the dog (Fig. 16) was essentially similar to that described recently by Fawcett and McNutt in the cat ventricle (16). Throughout most of its length, the intercalated disc was formed by intermediate junctions; desmosomes were less numerous. Gap junctions were usually limited to the most peripheral regions of the intercalated disc. At higher magnifications, ventricular gap junctions were basically similar to gap junctions of Purkinje and transitional cells (Fig. 19).

#### DISCUSSION

This correlative study demonstrates the presence of transitional cardiac cells at the junctional region between Purkinje and ventricular fibers. Both the electrophysiological and the cytological features of the transitional cells are distinct enough to justify their designation as a separate cellular entity of the terminal region of the conductive system in the dog heart.

The electrophysiological properties of the transitional cells are quantitatively different from those of Purkinje and myocardial cells. The small resting membrane potential, the slow rate of depolarization, and the reduced amplitude of the responses have been consistently observed in this and in other studies (1-4, 7). These characteristics would confer, according to the electrical theory (10, 12, 21), a reduced safety margin for the propagation of impulses across transitional cells and for the corresponding excitation of adjacent ventricular cells. In fact, at the transitional region, conduction takes place under critical conditions as demonstrated by the effects of variables of different nature, such as high frequencies of stimulation,

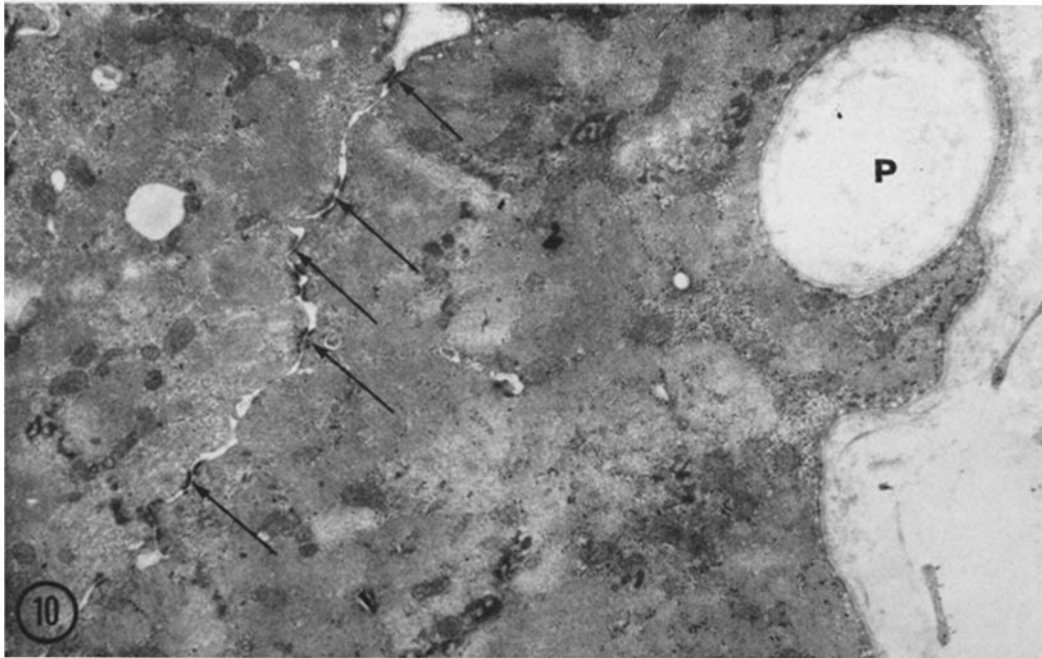


FIGURE 10 Low magnification micrograph of transitional cells. A regular perforation (*P*) produced by the recording micropipette is seen in a transverse section. Adjacent cells are joined only by a few intermediate junctions (arrows).  $\times 10,000$ .

low concentrations of external sodium, the use of calcium-free perfusing solution, anoxia, and low temperature. All these modifications lower the rate of rise and the amplitude of the transitional potentials, and, as a consequence, the conduction velocity of the impulses traveling along transitional cells is reduced (4 and 7). When one takes into account these electrophysiological properties, it seems likely that transitional cells would be responsible for the delays and blockages of propagation that occur preferentially at the P-V junctional region.

The morphological study of serial sections from the P-V regions allowed us to identify those cardiac cells which generated the transitional type of action potential described above. The technique used in the present study for the identification of impaled cells was based on the detection of the hole left by the shaft of the recording micropipette in a cell (11). Since several subendocardial cells from each preparation were usually impaled before a transitional action potential was recorded, only after a transitional cell was identified was the micropipette introduced farther down two to five

cell layers. The latter procedure prevented the detachment of the shaft of the micropipette during fixation, thus facilitating the location of the cells under study. In subsequent experiments, the subendocardial location of transitional cells, their small diameter, and the absence of intercalated discs were used as criteria for their identification in preparations that were neither perfused nor explored with the recording micropipette. The obtaining of consecutive thin sections immediately after a transitional cell was detected with the light microscope allowed us to study the fine structural features of the particular cell from which the transitional action potential was recorded in each preparation. In some instances, the cell architecture was disturbed in cells transpierced by the thick segment of the micropipette (Fig. 3); in other cases, the cell membrane sealed itself around the tip of the micropipette, leaving the cytoplasmic components of the transitional cell apparently unaltered even when observed at the ultrastructural level (Fig. 10).

The existence of a "transitional" type of cardiac cell in the terminal region of the conducting sys-

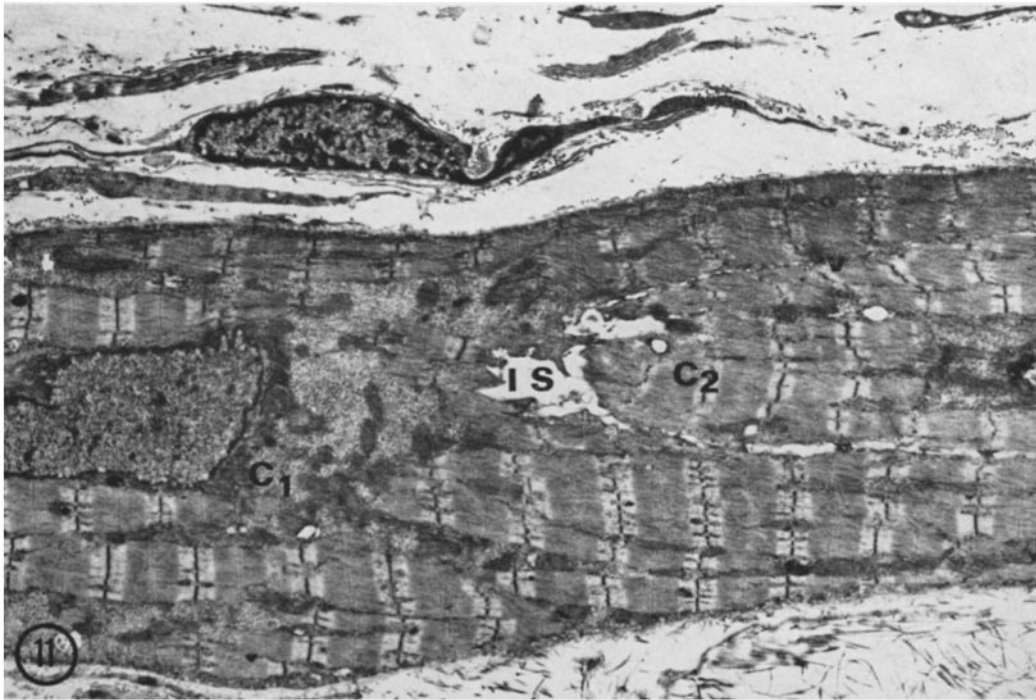


FIGURE 11 Longitudinal section of transitional cells, showing the small diameter of the cell, the abundance of contractile material, and the sparsity of junctional elements between two adjoining cells ( $C_1$ ,  $C_2$ ). Notice the large intercellular space ( $IS$ ) with numerous collagen fibers.  $\times 5,000$ .

tem of the mammalian heart was suggested earlier by the histological studies of Truex (35). However, definite identification of cellular entities different from both Purkinje and myocardial cells was not achieved with the methods used by that author. In the present study, the use of consecutive thick and thin sections for light and electron microscopy, respectively, permitted the characterization of the transitional cells. In both Purkinje and transitional cells, the distribution of the contractile material is more irregular than in typical "working" myocardial cells (16); mitochondria are small and irregularly distributed, transverse tubules are lacking, and glycogen granules tend to form large deposits seldom seen in ventricular cells. On the other hand, the small diameter and the lack of intercalated discs are characteristics of transitional cells. Furthermore, the peripheral elements of the sarcoplasmic reticulum, so prominent in Purkinje cells, are not seen in the transitional elements.

In turn, transitional cells differ from ventricular fibers in that they lack the membranous compo-

nents of the sarcoplasmic reticulum, transverse tubules, and intercalated discs. In addition, the typical subendocardial location, the fact that sarcomeres are rarely seen in register over the entire width of the cell, and the formation of large intermyofibrillar or paranuclear deposits of glycogen also contribute to the differentiation of transitional cells.

The particular cytological traits of transitional cells are likely to be related to some electrophysiological features of the P-V region, such as the slow conduction rate and the susceptibility to block. Among the significant structural characteristics, those that may determine the slow rate of conduction of transitional cells are: (a) the absence of a transverse tubular system; (b) the small diameter of the cell; and (c) the sparsity of specialized intercellular junctions.

The absence of transverse tubules in transitional cells could be interpreted, following Girardier (17), as a reflection of the small diameter of the cell. He considered that in both skeletal and cardiac muscle

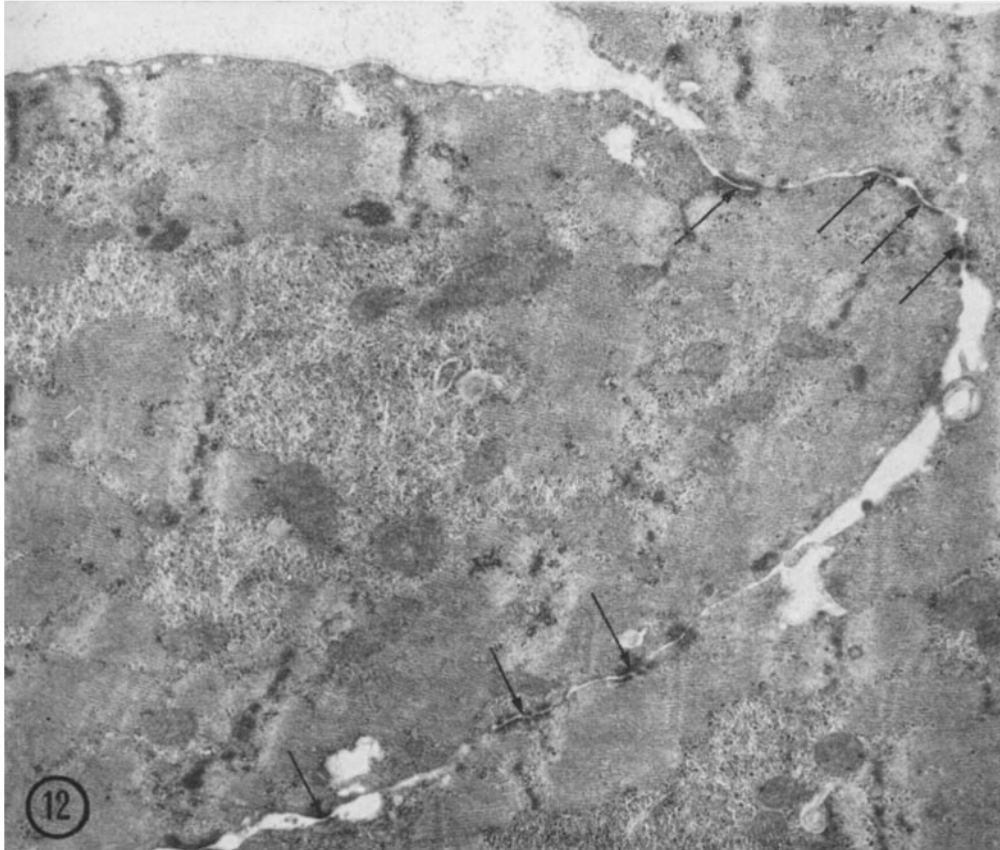


FIGURE 12 Longitudinal section of a transitional cell seen at higher magnification. At a v-shaped junctional region only a small number of intermediate junctions are present (arrows). In the remainder of the junctional region, the intercellular space is irregularly widened.  $\times 25,000$ .

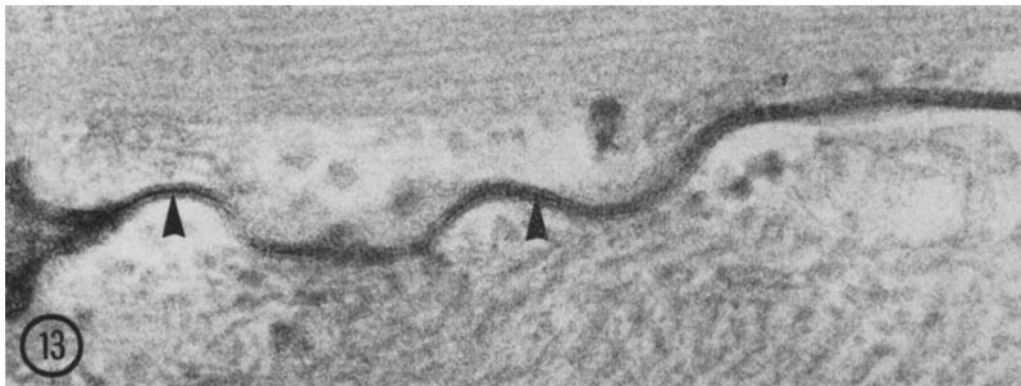
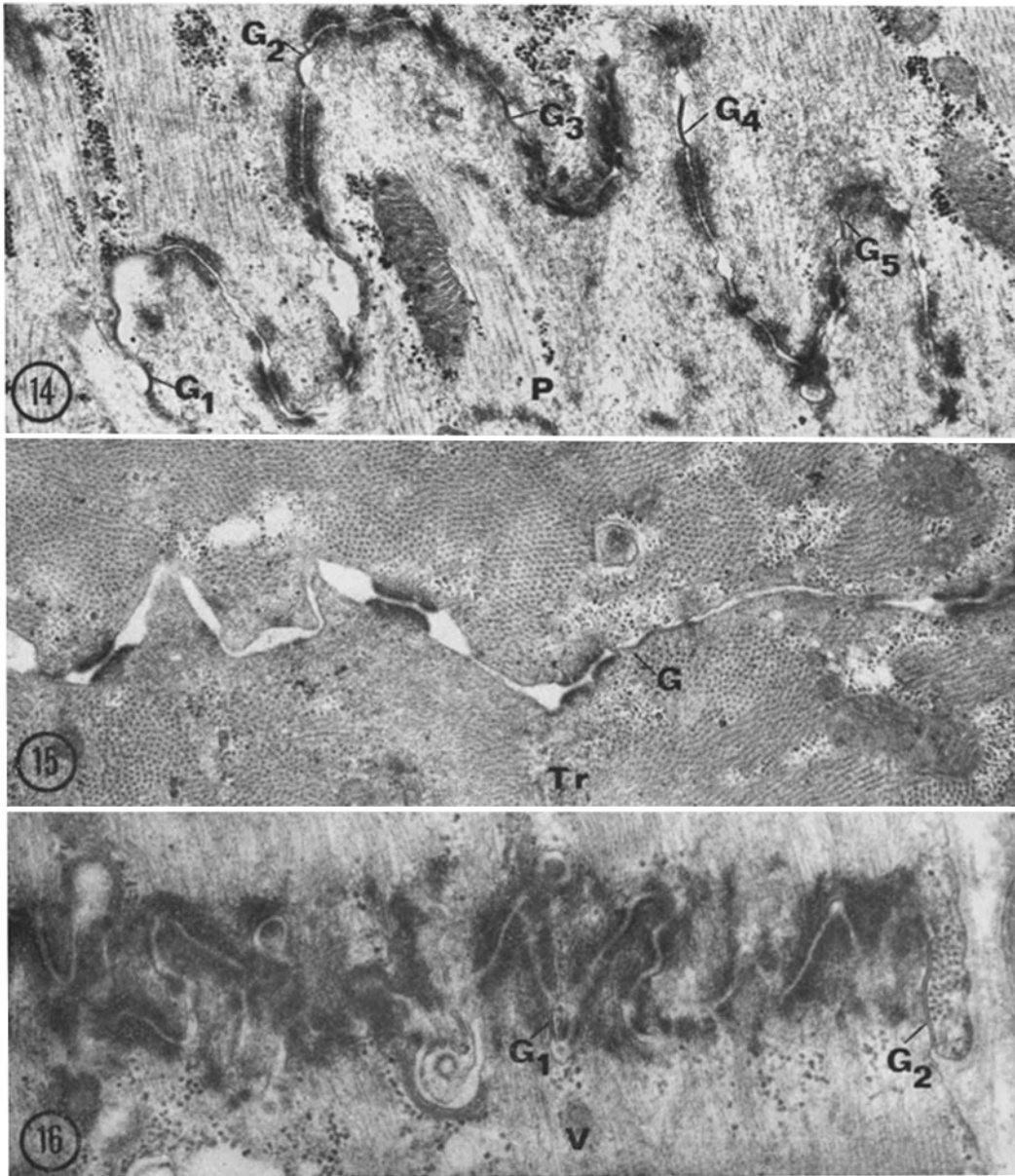
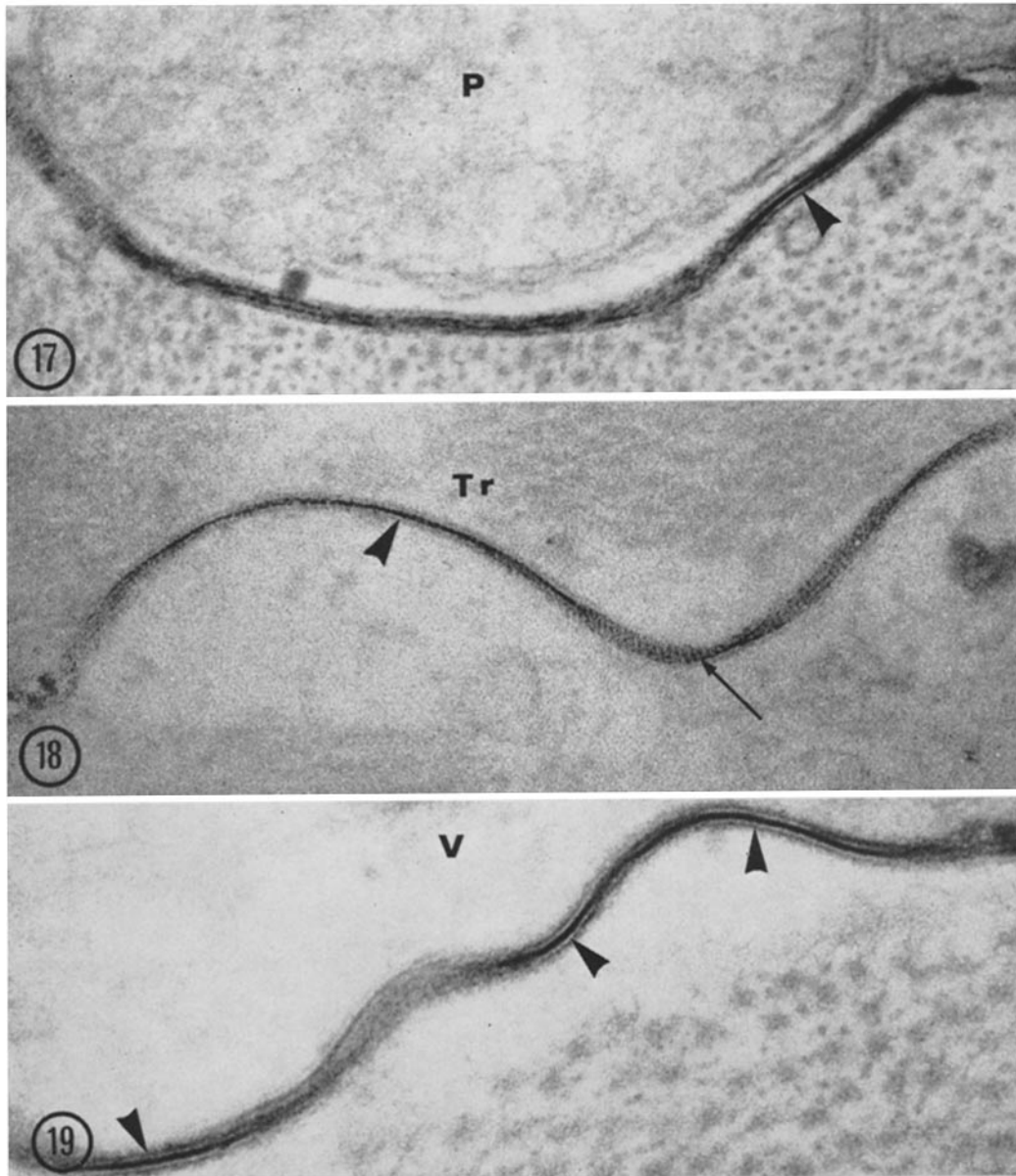


FIGURE 13 High magnification of a gap junction from a Purkinje cell. With standard techniques, gap junctions appear to be formed by fusion of the outer leaflets of adjoining cell membranes. However, ruthenium red permeates the intermediate region of the junction which appears as a dense thin line (arrowheads).  $\times 120,000$ .



FIGURES 14-16 Specialized intercellular contacts joining end-to-end Purkinje (*P*), transitional (*Tr*), and ventricular (*V*) cells. The intercalated disc of Purkinje cells is formed by a succession of gap junctions, desmosomes, and intermediate junctions; gap junctions are particularly numerous in longitudinal sections ( $G_1 - G_5$ ). In contrast, transitional cells (*Tr*) are joined by only a few intermediate junctions and by an occasional small gap junction (*G*). The intercalated disc of ventricular cells (*V*) is formed mainly by intermediate junctions; gap junctions ( $G_1 - G_2$ ) are usually seen in the peripheral regions of the intercalated disc. Figs. 14-16:  $\times 21,000, 30,000,$  and  $30,000,$  respectively.



FIGURES 17-19 High magnifications of gap junctions after treatment with lanthanum hydroxide. The width of the intermediate line (arrowheads) is similar in gap junctions of Purkinje (*P*), transitional (*Tr*), and ventricular cells (*V*). A periodic substructure is seen when the junction is cut in a tangential plane (arrow). Figs. 17-19:  $\times 160,000$ ,  $120,000$ , and  $160,000$ , respectively.

cells, a critical cell size exists below which no transverse or sarcoplasmic reticulum is present. However, the lack of a T system in transitional cells does not appear to be related to the cell diameter, since we have observed a prominent T

system in ventricular cells from the papillary muscle, some of which have approximately the same diameter as transitional cells. Furthermore, in a comparative study of atrial and ventricular myocardial cells, Fawcett and McNutt (16, 27)

found that the presence of T tubules is not necessarily related to the diameter of the cell. The hypothesis that the occurrence of the T system tends to slow conduction by increasing membrane capacitance has been strengthened by the finding of a lack of these elements in Purkinje (32) and auricular fibers (27) in which the propagation of impulses is faster than in the ventricular cells. We have confirmed in the present study the absence of transverse tubules in typical Purkinje cells from the false tendon. However, transitional cells, which have characteristically a slow conduction velocity (4), are also devoid of this system. Hence, a relative or absolute deficiency of a T system should not be taken alone as a reliable morphological index of the velocity of propagation in cardiac cells.

Another cytological characteristic of transitional cells which would modify, according to the cable theory (22), the rate of conduction of action potentials across the P-V junctional regions is the small cross-sectional area of transitional cells. In fact, Purkinje fibers from the false tendon, being noticeably larger in diameter, conduct faster than the slender transitional cells (4).

At the present time, there is considerable evidence that the spread of excitation in cardiac muscle cells occurs through low-resistance intercellular pathways (36, 37) represented morphologically by intercellular junctions in which the plasma membranes of adjacent cells are in close apposition. Until recently, these specialized intercellular contacts were considered to be formed by fusion of the outer membrane leaflets of converging cell membranes. Accordingly, the terms tight junctions, fascia occludens (16), or nexus (5) have been used in the literature. However, with the use of uranyl staining *en bloc* (14), a narrow intercellular gap approximately 18 Å wide has been detected in the "nexuses" of mouse cardiac cells (30). In addition, this gap has been found to be freely permeable to lanthanum solutions (30), demonstrating the continuity of the narrow junctional intercellular space with the extracellular space. Therefore, we consider that the term gap junction (9, 30) describes better the fine structural features of the regions of close membrane apposition found at the intercalated discs of cardiac cells. Gap junctions with similar characteristics have also been described in the central nervous system (9) and in a variety of epithelial tissues (26). A peculiar macromolecular arrangement has been found at the outer membrane leaflets of gap junctions in

mouse cardiac cells, in which lanthanum delineates an hexagonal pattern with a center-to-center distance of 90 Å (30). The hexagonal pattern is evident in gap junctions from other tissues, as demonstrated not only with lanthanum but also with negative staining (19) and with the freeze-etching technique (34). This hexagonal pattern may be related to the presence of phospholipids (19). The present study demonstrates that Purkinje and transitional cells of the dog heart are also joined by gap contacts. The width of the intermediate line stained with lanthanum and the characteristics of the hexagonal pattern were similar in gap junctions of Purkinje, ventricular, and transitional cells.

The investigation of the effects of hypertonic solutions on the rate of conduction of cardiac cells and on the ultrastructure of the intercalated disc has suggested that gap junctions (nexuses) are sites of electrical coupling (13, 28). Desmosomes and intermediate junctions (fasciae adhaerentes) may also represent pathways of low resistance since they are present in the hearts of some species (fish, frog, chicken) which are devoid of gap junctions (33, 38). When our results are taken into account, the extent and distribution of the various intercellular contacts which join cardiac cells are likely to influence the conduction rate of the action potential. This possibility is based on the observation that the transverse segment of the intercalated disc of Purkinje cells contains considerably more gap junctions than that of "working" ventricular cells, which have a lower conduction rate. On the other hand, transitional cells having characteristically a low conduction rate (4) were found to be joined by few intercellular contacts which do not form a continuous intercalated disc. These observations suggest that a quantitative variation in the distribution of intercellular junctions may be one of the structural factors related to the various conduction rates recorded in different regions of the conduction system of the heart.

Work already in progress in our laboratories indicates that transitional cells are present not only in the P-V region, but also in other parts of the conductive system of the mammalian heart in which the conduction of impulses is delayed and from which transitional potentials have been recorded under different experimental conditions (1, 2).

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