CARDIAC MUSCLE

A Comparative Study of Purkinje Fibers and Ventricular Fibers

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ABSTRACT

With light and electron microscopy a comparison has been made of the morphology of ventricular (V) and Purkinje (P) fibers of the hearts of guinea pig, rabbit, cat, dog, goat, and sheep. The criteria, previously established for the rabbit heart, that V fibers are distinguished from P fibers by the respective presence and absence of transverse tubules is shown to be true for all animals studied. No evidence was found of a permanent connection between the sarcoplasmic reticulum and the extracellular space. The sarcoplasmic reticulum (SR) of V fibers formed couplings with the sarcolemma of a transverse tubule (interior coupling) and with the peripheral sarcolemma (peripheral coupling), whereas in P fibers the SR formed only peripheral couplings. The forms of the couplings were identical. The significance, with respect to excitation-contraction coupling, of the difference in the form of the couplings in cardiac versus skeletal muscle is discussed together with the electrophysiological implications of the differing geometries of bundles of P fibers from different animals.

INTRODUCTION

The difficulties confronting the worker concerned with cardiac muscle, physiologist and morphologist alike, are mainly due to the quasi-syncytial nature of cardiac muscle; the interdigitation and overlapping of cells complicates considerably the topographic relationship of one cell to another. It may be for this reason that comprehensive structural studies of heart muscle, matching or even approaching those of skeletal muscle in their usefulness for structure–function correlation, are missing from the literature (1–8). Another reason may be that advances in fixation for electron microscopy have been made only rather recently.

Concentrating on structure–function correlation in heart muscle, we have recently analyzed a strand of rabbit cardiac muscle (9) as well as a Purkinje fiber of the goat (10). In the course of these analyses we have made two observations of strategic importance that have pointed the way for the present investigation. One of these observations had to do with the frequency and geometric distribution of regions of close apposition between cardiac cells. The other observation was that there were two distinct populations of muscle cells in the ventricle. The cells of one population had transverse tubules while the cells of the other did not. These latter cells were topographically and electrophysiologically Purkinje fibers. Although from their first description by Purkinje (11) until recently the Purkinje fibers have evaded clear structural definition by either light (12) or electron microscopy (13–20), it has now become evident that the fundamental structural difference between the Purkinje fibers and ventricular fibers resides in the respective absence or presence of transverse tubules. The objectives of this paper are (*a*) to

present evidence that the original observation made on the Purkinje fibers of the rabbit holds true also for the goat, sheep, dog, guinea pig, and cat and (*b*) to discuss the functional characteristics of Purkinje fibers *versus* ventricular fibers on the basis of their structural differences. With respect to the cell apposition in the case of Purkinje fibers, it will be shown that a variation exists in different animals which seems to correlate with the size of the animal, that is to say, with the relative size of the hearts. From these comparative structural observations certain conclusions will be drawn concerning the applicability of these preparations for the sucrose-gap and voltage-clamp techniques (21–23).

DEFINITIONS, CRITERIA, AND DIMENSIONS

Purkinje Strand and Purkinje Fiber

We have chosen the name "Purkinje fiber" (P fiber) for heart muscle cells that do not have transverse tubules, and the name "Purkinje strand" (P strand) for bundles of such fibers. Heart-muscle cells that have transverse tubules we call "ventricular fibers" (V fibers) and bundles thereof "ventricular strands" (V strands). Comparative studies have shown that papillary muscle corresponds to ventricular muscle elsewhere.

Sarcoplasmic Reticulum

The sacroplasmic reticulum (SR) is described in terms of tubules that vary between approximately 20 and 60 mμ in diameter. In our previous paper (9) we retained the term terminal cisternae for the compartments of the SR that are a part of triads, diads, etc. for historical and comparative reasons and to avoid confusion. However, we have decided not to use this term, from now on, because in heart muscle, as opposed to skeletal muscle, these "cisternae" rarely look like cisternae and are certainly not terminal. In the present paper we shall refer to this specialized compartment of the SR as the "junctional SR."

Transverse Tubules

These are large, true tubules of sarcolemma, continuous with the extracellular space and vested with a clearly visible basement membrane. The transverse tubules, averaging approximately 0.2 μ in diameter, traverse the muscle cells transversely, i.e. at right angles to the longitudinal axis of the sarcomeres, and are located at the Z lines in heart muscle. They correspond to the central component or intermediate vesicles of the triads (24).

Sarcolemmal Invaginations

The term is applied to sarcolemmal folds of varying geometry that extend into the cell. They are not too common, and there was never any doubt as to their nature because of their large, irregular dimensions, and the fact that a topographic relationship to Z lines was seen only occasionally.

Sarcolemma

This is the plasma membrane, or cell membrane, of the individual muscle cell that separates the cell physically from the environment. Since the sarcolemma of heart muscle is always vested with a basement membrane on the side facing the extracellular space, in this paper the term sarcolemma refers to the plasma membrane plus basement membrane.

Couplings

The terms triads, diads, dyads, and pentads refer to the numerical relationship between terminal cisternae and transverse tubules (25–27), and there is good evidence that these structures are important for at least one step in excitation-contraction coupling (28). The functional unit seems to be the very close junction of a portion of the SR with a portion of the sarcolemma. However, in heart muscle these units also occur at the periphery of the cell, that is to say, unassociated with transverse tubules. Therefore, in these instances, the terms triads, dyads (diads), and pentads do not, *sensu stricto*, apply. Consequently, in the present paper this functional unit is referred to as coupling (9). As a matter of general topographic distribution, couplings occurring at the outer sarcolemmal envelope of the cell are referred to as "peripheral couplings," e.g. in P fibers, as opposed to "interior couplings" that are always associated with transverse tubules, i.e., in the framework of triads, dyads, etc. Invariant features of the coupling are the "junctional granules" (central membrane of reference 9), located in the center of the junctional SR. It is by the junctional granules, which are only seen in the junctional SR, that the couplings of cardiac

muscle can be easily identified. An additional characterizing feature of the coupling is the presence of periodic processes or densities between the junctional SR and the sarcolemma (9).

Cell Junctions

The nomenclature of Farquhar and Palade is used here (29, 30). The term zonula occludens refers to the region of close approximation of the plasma membranes of adjacent heart muscle cells which are parallel to the longitudinal axis of the myofibrils. No consideration was given here to the question whether a gap exists between the two unit membranes constituting the zonula occludens (31). In any case, we assume this particular junctional complex to be of low electrical resistance. Macula adherens refers to small, more isolated structures and is synonymous with the desmosome. The intercalated disc refers to the zigzag portion of cell apposition which is so characteristic of heart muscle. The intercalated disc is represented by the zonula adherens only. It occasionally alternates with maculae adherentes and longitudinally arranged zonulae occludentes as well as with simple appositions of the two plasma membranes which sometimes dilate into relatively large pockets of extracellular space. Neither these pockets nor the zonula occludens are, *sensu stricto*, part of the intercalated disc. The term intercellular cleft is used whenever reference is to be made to intercellular space that is characterized by its narrow width (approximately 20–30 mμ) over great lengths, e.g., in the P strands of the ungulates.

MATERIALS AND METHODS

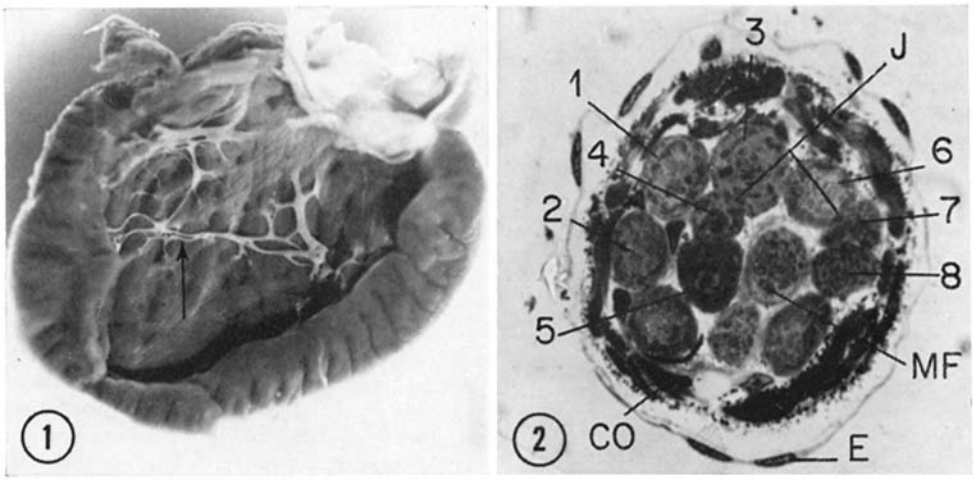
The P strands and papillary muscles were obtained as previously described in detail (9). The sheep, goat, and dog were sacrificed by exsanguination under anesthesia (pentobarbital sodium) and the guinea pig and rabbit by a blow on the neck; in the case of the cat, the heart was removed under anesthesia. The P strands and the papillary muscles were fixed by immersion in 4% glutaraldehyde (32) buffered with 0.05 M phosphate buffer at pH 7.4 for from 2 to 24 hr at room temperature. In the case of the dog, the heart was perfused with glutaraldehyde via the coronary arteries. After having been washed in distilled water for 12 hr, the P strands were postfixed in osmium tetroxide (33) and embedded in Maraglas. After proper alignment, the tissue was cut in longitudinal and transverse directions. The sections, cut with glass knives on a Porter-Blum ultramicrotome MT-1, and suspended on naked 200- and 300-mesh copper grids, were stained with uranyl acetate in 50% ethanol followed by lead citrate (34), and then viewed with either the RCA EMU-3F or the Zeiss EM-9. Of the P strands of each animal, thick, transverse sections (approximately 1 μ) were prepared for light microscopy and stained essentially according to the methods of Richardson (35), and then viewed with a Leitz Ortholux microscope. In the case of one goat strand, 100 consecutive thick serial sections were cut and processed as previously described (9). Thin sections adjacent to the first and last of these sections showed that no reliable estimate with respect to the presence or absence of zonulae occludentes could be made from the light microscopic pictures as has been possible with the P strand of the rabbit (9). However, the serial sections turned out to be revealing in another aspect of the geometry of the P strand. For this reason, strategic sections were chosen to represent the course of the P strand over a distance of about 100 μ. The ATPase reaction as illustrated in Figs. 46 and 47 has been described in detail elsewhere (36).

RESULTS

Light Microscopic Observations

Cross-sections through the P strands from the different animals (Figs. 1–6) showed the following differences and similarities.

GENERAL OBSERVATIONS: The P fibers were rather loosely assembled in the cat, rabbit (Fig. 2), and guinea pig (Fig. 3). They were very tightly packed in the goat and sheep, so much so that, without electron microscopic information, the impression of large, single P fibers might be created (Figs. 5 and 7) whereas actually they were P strands. In their general arrangement and appearance, the P fibers in the dog resembled those in the cat, rabbit, and guinea pig although they were much more tightly packed in the dog. The P fibers showed great variation in their individual diameters in all animals, including the rabbit; this is partly because they fuse with one another via junctional complexes. In an earlier report (9) it was shown that a reasonably accurate estimate of the number and distribution of junctional complexes could be made in an approximately 200 μ long segment of a P strand of the rabbit. In the cat and guinea pig, junctional complexes between two cells can also be seen, while in the dog they were more difficult to visualize (Fig. 4). This was also true for the goat and sheep, although actually the location of the cell borders of two adjacent cells was easier to estimate because the myofibrils, readily visualized with the light microscope, were almost exclusively located



Key to Symbols

A, zonula adherens
B, basement membrane
C, coupling
CM, junctional granules
CO, collagen
D, macula adherens
E, endocardium
EC, endothelial cell
F, fibrocyte
G, extracellular space
Gl, glycogen
GO, Golgi complex
I, I band
IC, intercellular cleft
ID, intercalated disc
J, junctional complex
JS, junctional SR
M, M line
MF, myofibril
Mi, mitochondrion
MS, special mitochondrion
N, nucleus
NL, nucleolus
O, zonula occludens
P, pinocytotic vesicle
Pi, pit
S, sarcolemma
Si, sarcolemmal invagination
SR, sarcoplasmic reticulum
T, transverse tubule
Z, Z line

FIGURE 1 Cat heart. The P strands are clearly visible as a network of fibers of varying thickness crossing the ventricular cavity. The strand marked with an arrow was used for light and EM studies. Figs. 2–6 represent light microscopic illustrations of cross-sections of P fibers of several animals. The P fibers of the cat, not shown here, are identical with those of the rabbit. The P strand of the rabbit has been the object of an extensive study elsewhere (9).

FIGURE 2 Rabbit heart, P strand, cross-section. The strand is separated into individual fibers, e.g. fiber *1*, *2*, and fibers joined by junctional complexes, e.g. *3*, *4*, *5*, and *6*, *7*, *8* (see reference 9). The whole strand is surrounded by a dense layer of collagen (*CO*) which, in turn, is covered by endothelium. *E*, endocardium. The individual fibers are rather loosely joined. Note thin sheath of collagen as compared to that in Fig. 7. × 900.

subjacent to the sarcolemma (Figs. 5 and 6). The P strands were always surrounded by a considerable amount of collagen with fibrocytes and Schwann cells intermixed. All structures were ultimately covered by endocardial endothelium. The number of myofibrils in the P fibers of most animals studied was considerable, except in the goat and sheep in which they were small in number and almost exclusively located in the extreme periphery of each cell. Consequently, and in the absence of other discrete differences, a safe distinction between P fibers and V fibers is possible, with the light microscope, only in the goat and sheep (and presumably in all larger hearts; see Discussion). The glycogen content of V fibers compared with P fibers is altogether unreliable as

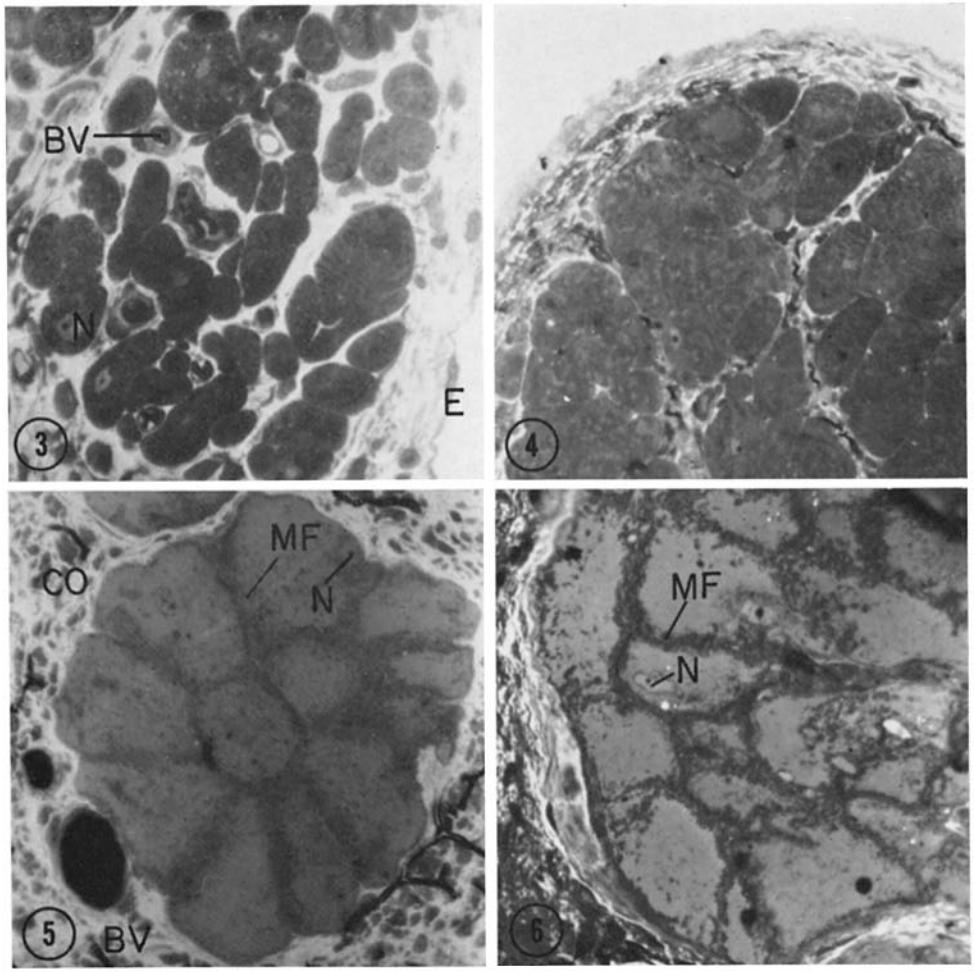


FIGURE 3 Guinea pig, P strand, cross-section. Similar to Fig. 2. The strand is larger and, therefore, contains several blood vessels (*BV*). The myofibrils are often arranged in bands. × 400.

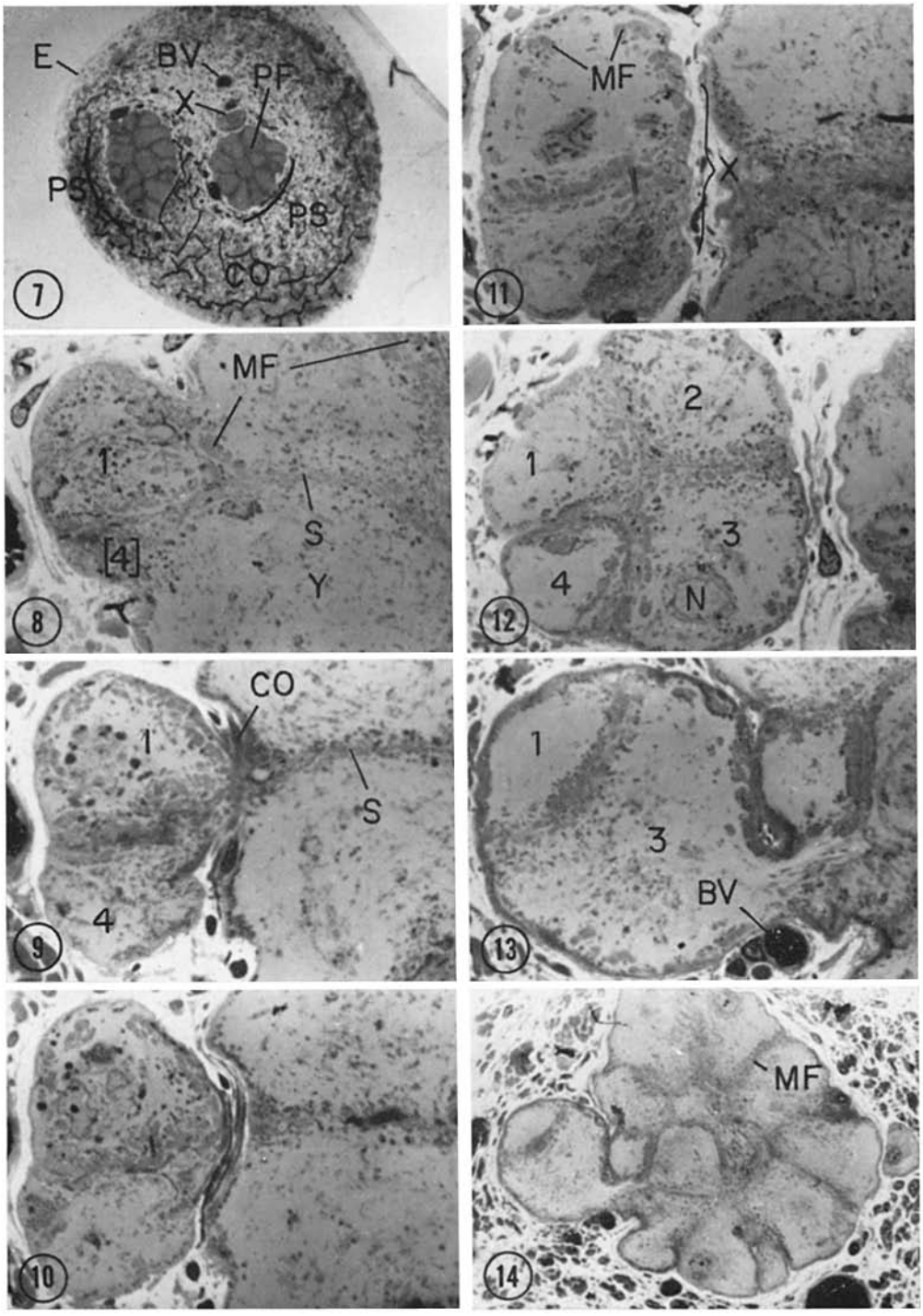
FIGURE 4 Dog, P strand, cross-section. Similar to Figs. 2 and 3, but the fibers are more closely joined. The arrangement of the myofibrils is similar to that in the guinea pig (Fig. 3). × 400.

FIGURE 5 Goat, P strand, cross-section (see Fig. 7). The P fibers are very densely joined. The myofibrils (*MF*) are almost all in the periphery of the individual fibers and are close to the narrow intercellular cleft seen with the EM. Individual fibers may break loose and later rejoin the main bundle (see Figs. 7–14). Blood vessels (*BV*) are always outside the strands. × 370.

FIGURE 6 Sheep, P strand, cross-section. The light microscopic picture is almost identical with that of the P strand of the goat. × 370.

a safe differential criterion. The nuclei were usually situated in or close to, the center of both P fibers and V fibers. P fibers presumably may have two nuclei (12). We can confirm this for the P fibers of the goat as revealed by serial sections (thick sections viewed with the light microscope).

SERIAL SECTIONS THROUGH A P STRAND OF THE GOAT: Representative specimens out of a total of 100 serial sections (each approximately 1 μ thick) are shown in Figs. 7–14. A P



FIGURES 7–14 These represent samples of 1 μ serial sections through an approximately 100 μ length of P strand of the goat. Beginning with Fig. 8, it can be seen that P fiber *1* becomes progressively sequestered from the main bundle (see Fig. 7, *X*). On the way, until finally rejoining the main bundle, the fiber changes into *2*, *3*, and *4* P fibers and, finally, back into two fibers (Figs. 13, 14). Figs. 7 and 14 correspond to Figs. 11 and 13, respectively; they demonstrate the topographic relationships to the parent structure and, in the case of Fig. 7, also to a sister P strand (*PS*). Note thick sheath of collagen in Fig. 7 (see Fig. 2) which in turn would form a thick unstirred layer with respect to diffusion. Fig. 7, × 80; Figs. 8–13, × 770; Fig. 14, × 320.

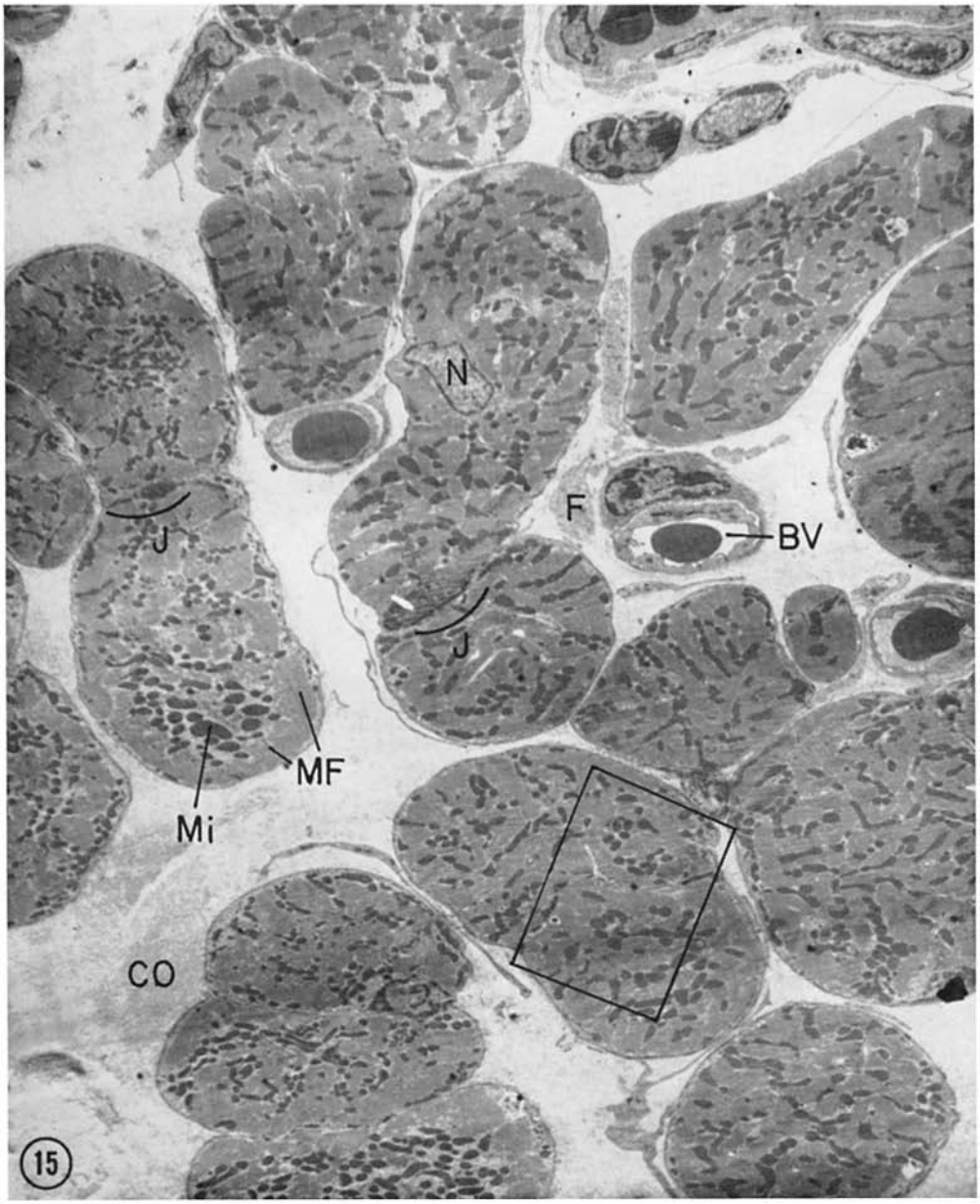


FIGURE 15 Guinea pig P strand, transverse section. As in the rabbit, the fibers are much more loosely joined compared with those of the sheep and goat (see Figs. 2–6). However, close apposition by the characteristic junctional complexes (*J*) is common. The fibers are filled to capacity with myofibrils. The intercellular space contains capillaries (*BV*), fibrocytes (*F*), and collagen (*CO*). × 2300.

strand (not attached to leaflets of cardiac valves), similar in general topography to that in the cat (Fig. 1), is shown in Fig. 7 to contain two large (*PS*) and one small P strand (*X*) (see Fig. 11). P fiber 1, which in Fig. 8 is still part of the main P strand, separates from the latter in Figs. 9 and 10, while the region *4* in Fig. 8, which is still a part of P-fiber *Y* of the main P strand, becomes clearly

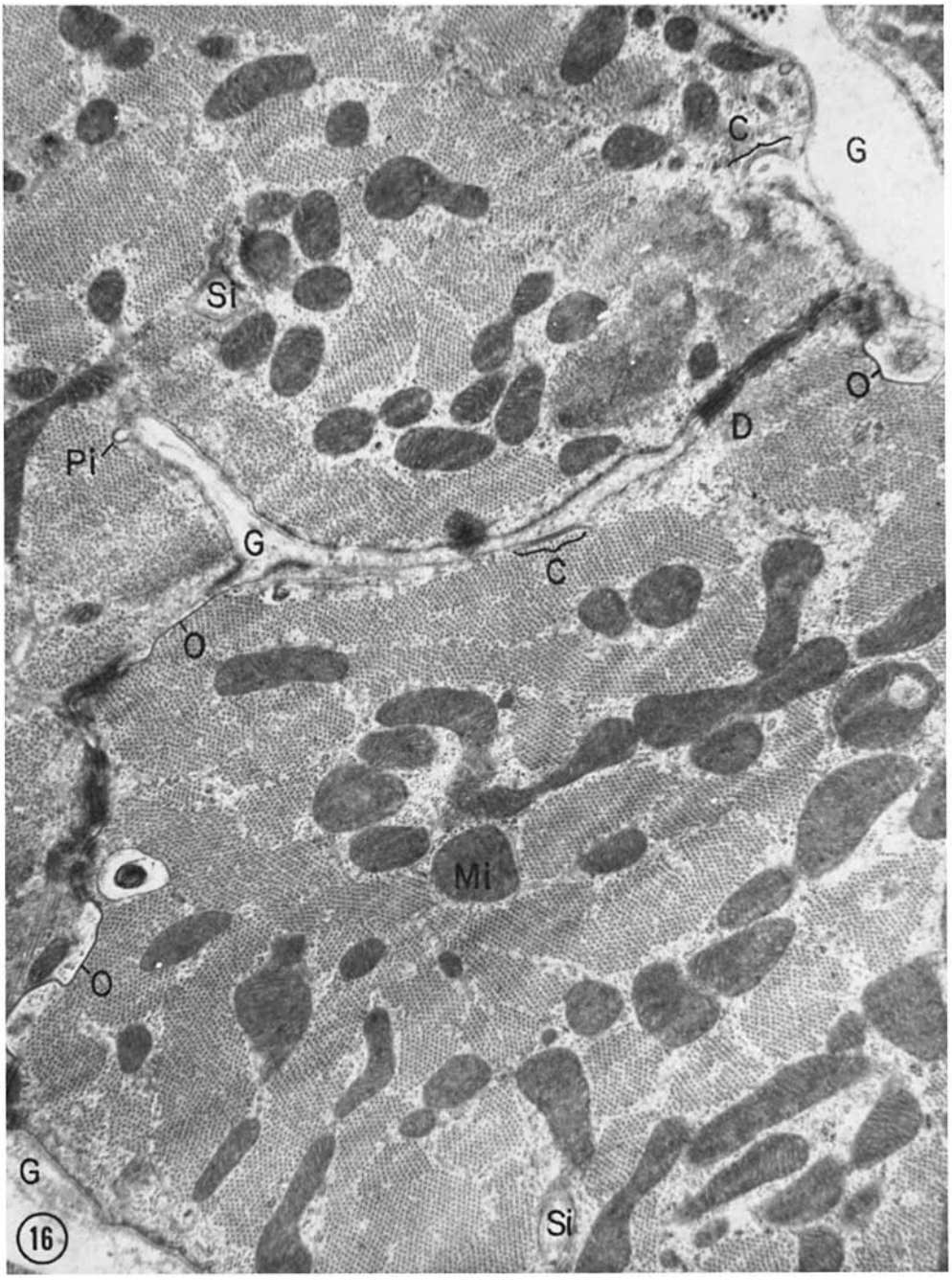


FIGURE 16 Higher magnification of marked area of Fig. 15. The profiles marked *Si* are interpreted as sarcolemmal invaginations that, unlike transverse tubules, run parallel to the longitudinal axis of the myofibrils in this section. *Pi*, pit (37). × 21,500.

separated from P-fiber *Y* in Fig. 9. The newly established sister P strand (*X* in Fig. 11) has acquired a third P-fiber in Fig. 11, and a fourth in Fig. 12; finally it reduces to two P fibers and simultaneously, by rejoining the main P strand, loses its identity as an individual P strand. A similar separation and rejoining of several other P fibers of this same main P strand was observed.

Electron Microscopic Findings

PURKINJE STRANDS: In their ultrastructural morphology the P strands in the guinea pig and cat were essentially like those in the rabbit, analyzed in detail elsewhere (9), while the P strands of the dog, goat, and sheep were somewhat different (see below). Briefly, in the first group of animals, the P fibers, as well as the V fibers, were joined by junctional complexes (zonula occludens, zonula adherens, macula adherens) over varying distances (Figs. 15, 16); occasionally they formed what is generally referred to as the "intercalated disc" (see Definitions, Criteria, and Dimensions). Except for the junctional complexes proper, the intercellular space between individual P fibers was almost always large, in the order of 1 μ or more (Figs. 2, 3, 15), quite in contrast to the interfiber space in P strands of the ungulates. The myofibrils had Z and M lines, as well as A and I bands. H bands were not observed under the conditions of the present investigation. The sarcoplasmic reticulum was sparse throughout. No transverse tubules were seen, but there were numerous peripheral couplings (Fig. 16, *C*). There were fewer mitochondria between the individual myofibrils than in the V fibers, but elsewhere there were often large aggregates of mitochondria (Fig. 15). As a rule, two kinds of mitochondria were observed. One population, the more numerous, was similar to that seen in V fibers, while the other, exceedingly rare in V fibers, consisted of elongated, thin mitochondria that had only one central crista (9). Looking like doughnuts on cross-section, these mitochondria were quite similar to those present in the axons of adjacent autonomic nerve fibers. The latter were common constituents of the intercellular space of P strands, quite contrary to V strands in which only an occasional nerve fiber was found. Myoneural junctions were absent. "Pits," as described by Roth and Porter (37), were regularly seen at the sarcolemma (Fig. 16).

What has just been said about both the P strand and the P fibers of the rabbit, guinea pig, and cat also holds true for those of the goat, sheep, and dog, with the following qualifications. As has already been shown with the light microscope, in the goat and sheep (Figs. 5–14) the myofibrils were relatively few in number and small in size; they were located chiefly in the periphery of the P fiber (Figs. 17, 22). The SR was sparse (Fig. 26). Occasionally intercalated discs were formed (Fig. 19) and the Z lines were associated with tubules of the SR (Fig. 20). The remaining portions of the cytoplasm contained an assortment of fibrils of rather constant width (7–10 mμ) and varying length. Interspersed were ribosomes and glycogen granules as well as an occasional tubular fibril (microtubule, reference 38). Adjacent P fibers of the goat and sheep were joined together by the same forms of junctional complexes as were seen in all other P strands and V strands. However, as in another ungulate, the steer (17), the maculae adherentes dominated the picture, so that adjacent fibers appeared to be spot-welded together (Figs. 17, 18, 21, 22). The intercellular clefts (see Definitions, Criteria and Dimensions) were of remarkably constant width (20–30 mμ) (Figs. 18, 22), except for an occasional region that was wider (Fig. 21). Zonulae occludentes were few and short. Numerous peripheral couplings were present at the free sarcolemma as well as at the sarcolemma of the intercellular clefts (10). Collagen was not seen in the clefts.

In the dog, apposition of adjacent cells in some areas also showed the same spot-welding effect (Figs. 23, 25) that is typical for the ungulates but not prominent in any of the other animals studied. Significantly, in the dog the intercellular cleft between two fibers, from one macula adherens to another, was also small and very constant.

Ventricular Fibers (Papillary Muscle)

GENERAL OBSERVATIONS: In suitable sections the myofibrils of V fibers and P fibers were well lined up (Figs. 24, 28, 31), and the sarcolemma followed a scalloped course around prominent mitochondria within the cytoplasm (Figs. 26, 27, 30, 31). However, whereas in the V fibers the sarcolemmal indentations usually signified the origins of transverse tubules, in the P strand these indentations did not continue farther as transverse tubules. As can be seen, longitudinal sections through a P strand (Fig. 28) and a skeletal muscle (Fig. 29) looked quite similar at low magnification, except perhaps for the size and location of

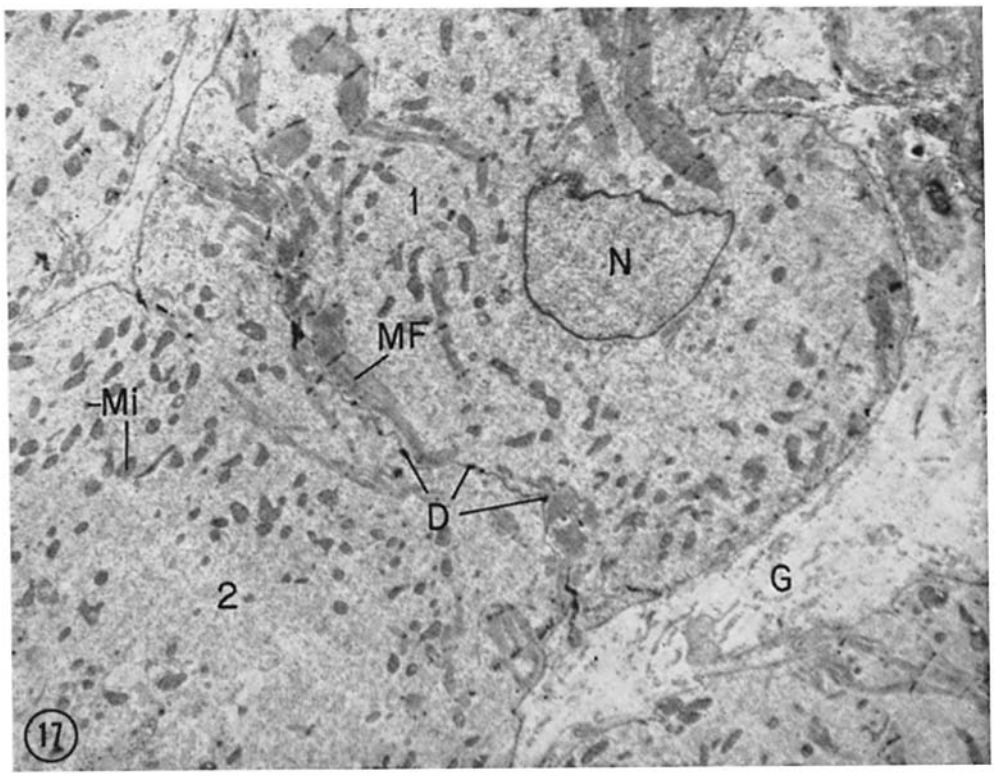


FIGURE 17 Goat, P strand. Two fibers (*1*, *2*) joined by junctional complexes are in the process of separating from the main strand. Note the "spot-welding" effect created by the maculae adherentes (*D*). The cytoplasm is rather clear and contains glycogen, some granules that appear to be ribosomes, many small irregular fibrils, and very occasionally a structure resembling a Golgi complex. The myofibrils (*MF*) are usually in the periphery of the cell. There are many mitochondria (*Mi*); among them are many very small ones with only one central crista which are quasi doughnut-shaped on cross-section. × 3800.

the mitochondria. At higher magnifications, however, triads with the centrally situated profile of a transverse tubule became apparent in skeletal muscle (Fig. 29 shows the location of one, although it is hardly visible at this magnification), whereas no such structures were ever observed in the P fibers. In contrast, V fibers had numerous transverse tubules that, it should be stressed, always had a large diameter and always were vested with a prominent basement membrane (36). All cells were connected by the usual junctional complexes, with intercalated discs being quite prominent (Fig. 32). The myofilaments, including their band patterns, were indistinguishable from those in P fibers and in skeletal muscle. The cytoplasm always contained varying amounts of glycogen granules, an occasional small fragment of rough endoplasmic reticulum, an occasional lipid droplet and Golgi complex, some tubular fibrils here and there (34), and very rarely microvesicular bodies, myelin figures and lysosomes. Sarcolemmal pits were often observed (37).

SARCOPLASMIC RETICULUM: Peripheral (Figs. 33, 34) and interior couplings (Figs. 31, 38, 41, 43) were very common. Often more than one peripheral coupling per sarcomere length was encountered. The SR formed interconnected networks that were frequently more intricate in the region of the Z lines (Figs. 32, 39, 43). Whereas the SR was quite prominent in all animals studied, it seemed to be more so in the guinea pig (Figs. 39, 40). Subsarcolemmal meshes of the network very often enclosed "pinocytotic" vesicles (Fig. 40). No terminal cisternae, *sensu stricto*, were observed. Rather, components of the SR network would periodically become junctional SR as part

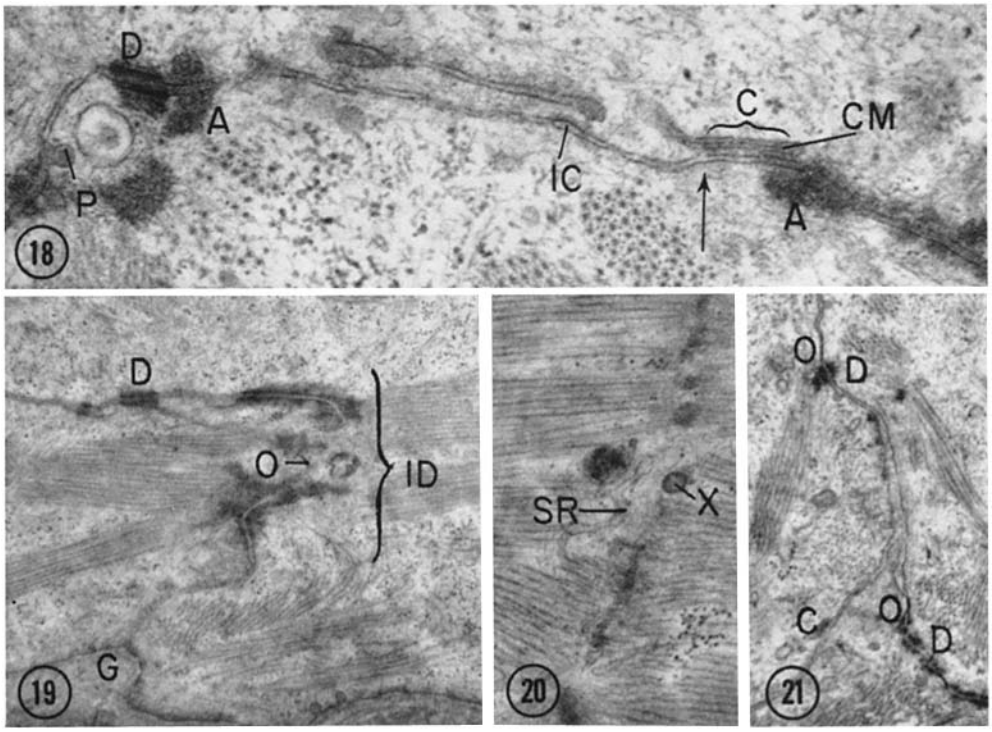


FIGURE 18 Goat, P strand. Junctional complexes between two fibers. Note macula adherens at *D*, and small and large zonulae adherentes (*A*). At *C* a coupling with the typical junctional granules (*CM*). Pinocytotic vesicles (*P*) contain dense material. The plasma membranes between two cells are separated by a quite constant cleft (*IC*) of about 300 A which rarely widens to about 600–800 A (see Fig. 21). Note tubular extension of plasma membrane toward the juctional SR of the coupling at arrow. × 50,800.

FIGURE 19 Goat, P strand. The junctional complexes between two cells form an intercalated disc (*ID*), typical in heart muscle as opposed to skeletal muscle. Note small zonula occludens at *O*. × 22,600.

FIGURE 20 Goat, P strand. The illustration shows a sample of the very sparse sarcoplasmic reticulum (*SR*). The structure at *X* is possibly a pit. × 28,500.

FIGURE 21 Goat, P strand. Cell junction between three cells. Note spot-welding effect by maculae adherentes (*D*). A few zonulae occludentes (*O*) are present. The width of the extracellular space between the plasma membranes is rather uniform except in the center of the illustration. Coupling at *C*. × 20,800.

of a coupling without changing their morphology, except for the acquisition of junctional granules.

That portion of the SR which was associated with the Z line was usually oriented in a transverse direction and may be shown to extend from one adjacent myofibril to another (Fig. 42). As a result, this portion of the SR may, incorrectly, be interpreted as a transverse tubule. However, the transverse tubules and the transversely oriented SR were quite different in morphology (Figs. 42, 43), and the confusing relationship between the two tubular systems became quite clear when both systems happened to be cut in one plane side-by-side (Fig. 43). No unequivocal, direct, open connection between the extracellular space and the SR was ever encountered.

TRANSVERSE TUBULES: In the absence of serial thin sections we are unable to state whether the transverse tubules branch, intersect, or do both.[1] As has already been mentioned, V fibers contained many transverse tubules; the tubules were large, though varying in size, and all were

[1] In Fig. 4 of their paper (36) Sommer and Spach have erroneously interpreted a very narrow intercellular space as a branching transverse tubule.

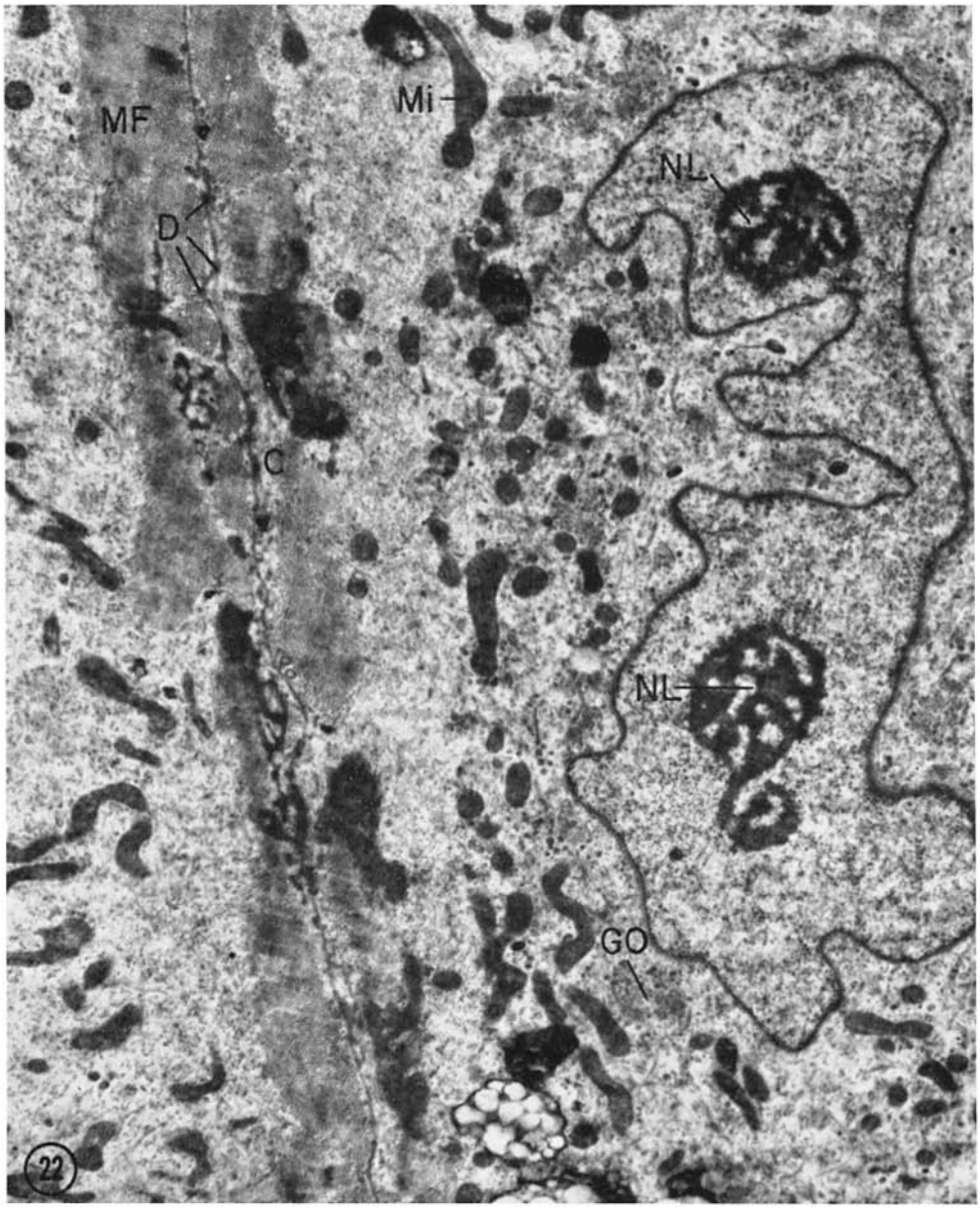


FIGURE 22 Sheep, P strand. Two P fibers are joined by junctional complexes with characteristic spot-welding effect. The myofibrils (*MF*) are most often located adjacent to the sarcolemma. The remainder of the cytoplasm is similar to that of the P fiber of the goat. Occasionally two nucleoli (*NL*) per nucleus are seen. × 9000.

vested with a prominent basement membrane. It was this latter material that often proved most helpful in distinguishing transverse tubules from the SR (Figs. 35, 37, 42, 43). For example, the subsarcolemmal cisternal profile in Fig. 35 (*Si*), actually containing basement membrane-like material, might have suggested that this profile belonged to the SR. However, Fig. 35 (*Si*) shows

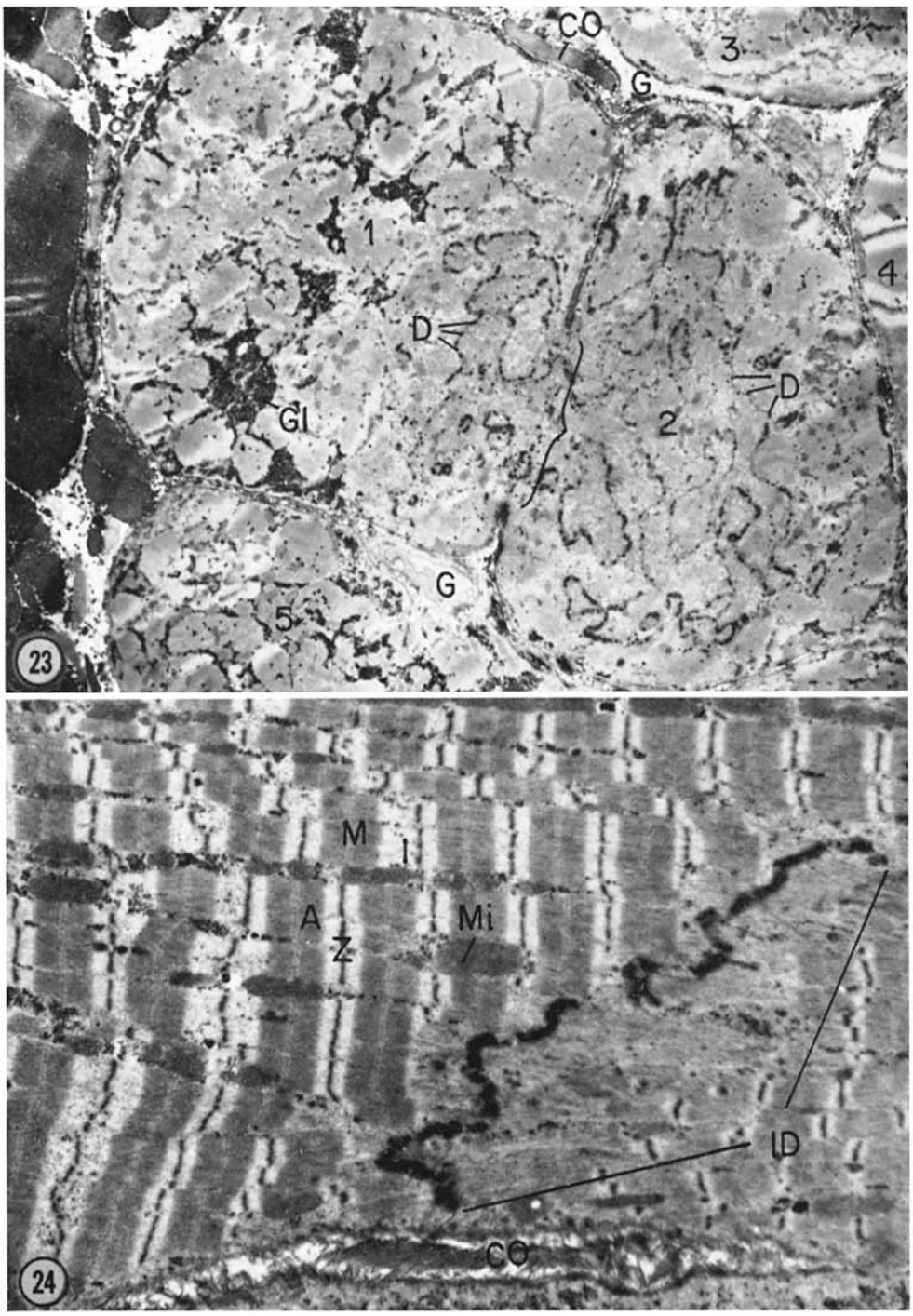


FIGURE 23 Dog, P strand. Low-power illustration of junctions between five P fibers: *1*, *2*, *3*, *4*, *5*. Large intercellular space (*G*) between all but cells *1* and *2*. The center portion (bracket) of cells *1* and *2* is joined by junctional complexes extending in a meandering fashion deep into either cell. Note prominent spot-welding effect created by maculae adherentes (*D*). The cytoplasm is filled to capacity with myofibrils. Glycogen is abundant (*Gl*). × 2800.

FIGURE 24 Dog, P strand, longitudinal section. Note extended sarcomeres, Z and M lines, and I and A bands. There is a typical intercalated disc between arrows. The mitochondria (*Mi*) are not so numerous as in ventricular muscle (see Fig. 31) but are in the same location. Note the variations in sarcomere length. × 5200.

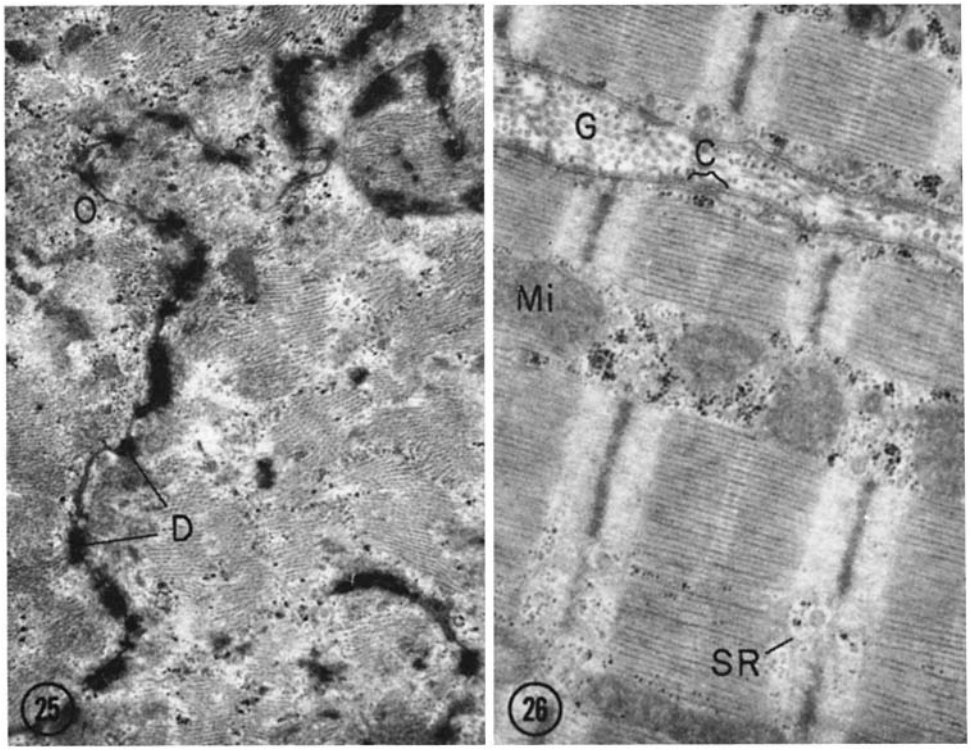


FIGURE 25 Dog, P strand, cross-section. Both fibers abound in myofibrils. The junctional complexes give a spot-welding effect (*D*). The extracellular space is rather uniform in width. Zonulae occludentes (*O*) are few and far between. × 17,500.

FIGURE 26 Dog, P strand, longitudinal section. Note sarcoplasmic reticulum (*SR*) at Z lines, and the peripheral coupling at *C*. × 21,700.

clearly that, at a suitable plane of sectioning, small invaginations of the sarcolemma would account for a profile such as that shown in Fig. 35 (*Si*). Very frequently, pinocytotic vesicles arising from transverse tubules were found at the Z lines (Figs. 20, 32, 38). A few vesicles appeared "empty" whereas most contained dense material (Figs. 32, 40) or small dense granules measuring approximately 6 mμ in diameter (fig. 40). The pinocytotic vesicles, usually presenting as round structures, always were found to have a wide neck, but depending on the plane of section they occasionally appeared completely separated from their origin. Larger invaginations of sarcolemma, including possible true duplications of transverse tubules, were often observed (Figs. 38, 43). Occasionally an odd, isolated structure defied interpretation; for example, the mitochondrial extension in Fig. 38 might have been misinterpreted had it been seen disconnected from its parent structure. Finally, small and empty-looking tubular structures connected with the extracellular space were observed (Fig. 44). Elsewhere, small, tubular profiles connected with larger ones were seen (Fig. 45). However, whether these structures were tubules or sheets of apposed sarcolemma could not be decided. At any rate, in both instances the tubular profiles were interpreted as peculiar infoldings of the sarcolemma enclosing extracellular space. On the other hand, another interpretation is imaginable for the structures of Fig. 44 if, by chance, the opening of the tubular profiles to the extracellular space had been missed by the level of sectioning. In this case these profiles might have been erroneously assigned to the SR (see Discussion).

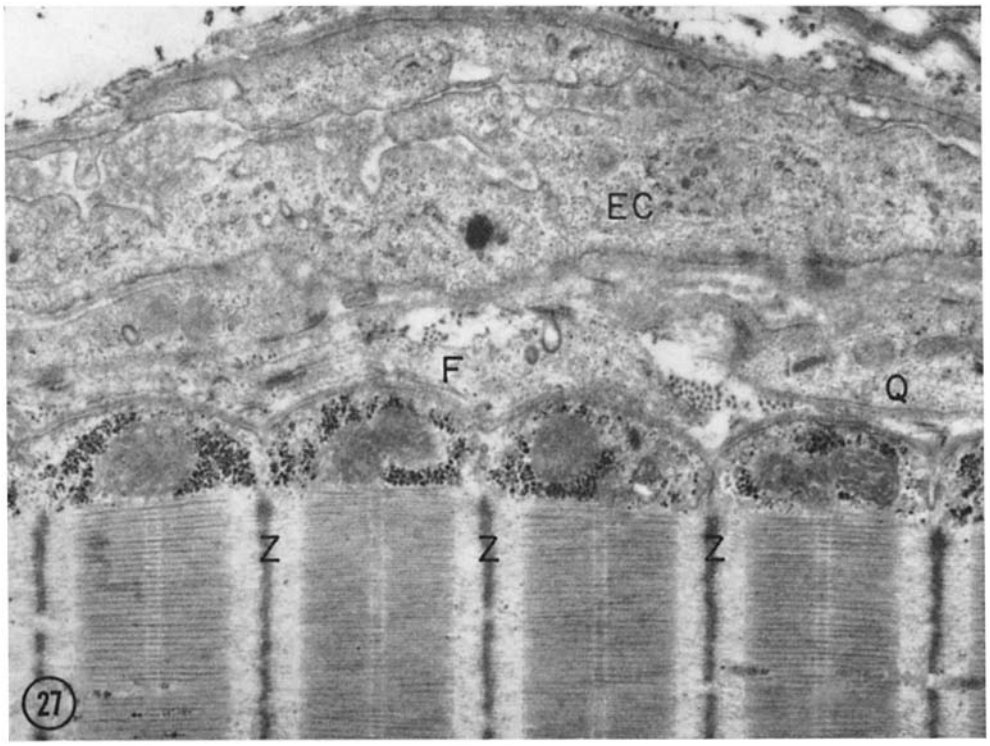


FIGURE 27 Dog, P strand. The sarcolemma shows an indentation at each Z line. Mitochondria and glycogen fill the space between these indentations. In the extracellular space are nerve fibers (*Q*), fibrocytes (*F*), and a capillary with an endothelial cell (*EC*). × 19,000.

DISCUSSION

Perhaps the most important result of this comparative study on several mammalian hearts is that the P fibers of the heart can now, for the first time, be clearly defined ultrastructurally as cardiac fibers that have no transverse tubules. Except for the transverse tubules and one special form of mitochondrion (9), the two populations of heart muscle fibers, i.e. P fibers and V fibers, are qualitatively similar.

Since its demonstration by Andersson-Cedergren (39), the transverse tubule system of skeletal muscle has played a prominent role in attempts to explain some of the physiologic properties of muscle during contraction. If there were any doubts left, the tracer studies with ferritin by Huxley (40) and Page (41) have firmly established, in skeletal muscle, the direct connection of the transverse tubules with the extracellular space. That a transverse tubular system also exists in other muscles was convincingly demonstrated morphologically by Franzini-Armstrong and Porter (42) and others (43, 44). Comparable observations have been reported with respect to the transverse tubules in heart muscle (4–6, 45–47), including the elegant tridimensional demonstration of their origin at the cell surface (48).

Gordon and Blumberg (49) found lead-phosphate reaction product in transverse tubules of heart muscle but not in transverse tubules of skeletal muscle; this suggested to them that the transverse tubules of heart muscle were intrinsically different from those of skeletal muscle, at least as regards the presence of a certain phosphatase. An alternate explanation of these findings seems to be more likely (36). In contrast to the small, delicate lead-phosphate reaction product observed in other sites (myofilaments, couplings, zonula occludens), that reaction product arising from the pinocytotic vesicles of endothelial cells is usually large and clumped (Figs. 46, 47). While these large clumps have easy access, by simple

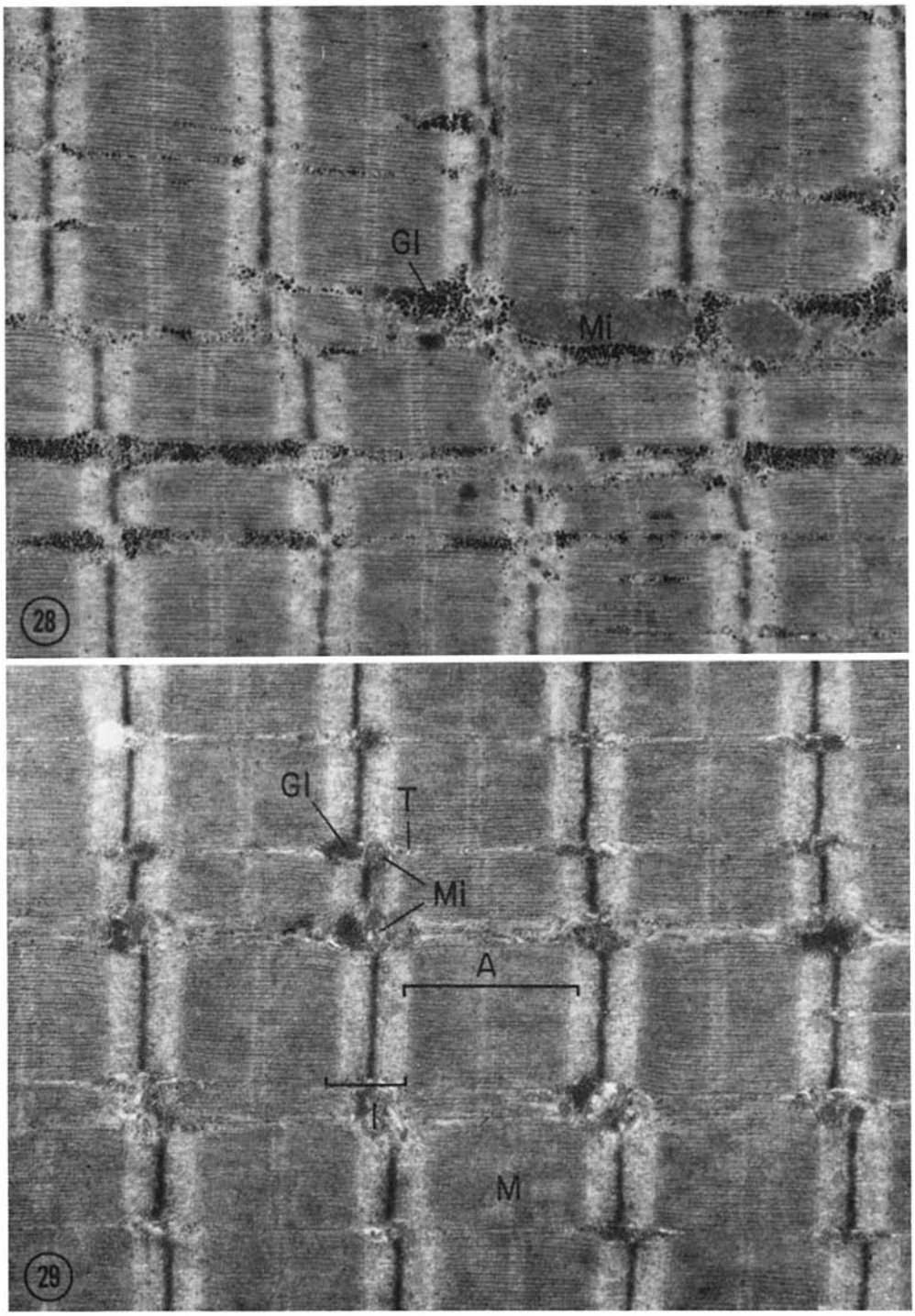


FIGURE 28 Dog, P fiber, longitudinal section. There are no transverse tubules, in contrast to ventricular muscle (see Fig. 31). Mitochondria (*Mi*) are few in number. Glycogen (*Gl*) is prominent. Note the great similarity of the general structural arrangement of the myofibrils to that of rabbit skeletal muscle shown for the purpose of comparison in Fig. 29. × 19,500.

FIGURE 29 Rabbit, skeletal muscle, longitudinal section (see Fig. 28). There are few mitochondria (*Mi*) close to the Z line as well as glycogen (*Gl*). The transverse tubules (*T*) are at the A–I junction. × 19,500.

diffusion, to the wide (0.2 μ) transverse tubules of V fibers (Fig. 46), such is not the case in skeletal muscle, in which the transverse tubules are narrow, measuring only about 25 mμ in diameter. Studies with ferritin as a tracer also have been performed recently in cardiac muscle to show continuity of transverse tubules with the extracellular space (50). The transverse tubules in the P fibers of the cat shown recently by Page (20) are most likely sarcolemmal invaginations similar to those shown in Fig. 16.

Heart muscle is composed of individual cells that are joined by junctional complexes, that is to say, the zonula occludens, zonula adherens and macula adherens. The so-called intercalated disc is a broad term derived from light microscopy that is applicable also to electron microscopy, with the qualification mentioned above (see Definitions, Criteria, and Dimensions). The intercalated disc, i.e. the zonula adherens, is oriented at right angles to the longitudinal axis of the myofibrils, and it is often interrupted by zonulae occludentes of varying lengths (Fig. 32) that are always oriented parallel to the longitudinal axis of the myofibrils. Frequently, a zonula adherens may be replaced over short distances by small pockets of intercellular space which Forssmann and Girardier interpreted as open connections between the sarcoplasmic reticulum and the intercellular space of the intercalated disc (see Fig. 10 of reference 3). Couplings may be found at those pockets but, since couplings never occur at junctional complexes such as the zonula adherens, the statement of Page concerning the presence of couplings at the intercalated disc of cat Purkinje fibers (20) should be qualified in accordance with the definition of the intercalated disc. Moreover, the intercalated disc is presumably subjected to considerable shearing stresses, and therefore it is no surprise that cellular intussusceptions would occasionally appear here as well as elsewhere in cardiac muscle cells that may give rise to strange membranous structures. Page reports at great length on one system of such structures but interprets them as structures *sui generis* which he calls ovoid bodies (51).

The sarcoplasmic reticulum in V fibers and P fibers is a continuous network of tubules, contiguous from sarcomere to sarcomere. It is more prominent than is commonly believed, especially in the guinea pig V fibers (Figs. 39, 40). The width of the tubules is rather constant within moderate limits of variation (20–60 mμ). As Simpson already suspected (47), the SR network seems to have a transverse component, possibly a complete ring, surrounding the whole myofibril, which is continuous with similar transverse components of adjacent myofibrils (Figs. 42, 43). As in skeletal muscle, the SR of V fibers forms couplings with the transverse tubules but without cisternal dilations of the SR. In addition, in contrast to the SR in mammalian skeletal muscle, the SR in V fibers and P fibers forms peripheral couplings. The SR of cardiac muscle, including the junctional SR of the couplings, and the SR of skeletal muscle have been shown to have ATPase activity (36, 52–58), and some authors have related this ATPase activity to the Ca-pump ATPase (59, 60). Recent studies in Hasselbach's laboratory, however, have raised some doubts on this interpretation of these cytochemical findings (61, 62).

Of particular interest are the junctional granules found in the junctional SR which we feel represent the homologue of the granular material observed in the terminal cisternae of skeletal muscle (26, 63). In skeletal muscle, the SR, especially the terminal cisternae, have been shown to contain calcium (64–67). Radioautographic studies of histologically fixed skeletal muscle suggest that calcium is stored in the terminal cisternae from which it is released on excitation (64). In cardiac muscle the calcium storage capacity is considerably less than it is in skeletal muscle (68, 69). This may be related to the fact that the volume of the junctional SR of heart muscle is much less than the volume of the terminal cisternae of skeletal muscle and that the granular material seen in the junctional SR of heart muscle is considerably less prominent than it is in the terminal cisternae of skeletal muscle. While the nature of this material is obscure, it is not unreasonable to speculate that it may have something to do with the binding of calcium and especially with the release of calcium incident to the arrival of an action potential. The sarcotubular vesicles of rabbit skeletal muscle contain a large amount of amino sugars (70) which may be significant in view of the fact that mucopolysaccharides have a considerable affinity for calcium (71). The work of Marler and Davidson (72) shows that the protein-polysaccharide–complex chondroitin sulfate A strongly binds calcium. The nature of that binding is such that, short of irreversible cleavage of the native protein polysaccharide complex, calcium cannot be freed from it, except, significantly, by the application of an electric field which effects a reversible release of

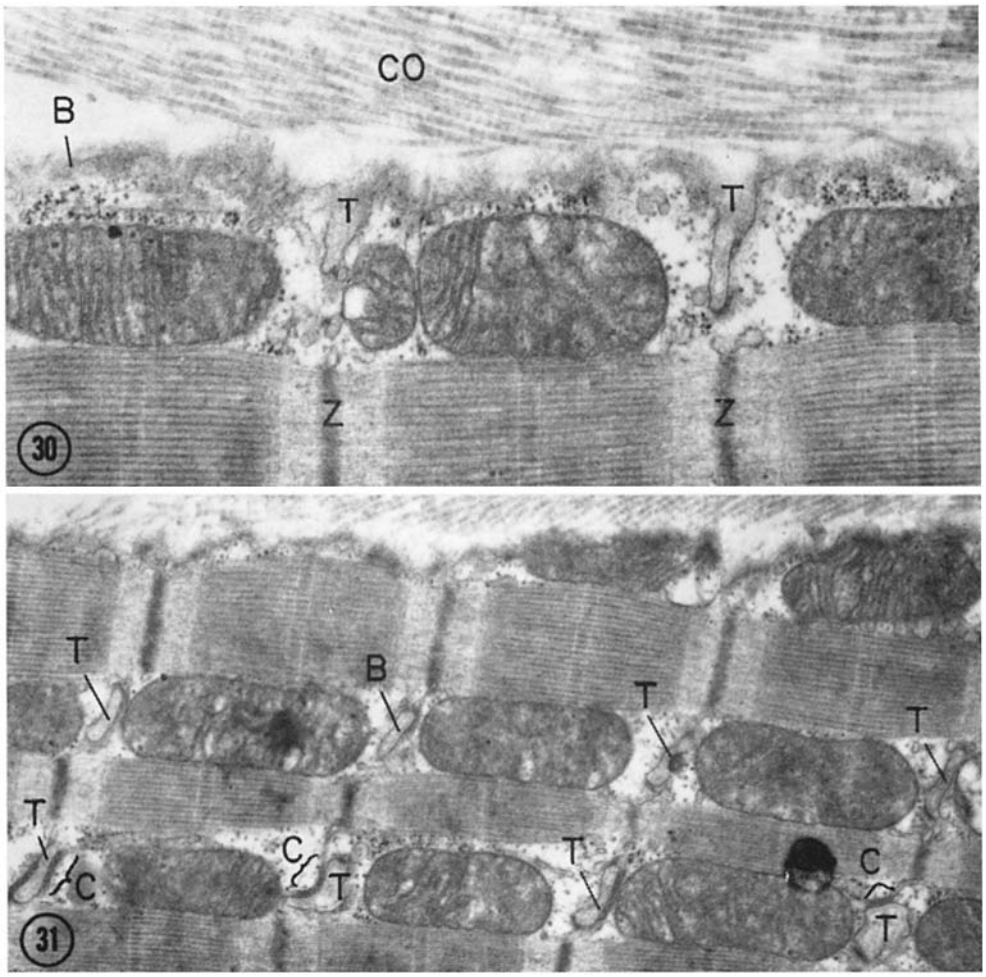


FIGURE 30 Rabbit, V strand (papillary muscle). Prominent indentations of the sarcolemma at the Z lines are seen (*T*). Note basement membrane (*B*). × 37,300.

FIGURE 31 Rabbit, V strand. Note cross-sections of transverse tubules (*T*) at the Z lines, each showing its basement membrane (*B*) (see Figs. 26–28). Each transverse tubule forms at least one coupling (*C*) with the junctional SR at the level of this section. × 20,300.

calcium.[2] This finding opens up the attractive possibility that chondroitin sulfate A may act as the transducer for the reversible calcium release during excitation-contraction coupling (See Note Added in Proof, page 524).

For reasons that will be discussed below, Forssmann and Girardier, from their studies of rat heart (3), suggested that the calcium content of the SR in heart muscle is continuous with the calcium content of the extracellular space. In support of this assumption they quote the work of Lüttgau and Niedergerke (73). However, the observations of Lüttgau and Niedergerke were made on the frog heart which structurally is quite different from mammalian heart,[3] and the morphologic differences between frog and mammalian heart show that extrapolation to mammalian heart

[2] Davidson, E. 1967. Personal communication.

[3] Sommer, J. R., and E. A. Johnson. Frog heart. Data in preparation.

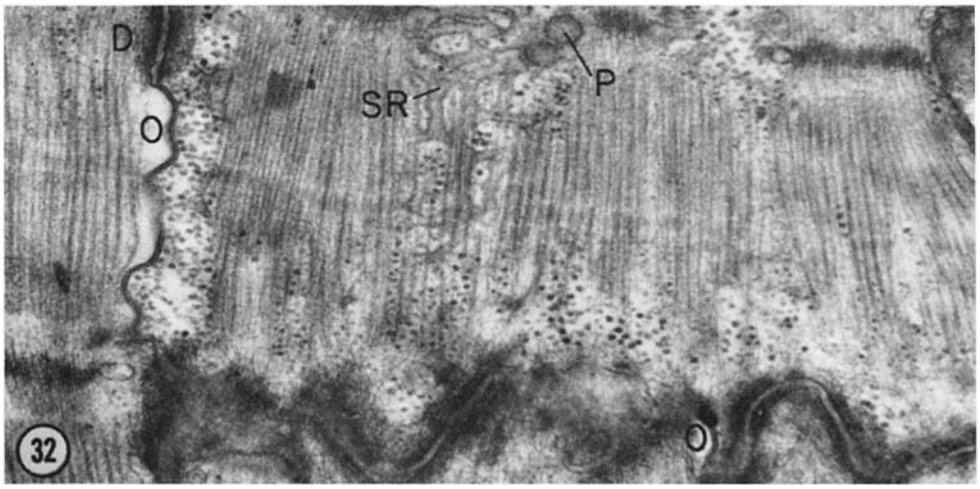


FIGURE 32 Rabbit, V strand. The junctional complexes in papillary muscle are shown which are similar to those in the P strand. Zonula occludens (*O*), macula adherens (*D*), zonula adherens (*A*). Note the rather well-developed SR (*SR*) and presumable pinocytotic vesicles at the level of the Z line. × 45,600.

of the behavior of the frog heart muscle cannot be made safely. Indeed, the difference in structure may give some insight into known differences between frog and mammalian (e.g. rabbit) hearts, namely in the relationship between the force of contraction of ventricular muscle and the rate and pattern of stimulation (74, 75).

For a number of reasons, the question arises whether the tubules of the sarcoplasmic reticulum are open to the extracellular space. For skeletal muscle this has been generally answered in the negative, especially after the use of ferritin as a tracer (40, 41). In heart muscle a rather convincing opening between an intracellular cisternal structure and the transverse tubule was shown by Sommer and Spach (Fig. 1 of reference 36). However, the possibility that this intracellular structure was a pinocytotic vesicle could not be excluded, even though, then as now, we have never seen a pinocytotic vesicle with such a small neck. Nevertheless, that this cisternal profile belongs to the SR appears to be even more in doubt now, since the wall of the structure shown in that paper apparently participates in a coupling-like apposition with a component of the SR, a situation that we have never observed elsewhere. An apparent opening between a component of the SR (junctional SR, in this instance) and the extracellular space as seen in Fig. 34 was found only in three instances out of several hundred electron micrographs. Even so, in all instances, the demonstrated opening between these two compartments, e.g. Fig. 34, is uncertain if one considers the haze across the opening which may represent a tangential section through a membrane. Moreover, even if it were a true opening, one cannot rule out an artifact, such as a tear, that may have come about in the process of tissue preparation. Although we have specifically searched for it, we have not discovered any evidence of a permanent, consistent, free communication between the sarcoplasmic reticulum and the extracellular space in heart muscle. Nevertheless, as has already been pointed out (9), the evidence with respect to the processes of the junctional SR and the apposing sarcolemma (9, 76, 77) perhaps may not exclude the possibility that a shortlived, transitory opening to the extracellular space may exist. A biological precedence for a reversible open contact between smooth endoplasmic reticulum and a surface membrane may be found in the analysis of the nephridial system of *Paramecium* by Schneider (78).

In view of the extreme paucity of morphologic evidence for continuity between the SR and the extracellular space, albeit tenuous evidence at that, the extraordinary findings of Forssmann and Girardier (3) are, indeed, astounding. These authors, using potentially elegant techniques, i.e. ferritin as tracer and serial sections, propose to have demonstrated that the SR communicates

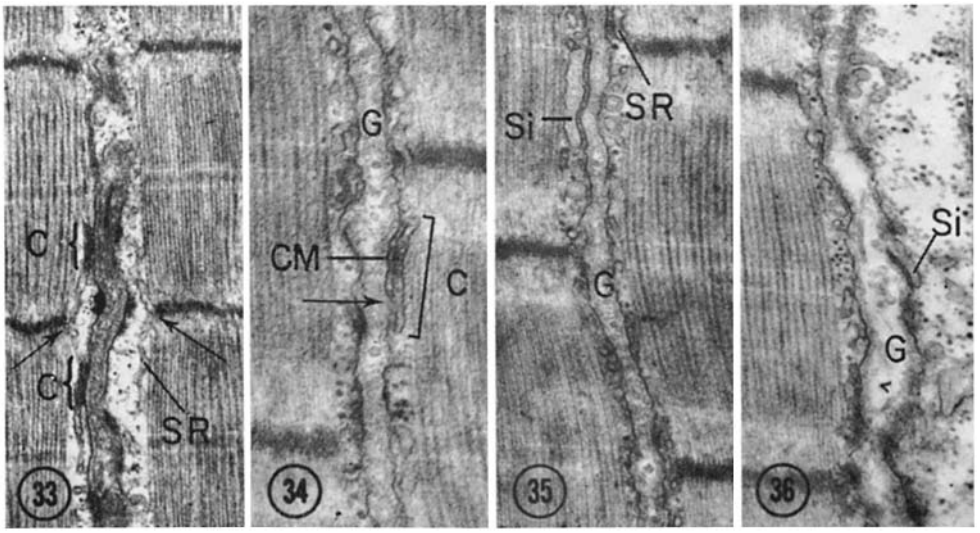


FIGURE 33 Guinea pig, V strand. Two peripheral couplings (*C*) are shown, the SR components of which (junctional SR) converge in the characteristic V fashion upon the Z line (arrows). The SR in the adjacent fiber is arranged likewise (*SR*), although the couplings are out of the plane of sectioning. Often two peripheral couplings can be visualized in one sarcomere. Peripheral couplings are usually located at a level corresponding to A–I junctions. Where the couplings form triads or diads (interior couplings), they are always at the Z line in heart muscle. × 30,700.

Figs. 34–38 demonstrate some structural features that may lead to misinterpretations, especially with respect to the question as to whether or not the SR is continuous with the extracellular space even when tracers are employed.

FIGURE 34 Rabbit, V strand. Intercellular space bounded by two muscle fibers. The junctional SR of the coupling (*C*) may give the impression of being continuous with the extracellular space (*G*) at the arrow (but see Discussion). × 62,400.

FIGURE 35 Rabbit, V strand. The tubule at *Si* is presumably an invagination of sarcolemma (note basement membrane) which may be mistaken for SR (*SR*) (see Fig. 36). × 36,100.

FIGURE 36 Rabbit, V strand. Invagination of extracellular space (*Si*). If cut differently, it may appear as though it were SR (see text) (see Fig. 35). × 36,100.

with the extracellular space in a truly massive way, from which they conclude, for example, that the calcium within the SR is continuous with the calcium in the extracellular space. One must realize that tracer studies generally are fraught with difficulties, some of which are related to the rate of diffusion of the tracer molecule which, in turn, is dependent on the size and other properties of that molecule. Other difficulties are related to cell preservation and the ever-puzzling question, whether tracer molecules are being taken up actively into preexisting intracellular structures (79). Nevertheless, taken with these qualifications, tracer studies are very revealing, provided that safe, independent, new, or already existing criteria are applied for characterization of the different compartments that one proposes to study. Forssman and Girardier show ferritin in the T system and in what they call the L system, but they do not explain on what basis they distinguish the two. In fact, the two structures shown in Fig. 2 of reference 3 can be interpreted, without effort, as parts of the T system or invaginated extracellular space. Similarly, in that illustration two other membranous profiles, quite alike in appearance, topography, and, one suspects, nature, were interpreted by the same authors as a subsarcolemmal cisterna and a transverse tubule, respec-

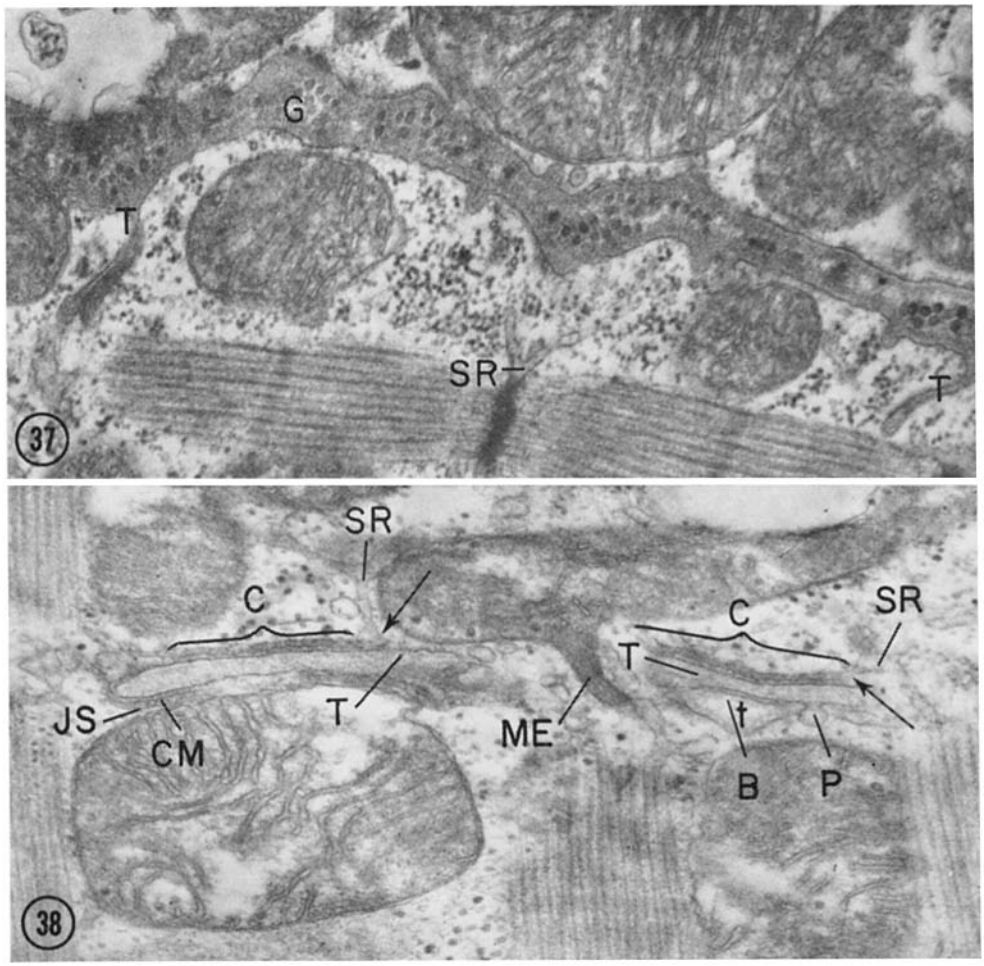


FIGURE 37 Sheep, V strand. Transverse tubules extend from the extracellular space downward. Note the dense basement membrane material with which they are filled. In contrast, the SR has a rather clear lumen (*SR*) and has the typical V shape as two tubules fuse at the Z line (see Figs. 33, 39, 42). × 48,200.

FIGURE 38 Rabbit, V strand. This figure illustrates the great variety of membranous structures that one may encounter about the Z line. Transverse tubules (*T*), couplings (*C*), sarcoplasmic reticulum (*SR*), basement membrane (*B*), central densities (*CM*), pinocytotic vesicle (*P*), mitochondrial extension (*ME*). The profile (*t*) is in a position of a terminal cisterna with respect to the transverse tubule (*T*). However, in it a sarcolemmal basement membrane (*B*) is clearly visible and neither central densities nor regular cisternal processes can be identified between it and the transverse tubule (*T*). × 30,500.

tively, both, to be sure, containing ferritin. Since nowhere in their paper do these authors manage to properly define the compartment they have named, their paper may safely be disregarded as evidence for continuity between the SR and the extracellular space.

Recent attempts to voltage clamp a short (1–2 mm) segment of a bundle of P fibers from the sheep have been made by Deck and Trautwein (80), Deck et al. (81), and McAllister and Noble (82). Those workers have used intracellular microelectrodes, one to inject current at a point in one fiber and the other to record the membrane potential (at a point).

Deck et al. (81) and McAllister and Noble (82) discuss the limitations of this kind of voltage-clamp

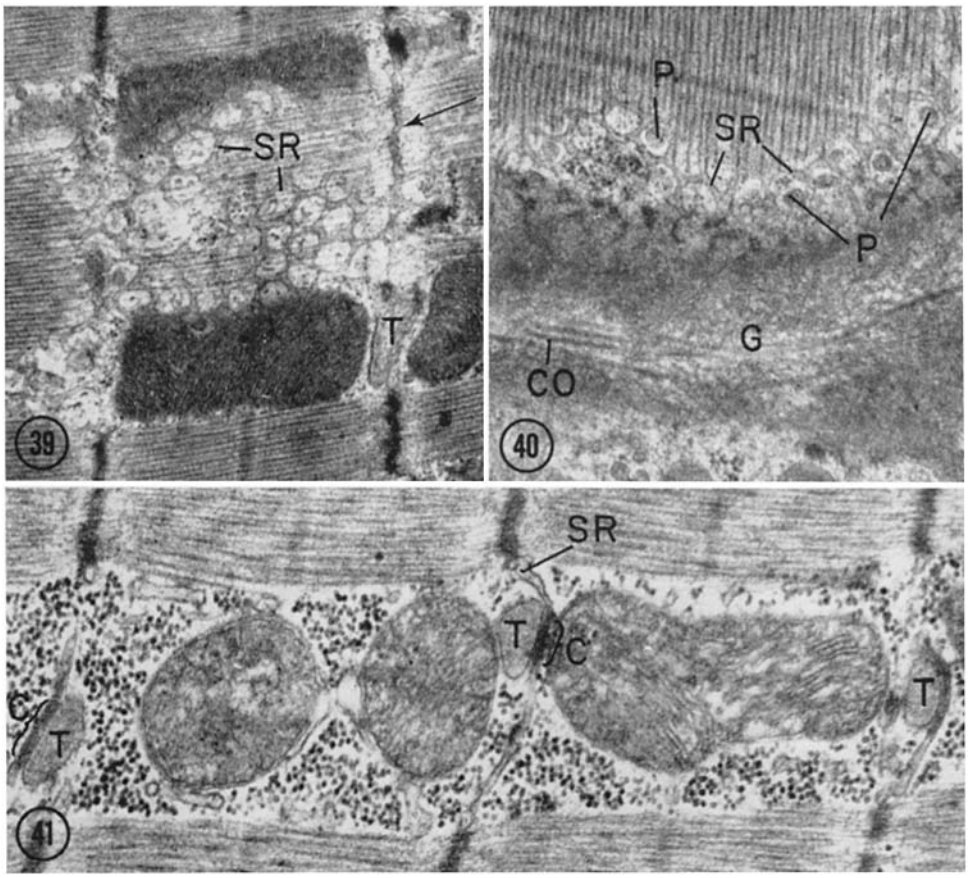


FIGURE 39 Guinea pig, V strand. Note the extensive sarcoplasmic reticulum (*SR*) which has a component that runs transversely across the sarcomere at the Z line (arrow). × 30,200.

FIGURE 40 Guinea pig, V strand. Note the extensive sarcoplasmic reticulum (*SR*) which, in its meshes, contains round profiles, pinocytotic vesicles (*P*), some of which contain granular electron-opaque material. × 26,900.

FIGURE 41 Sheep, V strand. The transverse tubules (*T*) are approached by components of the SR (*SR*) coming from the Z lines to form couplings (*C*). The portion of the SR that is part of the coupling is the junctional SR. × 40,100.

technique. In particular, it is not possible to clamp the membrane potential during the intense sodium current which generates the initial spike of the action potential: on the one hand, not enough current can be made to flow through the intracellular microelectrode and, on the other hand, the length constant of the fibers decreases in these circumstances and becomes very much less than the length of the preparation. Furthermore, only when the membrane capacity is a simple capacitor with negligible series resistance can the voltage-clamp technique clearly separate the capacitive and ionic currents. In such a case, the capacitive current flows only during the time taken to establish the constant clamp potential. It is now clear, however, that the membrane capacitance of P fibers of the sheep and goat is not so simple (83) and that it resembles the skeletal muscle membrane capacitance (84), in that a considerable fraction, about 80%, is in series with an appreciable resistance. Fozzard (83) suggested that this membrane was in the form of transverse

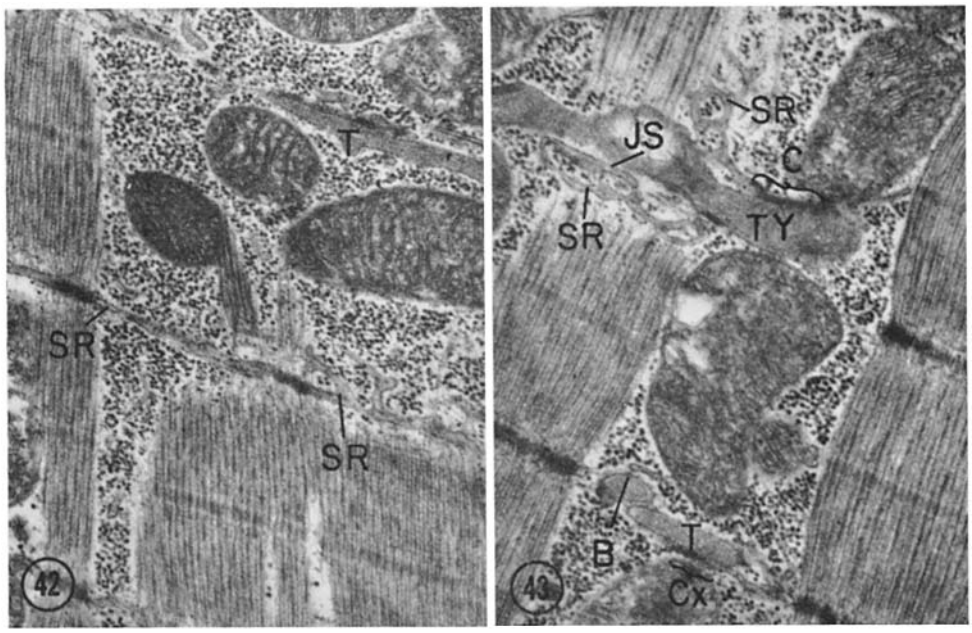


FIGURE 42 Sheep, V strand. This figure illustrates the similarity of position but difference of structure of transverse tubule (*T*) and some components of the SR at the level of the Z line (*SR*). × 32,800.

FIGURE 43 Sheep, V strand. A large transverse tubule (*TY*) runs across the Z-line region. Adjacent to it are branching structures of the SR, some of which run parallel to the direction of the transverse tubule. One of these SR complexes forms a coupling (*C*). The other one also seems to include junctional SR (*JS*). Another coupling at *Cx*. × 34,400.

tubules, the resistance in series with it being the resistance represented by the core of the tubules. The electron micrographs presented in the present paper show no evidence of transverse tubules in the goat and sheep P fibers. It would appear that in the P strand of these animals the membrane in series with a resistance resides in the narrow (~300 A) clefts between the tightly packed fibers. In this case the capacitive current will flow for several milliseconds, i.e. the time taken for the membrane in series with the resistance to charge or discharge to the new potential. That is to say, although the potential across the surface membrane, recorded by the voltage microelectrode, can be made to change instantaneously, the potential across a considerable portion of the total membrane area changes slowly. Again, a similar prolongation of membrane capacitive current arises from the fact that the membrane current is applied to the preparation at one point. Although the preparation is short compared to the DC length constant so that the membrane forming the periphery of the bundle would be reasonably polarized uniformly in the steady state, this condition would not be attained immediately after the application of a step change in voltage since the capacity of membrane areas at a distance from the current electrode has to be charged or discharged through the series resistance formed by the intracellular fluid. A large fraction of the total capacity of the preparation, therefore, will be charged or discharged through two resistances, the intracellular resistance and the extracellular cleft resistance in series with the greater part of the membrane capacitance. It is thus impossible to separate the ionic and capacitive currents during the first several milliseconds (up to 20–40 msec) (82) after a change in clamp potential. It can be seen that these limitations result from (*a*) the method and (*b*) the preparation.

Changing the method to the sucrose-gap technique (21–23) would avoid one of these difficulties, in that the controlling current is not injected at a point in one fiber but is, in effect, injected into

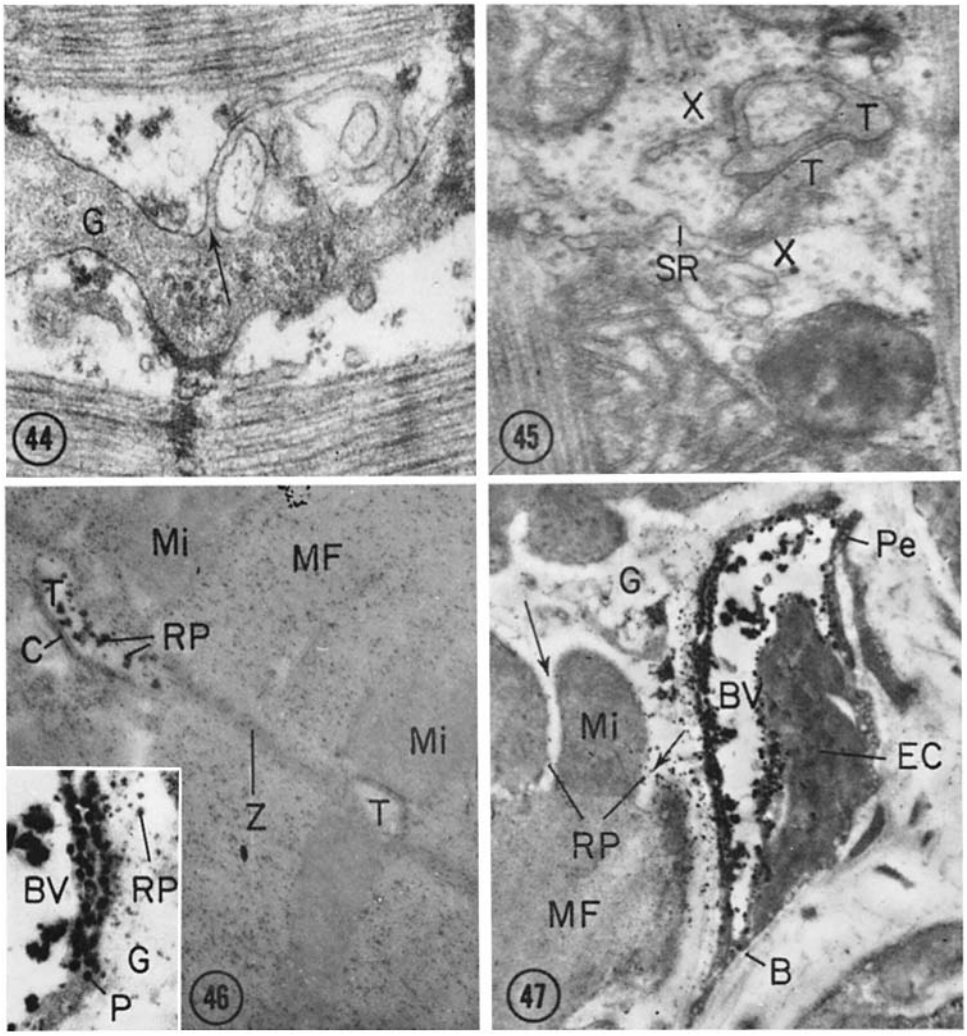


FIGURE 44 Guinea pig, V strand. Continuity of the extracellular space with a tubular system in the cytoplasm is shown (arrow). These are presumably sarcolemmal invaginations, not necessarily tubules, and unrelated to the SR, that may lead to misinterpretations when extracellular tracer substances, e.g. ferritin, are found in them (see reference 3). × 43,000.

FIGURE 45 Guinea pig, V strand. A similar convolution of presumably sarcolemmal profiles, possibly transverse tubules (*T*), which seem to form couplings with the SR (*SR*) at two places (*X*). × 45,000.

FIGURE 46 Dog, V strand. Wachstein-Meisel reaction for ATPase activity. Delicate lead phosphate precipitates, the reaction product (*RP*) resulting from the enzymic cleavage of ATP in the presence of lead ions is rather diffusely spread over the myofibrils (*MF*) and sharply localized in the junctional SR of the couplings (*C*). In contrast to these delicate precipitates are the large, coarse clumps (see inset, *RP*) of reaction product in one of the transverse tubules (*T*). As judged by the morphology, these clumps evidently come from the perivascular space and, presumably, from the pinocytotic vesicles of the endothelial cells. Lumen of blood vessel, *BV*. × 34,000.

FIGURE 47 Dog, V strand. Wachstein-Meisel reaction for ATPase activity. The wall of an endothelial cell (*EC*) has numerous pinocytotic vesicles filled with reaction product, while others appear emptied (*Pe*). The clumps of reaction product, especially to one side of the blood vessel (*BV*), decrease in number with increasing distance from the blood vessel, which is consistent with motion by diffusion. Both arrows point into sarcolemmal invaginations that contain reaction product (*RP*) and that may be, in fact, transverse tubules. The myofibrils (*MF*) display stippling by very delicate reaction product. × 20,000.

all fibers. However, among other problems there remains the problem of the resistance of the extracellular space in series with some of the membrane of the fibers in the sucrose gap. It is clear from the histology presented here that, because of the narrow clefts separating many of the fibers in the goat and sheep P strand, these bundles are the least suitable of the ones examined as a preparation for study in the sucrose gap.

An estimate of the effects of the resistance in series with the membrane in the clefts can be calculated by using the one-dimensional cable theory. Let us take the case where the transmembrane potential of the surface sarcolemma, i.e. that of the membrane just outside the mouth of the cleft, is driven to a certain value, E_s, by passing current through an intracellular microelectrode. The transmembrane potential of the membrane in the cleft, E_T, will deviate from this value because of the voltage drop across the series resistance of the material (Krebs' solution) in the cleft. Equation 1 gives the change in internal potential with reference to the external potential (zero) as a function of distance from the site of injection of current into one end of a cable terminated at a distance $X = L$ by an arbitrary resistance, R (for discussion of transmission line theory, see references 87, 88):

$$E_T(x) = E_s\left(\frac{R\cosh\left(\frac{L-X}{\lambda}\right)+z\sinh\left(\frac{L-X}{\lambda}\right)}{R\cosh\frac{L}{\lambda}+z\sinh\frac{L}{\lambda}}\right), \quad (1)$$

where z is the input resistance of the unterminated cable given by $\sqrt{r_m r_i}$ (r_m is the membrane resistance times unit length and r_i is the core resistance per unit length) and λ is the length constant given by $\sqrt{r_m/r_i}$. For the case of a cleft, the steady state solution for $E_T(x)$, (x, distance down the cleft) is equivalent to that of a one-dimensional cable terminated at a distance $x = L$ by an infinite resistance, i.e. the longitudinal current at $x = L$ is zero $(dE_T/dx)_{x=L} = 0$, where L is the distance to the center of the cleft. E_T as a function of distance from the center of the cleft to its opening on the other side of the strand will be simply the mirror image of this solution.

For the case where $R \to \infty$, equation 1 reduces to

$$E_T = \frac{E_s \cdot \cosh\frac{L-X}{\lambda}}{\cosh\frac{L}{\lambda}} \quad (2)$$

where λ in this case equals $\sqrt{R_m\, d/2R_i}$ and where R_m is the specific membrane resistance (Ω cm^2) and R_i is the resistivity of the material in the cleft, i.e. of Krebs solution (50 ohms cm), and d is the width of the cleft in centimeters.

$E_T(x)$ is plotted out in Fig. 48 for various values of R_m. The value of d was 3×10^{-6} cm and that of L was 50 μ, i.e., a total length of 100 μ for the cleft. This length is somewhat lower than the average value for the diameter of the P strands of the sheep that were used by Deck et al. (70–200 μ) (81). It can be seen from the figure that, except for values of R_m of 10,000 ohms cm^2 and over, E_T falls off considerably with distance down the cleft so that even in the steady state during voltage clamp the transmembrane potential of the cleft membrane would deviate seriously from that across the free surface membrane the potential of which (at a point) is being clamped.

For the case of the P strand of the rabbit where the total length of the cleft separating the fibers should not exceed 10 μ, i.e. L is 5 μ, and where the minimum gap is of the order of 1 μ, i.e. d is 1 μ, except where the fibers are forming junctional complexes, E_T, at the center of the cleft, ($x = L$) is not considerably different from E_s, except for for values of R_m of the order of 1 ohms cm^2. In this case, E_T at $x = L$ is 0.887 E_s. Thus, since the rabbit P fibers, unlike skeletal muscle, do not have transverse tubules, it would appear that none of the membrane of the fibers is in series with a significant resistance and that, in this respect, this preparation should be suitable for study with the sucrose-gap technique.

The average over-all diameter of rabbit P and V fibers is the same, 10 μ (87). All other factors being equal, fibers which have transverse tubules (V fibers) should differ in certain electrical characteristics from those fibers that do not have transverse tubules, namely, in input resistance and in the conduction velocity of an action potential.

An estimate can be made of the added amount of membrane in the form of transverse tubules per sarcomere in V fibers. If one assumed that the myofibrils within the fiber have an average diameter of 1 μ and if one took a fiber diameter of 10 μ, there would be approximately 30 myofibrils lying around the circumference of the fiber; hence if there were one transverse tubule per myofibril, there would be approximately 30 transverse tubules per sarcomere. At an average diameter of 200 mμ for the transverse tubule and a length

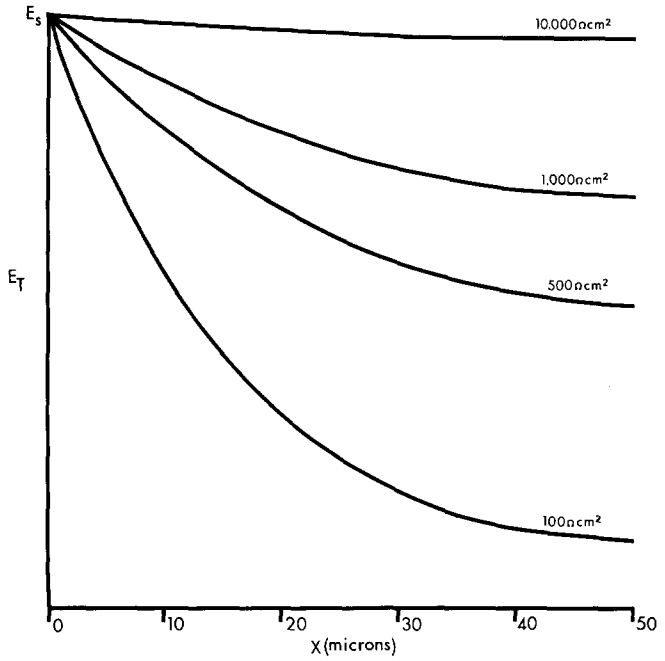


FIGURE 48 Current is injected into one fiber of a tightly packed bundle of fibers so as to displace the membrane potential just outside the mouth of the cleft between the fibers by an amount E_s. Ordinate, transmembrane potential of membrane in cleft E_T for various values of membrane resistivity; abscissa, distance down the cleft to the center (50 μ). Cleft width, 3×10^{-6} cm; resistivity of Krebs' solution in cleft, 50 Ω cm.

equal to the radius of the fiber, the area of membrane in the form of transverse tubules per sarcomere (2 μ long) would be approximately equal to 1.5 times the area of surface sarcolemma in a sarcomere 2 μ in length. That is to say, the area of sarcomere per unit length for rabbit ventricular muscle fiber would be 2.5 times the area of sarcomere per unit length for a P fiber. Hence, were the specific membrane resistance, myoplasmic resistivity, and the geometry of interconnections between fibers the same for the two types of fibers, the input resistance of V fibers would be less than that of P fibers, as is apparently the case (88). Furthermore, owing to the wide diameter and short length of transverse tubules in V fibers, the changes in transtubular membrane potential would follow closely those of the surface sarcolemma during an action potential. Evidence that this is so is provided by calculating the steady-state value of the change in transtubular potential at the inner end of the tubule for a given change in the transtubular potential just inside the mouth of the tubule. For this calculation we made use of equation 2 again and assumed that the tubule behaves passively as a leaky cable and that the external longitudinal resistance to current flow (in this case the myoplasm) is negligible compared to the resistance of current flow down the core of the tubule. For a tubule of radius 100 mμ (10^{-5} cm) and length 5 μ ($L = 5 \times 10^{-4}$ cm) specific membrane resistance R_m of 1,000 ohms cm² and resistivity of the material in the core (Krebs' solution) of 50 ohms cm, the value of λ is 100 μ; ($\lambda = \sqrt{r_m/r_i}$) where r_m equals membrane resistance times unit length and r_i is the core resistance per unit length. The transtubular membrane potential E at $X = L = 5$ μ is 0.999 of E at $x = 0$. Even if one takes the low value of R_m of 10 ohms cm² giving a value for $\lambda = 10$ μ, E at $x = L = 5$ μ is 0.887 the value of E at $x = 0$.

Since the conduction velocity varies inversely with the product of the myoplasmic resistance per unit length and the membrane capacitance per unit length of the fiber, the conduction velocity of P fibers, all other parameters being equal, should be greater than that of V fibers. Draper and Mya-Tu (87) found the conduction velocity in dog P fibers to be approximately four times the conduction velocity in dog V fibers. Part of this increase could then be accounted for by the absence of transverse tubules in the P fibers.[4] In the P strands of the sheep and goat (and to some extent in those of the dog), where a considerable proportion of the total membrane capacitance would be effectively isolated by the series resistance of the clefts during

[4] We have recently found that in dog atrial muscle there are transitions from fibers without transverse tubules to fibers having a few to fibers displaying transverse tubules at almost every Z line.

periods of high membrane conductance, the remaining membrane would depolarize more rapidly, and the propagation velocity of the action potential would tend to approach that of a fiber of a diameter equal to that of the over-all diameter of the P strand. Perhaps because of the relatively large size of the heart the need for a higher conduction velocity in the P strands has been met in this way by tight packing of the fibers. Indeed, the density of packing of the fibers seems to vary with the size of the heart as shown in Figs. 2–6. The transition from the loose packing of the fibers in the strand of the relatively small guinea pig heart to the tight packing in the larger goat and sheep P fibers is quite striking.[5]

Prolonged currents injected into single fibers of a sheep P strand cause a change in the quiescent membrane potential (82). McAllister and Noble (82) attributed these changes to changes in the potassium concentration immediately outside the cell membrane, presumably in a space, which the authors suggest might be in the intercalated disc or in the transverse tubules, that equilibrates slowly with the extracellular fluid. To account for their results, the authors concluded that the diffusion of potassium ions over such a space into the extracellular fluid must be rate limited over a considerable distance and not only at one point. In the electron micrographs of the sheep P strand a space having these properties would be the deep, narrow clefts separating the tightly packed fibers. It would appear, therefore, that this phenomenon would not occur in a P strand similar to that of the rabbit, in which the fibers are not tightly packed and in which relatively large spaces separate the fibers when the fibers are not forming tight junctions.

Dudel et al. (85) have recently studied the current-voltage relationships of sheep P fibers by using short lengths of P strands and intracellular microelectrodes. With the strand in sodium-free solution they found an inward ionic current in the membrane potential range of -40–$+10$ mv (inside with respect to outside). Since this current was obtained in sodium-free solution, they attribute the current to some ion other than sodium but could not identify the ion.

A possible explanation of this inward current in sodium-free media is that this current is, in fact, sodium current, especially since the time course of the current and the characteristics of its threshold and inactivation were similar to those of the inward-going sodium current in normal sodium media. The removal of sodium ions from the narrow clefts between the fibers of the sheep and goat P strands might be difficult, particularly if the membrane has an active sodium pump. Indeed a pump would be necessary to maintain a constant ionic concentration in such a narrow cleft. The volume of a 30 mμ gap separating 1 cm^2 of membrane from another such membrane area is 3×10^{-6} cm^3, which would contain 4.5×10^{-10} mole of sodium ions (assuming the sodium concentration to be 150 mM). If the sodium entry per action potential were the same as it is in frog skeletal muscle, 1.5×10^{-12} mole cm^{-2} (90) then in the absence of the pump approximately 5 action potentials would deplete this space by one-third of its sodium content. Although diffusion down the clefts through the extracellular space would diminish this depletion to some extent, these clefts can be as deep as 50 μ and the diffusion time constant would be in the range of a second or two, if one assumes the sodium-diffusion constant to be the same as in water.

Because of these geometrical considerations, a possible explanation for the inward current in sodium-free media is that, owing to the active extrusion of sodium ions into the narrow clefts, the sodium ion concentration in the clefts, with the strand in sodium-free external media, may be high in spite of diffusion along the clefts to the outside extracellular space. For some time this loss by diffusion could be replaced by the pump from the relatively large store of intracellular sodium. Thus, although in sodium-free media the shift in equilibrium potential of sodium ions across the surface membrane would be as expected, the shift for the membrane deep in the clefts might be little changed (at least until the intracellular sodium ion store became depleted or the pump stopped). Thus, these membranes would exhibit an inward sodium current on depolarization and, hence, an apparent nonsodium inward current would be

[5] Through the courtesy of Dr. Black-Schaffer, Department of Pathology of the University of Cincinnati, Ohio, we have had the opportunity to study with the light microscope the P fibers of an elephant and a whale. The P fibers of both animals were, indeed, very similar to those of the goat and sheep rather than to those of the smaller animals. It should also be pointed out that the large, thick, usually brown P strands of sheep, although composed almost entirely of V fibers, appear by microscopic examination also to contain P strands.

recorded in sodium-free media. Although this explanation may be incorrect, it should be excluded before the inward current observed in sodium-free media is attributed to a nonsodium current, particularly since the nature of the ion is obscure, Dudel et al. having excluded the possibility that the ion is calcium (89).

Although the sheep and goat P strands have been assumed to behave electrically as a single leaky capacitive cable (81, 83, 91, 92), our light and electron microscopic study offers no evidence in support of this assumption as our previous study failed to justify such an assumption for the rabbit P strand. In the latter, there is no unique relationship between the input resistance and membrane resistance. At high values of membrane resistance the input resistance of a fiber in a bundle approaches the input resistance of all the fibers in parallel, i.e., it behaves like a single cable. However, as the membrane resistance is decreased, the input resistance changes to one which is solely dependent on the geometry and the electrical properties of the individual fiber into which the electrode is inserted (9). We have no evidence that the behavior would be any different for the sheep and goat P strands except that it would be further complicated by the large area of membrane in series with an extracellular resistance (that of the clefts), and by the splitting and reforming of the strand into daughter strands. In view of the continued popularity of the sheep and goat P strands as preparations for the study of the electrical properties and ionic fluxes in cardiac muscle, we should like to stress that the possibility should be assessed beforehand whether the deep, narrow clefts between fibers and the splitting and reforming of the strand might complicate the interpretation of the experimental results.

P fibers of the goat and sheep in sodium-free bathing solutions exhibit a decrease in membrane conductance on depolarization from the resting potential to about −20 mv (inside with respect to outside) (82). This effect has been attributed (82) to the anomalous potassium rectification which had previously been described in frog skeletal muscle (93–96). Adrian and Freygang (96) postulate that an intracellular compartment (presumably the transverse tubular system) which opened to the extracellular space was involved with the phenomenon of anomalous rectification in this tissue. The selective ionic permeability of the membrane of this compartment was considered to be different from that of the surface sarcolemma. Since P fibers of the sheep and goat do not have transverse tubules, if a similar model were used to account for anomalous rectification in these fibers, the membrane of the clefts would have to behave like the transverse tubular membrane of skeletal muscle. Although this assumption at first sight seems reasonable, our observations of serial sections indicate that an additional assumption would be required. As is shown in Fig. 1, some fibers at one point in the strand are separated by a narrow cleft, whereas farther down the strand they separate. Hence, to preserve the hypothesis, one would have to assume that, when adjacent fiber membranes approach one another to become separated by a narrow cleft, the ionic permeability of the membranes must change.

Müller (97) has attempted to produce localized contraction of single sarcomeres of sheep and dog trabeculae (*Trabekelmuskel*) by passing electrical current through a micropipette, the tip of which was pressed against the surface sarcolemma in the region of the A or I bands. He failed to obtain a localized contraction at the tip in either location, in contrast with the previous results of similar experiments by Huxley and Taylor (28) with frog skeletal muscle. It is possible that the fiber of the dog trabecula could have been a P fiber, in which case the explanation of the failure to produce localized contraction of sarcomeres is that the fiber had no transverse tubules. However, in the case of the sheep trabecula, if the fibers were rich in myofibrils, it is most likely that they were ventricular fibers and would therefore have had transverse tubules. The reason for Müller's failure to elicit a localized contraction in these fibers is thus obscure. The only contractile response he observed in response to the current was a contraction of all the sarcomeres in the microscope field. Müller does not say whether the all-or-none response was the result of an action potential being initiated by the applied current. Were this not the case, then a possible explanation lies in the fact that the sarcoplasmic reticulum in sarcomeres of both P and V fibers is continuous with the sarcoplasmic reticulum of adjacent sarcomeres by extensive connections. Perhaps the activation of calcium release from the SR of one sarcomere spreads to other sarcomers so that localized activation cannot be initiated.

NOTE ADDED IN PROOF: The recent cytochemical demonstration in the SR of acid mucopolysaccharides

by Philpott and Goldstein (Philpott, C. W., and M. A. Goldstein. 1967. *Science.* **155:** 1019.) and Daugherty (Daugherty, W. J. 1967. *J. Cell Biol.* **35,** 2, Pt. 2:66.) lends added support to the suggestion that chondroitin sulfate A may be the transducer for excitation-contraction coupling and reversible calcium storage.

This investigation was, in part, presented at the Second International Biophysics Congress, Vienna, Austria, 5–9 September 1966 (Abstract No. 500) and, in part, at the Seventh Annual Meeting of the American Society for Cell Biology, 13–15 November 1967, Denver, Colorado (Abstract No. 127).

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