THE ULTRASTRUCTURE OF THE CAT MYOCARDIUM

II. Atrial Muscle

N. SCOTT McNUTT and DON W. FAWCETT

From the Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115. Dr. McNutt's present address is Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts 02114

ABSTRACT

The ultrastructure of the cells specialized for contraction in the atrium and ventricle of young adult cats are compared. The cells specialized for conduction are not included. In addition to possessing distinctive atrial granules, the cells of the atrium are smaller in diameter (5–6 μ) than ventricular cells (10–12 μ) and have strikingly fewer T tubules. These latter differences are discussed in terms of their possible significance for the rate of conduction of the action potential. It is suggested that the very small number of T tubules in atrial cells may compensate for the small cell diameter, and thus permit rapid conduction of the action potential across the surface of the atrium. Coated dense vesicles found in association with the sarcoplasmic reticulum at the level of the Z line in ventricular muscle are more evident in atrial cells. In the virtual absence of T tubules in atrial cells, the subsarcolemmal cisternae of the sarcoplasmic reticulum are almost exclusively at the cell periphery. The ends of the cells and their processes in ventricular muscle are rectilinear with the interdigitated portions of the intercalated discs oriented transversely, whereas those of the atrium are often oblique to the myofilament axis. This difference may be related to the lower mechanical tension on atrial cells.

INTRODUCTION

Physiological differences between atrial and ventricular cardiac muscle (46, 71) suggest that there may also be associated differences in the fine structural organization of their cells. However, in their classical paper on cardiac muscle, Porter and Palade (61) did not note significant differences between atrial and ventricular muscle in the rat. There have since been many studies of ventricular muscle (49, 51, 53, 70, 77), but few have devoted specific attention to the atrium. There have been studies of turtle atrium (21), of frog atrium (3), and of the cardiac muscle in the pulmonary veins (41, 42). A population of granules has been described as specific for atrial muscle cells (38, 44). Also, special study has been made of the sinoatrial node (79). However, to our knowledge, there has not been a general description of the fine structure of the cells specialized for contraction in mammalian atrial muscle that would permit a detailed comparison with the fine structure of similarly specialized cells in ventricular muscle.

This paper is the second of a series devoted to the cat heart. In the companion paper (20), the ultrastructure of the ventricular muscle was presented in considerable detail. So as to avoid unnecessary repetition of description, emphasis will be placed

on the structural differences between atrial and ventricular papillary muscle. Those features common to both will be discussed when they are more clearly demonstrated in the atrium.

MATERIALS AND METHODS

Ten young adult cats were anesthetized with chloroform or pentobarbital, and the hearts were excised. The hearts were dissected in a bath of bicarbonate-buffered physiological salt solution (Table I), oxygenated with 95% O_2-5% CO_2. Spontaneous contractile activity of the right atria was preserved in this medium. The persistence of spontaneous activity was variable in left atria. The atrial appendages were cut from the atrial walls, were opened with scissors, and spread to form flat sheets. The smooth-walled areas of the atria were then dissected from the remainder of the hearts. The sinoatrial nodes and interatrial septa were not examined in this study.

TABLE I

Physiological Salt Solution
(Buffered at pH 7.4 with 95% oxygen-5% CO_2)

Cations	(Meq./liter)	Anions	(Meq./liter)
Na^+	135	Cl^-	97
K^+	5	$HCO_3^=$	24
Ca^{++}	3	$PO_4^=$	1
Mg^{++}	2	$SO_4^=$	2
		Pyruvate$^-$	10
		Acetate$^-$	10

Atrial muscle was fixed by immersion either as freely floating pieces, as pieces stretched in a physiological apparatus (5), or as strips tied to lengths of applicator sticks. Three fixative solutions were used at ice-bath temperatures for a duration of 2 hr: Karnovsky's fixative employing a glutaraldehyde-paraformaldehyde mixture (40); 6% glutaraldehyde buffered with 0.1 M sodium cacodylate pH 7.4; and 6% glutaraldehyde buffered with modified Krebs-Ringer solution oxygenated with 95% O_2-5% CO_2. After initial glutaraldehyde fixation, the tissues were rinsed for 2 hr in 0.1 M cacodylate buffer (0.05 M in $CaCl_2$). The further processing of tissue for electron microscopy was identical to that described previously for cat ventricular muscle (20, 81).

Cross-sectional diameters of the subendocardial muscle cells were determined by light microscopic examination of 1-μ sections of the Epon-embedded tissue stained with toluidine blue. These measurements were confirmed by electron microscopy. Although the cells in the ventricular papillary muscle are generally parallel, the orientation of the atrial cells varied greatly. Since there was only a single axis of stretch, a small proportion of the cells in any given preparation met the requirements for measurement by being stretched to a sarcomere length of 2.2 μ. After determining the region of such stretch, this same block was turned 90° and cut in transverse section. Because of the variability in fiber orientation, it did not seem profitable to determine frequencies of occurrence of various cell diameters in the entire population of cells.

OBSERVATIONS

Cell Size

The atrial cells studied tended to be smaller in diameter than ventricular cells when both were stretched to approximately the same sarcomere length, 2.2 μ (Fig. 1). The atrial cells averaged 5–6 μ in diameter, while right ventricular papillary muscle cells averaged 9–10 μ in diameter. The great majority of cells fell within these ranges, but individual atrial cells were occasionally encountered that had a diameter up to 11 μ, and rare individual ventricular cells from papillary muscle had diameters as small as 6 μ or as large as 20 μ.

Organization of the Contractile Elements

In the atrial cell there are two interdigitating sets of myofilaments as in other striated muscle (29, 36, 37). The myofilaments appear identical in dimensions and arrangement with those described for ventricular muscle (20). Also, as in the ventricle, the myofilaments are not aggregated into discrete units which could be called myofibrils but instead are distributed continuously throughout the fiber cross-section forming essentially a single large bundle (Fig. 2). Approximately midway along the length of the cell, the myofilaments diverge to bound a fusiform central area of sarcoplasm containing the elongate nucleus. In the atrium, these conical regions of sarcoplasm at the poles of the nucleus have a much more prominent Golgi complex than is present in the ventricle. Associated with this Golgi complex are numerous dense granules, vesicles, and mitochondria (Figs. 2 and 7). Occasionally in the very small atrial cell, the nucleus is eccentrically located with only a small rim of sarcoplasm containing myofilaments (Figs. 5 and 6). Numerous small clefts and fusiform spaces within the bundle of myofilaments are filled by mitochondria which in atrial muscle appear to occupy a somewhat greater proportion of the cross section than they do in ventricular

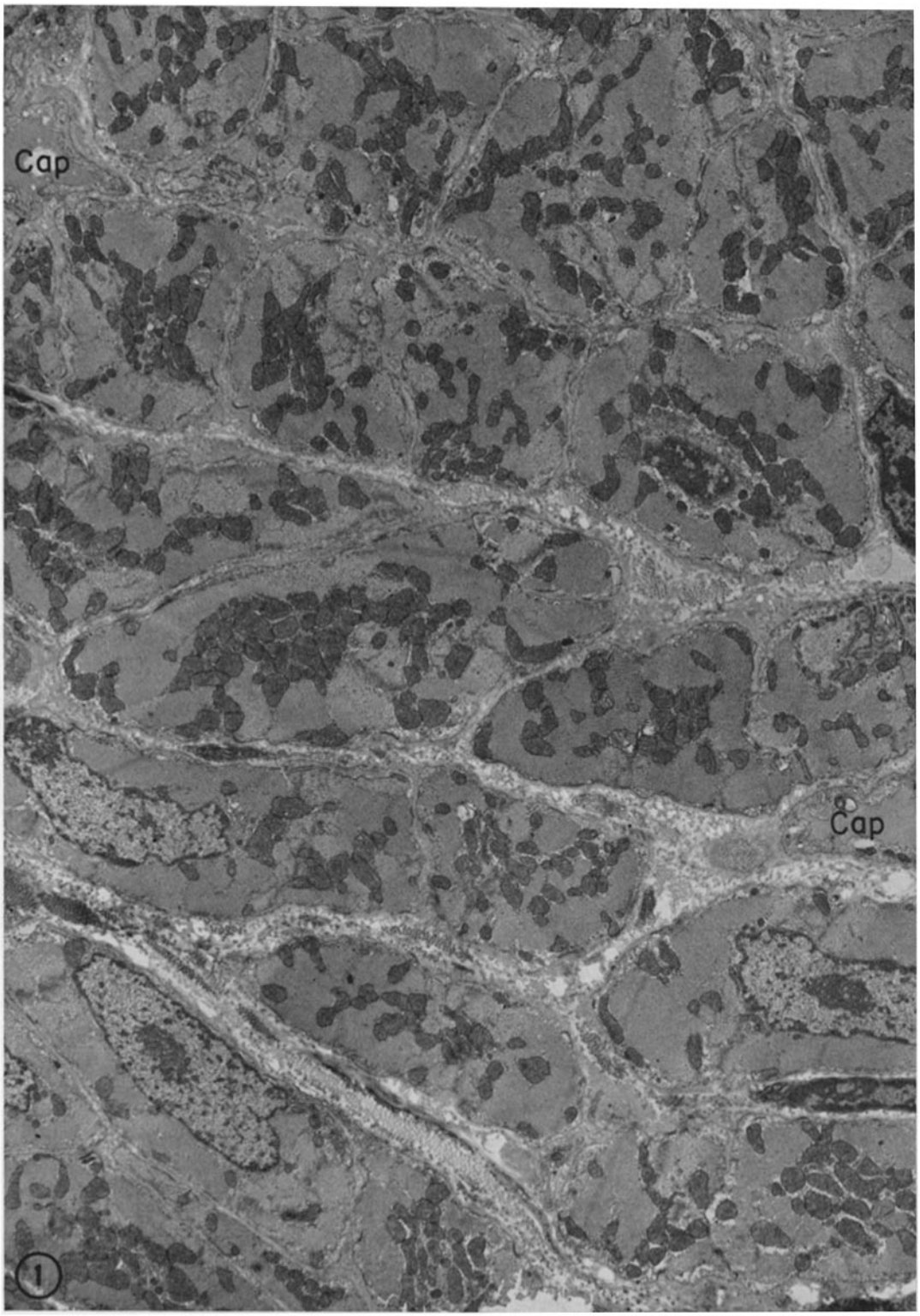


FIGURE 1 The general histological organization of the atrial pectinate muscle is illustrated in this low-magnification electron micrograph of the muscle fibers in transverse section. The fibers are of small diameter, and they are closely packed. The interstices among them are occupied by collagen embedded in a moderately dense ground substance. Portions of two capillaries (*Cap*) are included in the field. × 5000.

muscle (Figs. 2–4). In ventricular muscle, the clefts containing mitochondria are separated by areas of myofilaments a micron or more in width, whereas in atrial muscle the layer of myofilaments intervening between groups of mitochondria is only 0.2–0.5 μ thick. Short rows of circular profiles of sarcotubules may extend from one cleft to another, thus delimiting myofibril-like areas of irregular outline (Fig. 2), but fascicles of myofilaments are not completely surrounded by a network of sarcotubules as are the myofibrils of the common phasic type of skeletal muscle (34, 57).

Owing to the unity of the mass of myofilaments, there is a strong tendency for the striations to be in register across the width of the cell. This alignment of sarcomeres is particularly evident in longitudinal sections but is also detectable in transverse sections which tend to exhibit the same pattern of punctate cross-sectional profiles of myofilaments throughout. Despite this prevailing consistency in arrangement, neighboring areas within the myofilament mass may occasionally show different myofilament patterns (Figs. 2 and 5). The occurrence of contiguous areas of different cross-sectional appearance suggests that the filaments of one area are, to some extent, capable of moving independently of the neighboring areas within the coherent myofilament bundle.

Longitudinal sections of stretched atrial cells (Figs. 7–9) show a banding pattern indistinguishable from that described in the companion paper on cat papillary muscle (20), and in many other studies of striated muscle (6, 8, 32, 37, 38, 51, 60, 75, 77). As noted by Stenger and Spiro (78), occasional images are seen in which the Z line has thick longitudinal linear densities suggesting filaments passing straight through the Z line. However, these appearances are rare compared to images of filaments diverging from the ends of offset actin filaments, and they are probably attributable to optical superimposition of structures as suggested by both Franzini-Armstrong and Porter (24) and by Kelly (43).

Broad Z lines with a periodicity approximating that of crystalline tropomyosin, similar to those previously described in ventricular muscle (19), were rarely seen in atrial cardiac muscle. These widened Z lines are difficult to bring into accord with any of the models of Z line structure offered to date (24, 43, 45, 65).

The extrafibrillar material of the Z lines resembles in density and in texture the dense substance seen at desmosomes and at fasciae adherentes the site of attachment of the myofilaments to the plasma membrane at the intercalated disc. This dense component is often continuous from the Z lines at the lateral margin of the contractile material to the dense layer of lateral junctional complexes between cells (21, 47) (Fig. 7).

The Sarcolemma and the T Tubules

The cell is enclosed by a membrane coated on its outer aspect by a fibrillar layer approximately 500 A thick which is thought to consist of protein-polysaccharide (4, 63). The term sarcolemma is used here to designate the 90 A trilaminar unit membrane which is commonly considered to be the anatomic boundary between the intracellular and extracellular compartments. The substructure of the layer of coating material on the sarcolemma of atrial cells appears identical to that on ventricular sarcolemma.

Two types of vesicular invaginations of the sarcolemma are encountered. One type is 300–500 A in diameter and has the appearance typical of smooth-surfaced caveolae involved in micropinocytosis (56). The other type is larger, 500–1,000 A in diameter, and has a finely fibrillar material coating the cytoplasmic surface of its trilaminar membrane. This coating is 150–200 A thick and often has a delicate radially striate appearance. Larger vesicles of this type correspond to the "coated vesicles" or "bristle coated vesicles" thought to be associated with protein transport in insect oocytes, and in epithelial cells of a number of invertebrate and vertebrate species (1, 2, 26, 68, 69).

One of the principal differences between atrial and ventricular myocardium of the cat heart is in the degree of development of the T-system. In ventricle, T tubules are abundant (20, 53, 55, 70) and tend to occur in rows with their openings onto the cell surface at the level of the Z lines (64). They are present in ventricular fibers irrespective of fiber diameter. In the atrium of old cats in which the cells may range up to 11 μ in diameter, T tubules are seen from time to time, but they are quite rare compared to their occurrence in the ventricle; in young cats they are entirely lacking in most electron microscopic fields even at low magnification. When T tubules are observed in the atrium, they have all of the features typical of those of the ventricle. They are 1500–2000 A in diameter, and their limiting membrane has a fibrillar coating on

the luminal surface similar to that on the sarcolemma at the periphery of the cell (Fig. 14).

If atrial cells are allowed to contract on fixation, then the sarcolemma tends to become scalloped, forming indentations at the Z lines and bulges over the A bands. These superficial indentations of the sarcolemma are not to be confused with true T tubules. The latter penetrate well beyond the most superficial myofilaments; they present round or oval profiles in cross-sections, and do not disappear when the muscle is optimally stretched before fixation.

Fine Structure of the Common Cell Organelles

The atrial cell nuclei do not appear to differ from those of the ventricle and need no detailed description here (20). The Golgi complex occupies a prominent position in the conical caps of sarcoplasm at the ends of the nucleus (Fig. 7). It has been well studied by Jamieson and Palade (38) in a number of species including the cat. It is less intimately associated with the perinuclear cisterna than is its counterpart in the ventricle and consists of several stacks of flat, or somewhat dilated saccules or cisternae and numerous small vesicles. A dense homogeneous material can be found in small foci in the Golgi cisternae and within some of the small vesicles associated with them. These vesicles with a dense content appear to arise in the Golgi complex and progressively enlarge until they attain a diameter of 0.3–0.4 μ (Figs. 7–9). They were described by Jamieson and Palade as "specific atrial granules" because they are found only in the atrium. The limiting membrane is often separated from the dense content by a clear zone 100–200 A wide. A crescentic layer of fibrillar material 150–200 A thick often coats an area on the cytoplasmic surface of the membrane limiting the granule.

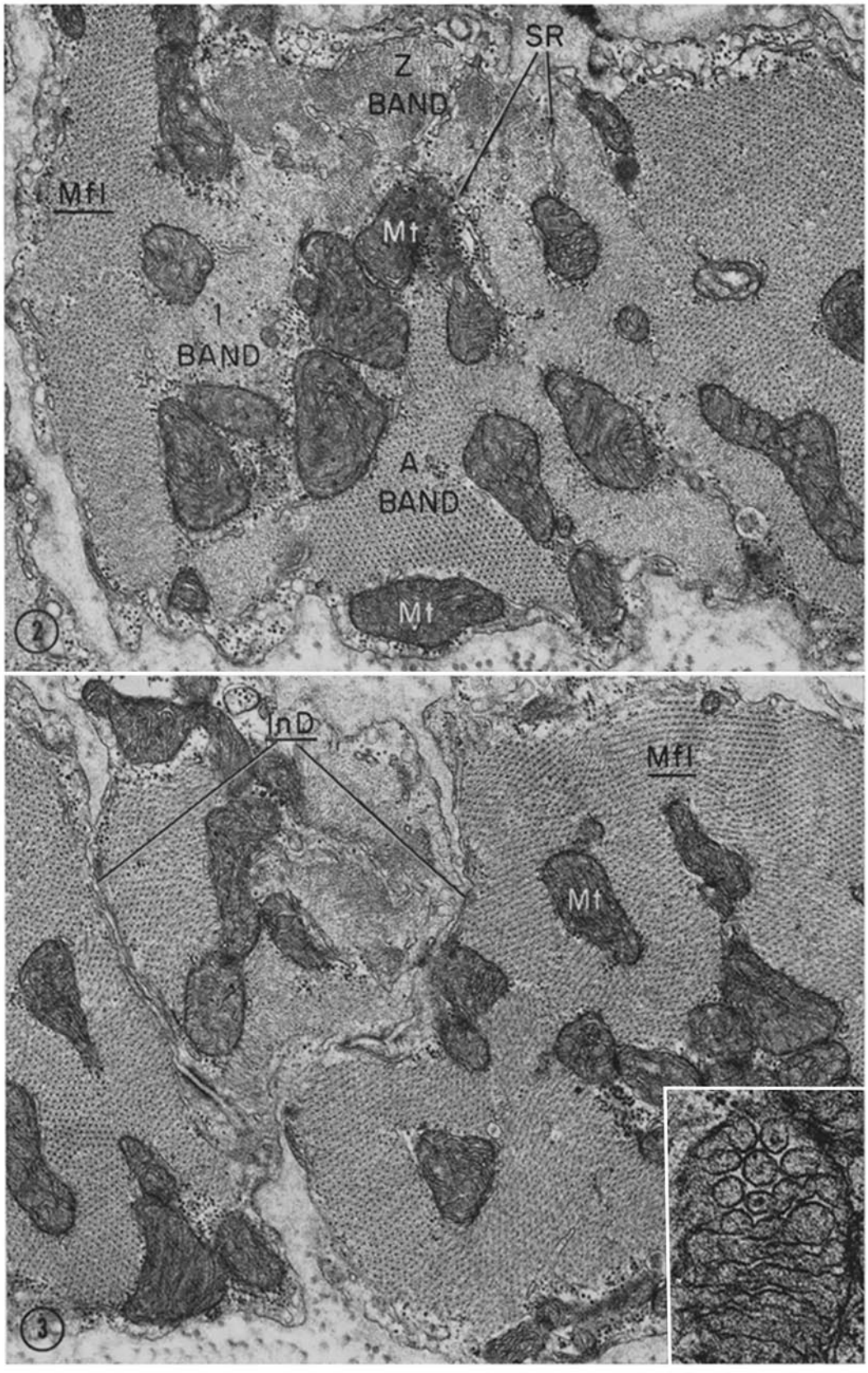
The other vesicles associated with the Golgi complex are of the same kind as those described for the ventricle and are present in similar abundance. These include (*1*) small vesicles 500–800 A in diameter with clear centers and with a 150-200 A radially striated coating on their outer surface (Fig. 9), an appearance typical of the family of "coated vesicles" or "bristle coated vesicles" (26, 69), and (*2*) multivesicular bodies 0.5–0.8 μ in diameter. Larger, irregularly shaped, membrane-bounded deposits of lipofuscin pigment (Fig. 7) (23) are uncommon in the young cats employed in the present study, but are abundant in older animals.

Small dense particles are present in great abundance in the sarcoplasm of atrial cells and are both attached to membranes and free in the cytoplasm. Those attached to membranes and occurring in chains or whorls are identified as typical polyribosomes. Those free in the matrix, however, present greater difficulties in identification because the size range of glycogen particles (150–300 A) in cardiac muscle overlaps that of ribosomes (150–200 A). The vast majority of dense particles free in the sarcoplasm are large enough and stain densely enough to be regarded as glycogen (66). It is not possible, however, to make any definite statement about the number or distribution of free ribosomes in the population of small particles.

Lipid droplets which are common in ventricular cells are rarely encountered in the atrial cells, but when present, they are 0.3–1.0 μ in diameter and usually closely associated with mitochondria. The less frequent occurrence of lipid droplets in atrial cells might possibly be related to a lower requirement for storage of energy-rich material.

The mitochondria, like those of the ventricular muscle, are densely packed in the cones of juxtanuclear sarcoplasm and aligned end to end in clefts in the myofilament mass. Their matrix is rather dense and contains numerous 250–300 A matrix

FIGURES 2 and 3 In transverse sections, the contractile material is identified by the punctate cut ends of the myofilaments (*Mfl*). The myofilaments form a single bundle that extends throughout the cross-section without clear demarcation of separate myofibrils. The continuity of the bundle is interrupted only by irregular clusters of mitochondria (*Mt*) and a few circular profiles of sarcoplasmic reticulum (*SR*). The bands of myofilaments are not all precisely in register since the pattern characteristic of Z bands (*Z*), I bands (*I*), and A bands (*A*) are visible in the same cross-section. T tubules are absent. The mitochondria have foliate cristae of variable orientation which often show zigzag angulations or honeycomb configurations (see inset). × 30,000.


SR
Z
BAND
Mfl
Mt
I
BAND
A
BAND
Mt
2
InD
Mfl
Mt
3

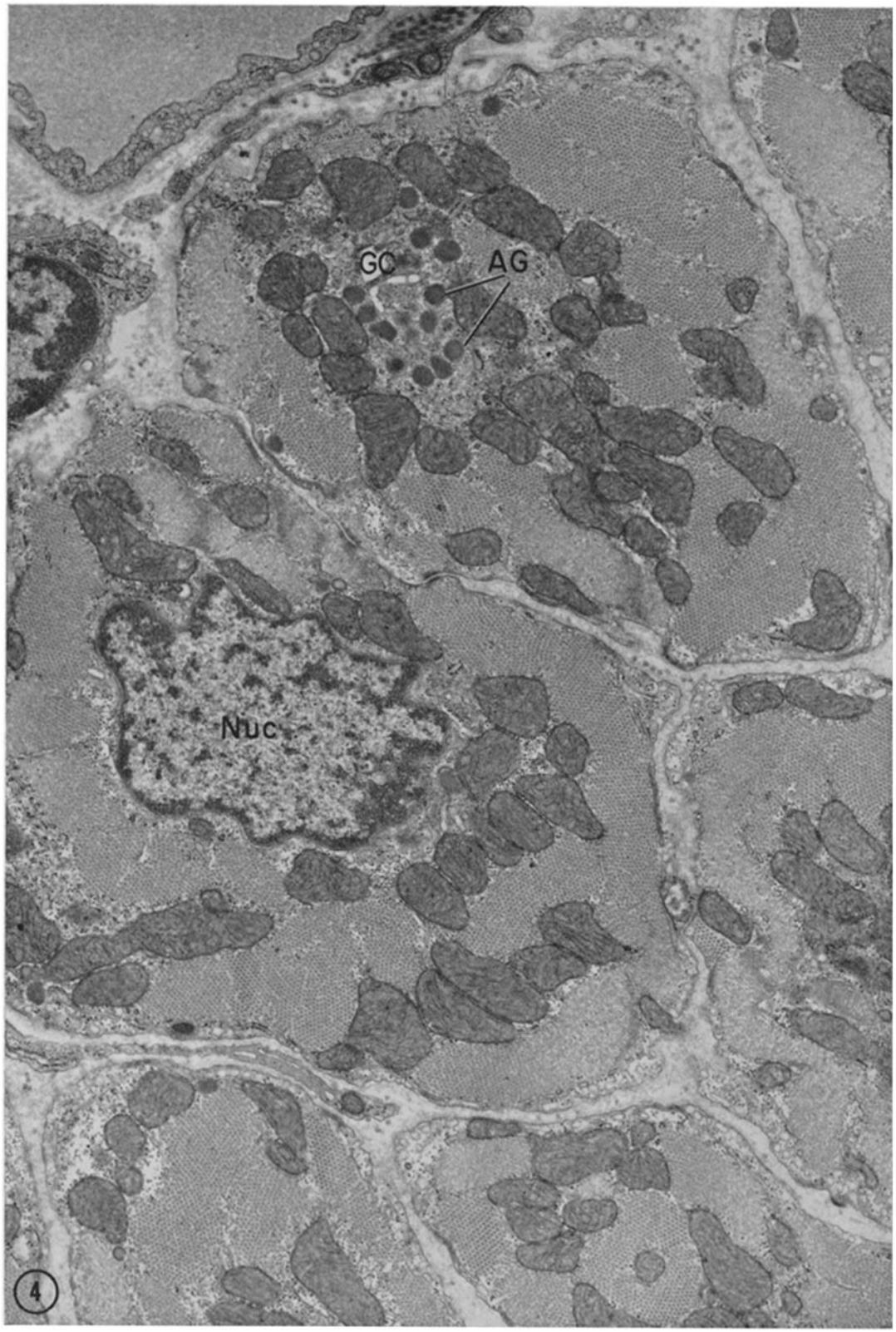
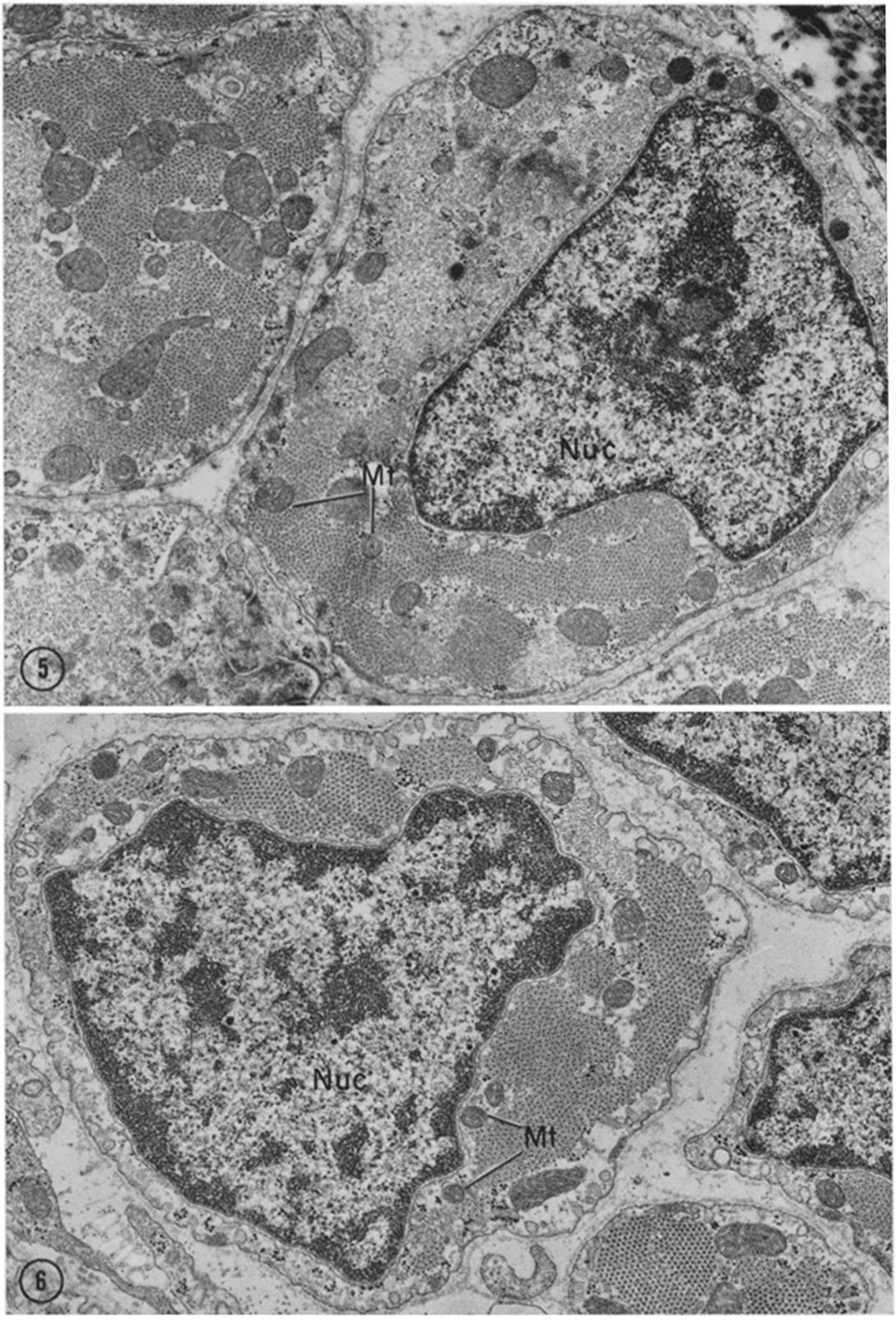


FIGURE 4 Transverse sections of atrial cells showing, in one, the centrally placed nucleus (*Nuc*), and in the other, a juxtanuclear Golgi complex (*GC*) containing a number of specific atrial granules (*AG*), and surrounded by mitochondria. Notice again that no T tubules are present. × 14,500.



FIGURES 5 and 6 Micrographs of typical atrial cells in transverse section through the nucleus (*Nuc*). The small cells have only a very thin rim of myofilaments around the nucleus. The mitochondria (*Mt*) are also very slender, rather evenly spaced, and occasionally contain only a single longitudinal crista. No T tubules are present. × 18,500; × 22,000.

granules. The internal membrane structure is not significantly different from that described for ventricular mitochondria. Long cristae with periodic angulations usually run transversely but may exhibit spiral configurations, and the angulations of adjacent cristae may fuse forming a "honeycomb" pattern (Figs. 2 and 3). The mitochondria are quite pleomorphic and often have long finger-like projections. These projections were first noted by Jamieson and Palade (38) in atrial cells and are similar to those illustrated and described in greater detail in the companion paper on the ventricular myocardium (20). These slender processes may be 2–6 μ in length, but usually have a diameter of 0.1 μ or less. They often extend into crevices and clefts in the myofilament mass. They are frequent in atrial cardiac muscle cells (Figs. 5 and 6) and may facilitate rapid atrial contractility by insuring an even distribution of the ATP-generating system to all parts of the mass of myofilaments.

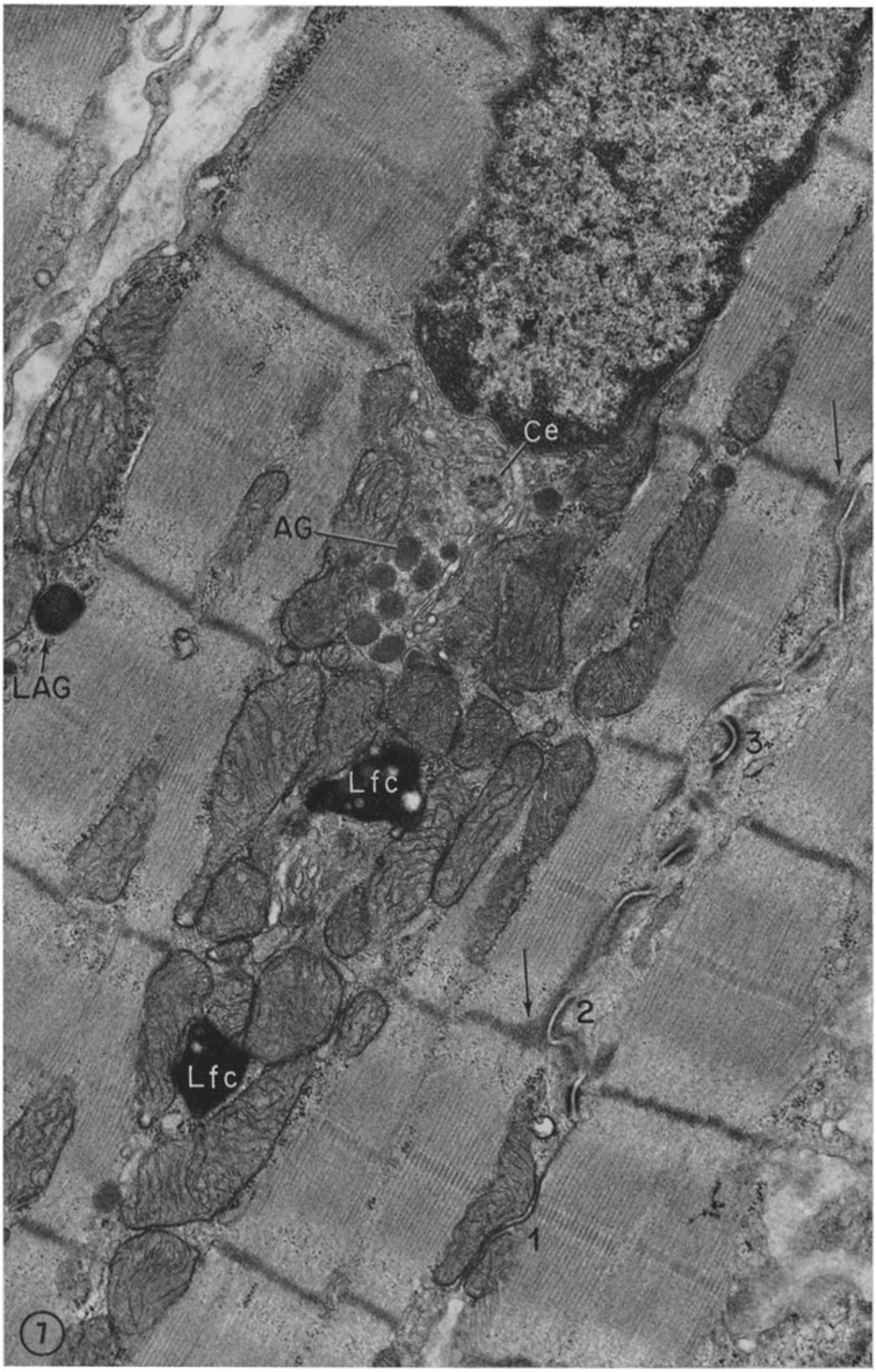
The Sarcoplasmic Reticulum and the Subsarcolemmal Cisternae

Since atrial cardiac muscle fibers have a small diameter and no regularly shaped, discrete myofibrils, one seldom obtains sections that present an extensive surface view of the sarcoplasmic reticulum. However, it is evident from the pattern observed in many limited areas that there is no prevailing longitudinal orientation of the sarcotubules as there is in skeletal muscle (17). Though occasional tubules pursue a longitudinal course for a limited distance, the great majority are relatively short, randomly oriented links of a loose network (Fig. 10) and sarcotubules are found crossing any of the bands in any direction. Working with species other than the cat, Simpson and Oertelis have described a special thin tubular element of the reticulum, the Z tubule, which is said to encircle the "myofibrils" at successive Z lines (70). The appearance of separate myofibrils in their micrographs is, we suggest, a consequence of swelling during specimen preparation which has produced clefts separating the myofilament bundle into myofibril-like units. In cat heart muscle fixed by the methods employed in the present study, no special Z tubule is identified. Usually, the tubules overlying the Z line are of the same diameter as tubules over other bands. Moreover, in cross-sections at the level of the Z line, where one might expect an encircling Z tubule, one does not find any tubule coursing circumferentially for more than a fraction of a micron.

As in the ventricle, the tubules of the sarcoplasmic reticulum have local specializations which establish a very close relationship to the sarcolemma. These specializations take the form of flattened saccules parallel to the sarcolemma and separated from it by a 150–200 A interspace which characteristically has a beaded appearance due to the presence of ill-defined periodic densities. Whether these densities represent serrations of one of the membranes or extramembranous material is not clear. With some fixatives, the flattened saccule contains a dense line in the middle of its 200 A lumen. The nature of the association between the reticulum and the cell surface in cardiac muscle is identical whether the contact is made with the sarcolemma at the periphery of the fiber or with that bounding the T tubules. Since T tubules are very uncommon in the atrium, the saccules of the reticulum occur almost exclusively at the periphery of the fibers, whereas in the ventricle they are abundant in both locations. Those at the periphery do not seem to have any preferential localization with respect to the cross-bands of the underlying contractile mass (Fig. 11).

Before the continuity of the transverse tubules with the cell surface was established, the term "dyad" was often applied to the pair of profiles consisting of a T tube and the closely apposed flattened saccule of the reticulum. This seemed

FIGURE 7 A typical atrial cell in longitudinal section. The cone of sarcoplasm at the end of the nucleus contains a centriole (*Ce*), a Golgi complex with associated specific atrial granules (*AG*), large atrial granules (*LAG*), and lipofuscin pigment (*Lfc*). The side-to-side junction with the adjacent cell exhibits all three types of local junctional specializations, the fascia occludens (*1*), fascia adherens (*2*), and macula adherens (*3*). The continuity of the dense extrafibrillar component of the Z bands with the subsarcolemmal density of the fasciae adherentes is clearly shown at the arrows. Note the absence of T tubules. × 20,000.


Ce
AG
LAG
Lfc
Lfc
1
2
3
7

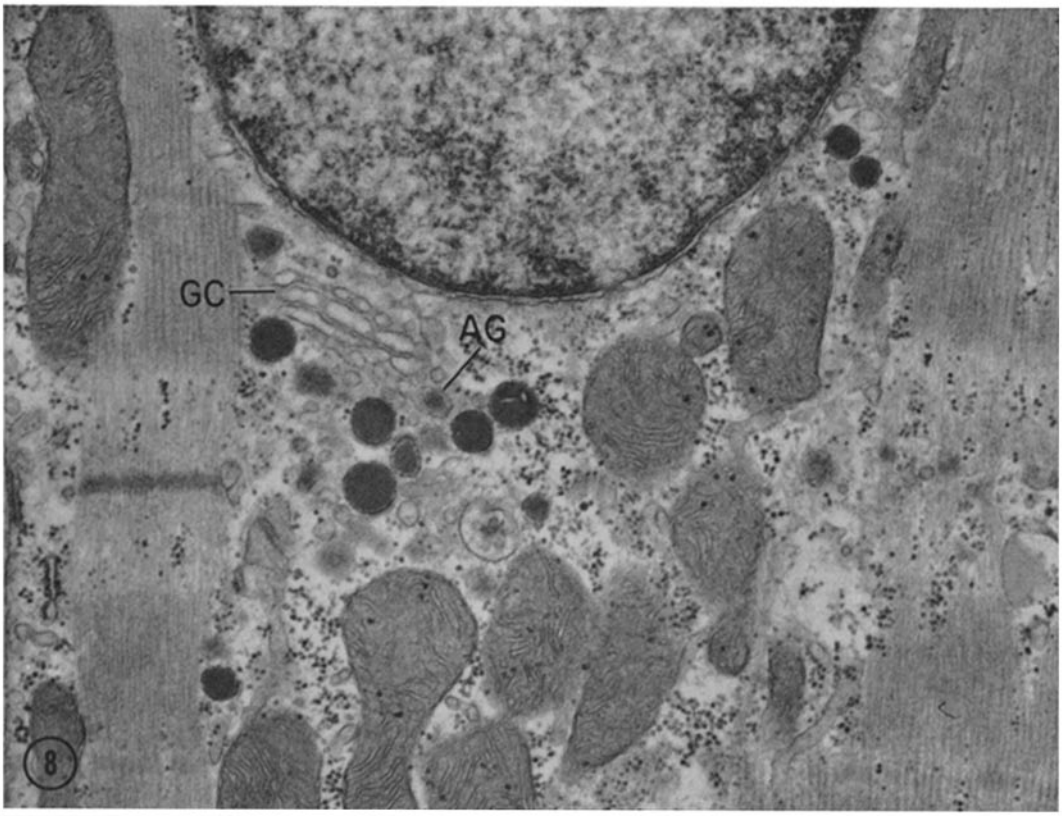
appropriate by analogy with the "triads" of skeletal muscle. With the realization that the T tube is an interiorized part of the sarcolemma and with the demonstration of a similar relationship of the saccules of reticulum to the T tubules and to the peripheral sarcolemma, it has become desirable to have a single term applicable to the saccules of reticulum in either location. We have, therefore, chosen to call these flattened saccules "subsarcolemmal cisternae" because of their superficial resemblance to the "subsurface cisterns" previously described for certain cells in the nervous system (67) and to those cisterns found as part of the junctional complexes of Sertoli cells in the testis (22). This is not to imply that the functional significance of all of these cisterns is the same, but the morphological relations are sufficiently similar to warrant a comparable descriptive term.

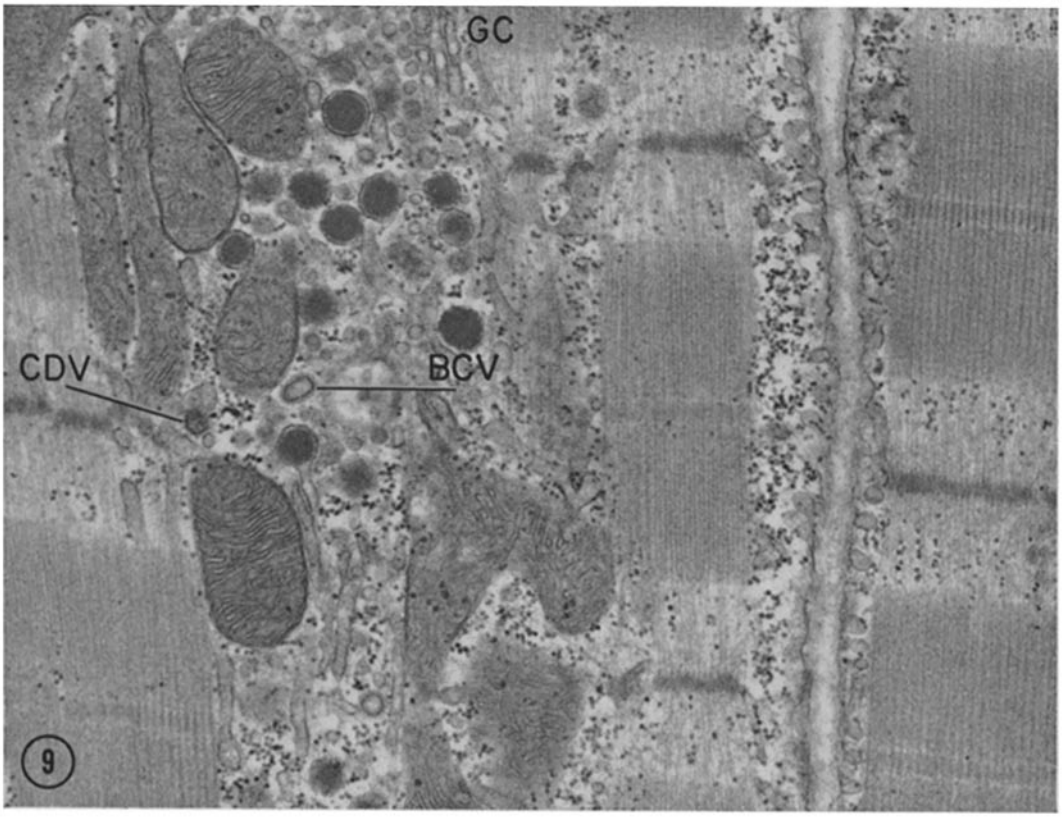
The sarcoplasmic reticulum is composed predominantly of tubules which do not have associated ribosomes. However, short segments may bear polyribosomes in typical rows or whorls. These granular segments may be found in any part of the system. They may represent transient attachment of polyribosomes to the membranes of the reticulum or local sites of synthesis of membrane precursors that provide for extension or renewal of the system as suggested by the work of Dallner, Siekevitz, and Palade (10). With rather striking regularity in the atrium, polyribosomes were found on the membranes of the reticulum adjacent to the subsarcolemmal cisternae (Fig. 11). This frequent association of polyribosomes with the reticulum near the subsarcolemmal cisternae may not be fortuitous and is in need of further investigation since the involvement of these regions in excitation-contraction coupling (9) may entail increased membrane renewal or other synthetic activities.

Coated Vesicles and the Reticulum

Very frequently associated with the sarcoplasmic reticulum in the immediate vicinity of the Z band is a special type of coated vesicle that has a moderately dense content (Fig. 12). These vesicles are not identical with the coated vesicles described by other investigators and thought to be involved in protein transport (2, 26, 69) (Figs. 13 and 14). The typical protein transport vesicle is said to be 800–1000 A in over-all diameter with a cytoplasmic coat 200 A thick apparently consisting of delicate spines that give it a radially striated appearance, and with a lumen that usually appears clear or of very low density. The vesicles associated with the Z line, on the other hand, are slightly smaller, 500–800 A in diameter, and their 100–150 A coat does not have a distinct radially striated appearance. In very thin sections at high magnification, their lumen is seen to contain a flocculent precipitate of cohering fine granules 30–70 A in diameter (Fig. 13). Both the coating and granular content of these vesicles contribute to their characteristic dense appearance. Despite the minor differences between these vesicles and the typical protein transport vesicles, it is suggested that the vesicles described here also be considered as one type of "coated vesicle." Though these vesicles are present in considerable numbers at the Z lines of ventricular muscle, they are less conspicuous there because of the presence of profiles of T tubules at the same level. They are more evident, and possibly more numerous, in atrial cells. The coated dense vesicles are clearly associated with the sarcoplasmic reticulum. Occasionally, several vesicles are found adjacent to one another along the same Z line (Fig. 12). Their shape is usually the same in all planes of section. In favorable sections of both atrium and ventricle, some of the coated dense vesicles are clearly seen to be continuous with the smooth membrane of the reticulum by a short neck through which the lumen of the vesicle communicates with the interior of the reticulum. Rarely, local dilatations of sarcotubules have a coated wall identical to that of a coated vesicle and have dense granular material in the lumen (Fig. 12). The occurrence of free coated vesicles, vesicles communicating with sarcotubules via narrow necks, and dilated segments of sarcotubules having a coat and a luminal density suggests that these forms represent various stages in a dynamic process in which coated dense vesicles

FIGURES 8 and 9 Two micrographs of Golgi areas of atrial cells showing the condensation of dense material in vesicles associated with the Golgi complex (*GC*) to form the specific atrial granules (*AG*). Bristle-coated vesicles (*BCV*) are abundant and may be compared to the coated dense vesicle (*CDV*) associated with the reticulum. × 30,000.


GC
AG
8


GC
CDV
BCV
9

either become incorporated into or arise from the sarcoplasmic reticulum in the region of the Z line. The rather specific localization of the coated vesicles to the reticulum at this one level clearly suggests their functional role in the physiological relationship of the sarcoplasmic reticulum to the contractile filaments. Although their localization near the Z line may conceivably be fortuitous, it is tempting to speculate that they are related to the contractile process, and possible to calcium metabolism, since this is the most clearly defined function of the sarcoplasmic reticulum (9, 11, 30).

Cell-to-Cell Junctions

At the light microscopic level, atrial cells are long, thin cylinders that branch and are interconnected end to end at intercalated discs which are smaller than those in the ventricle (Fig. 15). In electron micrographs, the junctions between the ends of atrial cells are usually typical steplike intercalated discs with the four types of junctional specializations previously described for ventricular muscle (18, 20). As in the ventricle, the intercalated disc in the atrium can be subdivided into transverse and longitudinal segments. On the transverse segments are junctional specializations of the types called fascia adherens and macula adherens having an intercellular gap of approximately 300 A (15, 16). Specializations of the fascia occludens type are present on the longitudinal segments of the disc, and maculae occludentes are found on both transverse and longitudinal segments. The boundary between cells in ventricular muscle is quite rectilinear, running alternately perpendicular and parallel to the axis of the fibers (20, 52). In atrial muscle the intercalated discs show more variation in their course across the fiber, sometimes running obliquely for considerable distances (Fig. 16). Maculae adherentes are particularly numerous along the sides of the atrial cells. Since the pressures developed in the lumen of the atrium are much less than those developed in the ventricle, the less rectilinear outline of the atrial cell boundaries may be related to the decreased mechanical tension on atrial cells.

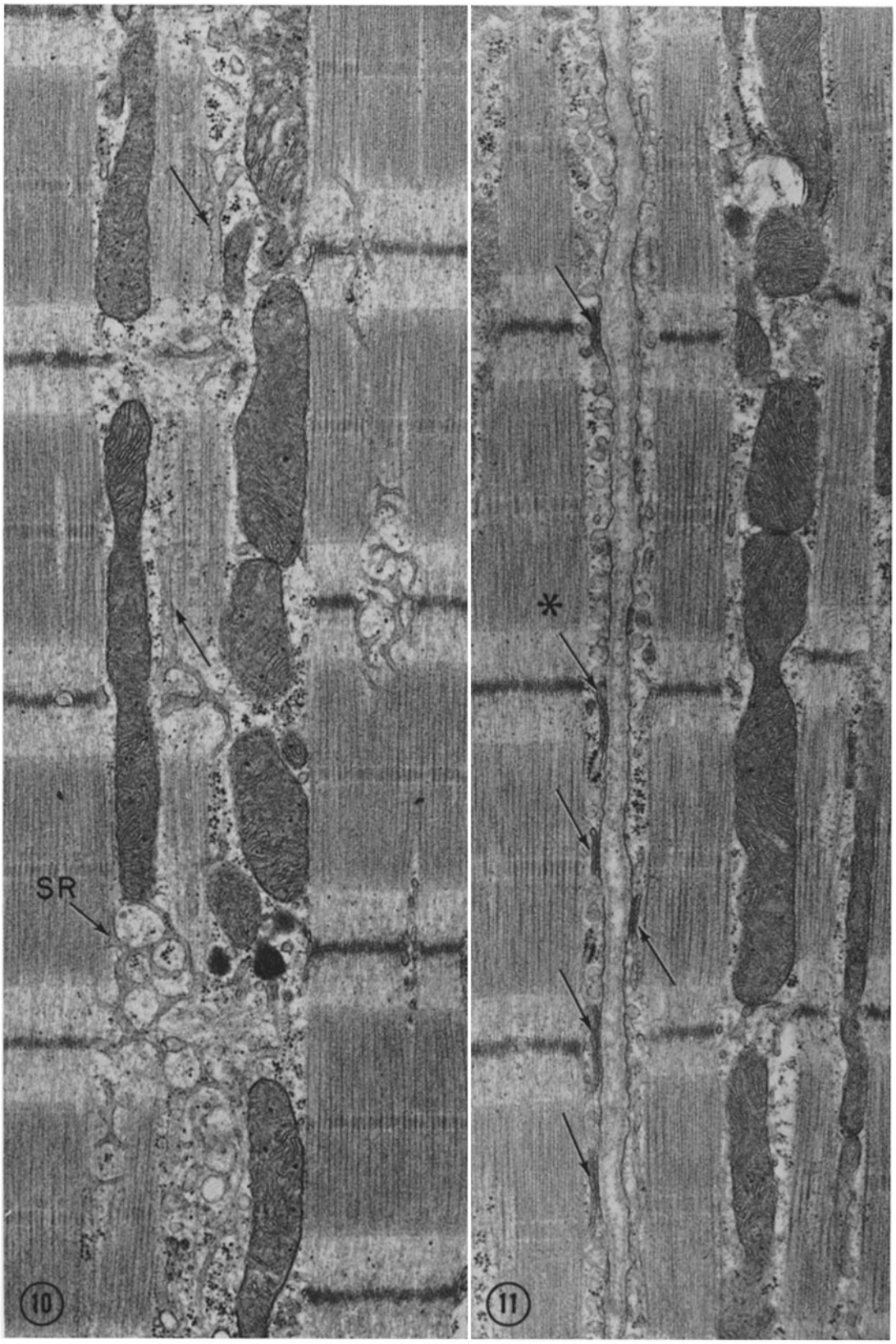
Whether on the ends or on the sides of the cell, much of the membrane involved in the junctions is covered on its cytoplasmic surface by a densely staining mat or meshwork of fibrils. In the transverse portions of the intercalated disc, the actin filaments insert directly into this subsarcolemmal feltwork of fine filaments. In the longitudinal portions of the disc, however, the dense matrix of the fibrillar mat of fasciae adherentes often is continuous with the dense material of the Z lines (Fig. 7). This feature was first reported by Fawcett and Selby (21) for the turtle atrium. In the mammal, too, this continuity between Z lines and fasciae adherentes appears more common in the atrium than in the ventricle. Occasionally, a dense subsarcolemmal mat of fine fibrils is seen in continuity with the dense material of Z lines at sites not associated with a specialized junctional region. These accumulations of dense material appear to be sites of lateral bonding of the sarcolemma to the underlying myofilament bundle. They are largely responsible for the scalloped appearance of the sarcolemma in contracted atrial muscle.

DISCUSSION

Two anatomical differences between cat atrial and ventricular contractile cells are emphasized. Atrial cells tend to have a smaller average diameter and to have very few T tubules compared to ventricular

FIGURE 10 Micrograph showing tubules of the sarcoplasmic reticulum (*SR*) and their relations to the cross-banding pattern. The reticulum is a loose meshwork of tubules and has no local specialization in relation to any of the bands. A frequent site of transverse orientation of elements of the reticulum coincides with the Z line but the tubules are short and of the usual diameter. No so called Z tubules are identifiable. Occasional rather straight longitudinal tubules are also present (see arrows). × 25,000.

FIGURE 11 Micrograph illustrating subsarcolemmal cisternae of the reticulum. Six specialized flattened saccules of the reticulum (see arrows) may be seen in this field abutting against the inner surface of the sarcolemma at the periphery of the cell. These cisternae are random in their distribution with respect to the cross-banded pattern of the myofilaments. They appear more numerous at the periphery of atrial cells than in ventricular cells since atrial cells have very few T tubules. Ribosomes are frequently attached to the membrane of the reticulum near these cisternae (see asterisk). × 27,000.


SR
10
*
11

cells. Both structural features result in a difference in the total membrane surface area and in the degree to which the extracellular space interdigitates with the intracellular space. These two differences might be interpreted as supporting the statement of Girardier (28) that there appears to be a critical size of fiber below which no T tubules or sarcoplasmic reticulum are found. However, on further consideration, the situation clearly is more complex because T tubules may be absent in an atrial fiber of the same diameter as a ventricular fiber that possesses them. Moreover, small brances of ventricular cells often contain T tubules (20). From these observations it seems that when fibers from different chambers are compared, the presence or absence of T tubules cannot be predicted from the cell diameter alone. Thus the abundant T tubules in ventricular muscle and the few in atrial muscle are conditions that do not appear to be imposed solely by the requirement for maintenance of a particular surface-to-volume ratio, but represent one of several inherent differences in the cells of the two chambers.

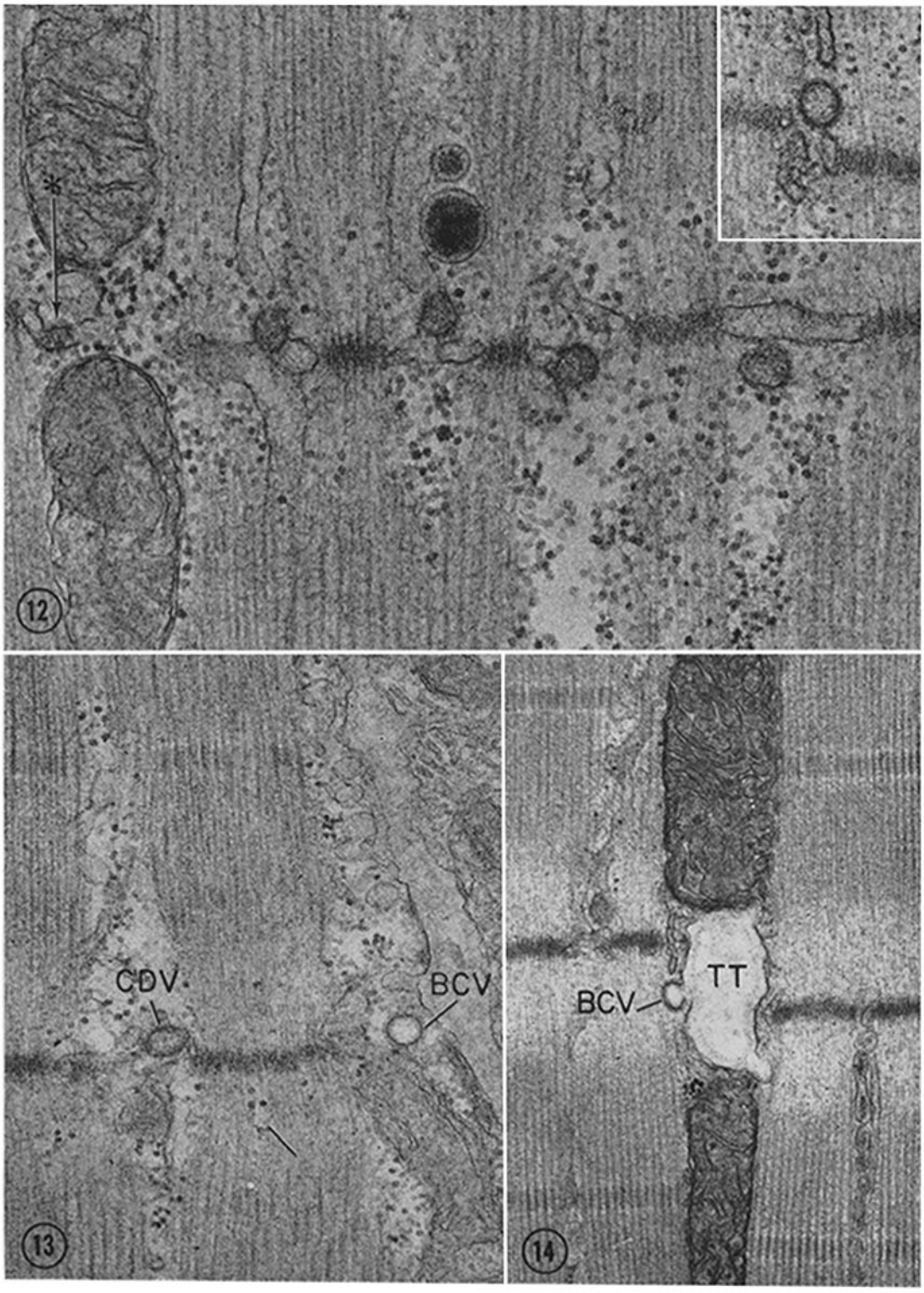
Much attention has been focused on the biochemical mechanisms by which the membrane depolarization wave sets off a series of biochemical events leading to activation of the sliding filaments. The very elegant correlative physiological and ultrastructural work on skeletal muscle implicates the T tubule in carrying the membrane depolarization from the cell periphery to the myofilaments in the cell interior (25, 27, 35, 57, 58, 59). The membrane depolarization of the transverse tubules is presumed to cause release of ionic calcium into the myofilaments, and this calcium in turn activates the sliding mechanism of contraction (9, 50, 54, 82, 84, 85, 86). The calculations of A. V. Hill (31) stress the importance of stored calcium for rapid contractility of skeletal muscle fibers, which are usually quite large in diameter compared to cardiac muscle fibers.

It is probable that the general outlines of excitation-contraction coupling are the same for skeletal and cardiac muscle, but some details of the mechanisms may differ (80). For example, the marked dependence of cardiac muscle on extracellular calcium ion concentration stands in striking contrast to the ability of skeletal muscle to maintain contractility over a wide range of extracellular calcium ion concentrations (12, 74). The rapidity with which fluctuations in the calcium ion concentration affect the absolute force of cardiac muscle contraction and the rate of development of this force suggests that the pool of calcium utilized is in rapid equilibrium with the extracellular space (54, 83). Weidmann conducted an experiment on a reptilian heart which suggested that the calcium utilized for each contraction is probably obtained from the extracellular space itself (83). Electron microscopic studies on reptilian hearts have shown an absence of T tubules (21, 47). The reptilian hearts studied by these investigators are also composed of very small cells with estimated diameters of approximately 3 μ for the turtle atrium (21) and of 5–6 μ for the snake ventricle (47). The application of Hill's calculations to heart muscle by Nelson and Benson (53) indicated that calcium storage may not be necessary for a cylindrical mammalian car-

FIGURE 12 Six examples of the coated dense vesicles associated with the reticulum at the Z lines illustrating the variety of their shapes. On the far left, a short tubule has a focal dense content and a coated membrane (see asterisk). The three central vesicles may either represent fusiform swellings of the reticulum in transverse section or separate vesicles. On the far right, a coated dense vesicle is seen adjacent to a sarcotubule. The inset at upper right shows the usual appearance of a vesicle near the Z line and apparently not in continuity with other membranes. The granular luminal content and 150 A coat on the outer surface of the membrane can be seen. Such a variety of forms suggests a dynamic process involving these vesicles and the reticulum. × 67,000.

FIGURE 13 A micrograph allowing a direct comparison of a coated dense vesicle (*CDV*) with a bristle-coated vesicle (*BCV*). The coated dense vesicle has a content of appreciable density while the nearby bristle-coated vesicle is clearly devoid of stainable contents. The interrelationship of these two types of coated vesicles is not clear. See also Fig. 9. × 27,000.

FIGURE 14 A micrograph illustrating one of the very rare T tubules (*TT*) encountered in an atrial cell and the opening of a bristle-coated vesicle (*BCV*) onto its lumen. × 27,000.


12
CDV
BCV
13
BCV
TT
14

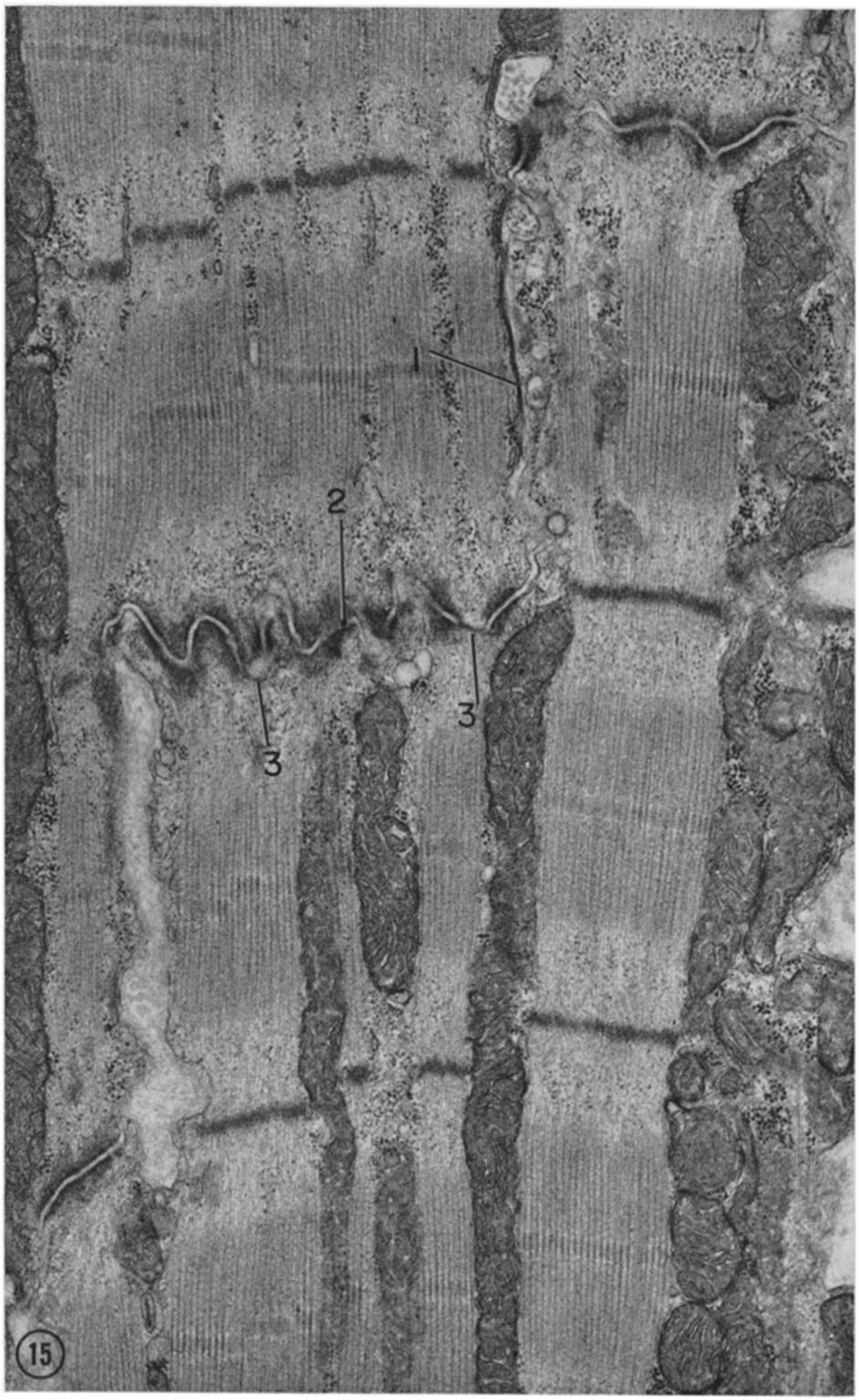


FIGURE 15 A longitudinal section of atrial muscle including a typical rectilinear intercalated disc consisting of interdigitated transverse and relatively straight longitudinal segments. A moderately extensive fascia occludens is shown on the longitudinal portion of the disc (*1*). For the most part, the transverse portion of the disc consists of junctions of the fascia adherens type (*2*) but two maculae occludentes (*3*) are also present. × 23,000.

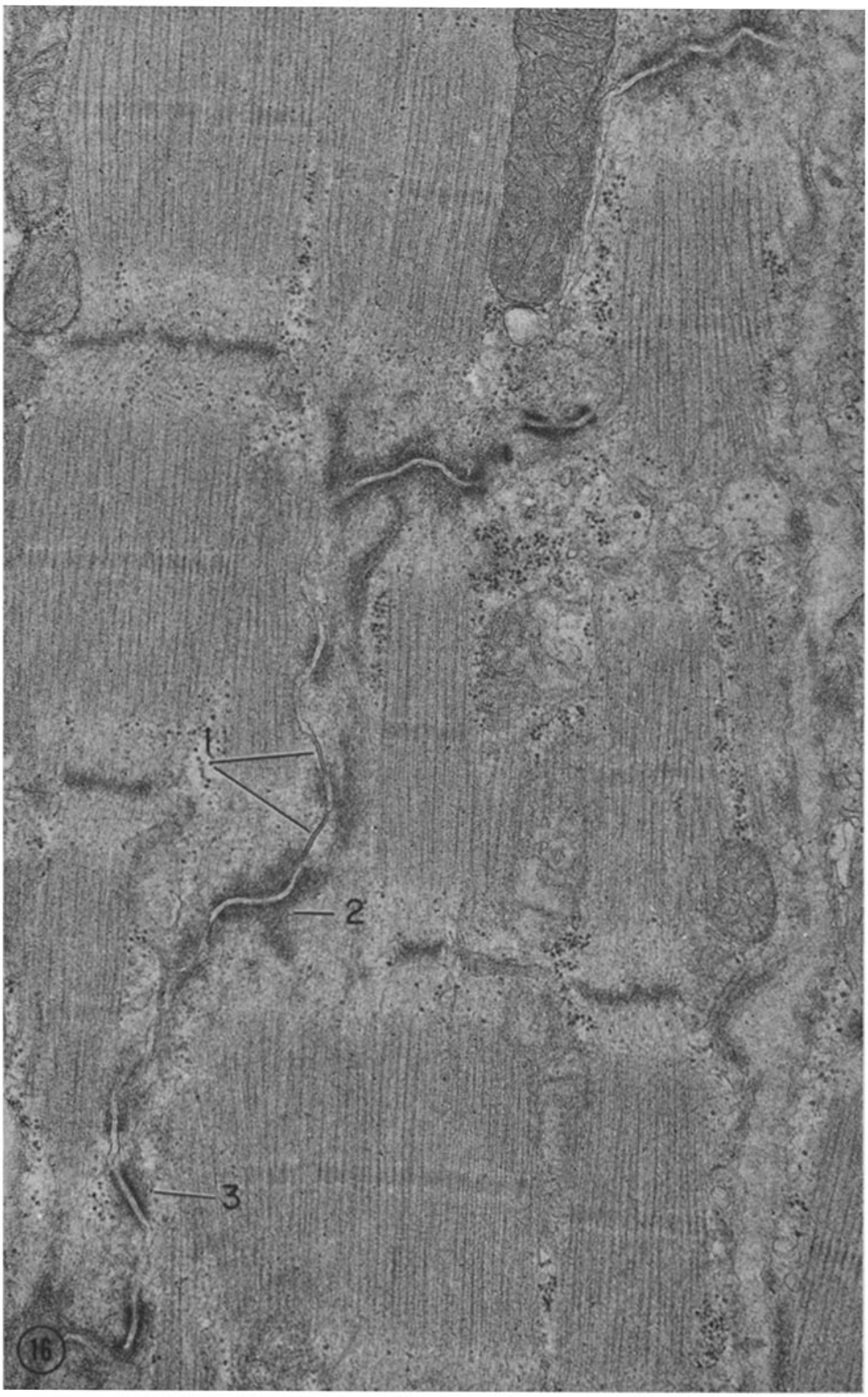


FIGURE 16 Micrograph of one of the oblique intercalated discs which are more frequent in the atrium than in the ventricle. The fasciae adherentes (*2*) are less extensive, but the fasciae occludentes (*1*) and maculae adherentes (*3*) appear similar to those in the rectilinear disc illustrated in Fig. 15. × 30,000.

diac muscle cell with a diameter as great as 20 μ. Thus it seems evident for heart muscle that diffusion may be an adequate mechanism for the calcium flux from the extracellular space to the myofilaments during activation. The above discussion only considers the cardiac cell as a cylinder and does not take into account either branching of the cell or the presence of T tubules in ventricular cells and their absence in atrial cells. If there is time enough for calcium to diffuse over the small distance from the extracellular space into the myofilaments during activation of the atrium, then there seems no reason to believe that the presence or absence of a T system would have any physiological significance in relation to calcium diffusion for a cell as small as the atrial cell.

Several physiologists (48, 62, 72) have reported a faster rate of conduction of the action potential across the surface of the atrium than across the "working" ventricular myocardium. Although total membrane surface would be one factor in conduction rate, one must evaluate all of the anatomical and biochemical factors which affect the rate of conduction in order to determine which factors are simply permissive and which are truly rate limiting. Possible intrinsic membrane differences must be taken into consideration. In addition to the cross-sectional areas of the cells, the internal resistances of the cytoplasm to ion flow would have to be compared. Only if all of these factors were determined could the differences in the total area of membrane surface really be evaluated with respect to their effects on conduction rate.

Physiological measurement of conduction rates in cardiac muscle presents two difficulties which are not a serious problem with skeletal muscle or nerve fibers. Since single cardiac muscle fibers are not easily studied because of their small size and complex relations, physiological measurements are usually made on populations of cells. Therefore, the efficiency of cell-to-cell electrical coupling, as well as the question of uniformity in rate of conduction of an action potential throughout the population of cells studied, become extremely important considerations. Although this study is not adequate to establish the cause of rapid conduction in the atrium, it does add two morphological factors that might well influence the conduction rate of the action potential across the surface of the atrial cell. In cardiac muscle of similar cytoplasmic compositions but varying cross-sectional areas, a large cell would be expected to conduct more rapidly than a small cell (33). Therefore, the small cross-sectional area of the atrial cell would tend to slow conduction of the action potential. The effect of T tubules on the rate of conduction of the action potential across the peripheral cell surface can be better understood if one considers two cells with the same cross-sectional area, one a ventricular cell with T tubules and the other an atrial cell lacking T tubules. In the ventricular cell, the T tubules would be expected to add to membrane capacitance as they have been shown to do in skeletal muscle (13, 14, 25, 57). This increase in total membrane capacitance would tend to slow conduction by adding to the area of membrane which must be depolarized before an action potential would be propagated across the fiber surface (33). Therefore, the absence of T tubules in the atrial cell might be expected to speed conduction of the action potential because there would be less membrane to depolarize. Other factors permitting, a wave would, therefore, tend to move faster across the surface of the atrium. It is tempting to speculate that the absence of T tubules is important in speeding conduction over the surface of the atrium where conduction otherwise might be quite slow owing to the small cell diameter.

The absence of T tubules in certain trabeculae carnae has been discussed by Johnson and Sommer as causing the conduction rate in the strands to be faster than in ordinary ventricular muscle (39, 73). While this speculation seems reasonable, they designate these muscle fibers lacking T tubules as Purkinje fibers or "P-fibers." This designation ignores the large cell diameter, paucity of myofilaments, small mitochondrial size, abundance of glycogen, and other defining characteristics of the classical Purkinje fibers, as exemplified in the fibers of the atrioventricular bundle. Several of these additional specializations, which are not present in the strands described by Johnson and Sommer, may also contribute to the rapid conduction rates of Purkinje fibers recorded in the literature (83). Certainly the absence of T tubules does not, in itself, define either "Purkinje fibers" or a rapid conduction system, since T tubules are reported here to be absent from the cat atrium and they have previously been reported to be lacking in frog atrium (3), frog ventricle (76), turtle atrium (21), snake ventricle (47), and some cells of the dog atrium (73).

Owing to the great quantitative difference in the abundance of T tubules between cat atrial and ventricular papillary muscle, the kitten heart will continue to provide physiologists with favorable preparations for testing hypotheses concerning the influence of the T tubules on their measurements. The differences may prove to be less in other species (7) and may require more quantitative electron microscopic techniques for their demonstration than have been used in this study.

This study was supported by research grant GM-06729 and training grant G-406 from the Institute of General Medical Sciences, United States Public Health Service.

The authors wish to acknowledge their indebtedness to Dr. John R. Blinks and Dr. Jean P. Revel for their advice, assistance, and criticism throughout this study. The assistance of Miss Helen Deacon in typing the manuscript is gratefully acknowledged.

Received for publication 26 July 1968, and in revised form 20 January 1969.

REFERENCES

1. ANDERSON, E. 1967. A study of the specialization of the oolemma during oocyte differentiation in the roach *Periplaneta americana*. *J. Cell Biol.* **35**:3A. (Abstr.)
2. ANDERSON, E. 1967. Observations on the uptake of horseradish peroxidase by developing oocytes of the rabbit. *J. Cell Biol.* **35**:160A. (Abstr.)
3. BARR, L., M. M. DEWEY, and W. BERGER. 1965. Propagation of action potentials and the structure of the nexus in cardiac muscle. *J. Gen. Physiol.* **48**:797.
4. BENNETT, H. S. 1963. Morphological aspects of extracellular polysaccharides. *J. Histochem. Cytochem.* **11**:14.
5. BLINKS, J. R. 1965. Convenient apparatus for recording contractions of isolated heart muscle. *J. Appl. Physiol.* **20**:755.
6. BRAUNWALD, E., J. ROSS, JR., and E. H. SONNENBLICK. 1967. Mechanisms of contraction of the normal and failing heart. *New Engl. J. Med.* **277**:794, 853, 910, 962, 1012.
7. BURDETTE, W. J., and T. P. ASHFORD. 1965. Structural changes in the human myocardium following hypoxia. *J. Thorac. Cardiovasc. Surg.* **50**:210.
8. CARNEY, J. A., and A. L. BROWN. 1966. An electron microscopic study of canine cardiac myosin and some of its aggregates. *J. Cell Biol.* **28**:375.
9. CONSTANTIN, L. L., and R. J. PODOLSKY. 1965. Calcium localization and the activation of striated muscle fibers. *Fed. Proc.* **24**:1141.
10. DALLNER, G., P. SIEKEVITZ, and G. E. PALADE. 1966. Biogenesis of endoplasmic reticulum membranes. I. Structural and chemical differentiation in the developing rat hepatocyte. *J. Cell Biol.* **30**:73.
11. EBASHI, S., and F. LIPMANN. 1962. Adenosinetriphosphate linked concentration of calcium ions in a particulate fraction of rabbit muscle. *J. Cell Biol.* **14**:349.
12. EDWARDS, C., J. M. RITCHIE, and D. R. WILKIE. 1956. The effect of some cations on the active state of muscle. *J. Physiol. (London).* **133**:412.
13. EISENBERG, R. S., and P. W. GAGE. 1967. Frog skeletal muscle fibers: changes in electrical properties after disruption of transverse tubular system. *Science.* **158**:1700.
14. FALK, G., and P. FATT. 1964. Linear properties of striated muscle fibers observed with intracellular electrodes. *Proc. Roy. Soc. London, Ser. B.* **160**:69.
15. FARQUHAR, M. G., and G. E. PALADE. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* **17**:375.
16. FARQUHAR, M. G., and G. E. PALADE. 1965. Cell junctions in amphibian skin. *J. Cell Biol.* **26**:263.
17. FAWCETT, D. W. 1961. The sarcoplasmic reticulum of skeletal and cardiac muscle. *Circulation.* **24**:336.
18. FAWCETT, D. W. 1966. An Atlas of Fine Structure. The Cell. Its Organelles and Inclusions. W. B. Saunders Co., Philadelphia.
19. FAWCETT, D. W. 1967. The sporadic occurrence in cardiac muscle of anomalous Z bands exhibiting a periodic structure suggestive of tropomyosin. *J. Cell Biol.* **36**:266.
20. FAWCETT, D. W., and N. S. MCNUTT. 1969. The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. *J. Cell Biol.* **41**:1.
21. FAWCETT, D. W., and C. C. SELBY. 1958. Observations on the fine structure of the turtle atrium. *J. Biophys. Biochem. Cytol.* **4**:63.
22. FLICKINGER, C., and D. W. FAWCETT. 1967. The junctional specializations of Sertoli cells in the seminiferous epithelium. *Anat. Rec.* **158**:207.
23. FRANK, A. L., and A. K. CHRISTENSEN. 1968. Localization of acid phosphatase in lipofuscin granules and possible autophagic vacuoles in interstitial cells of the guinea pig testis. *J. Cell Biol.* **36**:1.
24. FRANZINI-ARMSTRONG, C., and K. R. PORTER.

1964. The Z disc in skeletal muscle fibrils. *Z. Zellforsch.* **61**:661.

25. FREYGANG, W. H. 1965. Tubular ionic movements. *Fed. Proc.* **24**:1135.
26. FRIEND, D. S., and M. G. FARQUHAR. 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* **35**:357.
27. GAGE, P. W., and R. S. EISENBERG. 1967. Action potentials without contraction in frog skeletal muscle fibers with disrupted transverse tubules. *Science.* **158**:1702.
28. GIRARDIER, L. 1965. The problem of the inward spread of excitation in skeletal and heart muscle cells. *In* Electrophysiology of the Heart. B. Taccardi and G. Marchetti, editors. Pergamon Press, Oxford. 53.
29. HANSON, J., and J. LOWY. 1963. The structure of F-actin and of actin filaments isolated from muscle. *J. Mol. Biol.* **6**:46.
30. HASSELBACH, W. 1964. Relaxation and the sarcotubular calcium pump. *Fed. Proc.* **23**:909.
31. HILL, A. V. 1949. The abrupt transition from rest to activity in muscle. *Proc. Roy. Soc. London, Ser. B.* **136**:399.
32. HODGE, A. J., H. E. HUXLEY, and D. SPIRO. 1954. Electron microscope studies on ultrathin sections of muscle. *J. Exp. Med.* **99**:201.
33. HODGKIN, A. L. 1964. The conduction of the nervous impulse. C. C. Thomas, Springfield, Illinois.
34. HOYLE, G. 1967. Diversity of striated muscle. *Amer. Zool.* **7**:435.
35. HUXLEY, A. F., and R. E. TAYLOR. 1958. Local activation of striated muscle fibers. *J. Physiol. (London).* **144**:426.
36. HUXLEY, H. E. 1957. The double array of filaments in cross-striated muscle. *J. Biophys. Biochem. Cytol.* **3**:631.
37. HUXLEY, H. E. 1963. Electron microscopic studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **7**:281.
38. JAMIESON, J. D., and G. E. PALADE. 1964. Specific granules in atrial muscle cells. *J. Cell Biol.* **23**:151.
39. JOHNSON, E. A., and J. R. SOMMER. 1967. A strand of cardiac muscle. Its ultrastructure and the electrophysiological implications of its geometry. *J. Cell Biol.* **33**:103.
40. KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**: 137A (Abstr.)
41. KARRER, H. E. 1959. The striated musculature of blood vessels. I. General cell morphology *J. Biophys. Biochem. Cytol.* **6**:383.
42. KARRER, H. E. 1960. The striated musculature of blood vessels. II. Cell interconnections and cell surface. *J. Biophys. Biochem. Cytol.* **8**:135.
43. KELLY, D. W. 1967. Models of muscle Z-band fine structure based on a looping filament configuration. *J. Cell Biol.* **34**:827.
44. KISCH, B. 1963. A significant electron microscopic difference between the atria and the ventricle of the mammalian heart. *Exp. Med. Surg.* **21**:193.
45. KNAPPEIS, G. G., and F. CARLSEN. 1962. The ultrastructure of the Z disc in skeletal muscle *J. Cell Biol.* **13**:323.
46. KOCH-WESER, J., and J. R. BLINKS. 1963. The influence of the interval between beats on myocardial contractility. *Pharmacol. Rev.* **15**: 601.
47. LEAK, L. V. 1967. The ultrastructure of myofibers in a reptilian heart: the boa constrictor. *Amer. J. Anat.* **120**:553.
48. LEWIS, T., J. MECKINS, and P. D. WHITE. 1914. The excitatory process in the dog's heart. Part I. The auricles. *Phil. Trans. Roy. Soc. London, Ser. B.* **205**:375.
49. LINDNER, E. 1957. Die submikroskopische Morphologie des Herzmuskels. *Z. Zellforsch.* **45**:702.
50. LÜTTGAU, H. C. 1965. The role of calcium ions in excitation-contraction coupling. *In* Electrophysiology of the Heart. B. Taccardi and G. Marchett, editors, Pergamon Press, Oxford. 87.
51. MOORE, D. H., and H. RUSKA. 1957. Electron microscope study of mammalian cardiac muscle cells. *J. Biophys. Biochem. Cytol.* **3**:261.
52. MUIR, A. R. 1957. Electron microscope study of the embryology of the intercalated disc in the heart of a rabbit. *J. Biophys. Biochem. Cytol.* **3**:193.
53. NELSON, D., and E. S. BENSON. 1963. Structural continuities of the transverse tubular systems of rabbit and human myocardial cells. *J. Cell Biol.* **16**:297.
54. NIEDERGERKE, R. 1959. Calcium and the activation of contraction. *Experientia.* **15**:128.
55. PAGE, E. 1962. Cat heart muscle *in vitro*. III. The extracellular space. *J. Gen. Physiol.* **46**: 201.
56. PALADE, G. E. 1960. Transport in quanta across the endothelium of blood capillaries. *Anat. Rec.* **136**:254.
57. PEACHEY, L. D. 1965. The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. *J. Cell Biol.* **25**:209.
58. PEACHEY, L. D. 1965. Transverse tubules in excitation-contraction coupling. *Fed. Proc.* **24**: 1124.
59. PEACHEY, L. D. 1966. The role of transverse

tubules in excitation contraction coupling in striated muscle. *Ann. N. Y. Acad. Sci.* **137**:1025.

60. PEPE, F. A. 1967. The myosin filament. I. Structural organization from antibody staining observed in electron microscopy. *J. Mol. Biol.* **27**:203.
61. PORTER, K. E., and G. PALADE. 1957. Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. *J. Biophys. Biochem. Cytol.* **3**:269.
62. PUECH, P., M. ESCLAVISSAT, D. SODI-PALLARES, and F. CISNEROS. 1954. Normal auricular activation in the dog's heart. *Amer. Heart J.* **47**: 174.
63. RAMBOURG, A., and C. P. LEBLOND. 1967. Electron microscope observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. *J. Cell Biol.* **32**:27.
64. RAYNS, D. G., F. O. SIMPSON, and W. S. BERTAUD. 1967. Transverse tubule apertures in mammalian myocardial cells: surface array. *Science.* **156**:656.
65. REEDY, M. K. 1964. Discussion in *Proc. Roy Soc. London Ser. (Biol.)* **160**:458.
66. REVEL, J. P., L. NAPOLITANO, and D. W. FAWCETT. 1960. Identification of glycogen in electron micrographs of thin tissue sections. *J. Biophys. Biochem. Cytol.* **8**:575.
67. ROSENBLUTH, J. 1962. Subsurface cisterns and their relationship to the neuronal plasma membrane. *J. Cell Biol.* **13**:405.
68. ROSENBLUTH, J., and S. L. WISSIG. 1964. The distribution of exogenous ferritin in toad spinal ganglia and the mechanism of its uptake by neurons. *J. Cell Biol.* **23**:307.
69. ROTH, T. F., and K. R. PORTER. 1964. Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* L. *J. Cell Biol.* **20**:313.
70. SIMPSON, F. O., and S. J. OERTELIS. 1962. The fine structure of sheep myocardial cells; sarcolemmal invaginations and the transverse tubular system. *J. Cell Biol.* **12**:91.
71. SJÖSTRAND, F. S., E. ANDERSSON-CEDERGREN, and M. M. DEWEY. 1958. Ultrastructure of intercalated discs of frog, mouse and guinea pig cardiac muscle. *J. Ultrastruct. Res.* **1**:271.
72. SODI-PALLARES, D., and R. M. CALDER. 1956. The activation process of the human heart. *In* New Bases of Electrocardiography. C. V. Mosby Company, St. Louis. 358.
73. SOMMER, J. R., and E. A. JOHNSON. 1968. Cardiac muscle. A comparative study of Purkinje fibers and ventricular fibers. *J. Cell Biol.* **36**: 497.
74. SONNENBLICK, E. H. 1962. Force-velocity relations in mammalian heart muscle. *Amer. J. Physiol.* **202**:931.
75. SPIRO, D., and E. H. SONNENBLICK. 1964. Comparison of the ultrastructural basis of the contractile process in heart and skeletal muscle. *Circ. Res.* **15** (Suppl. 2):14.
76. STALEY, N., and E. S. BENSON. 1966. Some unique ultrastructural features of frog ventricular muscle. *J. Cell Biol.* **31**:112A. (Abstr.)
77. STENGER, R. J., and D. SPIRO. 1961. Ultrastructure of mammalian cardiac muscle. *J. Biophys. Biochem. Cytol.* **9**:325.
78. STENGER, R. J., and D. SPIRO. 1961. Structure of the cardiac muscle cell. *Amer. J. Med.* **30**: 653.
79. TRAUTWEIN, W., and K. UCHIZONO. 1963. Electron microscopic and electrophysiologic study of the pacemaker in the sino-atrial node of the rabbit heart. *Z. Zellforsch.* **61**:96.
80. VAN DER KLOOT, W. G., and B. DANE. 1964. Conduction of the action potential in the frog ventricle. *Science.* **146**:74.
81. VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407.
82. WEBER, A., R. HERZ, and I. REISS. 1964. The regulation of myofibrillar activity by calcium. *Proc. Roy. Soc. London Ser. B.* **160**:489.
83. WEIDMANN, S. 1952. The electrical constants of Purkinje fibers. *J. Physiol. (London).* **118**:348.
84. WINEGRAD, S. 1965. Autoradiographic studies of intracellular calcium in frog skeletal muscle. *J. Gen. Physiol.* **48**:455.
85. WINEGRAD, S. 1968. Intracellular calcium movements of frog skeletal muscle during recovery from tetanus. *J. Gen. Physiol.* **51**:65.
86. WINEGRAD, S., and A. M. SHANES. 1962. Calcium flux and contractility in guinea pig atria. *J. Gen. Physiol.* **45**:371.