LAMELLAR JUNCTIONAL SARCOPLASMIC RETICULUM

A Specialization of Cardiac Sarcoplasmic Reticulum

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This report deals with a description of an assembly of lamellar structures that have a morphology indistinguishable from that of cardiac junctional (1) and extended junctional (2) sarcoplasmic reticulum (SR). Briefly, junctional SR, a normal component of all myocardial cells, is that specialized portion of the SR which is periodically attached to sarcolemma by processes (junctional processes) in the I regions of the sarcomere to form peripheral and interior couplings (the former at surface sarcolemma, the latter at transverse tubules); extended junctional SR is morphologically similar to junctional SR except that it occurs throughout the interior of the myofiber with no immediate sarcolemmal contact. The latter exists in both continuous form (with direct extension from the junctional SR of couplings) and discontinuous form (with segments of free SR interspersed between it and the junctional SR of peripheral couplings) and is particularly prominent in the fast beating hearts of certain birds (2). Lamellar junctional SR represents stacks or multiples of junctional SR, which is an anatomically discrete single structure. The existence of similar profiles in mammalian atrial and Purkinje fibers has been mentioned briefly before (3). The present material, however, represents ventricular fibers and offers an opportunity to describe and illustrate the lamellar junctional SR in great detail. In as much as the lamellar junctional SR is indistinguishable in its component parts from the junctional and extended junctional SR of cardiac muscle, it seems appropriate to make the present observations known to a wider audience interested in the ultrastructure of striated muscle. In addition, the present findings are relevant when one

considers the identity of the 'lamellar inclusions' (4) or 'helical complexes' (5) that are found in denervated skeletal muscle and the nature of which is quite obscure.

MATERIALS AND METHODS

Our own observations are based on the electron microscope examination of eight lamellar arrays from cardiac fibers of two adult opossums. The hearts were perfused with cacodylate-buffered glutaraldehyde, postfixed with osmium tetroxide, contrasted with uranyl acetate in block, and processed conventionally from there. The cardiac tissue from the bat, hummingbird, and zebra finch was processed similarly and was added here for comparative reasons which seem essential to our arguments. The measurements are the average of at least 10 determinations from representative areas of several arrays (including the array shown in Fig. 1). The microscope and enlarger were calibrated with a Fullam crossed lines grating replica (E. F. Fullam, Inc., Schenectady, New York) containing 54,864 lines per inch, and care was taken to eliminate magnetic hysteresis of the lenses before exposing the photographic plate.

RESULTS

The structures varied widely in overall dimensions but in general presented as rectangular lamellar arrays of membranes (Figs. 1–3). Similar profiles, albeit occurring individually, are seen when the flat cisternae of mammalian cardiac junctional SR [terminal cisternae (6, 7)] are sectioned transversely (Figs. 4 and 5). While we do not have definitive information concerning the third dimension, in one instance an array could be followed through four consecutive thin sections (a distance of approximately 2,400 Å). Appreciable depth of the flat sacs would explain why so much of each array remained visible within the plane of each section (Figs. 1–3). No predilection for a particular

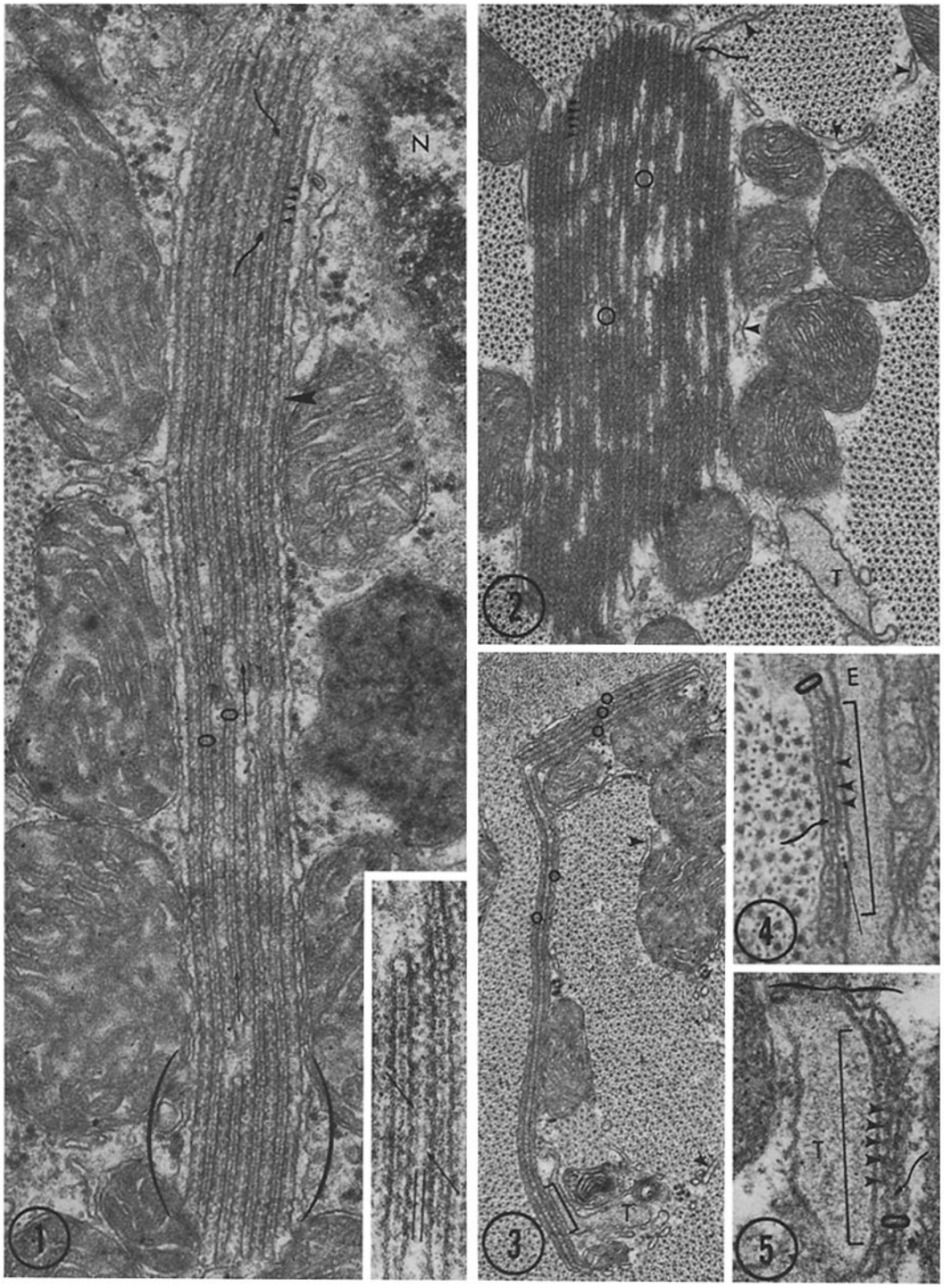
FIGURE 1 Opossum, left ventricular free wall. A large array of lamellar junctional SR (flat ring circumscribes one lamella) composed of tiers of flattened cisternae or lamellae, each of which is indistinguishable from the junctional SR normally occurring in cardiac muscle (cf. Figs. 4–7), as well as from the extended junctional SR normally occurring in bird hearts (Figs. 6, 8). The junctional processes (small arrow heads) are mostly in register with processes from adjacent lamellae (curved brackets) but less so from one side of a lamella to the other, and when almost touching, the junctional processes form circular profiles between adjacent ones that contain an electron-dense spot in the center (between curved arrows). The junctional processes appear, by and large, to be square and have an approximate spacing of 320 Å center-to-center. The free ends in the interlamellar gap have thin lateral linear extensions that may or may not make visible contact with similar extensions from an adjacent process (inset). The junctional granules form rather continuous central membranes bisecting the lamellae (thin arrows) except in the most peripheral lamellae where the presence of such a central membrane (large arrow head) is variable. These peripheral lamellae also do not carry junctional processes on their outer lamina facing away from the array's interior. Nucleus, *N*. × 58,500 (approx.). Inset: the locations of linear lateral extensions of junctional processes (arrows) are emphasized by parallel black lines. × 107,000 (approx.).

FIGURE 2 Opossum, left ventricular papillary muscle. Lamellar junctional SR. The array shows tiers of flattened cisternae or lamellae (circles). Junctional processes (small arrow heads) occurring in register with each other emphasize scalloping of the subjacent membrane. Central membranes (junctional granules) are present. One lamella (curved arrow) is continuous with a tubule of free SR (large arrow heads). Transverse tubule, *T*. × 32,250 (approx.).

FIGURE 3 Opossum, left ventricular free wall. A low-power view of another array of lamellar junctional SR in which one of two lamellae (circles) forms a coupling (bracket) with a transverse tubule (*T*). The circled lamellae are identical to those shown in more detail in Fig. 1. Small arrow heads point to tubules of the free SR. × 29,100 (approx.).

FIGURE 4 Opossum, left ventricular free wall. Peripheral coupling (bracket) with junctional SR (flat ring), junctional processes (small arrow heads), and junctional granules (central membrane, curved arrow). Two small dotlike densities, presumably actin filaments, are seen in the junctional gap between junctional SR and sarcolemma (thin arrow). Extracellular space, *E*. × 68,250 (approx.).

FIGURE 5 Bat, left ventricular free wall. An interior coupling (large bracket) forming a 'dyad' (flared bracket) with a transverse tubule (*T*). Junctional SR (flat ring), junctional granules (curved arrow), and junctional processes (small arrow heads). × 91,000 (approx.).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area of the cell could be established, one array occurring in the perinuclear area and the others in various areas between this central location and the periphery of the cell.

Each lamella of the array was approximately 260–270 Å in width and was bounded by a limiting membrane of "unit membrane" configuration. Continuity between individual lamellae was not seen but cannot be ruled out. The center of most of the lamellae was bisected by an electron-dense line (Figs. 1–3) corresponding to the central membrane [or junctional granules (8)] of cardiac junctional and extended junctional SR (cf. Figs. 1–3 with Figs. 4–8). The presence of such a membrane in the most peripheral lamellae of the larger arrays was variable (cf. Figs. 1 and 2).

The interlamellar gap varied between 310 and 350 Å. Bilateral rows of conical to square processes (junctional processes) with a 320–330-Å periodicity extended from the limiting membrane of each lamella towards the processes of adjacent lamellae within the array. The limiting membrane of the most peripheral lamellae facing the cytoplasm was essentially without junctional processes (Fig. 1). Such processes are similar to the junctional processes seen in the junctional SR of bird and mammalian hearts (Figs. 4–7) and in the extended junctional SR of bird hearts (Figs. 6, 8). Like the processes or SR feet (9) in the interior (9, 10) and peripheral (11) couplings of skeletal muscle, the projections ocasionally revealed thin lateral extensions forming an electron-dense line (Fig. 1). In the present instance as opposed to the findings in skeletal muscle (10), there were two such lines reflecting the double row of opposing junctional processes. Analogous to the morphology of junctional SR of skeletal muscle (9), the membranes of the lamellae were scalloped in register with their processes (Fig. 1). In one instance, a bilamellar extension of an array appeared to form a coupling with a transverse tubule (Fig. 3), and at least one lamella was continuous with a tubule of free SR (Fig. 2).

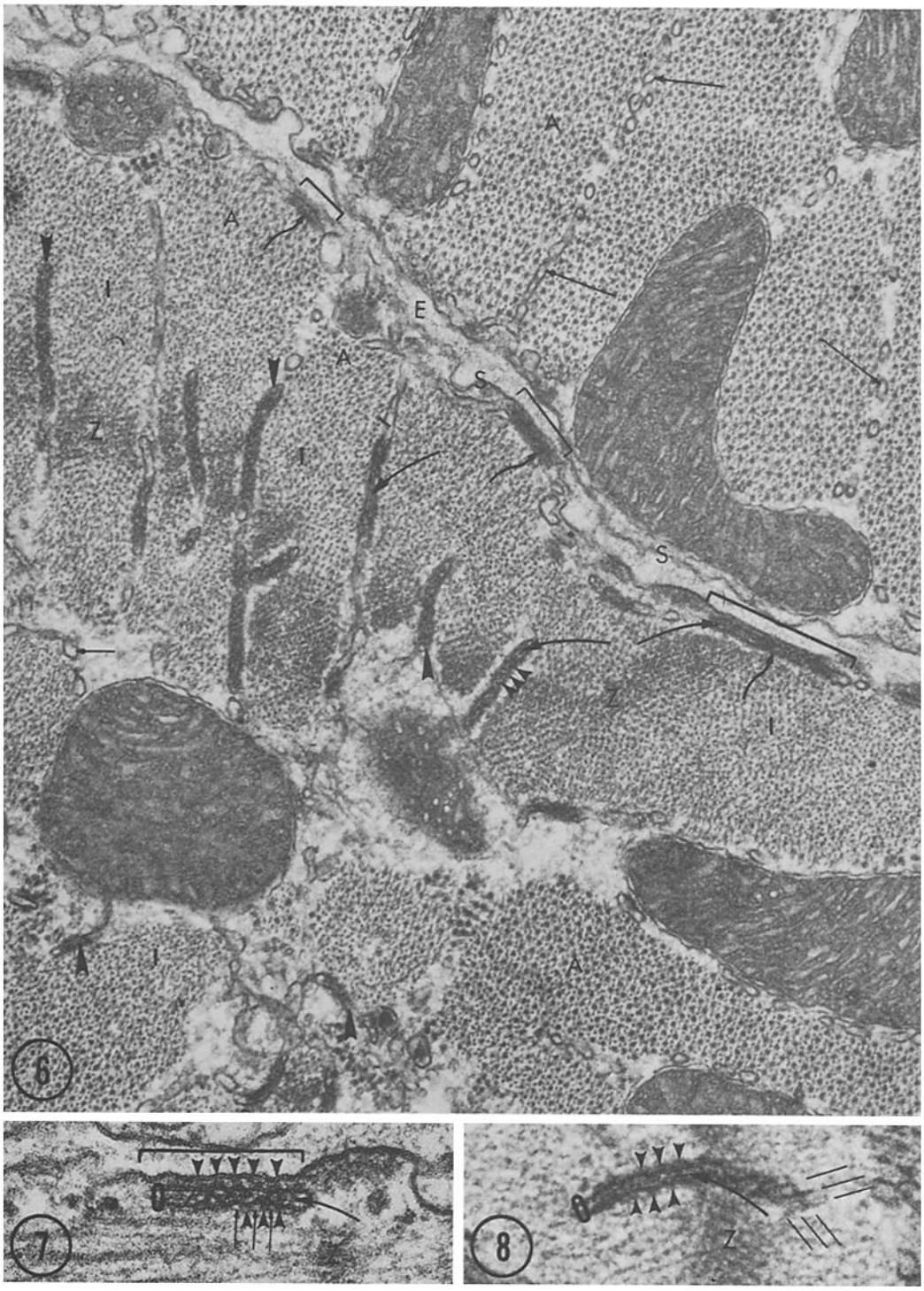
DISCUSSION

Anatomically, the lamellar structures are identical to cardiac junctional (1) and extended junctional SR (2). That congruency is made complete by the observation that at least one of the lamellar profiles is continuous with free SR (Fig. 2). At the moment, and for want of a better explanation, we look at the lamellar junctional SR as a form of hyperplasia or excessive growth of junctional SR (12). Its very occurrence suggests that junctional SR can be duplicated independent from the free SR tubules without losing its intercalated position within that network, thus providing additional evidence that junctional SR is a specialization, *sui generis*, of the SR. Although the individual lamellar structures are closely apposed, the conical

FIGURE 6 Hummingbird, left ventricular free wall. Junctional SR (small curved arrows) forms several peripheral couplings (brackets) with the surface sarcolemma (*S*) and also exists deep in the interior of the fiber as extended junctional SR (large arrow heads) at the Z–I region (*Z*, *I*). Junctional granules, mostly in the form of a quasi membrane (central membrane, large curved arrows), are prominent in both junctional and extended junctional SR and are shown in greater detail in Figs. 7 and 8. Extended junctional SR carries junctional processes (small arrow heads). Tubules of free SR (straight arrows) at A bands (*A*). Extracellular space (*E*). × 42,000 (approx.).

FIGURE 7 Zebra finch, left ventricular free wall. A peripheral coupling (bracket) is composed of junctional SR (flat ring), junctional granules forming a quasi central membrane (curved arrow), junctional processes (small arrow heads), and peripheral sarcolemma. The symmetrical disposition of junctional processes on either side of the junctional SR is especially prominent in birds with very fast heart rates, while in other animals the junctional processes are usually seen prominently on the sarcolemmal side of the junctional SR; in skeletal muscle of higher animals they are only on that side. The thin arrows show the linear lateral extensions of the junctional processes. × 87,450 (approx.).

FIGURE 8 Hummingbird, left ventricular free wall. A portion of extended junctional SR (flat ring) showing the central membrane (curved arrow) and junctional processes (small arrow heads). The thin black lines indicate the pattern in which the junctional processes and their lateral linear extensions are disposed. It is a square lattice the skew of which may account for the quasi helical pattern that sometimes is seen. (cf. Fig. 2). × 85,500 (approx.).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junctional processes are fully differentiated as are the corresponding dimples on the luminal side of the flattened cisternae. This arrangement detracts somewhat from the notion that conical images of the junctional processes are created, at least in part, by pull exerted at points of attachment (as for example to sarcolemma at the couplings), and it adds further doubt to the idea that their presence is solely a function of sarcolemmal contact (2). Finally, the lamellar junctional SR brings into full view the symmetrical disposition of the membranes forming the junctional and extended junctional SR of cardiac muscle. Such symmetry is seen particularly well in fast beating bird hearts (Figs. 6–8) where it occurs in the absence of immediate sarcolemmal contact (i.e., on the cytoplasmic surface of the peripheral couplings and on both surfaces of the extended junctional SR). By way of contrast, in the junctional SR (terminal cisternae) of skeletal muscle, junctional processes are ordinarily seen only on the surface facing the sarcolemma, while the cytoplasmic surface lacks them. Apparent exceptions occur with pentads and higher multiples of interior couplings, but in such instances the symmetry develops in the presence of adjacent sarcolemmal contact (i.e., with transverse tubules). This is an important observation, for if one attaches functional significance, as one should, to such discrete structural differentiations of the membranes of the junctional SR (i.e., presence of junctional processes vs. none), then the ratio between specialized surface area to volume of the junctional SR of cardiac vs. skeletal muscle will be greatly affected. In this we may recognize a fundamental difference between the junctional SR of cardiac muscle and that of skeletal muscle (2).

In comparing these specializations of cardiac junctional SR to the tubular aggregates reported in skeletal muslce biopsies from patients with a variety of disease processes (e.g., 13), there is no morphologic similarity. On the other hand, while some differences exist, there is convincing similarity between the lamellar junctional SR and the 'lamellar inclusions' (4) or 'helical complexes' (5) observed in denervated skeletal muscle. Notable differences concerning the latter include the absence of a discrete central membrane, greater width of the individual lamellae and, significantly, in contrast to the lamellar junctional SR, the junctional processes of adjacent lamellae produce a chessboard effect of considerable regularity; that is to say, the opposing processes are not in register with each other. Notwithstanding such differences, a striking similarity remains with respect to the processes proper and their lateral continuous and discontinuous linear extensions (compare Fig. 1 with reference 4; Figs. 7 and 8). Thus, there is no apparent morphologic difference in the junctional processes of either the lamellar junctional SR, the 'lamellar inclusions' (4), the 'helical complexes' (5), or the SR feet of skeletal muscle (9, 10). For the moment, therefore, it may be stimulating to consider all these structures potential homologues, an especially intriguing idea since it would add another sophisticated structural differentiation that cardiac and skeletal muscle share.

Whether lamellar junctional SR is an infrequent but normally occurring component of the myocardial cell with a specific function, or represents an abnormality in development of junctional SR, we cannot say.

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