CHICKEN CARDIAC MUSCLE

Its Elusive Extended Junctional Sarcoplasmic Reticulum and Sarcoplasmic Reticulum Fenestrations

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Selective comparative investigations into the ultrastructure of cardiac muscle have yielded much information regarding the existence in cardiac muscle of a number of discrete subcellular components that are common to the hearts of most, if not all, of a variety of different vertebrates.

Within the limits of the subject matter of our investigation, it has been our experience that in accordance with the old adage *natura non saltat*, species differences of the anatomy of cardiac muscle are more the result of quantitative modification of existing structural components than jumps of innovative differentiation.

We were puzzled, therefore, when we discovered in the hearts of certain birds what at first seemed to be a unique structural differentiation of the sarcoplasmic reticulum (SR) (1), and which we had not noticed earlier in chicken cardiac muscle (2). In chicken cardiac muscle we had, nevertheless, described the presence of electron-opaque material of varying appearance inside short segments of tubular or circular membranous profiles and, although no further distinction between different forms of that membrane-enclosed electron-opaque material was attempted, we did point out at that time that tubules of SR containing electron-opaque material were quite frequently situated near Z lines. In the course of a subsequent analysis of extended junctional sarcoplasmic reticulum (EJSR) in the hearts of the finch and hummingbird (1) we were reminded of these earlier observations in the chicken, and we decided to take another look at chicken cardiac muscle in the light of our newly won knowledge.

The intent of the present communication is to show the existence, albeit rudimentary, of EJSR in chicken cardiac muscle. The argument rests on the presentation of comparative anatomical evidence showing that certain morphologic components of chicken cardiac muscle cells have the same ultrastructure as the EJSR in other birds, differing from the latter only quantitatively. In addition, we found that the SR of chicken cardiac muscle seems to have fenestrations not unlike those occurring in skeletal muscle.

MATERIALS, METHODS, AND TERMINOLOGY

Young and adult chickens were anesthesized with ether, the still beating hearts were removed, immersed in glutaraldehyde, and small pieces cut from these hearts were processed for electron microscopy as previously described (1). Sections were viewed with the JEM 100-B or the AEI EM-6B electron microscopes. All magnifications are approximations within the limits of routinely calibrated electron microscopes and routine photographic processing. The illustration of Fig. 1, included here for purposes of comparison, was obtained from material (hummingbird) processed in connection with a previous study (1).

The term EJSR was first used to describe certain morphologic specializations of SR in the hearts of birds that have a very fast heart rate (1). The justification for this terminology, then as now, derives from the fact that these morphologic specializations of SR are (*a*) indistinguishable from the junctional SR of couplings (peripheral and interior), except for the lack of sarcolemmal contact, and that, (*b*) they sometimes appear as direct extensions (continuous form) of junctional SR of the couplings extending into the interior of the cells in the Z-I region, although more commonly they may be separated from the junctional SR of couplings by segments of smooth SR (discontinuous form). It is the latter form which prevails in chicken cardiac muscle.

We use the term "junctional SR" to describe what is called terminal cisternae in skeletal muscle, and sometimes referred to as subsarcolemmal cisternae in cardiac muscle. It is joined with the sarcolemma via periodic densities, the junctional processes, and contains electron-opaque material, the junctional granules. The structural unit composed of junctional SR and sarcolemma, we call coupling in both skeletal and cardiac muscle. Peripheral couplings[1] are exclusively at the surface sarcolemma of the cells, interior couplings exclusively at transverse tubules.

RESULTS AND DISCUSSION

As in mammals (3), in the chicken the SR formed rather extensive networks surrounding the contractile material. In the SR at the center of the sarcomere (Fig. 2, open arrow), small circular profiles were seen that were identical in appearance to the fenestrations which have been described in a similar location in skeletal muscle (4, 5). Z tubules (6) were present. Transverse tubules were absent. Peripheral couplings were numerous and were usually located very close to the Z lines, one on either side. Membranous profiles containing electron-opaque material were common near the Z line and among them there was one group that contained electron-opaque material displaying a convoluted, sometimes cribriform pattern (Fig. 2, cf. Fig. 1), or a row of often connected electron-opaque dots (central membrane, 3) (Fig. 3, curved arrows). It was this group which was indistinguishable from EJSR of other birds (Fig. 1, cf. reference 1). The membranous envelopes con-

[1] We have recently found peripheral couplings in the pectoral muscle of the finch, a vertebrate skeletal muscle.

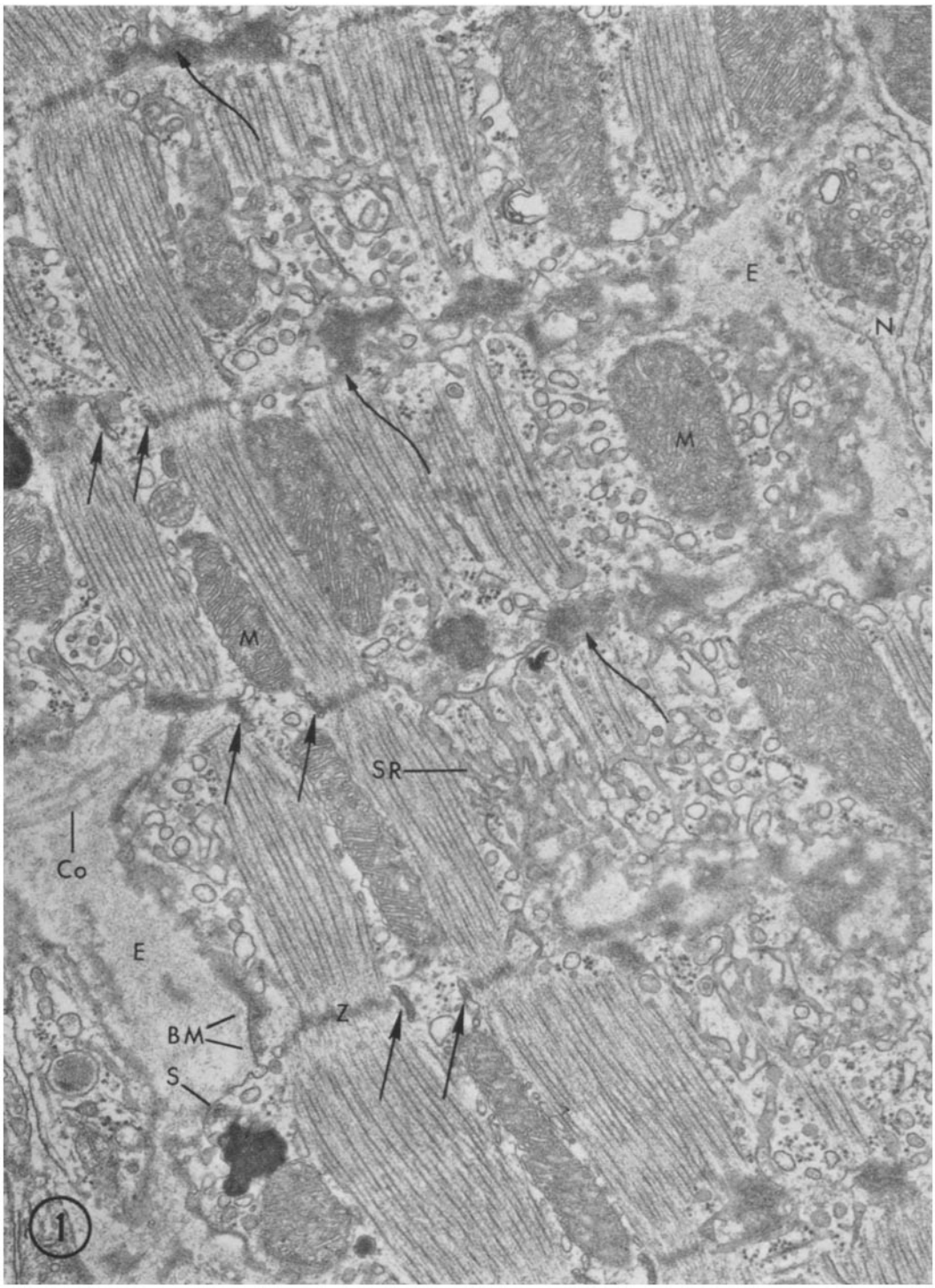
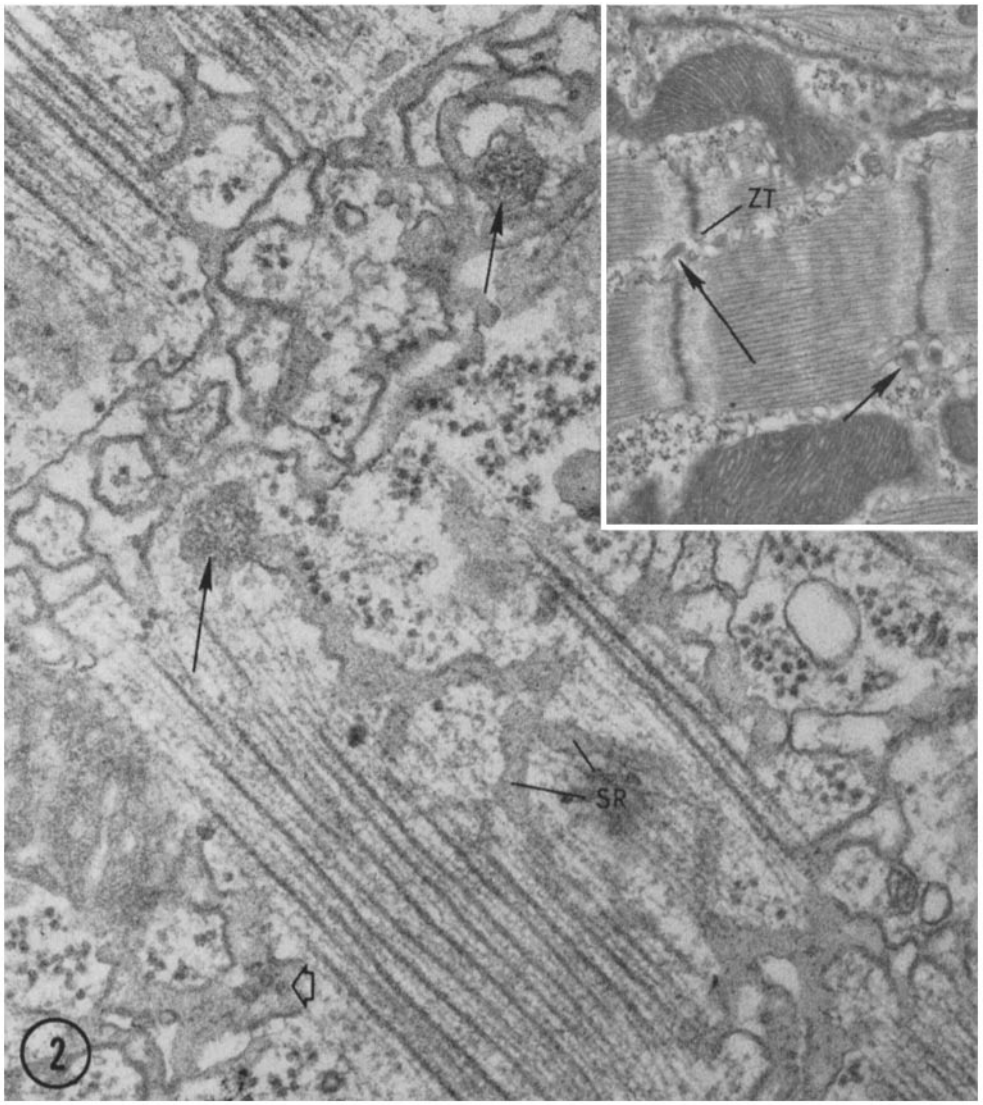


FIGURE 1 Longitudinal section of a mature hummingbird cardiac muscle fiber, showing the SR as a branching network of tubules. EJSR is seen in the interior of the fiber over the Z-I region. Depending on the plane of sectioning, the EJSR appears as elongated profiles (straight arrows) containing junctional granules, or as broad profiles (curved arrows) in which the electron-opaque material has a cribriform appearance. Note continuity between the EJSR and the free SR (*SR*). *BM*, basement membrane; *Co*, collagen; *E*, extracellular space; *M*, mitochondrion; *N*, nerve; *S*, sarcolemma; Z, Z line. × 24,000, approximately.


ZT
SR
2
MT
SR
3

taining the electron-opaque material, and which were continuous with the free, i.e., unspecialized, SR membranes (Figs. 2 and 3) formed polymorphous structures in most planes of sectioning displaying scalloped limiting membranes.

In other birds (Fig. 1) the EJSR, at least in one plane, usually presents as an elongated membranous profile having junctional processes and junctional granules (3). Identical oblong profiles were seen in the chicken, though rarely (Fig. 3, curved arrows). "Coated vesicles" (7) were present at Z lines, underneath the sarcolemma and around the Golgi region. It may, at times, be difficult to make a distinction between coated vesicles fused to the SR, and the EJSR proper (Fig. 3, arrow). In most instances, however, that distinction can be made, and profiles as shown in Fig. 3 (curved arrows) remove any doubt as to the occurrence in chicken cardiac muscle of EJSR as it has been defined structurally in other birds (2).

The present investigation thus confirms our earlier suspicions that chicken cardiac muscle, like cardiac muscle of other birds, contains EJSR. Its presence in the chicken, if only in rudimentary form, reasserts the structural homogeneity to be expected within one phylogenetic class. Our investigations have also been informative in that they have allowed us to at least categorize, by morphologic analogy, one of the several types of membrane-enclosed electron-opaque material found in chicken hearts. The structural congruency of EJSR and junctional SR of couplings is important. It suggests that, since EJSR by definition has no sarcolemmal contact, at least one function of junctional SR may not require sarcolemmal contact. This gives further cause to reexamine the presumed participation of the couplings in the process of excitation-contraction coupling, per se (8), and to ponder alternative roles for them during the contraction-relaxation cycle. The morphologic congruency between EJSR and junctional SR of couplings also suggests that, since all of the avian hearts studied lack transverse tubules, the EJSR, even in the absence of sarcolemmal contact, may play a functional role analogous to that of the junctional SR of interior couplings in mammalian cardiac muscle, where the junctional SR makes contact with sarcolemma (that of the transverse tubules). So far there has been no reason to posit different roles for peripheral vs. interior couplings. The rudimentary development of EJSR in chicken cardiac muscle as compared with its massive development in other birds (Fig. 1) raises the possibility that the quantity of EJSR found in individual hearts is related to the respective rates of contraction of which these hearts are capable (1). The occasional similarity between the rudimentary EJSR in the chicken and coated vesicles (7) deserves, perhaps, some further comment. The coated vesicles, except for their fuzzy, sometimes spiny layer on the outside of the membranous envelope (hence: coated vesicle), always present as smooth-round or, when fused with the SR, smooth-oval profiles. The membranous envelopes of rudimentary EJSR, in contrast, are scalloped (Figs. 1 and 2), and do not have the fuzzy, sometimes spiny outside layer. The fact that coated vesicles may fuse with, or bud from, the SR has recently been stated to suggest a dynamic anatomical relationship between the two membrane systems (7). Indeed, the evidence in the case of the coated vesicles does foster such speculation; structures presumed to be fused with the SR often do show considerable anatomical similarity to the coated vesicles. In the case of the rudimentary EJSR, however, there is nothing

FIGURE 2 Cribriform electron-opaque material (arrows) within SR tubules at the Z line in adult chicken. Note the striking similarity between this material and that seen in Fig. 1. The profiles in the SR at the center of the sarcomere (open arrow) appear identical to the SR fenestrations which have been described at the M line in skeletal muscle. *Inset:* Low power view showing two different appearances of EJSR (arrows) in the chicken, depending on the plane of sectioning (cf. Figs. 1 and 3). *ZT*, Z tubule. × 22,000, approximately.

FIGURE 3 5-day old chick. Elongated membranous profiles (curved arrow) contain electron-opaque granular material. The structures are indistinguishable from the junctional SR of couplings and the EJSR of other birds. Straight arrow points to what may be a coated vesicle. Arrow head shows a circular membranous profile containing very homogeneous electron-opaque material which is clearly not related to the structures under study in this report. *MT*, microtubule. × 49,000.

to remind one of coated vesicles except for the fact that both EJSR and coated vesicles contain electron-opaque material, and that coated vesicles are often at the Z line where EJSR is always located. Structural profiles such as seen in Fig. 3 (curved arrows) defy comparison with coated vesicles.

The circular profiles in the SR at the mid-portion of the sarcomere are presumably fenestrations as they are seen in skeletal muscle. They have not been described in cardiac muscle before.

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