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The density attached to the inside surface of the apposed sarcoplasmic reticular membrane in vertebrate cardiac and skeletal muscle fibres

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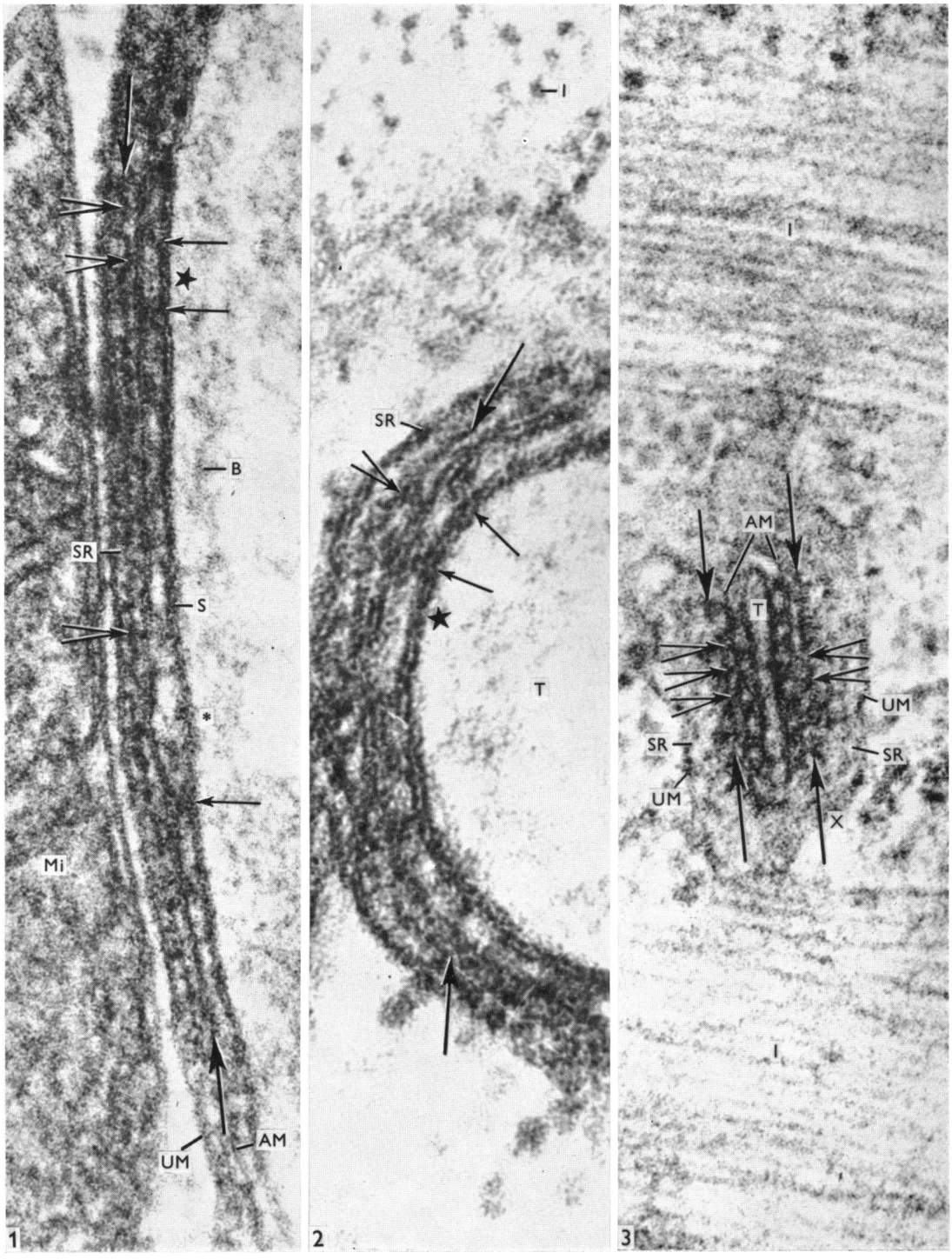
INTRODUCTION

Several resemblances between sarcoplasmic reticulum (SR) in cardiac and skeletal muscle fibres of vertebrates have been observed. In some instances the similarities are more obvious when skeletal muscle fibres of foetal and newborn animals are used for comparison with cardiac fibres of adults. The network of tubules formed by SR in adult rat cardiac fibres (Porter & Palade, 1957) looks very much like the network of SR tubules found in foetal and newborn rat skeletal muscle fibres (Walker, Schrodt & Bingham, 1968). In both types of fibres the network is continuous throughout the sarcomere and in successive sarcomeres. Tubules continuous with the network of SR tubules encircle the fibril at the Z line level in cardiac (Simpson & Rayns, 1968; Edge & Walker, 1970) and in skeletal (Walker, *et al.*, 1968) muscle fibres. Invaginations of the sarcolemma form transverse tubules (T) in cardiac (Lindner, 1957; Simpson & Oertelis, 1961) and skeletal (Franzini-Armstrong & Porter, 1964) muscle fibres. SR shows apposition to T and to the sarcolemma in cardiac fibres (Johnson & Sommer, 1967) and in foetal and newborn rat skeletal muscle fibres (Walker, Schrodt & Bingham, 1969; Schiaffino & Margreth, 1969).

Conversely, certain characteristics of SR have been reported for one fibre type and not for the other. For example, a characteristic width of the space between apposed SR and T has been reported for skeletal muscle fibres, but a quite variable space has been reported for cardiac fibres. A linear density is consistently found in sections of apposed SR of cardiac fibres but a similar density has not been reported for skeletal muscle fibres. Andersson-Cedergren (1959) observed a space about 100 Å (10 nm) wide between apposed membranes of SR and T in mouse leg muscles. This space width has been found in numerous investigations on other vertebrate skeletal muscles. On the other hand, a space 150–200 Å (15–20 nm) wide is found between membranes of apposed SR and the sarcolemma or T of cat cardiac fibre (Fawcett & McNutt, 1969). Simpson & Rayns (1968) expressed the distance between apposed SR and T in ferret cardiac fibres as about 250 Å (25 nm) from centre to centre of apposed membranes. If 7·5 nm is allowed for membrane thickness included in the measurement the width of the space between apposed membranes is about 17·5 nm. Revel (1962) observed densities traversing the space between apposed SR and T in bat cricothyroid muscle. This observation has been confirmed in many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subsequent investigations on vertebrate skeletal muscle. Conversely, descriptions of densities within the space between apposed SR and T or the sarcolemma of cardiac fibres vary widely. Johnson & Sommer (1967) observed densities traversing the space in the rabbit. Simpson & Rayns (1968) occasionally found an electron-dense line within the space and parallel to the apposed membranes in the ferret. They never found cross bridges like those reported by Revel (1962). Fawcett & McNutt (1969) found periodic densities in the space that seemed to be no more closely associated with one apposed membrane than the other in cat cardiac fibres.

The intraluminal content of apposed SR exhibits more intense staining than the remainder of SR in cardiac and skeletal muscle fibres of vertebrates (Porter & Palade, 1957). Porter (1956) was the first to point out that the SR of skeletal muscle fibres is in general apparently devoid of structural material, the notable exception being SR apposed at T. He observed that apposed SR contains a finely fibrous material. The intraluminal content of apposed SR in skeletal muscle fibres has been variously referred to as opaque material (Andersson-Cedergren, 1959), granular or vesicular elements (Reger, 1961), a fine meshwork interpreted as a precipitate (Revel, 1962), amorphous-looking material (Huxley, 1964), diffuse granular material (Peachey, 1965), membrane-like structures (Walker & Schrodt, 1965), and flocculent material (Kelly, 1969). In studies on cardiac fibres of numerous mammals a dense wavy line is found midway between and parallel to the limiting membranes of apposed SR (Johnson & Sommer, 1967; Sommer & Johnson, 1968). An electron-dense line midway between limiting membranes of apposed SR is a consistent finding in ventricular muscle fibres of the ferret (Simpson & Rayns, 1968). Fawcett & McNutt (1969) observed a 30–50 Å (3–5 nm) dense line in the centre of the lumen of apposed SR in cat cardiac fibres.

Electron microscope studies have shown resemblances of the network of tubules formed by SR in cardiac and skeletal muscle fibres. The differences in findings related to apposed SR suggested the need for further electron microscopic study. The work

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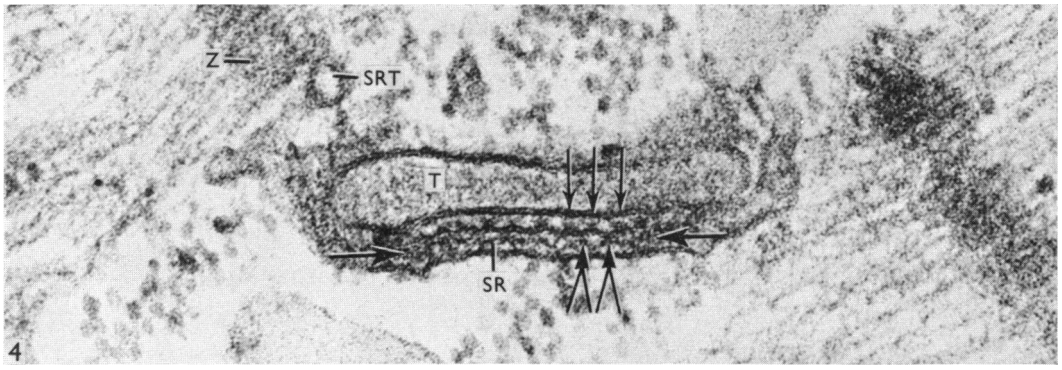
Fig. 1. Electron micrograph of a longitudinal section of dog cardiac muscle fibre showing sarcoplasmic reticulum (SR) apposed to the sarcolemma (S). The star designates a typical width (about 10 nm) of the space between apposed membranes and the asterisk is placed beside an atypically wide space. The large arrows point toward the central density within SR. The small arrows in the right side of the micrograph point toward bridges across the space between apposed membranes of SR and S. The double-stemmed arrows in the left side point toward connexions between the apposed SR membrane (AM) and the central density. The connexions between the central density and the unapposed SR membrane (UM) are visible but not labelled. B, Basement membrane; Mi, mitochondrion. × 175 000.

Fig. 2. Cross-section of dog cardiac fibre showing SR apposed to a transverse tubule (T). I, cross-section of I-band filament. Other labels as in Fig. 1. × 300 000.

Fig. 3. Longitudinal section of rat extensor digitorum longus muscle fibre showing a longitudinal section of a triad. The large arrows are directed toward coextensive densities near and parallel to the inside surface of apposed SR membranes (AM) facing the transverse tubule (T). The double-stemmed arrows point toward connexions between apposed membranes and the densities parallel to them. Six bridges between apposed membranes of T and SR are shown on the left side of T and seven similar bridges are shown on the right side. The unapposed SR membranes (UM) are the parts of the SR membranes not facing T. Electron-dense material between the densities (large arrows) and the unapposed SR membrane is indicated by the X placed below SR in the right side of the micrograph. I, I band. × 160 000.



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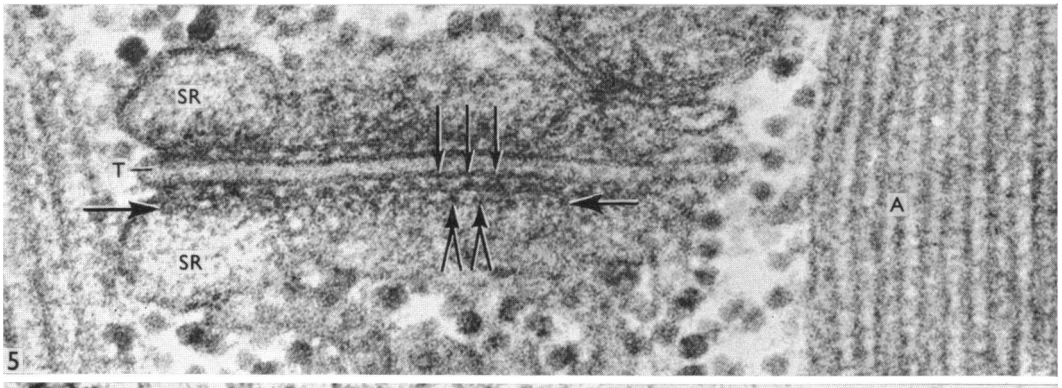

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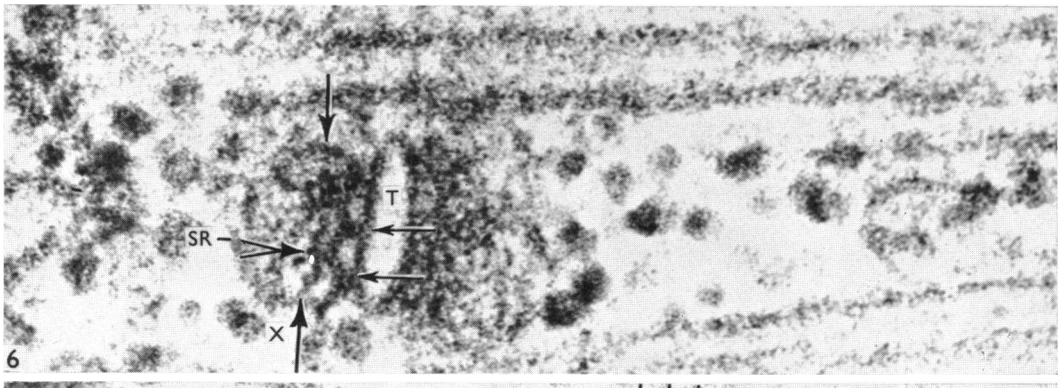

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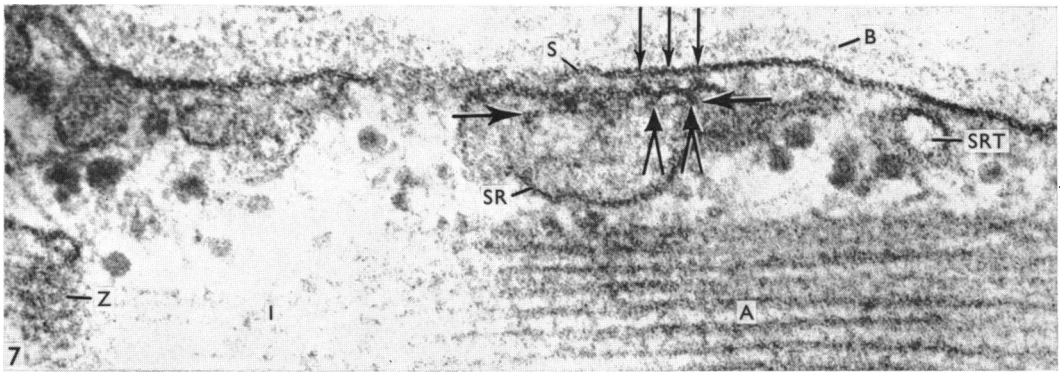

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reported here is concerned with the width of the space between apposed membranes and with the density attached to the inside surface of the apposed SR limiting membrane.

METHODS

Dogs were used for examination of cardiac fibres and dogs, rats and frogs were used for examination of skeletal muscle fibres. Dogs were anaesthetized with sodium pentobarbital. The hearts were exposed and removed from the animals. Small bundles of papillary muscle fibres and skeletal muscle fibres were dissected, extended slightly and tied to Plexiglas stays, fixed in 3 % glutaraldehyde by the method of Sabatini, Bensch & Barrnett (1963) and postfixed in OsO_4 by the Palade method as modified by Caulfield (1957). The fibres were dehydrated for 10 min in 50 % ethanol and then placed overnight in 70 parts absolute ethanol and 30 parts 1 % aqueous solution of uranyl acetate. After completion of dehydration with 95 % and 100 % ethanol the fibres were embedded in Maraglas and sectioned with an LKB microtome. The sections were triple-stained with lead, uranyl acetate and lead. A modification of the method of Reynolds (1963) was used for lead staining with the duration of the triple-stain sequence varying from 5, 3, 5 to 10, 5, 10 min. Sections exhibiting grey or light silver interference colours were examined with a Siemens Elmiskop I-A electron microscope. In a few preparations of cardiac and skeletal muscle fibres 4 % formaldehyde containing 1 % $CaCl_2$ was substituted for 3 % glutaraldehyde as the initial step of fixation.

RESULTS

The membranes of SR, T and the sarcolemma in striated muscle fibres are composed of two dense layers separated by a less-dense layer. The total membrane thickness is about 7·5 nm with the thickness of each of the three layers being about equal. The space between apposed membranes of SR and T or of SR and the sarcolemma in dog cardiac fibres is about 10 nm wide and this space is traversed by bridges (small arrows in Figs. 1, 2 and 4). Frequently, the bridges that traverse the space

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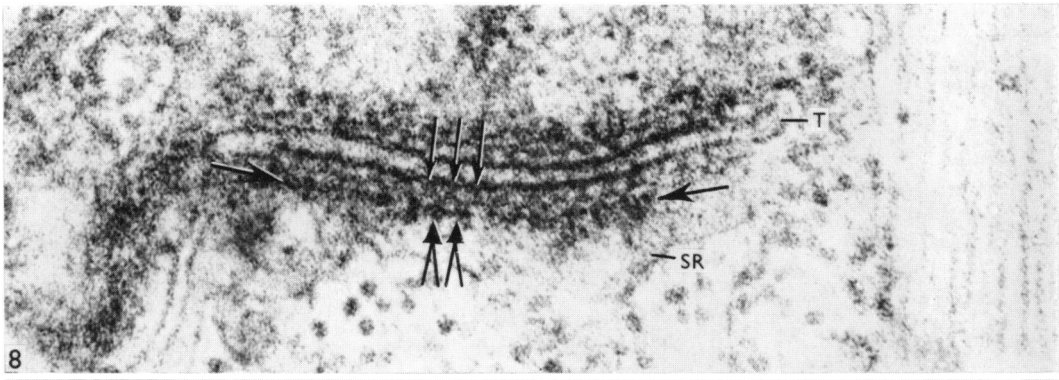
Fig. 4. Longitudinal section of dog cardiac fibre showing SR apposed to T. Bridges between T and SR (small arrows) are regularly spaced. Connexions between the central density and the apposed SR membrane (double-stemmed arrows) are also rather regularly spaced. The waviness of the central density (large arrows) appears to be associated with connexions. SRT, Cross-section of a tubule in the SR network of tubules; Z, Z line. × 120 000.

Fig. 5. Longitudinal section of dog cricothyroid muscle fibre showing longitudinal section of a triad. The positions of bridges (small arrows) between apposed membranes of T and SR alternate with positions of connexions (double-stemmed arrows) between the coextensive density (large arrows) and the apposed SR membrane. A, A band. × 125000.

Fig. 6. Longitudinal section of dog cricothyroid muscle fibre showing cross-section of a triad. Electron-dense material between the coextensive density (large arrows) and the unapposed SR membrane is indicated by the X placed below the apposed SR. Other labels as in Fig. 5. × 200 000.

Fig. 7. Longitudinal section of a 10-d-old puppy cricothyroid muscle fibre showing SR apposed to sarcolemma (S). The coextensive density (large arrows) is very faint. However, the connexions (double-stemmed arrows) between this density and the apposed SR membrane and the bridges (small arrows) between SR and S are quite distinct. B, Basement membrane; Z, Z line; I, I band; A, A band; SRT, cross-section of a tubule in the SR network of tubules. × 125 000.



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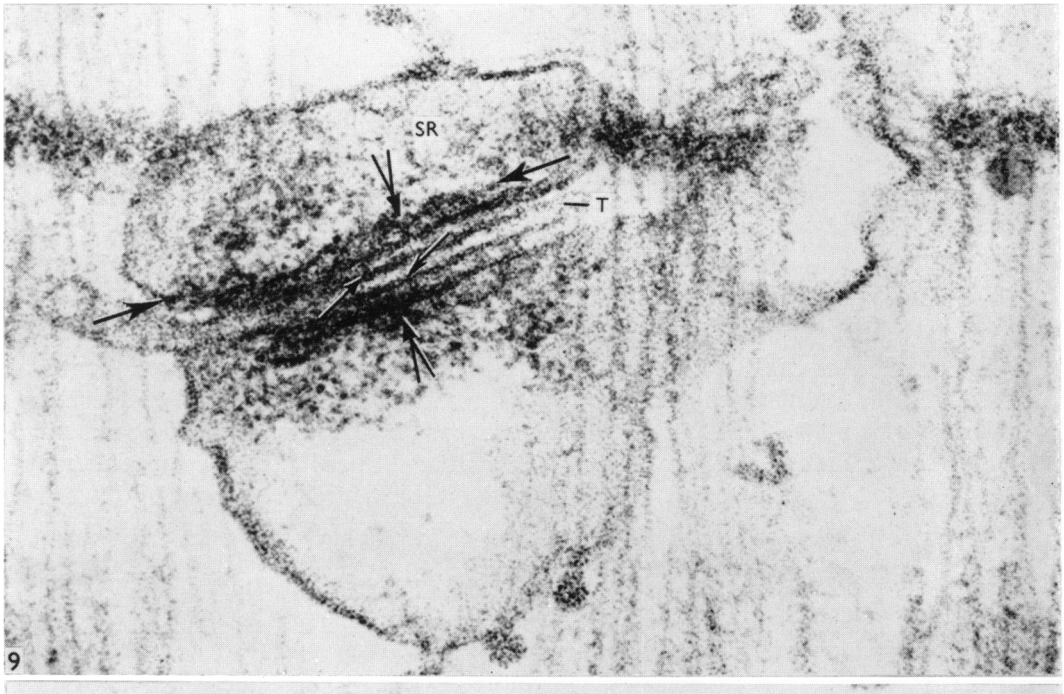

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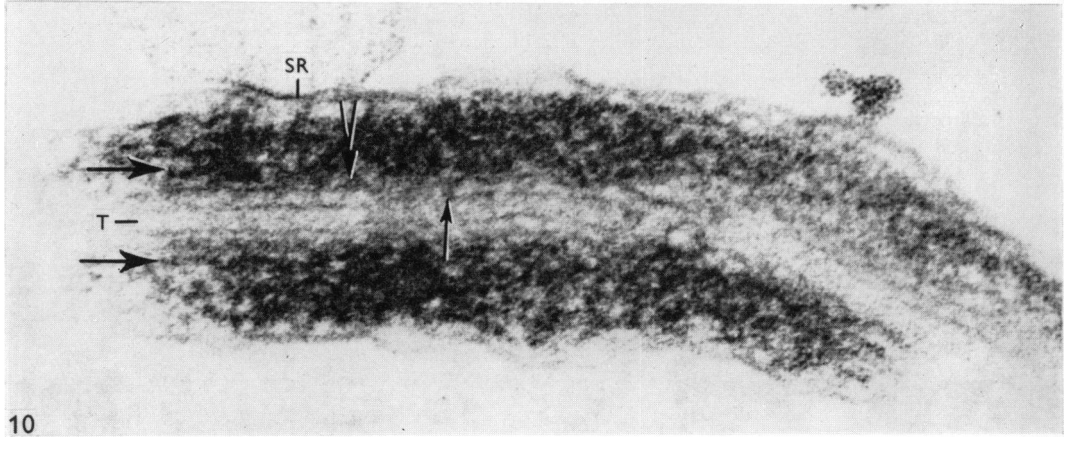

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between T and apposed SR in cardiac fibres are not regularly spaced (Fig. 2), but a rather regular spacing of bridges is not infrequently found (Fig. 4). In skeletal muscle fibres of the dog and the rat the space between apposed membranes of SR and T is also about 10 nm wide and the periodicity of bridges across this space is remarkably constant in favourable sections (Figs. 3, 5).

The apposed SR in cardiac fibres is widely expanded in a direction parallel to the plane of apposition and the extent of expansion along the sarcolemma is indicated by the observation of apposed SR segments more than 1 μm long in transverse and longitudinal sections of fibres (Fig. 1). Conversely, the expansion of apposed SR perpendicular to the plane of apposition is always sharply limited. The distance between the SR limiting membranes varies from 15 to 25 nm and the outer dimension of the apposed SR varies from 30 to 40 nm. It is possible that the expansion perpendicular to the plane of apposition is limited by short connexions between the inside surfaces of SR membranes and a central density (large arrows in Figs. 1 and 2) within SR. The short connexions between the part of the SR membrane not apposed to T or the sarcolemma (UM in Fig. 1) resemble the connexions between the part of the SR membrane apposed to T or the sarcolemma (AM in Fig. 1). It is the connexions between the central density and the apposed SR membrane (double-stemmed arrows in Figs. 1 and 2) that seem to be comparable to connexions within apposed SR of skeletal muscle fibres.

To visualize the dimensions of the central density in cardiac fibres the limiting membranes of apposed SR can be regarded as the walls of an envelope. An unfolded sheet of paper inserted into the envelope would represent the central density. Any random section of the envelope and the enclosed sheet of paper would show a profile of the paper as a line midway between and parallel to the walls of the envelope. Similarly, any random section across apposed SR reveals a dense line between the limiting membranes of SR (large arrows in Figs. 1 and 2). Thus it can be seen that the length and breadth of the central density and the limiting membranes of apposed SR are coextensive.

In sections of apposed SR in skeletal muscle fibres a linear density is found near

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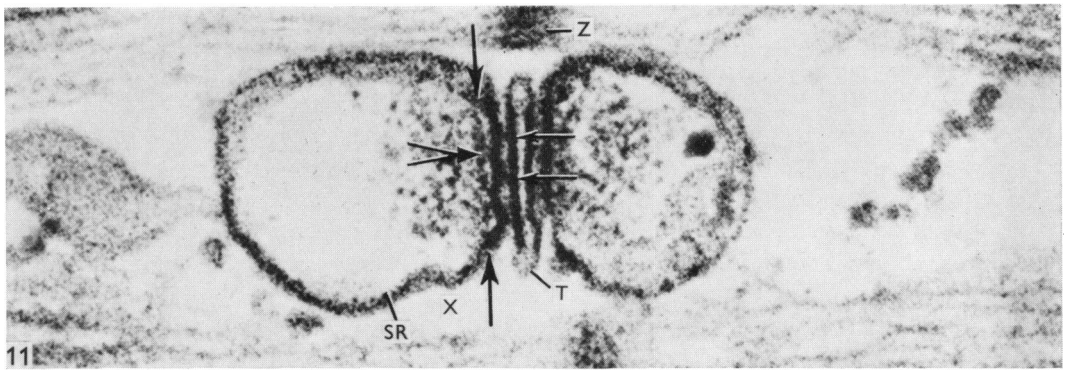
Fig. 8. Longitudinal section of rat extensor digitorum longus muscle fibre showing a longitudinal section of a triad. The coextensive density (large arrows) parallel to and near the apposed SR membrane is very intensely stained. The connexions (double-stemmed arrows) between this density and the apposed SR membrane are in positions that alternate with the positions of bridges (small arrows) between T and SR. × 120 000.

Fig. 9. Longitudinal section of frog sartorius muscle fibre showing a plane of section passing slightly oblique to the longitudinal axis of the triad. The coextensive densities parallel to and near the inside surface of apposed SR membranes are very wavy. Connexions between the densities and the apposed SR membranes are visible at a few points and are indicated by double-stemmed arrows at two points. Bridges between T and SR are indicated by small arrows at two points. The apposed SR in the bottom of the micrograph shows a large electron-lucent space. Z, Z line. × 150 000.

Fig. 10. Longitudinal section of frog sartorius muscle fibre showing longitudinal section of a triad. Formaldehyde and $CaCl_2$ were used in the first step in fixation of this fibre. The large arrows are directed toward coextensive densities parallel to and near the apposed SR membranes. The double-stemmed arrow is directed toward a connexion between a coextensive density and the apposed SR membrane and the small arrow is directed toward a bridge between T and SR. It can be seen that details of structure are poorly preserved when this method of fixation is used. × 150 000.



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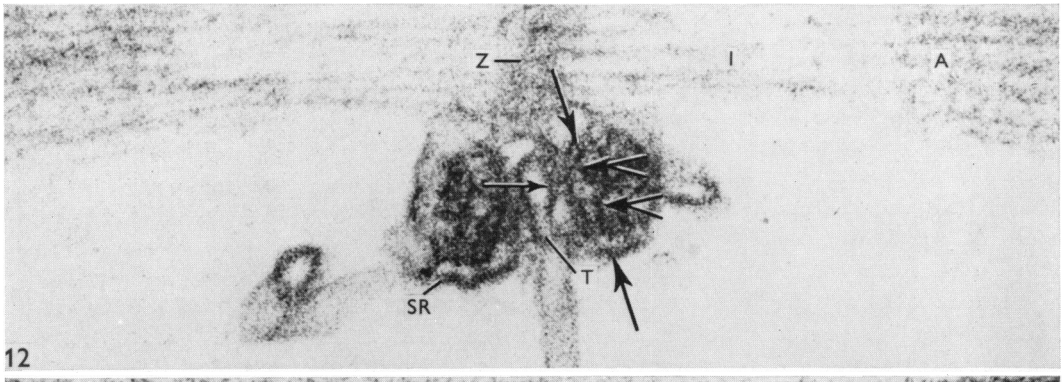

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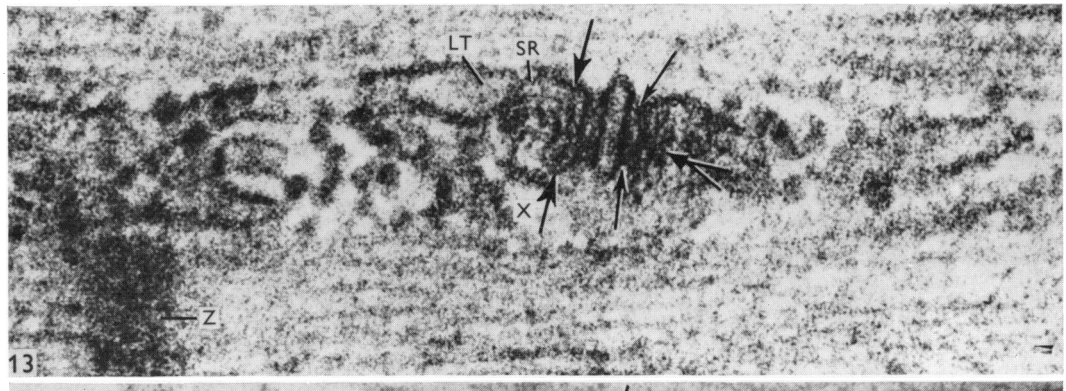

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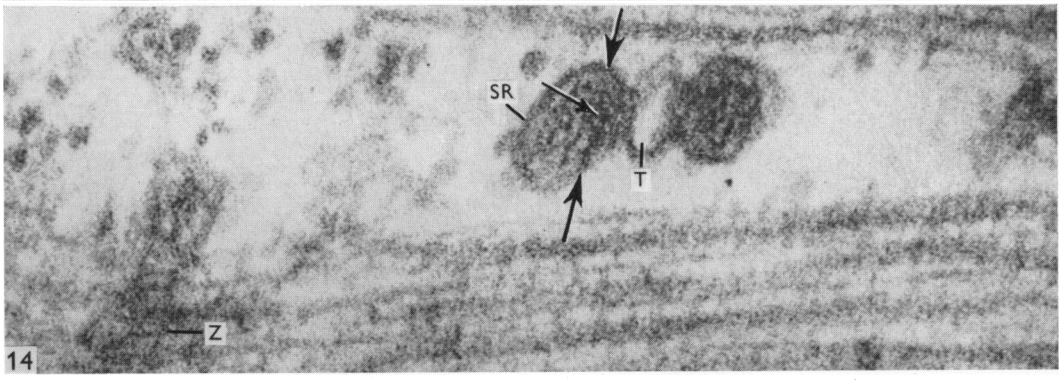

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and parallel to the inside surface of the apposed SR membrane (large arrows in Fig. 3). The location suggests that the linear density might be comparable to the central density in cardiac fibres. To visualize the dimensions of the material shown as a linear density in longitudinal sections of triads in skeletal muscle fibres (Figs. 3, 5) it is necessary to examine cross-sections of triads. Cross-sections of triads reveal a linear density (large arrows in Fig. 6) parallel to the part of the SR limiting membrane apposed at T. The distance of the linear density from the inside surface of the apposed SR membrane is equal in longitudinal sections and cross-sections. In both instances the position of bridges between T and apposed SR alternate with the position of bridges between the linear density and the apposed SR membrane. It seems certain that the linear density viewed in triad cross-sections is a measure of the breadth of electron-dense material displaying a linear profile in longitudinal sections of triads (Figs. 3, 5). If this assumption is granted the material shown as a linear density in longitudinal and cross-sections of triads is in fact a sheet of electron-dense material coextensive with and parallel to the inside surface of the apposed SR membrane.

The connexions between the central density and the apposed SR membrane in cardiac fibres may show irregular (Figs. 1, 2) or regular (Fig. 4) spacing. The regular spacing of connexions is associated with regular spacing of bridges. In longitudinal sections of skeletal muscle triads the connexions between the coextensive density and the apposed SR membrane are regularly spaced and the position of connexions along the inside surface of the membrane alternates with the position of bridges along the outside surface of the membrane (Figs. 3, 5). The alternating positions of connexions and bridges are closely associated with a scalloped appearance of the apposed SR membrane in skeletal and in cardiac fibres (Figs. 3–5). Although the coextensive density in skeletal muscle is not located in the centre of apposed SR its distance from the apposed SR membrane is about equal in skeletal and cardiac muscle fibres (cf. Figs. 1, 3, 5).

In skeletal muscle fibres of puppies and young rats the SR is frequently apposed to the sarcolemma. In some instances, the position of bridges between the sarcolemma and the apposed SR membrane alternates with the position of connexions between the apposed SR membrane and the coextensive density parallel to this membrane (Fig. 7). This alternation is accompanied by a scalloped appearance of the apposed

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Fig. 11. Longitudinal section of frog sartorious muscle fibre showing a cross-section of a triad. Swelling in the apposed SR in the left side of the micrograph is indicated by a large electron-lucent space. The electron-dense material between the coextensive density (large arrows) and the electron-lucent space is indicated by the X placed beneath the apposed SR. Z, Z line. × 150 000.

Fig. 12. Longitudinal section of frog sartorius muscle fibre in which formaldehyde and $CaCl_2$ were used in the first step of fixation. The apposed SR is filled with a network of dense sheets. Z, Z line; I, I band; A, A band. × 150 000.

Fig. 13. Longitudinal section of dog cricothyroid muscle fibre showing a cross-section of a triad and a short segment of a longitudinally oriented tubule (LT) of the SR network of tubules. Electron-dense material between the coextensive density (large arrows) and the unapposed SR membrane is indicated by the X placed beneath the apposed SR. Z, Z line. × 150 000.

Fig. 14. Longitudinal section of a dog cricothyroid muscle fibre used for purposes of comparison with Fig. 13. Formaldehyde and $CaCl_2$ were used in the first step of fixation. It can be seen that the dimensions of apposed SR are similar to the dimensions of apposed SR in Figs. 13 and 6. The apposed SR is filled with a network of dense sheets that tend to obscure the coextensive density (large arrows) parallel to and near the apposed SR membrane. Z, Z line. × 150 000.

SR membrane. In other instances the spacing of bridges and connexions is irregular and alternation and the scalloped appearance are absent. The variation in spacing from regular to irregular might be due to variation in the plane of the section passing through the apposed SR and sarcolemma.

Coextensive densities like those observed in sections in apposed SR of dog cricothyroid muscle fibres are found in all vertebrate muscle fibres examined. In the bottom of Fig. 8 a very intensely stained coextensive density in apposed SR of rat extensor digitorum longus is illustrated (large arrows). Connexions traversing the electron-lucent space between this density and the apposed SR membrane are clearly indicated (double-stemmed arrows). The alternation of connexions and bridges (small arrows) along the apposed SR membrane and the scalloped appearance of this membrane are also illustrated.

The use of 3 % glutaraldehyde for the initial step in fixation of frog muscle fibres frequently produces swelling of apposed SR. The swelling is occasionally accompanied by uneven distribution of electron-dense material within SR. Nevertheless, a density parallel to and near the inside surface of the apposed SR limiting membrane is usually found (large arrows in Fig. 9). Bridges appear to form connexions between the density and the apposed SR limiting membrane. To avoid swelling of apposed SR in frog muscle fibres 4 % formaldehyde containing 1 % $CaCl_2$ has been used for the first step of fixation. The dimensions of apposed SR are much smaller when formaldehyde is substituted for glutaraldehyde. The limiting membranes of T and SR are rather faintly stained and poorly preserved when this substitution is made but they can be seen by close inspection. A density parallel to the inside surface of the apposed SR limiting membrane (large arrows in Fig. 10) is visible but it is not prominent. Its prominence is obscured by very intensely stained material within the remainder of apposed SR. This material appears to be in the form of a network of interwoven sheets of density. A network of dense sheets like that illustrated in Fig. 10 is also found within apposed SR in longitudinal sections of triads from formaldehyde-fixed skeletal muscle fibres of dogs and rats.

Although SR apposed to T in skeletal muscle fibres is continuous with longitudinally oriented tubules of the SR network many cross-sections of triads are in planes that do not show these continuities (Figs. 6, 11). These cross-sections illustrate the expansion of apposed SR in a direction perpendicular to the plane of apposition. The average value for dog cricothyroid fibres is about 75 nm and is in agreement with the findings of Revel (1962) for bat cricothyroid fibres. The average value for glutaraldehyde-fixed frog sartorius fibres is almost 220 nm and is in agreement with observations of Peachey (1965) on frog sartorius fibres. Occasionally the apposed SR (lateral elements) of the triad are unequal in size and the larger lateral element shows a large electron-lucent space (Fig. 11). This space is distal to the apposed part of the lateral element. The proximal part of the lateral element contains a dense line flanked by irregularly distributed dense material. Cross-sections of frog muscle triads fixed in formaldehyde show much smaller lateral elements than those fixed in glutaraldehyde (Fig. 12). Densities are seen near the apposed SR limiting membrane and the remainder of the apposed SR is filled with a network of dense sheets. The dimensions of apposed SR are usually only slightly reduced when formaldehyde is substituted for glutaraldehyde in the first step of fixation of dog and rat skeletal muscle fibres and

electron-dense material is seen throughout the apposed SR with both methods of fixation (Figs. 13, 14). However, the electron-dense material between the coextensive density and the unapposed SR limiting membrane frequently exhibits disconnected segments of dense lines (X in Figs. 3, 6, 13). In the formaldehyde-fixed fibre a network of dense sheets is equally distributed in apposed SR (Fig. 14).

DISCUSSION

The diagrams in Fig. 15 illustrate the similarities of bridges, connexions and coextensive densities at the sites of SR and T apposition in cardiac and skeletal muscle fibres. The typical narrow zone of apposition as viewed in a cross-section of SR and T at the triad level in mammalian skeletal muscle fibres is shown in Fig. 15 (c). Although the number of bridges visible in an apposition zone may be two or three the usual number is two. Now a plane of section that includes an extensive longitudinal section of a triad in the mammalian muscle fibre is rather precisely oriented along the longitudinal axis of the triad. These extensive longitudinal sections show alternation of bridges between T and SR with connexions between the co-extensive density and the apposed SR membrane as illustrated in Fig. 15 (*b*). The coextensive densities shown as lines in Fig. 15 are in fact sheets of electron-dense material appearing as linear images in sections of apposed SR.

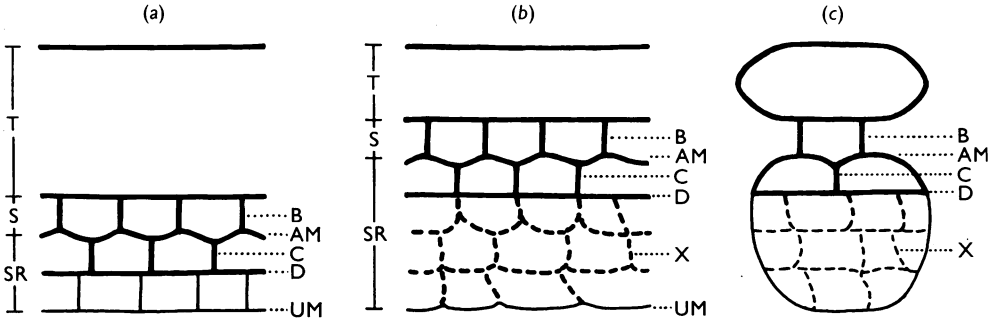

Fig. 15. The bold solid lines in the diagrams represent comparable structural characteristics at sites of SR and T apposition in cardiac and skeletal muscle fibres. (*a*) Section along the longitudinal axis of T in a cardiac muscle fibre. (*b*) Section along the longitudinal axis of T in a skeletal muscle fibre. (*c*) Cross-section of T and one of the lateral elements of the triad in a skeletal muscle fibre. S, Space about 10 nm wide between SR and T; B, bridges traversing the space between SR and T; AM, the apposed part of the SR membrane; D, a sheet of electron-dense material coextensive with AM; C, connexions between D and AM; UM, the unapposed part of the SR membrane; X, broken lines between D and UM representing electron-dense material with unknown spatial arrangement.

The observations in the present investigation indicate that the width of the space (S in Fig. 15) separating surface membrane from the apposed SR at its sites of apposition is about equal in cardiac and skeletal muscle fibres of vertebrates. The space is traversed by bridges (B in Fig. 15) in both fibre types. The observation that the space between apposed SR and T or the sarcolemma is about 10 nm wide in cardiac and skeletal muscle fibres raises a question about the 15–20 nm width of this

space reported in previous investigations (Simpson & Rayns, 1968; Fawcett & McNutt, 1969). It is suggested that the extensive expansion of apposed SR parallel to the plane of apposition presents a condition conducive to disruption of bridges traversing the space and to separation of apposed membranes while the fibre is being prepared for examination. An example of excessive distance between apposed membranes is designated by an asterisk in Fig. 1.

The distance between apposed membranes of SR and T is about 7·5 nm at the positions of bridges (Walker & Schrodt, 1966). The most reliable measurements are obtained at points where the three layers of the SR membrane and the three layers of the T membrane are clearly visible at the level of a bridge. In the present study the 10 nm space width is the average distance between apposed membranes at the level of areas interspersed between the bridges. It can be seen that the distance is somewhat less at the level of bridges. Wide variations in width and appearance of densities in the space between apposed membranes have been observed. A recent report (Kelly, 1969) shows dimples on the external surface of apposed SR membranes in skeletal muscle of young newts and adult frogs. These dimples may or may not show contact with the membrane of the T tubule and they may or may not be accompanied by evagination of the apposed SR membrane. Observations that projections from the external surface of SR membranes and bridges between apposed membranes often show similar periodicities in foetal leg muscle fibres of rats led to the suggestion that the projections may be involved in formation of bridges between apposed membranes (Walker, *et al.* 1969).

There is substantial evidence that apposed SR in foetal and newborn rat skeletal muscle fibres is formed by expansion of tubular segments in the SR network (Walker & Schrodt, 1967, 1968; Schiaffino & Margreth, 1969). It is not known with certainty whether the expansion of tubule segments occurs before or after apposition. Structures with the appearance of SR tubules are occasionally found apposed to the sarcolemma in these fibres. On the other hand, structures showing the expansion characteristic of apposed SR are never found unless the SR is indeed apposed to T or the sarcolemma (Fig. 5 in Walker, *et al.* 1969). These observations suggest that apposition of SR tubules to T or the sarcolemma is prerequisite to development of the unique structural characteristics in apposed SR.

The densities exhibited in the present investigation suggest that two unique structural characteristics of apposed SR are shared by cardiac and skeletal muscle fibres. First, the density (D in Fig. 15) parallel to and near the apposed SR membrane is coextensive with this membrane. Second, the electron-lucent space between this density and the apposed SR membrane is traversed by numerous densities that appear to be connexions (C in Fig. 15). The existence of connexions between the coextensive density and the apposed SR membrane of cardiac and skeletal muscle fibres is suggested by three lines of support derived from examinations of skeletal muscle fibres. First, the distance between the density and the membrane is usually shortest at points showing the apparent connexions. This observation indicates a holding force exerted by connexions. Secondly, a remarkably regular spacing of the presumed connexions is frequently seen. This regular spacing indicates the presence of developed structures. It seems unlikely that granular material would aggregate in such orderly fashion during preparation of the fibre for electron microscope examination. Thirdly

the coextensive density retains its position parallel to and near the apposed SR membrane in apposed SR showing evidence of marked swelling (Figs. 9, 11). This observation indicates that the density is held in its position by structures attached to the membrane.

It seems likely that the absence of expansion perpendicular to the plane of apposition in apposed SR of cardiac fibres is imposed by formation of short connexions between the central density and the unapposed SR membrane as illustrated in Fig. 15 (*a*). In skeletal muscle fibres abundant electron-dense material is seen between the coextensive density and the unapposed SR membrane (X in Fig. 15). Although the appearance of this material as unconnected segments in triad sections suggests that it might be associated with expansion of apposed SR in a direction perpendicular to the plane of apposition, methods of preparation must be improved before the possible structural nature of this material can be adequately investigated. Sections of apposed SR in formaldehyde-fixed fibres exhibit this material as an interwoven network of dense sheets. However, these dense sheets do not reveal a regular structural pattern. Unlike the coextensive density, the spatial arrangement of material in the network cannot be measured with present methods.

Fawcett & McNutt (1969) suggested that the central density in apposed SR of cardiac fibres is the result of condensed content within SR. This suggestion does not seem applicable to the findings reported here. In the first place, the coextensive density in skeletal muscle fibres is not centrally located and therefore is not in a position that one would expect to find condensed content. Secondly, the connexions between the coextensive density and the apposed SR limiting membrane in cardiac and skeletal muscle fibres (double-stemmed arrows in Figs. 1–14) indicate that this density is an integral part of developed structures.

SUMMARY

The fine structures at the sites of SR apposition and within apposed SR of vertebrate cardiac and skeletal muscle fibres were examined with an electron microscope. A space about 10 nm wide separates the apposed SR limiting membrane and the membrane of T or the sarcolemma in both fibre types. This space is traversed by densities that appear to form bridges between apposed membranes. The fine structures within apposed SR exhibit two unique structural characteristics shared by both fibre types. First, a density near the inside surface of the apposed SR membrane is coextensive with the membrane. Secondly, numerous densities appear to form connexions across the electron-lucent space between the coextensive density and the membrane.

It is suggested that apposition of undifferentiated tubule segments in the SR network of tubules precedes development of structures within apposed SR. The subsequent development of unique structural characteristics within apposed SR is dependent upon this apposition.

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