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The transverse tubular system of rat myocardium: its morphology and morphometry in the developing and adult animal

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Summary. The three dimensional arrangements of the T system in the developing and adult animal were investigated by means of high voltage electron microscope stereoscopy using Golgi treated materials. The rat myocardial T system was composed of three major group elements: the transverse tubules, longitudinal tubules and flattened cisternae, which were classified according to their orientation and to their morphological features. It was found, as the growth of the rats proceeded, that the longitudinal tubules increased in number and that the transverse tubules were arranged more regularly and densely at the level of the z band. The flattened cisternae transiently increased in number during the 2–9 weeks, and then decreased gradually. Electron microscopy also revealed that all the transverse, longitudinal tubules and flattened cisternae of the T system had the chance of forming a coupling with the sarcoplasmic reticulum irrespective of its morphology and orientation to the myofibrils. Quantitative analysis of the rat T system from the stereo images indicated that the surface area (0.299 $\mu m^2/\mu m^3$) was considerably greater than previously reported.

Key words: Rat heart – T system – Morphology – Morphometry

Introduction

Among vertebrates, the transverse tubular system (T system) is only characteristic for the heart cells of mammals (Sommer and Johnson 1979). The T system, continuous with the surface plasma membrane, invaginates into the cell and forms an intimate contact with the sarcoplasmic reticulum (SR). Though the functional role of the T system in the cardiac muscle is far from being clear, the structural relationships between the T system and the SR suggest that the T system is presumably involved in the coupling of excitation and contraction (E–C coupling) by providing a pathway for the inward spread of the electrical signals (Forssman and Girardier 1970; Sommer and Waugh 1976; Sommer and Johnson 1979; Forbes et al. 1984). Previous study has revealed that the T system appears when the average diameter of the mammalian cardiac cells is greater than 7–8 μm (Hirakow 1970). It has also been pointed out that the surface-to-volume ratio (membrane areas of external sarcolemma + T system/unit cell volume) keeps pace with hypertrophy in the rat heart (Page and McCallister 1973; McCallister and Page 1973) and that the T system probably permits the hypertrophy of cells to 30 μm without incurring prohibitive delay in the E-C coupling (Sommer and Johnson 1979). The T system, probably bearing some relationship to the size of the mammalian cardiac cells, is necessary to ensure synchronous contraction of the whole population of myofibrils of one cell without the delay between excitation and contraction (Forssman and Girardier 1970; Forbes et al. 1984).

Systematic analysis of the morphological development of the T system presumably contributes to the understanding of the functional maturation of mammalian heart cells.

Observation of thick sections under a high voltage electron microscope (HVEM) provides three dimensional information on the cellular fine structure with reasonable resolution (Hama and Porter 1969). The morphology of the T system of the cardiac muscle under the HVEM has been described in mice (Sommer and Waugh 1976; Sommer and Johnson 1979; Yamada and Ishikawa 1981; Forbes et al. 1984). In the present study, the development in the three-dimensional arrangement of the T system of rat hearts, contrasted selectively by the modified Golgi method, (Franzini-Armstrong and Peachey 1982) was observed by recording stereo pair images using the HVEM. Furthermore, the length, volume and surface area of the T system were directly measured from the stereo images, using a Luzex 5000 stereo image analysis system.

The SR forms a specialized complex (coupling) with both the sarcolemma and its extension, the T system. Though the precise functions of the couplings are still in doubt, there are junctional processes in thin sections at the coupling regions of the SR making close contact with the cytoplasmic surface of the T system, and these processes are envisaged as forming a possible pathway for the E-C coupling (Forbes and Sperelakis 1977). Previous studied revealed that the wide spectrum of the coupling morphologies can exist even within the myocardial tissue of a single species (Forssmann and Girardier 1966; 1970; Forbes and Sperelakis 1977). The fine structures of the coupling in rat hearts were also examined in thin sections to determine the relationship between the orientation and morphology of each element of the T system seen in the Golgi preparation and the SR.

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Materials and methods

Sixty five male and female albino rats (Wistar) aged between 2 days and 23 weeks, weighing 7.0 g to 550 g, were used.

Initial fixation. Each rat was anesthetized with an intraabdominal injection of sodium pentobarbital (5 mg/100 g). After anesthesia, the heart was perfused with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) by inserting a needle into the left ventricle at the apex to prevent the supercontraction of myofibrils. After perfusion, the heart was removed and immersed in the same fixative at room temperature for 2 h. Pieces of myocardial tissues were dissected out from the left ventricle. Thin section electron microscopy: The specimens were postfixed in 2% OsO_4 in 0.1 M sodium cacodylate buffer for 1 h, block-stained with 2% uranylacetate, dehydrated in an ascending series of alcohol, and embedded in an epon araldite mixture. Some of the specimens were rinsed in 1% tannic acid in a 0.05 M sodium cacodylate buffer for 40 min after osmification. Thin sections were cut on a Porter-Blum MT-2 ultramicrotome, mounted on copper grids, double-stained with 2% uranylacetate and 1% lead citrate, and examined in a JEOL 1200EX transmission electron microscope at 80 kV.

Golgi preparation. The modified Golgi method (Franzini-Armstrong and Peachey 1982) was used for the present study. After initial fixation with 4% glutaraldehyde, the specimens of 4×4 mm size were rinsed for 20 min in 3% $K_2Cr_2O_7$, and then kept in 3% $K_2Cr_2O_7$ containing 1% OsO_4 for 8 days at 4° C with intermittent agitation. The specimens were rinsed with 0.75% $AgNO_3$ (three changes), and kept in the same solution for 3 days at 4° C. The procedures were repeated with shorter durations, in 3% $K_2Cr_2O_7$ for 3 days and in 0.75% $AgNO_3$ for 2 days. Then they were dehydrated and embedded in an epon araldite mixture. Sections of 0.1, 1, 2, and 3 μm thickness were cut on a Porter-Blum MT-2 ultramicrotome. Thick sections were mounted on 100 mesh copper grids and examined in a Hitachi H 1250-M high voltage electron microscope (HVEM) at 1,000 kV.

HVEM observation. The thickness of the specimen and the azimuth and amount of the tilting which were the most suitable for the HVEM stereo views were determined by the size and structural complexity of the objective. In the T system of the ventricular myocardial cells, the tilting angle of $\pm 8°$ was the most appropriate angle for the stereo views. The 1 μm sections (Fig. 3) were not thick enough to gain the continuity of the three dimensional arrangement of the T system. The 2 and 3 μm thick sections (Figs. 4–11) revealed the good continuity of the T system in stereo views. As the sections became thicker, more information about the arrangement of the T system was provided, but it was found that in the case of observations of specimens thicker than 3 μm, the stereo analysis of the T system became difficult because of the superimposition of the images within the depth of the specimen. Stereo pairs of electron micrographs gave better three dimensional images of the T system in longitudinal sections of cardiac fibers than in transverse sections. The tilt axis was arranged to be slightly oblique to the long axis of the cell for the convenience of observation and measuring of the T system.

Methods of measuring the length, volume and surface areas of the T system. We developed an analysis system to get three dimensional quantities from stereo pair images. It consisted of the improvement of both the HVEM (Hama et al. 1983) and an image processing system (Hama et al. 1984).

In brief, stereo pair pcitures from the selected area were taken at 1,000 kV by tilting the stages by $\pm 8°$ with specially controlled tilting stages in which the accuracy was achieved within $\pm 0.1°$ at given tilting angles. The microscope was provided with a rotation free zoom system and the tilting axis was always fixed with the long axis of the photographic plate for accuracy and simplicity of the stereo setting.

A quantification from the stereo pair pictures was performed using a Japan Regulater Luzex 5,000 image analysis system. (Details of the optical system and image processing system are described elsewhere (Arii et al. 1984).) In brief, the procedure for image processing consisted of the following steps: 1) characteristics of images were extracted by an image processing of gray levels, 2) they were simplified into binary images, 3) the binary images were skeltonized, 4) XYZ coordinates were obtained from the parallax between the stereo binary images of $\pm 8°$ tilt conditions, and 5) quantification was determined using the values of the width of a binary image with some approximations of shapes. The accuracy of the system was checked by measuring the model of the stereo skeletonized patterns of parallel projection calculated by a computer (Arii et al. 1984). Three dimensional quantities of the length, volume and surface area of the T system in the unit volume of the cardiac cell were measured in 58 g rats (23 days) and 200 g rats (8 weeks) using stereo photos recorded at a magnification of $\times 5{,}000$ from 2 μm thick sections. The final magnification of the prints used for morphometry was $\times 13{,}000$. Measurement of the T system was made until the volume of the ventricular cells surpassed 1,000 μm^3. The thickness of the flattened cisternae in the T system was assumed to be 70 nm, which was almost equal to the average diameter of the rounded tubules.

Statistical examinations were made by analysis of the variance test for regional differences and Student's test for comparison of two sample means at the significance level of 0.99. Based on preliminary observations, a sample size of 10 cells was taken as adequate for the present analysis. Values represented the mean ± 1 standard deviation calculated from the sampling means.

Results

As observed by light microscopy, deposits of silver filled the extracellular space, and the T system elements were clearly visible in many ventricular myocardial cells at the z band level together with the longitudinal branches of the T system (Fig. 1). Thin sections (Fig. 2) cut from the silver impregnated tissue blocks were observed under the EM and showed good preservation of the cardiac muscle and deposits of silver selectively in the T system.

The development of the T system

In the ventricular cells of the neonatal 8 g rats (2–5 days after birth), virtually no definitive T system was detected in the silver impregnated specimen. The T system was poorly developed in the ventricular cells of rats of less than

Figs. 1–16. All specimens are taken from left ventricle of the rat

Fig. 1. A light micrograph showing the T system in the adult ventricular myocardial cell. The T system (*arrows*) is filled with silver chromate deposits. × 1,400

20 g. The definitive T system was first observed in the ventricular cells of 28 g rats (13 days) (Fig. 4). Short blind-ended tubules were observed to invaginate from the sarcolemma. They tended to direct transversely at the level of the z band. Longitudinal tubules running parallel to the cell axis were detected in some ventricular cells, but those interconnecting the transverse tubules were rarely observed even in 3 μm thick sections. The diameter of the tubules was 100–200 nm, varying irregularly along their length and frequently revealed a beaded configuration (Fig. 4).

In the ventricular cells of 38 g rats (17 days), the T system was still few in quantity and showed areas of flattened cisternae along its course.

The T system in 58 g rats (23 days) was more developed than that of 38 g rats, though it was still irregular in distribution (Fig. 5). The morphology of the T system varied among cells. In some cells, the T system consisted of a few transverse tubules which were aligned at the level of the z band. These tubules displayed a beaded profile and the adjacent transverse tubules were connected by the longitudinal tubules which often followed a twisted course with a relatively uniform diameter along their length. In other cells, they displayed flattened cisternae at various places. Some of the cisternae were located at the tips of the tubules.

In 120 g rats (31 days), the transverse tubules were seen running irregularly around the myofibrils at the level of the z band with an occasional occurrence of flattened cisternae and formed a loose z plate network transversely across the cell (Fig. 6a, b). There were some longitudinal tubules connecting the adjacent planes of the network.

In 180–200 g rats (6–7 weeks), the transverse tubules displayed a loose z plate network throughout the overall cell, and some longitudinal tubules connected adjacent z plate networks (Fig. 7a, b). A number of the flattened cisternae were randomly distributed along the transverse tubules and preferentially located at the transverse-transverse and transverse-longitudinal branching sites of the tubules. Some of the cisternae were detected at the tips and in the middle of the longitudinal tubules (Fig. 8). Though the cisternae varied considerably in size, they were either simple disc or ellipsoid shapes. The longer diameter of the cisternae was 200–1,600 nm. The transverse tubules showed a beaded configuration along the tubules and the diameter of the tubules varied 60–180 nm, but the longitudinal tubules had a relatively small and uniform diameter compared with the transverse tubules. The T system revealed two types of tubules irrespective of their orientation to the cell axis: a ribbon-like flattened type and a cylindrical type corresponding to the flattened and rounded tubules in cross section (Fig. 8). These two components occurred in alternate disposition along the T system.

As the development of the T system proceeded in 200–260 g rats (8–9 weeks), the transverse tubules were surrounded more densely at the level of the z band around each myofibrils (Fig. 9a, b). The longitudinal tubules gradually increased in number. The flattened cisternae were detected mainly at the branching sites of the T system, but they decreased in number in some ventricular myocardial cells. Furthermore, in a small number of cells, the T system morphologically resembled that observed in 23 w rat hearts.

In 300–350 g rats (12 weeks), the longitudinal tubules became more predominant in the T system. Flattened cisternae still existed, but decreased in number and their size became smaller as compared with those of the 8–9 w rats.

In 490–560 g rats (18–23 weeks), the T system was characterized by the presence of many longitudinal tubules (Fig. 10a, b). The transverse tubules were regularly arranged at the level of the z band and densely surrounded the myofibrils (Fig. 11a, b). Each myofibril was encircled by transverse tubules and accompanied by longitudinal ones. Few flattened cisternae were seen in the T system. Cylindrical tubules, 60–120 nm in diameter, occupied the larger part of the T system irrespective of the orientation of the tubules, and their diameters were uniform along the length.

A number of spotty regions were observed to be associated with the T system (Fig. 8), some of which may correspond to the smooth and coated pits seen in thin sections (see below).

The morphological differences in the three dimensional arrangement of the T system under the HVTEM were not recognized among the anterior, lateral and posterior walls of the left ventricle, septum and the right ventricle observed so far. The atrium and papillary muscles were not investigated in the present study.

The structural relationships between the T system and the SR

Though rarely, golgi etc. Golgi specimen showed simultaneous infiltration of the T system and SR, (Fig. 14). Thick

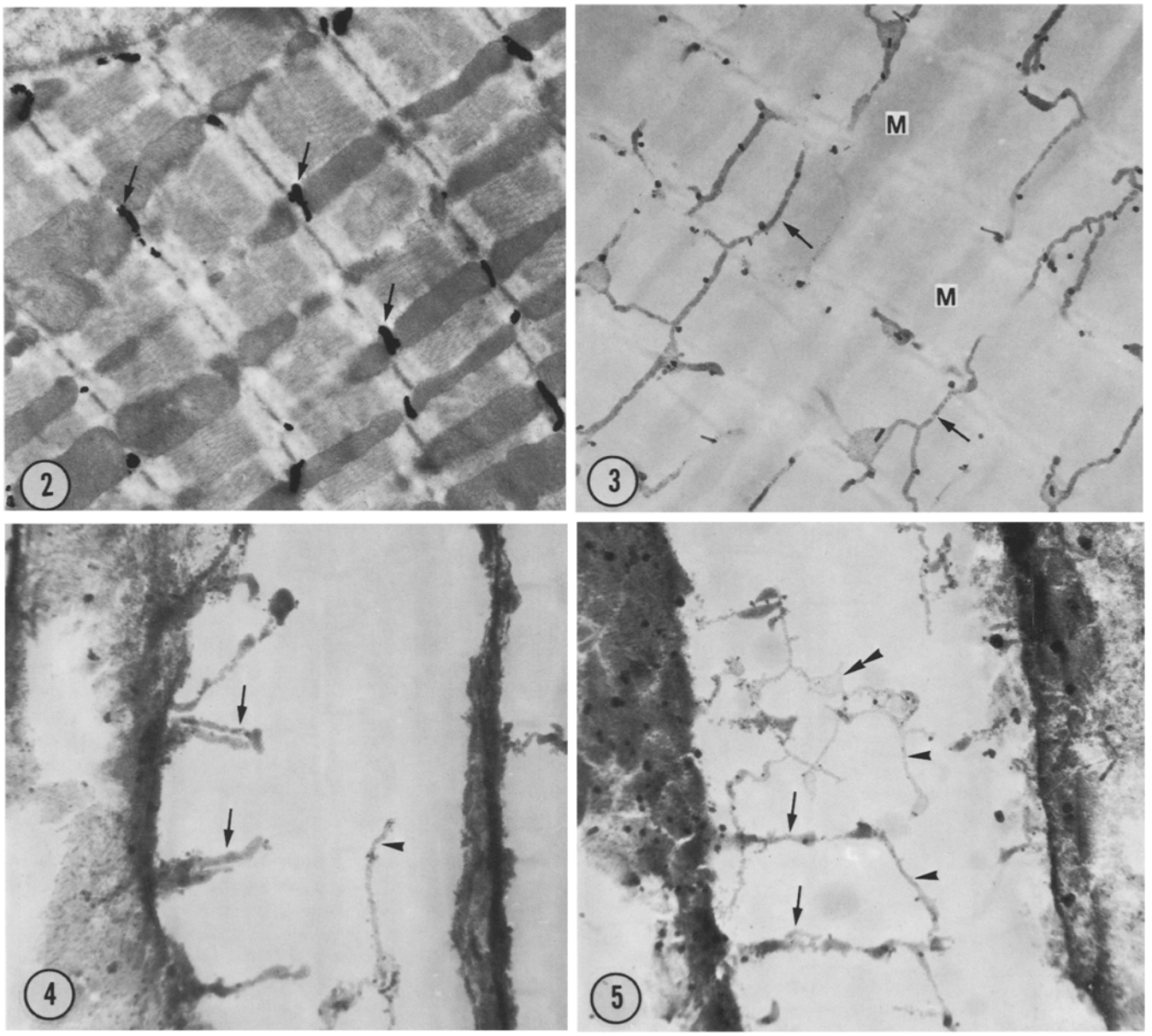


Fig. 2. An electron micrograph of the 0.1 μm thick section from a similar specimen as shown in Fig. 1. The T system (*arrows*) is shown as black profiles at the z bands. ×10,000

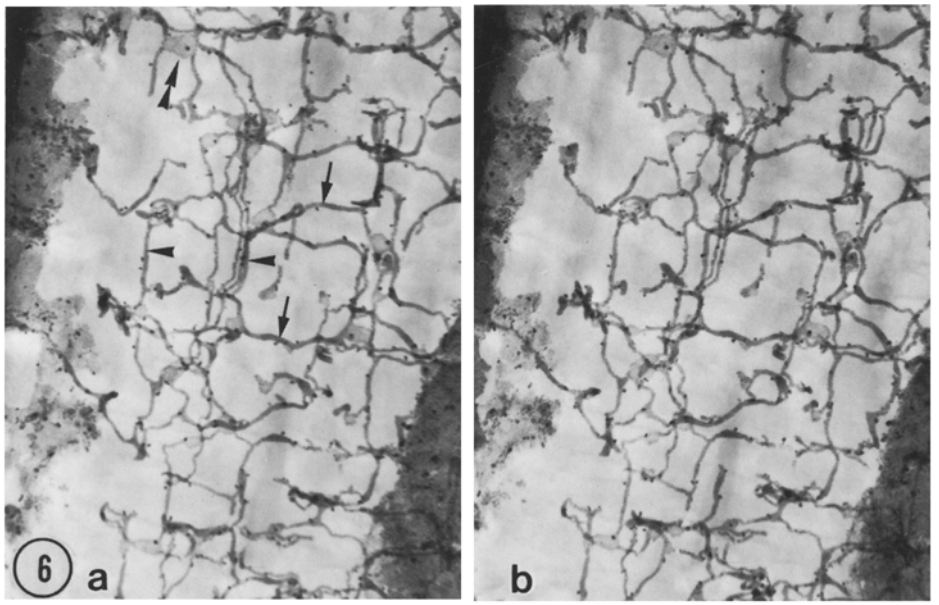
Fig. 3. A high voltage electron micrograph (HVEMG) of a 1 μm thick Golgi preparation. The continuity of the T system (*arrows*) is better demonstrated in 1 μm thick preparation than in the thinner (Fig. 1) one. *M* mitochondrion. ×10,000

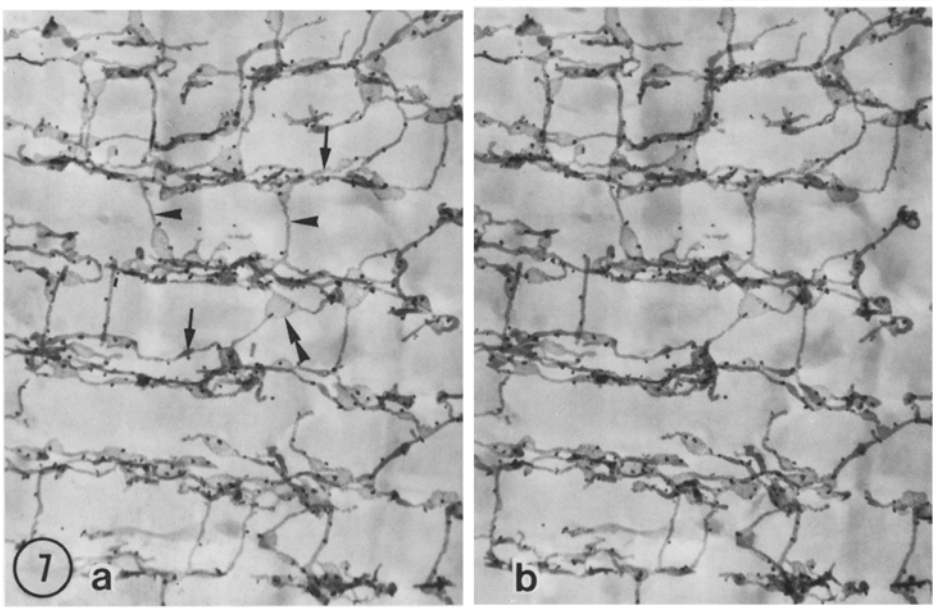
Fig. 4. A HVEMG of 3 μm thick section from a 28 g rat (13 days) left ventricule. T-tubules are poorly developed. The transverse tubules of the T system are indicated by *arrows*. A longitudinal tubule is indicated by *arrowhead*. ×9,800

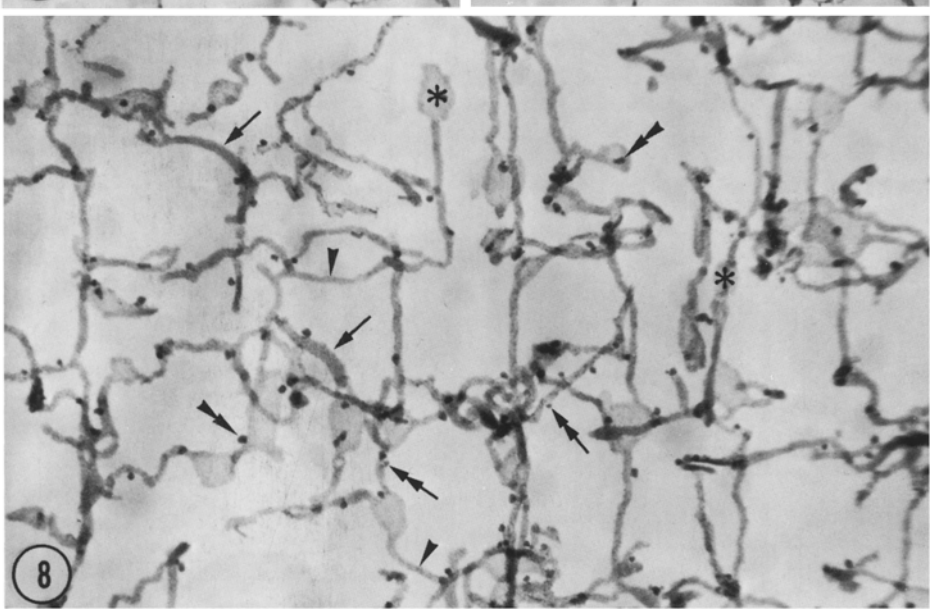
Fig. 5. 58 g rat (23 days) A HVEMG of the 3 μm thick section. The adjacent transverse tubules (*arrows*) are connected with the longitudinal tubules (*arrowheads*). The flattened cisternae are indicated by *double-arrowhead*. ×8,000

sections of these Golgi specimens revealed that the SR was closely associated with all elements of the T system irrespective of its orientation and morphological resemblances.

In thin sections of the ventricular myocardial cells of the 2–9 w rats, the flattened tubules were observed to form a coupling with the SR in both the longitudinal and transverse elements of the T system (Fig. 13). The rounded tubules were partly or completely surrounded by the junctional SR (Fig. not shown). The coupling of the flattened cisternae of the T system to the SR was confirmed by serial sectioning. The flattened cisternae of the T system were confirmed to form the coupling with the SR by serial sectioning (Fig. not shown). In thin sections, the T system of 23 w rats were recognized to be partly or completely surrounded by the junctional SR (Fig. 12). The T system component of the coupling was observed in thin section to remain relatively unchanged in diameter where it came into contact with the junctional SR.





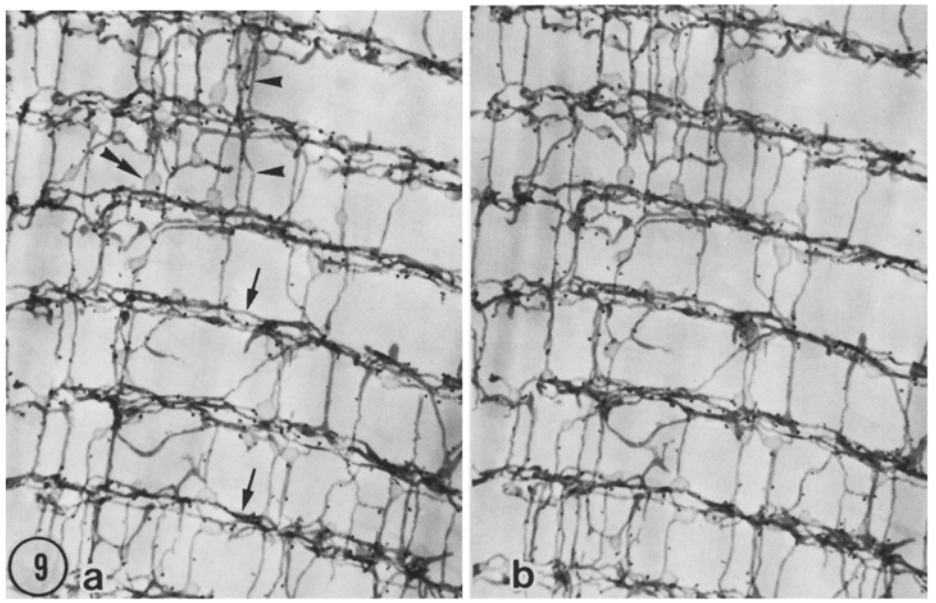


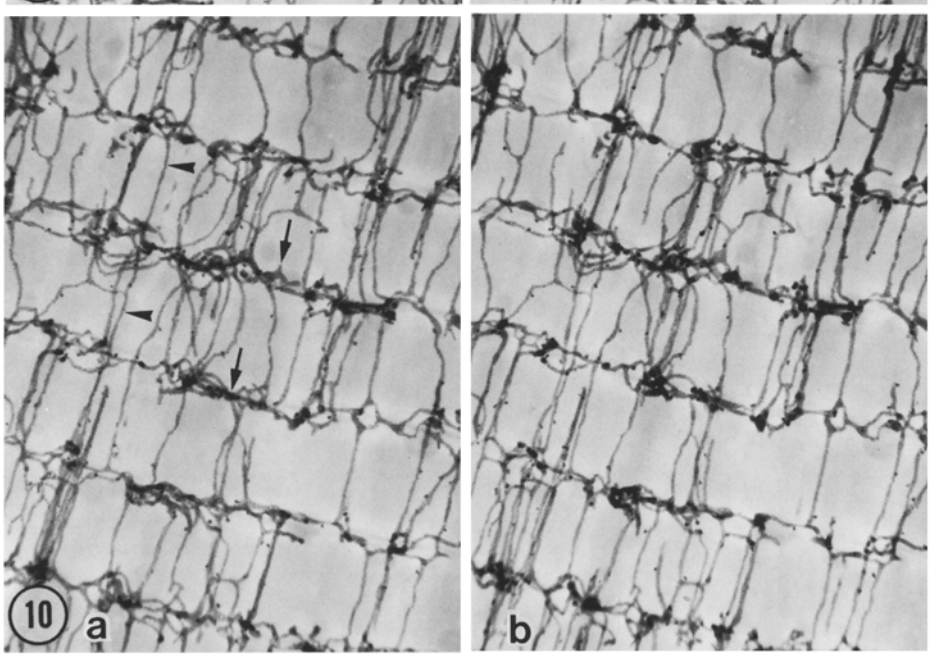
Figs. 6, 7, 9, 10, 11. Stereo pair HVEMGs showing the T system in the rat ventricular myocardial cell at different developmental stages. The specimens are 3 μm thick Golgi preparation. Tilting angles are ±8°

Fig. 6a, b. 120 g rat (31 days). The T system is accomplished as a loose three dimensional network at this stage. The T system consists of the transverse tubules (*arrows*), the longitudinal tubules (*arrowheads*) and the cisternae (*double-arrowhead*). ×5,600

Fig. 7a, b. 180 g rat (6 weeks). The transverse tubules (*arrows*) irregularly surround the z band of myofibrils and are connected by a small number of the longitudinal tubules (*arrowheads*). The cisternae are seen along the transverse tubules and vary in shape and size. They (*double-arrowhead*) are preferentially observed at the branching sites of the T system. ×5,700

Fig. 8. 200 g rat (7 weeks). The T system reveals two types of tubules: a ribbon-like flattened type (*arrows*) and a cylindrical type (*arrowheads*) corresponding to the flattened and rounded tubules in cross section. Some of the cisternae are found on the tip (*) and the middle (*) of the longitudinal tubules. A number of spotty regions (*double-arrowheads*) are associated with the T system, but palely stained ones (*double-headed arrows*) may be the expression of the smooth-surfaced micropinocytotic vesicles and/or coated pits. ×11,700





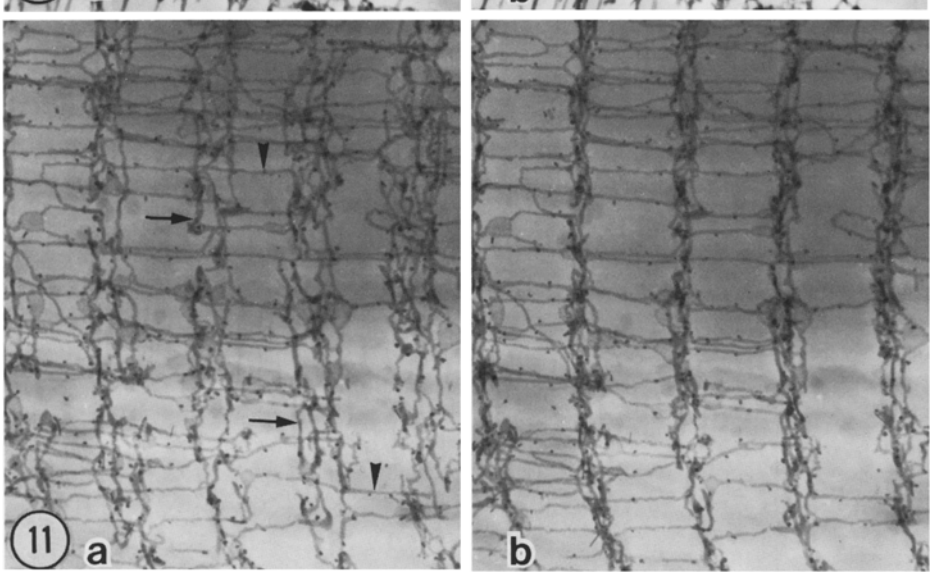


Fig. 9a, b. 240 g rat (8 weeks). The longitudinal tubules (*arrowheads*) gradually increase and the transverse tubules (*arrows*) surround the myofibrils more densely than those seen in Fig. 7. Some cisternae (*double-arrowhead*) are seen along the T system. × 5,600

Fig. 10a, b. 530 g rat (23 weeks). The transverse tubules (*arrows*) surround the myofibrils very densely and are connected by a number of longitudinal tubules (*arrowheads*). Few cisternae are observed. A larger part of the T system consist of cylindrical tubules. × 6,700

Fig. 11a, b. 530 g rat (23 weeks). The tilt axis is arranged to be perpendicular to the long axis of the cell. These stereo pair HVEMGs make it clearly visible that the transverse tubules (*arrows*) densely surrounded the myofibrils at the level of the z band. The longitudinal tubules are indicated by *arrowheads*. × 5,600

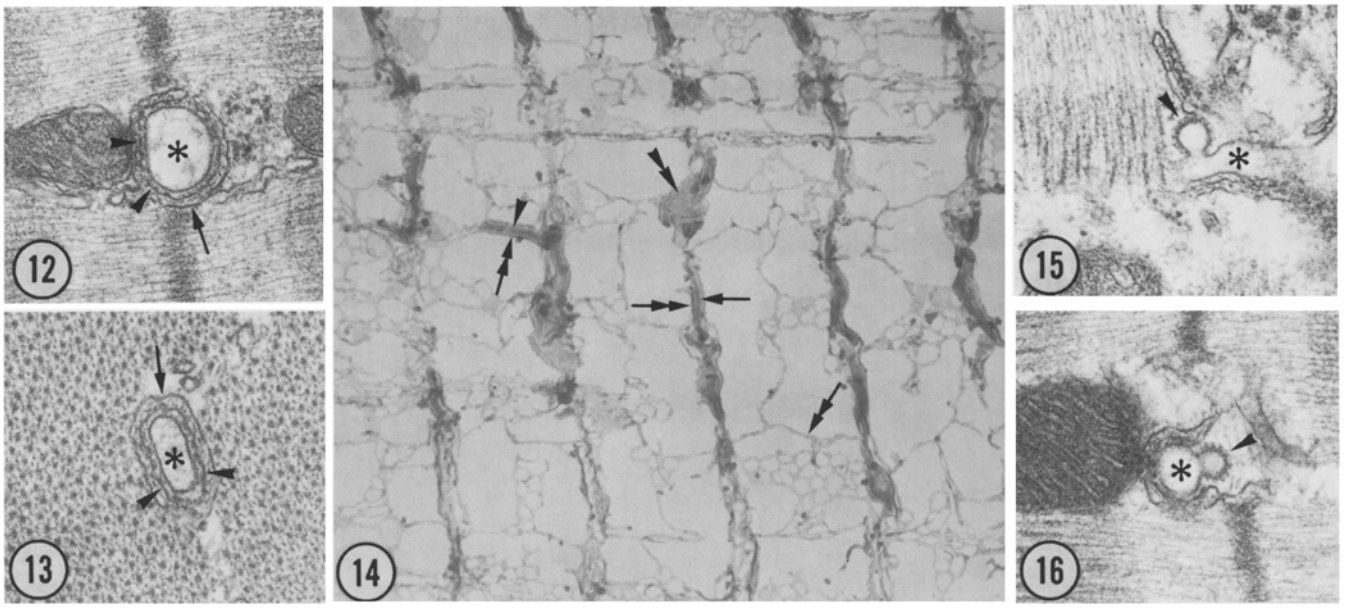


Fig. 12. A longitudinal section parallel with myofibrils, revealing a cross section of a transverse tubule (*) which forms the coupling with the SR (*arrow*). Junctional processes are indicated by *arrowheads*. × 52,000

Fig. 13. A transverse section of the myocardial cell discloses a cross section of a longitudinal tubule (*) studded with junctional processes (*arrowheads*) forming the coupling with the SR (*arrow*). × 52,000

Fig. 14. A HVEMG of a 1 μm thick section, which shows the simultaneous infiltration of the T system and SR. It reveals that all the transverse tubules (*arrow*), the longitudinal tubules (*arrowhead*) and the cisternae (*double-arrowhead*) are associated with the SR (*double-headed arrows*). × 10,000

Fig. 15. A longitudinal section of a ventricular wall. A coated pit (*arrowhead*) is seen to be associated with the T system (*). × 49,000

Fig. 16. A coated pit (*arrowhead*) is found at the free surface of the T system (*). × 49,000

Table 1

Rat body weight	58 g ($n=11$)	200 g ($n=10$)
Length $\mu m/\mu m^3$	0.435 ±0.063	0.830 ±0.114
Surface area $\mu m^2/\mu m^3$	0.169 ±0.022	0.299 ±0.035
Volume $\mu m^3/\mu m^3$	0.0040±0.00059	0.0075±0.0011

Our observation indicated that all elements of the T system have the potentiality of forming a coupling with the SR. It was unable to be determined from the present morphological study when and where the coupling between the T system and the SR was first formed.

Smooth-surfaced micropinocytotic vesicles associated with the T system (Forssman and Girardier 1966; Simpson and Rayns 1968), were detected in the present study (Fig. not shown). Coated pits were also found at the non-coupling regions of the T system in thin sections of our materials (Figs. 15, 16), and suggested the involvement of the T system in the exchange of the material. Most of the spotty regions seen along the T system in thick Golgi sections were artificial metal deposits based on comparison with conventional thin sections, but some of them, which were particularly palely stained (Fig. 8), might be the expression of the smooth-surfaced micropinocytotic vesicles and/or coated pits.

Measuring of the length, surface areas and volume of the T system

The length, surface area and volume of the T system at 23 days and 8 weeks were measured by using the stereo image analyzer described in Materials and methods. The data were summarized in Table 1. The length, surface area and volume of the T system per unit volume were expressed as $\mu m/\mu m^3$, $\mu m^2/\mu m^3$ and $\mu m^3/\mu m^3$ respectively.

These differences between 58 g and 200 g rat were statistically significant $P<0.01$.

Discussion

The present study showed the morphological differences in the three dimensional arrangement of the T system among rat hearts of different postnatal ages. Previous studies indicated that the development of the T system in the rat heart begins between 10 and 20 days postnatally (Schiebler and Wolff 1966; Hirakow and Gotoh 1975; Hirakow et al. 1980). The definitive T system was observed 13 days after birth in the present study. The size and shape of the T system is also known to change enormously during its development (Sommer and Johnson 1979). Our observation of thick sections revealed considerable variation in the morphology of the T system among the ventricular myocardial cells of the different ages. It became clear that as the development proceeded, the longitudinal tubules increased

in number, the transverse tubules were arranged more regularly and densely at the level of the z band, and the flattened cisternae transiently increased in number mainly along the transverse tubules during 2–9 weeks, but then decreased gradually. As a result, the ventricular myocardial cells of the 23 w rats were notable in that their T systems were far more regular in distribution, contour, and average diameter of its elements than those of the developing myocardial cells.

The T system in various striate muscles of the vertebrates was investigated under the HVEM, and revealed that the T system in developing striated muscles was quantitively less than in mature muscles and irregularly distributed with many longitudinal tubules. Characteristic flattened cisternae were found to be most numerous along the T tubules in newborn animals (Ishikawa and Tsukita 1983). Some differences in the distribution and form of the T system were also reported between red and white fibers in the striated muscles. In red fibers, the T system was less regularly arranged with more longitudinal tubules than were found in white fibers (Yamada and Ishikawa 1981; Ishikawa and Tsukita 1983). The T system in the cardiac muscle was somewhat more similar to that in red fibers than in white fibers. It displayed such structural similarities as flattened cisternae during its differentiation, although the T system in the cardiac muscle is characterized by a larger caliber and accompanying basement lamina (Sommer and Waugh 1976). The helicoidal arrangement of the T system, which was observed in the frog sartorius muscle (Peachey and Eisenberg 1978), was not confirmed in the cardiac muscle in the present study.

It has been postulated that the T system in cardiac muscle first appeared as caveolae and then formed slender tubules with an ununiform diameter along their length through caveolae proliferation and fusion. Enlargement of the T system occurred as a secondary step (Ishikawa and Yamada 1975). Their finding coincided with our present observation showing that the T system in cardiac muscle often displayed the beaded profiles in the early stage.

The functional role of the T system should be considered in at least the following two connections: in E-C coupling and in metabolic exchanges between the cells and their environment, although it has been argued in recent literature that the role of the T system in the cardiac muscle is not obvious in detail (Forssmann and Girardier 1970; Sommer and Waugh 1976; Sommer and Johnson 1979; Forbes et al. 1984). Müller demonstrated that the myocardium does not respond to a local stimulus by local contraction, as was shown by Huxley and co-workers in various skeletal muscles, but that contraction spreads over at least a few sarcomeres (Müller 1966). This peculiar response of cardiac muscle to local stimulation was supposed to be due to the more predominant development of the longitudinal tubules in the cardiac muscle seen in present material than that in the striate muscle. Forbes et al. (1984) proposed that the T system promotes the influx of Ca^{++} at multiple levels within each myocardial cell, thereby shortening the diffusion distance and time required to activate the contractile proteins, assuming that the voltage- and time-dependent Ca-Na slow channels of the myocardial cells were located in the membrane of the T system as well as in the surface sarcolemma. The morphological development of the T system in cardiac muscle, such as the increase of the longitudinal tubules and the regular arrangement of the transverse tubules at the level of the z band seen in adult rats, not only provided membrane augumentation in response to enlargement of cellular diameter, but was probably essential to ensure simultaneous initiation of contraction of the whole population of myofibrils by the even, simultaneous distribution of excitation along the length of the cardiac cell.

The stereo observation of 2 μm thick sections at the HVEM level permitted the visualization of the continuous network of the T system, and enabled direct measurement of the length, surface areas and volume of the T system. Quantitative analysis of the thin sections requires either a reconstruction from serial sections or application of stereological procedures with assumptions on the shape of the T system. The difficulty accompanying the stereological methods has been pointed out by some authers (Loud et al. 1965; Bossen et al. 1978). Theoretically, stereological methods give best statistical approximations of isotropic (randomly oriented) structures when random sampling, embedding and sectioning techniques are employed. Various parameters to be measured are affected by the degree of order present. For example, the estimation of surface area per unit cell volume is more affected by anisotrophy than is the volume fraction. However, the T systems were not evenly distributed throughout the cardiac muscle. This meant that the stereological measurement of the T system was subject to serious errors. Stereological studies on the cardiac muscles reported in the literature have avoided random sampling by making use of various mathematical formulae which under certain rigorously defined circumstances, may be applicable to anisotropic, i.e., ordered samples. It may be impossible to compensate for the anisotrophy of the structure in computing, as the extent of existing biological variations does not permit one to safely accept one configuration as a valid representation of the characteristics of the structures in question. The analysis system of the three dimensional quantities from stereo images of the depth dimension provided us with great advantages over the stereological procedures in these problems. This system needs neither serial sectioning nor large quantities of randomly oriented sections. Rats have been used most extensively for quantiative studies of the heart and the volume fraction of the T system determined for rats achieved similar values of approximately 1–2% (Page et al. 1971; Pager 1971; McCallister and Page 1973; Page and McCallister 1973; Page et al. 1974; Anversa et al. 1976; Hirakow et al. 1980). Most of the data have come from Page et al. (1971) who have reported the values for the volume and surface areas per cell volume of the T system $-0.012\ \mu m^3/\mu m3$ at $\times 32{,}000$ and $0.07\ \mu m^2/\mu m^3$ at $\times 9{,}000$ by the stereological procedures. Our value for the volume of the T system $-0.0075\ \mu m^3/\mu m^3$ was slightly less than the value given by Page et al., and on the other hand, our value for the surface areas of the T system $-0.299\ \mu m^2/\mu m^3$ was considerably larger than the value given by Page et al. The reasons for this discrepancy were not immediately apparent although some possible explanations come to mind. The diameter of the T system measured in the Golgi specimen was slightly less than that in thin sections probably because of the existence of the glycoprotein coat in the T system which prevented the depositing of the silver chromate close to the plasma membrane. As the silver deposits in the T system were measured in the present study, the values for the volume and surface areas of the T system might be subject to underestimation when compared with the case

of Page et al. who used a thin section preparation. However, our value for the surface areas of the T system was markedly larger than the value given by Page et al. This discrepancy was supposed to be due to the difference between the direct and stereological measurement. Mathematically, the surface-to-volume ratio of the rounded tubules was smaller than that of the flattened. Assuming that the T system consisted only of rounded tubules, the diameter of the T tubules was estimated from the following formula: $S=2\pi rl$, $V=\pi r^2 l$, $r=2V/S$ where r=half diameter, l=length, S=surface areas, and V=volume. The diameter calculated from our values for the volume and surface areas of the T system was 100 nm, and that from the values given by Page et al. was 686 nm. The diameter of Page et al. was much greater than ours, although our diameter was relatively similar to the values measured in thin sections. Pager (1971) stereologically estimated the values of the rat T system, selectively stained with lanthanum compound at ×40,000, and gave values of 0.01 $\mu m^3/\mu m^3$ and 0.25 $\mu m^2/\mu m^3$, which were closer to our results than the values given by Page et al. (1971). These discrepancies between the two values measured by the stereological method were supposed to arise from the difference in the assumption of the morphology of the T system and the techniques such as selective staining, and suggested the difficulty of the application of stereological methods for the measuring the ordered samples such as the T system.

The tissue examined has usually been fixed and otherwise processed for electron microscopy and, therefore, should not be directly compared with the living counterpart, unless rigorous precautions have been taken, e.g. quick freezing techniques which promise to make such comparisons more admissible. Eisenberg and Mobley (1975), for example, have shown tissue shrinkage of up to 45% during processing.

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