

# A STUDY OF THE T SYSTEM IN RAT HEART

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## ABSTRACT

The technique of extracellular space tracing with horseradish peroxidase is adapted for labeling the transverse tubular system (T system) in rat heart. In rat ventricular muscle the T system shows extensive branching and remarkable tortuosity. The T system can only be defined operationally, since it does not display specific morphological features throughout its entire structure. Owing to branching of the T system, a sizable proportion of the apposition between the T system and L system (or closed system) occurs at the level of longitudinal branches of the T system and is not restricted to the Z line region. The regions of apposition between the T system and L system are analyzed in rat ventricular muscle and skeletal muscle (diaphragm) and compared with the intercellular tight junctions (nexuses) of heart muscle by the use of a photometric method. The over-all thickness of the nexus is significantly smaller than that of T-L junctions in both cardiac and skeletal muscles. The thickness of the membranes of the T and L systems are not significantly different in the two muscles, but the gap between both membranes is larger in the heart. In atrial muscle the following two types of cells are found: (a) those cells with a well-developed T system in which the tubular diameter is quite uniform and the orientation predominantly longitudinal and, (b) cells with no T system, but with a well-developed L system. Atrial cells possessing a T system are richly provided with *specific* granules and show little micropinocytotic activity, whereas cells devoid of T system show intense micropinocytotic activity and few specific granules. The possible functional implications of these findings are discussed.

## INTRODUCTION

In a recent paper (7), we studied the sarcoplasmic reticulum of rat heart muscle by means of serial sections and ferritin diffusion tracing.<sup>1</sup> Our study showed that in rat heart the sarcoplasmic reticulum, and especially the tubules of the T system (transverse tubular system), are of great complexity. Our results left us with the question: Does the T system in heart muscle communicate with the L system, or is the T system simply ramified? With

the methods available at that time, we were unable to answer this question.

The introduction of a new method for tracing free diffusion in the extracellular space (2, 15, 28, 29, 33, 52) has made possible a fresh approach to the problem. Preliminary reports of this study have been published elsewhere (8, 9).

## MATERIALS AND METHODS

### *Untreated Tissue and Histochemical Controls*

The hearts of male and female rats weighing from 180 to 300 g were removed after perfusion-fixation according to a recently published method (10). All histochemical and permeability controls were done as described by Karnovsky (29).

<sup>1</sup>The term sarcoplasmic reticulum is used according to Porter and Palade (39); longitudinal tubules and terminal cisternae sealed off from the extracellular space are called L system. All open tubular profiles (intermediate vesicles [39]) are considered as belonging to the T system.

### Application of the Peroxidase

Three different methods were evaluated in succession.

(a) The first method consisted in injecting the peroxidase<sup>2</sup> (50–100 mg per animal) into the vena femoralis. The animals were sacrificed by decapitation at intervals ranging from 1–45 min after injection. Fixation was performed in vitro either according to Karnovsky (27), or in 6.5% glutaraldehyde buffered with 90 mM sodium phosphate at pH 7.4. After thorough rinsing in 0.2 M sodium cacodylate solution, the tissue was incubated in a solution containing 3,3'-diaminobenzidine and H<sub>2</sub>O<sub>2</sub> (100 ml Tris buffer of 50 mM; 100 mg 3,3-diaminobenzidine, pH 7.45–7.5; 0.1 ml H<sub>2</sub>O<sub>2</sub> added before incubation) after the method of Graham and Karnovsky (15). Finally, postfixation was carried out in buffered 1% OsO<sub>4</sub>.

(b) In the second method the peroxidase tracer was perfused via the abdominal aorta at a concentration of 0.5 mg/ml in Tyrode's solution at 35°C for 1–5 min. Perfusion-fixation was then performed as in the controls.

(c) The final method combined perfusion-fixation and intravenous injection of peroxidase. The aorta of the anaesthetized animal was dissected so as to enable the perfusion cannula to be introduced rapidly. 50–100 mg horseradish peroxidase were then injected into the previously exposed femoral vein. The perfusion cannula was inserted into the aorta just before fixation. After the inferior vena cava was opened, the fixative (2.3 % glutaraldehyde in 75 mM NaCl or 2% glutaraldehyde in 90 mM phosphate buffer) was injected rapidly, without preliminary washing. After 2–5 min the glutaraldehyde fixative was replaced by the Karnovsky fixative (27). When it was desired to

<sup>2</sup> Fluka or Sigma type IV.

fix within less than 1 min after peroxidase injection, the clamped perfusion cannula was inserted and remained filled with heparin (Liquemin®, Hoffmann-La Roche<sup>3</sup>) in the aorta while peroxidase was injected into the jugular or brachial vein. After fixing for 10 min and washing out by perfusion of 150 mM sodium cacodylate, thin slices of tissue were incubated and postfixed as indicated above (32).

### Observations

Our experiments have demonstrated that the third method was the most satisfactory, regularly resulting in homogeneous fixation. Furthermore, this method has the advantage of permitting an accurate timing of diffusion. This method gave a uniform distribution of the tracer when fixation was performed 2–45 min after peroxidase injection, and no difference in labeling could be detected by double-blind tests<sup>4</sup> when the tissue was harvested from 2 to 45 min after injection of the tracer. For this reason, all figures have been chosen from animals that were perfusion-fixed 5 min after injection of the tracer. A detailed time study will be published elsewhere (W. G. Forssmann, in preparation).

### Sectioning and Observation

Small fragments of myocardial tissue were dehydrated in alcohol and embedded in Epon (31). Sections were prepared for both phase-contrast

<sup>3</sup> Kindly supplied by F. Hoffmann-La Roche and Co. A. G., Basle, Switzerland.

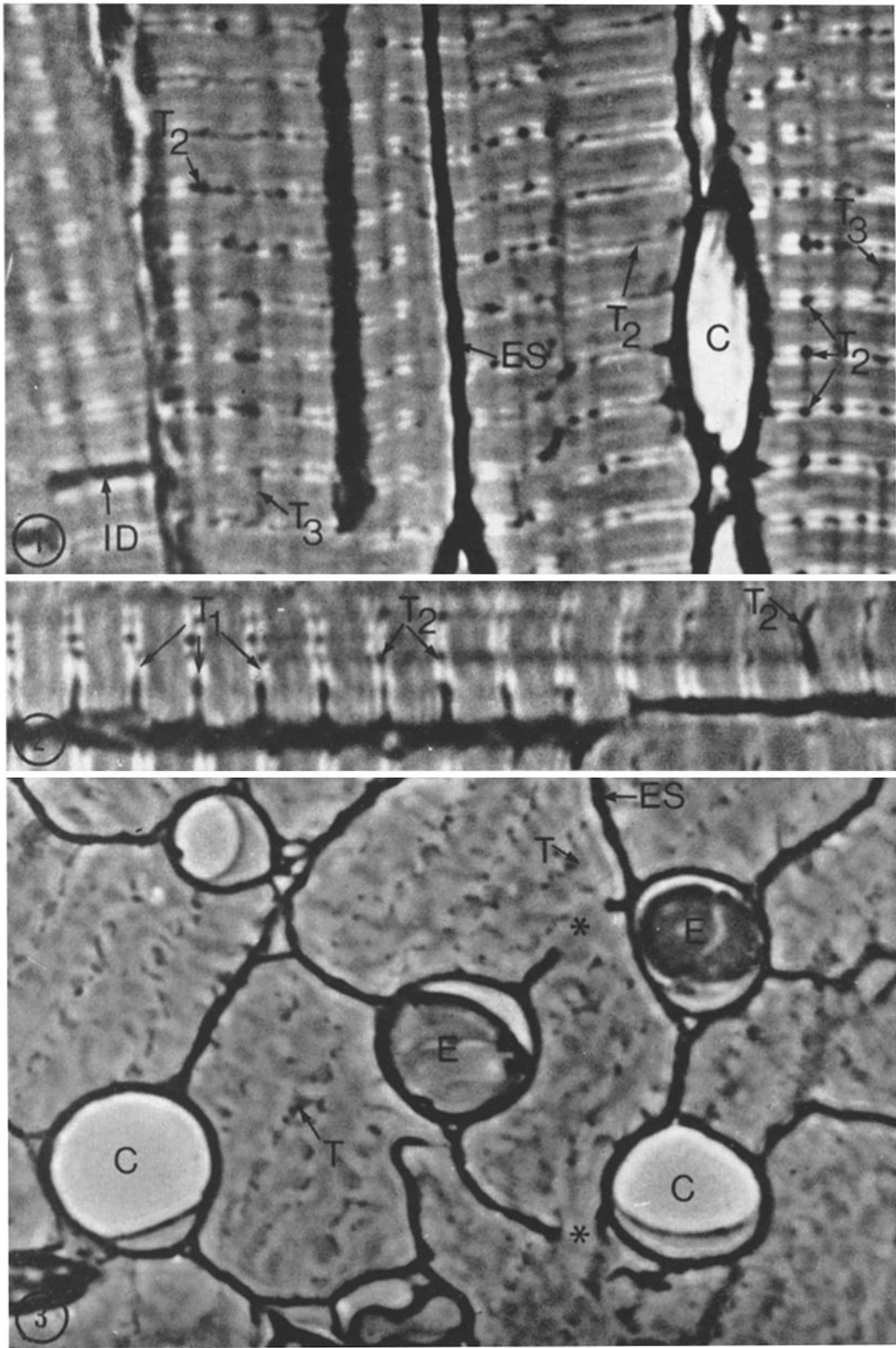
<sup>4</sup> Double-blind test means that one observer chose the example at the electron microscope and the other evaluated the results, but neither knew the time delay after peroxidase injection. Only a third observer knew the coded experiments.

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FIGURE 1 Phase-contrast micrograph of right ventricle after peroxidase injection. The black reaction product is seen in the extracellular space (ES) around the capillaries and in the T system of the sarcoplasmic reticulum. The tracer has been washed out of the lumen of the capillary (C) during perfusion-fixation. Tubules of the T system also run transversely at the level of the Z band (T<sub>2</sub>), with oblique or longitudinal branches (T<sub>3</sub>). The intercalated disc is also stained (ID). × 3,900.

FIGURE 2 Micrograph as in Fig. 1 showing two neighboring myocardial cells, one of which shows a series of invaginations of the T system in continuity with the extracellular space (T<sub>1</sub>). The T system inside the cell is also stained with the black reaction product (T<sub>2</sub>). × 4,200.

FIGURE 3 Micrograph as in Fig. 1 but transverse section through myocardial cells. The capillaries are round and washed out by perfusion (C) or contain erythrocytes surrounded by reaction product (E). Occasionally the extracellular space (ES) seems to be interrupted (\*), due to lateral nexuses that usually do not contain peroxidase. The T system (T) appears to be discontinuous (for explanation, see text). × 2,900.



microscopy and electron microscopy with Sorval and LKB microtomes. Light microscope observations were made with a Zeiss phase-contrast photomicroscope. After further staining with lead hydroxide (26), the ultrathin sections were observed with a Zeiss EM 9 or a Philips EM 300 electron microscope.

### Densitometry

Negatives of preparations of cardiac muscle and diaphragm were used. Material for these measurements was obtained by using perfusion-fixation and en bloc staining with uranyl acetate in 70% alcohol. The Philips EM 300 microscope was calibrated with a replica grid of 2157 lines/mm. All negatives used were taken during the same session with the same magnification ( $\times 51,220$ ) and with the same excitation voltage of the lenses of the electron microscope (except the objective). The densitometric tracings were recorded on a Chalonge microdensitometer with a slit of 30–300  $\mu$ . The slit was adjusted parallel to the membranes with a focusing microscope. The densitometer beam was made to pass through the T-L junction at points where the membranes appeared to have been cut perpendicularly, i.e. where the membranes displayed their maximum density and contrast. A preliminary goniometric study showed that it is correct to consider maximum density and contrast as a criterion of perpendicularity.

## RESULTS

### Tracing with Horseradish Peroxidase

#### VENTRICULAR MUSCLE

PHASE-CONTRAST MICROSCOPY: Figs. 1–3 show that with our method a homogeneous distribution of peroxidase is obtained. The black reaction product uniformly fills the extracellular space, which appears to be very narrow after perfusion-fixation. Some T system elements are clearly visible at the Z band (Figs. 1 and 2) as dense, beaded lines. Longitudinal branchings of this T system<sup>5</sup> are easily seen ( $T_3$  in Fig. 1), sometimes extending over several sarcomeres. Invaginations of the sarcolemma can also be observed at the level of the Z line, in some sections on several

<sup>5</sup> The following abbreviations of the T system are used in this paper: T, T tubules;  $T_1$ , invagination of T system;  $T_2$ , transverse T system;  $T_3$ , longitudinal T system;  $T_{3a}$ , longitudinal T system  $> 1000$  A;  $T_{3b}$ , longitudinal T system of about 1000 A;  $T_{3c}$ , longitudinal T system of 500–850 A;  $T_{3d}$ , longitudinal T system  $< 500$  A.

consecutive sarcomeres ( $T_1$  in Fig. 2). In transverse sections the T tubules can never be followed over a long distance (Fig. 3). This is probably due to the tortuosity of the tubes as can be observed in electron microscopy (see below).

## ELECTRON MICROSCOPY

THE T SYSTEM AT THE Z BAND LEVEL: The T system is not restricted to the Z-band level. It was found that the size, shape, and distribution of T tubules were far less uniform than in skeletal muscle (Fig. 4). Numerous ramifications were observed, mostly within the outermost third of the sarcomere, next to the Z band (Figs. 5–8). Occasionally the branchings appeared dichotomous (Fig. 7), although we often found two to three ramifications branching out at right angles to the T tubules over a short distance (Fig. 8).

The numerous branchings provided a partial explanation for the very frequent observation of several peroxidase-labeled profiles at the same Z band (Figs. 7, 9, and 10). Another reason for these multiple profiles may be the frequently tortuous path followed by the T tubules (Figs. 9–12). In places, the T tubules make hairpin bends and turn back on themselves (Figs. 11 and 12). In such instances, they may almost completely surround a nonlabeled tubular profile (Fig. 12). In the absence of labeling, the central element of such patterns certainly would have been interpreted as a T tubule, and the lateral profiles as cisternae belonging to the L system. The considerable tortuosity of the T tubules (Figs. 9–12, 14, 15) accounts for the fact that in transverse sections the tubules could seldom be followed over long distances (Figs. 3 and 18).

LONGITUDINAL T SYSTEM: Among the longitudinally oriented branches of the T system, four types can be distinguished.

(a) Tubules located where the Z lines of two adjacent myofibrils are not in phase. The diameter of these tubules is greater than 1000 A, and they are sometimes surrounded by empty profiles probably belonging to the L system ( $T_{3a}$  in Figs. 16 and 17). In untreated preparations these tubules are invested by a basement membrane and are consequently easily identified as belonging to the T system.

(b) Tubules located between two myofibrils having their Z lines in phase. Occasionally these tubules easily can be mistaken for elements of the

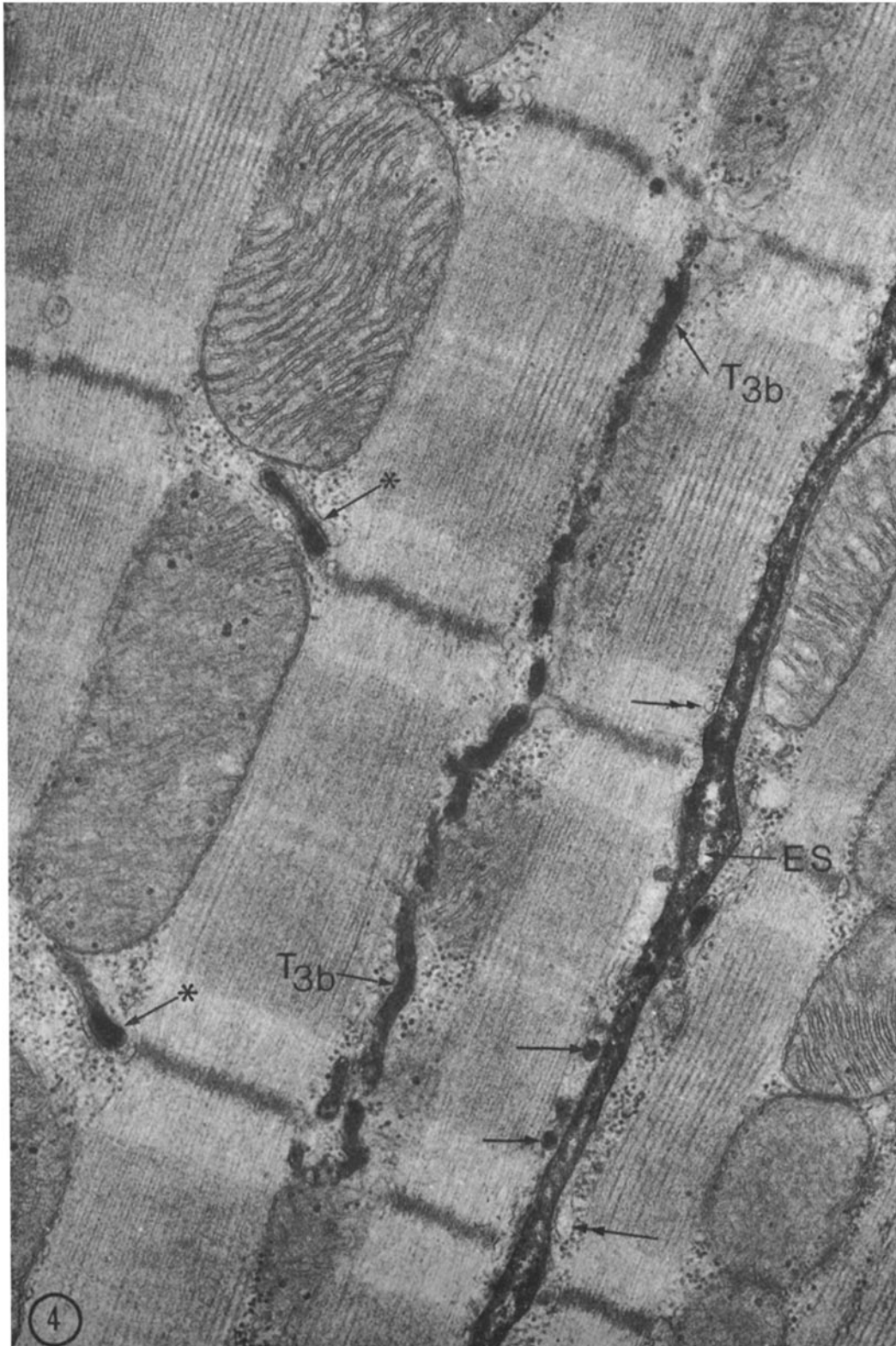


FIGURE 4 Electron micrograph of right ventricle revealing labeled T system and extracellular space (ES). The T system shows several transversely running tubules (\*), as well as longitudinally oriented profiles between myofibrils that are in phase ( $T_{3b}$ ). Under the sarcolemma we find labeled (arrows) and unlabeled (double arrows) cisternae.  $\times 36,000$ .

L system, since the basement membrane is not always clearly defined over the entire course of the tubule. The diameter of these tubules is about 1000 Å. In transverse sections these tubules are found next to the A band, and their cross-section is circular ( $T_{3b}$  in Fig. 18). The elements of the L system rarely surround them completely.

(c) Still easier to confuse with the L system are tubes with a mean diameter of 500–850 Å and a tortuous course ( $T_{3c}$  in Figs. 14 and 18). Seldom can empty profiles be found in their vicinity.

(d) Also observable are tracer-labeled tubes with a diameter of 300–500 Å ( $T_{3d}$  in Fig. 16). They are difficult to interpret, especially when, as in Fig. 16, elements of the L system seem to be continuous with the filled tubules. Such images are probably due to superpositioned artifacts in the 500 Å thick sections.

**MICROPINOCYTOSIS AND SUBSARCOLEMAL CISTERNAE:** Micropinocytotic vesicles were observed immediately beneath the sarcolemma and in the neighborhood of all membranes continuous with it. Thus, vesicles were also found at the intercalated disc and next to the T tubules (Figs. 6 and *inset*).

Yet another type of membrane structure was found in the subsarcolemmal area, consisting of elongated cisternae of markedly larger size than micropinocytotic vesicles (7). These are also unlabeled cisternae, belonging to the L system beneath the sarcolemma (Fig. 13).

#### ATRIAL MUSCLE

**GENERAL OBSERVATIONS:** The diameter of rat atrial cells is smaller than that of ventricular cells. Labeling with peroxidase revealed that in most of the cells, labeled tubules, i.e. T system, were either missing or poorly developed; however,

some atrial cells exhibited a highly developed network of these tubules. The cells that did possess a T system showed a homogeneous over-all distribution of T tubules.

**CELLS WITHOUT T SYSTEM:** The cells containing no T tubules seemed to be almost devoid of specific granules (25). These cells also showed an intense micropinocytotic activity (Figs. 19 and 20). Numerous vesicles labeled with the black reaction product were found along the sarcolemma. On the other hand, the bulk of the cytoplasm showed no pinocytotic vesicles at all. The vesicles, when detached from the sarcolemma, may lose their peroxidase reactivity. On the other hand, it could be that micropinocytotic vesicles do not migrate from the sarcolemma to the interior of the cell. The L system (unlabeled) is well developed in these cells.

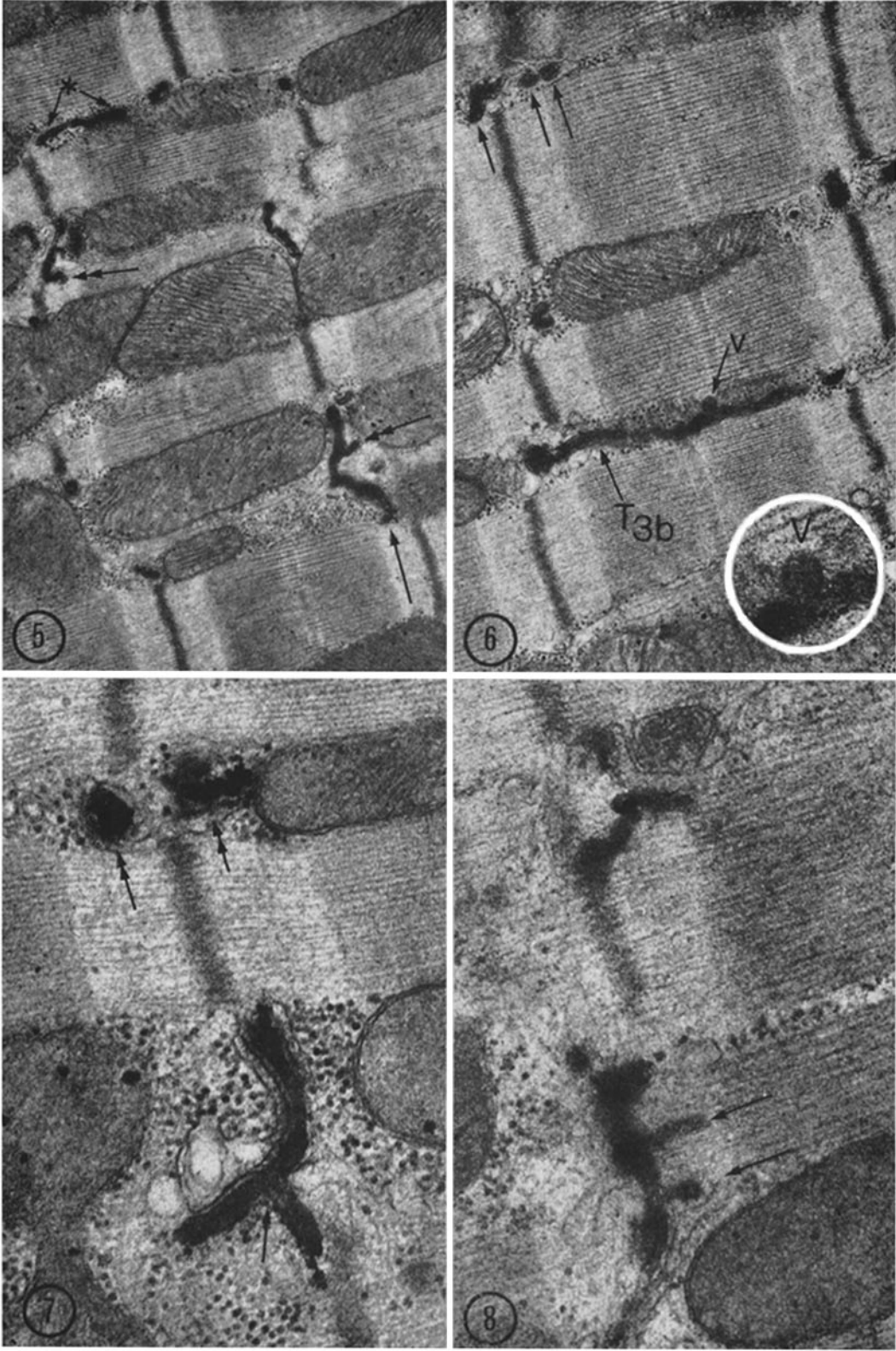
**CELLS WITH T SYSTEM:** These cells contained a network of small tubules predominantly oriented parallel to the myofibrils, along the longitudinal axis of the muscle fiber (Fig. 21). The diameter of these T tubules seemed constant at about 800 Å. In untreated preparations, a basement membrane could not be easily discerned in the T tubules and, as in some of the ventricular muscle T tubules, the distinction between T and L profiles was rather difficult. T–L junctions were found in these cells, most of them being comparable to the T–L junctions found along longitudinal T tubules in ventricular muscle. In general, the cells containing a considerable amount of labeled tubules showed a higher content of specific granules (25) (cf. Figs. 19 and 21).

#### *The Morphology of T–L Junctions*

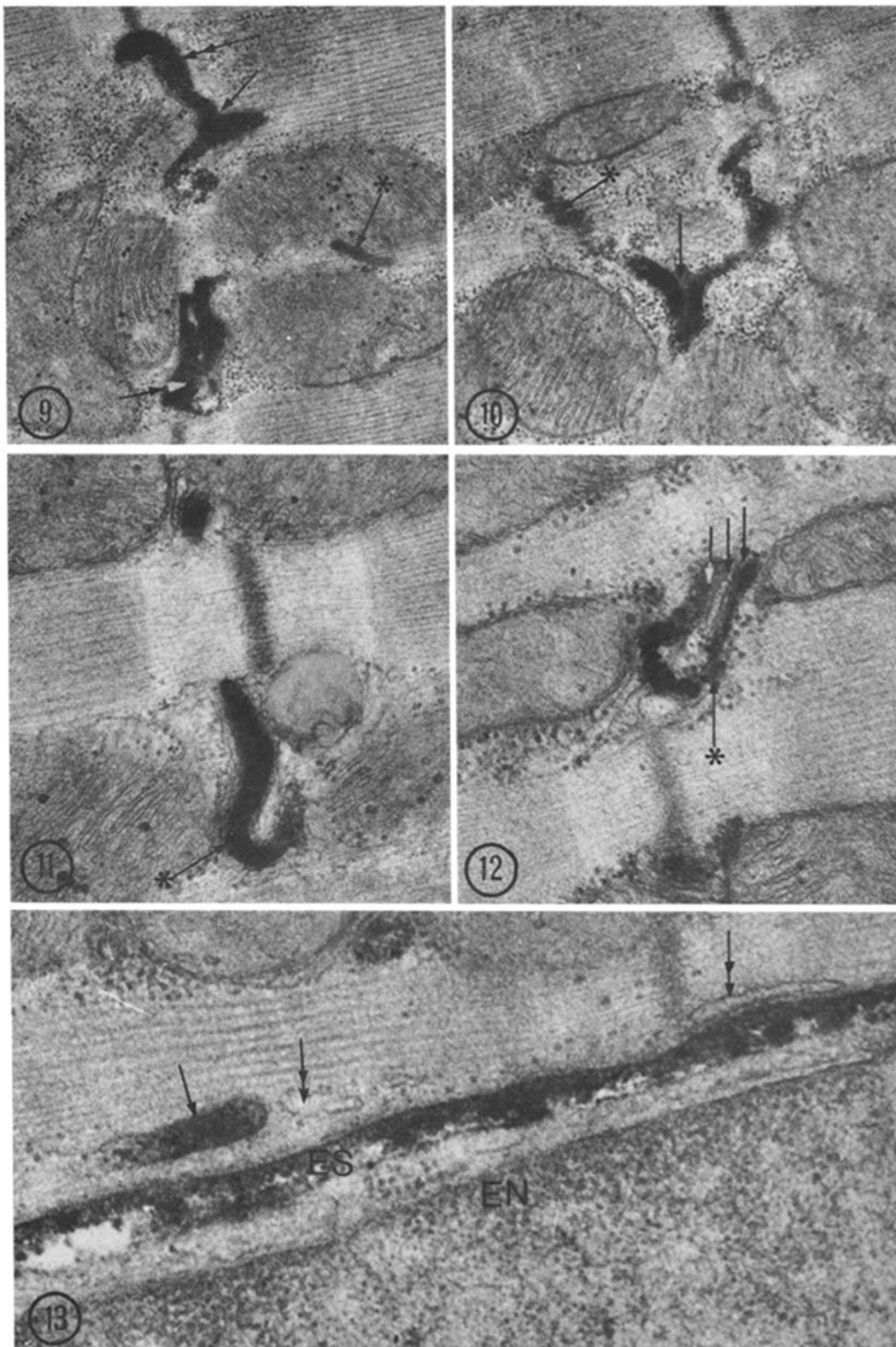
As pointed out above, in cardiac muscle the T tubules course alongside the L tubule for some distance, a situation that gives rise to regions of ap-

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**FIGURES 5–8** T system in right ventricle showing the complex structure of its tubules. Fig. 5 shows some tortuous T tubules (arrows) with lateral short branchings (double arrows). A longitudinal tubule is also labeled (\*).  $\times 18,000$ . Fig. 6 shows a longitudinal labeled tubule ( $T_{3b}$ ) traversing a whole sarcomere and a branch resembling a micropinocytotic vesicle; the latter is shown at a higher magnification in (*inset*, *v*). In the upper part of the figure three neighboring profiles are seen (arrows).  $\times 24,000$ ; *inset*  $\times 75,000$ . Fig. 7 shows branching of a tubule running alongside the Z band (arrow) and two profiles at same level (double arrow).  $\times 63,000$ . Fig. 8 shows two branches bearing a labeled tubule at right angles (arrows). These branches may be continuous with some of the longitudinal profiles.  $\times 63,000$ .



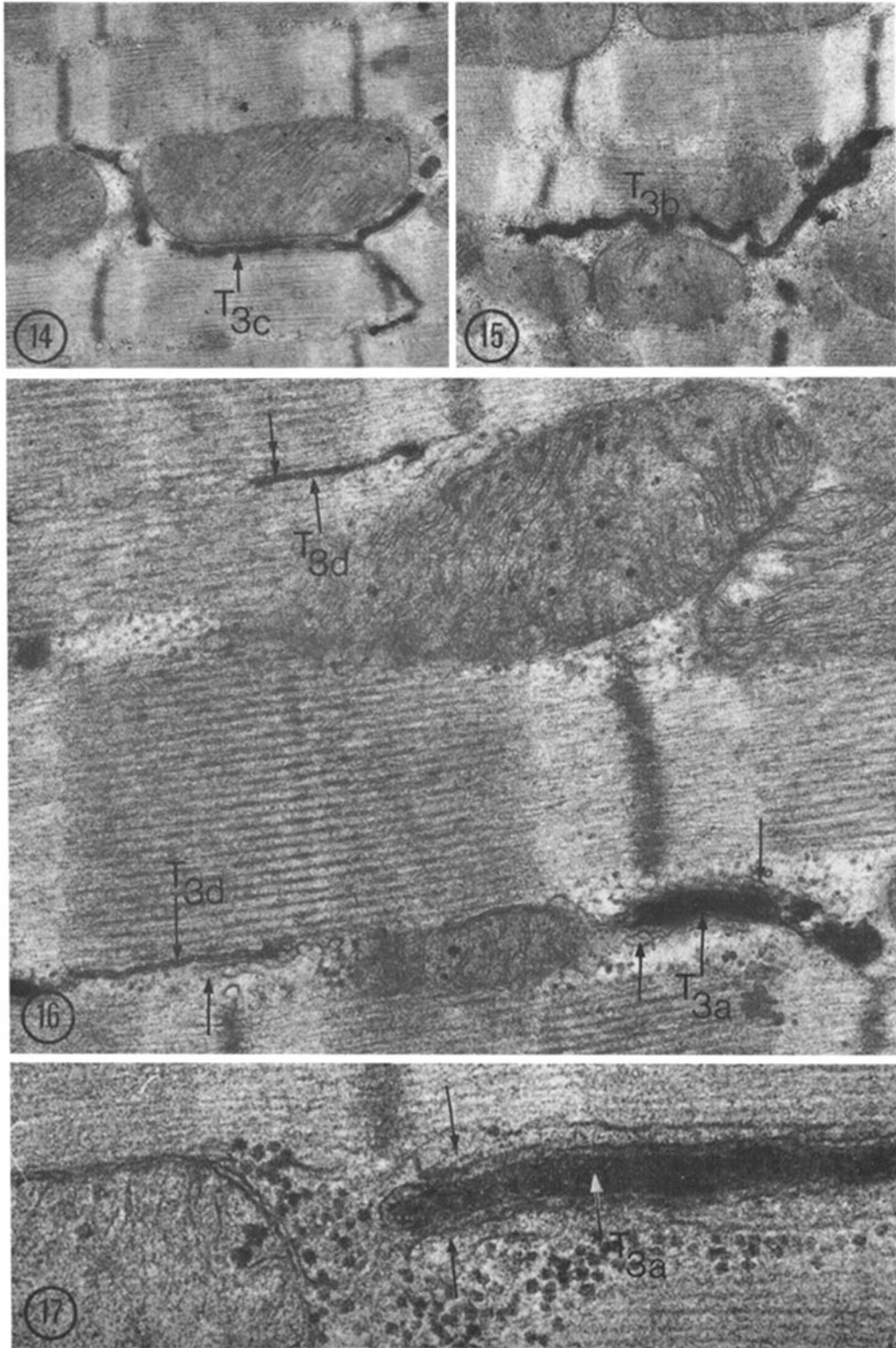




FIGURES 9-12 Electron micrographs of right ventricle illustrating the tortuosity of the T system lining the Z band. Branchings (arrows) and dilated regions (double arrows) are often seen. Tubules that would normally be interpreted as belonging to the L system can be seen in Figs. 9 and 10 (\*). Sometimes hairpin-like, labeled tubules (\*) may form triadoids as in Fig. 12 (three arrows). Figs. 9 and 10  $\times 20,000$ ; Figs. 11 and 12  $\times 19,000$ .

FIGURE 13 Right ventricle; organization of the T system near the sarcolemma. The cisternae near the sarcolemma are labeled (arrow) or unlabeled (double arrows). Endothelial cell, (EN); extracellular space, (ES).  $\times 56,000$ .





FIGURES 14-17 Different types of longitudinal T tubules seen in right ventricular cells. For explanation see text. Note especially the smallest tubules ( $T_{3d}$ ) in comparison to the thick ones ( $T_{3a}$ ). The T-L junctions are seen in Figs. 16 and 17 (arrows). One of the small T tubules in Fig. 16 seems to be continuous with an L tubule (double arrow); in fact, this may be due to superposition (see text). Figs. 14 and 15  $\times 19,000$ ; Fig. 16  $\times 46,000$ ; Fig. 17  $\times 70,000$ .

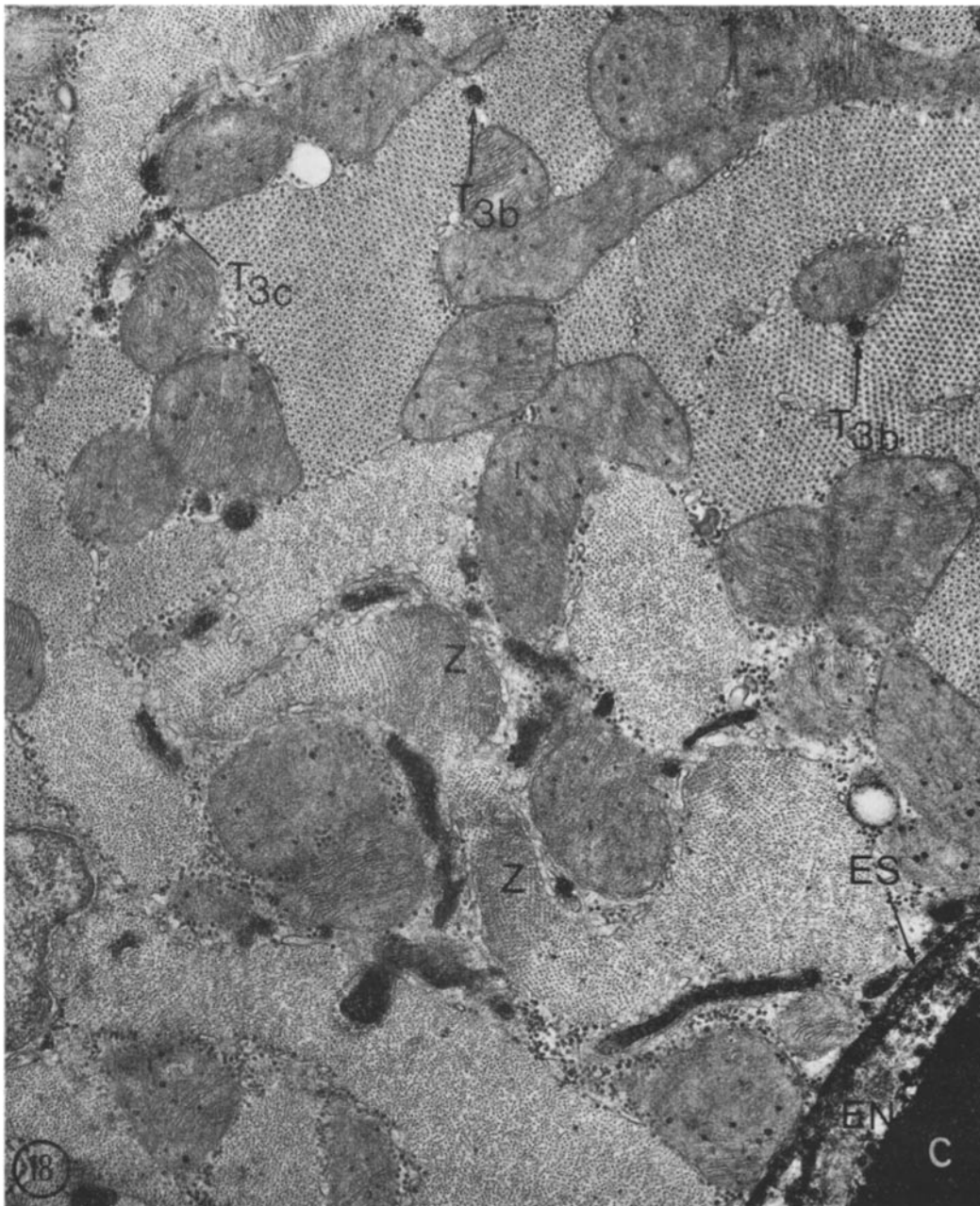
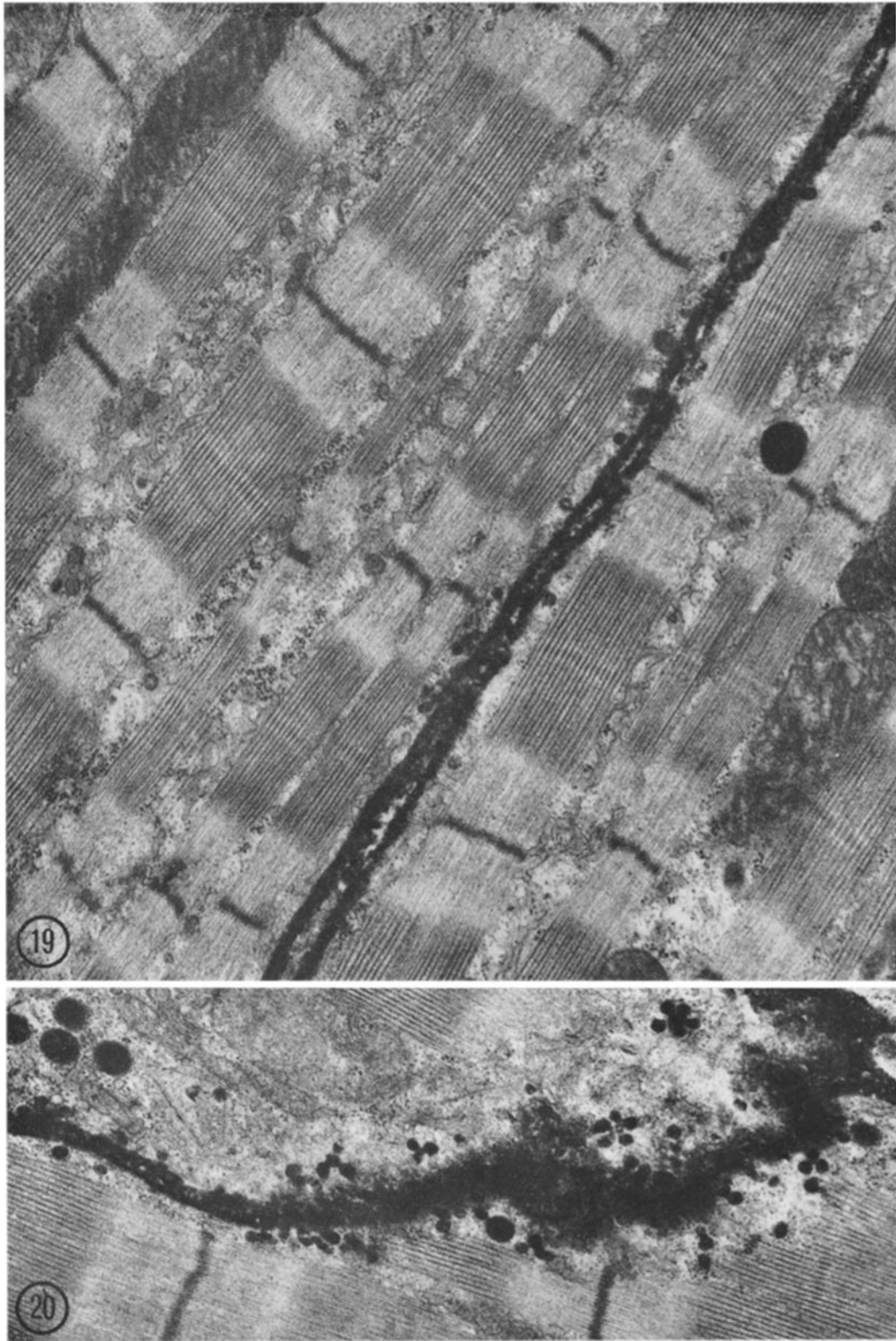


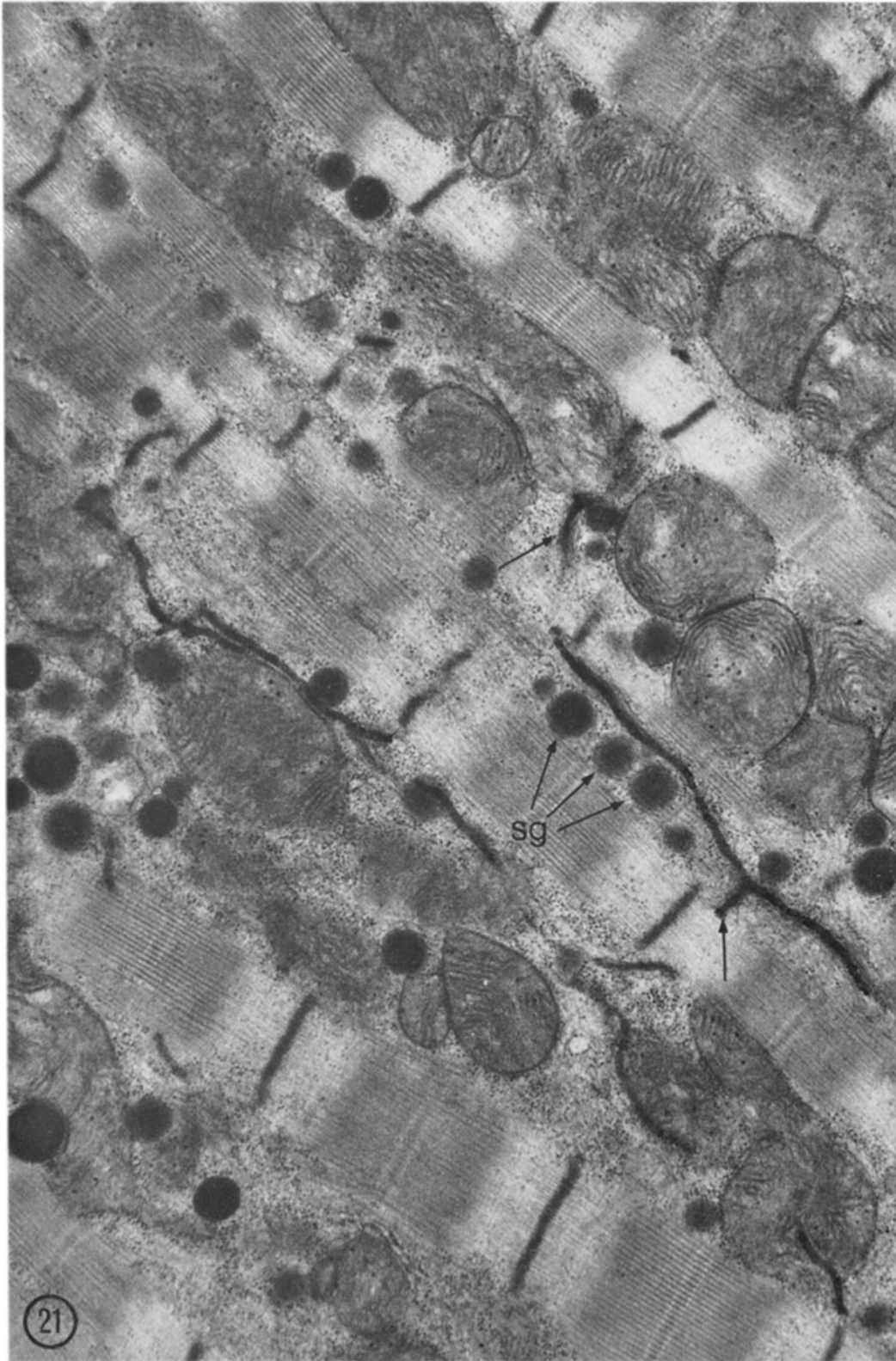
FIGURE 18 Right ventricular cell, transverse section; most of the T system is found at the level of the Z band (Z). Longitudinal tubules are also found that are of larger ( $T_{3b}$ ) and smaller ( $T_{3c}$ ) diameter. Endothelial cell, (EN); capillary lumen, (C); and extracellular space, (ES).  $\times 24,000$ .

position. The T tubules are much less regularly distributed than in skeletal muscle, where triads are found with quasi-geometrical regularity. In

the heart, T-L junctions are often situated at some point along a longitudinal profile of a T tubule and constitute longitudinal diadoids (Fig. 14) or tri-



FIGURES 19 and 20 Right atrium; cells showing no T system. A well-developed L system is seen in Fig. 19. Note the numerous micropinocytotic vesicles beneath the sarcolemma, especially in Fig. 20. Fig. 19  $\times$  23,500; Fig. 20  $\times$  26,500.



**FIGURE 21** Right atrial cell; most of the labeled T tubules run longitudinally along the myofibrils; a few are transverse (arrows). Numerous specific granules (*sg*) and mitochondria are found in these cells.  $\times 22,000$ .

TABLE I  
*Values of Total Membrane and Gap Thickness for the Nexus, Triad, and Triadoid*

	Total thickness	L membrane	T membrane	Gap
	<i>A</i>	<i>A</i>	<i>A</i>	<i>A</i>
Nexus	238			
	271			
	264			
	274			
	251			
	258			
	243			
	241			
	243			
	223			
	$250.6 \pm 5.09^*$			$\sim 20^\ddagger$
Triad	327	116	113	98
	330	128	131	70
	294	108	95	91
	286	98	103	86
	310	95	121	98
	325	93	116	116
	325	116	111	98
	284	100	95	88
	314	121	95	98
	309	95	111	103
	$310.8 \pm 5.44$	$107 \pm 3.86$	$109.1 \pm 3.82$	$94.6 \pm 3.83$
Triadoid	335	103	98	134
	330	113	128	88
	348	123	108	116
	365	113	108	144
	343	113	95	134
	312	126	85	101
	343	141	100	101
	314	116	95	103
	355	111	126	119
	299	116	85	98
	$334.4 \pm 6.58$	$117.5 \pm 3.29$	$102.8 \pm 4.71$	$113.8 \pm 5.88$

For explanation see text

\* Standard error of the mean

‡ Measured by micrometric method

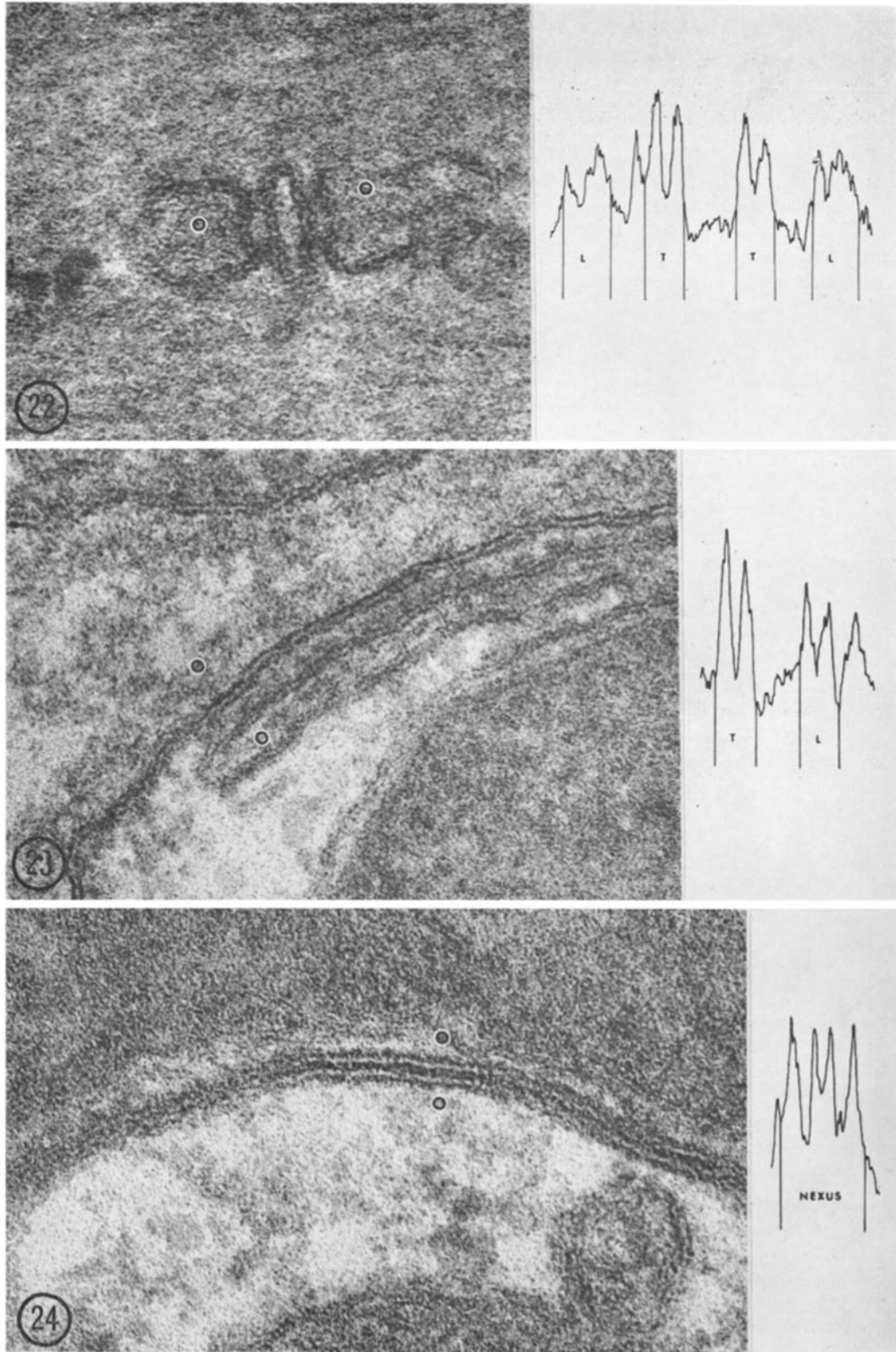
adoids<sup>6</sup> (Figs. 16 and 17). Noteworthy is the fact that such junctions can occur at any point along the T tubule, up to its origin.

Quantitatively, an interesting comparison can be made between T-L junctions in cardiac muscle

<sup>6</sup> The use of the terms diadoid and triadoid is justified by at least two facts: (a) T-L junctions in myocardium show no geometrical regularity, and (b) display variable membrane gap, in contrast to the diads or triads of skeletal muscle.

and their counterparts in skeletal muscle. Since the appearance of the junctions varies a great deal, with the fixation and staining techniques employed little can be expected from the values obtained for the absolute sizes of the structures under consideration. The comparison of these junctions is meaningful if both heart and skeletal muscles are taken from the same animal after perfusion-fixation, and provided the same staining method is applied to both tissues, and all the micrographs are taken during the same session, after calibration of the





FIGURES 22-24 Figures showing myocardial T-L junctions (Fig. 23) compared with T-L junctions of skeletal muscle (Fig. 22, diaphragm) and the tight junctions of the intercalated disc (Fig. 24, nexus). The densitometric tracings taken between the circles (o) are shown in the insets. For further explanation see text. Figs. 22-24  $\times 200,000$ .



electron microscope and densitometer. These conditions were met for all the data presented in Table I, which was based on documents such as those illustrated by Figs. 22–24.

The thickness of the cardiac intercellular tight junction (nexus) was our reference standard, for comparison with data obtained by other methods. With our technique, the over-all thickness of the cardiac nexus was 250.6 Å (Table I). This value is somewhat larger than most of the values found in the literature (see Fig. 3 in Revel and Karnovsky [42]). The difference may be due to our triple staining technique ( $\text{OsO}_4$ , followed by block impregnation with uranyl acetate, and by section staining with lead hydroxide). With this technique, no merging of membranes is observed in this type of junction. On the contrary, the membranes are separated by a gap approximately 20 Å wide (Fig. 24).

Table I shows that the over-all thickness of the T–L junction, in cardiac muscle (334.4 Å), as well as skeletal muscle (310.8 Å), was significantly larger ( $p < 0.001$ ) than that of the nexus, after identical preparative procedures. The mean thickness of the T–L junction was greater in heart muscle than in skeletal muscle ( $0.02 < p > 0.05$ ). The thicknesses of the T and L tubular membranes of heart muscle were not significantly different from that of their counterparts in skeletal muscle, so that the difference in over-all thickness of T–L junctions must be due to the larger gap in heart muscle (113.8 Å compared with 94.6 Å in skeletal muscle).

## DISCUSSION

Numerous papers have dealt with the structure of the T system in cardiac muscle (4, 7, 11, 13, 28, 34, 36, 37, 39, 44–49, 51, 52). The occurrence of sarcolemmal invaginations in register with the Z line and in continuity with transverse tubules invested with a basement membrane is well recognized. Thus the basement membrane has been used as an identification marker of the T system. The present results, however, show that, in myocardium, the T system can only be defined operationally, i.e. with the aid of diffusion tracers. After fixation and staining by routine electron microscopic techniques, the T system does not exhibit any characteristic morphological feature that can be found all along its tubules.

So defined, the T system of ventricular muscle consists of a ramified network of tubules that open

into the extracellular space. The tubes originate at the Z-band level, as larger-diameter channels ( $> 1000$  Å) lined by a basement membrane. The main stem gives rise to side-branches that run parallel to the myofibrils. Most of the branches are restricted to the I band, and are tortuous, with an irregular diameter. Occasionally, a branch can be seen coursing through the A band, and anastomosing with branches of the longitudinal T tubules of the other half of the sarcomere. The direction of the longitudinal T tubules is parallel to the axis of the fiber, whence their circular profiles in transverse sections. Some of these branches show diameters as small as 350 Å.

Under our experimental conditions, the inter-fibrillar space consistently revealed an anastomotic network of tubules similar to the L system of skeletal muscle, and devoid of tracer.

This work confirms the results reported earlier (7). The technical improvements that justify this paper have brought new data, among which the following should be pointed out: the penetration of the tracer into small-diameter tubules devoid of basement membrane is most probably not due to the post-mortem formation of channels connecting the T and L systems. This possibility deserved consideration when ferritin was used as the tracer, since the technique required exposing the tissue to the tracer for several hours. However, post-mortem changes are much less plausible with the more recent technique, since fixation is effected *in situ* a few minutes after injection of the tracer *in vivo*. Under these conditions the small-diameter tubules are distinctly labeled. If labeling of these tubules was a post-mortem artifact, one would expect the number of such labeled tubules to increase with increasing time between injection of the tracer and fixation. However, the distribution of the tracer is not significantly different with fixation between 2–45 min after injection (see Materials and Methods). This observation also rules out the hypothesis of intermittent connections between the T and L systems, unless the period of a complete cycle were greater than 45 min.

The functional role of the T system should be considered in at least the following two connections: (a) in excitation-contraction coupling and, (b) in metabolic interchanges between the cells and their medium.

The role of the transverse tubular system in excitation-contraction coupling has been dealt with in detail in recent literature (3, 11, 14, 17–24,

30, 35, 40, 43, 47, 54). The evidence, the majority of which is circumstantial, suggests that in skeletal muscle the T system synchronizes the contraction of the myofibrils throughout the entire cross-section of fast muscle fibers. The T tubules fulfill this function by providing a pathway for the inward spread of excitation; the precise mechanism of this inward spread is still controversial. Some authors reason in terms of an electrotonic depolarization of the T tubular membrane (43), while others favor the hypothetical shunting of part of the action current toward certain strategic loci of the contractile machinery, through points of the tubular membrane that display particular permeability features (11, 14, 40).

In the myocardium, the problem is made more difficult by the plexiform arrangement and the small size of the cells. As pointed out by Nelson and Benson (36), the very size of the cells makes unnecessary a specialized structure ensuring the inward spread of excitation, since diffusion of an activator, e.g. calcium ions, from the excited plasma membrane to the contractile machinery would be fast enough to afford synchronization of contraction. This suggestion would hold true provided the diffusion coefficient of the activator-calcium ions in the sarcoplasm was not too different from that of  $\text{Ca}^{++}$  in solution. In the case of calcium ions, this requirement does not seem to be satisfied, and one may reasonably assume that the diffusion of calcium ions in sarcoplasm is many orders of magnitude smaller than in free solution, since sarcoplasm contains numerous structures that are known to display a considerable avidity for that ion, e.g. the contractile proteins, the sarcoplasmic reticulum, and the mitochondria. The injection of calcium, through a microelectrode directly into striated muscle fibers of the crayfish, triggers contraction of a sharply circumscribed region in the immediate vicinity of the tip of the micropipette (41). Consequently, in spite of the small diameter of myocardial cells, a T system may be necessary to ensure synchronous contraction of the whole population of myofibrils of one cell.

A reversible swelling of the T tubules can be produced by immersion of fragments of ventricular tissue in hypertonic solutions (3, 11, 13) or by perfusion of the heart with such solutions. This swelling is associated with an equally reversible drop in conduction velocity, which suggests that the T

system does shunt an appreciable fraction of the action current (3). Similarly, Coraboeuf and Denoit (1) have suggested that the low conduction velocity of guinea pig heart muscle, compared with that of rat heart, was due to the larger diameter of the T tubules in the guinea pig.

Another indication of the possible involvement of the T system in excitation-contraction coupling can be found in the recent investigations of Müller (35). This author demonstrated that the myocardium does not respond to a local stimulus by local contraction, as was shown by Huxley and co-workers (18-24) in different types of skeletal muscle, but that the contraction spreads over at least a few sarcomeres. The peculiar response of cardiac muscle to local stimulation may possibly be due to the longitudinal branches of the T system.

Assuming that the electrical characteristics of the T tubular membranes are the same throughout the whole system, a ramified T system would be less efficient in electrotonically transmitting the action potential from the surface of the fiber to its interior than a nonramified T system would be, since at each fork the current loops would divide according to the ratio of the effective resistances of each branch, with a corresponding drop in current density at each fork.

On the other hand, if the T tubular membrane were electrically heterogeneous, i.e. possessed strategically situated low resistance sites, the density of current could remain high all the way to the core of the cell. It is tempting to speculate that these low resistance regions might be situated at the areas of apposition of the T and L systems. It has been shown that the cardiac sarcoplasmic reticulum, like the sarcoplasmic reticulum isolated from skeletal muscle, is capable of accumulating  $\text{Ca}^{++}$  (5, 6, 16, 17, 38, 53). Furthermore, it seems established that the ultimate link in the chain of events referred to as excitation-contraction coupling is the release of  $\text{Ca}^{++}$  into the sarcoplasm (43).

The mechanism by which the action potential triggers this release remains to be found. One possibility is that some kind of electrical connection exists between the T and L systems, which enables the action current to modify the permeability of the L system membranes. The current leaving a T tubule at such a T-L junction would split into two components, one of which would be shunted directly to the sarcoplasm through the gap separating the T tubule from the L tubule (shunt current),

while the other current component would cross the gap and flow through the L membrane (coupling current).

The magnitude of the coupling current would be inversely proportional to the effective resistance of the L membrane involved in the T-L junction, and consequently directly proportional to the T-L junction surface. On the other hand, the shunt resistance is independent of the surface area of apposed membrane, and depends only upon the width of the gap between the membranes and on the specific resistance of the gap material. It can be seen that the problem here is of the same type as that so often encountered by synaptologists (30). Simple calculations show that, in our ventricular preparations, since the surface area of T-L appositions is small relative to the width of the junctional gap (110 Å), practically all the current leaving the T tubules at the T-L junctions will bypass the L tubules. Thus, it is difficult to substantiate this direct current coupling hypothesis unless there exist between the two structures low resistance bridges, which our present techniques of fixation and preparation fail to preserve.

In a previous paper (7) we mentioned that one of the structural peculiarities of cardiac muscle, compared with other types of striated muscle, is that its highly developed T system makes contact with the majority of the mitochondria in a way that could make the mitochondria directly accessible to the extracellular milieu.

Stein and Stein (50) have radioautographically demonstrated that labeled oleic acid accumulates in esterified form in the lipid droplets of heart

muscle cells, without ever having been detected above the myofibrils. Thus the entry of free fatty acids into the cardiac cells is channeled. 15 sec after injection, radioactivity is found in esterified lipids localized over the sarcoplasmic reticulum and mitochondria. Esterification seems to occur in the diadoids.

Certain ramifications of the T system may represent a sort of permanent micropinocytosis. It is interesting to note the difference in micropinocytotic activity between turtle auricular muscle and rat ventricular muscle (12). In the turtle, which possesses a rudimentary T system, micropinocytosis is very active, whereas in the rat, the ventricular muscle, which has a highly developed T system, pinocytotic activity is apparently low. The present data show that the apparent intensity of micropinocytosis is inversely proportional to the development of the T system: atrial cells displaying an extensive network of T tubules are almost devoid of micropinocytotic vesicles, whereas cells of the same atrium, but without T tubules, contain numerous micropinocytotic vesicles. Ventricular muscle, on the other hand, consistently shows a more extensive T network than atrial muscle, and a much lower incidence of micropinocytotic vesicles.

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