A LABYRINTHINE STRUCTURE FORMED FROM A TRANSVERSE TUBULE OF MOUSE VENTRICULAR MYOCARDIUM

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INTRODUCTION

The transverse (T) tubules of mammalian myocardial cells and vertebrate skeletal muscle fibers both are invaginations into the cell interior whose membranes are continuous with the sarcolemma. Some pronounced differences exist between tubules from these two sources, however. One is the obvious disparity in size; the diameter of myocardial tubules may reach over 3,000 Å in the guinea pig (Sperelakis and Rubio, 1971), but T tubules of frog sartorius are only 260–280 Å in diameter (Freygang et al., 1964; Sperelakis and Schneider, 1968). Basal lamina lines the myocardial T tubules, but no evidence for its existence has been found along the walls of skeletal T tubules. The relationships between T tubules and sarcoplasmic reticulum (SR) appear morphologically similar in both muscle types, but on the basis of studies employing osmotic response (Birks and Davey, 1969) and electron-opaque tracer material (Rubio and Sperelakis, 1972), it has been concluded that the T tubule and SR terminal cisternae of frog sartorius are open to one another. Thus far, it appears that myocardial SR is a system closed to the intercellular space and T tubules (Forssmann and Girardier, 1970; Forbes et al., 1972). T tubules are absent in both muscle types in early embryonic stages. In chick skeletal muscle, SR is present in such early embryonic stages (Ezerman and Ishikawa, 1967; Shimada et al., 1967). Therefore, SR-T tubule contacts in the chick must arise after initial stages of T tubule formation. However, in embryonic rat skeletal muscle, the SR and T system form couplings during the early development of T tubules (Kelly, 1969, 1971). In the course of an ultrastructural study of mouse heart, a complex formation was found which supports the concept, already advanced by Jewett and Sommer (1970), that cardiac T tubule formation may come about in a manner similar to that proposed by Ishikawa (1968) for skeletal T tubules.

MATERIALS AND METHODS

Right ventricular wall from a white mouse (ICR strain, Flow Research Animals, Dublin, Va.) was

prepared for electron microscope examination by perfusion fixation with 3% glutaraldehyde in an aqueous solution (pH 7.2) of 3% dextran (mol wt 40,000–90,000) and 3% dextrose (Rostgaard and Behnke, 1965). After 5 min of perfusion, small blocks of tissue were fixed an additional 3 h, postfixed 1 h in 1% phosphate-buffered osmium tetroxide (Millonig, 1962), and stained en bloc for 30 min in a saturated aqueous solution of uranyl acetate. After dehydration in alcohols, the tissue was passed through propylene oxide, embedded in Epon 812 (Luft, 1961), and sectioned with a diamond knife. Thin sections were affixed to a 150-mesh copper grid; they were stained for 2 min with a saturated uranyl acetate solution in 50% acetone, and for 45 sec with 0.4% alkaline lead citrate (Venable and Coggeshall, 1965). The grid was examined in a Hitachi HU-11E-1 electron microscope, which was calibrated periodically against a replica of an optical grating.

RESULTS AND DISCUSSION

An unusual structure, apparently composed of a system of anastomosing tubules (500–800 Å in diameter) and vesicles (800–1,200 Å in diameter), was encountered in a thin section of one cell (Fig. 1). The SR forms several junctional complexes ("couplings") with this system; one of these complexes is shown at high magnification in the inset of Fig. 1. Three additional (nonconsecutive) sections were found which show details of the structure at successive levels of the cell (Figs. 2–4). The sections reveal that this labyrinthine mass is connected to a T tubule. This structure therefore appears homologous with those formed in cultured skeletal muscle (Ishikawa, 1968; Pappas et al., 1971). None of our sections reveals clear examples of either the ordered hexagonal or tetragonal patterns of tubule anastomosis found by Ishikawa. However, the overall appearance of the cardiac structure is quite similar to the less organized T tubular networks formed in young cultures of skeletal cells (Ezerman and Ishikawa, 1967); this suggests that its formation proceeds in a manner similar to that in cultured skeletal muscle. A possible difference in structural organization is that some of the 500–600 Å diameter "intertubular spaces" of the cardiac network may not constitute open cavities extending completely through the structure. Instead, they appear to end in depressions or "dimples" in the limiting membrane of the structure (double-headed arrows, Fig. 1). Therefore, much of the true three-dimensional structure of the mass might be similar to the surface covering of a golf ball, rather than to a fused network of discrete tubules. An alternate interpretation of the structure of the electron-opaque dimples is that some or all of them represent intratubular granule-like bodies. The three-dimensional architecture of the mass, particularly of the apparently open spaces (open arrows, Fig. 1), is not resolvable at this time, because of the unavailability of alternate serial sections.

In skeletal muscle, absence of innervation has been suggested to be responsible for the development of complex T tubule networks (Pellegrino and Franzini, 1963; Ishikawa, 1968; Schiaffino and Settembrini, 1970). Such an explanation cannot account for the presently-observed structure, since there was no loss of innervation. Furthermore, the cell in which the structure was found showed no signs of being otherwise abnormal or in an embryonic state. Skeletal muscle biopsies taken from patients afflicted with myotonic dystrophy show arrays of lattice-like membranous structures evidently derived from transverse tubules (Schotland, 1970). In cardiomyopathies, a common response of both the T tubules and sarcoplasmic reticulum is enlargement, and sometimes apparent fragmentation into vacuoles (Forbes and Sperelakis, 1972). However, to our knowledge, such an elaboration of T-tubular membranes has not been reported in any cardiomyopathy.

Invaginations of the sarcolemma into the myocardial cells are oriented not only transversely, but also longitudinally or axially with respect to the long axis of the myofibrils. In the guinea pig ventricle, an extensive interconnected latticework of transverse and axial tubules is present (Sperelakis and Rubio, 1971). Although axial tubules are encountered less frequently in the mouse heart, substantial numbers are present (Forbes, unpublished observations). Little information is available concerning the mode of formation of transverse and axial tubules of mammalian myocardial cells. T tubules originate in dogs during the period between birth and 8 wk of age (Jewett and Sommer, 1970; Bishop, 1972). The mechanism suggested by Ishikawa (1968) for formation of T tubules in skeletal muscle may also hold for myocardial cells. Ishikawa suggested that repeated formation of fused micropinocytotic-like vesicles, whose cavities are open to the intercellular space, forms the T tubules. The presence of the elaborate structure described here, and its structural continuity with an apparently fully-formed T tubule, suggest that T tubules are the first to

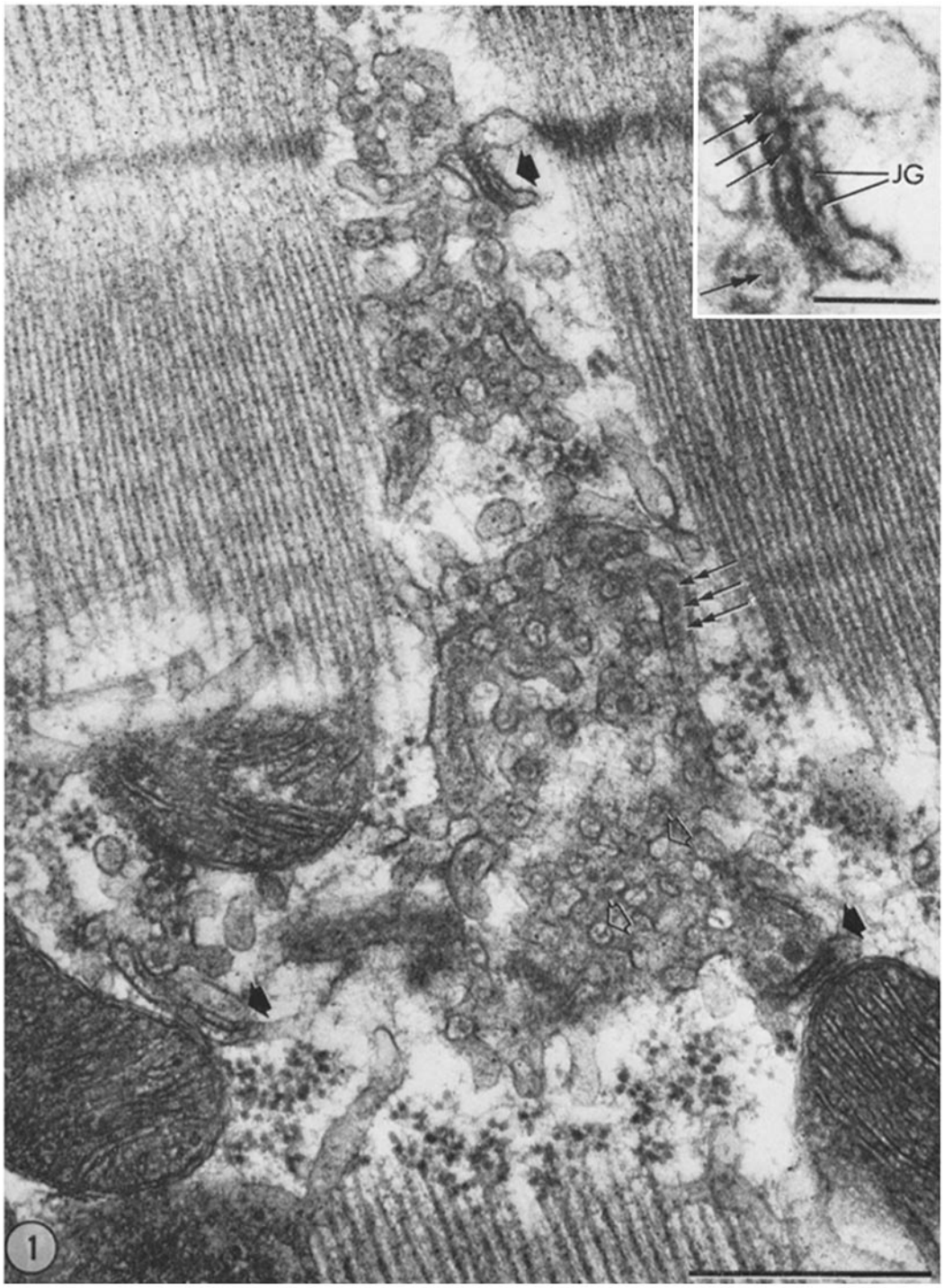
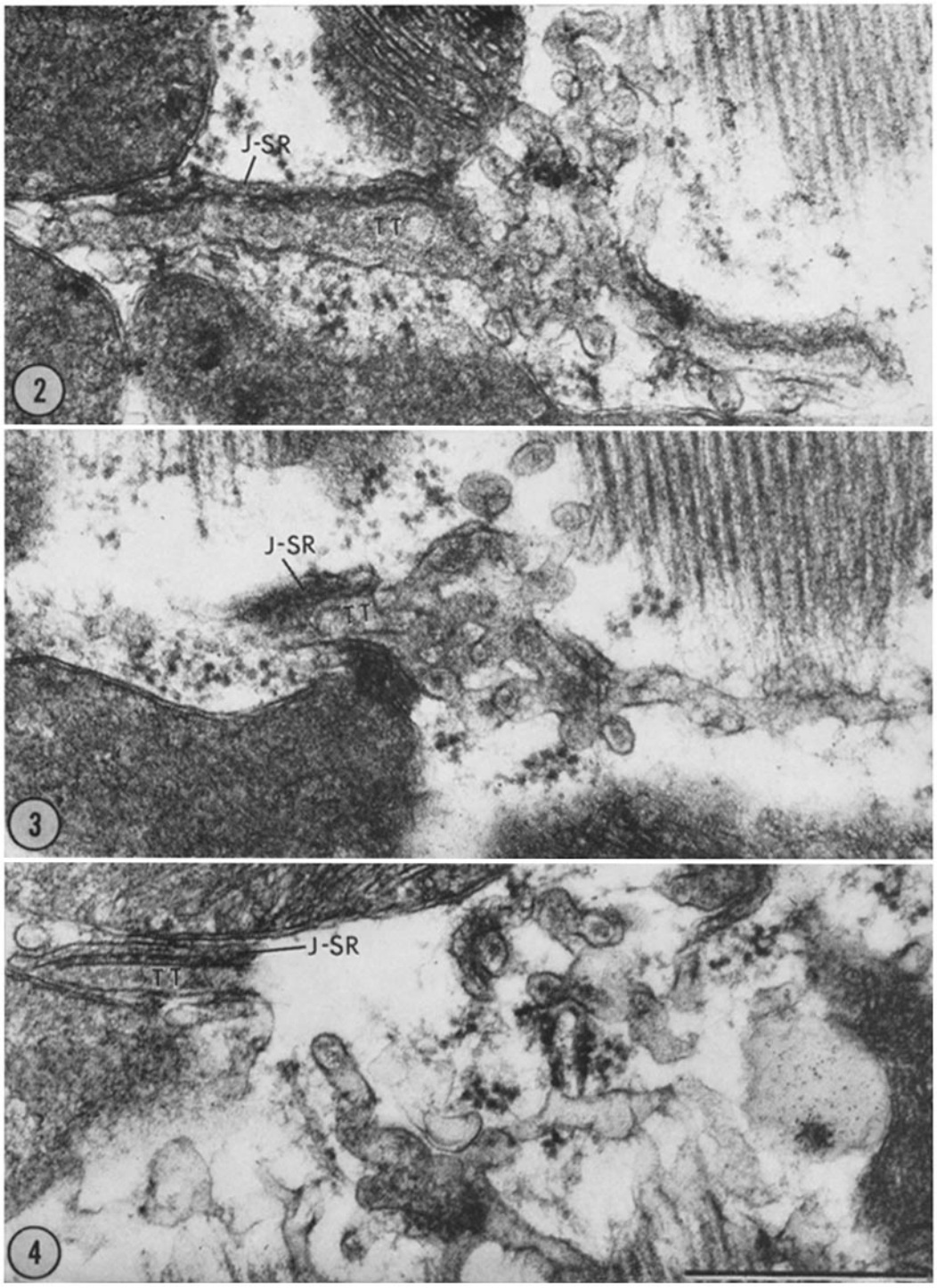


FIGURE 1 Elaborate structure found in a mouse ventricular myocardial cell. Three regions (short filled arrowheads) appear similar to couplings formed between SR and T tubules. The uppermost of these couplings is given at high magnification in the *inset* to show the junctional granules (*JG*) in the lumen of the SR junctional cisterna and the junctional processes (single arrows) which extend between the apposed SR and T tubule membranes. In the tubular mass, structures representing either depressions in the limiting membrane or intratubular dense granules are indicated by double-headed arrows. Other structures (short open arrowheads) may represent true intertubular spaces. Fig. 1, × 72,000; scale bar = 0.5 μm. *Inset*, × 180,000; scale bar = 0.1 μm.



FIGURES 2–4 Sections of the region shown in Fig. 1, taken at successive levels in the cell. In Figs. 2 and 3, a mass of tubules, apparently constituting part of the same structure seen in Fig. 1, is continuous with a normal-appearing T tubule (*TT*). The same coupling formed by a junctional SR cisterna (*J-SR*) with the T tubule is visible in all three figures. Fig. 4, scale bar = 0.5 μm. Figs. 2–4, × 72,000.

develop in the maturing heart cell, followed by axial tubules, which then may develop from the T tubules by a process of "budding-off" of vesicles at approximate right angles to the T tubule. In order to test the validity of such an hypothesis, it would be necessary to document the development of the T-axial tubular system in newborn mammals by use of electron-opaque tracers. Therefore, it appears that the structure described here may represent an aberrant example of axial tubule formation in a myocardial cell.

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REFERENCES

BIRKS, R. I., and D. F. DAVEY. 1969. Osmotic responses demonstrating the extracellular character of the sarcoplasmic reticulum. *J. Physiol. (Lond.).* **202**:171.

BISHOP, S. P. 1972. Effect of aortic stenosis on myocardial cell growth, hyperplasia and ultrastructure in neonatal dogs. Abstract of the Fifth Annual Meeting of the International Study Group for Research in Cardiac Metabolism, Winnipeg, Canada.

EZERMAN, E. B., and H. ISHIKAWA. 1967. Differentiation of the sarcoplasmic reticulum and T system in developing chick skeletal muscle in vitro. *J. Cell Biol.* **35**:405.

FORBES, M. S., R. RUBIO, and N. SPERELAKIS. 1972. Tubular systems of *Limulus* myocardial cells investigated by use of electron-opaque tracers and hypertonicity. *J. Ultrastruct. Res.* **39**:580.

FORBES, M. S., and N. SPERELAKIS. 1972. Ultrastructure of cardiac muscle from dystrophic mice. *Am. J. Anat.* **134**:271.

FORSSMANN, W. G., and L. GIRARDIER. 1970. A study of the T system in rat heart. *J. Cell Biol.* **44**:1.

FREYGANG, W. H., JR., D. A. GOLDSTEIN, D. C. HELLAM, and L. D. PEACHEY. 1964. The relation between the late after-potential and the size of the transverse tubular system of frog muscle. *J. Gen. Physiol.* **48**:235.

ISHIKAWA, H. 1968. Formation of elaborate networks of T-system tubules in cultured skeletal muscle with special reference to the T-system formation. *J. Cell Biol.* **38**:51.

JEWETT, P. H., and J. R. SOMMER. 1970. The left bundle branch, Purkinje network, and working muscle of the newborn dog: ultrastructure. (Abstr.) *Circulation.* **42**:III-79.

KELLY, A. M. 1969. Sarcoplasmic reticulum and the transverse tubular system in developing rat intercostal muscle. *J. Cell Biol.* **43**(2, Pt. 2):65 a (Abstr.)

KELLY, A. M. 1971. Sarcoplasmic reticulum and T tubules in differentiating rat skeletal muscle. *J. Cell Biol.* **49**:335.

LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.

MILLONIG, G. 1962. Further observations on a phosphate buffer for osmium solutions in fixation. *Proc. Congr. Electron Microsc., 5th.* **2**:P-8.

PAPPAS, G. D., E. R. PETERSON, E. B. MASUROVSKY, and S. M. CRAIN. 1971. Electron microscopy of the *in vitro* development of mammalian motor end plates. *Ann. N. Y. Acad. Sci.* **83**:33.

PELLEGRINO, C., and C. FRANZINI. 1963. An electron microscope study of denervation atrophy in red and white skeletal muscle fibers. *J. Cell Biol.* **17**:327.

ROSTGAARD, J., and O. BEHNKE. 1965. Fine structural localization of adenine nucleoside phosphatase activity in the sarcoplasmic reticulum and the T system of rat myocardium. *J. Ultrastruct. Res.* **12**:579.

RUBIO, R., and N. SPERELAKIS. 1972. Penetration of horseradish peroxidase into the terminal cisternae of frog skeletal muscle fibers and blockade of caffeine contracture by Ca^{++} depletion. *Z. Zellforsch. Mikrosk. Anat.* **124**:57.

SCHIAFFINO, S., and P. SETTEMBRINI. 1970. Studies on the effect of denervation in developing muscle. I. Differentiation of the sarcotubular system. *Virchows Arch. Abt. B Zellpathol. (Cell Pathol.).* **4**:345.

SCHOTLAND, D. L. 1970. An electron microscopic investigation of myotonic dystrophy. *J. Neuropathol. Exp. Neurol.* **29**:241.

SHIMADA, Y., D. A. FISCHMAN, and A. A. MOSCONA. 1967. The fine structure of embryonic chick skeletal muscle cells differentiated in vitro. *J. Cell Biol.* **35**:445.

SPERELAKIS, N., and R. RUBIO. 1971. An orderly lattice of axial tubules which interconnect adjacent transverse tubules in guinea-pig ventricular myocardium. *J. Mol. Cell. Cardiol.* **2**:211.

SPERELAKIS, N., and M. F. SCHNEIDER. 1968. Membrane ion conductances of frog sartorius fibers as a function of tonicity. *Am. J. Physiol.* **215**:723.

VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407.