

## SARCOLEMMAL SCALLOPING AT SHORT SARCOMERE LENGTHS WITH INCIDENTAL OBSERVATIONS ON THE T TUBULES

MARTIN HAGOPIAN and ELADIO A. NUNEZ. From the Department of Pathology, New York Medical College, and the Departments of Radiology and Medicine, Cornell University Medical College, New York 10029

### INTRODUCTION

It is now well established that the transverse tubular system (T system) in skeletal muscle communicates with the extracellular space. Moreover, it is also generally agreed that the T tubules act as a conducting mechanism for the spread of electrical activity from the surface to the interior of the cell. Experiments using tracers such as ferritin, lanthanum, and horseradish peroxidase confirmed the sarcolemmal invaginations (Huxley, 1964; Page, 1964; Revel and Karnovsky, 1967; Forssmann and Girardier, 1970). However, in fish skeletal muscle well-defined sarcolemmal invaginations which form the T system have been illustrated without the use of tracers (Franzini-

Armstrong and Porter, 1964). Furthermore Rayns et al. (1967) have shown external apertures of the T system in guinea pig cardiac muscle by the technique of freeze-etching for electron microscopy. In mammalian myocardium, T tubules are rarely seen directly joining the sarcolemma to any great length in longitudinal sections. Since T tubules are highly convoluted or tortuous near the surface of the myofibers in heart muscle, electron micrographs in past papers have only been suggestive. In the present study of the heart of active and hibernating bats (untreated and treated with reserpine), continuities of the T system to the base of the corrugated sarcolemma are clearly visible in the regions of the myocardium

where the sarcomeres are extremely shortened (less than  $1.5 \mu$ ). The deep sarcolemmal incursions appear to be correlated with the supercontracted sarcomeres and appear to be more prominent in bats injected with reserpine.

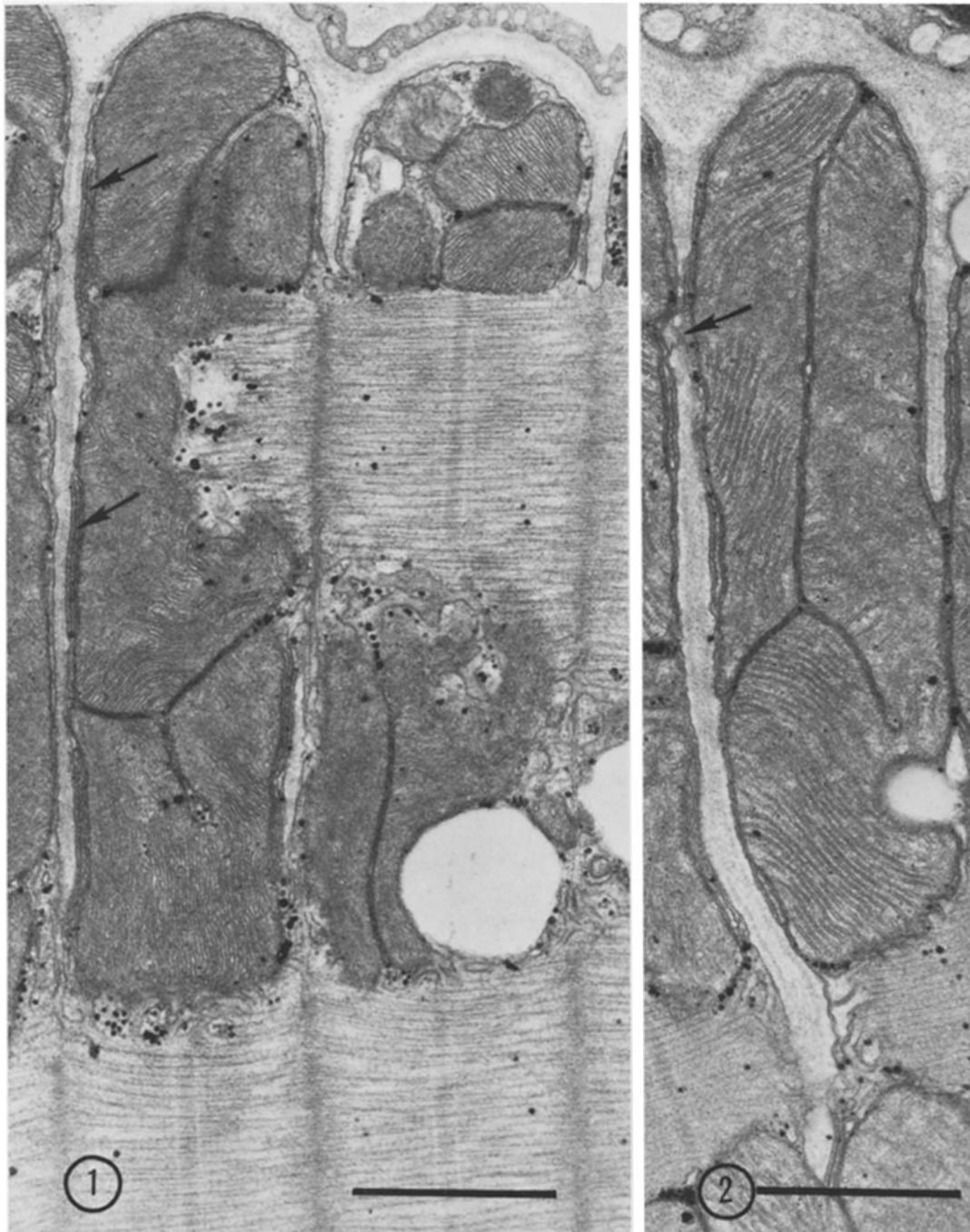
#### MATERIALS AND METHODS

Active, nonhibernating bats, *Myotis lucifugus*, were caught in their natural habitat in late October. In one group of four bats, reserpine (Serpasil, Ciba Ltd., Duxford, Cambridge, England) was injected intraperitoneally at low dosage ( $0.5 \text{ mg/kg}$ ) for 7 successive days. After 7 days the four treated animals and four normal active animals were anesthetized with ether, and the left ventricles were removed, diced, and prepared for electron microscopy. In another series of experiments, four late-hibernating bats (late March) were injected with reserpine ( $0.5 \text{ mg/kg}$ ) in a cold room ( $4^\circ\text{C}$ ). After 7 days the four treated and four hibernating bats were sacrificed and the cardiac muscle was immediately prepared for electron microscopy. The tissue was fixed in 6.25% glutaraldehyde in  $0.067 \text{ M}$  cacodylate buffer for 2 hr and then rinsed in ice-cold  $0.067 \text{ M}$  cacodylate buffer, pH 7.3, for around 12 hr. After postfixation in  $0.067 \text{ M}$  cacodylate-buffered 1% osmium tetroxide (pH 7.3) for 1 hr, the cardiac muscle was dehydrated and embedded in Epon. Thin sections were doubly stained with uranyl acetate and lead citrate and examined in a Philips EM 200 electron microscope.

#### RESULTS AND DISCUSSION

The ultrastructure of the bat heart is not unlike that of other mammalian hearts (Moore and Ruska, 1957; Stenger and Spiro, 1961; Fawcett and McNutt, 1969). When the myocardium is shortened, there are indentations of the sarcolemma in longitudinal sections at the Z-line levels of the band patterns. Dense material was never observed between the points of indentation opposite each Z line of the subjacent myofibrils as reported in some striated muscle studies (Simpson and Oertel, 1962; Hagopian and Spiro, 1967). Occasionally, in longitudinal sections of shortened muscle, portions of the invaginating sarcolemma have been noted in a series at Z-line levels between myofibrils. In the intermyofibrillar regions the thick-walled sarcolemmal invaginations or tubules are easily differentiated from the thinner-walled sarcoplasmic reticulum (SR). The SR in the bat myocardium, like that in other mammals, is essentially a closed system of anastomosing tubules with terminal cisternae surrounding each myofibril. The terminal cisternae of the SR are

flattened and have a close association with the T tubules at Z-line levels. If the association is only on one side of the T tubule, it is a dyad, whereas, if the intimate relationship is on both sides of T tubules, it is a triad. At times the sarcolemmal invaginations or T system in certain planes of orientation appear as longitudinally sectioned tubules running transversely to the myofibrils in the cell. In other cases identical tubules are visible near the surface of the myofiber and do not communicate with the extracellular space in the plane of section. Serial sections, however, demonstrate the continuity of the tubular lumen and the extracellular space. This indicates that the T tubules are very tortuous or convoluted near the surface of the myofiber. When pieces of tissue from the left ventricle have been placed directly into the fixative, however, there are on occasion areas of the myocardium which have undergone supercontraction, i.e., when the sarcomere lengths are less than  $1.5 \mu$ . When the muscle fiber is extremely shortened, the sarcolemma becomes deeply corrugated with rounded ridges alternating with deep grooves at each Z line. In longitudinal sections, the surface has a scalloped appearance. Some of these areas of supercontraction show the sarcolemma with its protein-carbohydrate coat invaginating deeply into the myofiber. In Fig. 1 the sarcolemmal incursion at the left of the micrograph has clearly penetrated approximately  $4.5 \mu$  at the Z-line level. The terminal cisternae of the SR are in close association with the sarcolemmal incursion to form dyads (arrows). It thus seems that a considerable portion of the incursion in Fig. 1 is a T tubule since it has penetrated the myofiber so deeply. The T tubule opens onto the cell surface in the depth of the circumferential groove. In longitudinal sections, the appearance of a groove and that of a tubule are identical. The occurrence of terminal cisternae in association with an incursion of the sarcolemma does not identify the latter as a T tubule because subsarcolemmal cisternae also occur at various points on the periphery of the fiber that would be drawn into grooves in the contracted muscle. Deep penetrations of the sarcolemma were never observed in the stretched heart muscle of bat. The upper sarcomere in Fig. 1 is about  $1.4 \mu$  in length, whereas the bottom left sarcomere is approximately  $1.3 \mu$ . In the sections examined,  $1.3 \mu$  is the maximum shortening length of the sarcomeres before disorganization of the myofila-



**FIGURE 1** A portion of a longitudinal section of the myocardium of the active bat illustrating a deep sarcolemmal invagination with its surface coat in a zone of supercontraction. The sarcolemmal groove with an opening into the T system is approximately  $4.5 \mu$  in length and is clearly defined. The sarcomere at the top of the micrograph is approximately  $1.4 \mu$  in length, whereas, the sarcomere at the bottom left is about  $1.3 \mu$  in length and is probably at its maximum shortening length. Arrows point to the close association between the terminal cisternae of the SR and the sarcolemmal incursion (dyads). Mark:  $1.0 \mu. \times 29,000$ .

**FIGURE 2** An oblique section of the myocardium of the active bat. The sarcolemmal invagination is deeper than in Fig. 1, but the communication with the extracellular space is interrupted by a portion of the SR (arrow). Mark:  $1.0 \mu. \times 29,000$ .



FIGURE 3 A micrograph of the surface of supercontracted myocardium of the reserpinized active bat. The sarcomeres in this longitudinal section are approximately  $1.4 \mu$  in length. The extreme indentations of the sarcolemma at the level of the Z lines are the profiles of the grooves in the normal scalloping of the extremely shortened muscle cell. Also note that the sarcolemma has little relationship with the terminal cisternae of the SR. Mark:  $1.0 \mu$ .  $\times 29,000$ .

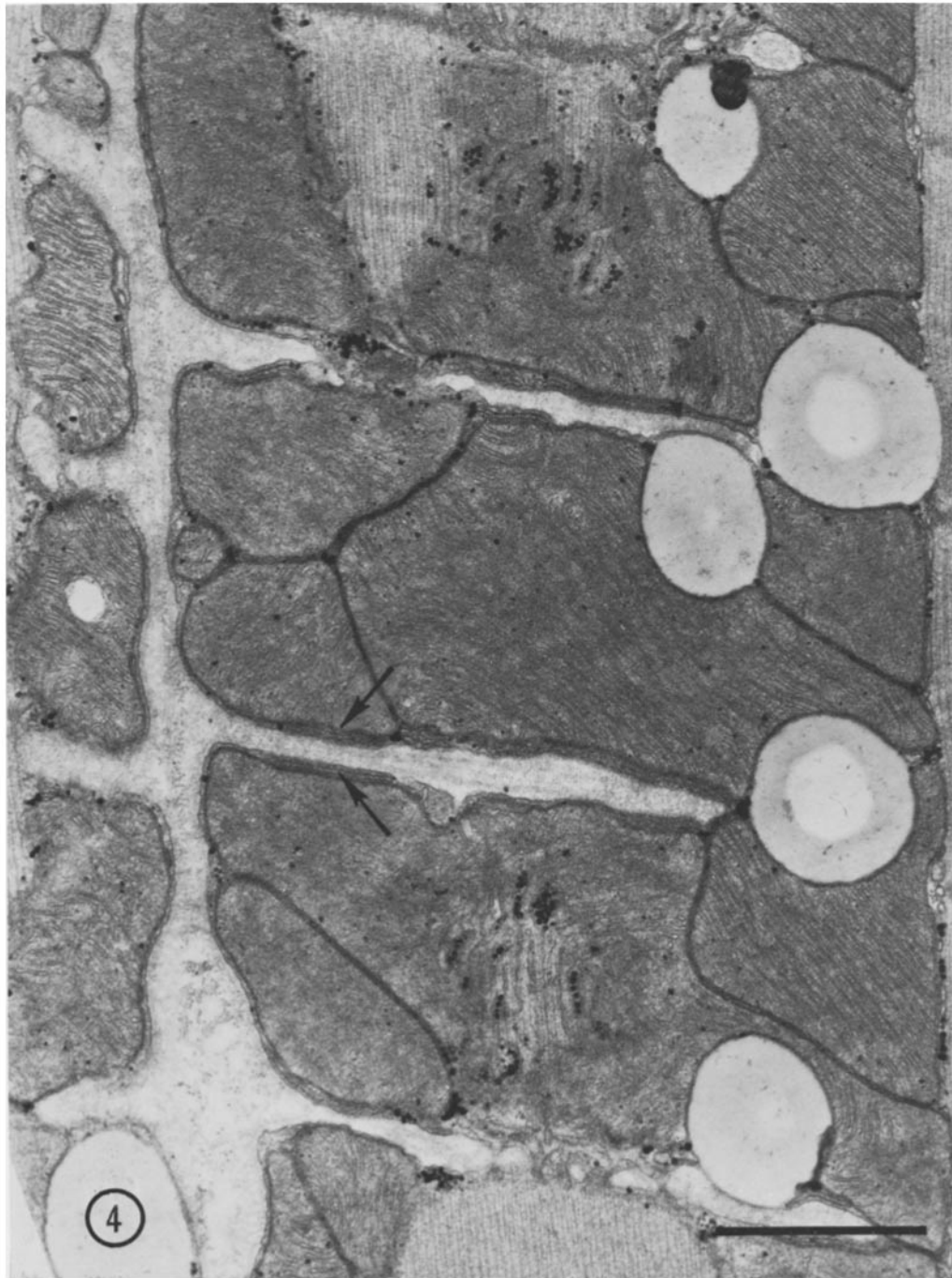


FIGURE 4 A portion of the surface of the myocardium of the reserpinized hibernating bat. In this view of an oblique section the sarcolemmal invaginations are widely spaced. The arrows point to the terminal cisternae of the SR on both sides of the surface membrane. Mark:  $1.0 \mu$ .  $\times 29,000$ .

ments. The myosin filaments measure about 1.6  $\mu$  in length and seem to penetrate the Z lines at sarcomere lengths of 1.5–1.3  $\mu$  (Figs. 1 and 3). In the same sarcomeres, note the dense zone on either side of the Z line. Fig. 2 illustrates a portion of an oblique section of the myocardial surface and shows that the sarcolemmal invagination in this plane of section is greater than the incursion in Fig. 1.

In reserpine-treated active and hibernating bats the zones of supercontraction in the myocardium are more frequent. Therefore, there are more areas of sarcolemmal scalloping and deep sarcolemmal penetrations into the myofiber. The morphological effects of reserpine which depletes norepinephrine (Chang, 1964; Roberts et al., 1965; Brodie et al., 1966) reveal in the treated active bats a large increase in the number and size of lipid droplets, an increase in glycogen granules, and some mitochondrial degeneration. In the treated hibernating bats the response in the myocardium is less effective. There is a block in the increase of lipid droplets, but there are an increase in glycogen granules and a focal degeneration in some mitochondria.<sup>1</sup> Fig. 3 depicts an area of supercontraction in the myocardium of the reserpinized active bat. The sarcomeres in the micrograph are approximately 1.4  $\mu$  in length, and there is an extreme scalloping of the sarcolemma in this region of the myofiber. Although some of the indentations penetrate 2.5–3.0  $\mu$  at the Z-line levels, the indentations are simply the profiles of the grooves at the Z-line level in the normal surface scalloping of the contracted heart muscle cell. In Fig. 3 the grooves have only a slight association with the terminal cisternae of the SR in this plane of orientation. Fig. 4 shows a portion of an oblique section of the myocardium of the treated hibernating bat. Because of the orientation, the grooves which possibly open into the T tubules are widely spaced. In this view, some terminal cisternae of the SR are in close association with both sides of the invaginations.

The communication between the T system and the extracellular space in the myocardium of the normal and reserpinized active and hibernating bats is clearly defined in the zones of supercontraction. This indicates that the convoluted or

<sup>1</sup> Hagopian, M., M. D. Gershon, and E. A. Nunez. 1972. An ultrastructural study of the effect of reserpine administration on the myocardium of active and hibernating bats (*Myotis lucifugus*). In preparation.

tortuous T tubules near the surface of the myofiber are straightened and are brought closer to each other as a result of the extreme shortening of the sarcomeres. It seems probable that the anastomosing tubules of the SR become folded or bent during supercontraction since the terminal cisternae of the SR are always attached or closely associated with the T tubules (dyads or triads) at all states of contraction. Furthermore, the myosin filaments (1.6  $\mu$  in length) appear to penetrate the Z lines in sarcomeres less than 1.5  $\mu$  in length, which is in agreement with a previous study of supercontracted glycerinated pectoral muscle of chick (Hagopian, 1970). Finally, the present findings on the T system, SR, and sarcomeres in the supercontracted zones of the bat heart are consistent with the model of contraction in striated muscle (see Huxley, 1965, for review).

The authors wish to express their gratitude to Mrs. Maria Demeri and Miss Joni Lingg for technical assistance, to Mr. Alfred E. Revzin for electronic management, and to Mr. Osmay Yalis for photographic assistance.

Dr. Eladio A. Nunez is the recipient of a Career Development Award (1-K4-GM, 29,958) from the National Institutes of Health.

This study was supported in part by United States Public Health Service Grants AM-12957 and HE-14713.

Received for publication 1 November 1971, and in revised form 9 December 1971.

## REFERENCES

- BRODIE, B. B., E. COSTA, A. DLABAC, N. H. NEFF, and H. H. SMOOKLER. 1966. Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. *J. Pharmacol. Exp. Ther.* 154:493.
- CHANG, C. C. 1964. A sensitive method for spectrophotofluorometric assay of catecholamines. *Int. J. Neuropharmacol.* 3:643.
- FAWCETT, D. W., and N. S. McNUTT. 1969. The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. *J. Cell Biol.* 42:1.
- FORSSMANN, W. G., and L. GIRARDIER. 1970. A study of the T system in rat heart. *J. Cell Biol.* 44:1.
- FRANZINI-ARMSTRONG, C., and K. R. PORTER. 1964. Sarcolemmal invaginations constituting the T system in fish muscle fibers. *J. Cell Biol.* 22:675.
- HAGOPIAN, M. 1970. Contraction bands at short sarcomere length in chick muscle. *J. Cell Biol.* 47:790.
- HAGOPIAN, M., and D. SPIRO. 1967. The sarcoplasmic

- reticulum and its association with the T system in an insect. *J. Cell Biol.* **32**:535.
- HUXLEY, H. E. 1964. Evidence for continuity between the central elements of the triads and extracellular space in frog sartorius muscle. *Nature (London)*. **202**:1067.
- HUXLEY, H. E. 1965. Structural evidence concerning the mechanism of contraction in striated muscle. *In Muscle*. W. M. Paul, E. E. Daniel, C. M. Kay, and C. Monckton, editors. Pergamon Press, Inc., Elmsford, N. Y. 3.
- MOORE, D. H., and H. RUSKA. 1957. Electron microscope study of mammalian cardiac muscle cells. *J. Biophys. Biochem. Cytol.* **3**:261.
- PAGE, S. 1964. The organization of the sarcoplasmic reticulum in frog muscle. *J. Physiol. (London)*. **175**:10P.
- RAYNS, D. G., F. O. SIMSON, and W. S. BERTRAND. 1967. Transverse tubule apertures in mammalian myocardial cells: surface array. *Science (Washington)*. **156**:656.
- REVEL, J. P., and M. J. KARNOVSKY. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. *J. Cell Biol.* **33**:C7.
- ROBERTS, J., S. EHRREICH, and B. LEVITT. 1965. Some aspects of the cardiac actions of reserpine and pronethol. *Fed. Proc.* **24**:1421.
- SIMPSON, F. O., and S. J. OERTELIS. 1962. The fine structure of sheep myocardial cells; sarcolemmal invaginations and the transverse tubular system. *J. Cell Biol.* **12**:91.
- STENGER, R. J., and D. SPIRO. 1961. The ultrastructure of mammalian cardiac muscle. *J. Biophys. Biochem. Cytol.* **9**:325.