

SIZE AND SHAPE OF TRANSVERSE TUBULE OPENINGS IN FROG TWITCH MUSCLE FIBERS

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The transverse (T) tubules of frog twitch fibers form a compartment communicating with the extracellular space, into which large molecules can diffuse (Endo, 1964; Page, 1964; Huxley, 1964) and from which small ions can escape with a half-time of 1–3 s (Hodgkin and Horowitz, 1960). Examination of the same fibers by electron microscopy, however, usually fails to provide direct evidence that the tubules are opened at the periphery of the fiber, and this, we think, is due to a combination of the following factors: (a) the openings are very small; (b) the course taken by the tubules at the periphery of the fiber is sinuous; (c) the openings are rare. The first two points are based on the assumption that the openings of the T tubules in frog fibers are similar to those described in muscles from a variety of other vertebrates (Hoyle et al., 1966; Page, 1968; Ashurst, 1969; Walker and Schrodt, 1965; Rayns et al., 1968). The assumption is confirmed by the observations presented here, as well as by the few previous descriptions of openings observed in frog muscles (Franzini-Armstrong, 1970; Peachey and Adrian, 1973). It is not clear why the T tubules in fibers of other vertebrates (Franzini-Armstrong and Porter, 1964; Kilarski, 1966; Goldstein, 1969), including frog tadpoles (Franzini-Armstrong, 1973), have fairly large, funnel-shaped mouths. The scarcity of openings ([c], above) has been proposed on the basis of local stimulation experiments by Huxley and Taylor (1958), who found that spots at which activation of the fibrils could be produced were placed at a distance of approximately 5 μm along the circumference of the fiber.

In this paper we report on a number of micrographs (nine in all) of frog fibers, collected in two

different laboratories over a period of several years, in which the plasma membrane can be clearly seen to invaginate into the fiber. From these pictures, it is possible to give a general description of the structure of the most peripheral portion of frog T tubules and of their openings. Our results are confirmed by reports from a third laboratory.¹

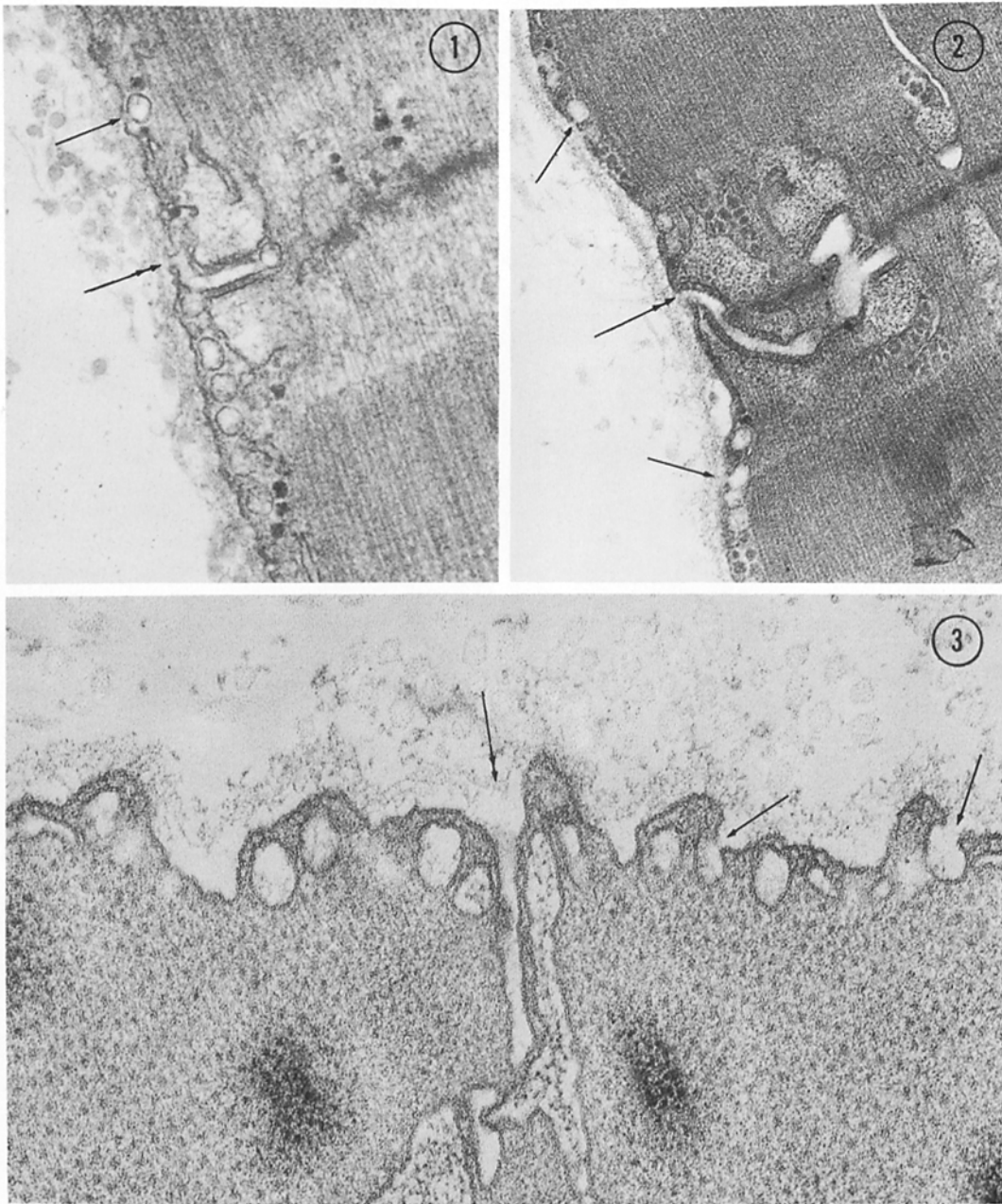
MATERIALS AND METHODS

Muscles from *Rana pipiens* were fixed in glutaraldehyde followed by osmium tetroxide, dehydrated in ethyl alcohol and propylene oxide, and embedded in Epon. Since the concentration of fixative and the composition of buffer varied, these are specified in each figure legend. One group of muscles (Figs. 4–7) had been transplanted to the chest and was reinnervated by a vagus nerve (Landmesser, 1971). This experimental procedure did not alter the structure of the sarcoplasmic reticulum and transverse tubules, nor the contractile and electrical properties of the muscle fibers (Landmesser, 1972). Sections were stained with uranyl acetate and lead citrate and examined in AEI 6B, AEI 801, and RCA EM3 electron microscopes.

RESULTS AND DISCUSSION

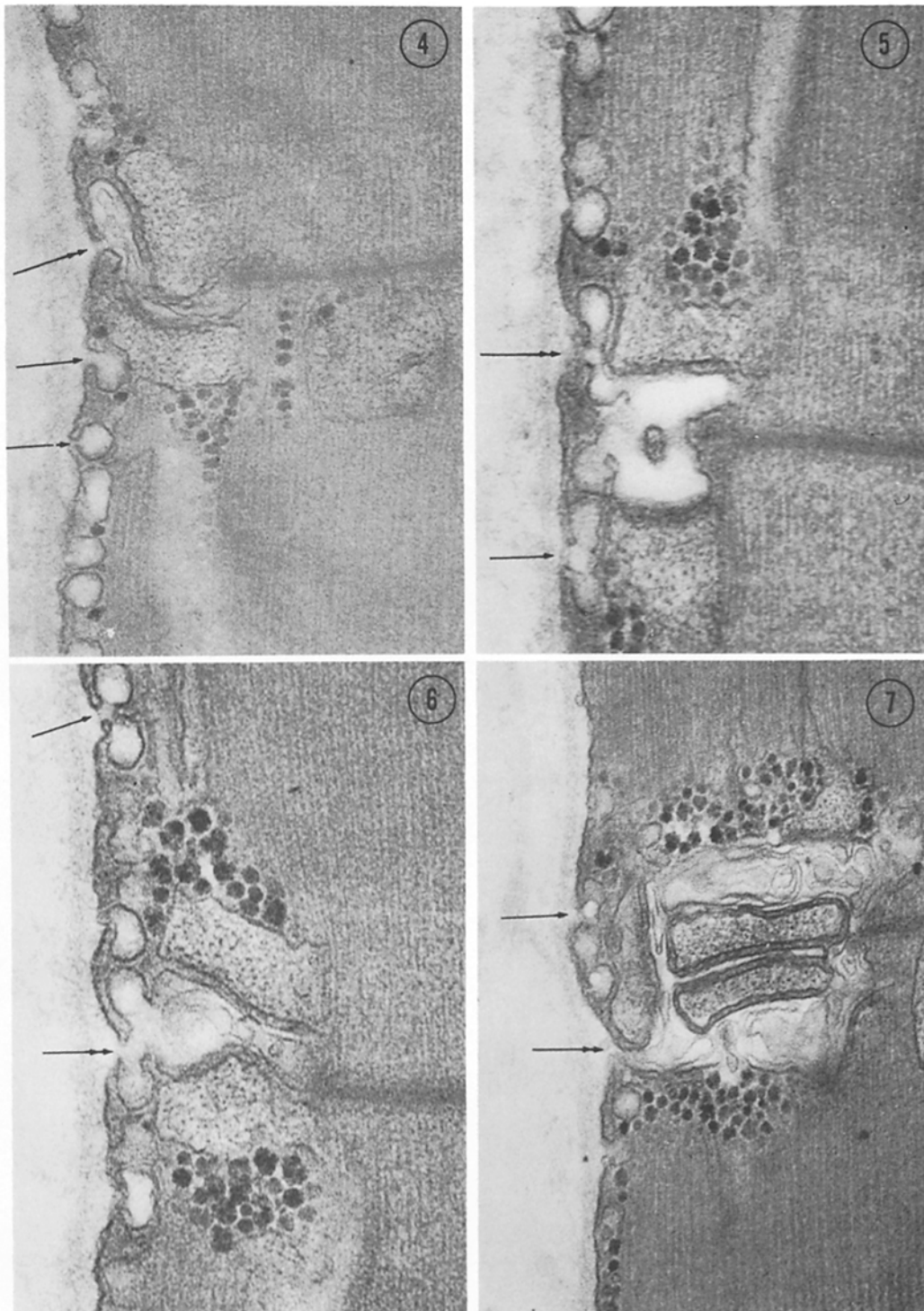
The entrance of the T tubules is a small cylinder, about 15-nm long and with a diameter of 17–22 nm (Figs. 1, 2, 4–7, double arrows. The size of the openings is given in the figure legends). No electron-dense material obstructs the mouth. The openings of the numerous caveolae, the small, spherical vesicles that lie immediately under the plasma membrane, are very similar in size and shape to those of the T system (Figs. 1–7, single

¹ L. D. Peachey. 1974. Personal communication.



FIGURES 1 and 2 Fibers from lumbricalis muscles fixed in 2% glutaraldehyde-phosphate buffer. In these and all figures, a double arrow points to the opening of a T tubule, a single arrow to the opening of a caveola. The sizes of the openings are: T tubule (T), 17 nm; caveolae (C), 17 nm, for Fig. 1; T, 20 nm; C, 17 nm, for Fig. 2. Fig. 1, $\times 56,000$. Fig. 2, $\times 48,000$.

FIGURE 3 Dilated T tubule's opening at the edge of a sartorius fiber. T, 28 nm; C, 18 nm (N = 3). Fixed in paraformaldehyde-glutaraldehyde (Karnovsky, 1965). $\times 90,000$.



FIGURES 4-7 Fibers from vagus-innervated sartorius fibers, fixed in 4% glutaraldehyde in phosphate buffer. Fig. 4, T, 20 nm; C, 19 nm (N = 10). $\times 57,000$. Fig. 5, T, 22 nm; C, 15-24 nm (N = 3), $\times 70,000$. Fig. 6, T, 23 nm; C, 23 nm (N = 2) $\times 76,000$. Fig. 7, T, 19 nm; C, 22 nm. $\times 63,000$.

arrows). The mean diameter of the caveolar openings, measured in a number of micrographs, is 19.7 ± 3 nm (mean \pm SD, $N = 45$). The opening of one T tubule, that we observed was larger than usual (28 nm, Fig. 3) and so were the openings of the caveolae in the same fiber, an effect which is perhaps attributable to the hypertonicity of the fixative (see Huxley et al., 1963). The similarity in size of the openings of the caveolae and of the T system unfortunately precludes the possibility of distinguishing the openings of the latter in freeze-fracture replicas of the plasma membrane and thus of counting their number (see Rayns et al., 1968). In sections, an opening can be identified as belonging to the T tubule only when continuity can be clearly shown to exist between the structure opening to the outside and a T tubule forming part of a triad. This happens only rarely. In addition, since the openings are small, they can be seen only in thin sections. The two factors combined may be sufficient to account for the paucity of convincing published evidence for T tubule openings.

In our limited number of observations, openings of the T tubules invariably occur a short distance away from the Z line, even when the lateral sacs of the triad accompany the T tubules to the very edge of the fiber. Thus, in cross sections of the fibers, visualization of the opening occurs only when the section is at the same angle as the final segment of the T tubule, a very unlikely event (Fig. 3). This longitudinal displacement of the opening is small and thus it would not have been detected in the local stimulation experiments of Huxley and Taylor (1958), which showed the active spots to coincide with the level of the Z line.

The most peripheral segments of the T tubules are variable in size and shape, and those observed here may be classified into three groups. (a) The triad does not reach the edge of the fiber (Fig. 1, see also Fig. 1 of Franzini-Armstrong, 1970). In this case, the terminal portion of the T tubule runs obliquely or longitudinally for a short distance (70–90 nm), independently of the elements of the sarcoplasmic reticulum, and in that region it has a diameter varying between 17 and 35 nm. Such a short segment of tubule with a restricted diameter, even if present at every opening of the T tubules, would not in itself appreciably slow the diffusion of solutes into the T tubules. Only two of a total of nine openings fall in this category. (b) The lateral sacs of the triad accompany the T tubules to the very edge of the fiber and there is no apparent change in the size and shape of the tubules until

they reach the opening (Figs. 2, 3). (c) The T tubules communicate with the outside via a large cisterna (Figs. 4–7), which in most cases can be identified to arise from the confluence of two to three caveolae (Figs. 4–6). Even where the peripheral dilation of the tubules occurs immediately under the plasma membrane, the opening has the same size as in case (a) and (b). Confluence of the T tubules with caveolae in frog fibers is more fully discussed by Zampighi et al.² (1974). Continuity between the two structures is very common during muscle differentiation, and it is thought that multiple invaginations of caveolae into the fiber is the mechanism by which T tubules are initially formed in some muscle fibers (Ezerman and Ishikawa, 1967; Schiaffino and Margreth, 1968). Other muscle fibers, besides the frog's, preserve a relationship between T tubules and caveolae in the adult (Rayns et al., 1968). The occurrence of dilated and convoluted shapes of the T tubules within a depth of two to three fibrils from the edge of the fiber is fairly frequent (Peachey, 1965; see Figs. 2, 4–6). And when T tubules appear dilated, which they frequently do near the sarcolemma, dense floccular material is often observed inside the lumen (Figs. 4, 6, 7).

The main conclusions from these observations are that the T tubules in frog fibers have a patent, small opening, and that occasionally the most peripheral segment of the tubule is restricted for a short distance, but more frequently a considerable dilation of the tubules occurs at the periphery of the fiber. Thus, unless some substance not visible in the electron microscope fills the openings, no barrier exists to the diffusion of solutes into the cavity of the T tubules.

One parameter is still needed to complete the description of the T system network in the frog, i.e. the spacing of the openings at the fiber's surface. As noted in the introduction, we think that part of the difficulty in seeing the openings in frog fibers is their scarcity. Since the openings of T tubules are of the same size as the openings of caveolae, the former cannot be identified by freeze-fracture.

SUMMARY

The openings of transverse tubules in frog twitch fibers are described. The tubules open to the extracellular space by a narrow neck, with an inner

² Zampighi, G., J. Vergara, and F. Ramon. 1974. Personal communication.

diameter of 20 nm. The most peripheral portion of the tubules is tortuous and has a variable diameter. The similarity in size of the openings of T tubules and caveolae and the meandering path of the tubules are sufficient to account for the paucity of observed openings.

This work was supported by United States Public Health Service grants NS10666, NS10338, and NS08893.

Received for publication 14 June 1974, and in revised form 3 October 1974.

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