

PORES IN THE SARCOPLASMIC RETICULUM

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The membranes limiting the tubules and vesicles of the endoplasmic reticulum appear, in general, to be of uniform thickness and without pores, discontinuities, or other specialization of structure. Some of these membranes support populations of ribosomes and are referred to as rough; others are particle free and are referred to as smooth. The membranous system in striated muscle fibers, the sarcoplasmic reticulum or SR, is mostly of the latter type. Small areas of it may support ribosomes, but these are relatively rare. In this respect, then, the SR is not exceptional. In other ways it is unique. There is, for instance, the remarkable organization in phase with the sarcomeric structure of the fibrils. Thus, opposite the I band and adjacent to the Z line, there are dilated "terminal" sacs; connecting with these and opposite the A band there are, usually, longitudinally oriented, slender sacs, and opposite the H band a more or less transversely oriented cisterna.

In addition, and even more unusual, are certain differentiations found in the membranes. These are so closely associated structurally with the components of the muscle fiber that a functional significance is implied, if not demonstrated. One of these, the subject of this report, appears as small pores or openings in the membrane of the SR cisterna located opposite the H band of the fibril. Such differentiations were described earlier by Porter and Palade (5) in the sarcoplasmic reticulum of *Amblystoma* tail muscles as "small, circular patches (ca 200 Å in diameter) of lower density in the membrane." It seemed doubtful to those authors that these light areas represented pores. This observation was essentially forgotten in the attention focused on other aspects of the SR. These structures remain, however, one of the most interesting features of the sarcoplasmic reticulum, especially in their peculiar localization opposite the H band. This prompts me to report

here a few additional observations made recently on their form, number, and distribution. These results are based on investigations of muscles from a variety of animals, fixed in different ways. Epon embedding of the tissue appears to provide a clearer and more consistent image than did the earlier methacrylate matrices.

Muscles from the tail myotomes of guppy, *Lebistes* sp., of newt, *Triturus viridescens*, of early and late stages of *Rana pipiens* tadpole, of mature *Rana catesbiana* tadpoles, skeletal muscles from adult *Rana pipiens*, and muscles from the swimbladder of the toad fish—*Opsanus tau*—were fixed in OsO₄ buffered with Veronal-acetate (Palade, 4). Tadpole tails were also fixed in 6.5 per cent gluteraldehyde buffered with 0.2 M Na cacodylate at pH 7.2, and postfixed in 1 per cent OsO₄ in water (essentially according Sabatini *et al.*, 6). In addition, some samples of frog skeletal muscles were frozen in CO₂ and allowed to thaw in Veronal-acetate buffered OsO₄.

All tissues were embedded in Epon 812 (Luft, 2). Sections were cut with a Porter-Blum microtome, stained with lead hydroxide (Karnovsky, 3), and observed in a Siemens Elmiskop I.

Fig. 1, a reconstruction from electron micrographs of tadpole tail fibers, helps to visualize the sarcoplasmic reticulum surrounding a single fibril. Starting from the "terminal sacs" at the I band level, long sacs run along the A band and join at the H band level in a flattened cisterna. The latter surrounds the fibril without interruptions. In all muscles observed, the H band cisterna is similarly well differentiated from the rest of the SR and occupies the interfibrillar spaces for the whole depth of the H band. A section tangential to the fibril thus cuts through the H band cisterna and provides a face view of the SR in the middle of the sarcomere. In such a view, as noted earlier (Porter and Palade, 5), numerous circular light areas are seen in the

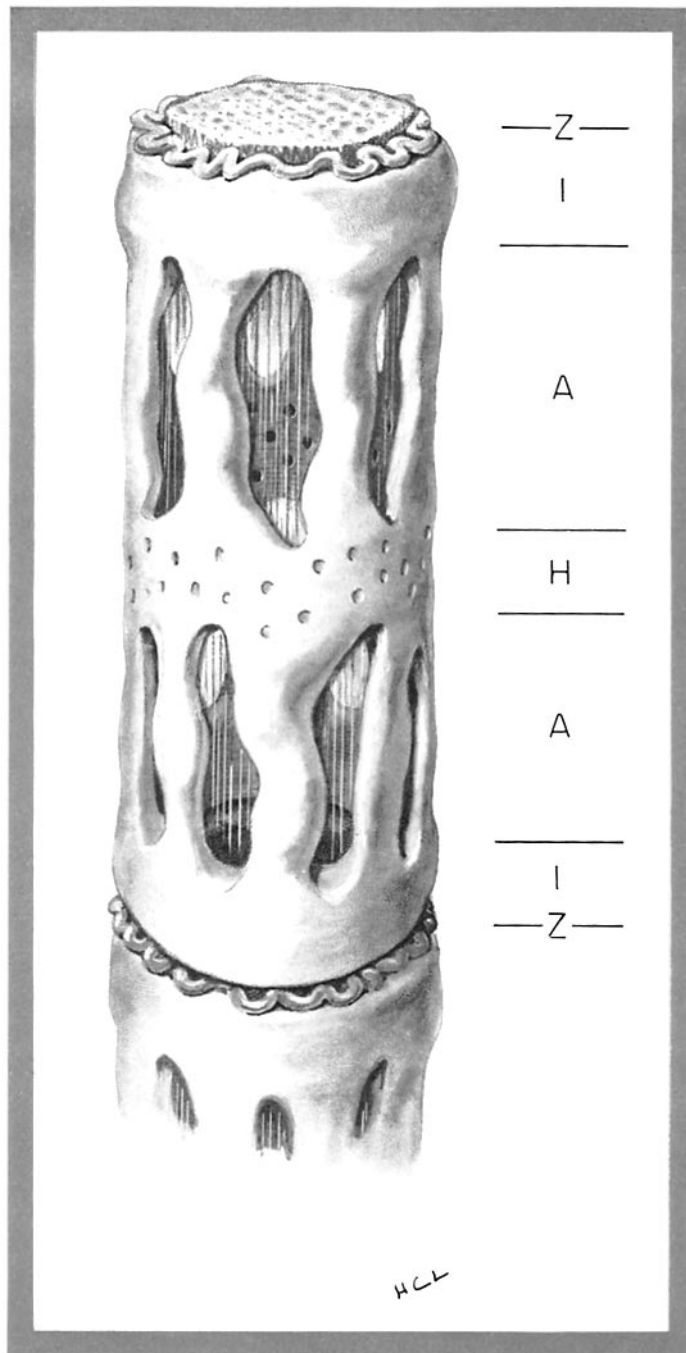


FIGURE 1 This drawing shows a tridimensional reconstruction of the elements of the sarcoplasmic reticulum surrounding one fibril over the length of one sarcomere. The reader must imagine that in the whole fiber other fibrils are adjacent to the one depicted, and share with it the SR layer. The triads are located at the Z line level. The bands of the fibril are marked at the side. The contractile material composing the myofibril is indicated by a few lines; this transparency allows a view of the reticulum on the other side of the fibril. The reconstruction is based on electron micrographs of sections from tadpole tail myotomes, but the basic features of the sarcoplasmic reticulum are the same in all the muscles investigated in this report.

Emphasis is put on the confluence of the A band cisternae into a common H band cisterna. The latter is oriented transversely to the long axis of the fiber and moves among the fibrils without interruptions.

Pores are depicted in the sarcoplasmic reticulum membrane at the H band level.

denser membrane (Fig. 2). These are irregularly disposed in the membrane and at times are very numerous (up to 20 have been counted in $0.09 \mu^2$ in a frog muscle).

Such differentiated spots in the membrane have now been seen in all types of striated muscle observed, independently of the state of contraction of the sarcomere and independently of the fixative used. It is difficult to state whether their number varies in the different kinds of animals, since the number that can be observed depends on the orientation of the membrane in the section.

These light areas are about 150 A in diameter and are surrounded by a dense border of about 70 A (Fig. 3), so that the over-all dimensions are slightly more than 200 A. Densitometric readings of the light areas in the center of the rings and of areas of the same dimensions in the surrounding sarcoplasm give the same values. Thus, the light areas appear to be pores or openings in the membrane and represent genuine interruptions in the continuity of the SR membrane. The reason for the existence of a dense border around the pores is not clear; it may be that an extra leaflet of lipoprotein is present around the rim of the opening.

Cross-sections through only the openings would be difficult to find, because to see them it would be necessary to have a section thinner than 150 A, the inner diameter of the openings. Even in "gray" sections, therefore, the pores would be masked by the underlying or overlying membrane edge. It is therefore impossible to study their relative disposition in the two facing sides of the H band cisterna. The author can say that, since a layer of sarcoplasmic reticulum is always shared by two adjacent fibrils, it is probable that pores are present on both sides of the H band cisterna.

Also, it is not possible at the moment to ascertain definitely the presence or absence of pores in muscles in which the SR does not form a flattened sac at the H band level, as in the case of vertebrate heart (5) and insect flight and fibrillar muscles (7, 8). In these fibers the form of the reticulum does not allow for a face view of its membrane, except over very small areas. The problem of the presence of pores where no H band cisterna is present remains open.

Apart from the marked difference in size, a similarity between the pores here observed and pores such as those cutting through the nuclear envelope (10) is to be excluded. The latter are

formed by a connection of the inner and outer membranes of the envelope and thus cut through the whole perinuclear cisterna and connect the cytoplasm with the nucleus. The content of the perinuclear cisterna is isolated from both. The structure of the nuclear pores is particularly evident in a section normal to the envelope, where a canal can be seen to run across the perinuclear cisterna. Nothing similar can be seen in the case of a section normal to the H band cisterna in striated muscle: in this the two sides are always independent and no structure crosses the space between them. The pores in the sarcoplasmic reticulum at the H band level are thus part of the membrane itself and connect the inside of the sarcoplasmic reticulum with the sarcoplasm.

We can ask whether the described structures in the membrane are found in striated muscle only, or in other cell types as well. In reply, one can only note that it seems rather improbable that the presence of similar structures in the endoplasmic reticulum of other cell types could have escaped the attention of the numerous researchers who have thoroughly investigated this component. Thus we can tentatively conclude that the pores are typical of striated muscle and, therefore, related to a particular function of this muscle, *i.e.* contraction. Their localization at the center of the sarcomere suggests a definite role during some phase of the contraction-relaxation cycle. Their relatively large size might allow the very rapid exchange of some substance between the SR cavity and the sarcoplasm, or *vice versa*.

Unfortunately, biochemical and physiological data on the events which take place at the H band level are scarce (1, 9) and it is difficult to relate them with the observed structure. At the moment, it is only possible to point out that a specific route of rapid exchange is opened, at this level, between the sarcoplasm and the cavity enclosed by the SR membrane.

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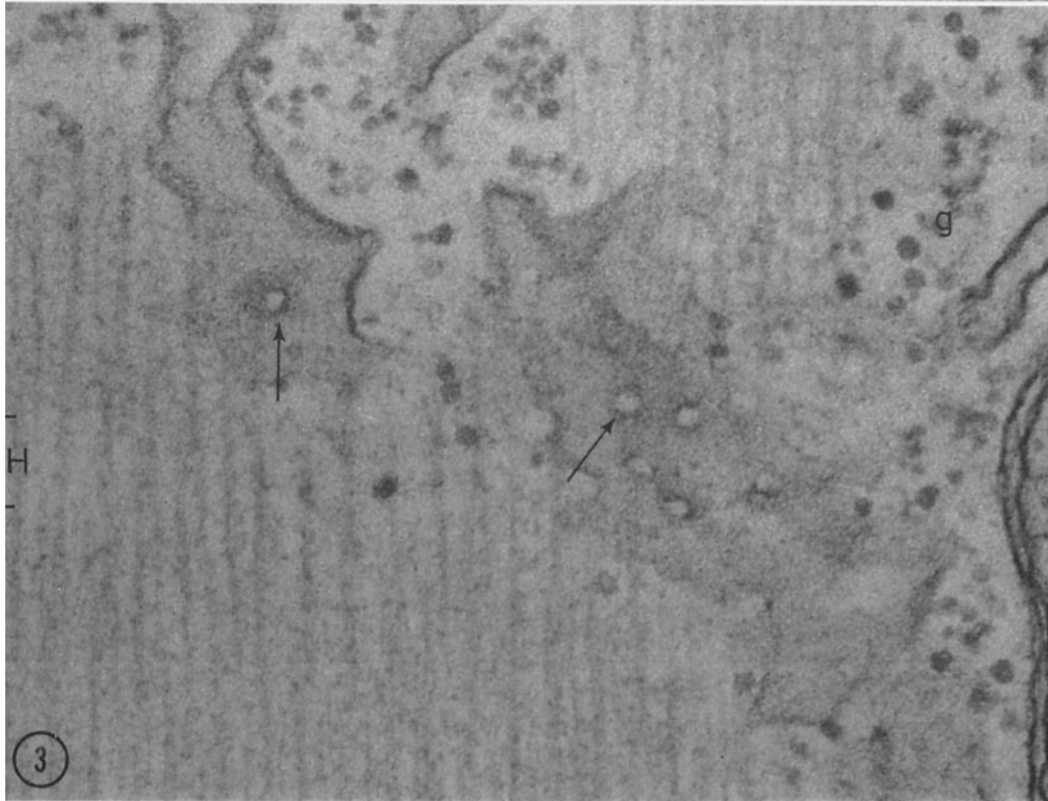
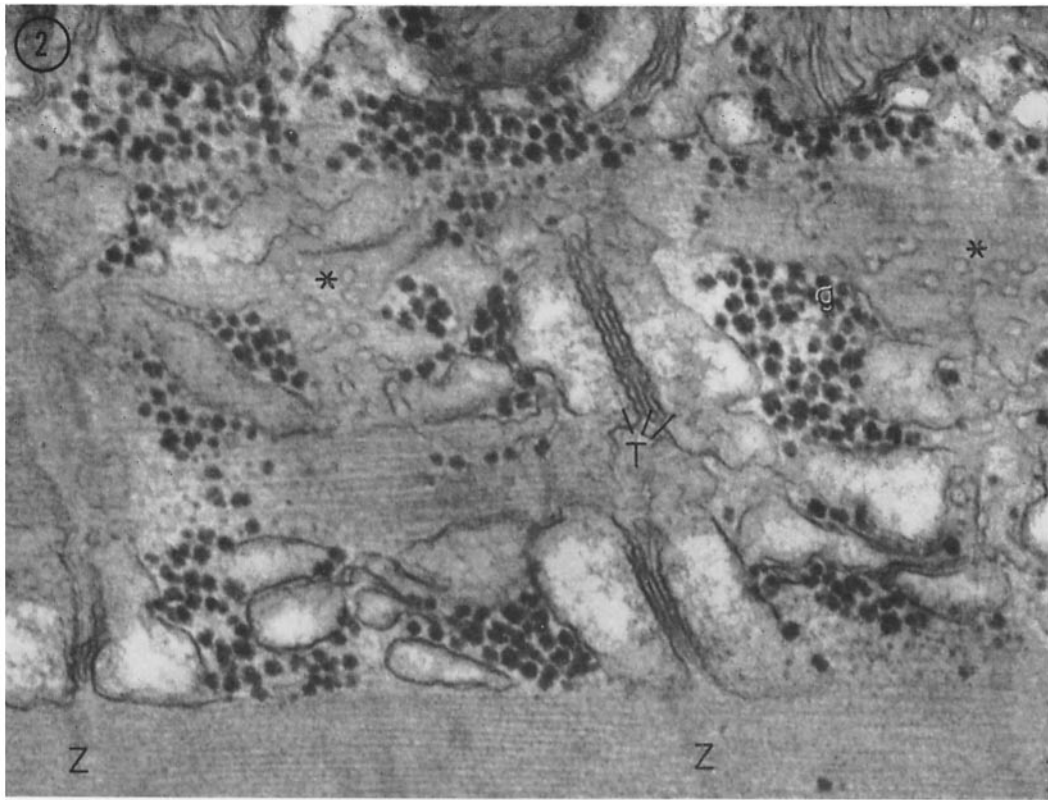


FIGURE 2 Longitudinal section of a newt (*Triturus viridescens*) tail fiber, fixed in Veronal-acetate-buffered osmium tetroxide. Asterisks mark the areas where the H band cisterna runs in the plane of the section. Numerous light areas or "pores" perforate the membrane. *T* = triad; *Z* = Z lines; *g* = glycogen. $\times 63,000$.

FIGURE 3 Shows a longitudinal section through a tadpole (*Rana pipiens*) tail myotome. Same fixation. The section cuts through the middle of the H band cisterna and part of the underlying fibril. Only one side of the cisterna is thus comprised within the plane of the section. The inside of the cisterna is toward the observer; on the outside of it run the filaments of the fibril.

Dense rims surround the pores which perforate the membrane limiting the sarcoplasmic reticulum at the H band level (arrows). The limits of the H band are marked at the left. *g* = glycogen. $\times 136,000$.

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