PURKINJE FIBERS OF THE HEART EXAMINED WITH THE PEROXIDASE REACTION

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The continuity of the transverse tubules in muscle with the extracellular space is of great physiological significance. Such a continuity is difficult to demonstrate morphologically, if, as in the case in skeletal muscle of the frog and other animals, the transverse tubules are very small or, as in the case of frog slow muscle (1), the tubules are not only very small, but are few in number. Nevertheless, the continuity of transverse tubules with the extracellular space has now clearly been demonstrated by using ferritin (1, 2) and the peroxidase reaction (3).

In recent communications (4, 5), we have pointed out that in the ventricles of mammalian hearts there are two populations of muscle fibers: those that have transverse tubules (ventricular fibers, V fibers), and those that do not. The latter we have named P fibers, since one of the places in which they are invariably found is the so called Purkinje network on the endocardial surface of the ventricles. Because the transverse tubules of cardiac muscle are large in diameter, their continuity with the extracellular space can be easily demonstrated on morphological grounds alone. Nevertheless, to confirm the absence of transverse tubules in P fibers, we performed tracer experi-

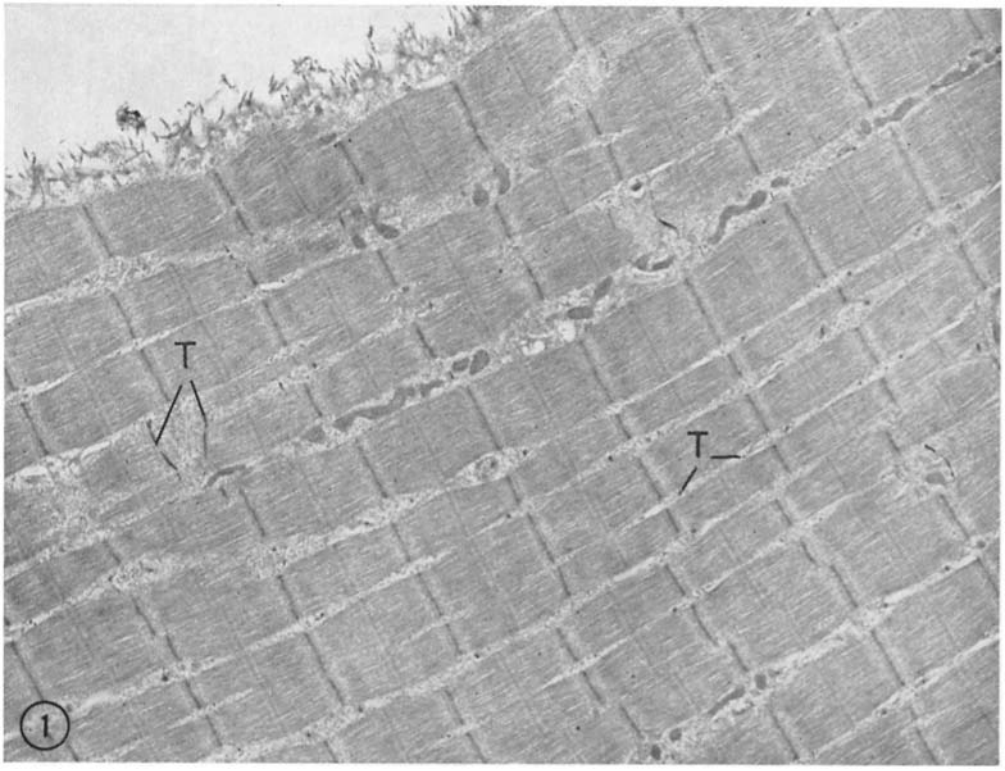

Figure 1 Rabbit psoas muscle. Epon. Exogenous peroxidase. Uranyl acetate. Note how deeply the exogenous peroxidase has penetrated into the cell, following the transverse tubules (*T*) by simple diffusion. Virtually all transverse tubules are marked. × 10,000.

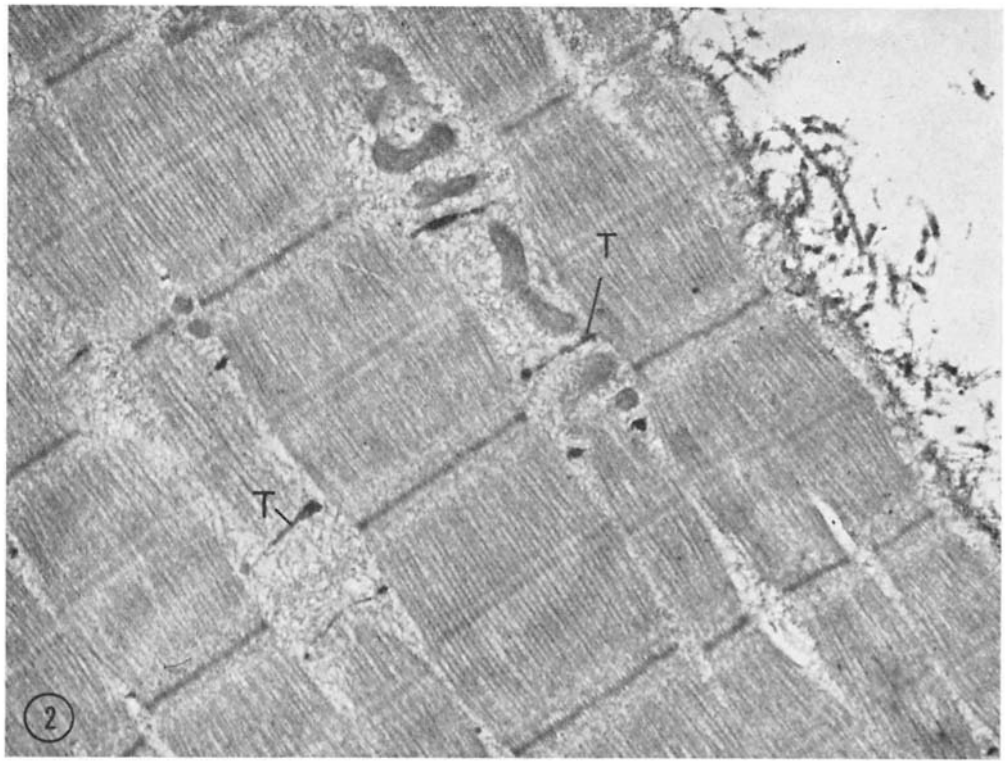

FIGURE 2 Rabbit psoas muscle. Epon. Exogenous peroxidase. Uranyl acetate. This higher magnification demonstrates that only the transverse tubules (*T*), but not the adjacent cisternae and tubules of the sarcoplasmic reticulum, are marked by the extracellular tracer. × 19,500.

ments with ferritin, but since they turned out to be inconclusive for reasons already discussed (4), the need for a more suitable tracer of extracellular space became apparent. Furthermore, additional morphological data have accumulated (unpublished observations) showing that in the atria of dog hearts there is great variation with respect to the number of transverse tubules per muscle fiber, ranging from fibers with no, or few, transverse tubules to those that have one for almost every sarcomere. Under such circumstances, a readily visible marker for transverse tubules would also be most desirable.

In the present paper, the horseradish peroxidase reaction was employed (6) to obtain further evidence for the absence of transverse tubules in the P fibers of mammalian hearts. In order to test the suitability of the method for very small transverse tubules, which exist in skeletal muscle and which might have been overlooked in our previous studies in P fibers (4, 5), we also studied rabbit psoas muscle.

MATERIALS AND METHODS

The method described by Graham and Karnovsky (6) was followed with minor modifications. A solution of 10 mg of horseraddish peroxidase (Type II, Sigma Chemical Company, St. Louis) in 0.5 ml of a 0.89% solution of NaCl was injected into the tail vein of white mice. 15 min later the mice were killed; the hearts were then rapidly removed and, after opening of the ventricles, were immersed in 4% phosphate-buffered glutaraldehyde (pH 7.2) containing 10% sucrose for 2 hr, and washed overnight in 0.05 M Tris-maleate buffer (pH 7.2). 40-μ-thick frozen sections of the ventricles were cut, and isolated P strands, papillary muscle, and skeletal muscle (see below) were excised and placed for 10 min into a solution of 3 mg/10 ml buffer of 3,3′-diaminobenzideine tetrahydrochloride (K & K Laboratories, Plainview, N. Y.) in 0.05 M Tris-maleate (pH 7.6), containing 0.01% H_2O_2. Afterwards, the tissue was briefly rinsed in the plain

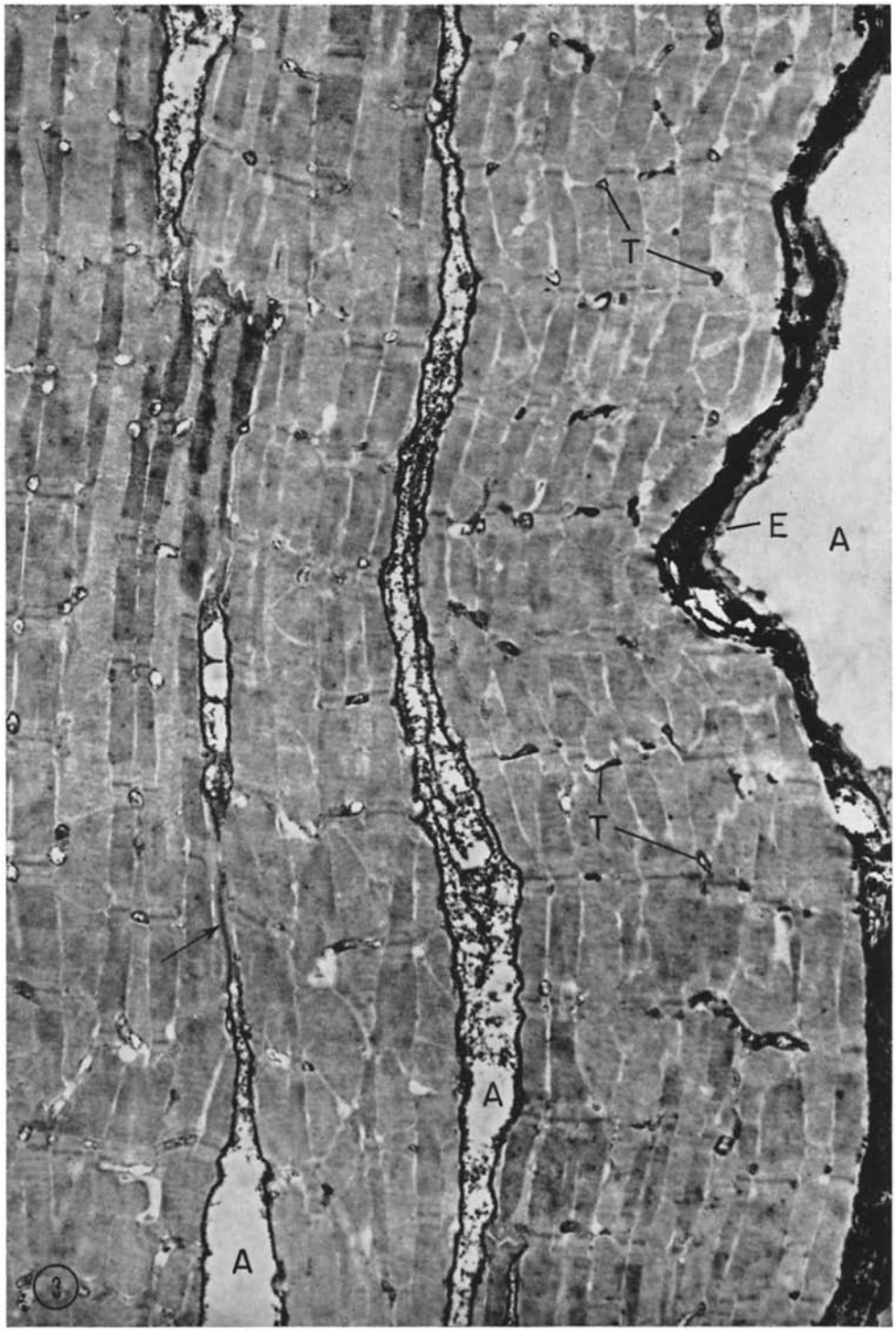

FIGURE 3 Mouse heart, papillary muscle. Exogenous peroxidase. Unstained longitudinal section. Almost all transverse tubules (*T*) contain the reaction product, although usually only those next to extracellular space are completely filled with it. Longitudinal components of the T system are sometimes suggested. However, these structures, in most instances, can clearly be shown to be intercellular space between two closely opposed cells (arrow). The low magnification was chosen to demonstrate more clearly the extent of the diffusion of the peroxidase and to show the large number of transverse tubules typical for V fibers. *E*, endothelium (endocardium in this case). *A*, extracellular space. × 7,600.

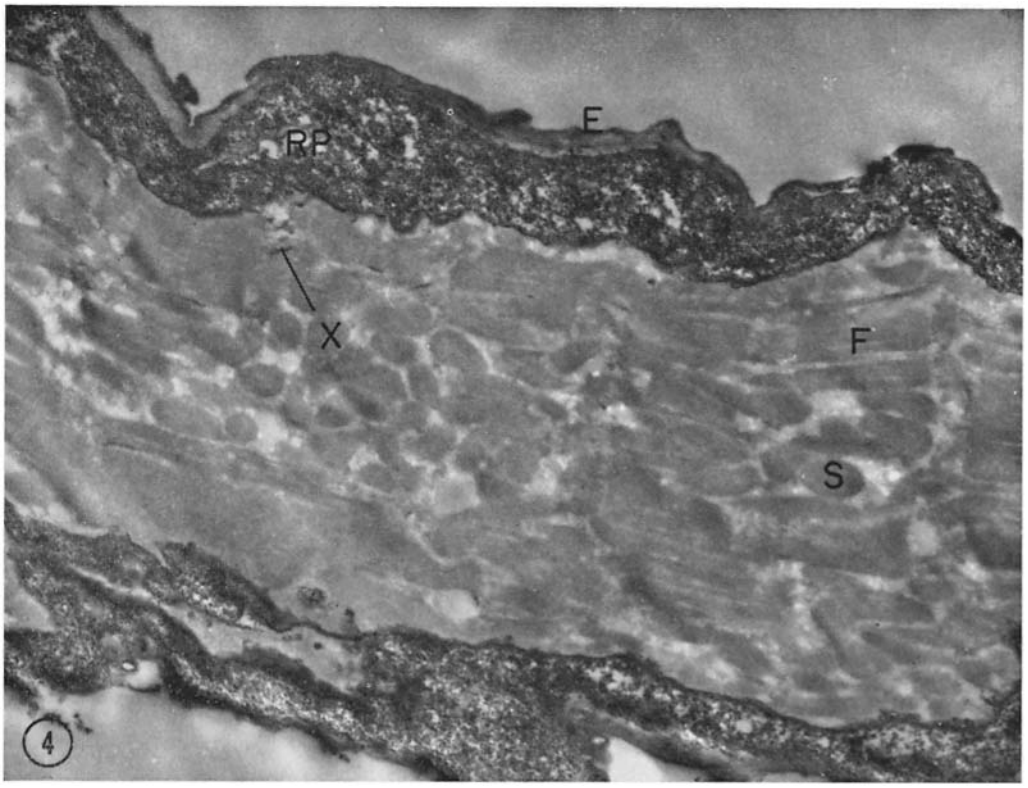

FIGURE 4 Mouse heart, isolated P fiber. Unstained longitudinal section. Exogenous peroxidase. The isolated P fiber, approximately 20 μ in diameter, is completely surrounded by endothelium (*E*, endocardium) and contains no blood vessels. There is no peroxidase marker anywhere within the fiber, except perhaps for the small spots, rarely seen, labeled *X*. This micrograph was selected for the purpose of showing them; *S*, mitochondria, *F*, myofibrils, *RP*, reaction product in extracellular space. × 11,000.

buffer, postfixed in osmium tetroxide for 2 hr, embedded in Epon, and processed for electron microscopy. Heavy metal stain was used when indicated in the legends.

Preparations of P Fibers, Papillary Muscle, and Skeletal Muscle

Under a dissecting microscope, P strands (i.e., small bundles of P fibers) were carefully freed from the base of the papillary muscles and the apex of the left ventricle of fixed hearts, so that minute networks of interconnected strands of P fibers were obtained. After embedding, individual strands of these networks measuring no more than 10–40 μ were carefully aligned for longitudinal sectioning. The papillary muscles were cut from their attachments and likewise processed without further sectioning.

A different procedure was followed for the preparation of skeletal muscle. Very small bundles (approximately 0.5 mm in diameter) from fresh rabbit psoas muscle were dissected and put for 10 min into a peroxidase solution (see above) through which air was continuously bubbled. After a brief rinse in buffer, the bundles were fixed in the same way as the cardiac tissue from the mouse. However, after fixation, the bundles were further dissected into smaller bundles of no more than approximately five muscle fibers. They were then processed with the other tissue.

Originally, we thought that the strands of P fibers could be isolated from the fresh heart, bathed in peroxidase, fixed, and then processed in the developing solution, especially so, since the smaller strands do not have blood vessels (4). However, this method gave a satisfactory peroxidase reaction only in skeletal muscle. For heart muscle, we found it necessary to inject the peroxidase intravenously. It should also be noted that the frozen sections of the ventricles were also quite unsatisfactory even after i.v. injection of

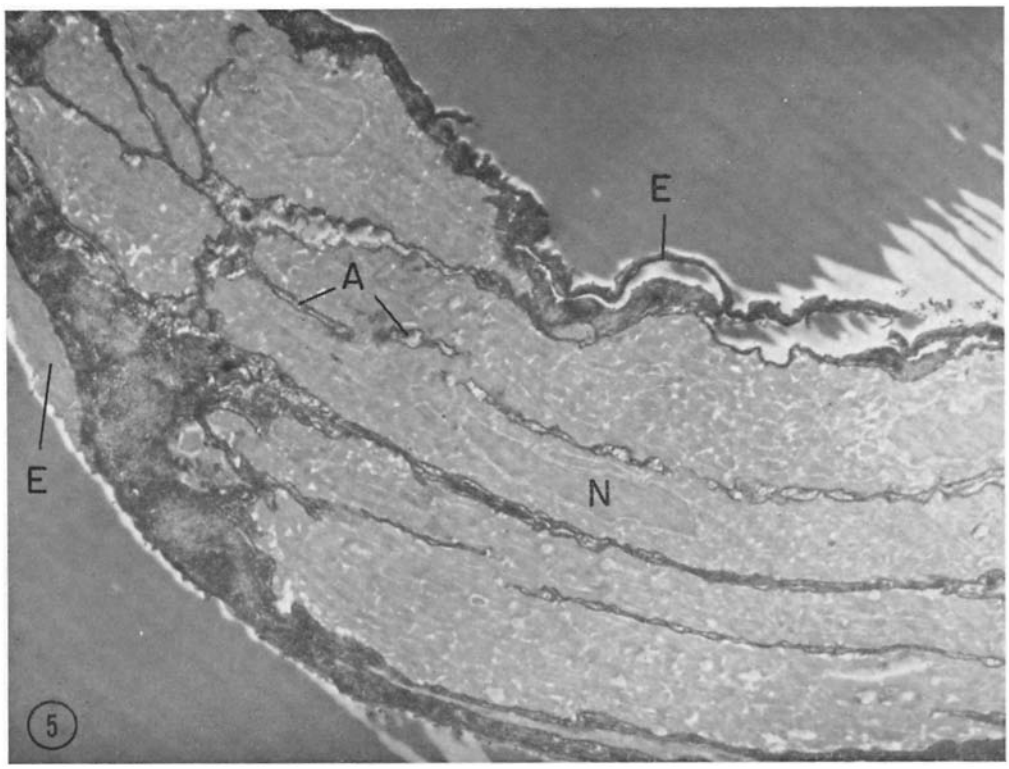

FIGURE 5 Mouse heart, isolated P strand cut longitudinally, unstained. Exogenous peroxidase. No extracellular marker can be seen within several cells, *E*, endothelium (endocardium, in this case). *N*, nucleus. *A*, extracellular space. × 2,800.

the peroxidase, but that the papillary muscle without sectioning gave the best results.

RESULTS AND DISCUSSION

The peroxidase reaction clearly traces the transverse tubules in both skeletal and ventricular muscle (Figs. 1–3). There is no evidence that the sarcoplasmic reticulum is in direct open contact with the transverse tubules or the extracellular space elsewhere. Although longitudinal components of the transverse tubular system were suggested in ventricular muscle, it is difficult, without serial sections, to rule out sarcolemmal invaginations or small segments of extracellular space between closely apposed cells (Fig. 3, arrow and Fig. 4). As one would have expected from the previous work on the P fibers (4, 5), there are virtually no stained tubules in the several P fibers so examined (Figs. 4 and 5), with the exception, perhaps, of the densities marked *X* in Fig. 4. As has already been discussed (5), even if these densities represent transverse tubules, they would not, because of their small number, be expected to have any influence on that electrical behavior of the P fiber, which was theoretically shown to be dependent, in part, on the absence of transverse tubules (5).

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