hibition in vitro of the reaction of RNA polymerase from *E. coli* (*3*); the decrease in protein synthesis was probably a consequence of the decreased production of messenger RNA. The rates of growth and of DNA synthesis were reduced to the same extent by $2 \times 10^{-4}M$ quinacrine. Others have stated that $4 \times 10^{-4}M$ quinacrine permitted DNA synthesis proportional to turbidity increases in cultures of *Bacillus cereus* (*10*).

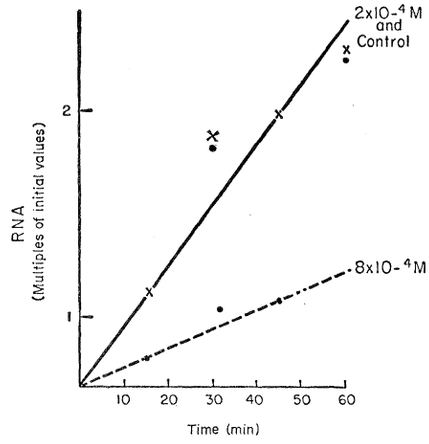

Fig. 4. Effect of quinacrine on RNA biosynthesis in *Escherichia coli*. Experimental design and procedures were the same as in Fig. 3, except that RNA was determined with an orcinol method (*9*).

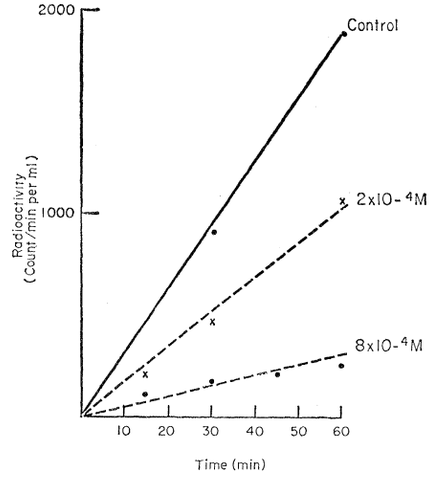

Fig. 5. Effect of quinacrine on protein biosynthesis in *Escherichia coli*. Incorporation of C^{14}-phenylalanine into bacterial proteins was measured by a membrane filter technique. Quinacrine (at indicated concentrations; none in control) and C^{14}-phenylalanine [5 μg/ml (specific activity 61 μc/μmole)] were both added to exponentially growing cultures at time zero. Samples were removed at intervals and immediately diluted with equal volumes of 10 percent trichloroacetic acid (containing 1 percent casamino acids). After 30 minutes at 2°C, the precipitates from the samples were collected on Millipore filters, washed first with 5 percent trichloroacetic acid (containing casamino acids), and then three times with chloroform to remove residual quinacrine. The filters were placed in a dioxane-based scintillation fluid, and radioactivities were counted in a Nuclear-Chicago liquid-scintillation counter.

Undiminished RNA synthesis and appreciable protein synthesis at the bacteriostatic concentration of $2 \times 10^{-4}M$ quinacrine accounted for the accumulation of large amounts of cytoplasmic material in the filaments after 24 hours (Fig. 2). This material was organized in distinct segments which were, however, not separated by cross septa. The fact that bacteria exposed to quinacrine failed to initiate events resulting in cell division may be related to the action of the drug upon DNA. By analogy, cells of *Escherichia coli* in which DNA has reacted with mitomycin C grow within shorter times to shorter filaments which exhibit structural characteristics similar to those we have observed in the filaments grown in the presence of quinacrine (*11*).

Biophysical studies on the nature of the quinacrine-DNA complex (*1–3*), inhibitions of DNA-dependent enzymatic reactions by the drug (*1, 3, 4*), as well as our study on the effects of quinacrine in whole bacterial cells are consistent with the view that the mechanism of biological action of the drug is the specific reaction of quinacrine with native, double-stranded DNA and that the resulting mode of action is an impairment of DNA replication and, at cytocidal concentrations, of RNA transcription. That quinacrine also exerts its antimalarial effect by acting on the nucleic acids of plasmodia has been postulated by Albert (*12*) and is supported by observations (*13*) that the drug inhibits the incorporation of P^{32}-orthophosphate into the nucleic acids of plasmodia.

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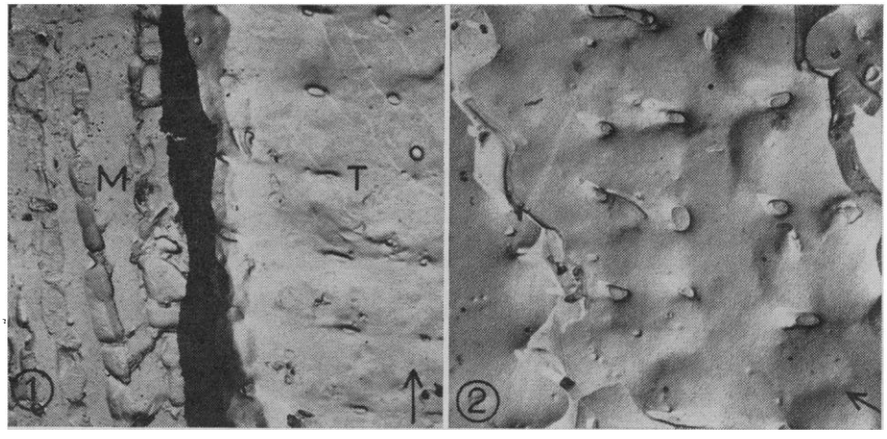
Transverse Tubule Apertures in Mammalian Myocardial Cells: Surface Array

Abstract. *The technique of "freeze-etching" tissues for electron microscopy has permitted observation of the external apertures of the transverse tubules. The apertures appear on the cell surface in approximately parallel rows, which can be interpreted as corresponding longitudinally to the spaces between the myofibrils and transversely to the Z regions of the myofibrils.*

On the basis of his well-known experiments on the inward conduction of excitation in striated muscle, Huxley (*1*) suggested that the simplest explanation of his findings was that a transverse tubular network must exist with its lumen communicating with the extracellular space. Continuity between the lumen of the transverse tubular system [T system (*2*)] and the extracellular space was first suggested on the basis of electron-microscopic evidence in mammalian cardiac muscle (*3*), and further observations have confirmed that such a continuity exists both in mammalian myocardium (*4*) and in many other types of striated muscle (*5*).

The electron-microscopic evidence of this continuity has hitherto been obtained from sectioned material. The development of "freeze-etching" (*6*) for electron microscopy has permitted a new approach to the study of the surface relationships of the tubular systems in muscle. We have studied guinea pig cardiac (papillary) muscle using this technique (*6*). The "freeze-etching" process was carried out with a Balzers freeze-etch apparatus (*7*) which



Figs. 1 and 2. Shadowed replicas of parts of longitudinally cut, freeze-etched myocardial cells from guinea pig. Direction of shadowing is indicated by arrows. Fig. 1. Part of cell surface membrane viewed from the outside of the cell. The apertures (*T*) of the transverse tubules lie in rows and appear as pits in the surface. Part of the interior of the same cell or a neighboring cell is also seen; the myofibril (*M*) and the columns of mitochondria indicate the long axis of the cell (× 7200). Fig. 2. Part of the surface membrane of another cell, this time viewed from the inside of the cell. The long axis of the cell is vertical, as in Fig. 1. The stumps of the tubules are seen projecting from the inner surface of the membrane (× 12,900).

is designed so that deep-frozen material can be fractured and etched and a replica made while being held at a low temperature in a high vacuum.

Specimens were plunged into liquid freon (*8*), cooled to −150°C with liquid nitrogen. The cooling rate of the specimen was approximately −100°C/sec. To prevent formation of ice crystals within the tissue, we perfused the muscle with glycerine (30 percent) in Ringer solution for not less than 15 minutes before it was frozen. The frozen sample, attached to a copper disc, was then transferred to the specimen stage of the freeze-etch apparatus, which was initially held at −150°C. While the apparatus was being pumped out to a pressure less than 1×10^{-5} torr, the temperature of the specimen was allowed to rise to −100°C. The fracturing was carried out with a microtome, the metal blade of which was cooled to −190°C. Etching was accomplished by the sublimation of water from the fractured surface, the structural details of which were thus caused to appear in relief. Shadowing with platinum-carbon enhanced the contrast of the specimen, and the whole fractured surface was preserved by carbon replication in the normal manner (*6*). The muscle tissue was removed by digestion in 40 percent chromic acid, and the replica was examined in an electron microscope.

The cutting of the frozen tissue is in fact a fracturing process (*6*), and the line of fracture partially follows natural lines of separation in the tissue. Thus when the cut is running in a direction which will graze a cell, the fracture will tend to follow the contour of the cell, exposing a surface which may be interpreted as being either the outer or inner aspect of the cell membrane, or a surface which was originally apposed to one of these, or even a new surface formed by cleaving the membrane along its center line (*9*).

From the interpretation of the shadowing, from the correlation with the fine structure established from sectioned material (*2–5*), and from other freeze-etch studies of both plant material (*10*) and animal cells (*11*), we believe that Figs. 1 and 2 represent, respectively, outer and inner aspects of the myocardial cell membrane. In Fig. 1, dense deposits of heavy metal have piled up on the side of the apertures farthest from the source of the shadowing (see arrow for direction of shadow-casting), thus indicating depressions in the surface.

In Fig. 2, on the other hand, a negative (that is, pale) shadow is cast beyond each of a number of structures, thus identifying these structures as projections upward from the surface rather than as depressions; the dense deposits of heavy metal have piled up against the nearer side of the projections. From their distribution and size, these projections are interpreted as the stumps of T-tubules broken off near the cell membrane.

The T-tubule apertures (whether viewed from outside or inside the cell membrane) are evidently arranged in rows that are in many places approximately parallel to each other in both a longitudinal and a transverse direction. It has been shown with sectioned material that in mammalian myocardial cells the T-tubules lie between the myofibrils at the Z regions (*4*); the distances between the rows of apertures in the present findings are in keeping with this arrangement of the T-tubules. In some places, however, the array of apertures is less regular, and sometimes no aperture is seen at a point where one might be expected.

With the freeze-etch method we have been able to demonstrate the surface relationships of the T-tubules in myocardial cells in a new and striking way. The method may be useful in the study of invaginations of the cell membrane in other cells.

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