and 1 hour in similarly buffered 1 percent osmium tetroxide, the tissue was dehydrated in a graded ethanol series and then passed through 1,3-epoxypropane to Epon resin. Sections were stained in aqueous 2 percent uranyl acetate at 60°C for 20 minutes and in aqueous 2.82 percent lead citrate, *p*H 12.0, at about 24°C for 7 minutes.

Strains J_1 and Si grew rapidly in PG 1 and produced zoospores in dilute salts, whereas G_1 grew more slowly and was never observed to produce zoospores. All observed hyphae of G_1, grown in PG 1 or dilute salts, contained mycoplasma-like bodies comparable to those shown in Figs. 1 to 3. Hyphae of strains J_1 and Si in both vegetative and sporulating phases did not contain such bodies.

The mycoplasma-like bodies found in the hyphae of strain G_1 were predominantly isodiametric with a diameter of approximately 0.4 μm. They were bounded by a single membrane which was indistinct but in certain cross sections was clearly resolved into a "typical" dark-light-dark membrane profile (Fig. 3). The matrix of the bodies was composed of variously osmiophilic granular material (Figs. 1 and 2). The more intensely stained bodies were more pleomorphic with frequent invaginations and membrane-lined cavities. The similar distinctive appearance of the contents of these invaginations and cavities and the membrane lining of the cavities suggest the possible origin of the cavities as occluded invaginations. Representatives of the entire range of the population also showed occasional fingerlike projections and profiles which are suggestive of a budding process (Figs. 1 and 2). The number of mycoplasma-like bodies per unit cytoplasm was variable; a typical high concentration is shown in Fig. 1. Subjective observation showed no pattern in the distribution of the areas of high or low population density throughout the hyphae, nor was any association detected between the bodies and other cellular organelles.

The true identity of the mycoplasma-like bodies is uncertain. Morphologically they most closely resemble certain mycoplasmas such as those shown in (*2*) and (*7*), although their membranes and possible ribosomes tend to be less distinct. This difference could be a function of the preparative procedures. The absence of these bodies from strains J_1 and Si suggests that they are not an essential part of this species' cellular machinery. For example, they are unlikely to be microbodies with which they are morphologically comparable. Because they are confined to a strain which grows relatively slowly (compared with J_1 and Si) and which lost the ability to produce zoospores (*4*), it is possible that they are agents which cause infective disease. No other structural differences which could account for the different performance of the various isolates were observed.

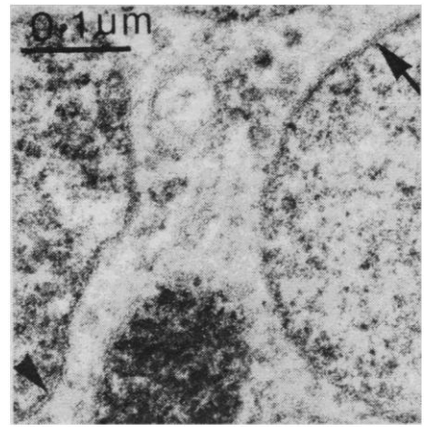

Fig. 3. Detail of two mycoplasma-like bodies showing the dark-light-dark profile of their bounding membranes (arrows).

If the structures reported above prove to be mycoplasmas, they represent the first report of such organisms in this group of fungi and possibly in the fungi as a whole. However, Schrantz (*8*) has shown comparable structures in an Ascomycete, *Scutellinia*. He considered them to be bacteria but there seems to be no valid reason for this assumption except the presence of a small amount of osmiophilic material around each particle, external to its membrane.

The possible presence of mycoplasmas in fungi is a finding of twofold major significance. Undetected mycoplasmas could cause serious problems of interpretation in physiological and biochemical studies, but also the potential for using mycoplasmas as agents for biological control of fungal pathogens should now be explored.

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Calcium Ion Release in Mechanically Disrupted Heart Cells

Abstract. *In cardiac muscle fibers which have had their sarcolemma disrupted intracellular stores of calcium ions can be released by the same chemical stimuli which cause their release from the sarcoplasmic reticulum of skinned skeletal muscle fibers. These stimuli are increases in calcium or caffeine concentrations and substitution of chloride for propionate or sodium for potassium in solutions bathing the fibers.*

Increases in the calcium concentration in the sarcoplasm of striated muscles initiate contraction (*1*). In skeletal muscle, the sarcoplasmic reticulum (SR) functions as an internal store of calcium and as the source of the Ca^{2+} which activates the contractile proteins (*2*). Due to differences in the structure of cardiac and skeletal muscle and the existence of a Ca^{2+} current across the sarcolemma during the cardiac action potential, the source of the calcium ions that initiate contraction in cardiac muscle is not clearly established (*3*, *4*).

Therefore it is important to know if chemical agents which cause Ca^{2+} release from the SR of skeletal muscle can also release Ca^{2+} from the SR in cardiac muscle in amounts sufficient to cause tension development. The Ca^{2+} sequestering abilities of skeletal SR have been studied (*5*); however, little is known about the mechanisms that cause Ca^{2+} release, the final event in a process that begins with the depolarization of the sarcolemma (surface membrane). Using single skeletal muscle fibers from which the sarcolemma had been re-

moved (skinned fibers), several investigators have initiated Ca^{2+} release from the sarcoplasmic reticulum following changes in the bathing medium surrounding these fibers (*6–9*). Specifically, Ca^{2+} can be released from the SR of skeletal muscle by: (i) changing the anion from propionate to Cl^- (*6, 8*); (ii) adding several millimolar caffeine (*7, 8*); and (iii) increasing the Ca^{2+} concentration (*7, 8*). The experiments described below demonstrate in cardiac muscle that Ca^{2+} release from intracellular stores and tension development can be triggered by the same chemical stimuli described above (*10–12*).

Cardiac muscle from rats killed by a blow on the head and from a monkey anesthetized with Vetalar (ketamine hydrochloride, Parke-Davis) and halothane was used. A piece of tissue from the apex of the heart was removed, cut into small pieces, and homogenized in a relaxing solution in a manner similar to the method of Fabiato and Fabiato (*10*). The relaxing solution contained a high concentration (7 m*M*) of the Ca^{2+} chelator ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA); *p*Ca = 9 (*13*). Small bundles of fibers 10 to 100 μm in diameter were then mounted in a force transducer, as has been described for skinned skeletal muscle fibers (*14, 15*). Tension development, which was interpreted as indicating an increase in Ca^{2+} concentration surrounding the myofibrils, was monitored as the bathing solutions surrounding the fiber bundles were changed. Tension in the cardiac muscle fibers was 20 to 50 percent of the tension in skeletal muscle fibers of comparable size. The results obtained from the primate tissue were qualitatively the same as those obtained from the rat.

In each experiment a small bundle of fibers was mounted in the force transducer, placed sequentially in a series of solutions (*15, 16*) designed to load internal stores with calcium, and finally immersed in a test solution. The protocol consisted of first placing the fiber bundles (Fig. 1, arrow 1 in all records) in a relaxing solution (high EGTA concentration, *p*Ca = 9, major anion propionate, ionic strength μ = 0.15) and then transferring them (at arrow 2) to a loading solution (7 m*M* EGTA, *p*Ca = 4.5, propionate, μ = 0.15) which caused contraction and simultaneous loading of internal stores with Ca^{2+}. At arrow 3 the bundles were moved to a lightly buffered solution with a low Ca^{2+} concentration (0.05 m*M* EGTA, *p*Ca = 6.4, propionate, μ = 0.170) which removed the high Ca^{2+} and EGTA concentration surrounding the SR and myofibrils and caused the fibers to relax. Under these conditions (low EGTA) any subsequent increase in the concentration of Ca^{2+} surrounding the myofilaments could be monitored as tension development. The test solutions (arrows labeled *Test*) were identical to the solution at arrow 3 except for the change in the major anion or cation, caffeine, EGTA, or Ca^{2+} concentrations, as noted for the particular record. For each test solution three sequential experiments were performed. In the first and last experiments the test solution contained a low EGTA concentration (or zero for the Ca^{2+}-induced contractures) and the appropriate change in ion or caffeine concentration (arrows labeled *Test*). In the second experiment of each sequence, the same test solution was used except that the EGTA concentration was raised to 7 m*M* to prevent released Ca^{2+} from binding to the contractile proteins (arrows labeled *Control*). Other control experiments indicated that tension development was not an artifact due to moving the fiber bundle through the air-solution interface (Fig. 1D, arrow labeled *Air*). All experiments were repeated after sodium azide (5 m*M*) had been added to the solutions.

In skinned skeletal muscle, suddenly substituting Cl^- for propionate as the major anion bathing the fiber will cause transient contraction (*8*). The proposed mechanism for this contraction is that the SR is electrically depolarized by this substitution, which causes Ca^{2+} to be released (*6, 8*). The first set of solution changes in Fig. 1A show that a similar change in the anionic medium bathing the fiber (from propionate to Cl^-) will also cause cardiac fibers to contract. The fiber bundle was relaxed, loaded (fiber contracted), and relaxed again in a solution lightly buf-

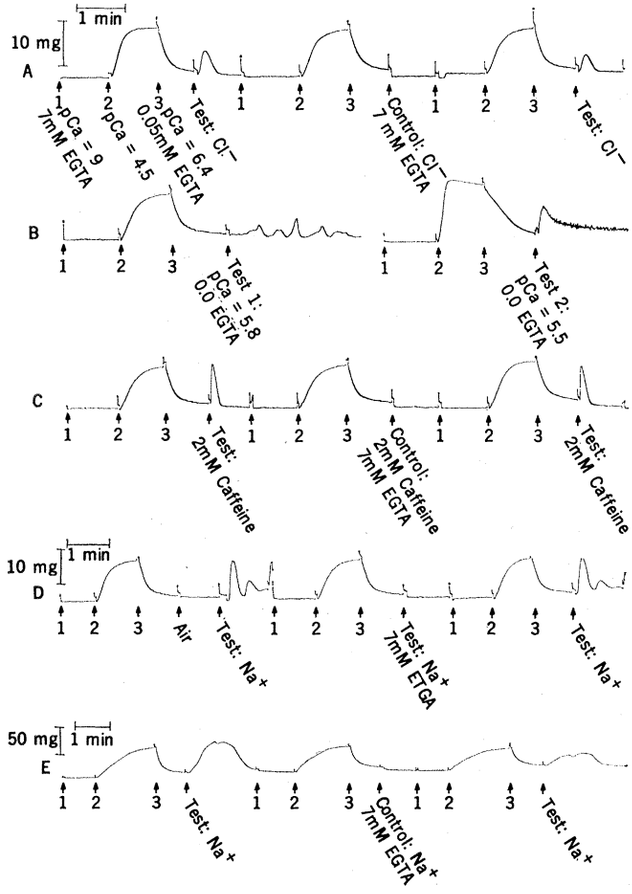

Fig. 1. Chemically induced contraction in mechanically disrupted rat ventricular muscle fibers (A to D) and skinned skeletal muscle fiber (E). The ordinate shows tension increasing upward and the abscissa shows time. Arrows indicate changes in bathing solutions. All solutions were calculated to have 2 m*M* $MgATP^{-2}$, 0.1 m*M* Mg^{2+}, 70 m*M* K^+, and 10^{-7} *M* H^+. The fiber was immersed in a relaxing solution at arrow 1, transferred to a Ca^{2+} loading solution at arrow 2, moved to a lightly buffered solution with low Ca^{2+} at arrow 3, and finally transferred to either a test solution or the control solution. Control solutions contained 7 m*M* EGTA and no Ca^{2+}. (A) Contraction produced by transferring the fiber bundle from a propionate solution to a test solution containing Cl^-. (B) Contraction produced by immersion into free Ca^{2+} solution at arrows Test 1 and Test 2. (C) Contraction produced by immersion into a test solution containing 2 m*M* caffeine. (D) Contraction produced by substituting Na^+ for K^+ in the test solution. At the arrow marked *Air*, the fiber bundle was moved through the air-solution interface, and then returned to the same solution. (E) Same protocol as in (D) except that a skinned fiber from the semitendinosus muscle of a frog (*Rana pipiens*) was used.

fered with EGTA at arrows 1, 2, and 3, respectively, as described above. The fiber bundle was then transferred to a test solution identical to the solution used at arrow 3 except that Cl^- was substituted for propionate as the major anion (low EGTA, $p\text{Ca} = 6.4$, Cl^-, $\mu = 0.170$). The increase in tension is not due to a direct effect of the change in anion on the contractile proteins (*17*). The transient increase in tension is qualitatively the same as that observed by Ford and Podolsky (*8*) for skinned skeletal muscle. The second series of solution changes in Fig. 1A (arrows labeled 1, 2, 3, and *Control*) shows that contraction caused by substituting Cl^- for propionate can be prevented by increasing the EGTA concentration from 0.05 to 7 m*M* in the test solution. The initial sequence of solution changes was then repeated.

Tension produced by "regenerative Ca^{2+} release" (Ca^{2+} release caused by sudden increases in the Ca^{2+} concentration surrounding the SR) described in skinned skeletal muscle fibers (*8*) has not previously been demonstrated in cardiac muscle [however, see (*10*)]. Figure 1B shows regenerative Ca^{2+} release (tension transient) caused by suddenly increasing the Ca^{2+} concentration in the myoplasm of cardiac muscle. The test solution was similar to the relaxing solution (arrow 3) except that there was no EGTA and the Ca^{2+} ion concentration had been raised (no EGTA, $p\text{Ca} \simeq 5.8$, anion unchanged, $\mu = 0.170$). After a short delay the fiber contracted spontaneously on being placed in the test solution.

The second sequence of solution changes in Fig. 1B is for a different fiber bundle. The muscle was transferred to a solution similar to the first test solution except that $p\text{Ca} \simeq 5.5$. The fibers spontaneously contracted and relaxed. Similar responses in skeletal muscle have been interpreted as reflecting regenerative Ca^{2+} release (*8*). Contraction could be prevented by raising the EGTA concentration in the test solution to 7 m*M*. In both of these fibers the Ca^{2+} concentrations in the test solutions are below contraction threshold since soaking these fibers for several minutes does not cause them to develop significant tension.

Figure 1C shows that tension transients can be initiated by immersing the fiber bundle in a test solution containing 2 m*M* caffeine (EGTA, Ca^{2+}, and anion concentrations unchanged).

Sodium ion causes Ca^{2+} release from cardiac fragmented SR (*11*). Substituting Na^+ for K^+ in the test solution (Fig. 1D) will cause the cardiac fiber bundle to contract. This response is also prevented by high concentrations of EGTA (second set of solutions) and is reproducible (third set of solutions). Similar results are obtained from skinned frog skeletal muscle transferred from potassium propionate to sodium propionate solutions (Fig. 1E).

There is ample evidence that these chemical stimuli act intracellularly rather than on any undisrupted surface membranes which may remain after homogenization. Fabiato and Fabiato (*10*), in the original description of the preparation used in this study, showed that the intracellularly measured transmembrane potential of the fiber bundles was generally near zero. They also showed that application of buffered Ca^{2+} solution caused the fiber bundles to contract, which suggests that the sarcolemma was permeable to the Ca_2EGTA complex. In addition, the solution changes in these experiments were made in such a way that they would be expected to hyperpolarize or have a negligible effect on the surface membrane potential of an intact cell. For instance, substituting the permeant anion Cl^- for the less permeant propionate would hyperpolarize the surface membrane. Thus, fibers whose surface membranes were intact and maintained a potential gradient would not be expected to contribute to the observed responses through depolarization of the sarcolemma. Further, when fibers were subjected to a similar procedure in which they were transferred from solutions containing Na^+ as the major cation to test solutions containing K^+ (a depolarizing solution for intact cells) no change in tension was observed. This suggests that if intact cells do exist after the homogenization, they do not contribute significantly to the observed tension transients. Finally, the time course of tension development and relaxation was directly comparable to that for skinned skeletal muscle fibers (see Fig. 1, D and E.).

The contractile responses to all stimuli could be prevented by high concentrations of EGTA (Fig. 1), which would be expected if the tension responses were caused by Ca^{2+} release and not by a direct effect of the solution changes on the contractile proteins (*8*). The addition of 5 m*M* azide, a poison that blocks Ca^{2+} uptake in mitochondria (*18*), to the bathing solutions has no effect on the results as described above. This fact and the similarity of the responses in heart and skeletal muscle (particularly the identical effect of caffeine) suggest that the source of Ca^{2+} that causes the tension response is the SR in heart muscle.

These data indicate for the first time that chemical stimuli which cause Ca^{2+} release from the SR in skinned skeletal muscle also cause enough Ca^{2+} to be released in cardiac muscle [which has significantly less SR than skeletal muscle (*4*)] to cause tension development. These findings show that the mechanism of Ca^{2+} release in skeletal muscle may also play a physiological role in cardiac muscle.

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12. Fabiato and Fabiato (*10*) showed that an increase in caffeine and Ca^{2+} concentrations will cause mechanically disrupted rat cardiac muscle fiber to spontaneously shorten. Palmer and Posey (*11*), using fragmented sarcoplasmic reticulum from rat heart, showed that Na^+ will cause Ca^{2+} release.
13. Abbreviation: $p\text{Ca} = -\log[Ca^{2+}]$, where the square brackets denote concentration.
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16. All bathing solutions were made by mixing varying amounts of KCl, NaCl, potassium propionate, sodium propionate, tris–adenosine triphosphate, tris-phosphocreatine, creatine kinase, EGTA, $MgCl_2$, magnesium propionate, $CaCl_2$, calcium propionate, imidazole, HCl, and propionic acid. The total amount of these chemicals added for each solution was adjusted to give constant, computed, free concentrations of 70 m*M* K^+ or Na^+, 2 m*M* $MgATP^{-2}$, 15 m*M* phosphocreatine anion, 3 mg of creatine kinase per milliliter, and 10^{-7} *M* H^+; the desired concentrations of Ca^{2+}, Mg^{2+}, and EGTA; the desired ionic strength; and to produce a solution with the appropriate major anion (Cl^- or propionate). The temperature was 24°C. Binding constants used in the computer program are listed in (*15*).
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