mc/kg and the interval between drug administration and killing was 24 hours for chickens and 3 hours for quail. The finding of grains along bone surfaces beneath osteoblasts but not along inactive and resorbing surfaces also argues against phagocytosis of the label, and provides an alternate explanation for the presence of grains in newly formed bone. That is, [^{3}H]acetazolamide may have been initially adsorbed on bone surfaces but, as shown by the quail data, was cleared from inactive and resorbing surfaces within 3 hours or less, while in areas of bone formation it was covered by layers of new bone before the loosely bound drug could be cleared completely.

The results of this study demonstrate that CA occurs in osteoclasts in physiologically significant quantities. This is in contrast to some earlier results with mammals (*16*) which indicated that chronic CA inhibition had no effect on bone growth or on blood calcium and phosphate. However, our results support more recent evidence (*1*, *2*) suggesting that CA may be important in bone resorption.

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18. We thank H. Schraer and R. Schraer for valuable discussion and criticism during the course of this work. Paper No. 4465 in the journal series of the Pennsylvania Agricultural Experiment Station. Supported in part by NIH grant AM 04362 and a National Science Foundation Traineeship.

25 June 1973; revised 23 October 1973

Mycoplasma-Like Structures in the Aquatic Fungus Aphanomyces astaci

Abstract. *The hyphae of a nonzoosporulating strain of* Aphanomyces astaci *contain numerous structures which most closely resemble published micrographs of certain mycoplasmas. Two normal strains of the same species do not contain these mycoplasma-like bodies.*

Numerous plant and animal diseases appear to be caused by mycoplasmas (*1*, *2*) but, although fungi frequently show various disease symptoms, only viruses have so far been reported as disease-causing agents of fungi (*3*). Unestam and Svensson (*4*) reported a strain (G_1) of the crayfish plague fungus, *Aphanomyces astaci*, which had lost the ability to produce zoospores in culture. If this defect was genetically controlled such a strain could yield information on the zoosporulation process when compared to normal strains. Alternatively, the defect could be due to an infective agent. In order to differentiate between these possibilities an ultrastructural investigation was initiated.

Isolates G_1, J_1, and Si (*4*) of *Aphanomyces astaci* Schikora (a member of the Oomycetes) were grown aseptically in liquid PG 1 medium (*5*) from infected PG 1 agar cubes at 25°C for 4 days. On this medium they only grow vegetatively. Zoosporulation was induced by transfer from PG 1 to "dilute salts" (*6*). Colonies at the appropriate growth stages were fixed by adding to the liquid growth medium an equal volume of 5 percent glutaraldehyde in M/15 Sorenson's phosphate buffer. After 5 minutes in this mixture at about 24°C the colonies were transferred to similarly buffered 5 percent glutaraldehyde for 65 minutes. After three 20-minute rinses in the buffer mentioned above

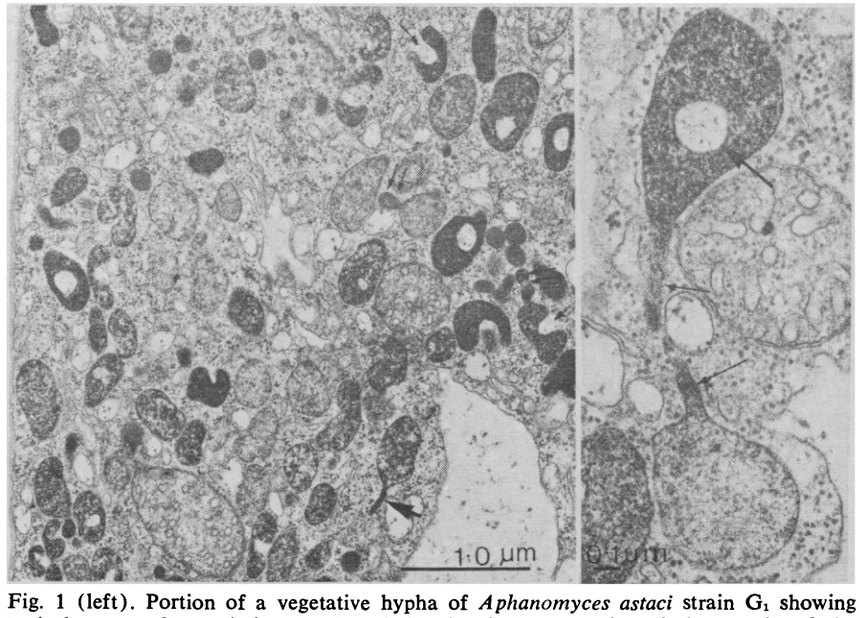

Fig. 1 (left). Portion of a vegetative hypha of *Aphanomyces astaci* strain G_1 showing typical range of morphology and variation in electron opacity of the matrix of the mycoplasma-like structures. Note frequent invaginations (small arrows), fingerlike projections (large arrow), and morphological suggestions of budding (double arrow). Fig. 2 (right). Detail of two mycoplasma-like bodies from a hypha of strain G_1 showing typical fingerlike projections (small arrows) and an enclosed cavity in the more electron-opaque body (large arrow). Serial sections verified that this cavity was enclosed and not an invagination comparable to those seen in Fig. 1.

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.

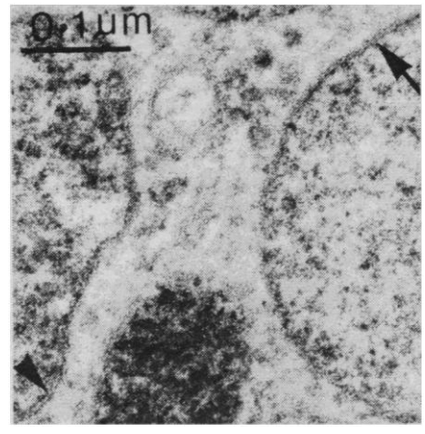

Fig. 3. Detail of two mycoplasma-like bodies showing the dark-light-dark profile of their bounding membranes (arrows).

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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20 August 1973; revised 2 October 1973

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-