Spoilage of grain and oilseeds by storage molds, fungi which attack seeds during storage and cause deterioration, amounts to over 1% of the world’s production (14). These losses include decrease in germinability, discoloration of seed, increase in free fatty acids, heating, mustiness, and production of mycotoxins (5). Previous studies concerning grain infected with storage fungi have been concerned with detection of these organisms or chemical changes which occur after infection. In this study electron microscopic examination of wheat (Triticum aestivum L., Pa 151X107) embryos was undertaken to determine the ultrastructural changes which may occur in wheat seeds infected with storage fungi of the Aspergillus glaucus group. The most striking change found in cells of infected tissue was the coalescence of lipid bodies (spherosomes) to form large masses of amorphous material. In addition, the protoplast was frequently withdrawn from the cell wall, and the plasmalemma appeared to be damaged. Aside from the ultrastructural changes in infected tissue, we report observations on the membrane-like boundary surrounding the lipid bodies (spherosomes). This boundary is ≥ half the thickness of mitochondrion membrane elements, and does not show convincingly the typical trilaminar structure observed in other biological membranes.

Wheat seeds were infected by the natural seed-borne mycoflora when placed in storage at 25 C and 75% relative humidity. After 26 weeks conidiophores of A. glaucus spp. were observed extending from the embryo end of some seeds. Control seeds were kept at -20 C. Small pieces of tissue were dissected from the embryonic axis of seeds, either dry or imbibed for 2 hr, fixed in glutaraldehyde followed by OsO₄, dehydrated in ethanol, embedded in Epon 812, and further processed for electron microscopy according to procedures reported earlier (2).

The bodies referred to as “lipid bodies” (spherosomes) generally range from 0.2 to 0.5 μm, but occasionally approach 1.0 μm in diameter or length, are spherical to ovoid and appear identical to bodies termed “spherosomes” in epithelial cells of barley scutellum (6, 18), coleohiza cells of Zea mays (9), aleurone cells of barley (16, 20), and hypocotyl parenchyma cells of Acer platanoides (8). It is not clear whether these bodies are the same as the bodies termed “spherosomes” in Campanula persicifolia guard cells (21) and fungal mycelia (1, 25). Except for their smaller size, they are similar in appearance to the bodies, termed “spherosome”, found in castor bean, cotton, peanut (13, 19, 26), and yucca (11). Sorokin (22) has criticized use of the term “sphero- Some” for these latter bodies, which she considers to be neutral storage lipid. We use the term “spherosome” only to conform with much of the literature concerning ultrastructure of seeds.

Prominent features of embryo cells from control wheat seeds are numerous mitochondria, protein bodies, a heavy population of ribosomes distributed uniformly throughout the cytoplasm, proplastids in the case of shoot cells, a large nucleus, and numerous spherosomes primarily around the periphery of the cytoplasm (Fig. 1A). The membranes of spherosomes can be compared with those of other organelles in the high magnification electron micrograph of Figure 1C. Whereas both outer and inner membranes of a mitochondrion and the single membrane of the plasmalemma show the expected unit membrane structure, the spherosome boundary appears to consist of one layer at this magnification. Other investigators have not demonstrated a trilaminar structure in spherosome boundaries (8, 13, 19, 26), nor do they present evidence, as is given in Figure 1C concerning its size and apparent single layer structure when compared to other trilaminar membranes. Figure 1C shows that the bounding layer of spherosomes is only 3.0 to 4.0 nm thick, thinner than that of mitochondrial elements (5.0-6.0 nm) and of the plasmalemma (7.5-9.0 nm). The character of the boundary suggests that these bodies might be oil or fat globules which stain more intensely around the border (7) than in the interior. However, two different types of experiments do not support this explanation: high speed centrifugation does not cause the spherosomes to coalesce (3, 15); the boundaries are not destroyed by extraction with lipid solvents prior to fixation (13, 19). Thus the spherosomal boundaries exhibit certain membrane-like properties but structurally do not resemble the typical biological membrane.

That the large amorphous masses in cells of infected tissue represent coalescence of spherosomes is deduced as follows. First, Figure IB (circle and arrow) shows the apparent coalescence of two or more spherosomes. Second, the amorphous mass has about the same electron opacity as the single spherosomes. Third, the number of single spherosomes is greatly reduced. Fourth, the amorphous masses are found in the areas where the individual spherosomes were located. The mechanism responsible for this coalescence of spherosomes is unclear. One explanation is that the spherosomal membrane is ruptured and the lipid contents of the organelle flow together to form the amorphous mass. A second possibility is that some fungal metabolite (lipase or surface-tension breaking compounds) liquifies the lipid and causes the coalescence of the spherosomes. A combination of both might be involved. The action of lipase would fit published data which show that seeds infected with storage fungi have higher amounts of free fatty acids than do noninfected seeds (5, 12). Whether the coalescence of the spherosomes shown here is related to the increase in free fatty acids remains to be established.

Frequently, the withdrawn plasmalemma of the infected tissues is ruptured, as demonstrated by bleb-like structures and actual disruptions of the membrane (Fig. 1B, insert). Poor fixation of
infected tissues is not the cause of the withdrawn and ruptured plasma membrane as shown by the good fixation of ribosomes and membranes of other organelles. Withdrawal of the plasmalemma from the cell wall also is found occasionally in control cells and might represent loss of turgor in dead cells. However, in such cases the plasmalemma did not appear to be ruptured as in cells of infected tissues. Deteriorated seeds are reported to leach more of their cellular constituents than nondeteriorated seeds, presumably because of an increase in membrane permeability (4, 10). The material found in the area between the protoplast and the cell wall probably represents material leached from the protoplast. The coalesced spherosomes and the withdrawn protoplast were found in both imbibed and nonimbibed infected seeds. Thus, the withdrawal of the plasmalemma and the leaching of material from the protoplast either occurred in storage or during fixation.

The rupture of the plasmalemma might be explained by fungal-produced phytotoxins which are reported to increase leaching of
cellular constituents (23, 24) and to affect membrane structure (17) of susceptible tissues.

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LITERATURE CITED