Extraction of Lipids from Cottonseed Tissue: V. Ultrastructural Effects of Extraction with Hexane-Acetic Acid[1]

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ABSTRACT

Cottonseed tissue was extracted with acidified hexane (hexane containing 2-25% acetic acid, v/v) and then examined with an electron microscope. In all cases, the contents of the oil-rich spherosomes were emptied and cytoplasm remained intact after lipid extraction. However, membranous elements of the cytoplasm appeared diffuse and disorganized. The possible effect of this disorganization of membranes in accounting for the greater amount of lipid extracted by acidified hexane than by hexane is discussed.

INTRODUCTION

Whereas a relationship between the composition of extracting solvents and that of extracted tissue lipid is well established, the relationship of these factors to cytological and ultrastructural changes that accompany solvent-extraction of tissues has not been investigated thoroughly. In previous communications (1-3), we showed that reserve lipids of cottonseed are stored as cytoplasmic particles called spherosomes, that spherosomal contents are depleted upon lipid extraction, and that cytoplasmic structures are disrupted upon thorough extraction of lipids by certain solvents. For example, extraction of cottonseed with hexane-acetone-water, which is a more efficient extracting medium than hexane (4-6), is accompanied by cytoplasmic disruption, whereas only spherosomal depletion results from extraction with hexane (3).

This paper presents results of electron microscopic examinations of cotyledonary tissues after extractions with acidified hexane, a solvent that extracts as much lipid from cottonseed as does hexane-acetone-water (6,7). Ultrastructural effects are compared with those previously observed (3,8-11) after extraction of tissues by other solvents.

EXPERIMENTAL PROCEDURES

Small pieces of dehulled cottonseed meats (*Gossypium hirsutum* L.), ca. 8 mm^3, were treated with hexane and mixtures of hexane-acetic acid (hexane containing 2-25% acetic acid, v/v) in the manner described previously (3). Hexane was spectrograde quality. The samples were rinsed in hexane and fixed in aqueous 2% $KMnO_4$. To avoid drastic changes from osmotic and imbibition forces, the samples gradually were converted from hexane to acetone and from acetone to water by use of a graded series of hexane-acetone mixtures and acetone-water mixtures, respectively. The pieces were fixed in permanganate, rinsed with water, serially dehydrated with a graded series of aqueous acetone, and embedded in Spurr's low viscosity plastic (12). Thin sections were cut with a diamond knife, poststained with uranyl acetate and lead citrate (13), and examined with a Philips EM-300 electron microscope.

Although hexane, hexane-acetone, and acetone are cytoplasmically nondisruptive solvents and tissues extracted by them appear ultrastructurally identical (3), we investigated the possibility that the use of these solvents prior to tissue fixation might produce artifacts. Samples of cottonseed were extracted with acidified hexane, rinsed in hexane, and fixed in 1% osmium tetroxide dissolved in hexane. Electron microscopic examinations of these tissues showed a morphological identicalness to those prepared as de-

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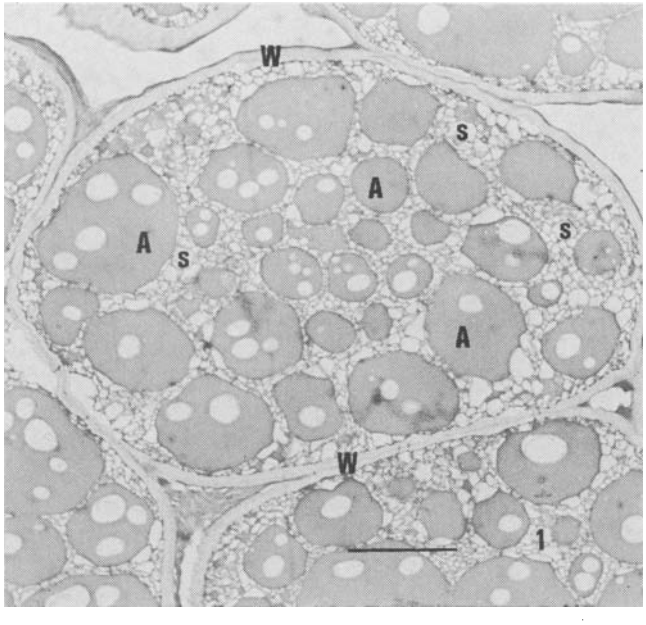

FIG. 1. Cotyledonary tissue of cottonseed extracted with hexane containing 25% acetic acid. S = spherosome, A = aleurone grain, and W = cell wall. Bar represents 5 μ.

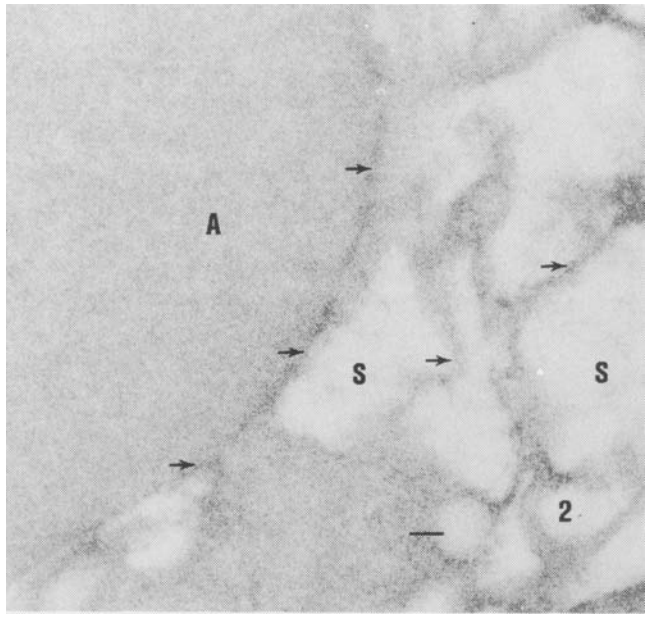

FIG. 2. Higher magnification of tissue shown in Figure 1. Arrows point to membranes. Bar represents 0.1 μ.

scribed above. However, tissues fixed in permanganate were preferred for study because membranes were much more clearly discernible with this fixative than with osmium tetroxide.

Cytological results were independent of the amount of acetic acid in hexane when at least 2% acetic acid was in the extracting medium. Electron micrographs of tissue treated with 25% acetic acid in hexane are depicted to illustrate that even the strongest concentration of acetic acid had a relatively mild effect upon the ultrastructure of the extracted tissue.

RESULTS AND DISCUSSION

Since acidified hexane extracts more lipoidal materials than does cytoplasmically nondisruptive hexane (7), the effects of acidified hexane upon the ultrastructure of cottonseed tissue were examined. Results from electron microscopic studies of cotyledonary tissue after extraction with this solvent (Fig. 1) showed that the cellular structure appeared morphologically similar to that extracted with hexane (3). The intactness of cytoplasmic structures and the apparent semblance to hexane-extracted tissue were unexpected. Instead, disruption of cellular structure like that produced by treatment with hexane-acetone-water (3), which extracts ca. the same amount of lipid as does acidified hexane (6,7), was expected. Additional electron microscopic examinations with high magnifications, however, indicated an ultrastructural disparity between tissues extracted with hexane and tissues extracted with acidified hexane. Figure 2 is an electron micrograph showing cytoplasmic membranes after treatment of tissue with acidified hexane. Membranes appeared fuzzy and diffuse rather than crisp and neat as in hexane-extracted tissue. This observation indicates that membranous elements in the cytoplasm became disorganized upon treatment with acidified hexane. Such disorganization readily allowed passage of solvents and solvent-lipid mixtures through membrane-bound, cytoplasmic structures. In this manner, lipids were extracted thoroughly without cytoplasmic disruption. In addition, the protogenicity (14) conferred on the solvent mixture by acetic acid aided penetration of extraspherosomal cytoplasm by the mixture. Somewhat inaccessible lipids, such as those associated with protein bodies (15), were then readily scavenged by the solvent mixture. Unfortunately, this aspect could not be evaluated by electron microscopic examinations.

Our results also indicate that the ultrastructural effects accompanying extraction of tissue with an oil-solvent are related more closely to the composition and properties of the solvent system than to the amount of lipid extracted by the solvent. In this regard, comparing the varying effects of lipid solvents on cellular ultrastructure is of interest. Electron microscopic observations of sectioned tissues have shown that extractions with acetone, diethyl ether, heptane, heptane followed by petroleum ether, hexane, hexane-acetone, or isooctane have essentially negligible effects upon the fine structure of membranes (3,8-10). Total collapse of membrane structure occurs upon treatment with chloroform-methanol, chloroform-methanol-water, or hexane-acetone-water (3,10,11). In the case of cottonseed, concomitant with this collapse is a significant increase in the amount of extracted lipids (3). However, treatment with acidified hexane results in maintenance of cytoplasmic structure but in disorganization of membrane elements (Figs. 1 and 2). Since the amount of lipid is quantitatively similar to the amounts extracted by cytoplasmic disruptive solvents (7), our results show that thorough extraction of tissue lipids always is accompanied by disorganization of membrane structure and sometimes by disruption of cytoplasmic structure.

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