Extraction of Lipids From Cottonseed Tissue: II. Ultrastructural Effects of Lipid Extraction[1]

T. P. HENSARLING, L. Y. YATSU and T. J. JACKS,
Southern Regional Research Laboratory,[2] New Orleans, Louisiana 70119

Abstract

Cottonseed tissue was extracted with chloroform-methanol-water, hexane-acetone-water, chloroform-methanol, hexane-acetone, hexane and acetone, and then examined with an electron microscope. In all cases, contents of oil-rich spherosomes were emptied and cell walls remained intact after lipid extraction. In addition, the two water-containing solvents obtained disruption of intracellular structure. Severalfold greater amounts of water-soluble phosphorus compounds were extracted with the water-containing solvents than with their nonaqueous counterparts.

Introduction

Storage oil in cottonseed appeared as an intracellular cytoplasmic emulsion with early techniques of light microscopy (1). Examinations with the electron microscope showed that oil is stored in intracellular organelles, called spherosomes, that are generally on the order of 2 μ in diameter and are bounded by limiting membranes composed in part of protein and phospholipid (2–4). Since spherosomes are the ultimate structural targets of oil-extracting procedures, cytological examination of the effect of oil solvents on the ultrastructure of cottonseed was of interest.

Experimental Procedures

Methods and Materials

Sections of dry cottonseed (*Gossypium hirsutum* L.) were placed over water in a Petri plate for 1 hr; smaller pieces, approximately 0.3 mm^3, were then easily excised from this pliable tissue. The pieces were dried over P_2O_5 in vacuo and placed into vials containing chloroform-methanol-water (CMW), chloroform-methanol (CM), hexane-acetone-water (HAW), hexane-acetone (HA), hexane (H) or acetone (A). Compositions of the solvents were reported in a previous paper (5). The capped vials, each containing about 5 ml of solvent and six pieces of tissue, were attached to a revolving vertical wheel to ensure continuous mixing. After four changes of respective solvents during a two-day period, the tissues were fixed for microscopic observation. To avoid artifacts produced by hydration of tissue that would occur in standard procedures of fixation, a method was developed to fix the tissue in the extracting solvent. The pieces were soaked overnight in 2% osmium tetroxide that was dissolved in the respective solvents used to extract the tissues. Tissue extracted with HAW, which is biphasic at room temperature, was fixed with 2% osmium tetroxide dissolved in the upper phase. Fortunately, osmium tetroxide was both soluble and stable in the solvents of interest. The fixed tissues were then thoroughly rinsed with the respective solvents, transferred to acetone in a stepwise manner consisting of gradually replacing solvent with acetone, and embedded in Maraglas (6). Tissues extracted and fixed in CM or CMW were gradually transferred to ethanol rather than acetone as above, then gradually transferred to propylene oxide, and finally embedded in Maraglas. Thus, the tissues were never in contact with water unless water was part of the extracting solvent.

Control tissue that was not treated with extracting solvents was fixed in fumes of osmium tetroxide and embedded in methacrylate.

[1] A preliminary report was presented at the Seventh Annual Symposium of the Louisiana Society for Electron Microscopy.
[2] So. Utiliz. Res. Dev. Div., ARS, USDA.

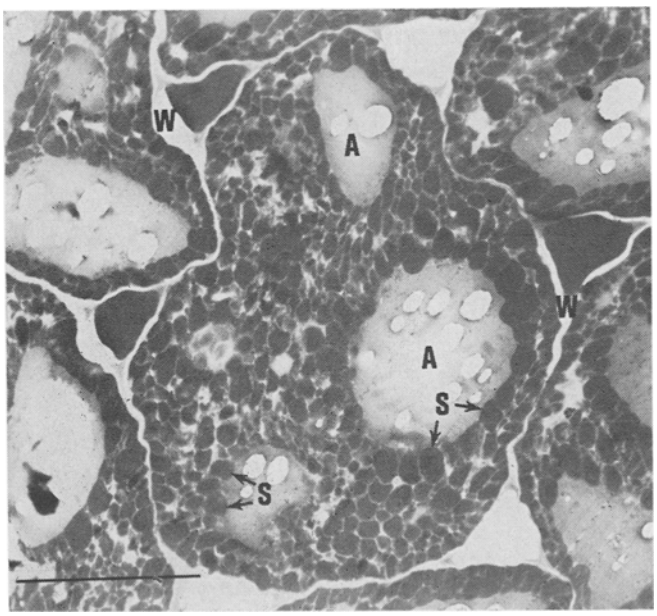

FIG. 1. Unextracted cotyledonary tissue of cottonseed. S, spherosome; A, aleurone grain; W, cell wall. In all Figures the bar represents 5 μ.

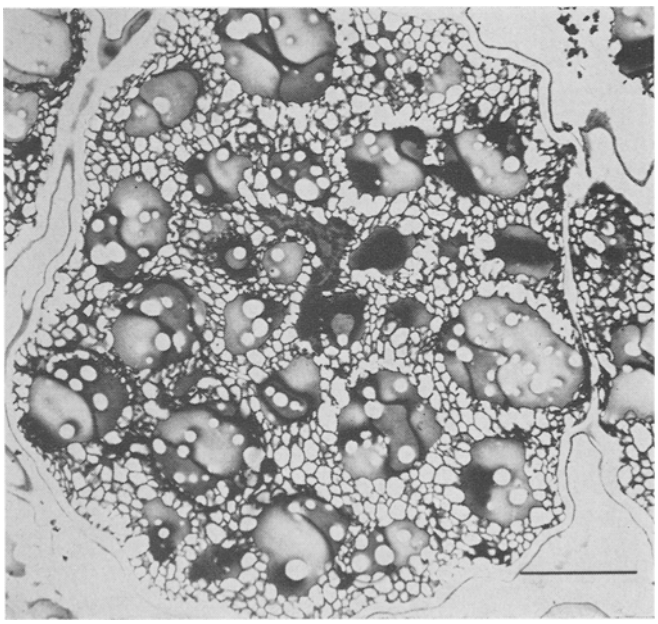

FIG. 2. Cotyledonary tissue of cottonseed extracted with chloroform-methanol.

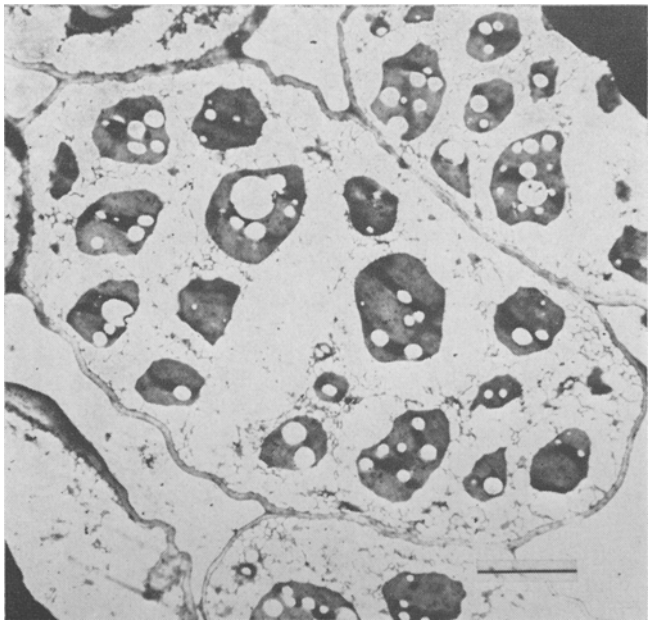

FIG. 3. Cotyledonary tissue of cottonseed extracted with hexane-acetone-water.

Thin sections were cut with a Porter-Blum microtome using a diamond knife, post-stained with uranyl acetate and lead citrate (7) and examined with a Phillips EM-100 or EM-200 electron microscope. Control tissue was not post-stained.

Procedures to extract lipid from flaked cottonseed, to remove water-soluble materials from lipid, and to determine phosphorus were described in a previous paper (5).

Results and Discussion

Figure 1 shows a portion of cotyledonary tissue of cottonseed that was not treated with extracting solvents prior to fixation. Since osmium tetroxide esterifies with fatty acids at unsaturated bonds (8), the intracellular sites of oil storage (spherosomes) are electron dense and appear dark in the micrograph.

When tissue was extracted with CM, HA, H or A, the cellular structure appeared similar to untreated tissue (Fig. 1) except that spherosomes were empty of oil and were electron-transparent after fixation with osmium tetroxide. Figure 2 shows CM-extracted tissue, which appeared structurally similar to tissues extracted with A, H or HA.

Extraction of tissue with CMW or HAW obtained disruption of intracellular structures without cellular rupture. Figure 3 shows disorganized cytoplasm in intact cells of tissue extracted with HAW. Tissue extracted with CMW appeared structurally similar to that shown in Figure 3.

TABLE I

Water-Soluble Phosphorus in Miscellae

Solvent	Phosphorus[a]
	μg
CMW	243 ± 5
CM	34 ± 0
HAW	804 ± 0
HA	78 ± 1

[a] Amount of phosphorus in oil before washing minus amount after washing. Oil was extracted from 2 g of tissue. Values represent means ± standard deviations from the means of three determinations.

Since CMW, CM and HAW extracted about 6% more neutral oil than HA, H and A (5), a relationship was expected between extraction of oil by the two groups of solvents and their effects on cellular structure. However, intracellular structures were disorganized in tissues extracted with CMW or HAW (Fig. 3) but not with CM. Instead, CM, which extracted a similar amount of neutral oil as HAW and CMW, produced structural effects similar to those from HA, H and A (Fig. 2). These observations indicate that cytoplasmic disruption does not necessarily accompany thorough extraction of oil from cottonseed.

Since disruption of intracellular cytoplasm occurred after extraction of tissue with water-containing solvents (CMW, HAW), it was of interest to determine some water-soluble materials in miscellae obtained from extraction with CMW, CM, HAW and HA. Miscellae were prepared from flaked cottonseed and washed with water as described previously (5). Very small amounts of miscellar materials were water soluble. However, determinations of phosphorus compounds before and after washing miscellae showed that the aqueous solvents extracted over sevenfold more water-soluble phosphorus compounds than their nonaqueous counterparts (Table I). Whether this occurrence was a cause or an effect of intracellular disruption has yet to be determined. Identifications of the phosphorus compounds in the miscellae are of future interest.

REFERENCES

1. Leahy, J., in "Cottonseed and Cottonseed Products," Edited by A. E. Bailey, Interscience Publishers, Inc., N.Y., 1948.
2. Jacks, T. J., L. Y. Yatsu and A. M. Altschul, Plant Physiol. *42*, 585–597 (1967).
3. Yatsu, L. Y., J. Cell Biol. *25*, 193–199 (1965).
4. Yatsu, L. Y., and T. J. Jacks, Amer. Tung News *20*, 6–7 (1969).
5. Jacks, T. J., L. Y. Yatsu and T. P. Hensarling, JAOCS.
6. Erlandson, R. A., J. Cell Biol. *22*, 704–709 (1964).
7. Venable, J. H., and R. Coggeshall, J. Cell Biol. *25*, 407–408 (1965).
8. Riemersma, J. C., Biochim. Biophys. Acta *152*, 718–727 (1968).

[Received November 19, 1969]