Extraction of Lipids from Cottonseed Tissue: VI. Ultrastructural Morphology of Isolated Pigment Glands

L.Y. YATSU, T.P. HENSARLING, and T.J. JACKS, Southern Regional Research Center[1], P.O. Box 19687, New Orleans, Louisiana 70179

ABSTRACT

Cottonseed pigment glands, isolated from the underflow fraction of the liquid cyclone process, were examined with an electron microscope. The glands were circumscribed by a layer of tangentially flattened cells. Subjacent to the flattened cells were partially lysed cells containing recognizable remnants of cell walls. Within the lumens or matrices of the glands were myriads of pigment spherules measuring 0.1-1.5 μ in diameter. These spherules remained within the glands, even though gland walls were ruptured mechanically. Since aggregates of pigment spherules devoid of gland walls were observed in the gland-rich fraction, it was concluded that maintenance of intact pigment glands was not a requisite for successful separation of gossypol from other cottonseed components with the liquid cyclone process. Avoiding dispersion of aggregated spherules from the glandular matrix was probably just as important as maintaining intact gland walls during mechanical separation of gossypol from other cottonseed components.

INTRODUCTION

Embedded within the tissues of glanded cottonseeds are myriads of small, black specks known as pigment glands. These glands are of great interest to the cottonseed industry since they contain gossypol and gossypol-related pigments (1-3). Gossypol is a polyphenolic pigment that, not only imparts undesired color to cottonseed oil and lessens the nutritive worth of cottonseed protein, but also is a poisonous principle per se (4).

Recently, a method was devised which separates pigment glands from other cottonseed constituents: the liquid cyclone process (LCP) (5-7). Basically, the process consists of differentially centrifuging comminuted seeds in hexane to separate nutritious protein from noxious pigment glands (the oil is reclaimed from the hexane). This process produces very high quality protein that is virtually free of gossypol contamination.

Since success of the LCP depends upon the fact that gossypol is sequestered in pigment glands whose structural integrity seemingly is maintained throughout processing, knowledge concerning structural aspects of the glands is important. In this communication, we describe the ultrastructural morphology of isolated pigment glands with special regard to structural relationships and cottonseed processing. In addition, previous misconceptions concerning pigment gland morphology are discussed.

EXPERIMENTAL PROCEDURES

The pigment gland-rich fraction (underflow) from an LCP fractionation of Texas high plains cottonseed was obtained from H. Gardner of this laboratory. Prior to LCP fractionation in hexane (Skellysolve B), the seeds had been dehulled and then dry-milled in an Alpine American Contraplex (sieveless wide chamber pin mill) with counter-rotating disks set at 2500 and 9500 rpm. Glands were isolated from the underflow fraction by flotation (8) in hexane and CCl_4, rinsed in hexane, dried, and stored in vacuo over silica gel.

Isolated glands were fixed in a 2% (v/v) aqueous solution of $KMnO_4$. This fixative did not cause glandular rupture, as did aqueous glutaraldehyde and osmium tetroxide (fixatives generally used in electron microscopy), and produced results that were superior to results with the osmium tetroxide fume fixation used by Moore and Rollins (9). The fixed glands were dehydrated serially in aqueous acetone mixtures and embedded in epoxy resin (10). Thin sections were cut with a diamond knife on a Sorvall Porter-Blum microtome and poststained with uranyl acetate and basic lead citrate (11). Stained sections were examined in a Philips EM-300 electron microscope.

RESULTS

Figure 1 is a low magnification electron-micrograph of pigment glands isolated from the underflow fraction of the LCP. Note that some glands were bounded by a single layer of compartments, whereas others had several layers. In micrographs showing multiple layers, the shapes of the compartments progressed from flat to ellipsoid as one moved distally from the glandular interior. Even at these low manifications, it was obvious that at least the distal compartments were actually cells (12). The compartments nearest the lumen of the gland were generally more electron-dense than the more distal cells. Within the lumen of the gland proper were myriads of grey to black entities which appeared stippled at these low magnifications. Note especially the glands with broken walls; the stippled con-

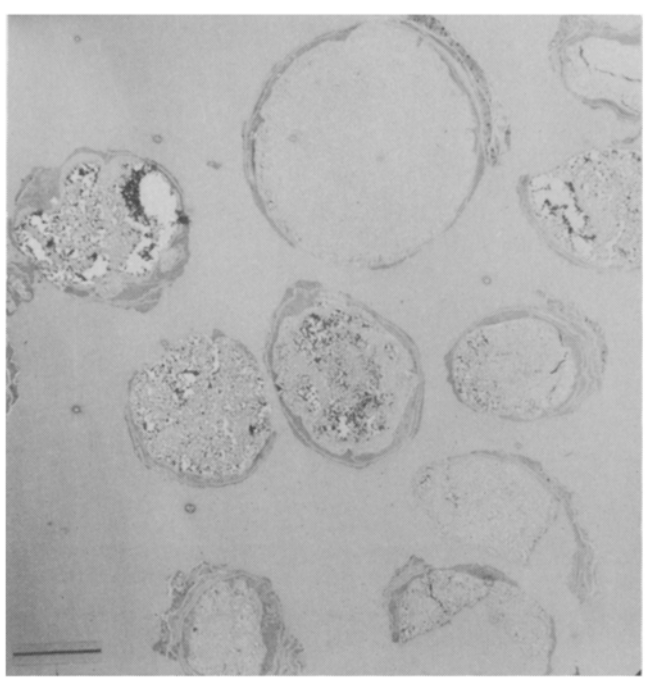

FIG. 1. Isolated pigment glands obtained from the "underflow" fraction of the liquid cyclone process. The glands are bounded by several layers of cells and are filled with material that stained grey to black. Note especially the broken glands which still contain glandular contents even though parts of the gland walls are absent. Marker indicates 50 μ.

[1]ARS, USDA.

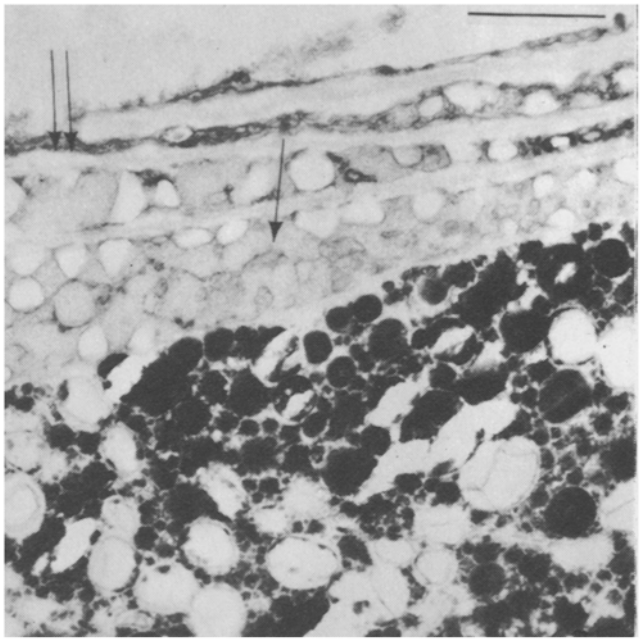

FIG. 2. A portion of a gland wall. Generally, cell walls do not completely encircle cells of the innermost layer; radial walls and the walls facing the lumen of the gland appear to have lysed. Organelles can be recognized in both the innermost cells and those adjacent to them, indicating the cellular nature of these compartments. The lumen of the gland is filled with darkly stained spherules. Marker indicates 1 μ.

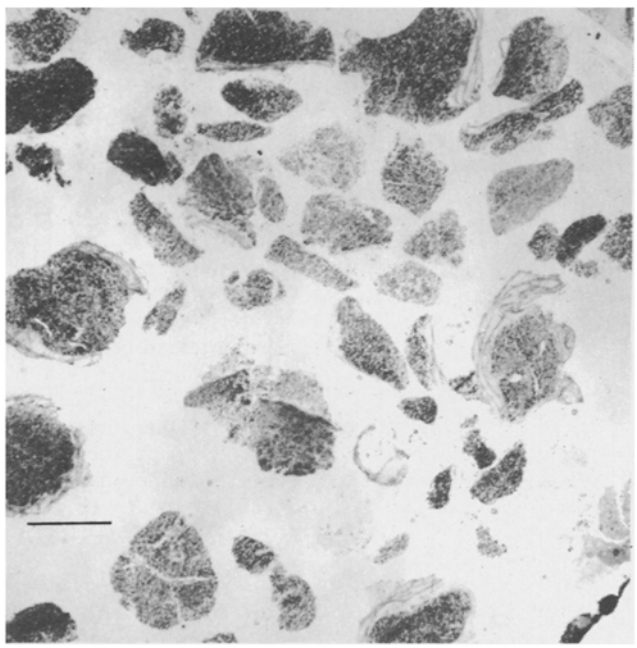

FIG. 3. The "fines" fraction of isolated pigment glands. The material in this fraction is composed of bits and pieces of glandular matrices with no intact glands present, yet the components have maintained an aggregate form rather than dispersed individual spherules. Marker indicates 50 μ.

tents were still present in the ruptured glands.

Figure 2 is a higher manification of a portion of a multiple layered gland wall. The double arrows point to the innermost layer of compartments which clearly is bounded by cell walls—these were undoubtedly the entities labelled "plates" by Boatner, et al. (13). Subjacent to this layer was often visible another layer of material (single arrow) which resembled the penultimate layer but which lacked cell walls. This was probably the mucilaginous layer to which early workers referred (14). In the lumen of the glands, material that appeared stippled at low magnification (Fig. 1) was seen to be grey or black spherules measuring 0.1-1.5 μ in diameter. These were similar to the spherical particles described by Moore and Rollins (9) and were undoubtedly "droplets" of gossypol and gossypol-related pigments.

In spite of broken walls, glandular contents were intact within the glandular matrices (Fig. 1). During preparations of glandular fractions for microscopic examinations, a fine, dark fraction was noted that settled somewhat slowly in hexane. Since this fraction appeared physically different from the other portion of the isolated gland fraction, it was examined with the electron microscope. Figure 3 shows that the material was composed of broken glands and aggregates of pigment spherules from glandular matrices.

DISCUSSION

Although the noxious pigment, gossypol, in glanded cottonseed has been a problem to processors, it is fortuitous that the compound is sequestered in neat intercellular packages called "pigment glands."

These glands appear to the naked eye merely as black specks, but, microscopically, they were revealed as round to ellipsoid structures measuring 100-400 μ in diameter (Fig. 1). The glands were bounded by structures that have been called "membranes" (14), "flattened cells" (2,3), or "plates" (9, 13).

Early workers described the gland walls as consisting of two concentric layers: an outer wall composed of tangentially flattened cells and an inner wall consisting of a layer of mucilaginous material in which traces of cell walls were still evident (14). This interpretation was corroborated by Stanford and Viehoever (3) who studied the ontogeny of the glands. They concluded that the glands arose lysigenously and that the surrounding cells became flattened as the glands swelled. The encircling layers of cells were believed somewhat mucilaginous "dissolving partially in water and cuprammonia, but not in alcohol, and giving no well defined cellulose reactions." This "mucilaginous layer" associated with the gland wall was probably partially lysed cells.

Boatner, et al., (13) who studied isolated glands histochemically concluded, on the other hand, that gland walls were not cells but were relatively thick, curved "plates." They proposed that the plates contained no proteins, lignin, nor pentosans and were coated on the outer surfaces with cutin. More recently, Moore and Rollins (9), working with the electron microscope, concluded that the plates "exist as flattened compartments, each of which has a complex internal structure." The "compartments" were believed to contain "more or less discrete lumps or blocks" of material extremely sensitive to osmium fixation. The difference in appearance of the substances within the lumens of the plates and of the glands suggested to these workers that gossypol might be contained in the interior of the gland, whereas gossypurpurin might be concentrated in the plates.

Our results show that the gland walls are composed of cells and recognizable cellular remnants and corroborate the conclusions of early light microscopists who believed glands were bounded by tangentially flattened cells (2,3,12). We suggest, therefore, that the terms, such as "mucilaginous layers," "compartments," and "plates," should be abandoned and that the term "cells" be used to describe gland walls.

Myriads of spherules, measuring 0.1-1.5 μ in diameter, were observed within the gland matrices (Figs. 1 and 2). These spherules have been seen in studies with the light microscope, as they extravasated from ruptured glands (9), and with the electron microscope (Fig. 3 and [9]). Failure of pigment spherules to be released from glanded cottonseed during grinding, blending, or storage in hexane slurries

is not necessarily because pigment glands are tough or resilient. For example, pigment spherules remained within glands even though gland walls were broken (Fig. 1). In still other instances, large aggregates of spherules totally devoid of gland walls were observed (Fig. 3). Thus, although mechanical rupture of pigment glands during cottonseed processing is undesirable, it is not especially critical, since the LCP is capable of separating gland fragments from the protein-rich fraction (overflow). For successful separation of gossypol from other constituents of the seed, maintenance of pigment spherules in undispersed aggregates is probably just as important as maintenance of intact gland walls.

ACKNOWLEDGMENT

H. Gardner provided the underflow of the liquid cyclone process from which pigment glands were isolated.

REFERENCES

1. Boatner, C.H., and C.M. Hall, Oil Soap 23:123 (1946).
2. Reeves, R.G., and J.O. Beasley, J. Agric. Res. 51:935 (1935).
3. Stanford, E.E., and A. Viehoever, Ibid. 13:419 (1918).
4. Adams, R., T.A. Geissman, and J.D. Edwards, Chem. Rev. 60:555 (1960).
5. Gastrock, E.A., E.L. D'Aquin, P.H. Eaves, and D.E. Cross, Cereal Sci. Today 14:8 (1969).
6. Vix, H.L.E., P.H. Eaves, H.K. Gardner, Jr., and M.G. Lambou, JAOCS 48:611 (1971).
7. Gardner, H.K., Jr., R.J. Hron, Sr., and H.L.E. Vix, Oil Mill Gaz. 78:12 (1973).
8. Boatner, C.H., in "Cottonseed and Cottonseed Products, Their Chemistry and Chemical Technology," Edited by A.E. Bailey, Interscience Publishers, New York, N.Y., 1948, p. 213.
9. Moore, A.T., and M.L. Rollins, JAOCS 38:156 (1961).
10. Spurr, A.R., J. Ultrastruct. Res. 26:31 (1969).
11. Venable, J.H., and R. Coggeshall, J. Cell Biol. 25:407 (1965).
12. Yatsu, L.Y., J. Cell Biol. 25:193 (1965).
13. Boatner, C.H., C.M. Hall, M.L. Rollins, and L.E. Castillon, Bot. Gaz. 108:484 (1947).
14. Bretfeld, V., J. Landw. 35:29 (1887).

[Received August 26, 1974]