trum may be compared with that of authentic tocopherylquinone (spectrum D, Figure 5). The identity of this fraction with α-tocopherylquinone was further substantiated by paper chromatography, employing a variety of solvent systems as presented in Table II.

TABLE II

Chromatographic Comparison of α-Tocopherylquinone and a Tocopherol Oxidation Product

Developing system	Rf Values		
	Authentic α-tocopherylquinone	Largest spot of unfractionated extract	Largest fraction from column
65% isooctane, ascending	0.67	0.66	.....
60% isooctane, ascending, paper 1	0.61	.....	0.59
60% isooctane, ascending, paper 2	0.62	0.63	.....
75% ethanol, ascending	0.44	.....	0.46
80% ethanol, ascending, paper 1	0.75	0.75	0.75
80% ethanol, ascending, paper 2	0.66	0.66	0.69
Ethylene glycol,monoethyl ether, n-propanol, methanol, water (35:10:30:25), descending	0.1	0.12	.....
Methanol, n-butanol, water (86:6:8) descending	0.70	0.68	0.69

The quinone fraction equalled 40–45% of the petroleum ether extract and was not more than 80% pure on the basis of $E^{1\%}_{1\,cm.}$ of 435 (15). On the basis of recovery values the amount of tocopherylquinone may be calculated as representing approximately 35% of the original weight of α-tocopherol. This is only slightly higher than the values of 25–30% reported by others (5,16). The presence of α-tocopherylquinone as the only major product of tocopherol oxidation is also in agreement with these authors.

The radioactivity of the quinone fraction, 23 c.p.m. per mg., was too low to be attributed to anything other than contamination by linoleic acid. The low level of radioactivity found in all the materials isolated indicates that any radicals which may have been incorporated into the postulated tocopherol-radical addition products were only fragments (without the labelled carboxyl group) of linoleic acid. An alternative explanation for the low level of activity would be based on the suggestion by Harrison and co-workers (8) that linoleic acid-tocopherol addition products might not be extractable from alkaline solution with organic solvents.

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New Information on the Morphology of the Gossypol Pigment Gland of Cottonseed

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Exploratory electron microscope studies of the cottonseed pigment gland demonstrate a complex internal structure in which discrete particles ranging in size from one micron to less than 0.2 micron diameter are held within a membranous mesh-like network. This structure is extremely sensitive to water, and it may be the rapid swelling of the network which results in the explosive release of pigment particles from the gland on exposure to moisture. Pigment particles are spherical, of a wide range of diameters, and exhibit no birefringence in the gland or when extruded. Calculations of specific surface based on sizes of particles and density of purified gossypol (1.34) indicate surface area per gram of gossypol particles of the order of 8 square meters. Further work is indicated to determine details of the structure of the platelets, which constitute the wall of the gland, and to establish the relationship of gossypol and gossypurpurin to morphological features within the gland.

THE TOXICITY of gossypol is still the subject of research in spite of major progress in the processing of cottonseed meal and oil to remove this deleterious component. To obtain a better understanding of the mode of occurrence of the pigment within its morphological reservoirs in the seed, a brief study of the internal structure of the gland has been made with the electron microscope.

Previous work by Von Bretfeld (12), Hanausek (8), Stanford and Viehoever (11), and Boatner and coworkers (4,5,6) had shown that the pigment glands are profusely distributed in the cotyledons and hypocotyl of the cottonseed; the sizes of glands vary considerably with different varieties of cotton. The glands are highly colored, ranging from yellow through orange and red to dark purple. When exposed to water, they immediately rupture and expel a stream of minutely divided particles, exhibiting Brownian movement, and leave a nearly transparent structure in the shape of the original gland. The smaller glands are usually spherical whereas larger glands are ovoid, measuring from 100 to 400 microns on the long axis. The rigid thick gland wall consists of from 5 to 8 irregularly shaped curved platelets,

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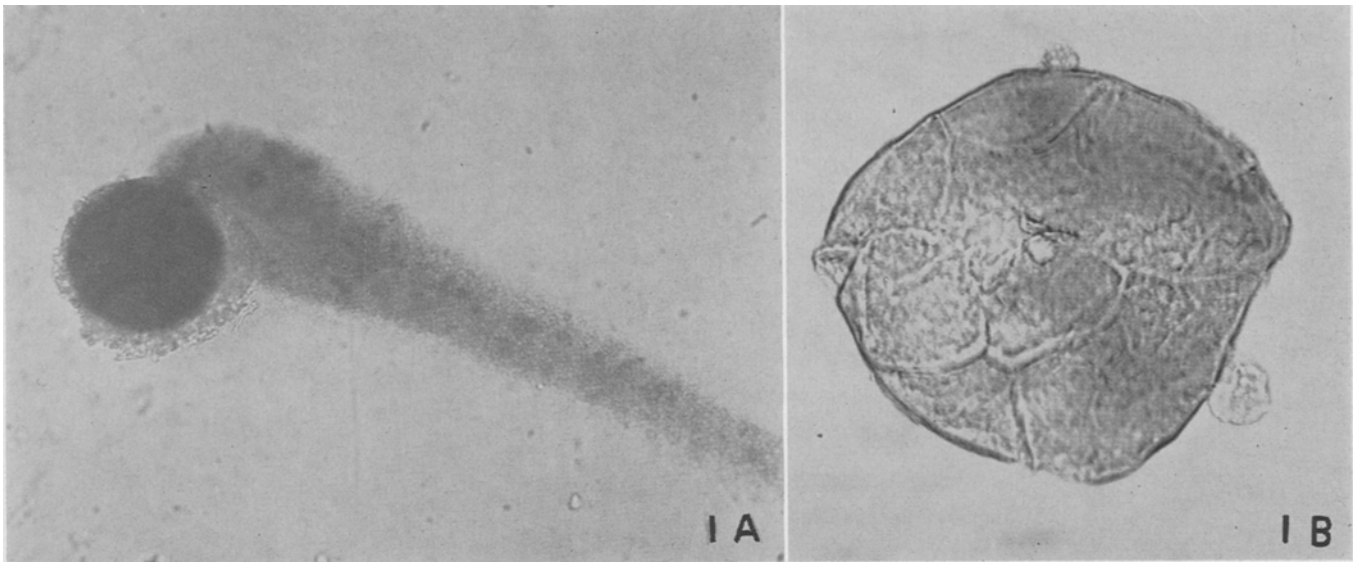

FIG. 1. Photomicrograph of whole pigment glands. A, in water, showing explosive extrusion of particles, mag. 226x; B; after extraction, showing separation of outer wall into platelets, mag. 364x.

which appear to be composed of cellulose impregnated with pectic materials, hemicelluloses, and other unidentified uronic acid derivatives. A layer of cutin surrounds the glands. The glands are resistant to mechanical rupture and to the action of most of a number of organic solvents. However the glands are very sensitive to water and are affected by the presence of traces of moisture in any of the organic or inorganic reagents. It has been estimated that 40 to 59% of the gland is wall, 35 to 50% gossypol, and 0.05 to 3% gossypurpurin, also that the glands in a single kernel make up 1 to 3% of its total weight (7,10,4).

The work of Boatner and coworkers published in 1947 (5) stated that the thick gland wall "encloses a gelatinous suspension of the pigments." No other knowledge of the internal arrangement of gossypol within the gland was possible within the limits of resolution of the light microscope, but, with the advent of the electron microscope, it has become possible to resolve a finer structure in the anatomy of the gossypol-containing bodies of the seed. The current investigation reveals a complex internal morphology in the glands and reports for the first time the existence of discrete particulate bodies enmeshed in a highly organized structure, from which globules of a wide range of sizes are extruded upon the rupture of the glands in water.

Experimental

Materials. Pigment glands were obtained from a commercial cottonseed sample of Deltapine cotton by hand-picking under a low-power, wide-field microscope the large glands from mature seed kernels. They were carefully cleaned with dissecting needles so that those selected were entirely free of adhering extraneous material.

Procedure. Free Particles. Glands were immersed in water where they quickly exploded, sending a stream of particles spurting from the opening in the wall (Figure 1a). A previously extracted empty gland (Figure 1b) shows separation of the platelets which constitute its outer wall. The extruded particles were mounted in water under a cover-slip and photographed at magnifications approximating 1,000 times the natural size. Other specimens of particles for examination with the electron microscope were mounted on carbon-coated, screen wire specimen grids, dried, shadowed with evaporated platinum at an angle of 3 to 1, and observed at magnifications of 9,000 x and up. From photographic enlargements of these electron micrographs, measurements were made of particle diameters and the range and frequency distribution of particle sizes were determined.

A sample of purified gossypol (9) was obtained, and its density was determined pycnometrically (3). By using the figure obtained and the specific surface area calculated from the measured particle sizes of extruded particles, the surface area in a gram of gossypol was estimated.

Gland Sections. Ultrathin sections of whole glands were prepared for electron microscope observations in the following manner. Specimens on a microscope slide were fixed by placing the slide over crystals of osmium tetroxide (OsO_4) (2) in a desiccator for 48 hrs.; the intact glands turned a deep black. To facilitate location of such small objects in the embedding for sectioning, a single colored synthetic fiber a few millimeters in length was placed as a marker with one end pointing to the gland on the microscope slide. A drop of partially polymerized methacrylate was allowed to set up on the slide in contact with these objects. Subsequently this sliver of hard polymer, containing the objects to be sectioned, was placed on top of a block of completely polymerized methacrylate in a gelatin capsule, and a small amount of the liquid embedding medium was poured into the capsule to ensure good adhesion of the specimen to the methacrylate block. The embedding medium was made up of 4 parts of butyl methacrylate, 1 part of methyl methacrylate, 0.5% of 2,4-dichlorobenzoyl peroxide (catalyst) with dibutyl phthalate as a softener.

Prior to embedment, some glands were extracted by immersion in acetone for 48 hrs. Others were extracted with water by flooding successively a number of times on a microscope slide. The extracted

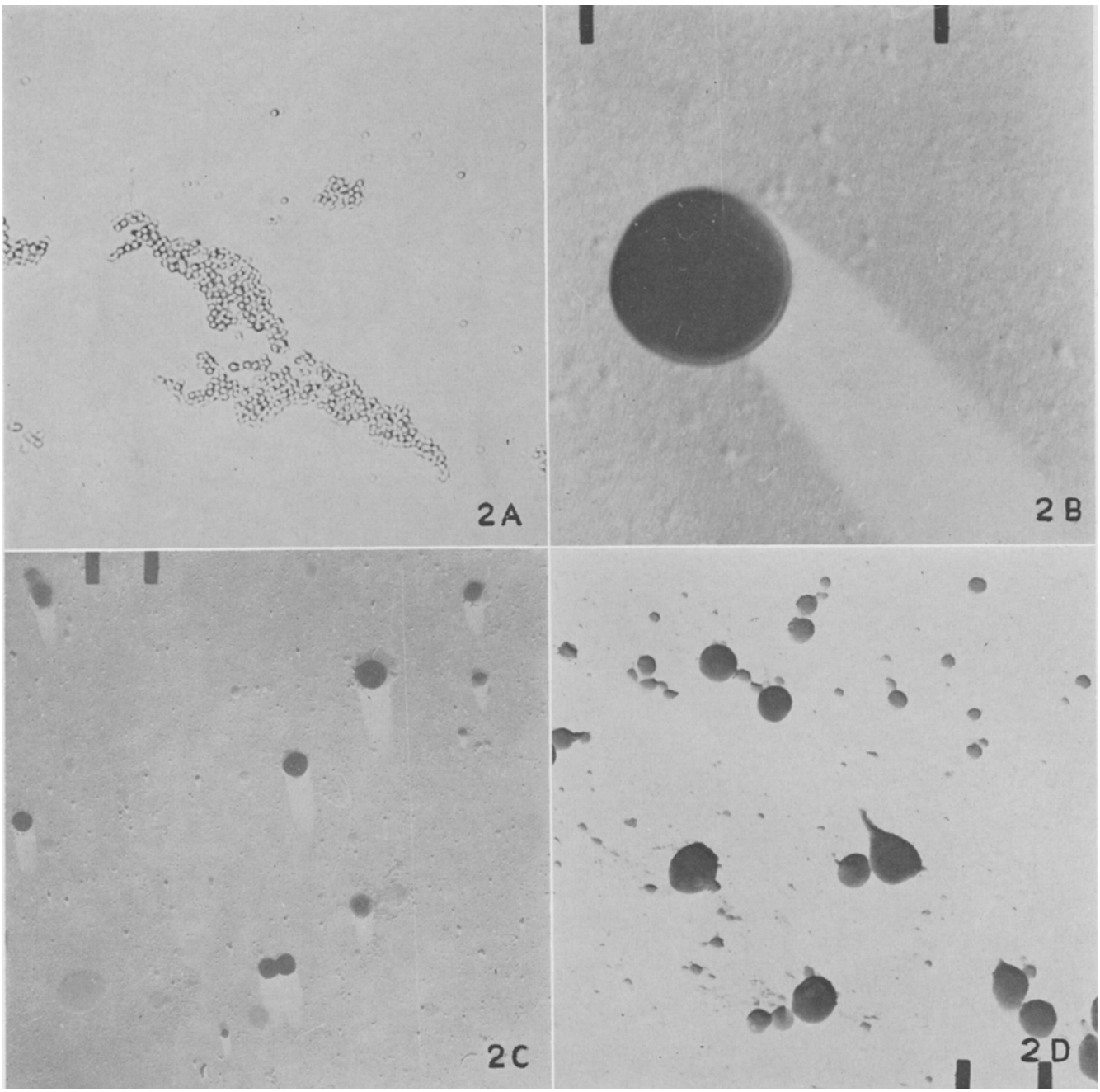

FIG. 2. Particles extruded from pigment gland upon exposure to water. A, photomicrograph of particles streaming out in water, mag. 700x; B, electron micrograph of single particles (platinum-shadowed), mag. 54,000x; C, electron micrograph of single particles from water suspension, mag. 9600x; D, electron micrograph of single particles after treatment with 2% aqueous NH_4OH, mag. 9600x.

glands were fixed with OsO_4 vapors and embedded in the same manner as the whole glands described above. Sections were cut on the Porter-Blum microtome with a diamond knife (1) bevelled to 48° and collected in the trough behind the knife on a 30% acetone solution. After drying, the sections were examined in the electron microscope.

Results and Discussion

Free Particles. The individual, free particles, extruded from the glands upon exposure to water (Figure 2a), were found to be uniformly spherical (Figures 2b, 2c) and to have a wide range of sizes, grading from approximately 1 micron in diameter to less than 0.2 micron. No birefringence could be detected when particles were examined between crossed nicols. The particles were stable to water but readily dissolved in acetone. They collapsed somewhat on exposure to dilute bases, such as 2% ammonium hydroxide. Figure 2d shows this apparent loss of surface tension and sphericity.

From photographs of the free-floating particles the diameters of 56 spheres were measured and grouped in ranges of 0.16 to 0.20 micron, 0.21 to 0.25 micron, etc. The midpoint of the group was used as the average diameter, and total surface areas and sphere volumes were calculated for each group (Table I). From the sums of the total group areas and volumes

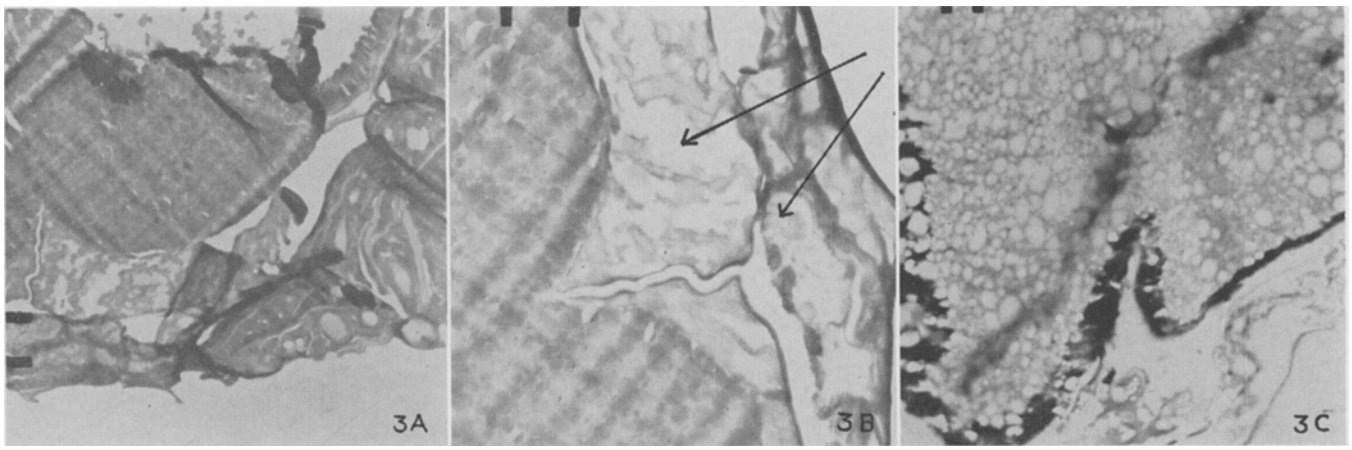

FIG. 3. Electron micrographs of cross-sections through portion of intact gland. A, showing overlapped platelets of outer wall and spherical particles in place in gland interior, mag. 3015x; B, arrows point to overlapped platelets in similar specimens, mag. 6332x; C, similar specimen after exposure to water, showing empty mesh, mag. 3015x.

TABLE I

Dimensions of Pigment Particles Extruded from Glands

Diameter, 1 x 10^{-4} cm. (midpoint of group)	No. of observations	Total volume 1x10^{-12}cm.^{3}	Total area 1x10^{-8}cm.^{2}
0.175	11	0.0309	1.0583
0.230	2	0.1274	0.3324
0.280	9	0.1034	2.2167
0.330	9	0.1693	3.0791
0.380	6	0.1724	2.7219
0.430	6	0.2498	3.4853
0.480	6	0.3474	4.3429
0.530	3	0.2338	2.6474
0.680	1	0.1646	1.4527
0.830	1	0.2994	2.1642
0.880	1	0.3568	2.4329
0.980	1	0.4928	3.0172
Sum.....	56	2.6335 x10^{-12}cm.^{3}	28.95 x10^{-8}cm.^{2}

Density (pycnometer) = 1.34 g./cm.^{3}
Total area of particles = 28.95 x 10^{-8} cm.^{2}
Total volume of particles = 2.63 x 10^{-12} cm.^{3}

$$\text{Specific surface} = \frac{\text{Total area}}{\text{Total volume}} = 11\text{x}10^4 \text{ cm.}^2/\text{cm.}^3$$

$$\text{Surface area/g.} = \frac{\text{Specific area}}{\text{density}} = \frac{11 \text{ x } 10^4 \text{ cm.}^2/\text{cm.}^3}{1.34 \text{ g./cm.}^3} = 8.2 \text{ m}^2/\text{g.}$$

a surface area of 11 x 10^4 cm.^{2} per cubic centimeter was calculated. The density of a sample of purified gossypol, determined pycnometrically in iso-octane (3), was found to be 1.34 g./cm.^{3} From these data, the maximum total reactive surface available in a gram of gossypol particles was calculated to be of the order of 8 square meters. It is to be remembered however that the gland contents probably consist of approximately 50% gossypol and that other components are present in varying amounts (6,10).

Gland Sections. Electron micrographs of ultrathin sections (*ca.* 1,000 Å thick) of the gland (Figure 3a) showed the interior of the intact ovoid to be packed with discrete spherical particles. The outside wall appears to be made up of the compartments which overlap along the edges. Von Bretfeld had described the gland wall as a "membrane," comprising two layers with the outer layer being made up of thin-walled, tangentially flattened cells and the inner layer consisting a mucilaginized material in which traces of cell walls could be discerned. Electron microscopy reveals that the structure is even more complicated than he had visualized.

In contact with the outer wall is a network of material tightly packed with the spherical particles. In specimens which had been well fixed with osmium (Figure 3b) the particles remained intact in the section even when floated on a 30% solution of acetone in the section trough of the microtome. But, wherever the osmium had not penetrated, the particles were immediately released, and the network was left empty as in Figure 3c. The bare sockets of the network corresponded in range of sizes to that of particle diameters.

The position and relative sizes of particles within the gland is clearly shown in the ultrathin section of a portion of an intact, osmium-fixed gland in Figure 4a. It is obvious that the occurrence of minute and of large particles is completely heterogeneous. There is no localization of small particles in any particular area of the gland. Particles within the gland have the same range of sizes as those previously measured in the free state after extrusion by water swelling of the gland to rupture. Figure 4b shows a similar randomization of sizes of holes in the empty network after the ultrathin section had been floated for some time on the aqueous acetone solution. This aggregate of voids is typical of the appearance of slices of the material which had been imperfectly fixed with osmium.

In glands which had been extracted with water, prior to embedding for sectioning, the outside wall of the gland remained intact but the empty membranous network within was partially torn and partially disintegrated. Figure 4c shows the situation encountered in ultrathin sections of these pre-extracted glands. In the case of glands which had been extracted with acetone prior to embedding, the only part remaining was the outer wall. It is thought that the delicate network structure of the internal mesh may have been broken up by surface-tension forces in the action of the solvent or may have been shattered by shrinkage during dehydration.

No attempt has yet been made to analyze the constitution of the intragland network, and it is not known at this point whether it is proteinaceous or cellulosic in character. Perhaps further microscopical and microanalytical work, including enzymolysis, could elucidate the composition of the glands and contribute to the solution of problems involved in gossypol extraction.

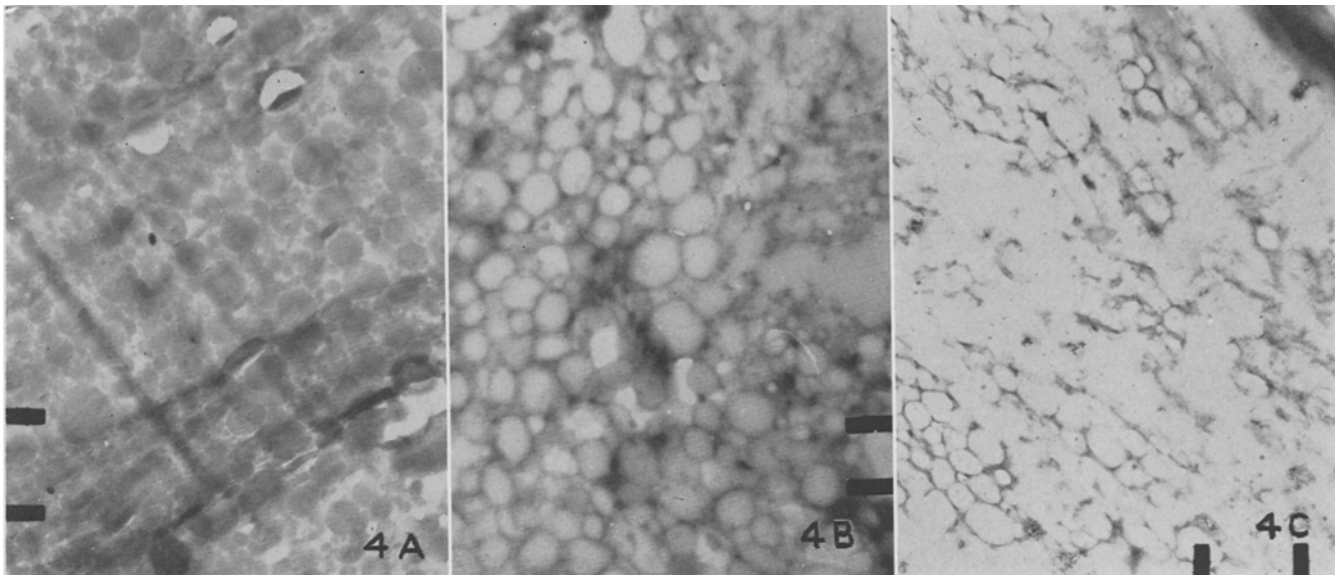

FIG. 4. Electron micrographs of sections of pigment glands, mag. 9024x. A, with globules intact; B, with globules dispersed by contact of section with water; C, section of gland extracted with water prior to embedment for sectioning.

The outside walls of the gland appear to consist of platelets which overlap each other at various intervals. These platelets exist as flattened compartments, each of which has a complex internal structure (Figure 5a). Within these platelets in more or less discrete lumps or blocks is a material extremely sensitive to osmium fixation (Figures 5b, 5c).

It has been suggested in the literature that the gossypurpurin may be concentrated in the outer wall of the gland and the gossypol deposited in the interior (11). No verification of this concept has been attempted, but the difference in structure suggests its possibility.

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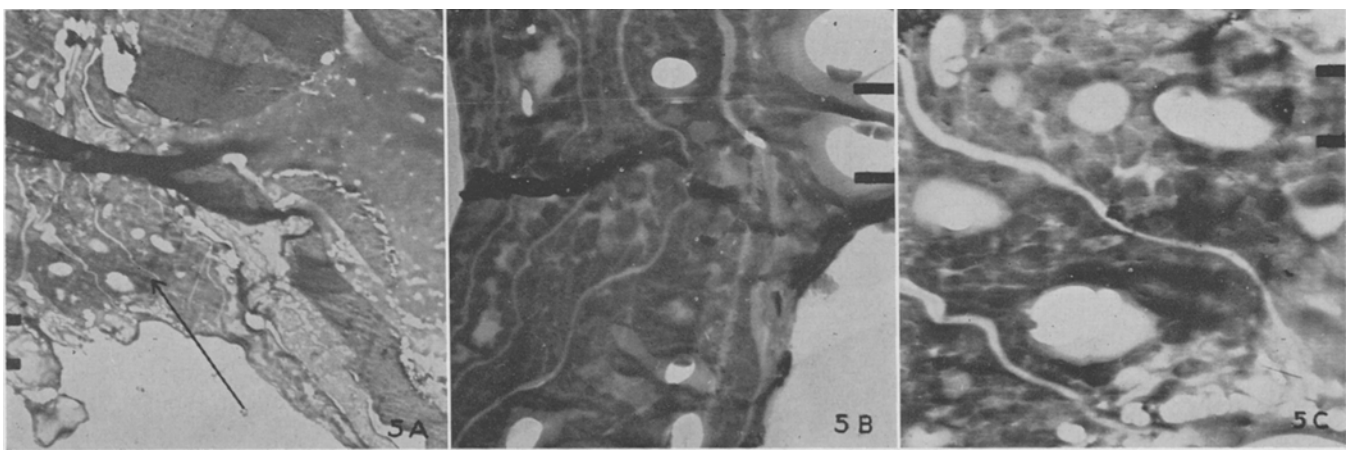

FIG. 5. Electron micrographs showing structure of outer platelets. A, section through portion of intact gland, arrow points to overlap of platelets, mag. 2994x; B, section through platelet, mag. 9585x; C, section through another area of similar platelet, mag. 6384x.