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ELASTIC-MATHEMATICAL THEORY OF CELLS AND MITOCHONDRIA IN SWELLING PROCESS. PART VI

ULTRASTRUCTURE OF MEMBRANE REGION IN EGG CELL OF SEA URCHIN (*STRONGYLOCENTROTUS PURPURATUS*) IN SUBELASTIC AND ELASTIC SWELLING

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SUMMARY

The ultrastructure of the egg cell of the sea urchin (*Strongylocentrotus purpuratus*) was electron-microscopically studied after the cell had swollen in hypotonic media of sea water. Owing to the exceptional osmotic conditions produced by these media, transformations occurred in the cell ultrastructure, which more clearly revealed structural details than when isotonic conditions are used in electronmicroscopy.

INTRODUCTION

The behavior of the cell membrane in the subelastic and elastic ranges was studied by means of swelling experiments[1,2], and along with these experiments an electronmicroscopic study was carried out to ascertain how the membrane structure behaves in various states of swelling of the cell[3].

When the cell is allowed to swell in hypotonic media, it is introduced to exceptional conditions. In electronmicroscopic studies one generally endeavors to maintain the conditions as close to normal as possible. "Exceptional conditions", especially when the deviation from the normal isotonic medium is considerable, have some interesting effects, however, on the structures of the cell. These effects occur only in those cell structures in which the influx of water is possible.

The accompanying electronmicrographs, besides complementing Part V of the series "Elastic-Mathematical Theory of Cells and Mitochondria in Swelling Process", serve the purpose of illustrating changes in egg cell structures (cortical granules, yolk granules, mitochondria, microvilli, *etc.*) of the sea urchin, *Strongylocentrotus purpuratus*, over a wide range of swelling media.

EXPERIMENTAL MATERIAL AND METHODS

The experimental arrangements have been explained in ref. 3, which also includes comments. The present electronmicrographs have been made in conjunction with the cell-swelling experiments so that the results in ref. 3 correspond to the states of swelling in the electronmicrographs.

RESULTS

The cell in different states of swelling

The transformations undergone by cell ultrastructures during subelastic swelling have proven, at least in some cases, to be physiologically reversible (*cf.*, footnote p. 547 in ref. 3). In subelastic and elastic swelling, the following general features are to be observed:

During subelastic swelling of the cell, the thickness of the cortical granule layer slightly increases, the granules swell and they are simultaneously deformed, resulting in oblate spheroids[3]. Also the yolk granules and mitochondria swell and the density of the microvilli decreases concurrently with the increase in the cell area. When the

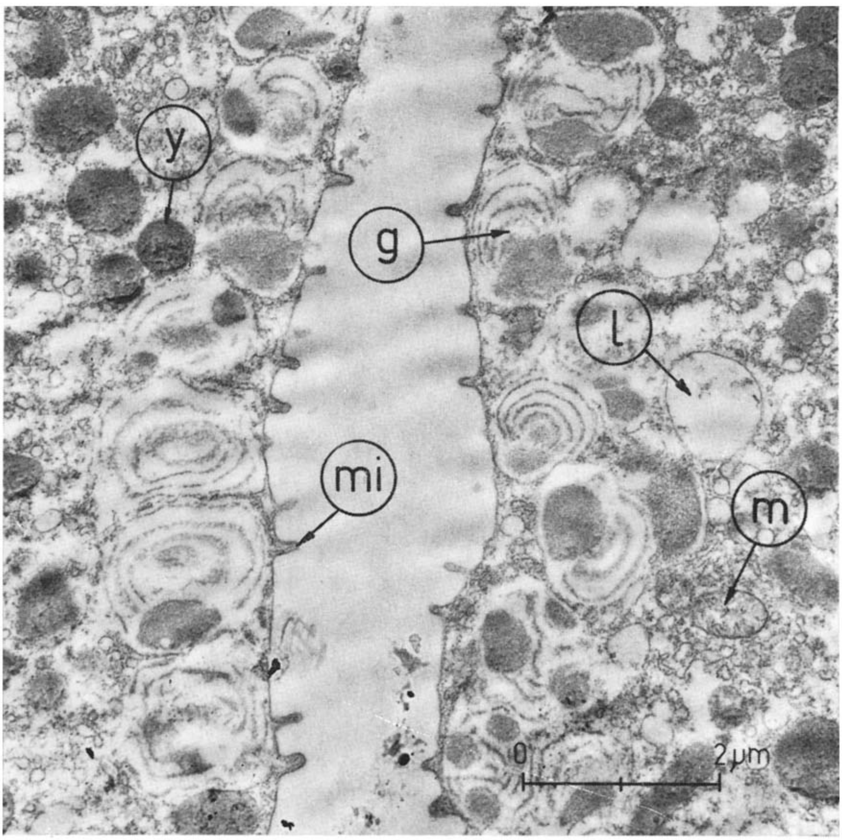

Fig. 1. The cell contour in 1.0-m. is macroscopically folded, or wavy (g = cortical granule, l = lipid body, m = mitochondrion, mi = microvillus, y = yolk granule).

upper limit of the subelastic range (V_E) is approached, spaces filled with water will have developed in the membrane region (= the space inside the cell near the membrane) and the cell contour takes on an even appearance as the macroscopic folds have straightened out.

Elastic swelling decreases the thickness of the granule layer and the granules are deformed, becoming increasingly oblate. The microvilli disappear and the cell contour appears stretched. In some mitochondria it is observed that the inner membrane has become detached and some yolk granules are broken. The number of water-filled spaces grows.

The following is a more detailed analysis:

1.0-m. The cortical granules form a single layer and a single granule apparently consists of eight shells*. The granules are connected by junctions, which probably occur only in the outer shells. The dark areas in the granules, visible in the electron-micrographs, are probably caused by the fact that, the thickness of the section being approximately 0.05–0.1 μm, parts of surrounding granules have also been sectioned and appear in the photograph. (The thickness of a granule shell is approx. 0.1 μm.)

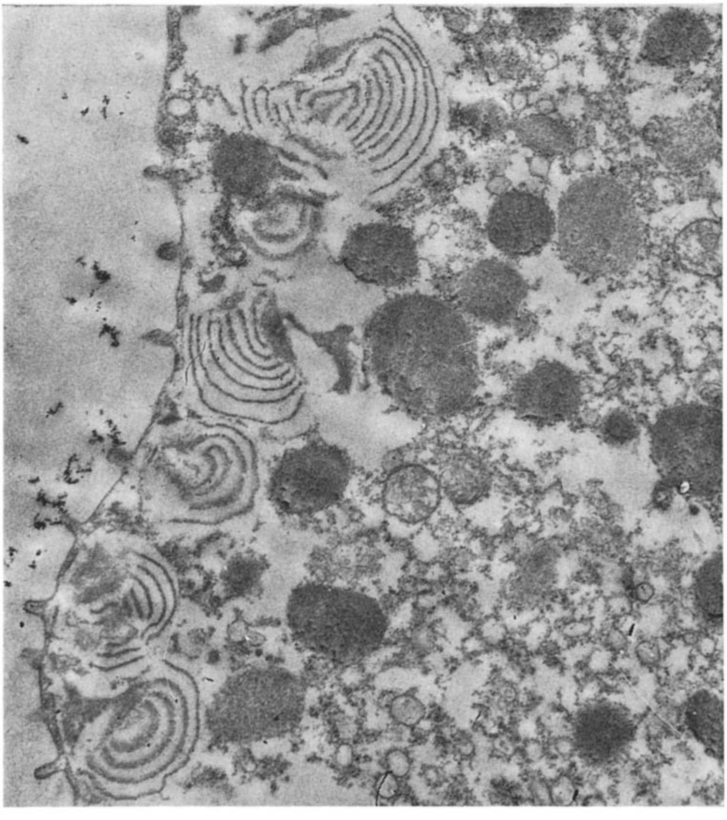

Fig. 2. Fluid spaces exist in the membrane region, since the diffusion in the cytoplasm is much slower than that through the cell membrane. The section plane goes through the center of a granule, eight shells being visible.

* *Strongylocentrotus droebachiensis* has 13 layers[4].

Some photographs reveal a jelly layer surrounding the cell, its thickness being the same as the height of the microvilli.

0.7-m. In Fig. 2 the granule net section shows eight shells of a granule. In the membrane region more water, carried by the influx, can be observed than in the inner cytoplasm. Some macroscopic waviness can still be observed in the cell contour, but the density of the microvilli has decreased. Swelling is observed to occur in certain components (*e.g.*, y, g, m) of the cytoplasm. By this state of swelling, it can be clearly seen that the water is not evenly distributed inside the cell. Based on this observation, the opinion that the diffusion in the cytoplasm takes place more rapidly than that through the membrane structure does not hold in this case.

0.5-m. In this medium the subelastic swelling of the cell and the granule has

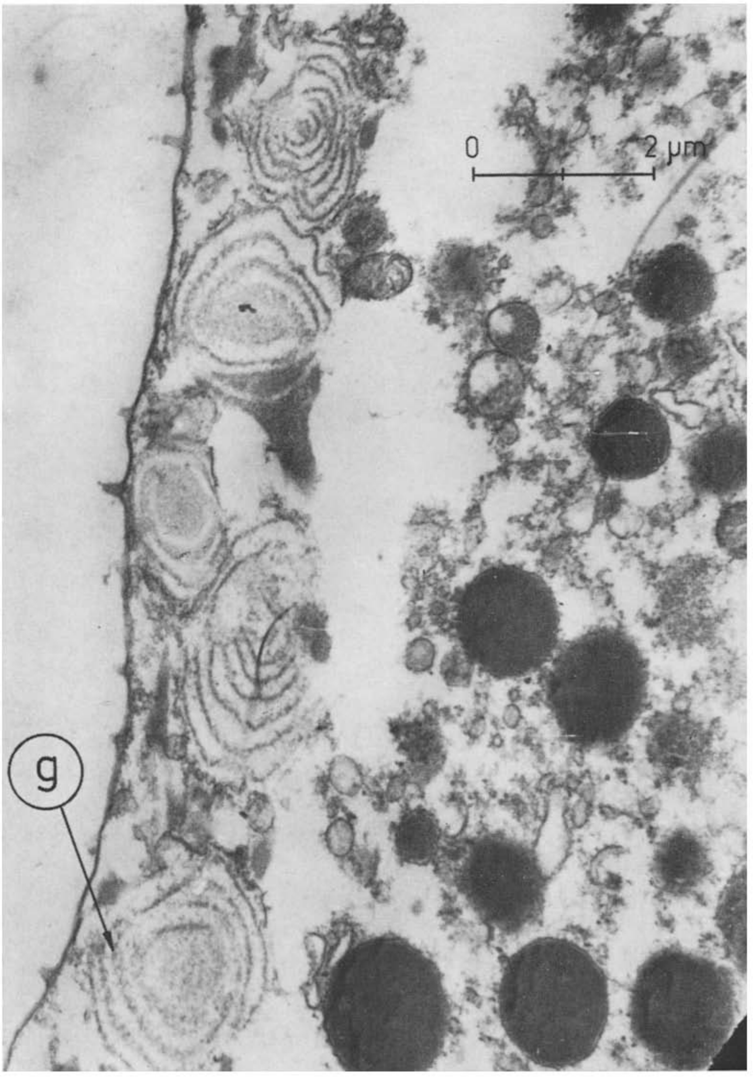

Fig. 3. The membrane region of the cell swollen in 0.5-m.

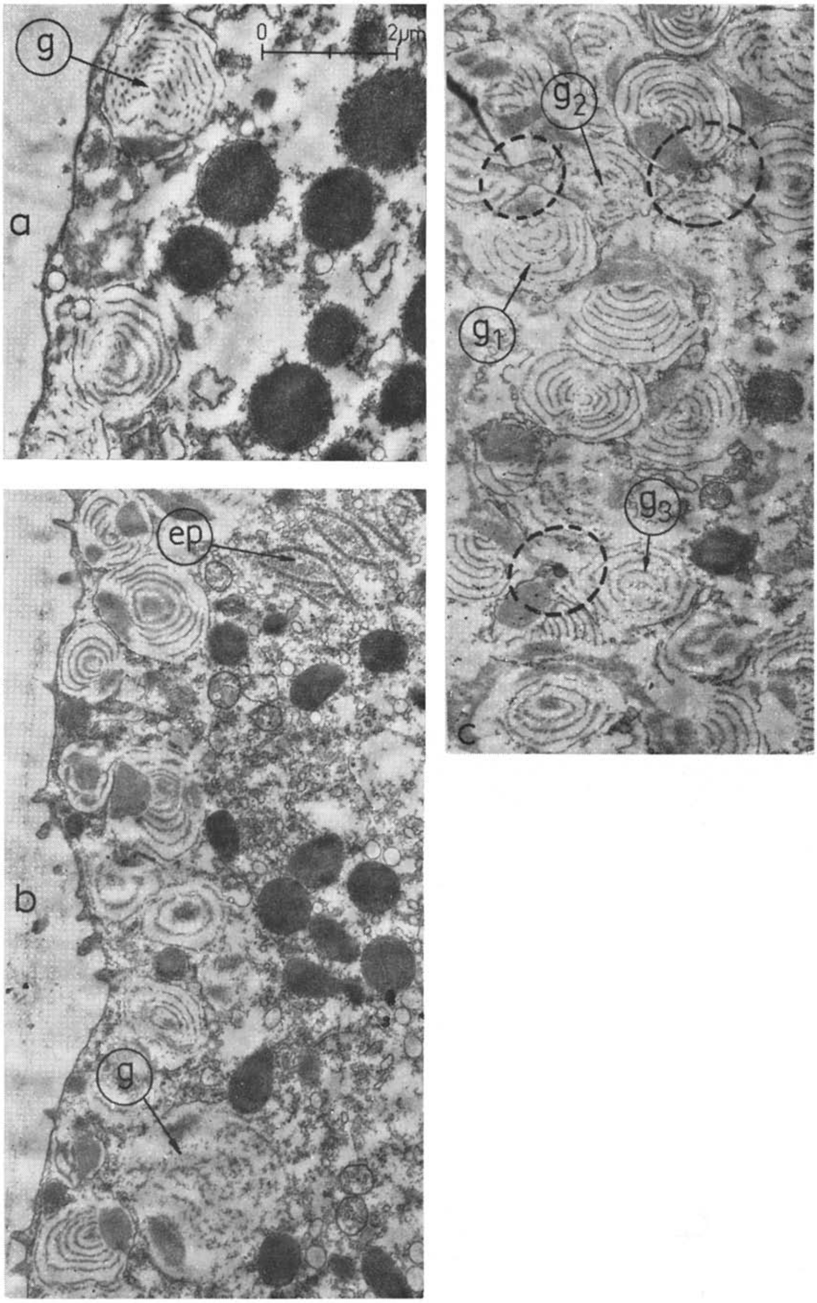

Fig. 4. The cells swollen in 0.45-m. (a) (g) just before granulolysis. The structure of the shells is clearly visible and perforated, owing to heavy stretching of the shells. (b) A completely granulolyzed granule (g), some endoplasmic reticulum (ep). (c) Cushion-form granules (g_1, g_2, g_3), some junctions of the granule net are encircled.

already ceased. The limit between the subelastic and elastic ranges is situated between 0.6- and 0.5-m. More precisely, this limit point can be seen in Figs. 1 and 2 (ref. 3) at an abscissa value of $1/\pi_E = 0.845$ cm²/dyne.

Some of the granules have obviously been oriented, concurrently with their swelling, in the direction tangent to the cell contour and been deformed to oblate spheroids. The outer surface of the cortical membrane appears to be stretched and it has become even. Granule (g) is already partially granulolyzed, its shell structure (especially the side facing the contour) appearing to be broken. The area of the swollen cell has increased and the microvillus density decayed. The osmosis causes the hydrostatic pressure difference in the cell during its elastic swelling between (1) the interior of the cell and the medium (p), (2) the interior of the cortical granule and the cell (p_{gc}) and (3) the interior of the granule and the medium (p_{gm}). In the subelastic range (up to $1/\pi_E$) $p = p_{gc} = p_{gm} = 0$, in the elastic range $p_{gm} > p_{gc} > p > 0$.

0.45-m. The pressure p (>0) produces a stress field on the cortical membrane, or on the granule net. The stresses[1] on the membrane tend to lengthen the granule in the direction tangent to the cell contour and simultaneously to flatten the granule (because $\nu \approx 0.5$) in the direction perpendicular to the cell contour, or h decreases. The pressure p_{gc} develops forces which, contrary to the ones mentioned above, tend to expand the granule in the direction of h, thus causing the granule to take on a spherical form. The elasticity of the granule causes the expansion of h to shorten the longer axis of the spheroidal granule. This deformation of the granule generates a stress field, which balances the stress field on the membrane caused by pressure p, as mentioned. When the characteristic yield limit of the granule is exceeded, the granule is broken (granulolysis). Fig. 4a shows a swollen granule (g) just before its granulolyzation. The perforated structure of the shells is revealed by this photograph.

From the calotte section micrographs (the section taken from the surface of the cell), it can be seen that both in the subelastic and elastic (see Fig. 4c) ranges the cross sections of the granules are more or less angular circles. In the subelastic range, this angularity is probably caused by crowding of the granules[3]. In the elastic range, the angularity remains and at several points of the granule groups three corners may be observed to be connected to each other. The calotte section micrograph of 1.0-m. does not make it unmistakably clear whether these corners are the junctions or just swollen parts of the granule. Fig. 4c, however, shows clearly that the corners are the junctions. The figure clearly reveals three junctions (g_1, g_2, g_3), and the fourth can be assumed to exist, judging by the shape of the granule. In a plane section it is obviously difficult to bring four junctions into view because of the curvature of the cell contour.

0.35-m. In ref. 3 the upper limit of the elastic range is assumed to be 0.35-m. Some local ruptures are observed in the granule membrane (Fig. 5a). Some of the yolk granules are partially broken and crystallites flow out. Possibly the crystallites form long prismatic rods[5], but they are not solidly crystallized.

The egg cells of *S. purpuratus* possibly have two different types of yolk granules, as is the case with *Limnaea stagnalis* L., which have been called complex and compact granules[5]. The present photographs reveal faintly discernible curved membranes inside most of the unbroken yolk granules. In Fig. 5a some yolk granules are freely drifting in 0.35-m. outside the cell. They seem to form groups and are thus possibly polarized.

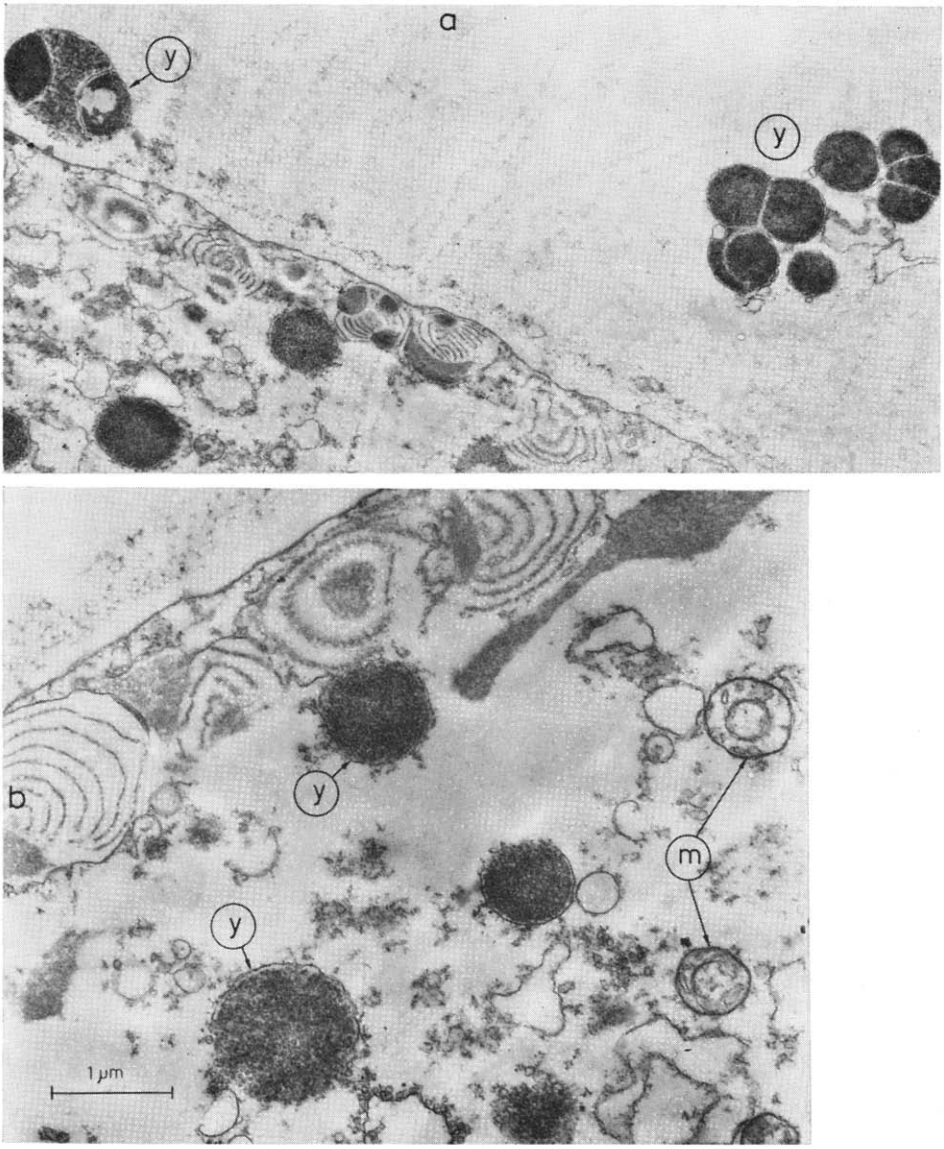

Fig. 5. (a) In 0.35-m. the elastic swelling of the cell is understood to have ceased. A part of the cortical granules, mitochondria and yolk granules has been destroyed. (b) The inner membrane of the mitochondrion has become loose and formed a continuous shell inside it. The crystal structure of yolk granules has split.

These granules show that a yolk granule consists of three compartments. This can be seen especially clearly in one of the yolk granules in Fig. 5a. In this granule the surrounding membrane has broken at the middle compartment, which is to be expected considering the distribution of stresses (Fig. 1 in ref. 1) and the structure of the yolk

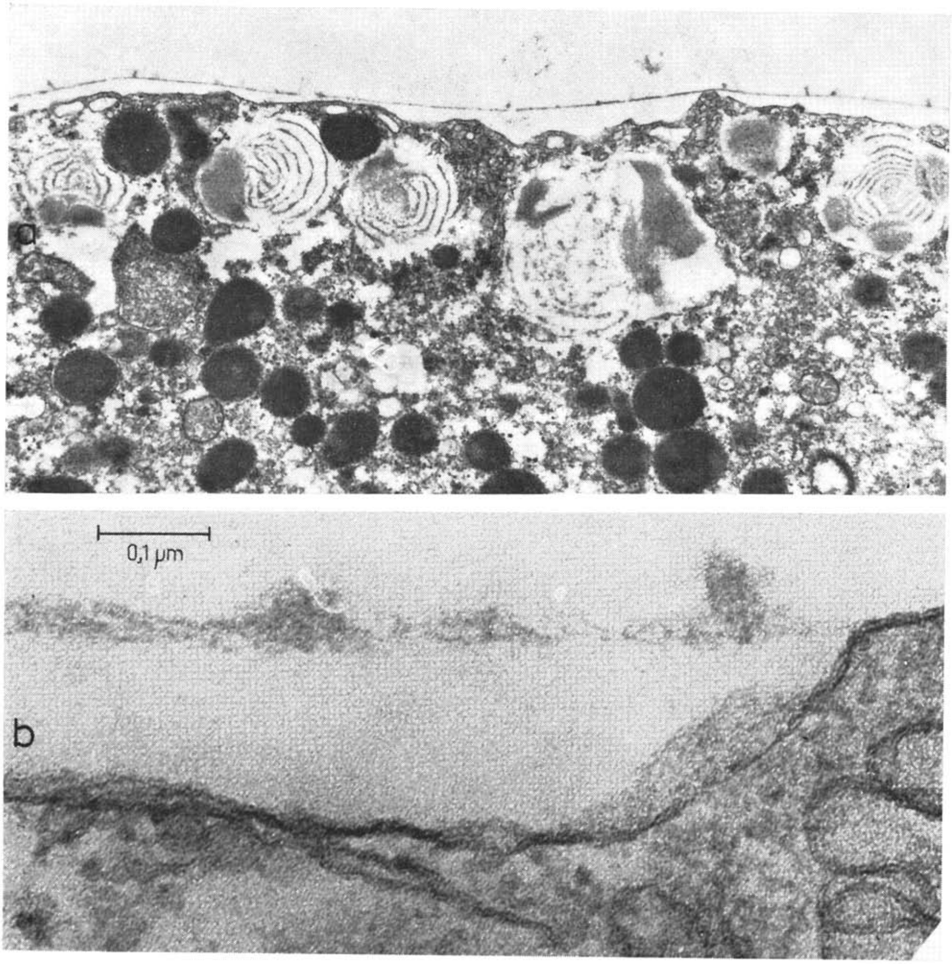

Fig. 6. (a) A cell returned from 0.25-m. to 1.0-m. The cortical granule membrane has become broken at several points and yolk granules have squeezed between cortical granules. (b) Two blebs produced by microvilli occur in the outer membrane; the inner membrane surrounds the cytoplasm.

granule (inner membranes). The section plane goes through some cortical granules so that the junctions are clearly visible.

Fig. 5b shows some crystallites which have leaked out of the yolk granules. The inner membrane (the cristae) of the mitochondrion has become deformed to produce a continuous shell inside the mitochondrion.

Returning from 0.25-m. to 1.0-m. When the cell is allowed to swell in the plastic range, it implies that some of its structures are deformed plastically; and when the cell is returned to 1.0-m., some irreversible features remain in the cell structure. Thus, depending on the stage of plasticity of different structures, details can be observed in electronmicrographs that otherwise might be hardly detectable. This method of study has been employed especially in an attempt to examine more accurately the phenomenon observed under a light microscope in swelling experiments, when the outer membrane of the collapsed cell[2] becomes loose after returning to 1.0-m.*,**.

Fig. 6a reveals that the granule chain has broken at several points and that yolk granules have squeezed into the interplasm between cortical granules. The broken granule has not been able to return. The cell is surrounded by at least two membranes, the outer one of which has in places become loose. Some of the yolk granules and mitochondria have, as indicated by the photographs, reverted to a geometric shape, which corresponds to that of Fig. 1. In Fig. 6a it can be seen that inside the outer membrane, the cytoplasm is surrounded by an inner membrane. This is clearly seen in Fig. 6b. Some photographs seem to show that the inner membrane consists of two different layers, but this has not been confirmed with certainty.

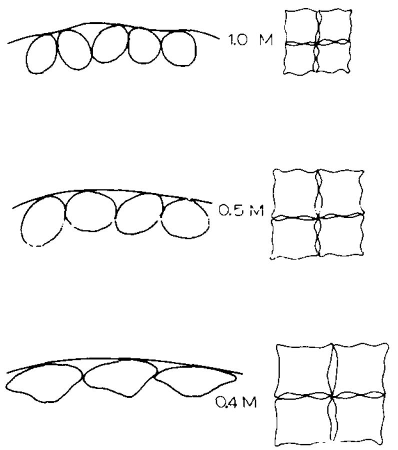

Fig. 7. The deformation of the granules at different stages of swelling. The sections in the plane tangent and perpendicular to the cell contour.

Some descriptive geometry of the cortical granule membrane

Based on numerous electronmicroscopic photographs, a series of drawings is reproduced in the following to depict the geometric deformation of the granule at different states of swelling (Fig. 7).

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* In shrinking experiments from 0.5-m. to 1.0-m. it has been observed electronmicroscopically that the outer membrane surrounding the cell is slightly deformed plastically at this stage so that it does not completely adhere to the surface of the shrunken cell but seems to be partially detached from it.

** The separation in ref. 6 has been made using distilled water.

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