# ISOLATION AND PROPERTIES OF SECRETORY GRANULES FROM RAT ISLETS OF LANGERHANS

## II. Ultrastructure of the Beta Granule

MARIE H. GREIDER, S. L. HOWELL, and P. E. LACY

From the Department of Pathology, Washington University, School of Medicine, St. Louis, Missouri 63110. Dr. Howell's present address is the School of Biological Sciences, University of Sussex, Brighton, England

ABSTRACT

Beta granules isolated from rat islets of Langerhans and subjected only to phosphotungstic acid had, in negatively stained images, a 50-A periodicity. This periodicity was also observed in thin-section profiles of beta granules in intact cells. In shadowed preparations, the granules were spherical in shape and had irregular edges and surface structure. The presence of such a periodicity in the beta granule indicates that its matrix may be composed of a crystalline material.

The isolation of a fraction of secretory granules from isolated islets of the rat pancreas (Howell, Fink, and Lacy, 1969) has made possible further characterization of the morphological properties of the isolated granules by methods not applicable to granules retained within the intact cell.

## MATERIALS AND METHODS

In most experiments, the washed pellet of the secretory granule fraction, prepared by density gradient centrifugation (Howell et al., 1969), was resuspended in 0.2 ml of a cold medium containing 160 mM KCl, 5 mM NaCl, 0.5 mM $MgCl_2$, and 5 mM sodium phosphate, pH 6.0, and maintained at ice-bath temperature while samples were withdrawn. Grids were prepared as soon as the pellet had been resuspended, since the insulin content of the granules becomes progressively solubilized with time after suspension (Howell, Young, and Lacy, 1969). All grids were prepared within 10 min of resuspension. In a single experiment, the pellet was resuspended in 2.5% glutaraldehyde in 0.15 M phosphate buffer, pH 7.5. In a further experiment, the pellet was resuspended in 0.3 M sucrose, buffered at pH 6.0 with 0.005 M phosphate buffer.

For negatively stained preparations, a small drop of suspension was placed on a collodion-carbon–coated 400-mesh copper grid, allowed to sit for 1 min, then drained by contact with filter paper. A drop of 2% phosphotungstic acid, pH 6.0, was placed on the grid and, after a minute, was drained in the same way. The first grid thus prepared was immediately placed in the vacuum chamber of the electron microscope; other grids were prepared and allowed to dry in a Petri dish containing a desiccant.

For shadow-casted preparations, after a drop of the suspension had been drained from the grid, the grid was flushed several times with distilled water for removal of salts. Some of the grids, while still wet, were subjected to osmium vapors for several minutes; others were allowed to dry in a Petri dish containing a desiccant. The grids were shadowed with platinum at an angle of 10°, or alternatively shadowed with platinum during continuous rotation of the grid (rotational shadowing).

Secretory granule pellets, isolated islets, and pancreatic tissue were fixed in 2.5% glutaraldehyde followed by osmium tetroxide solution, dehydrated in ethyl alcohol, and embedded in an epoxy resin according to Lockwood's procedure (Lockwood, 1964). The thin sections were stained with uranyl

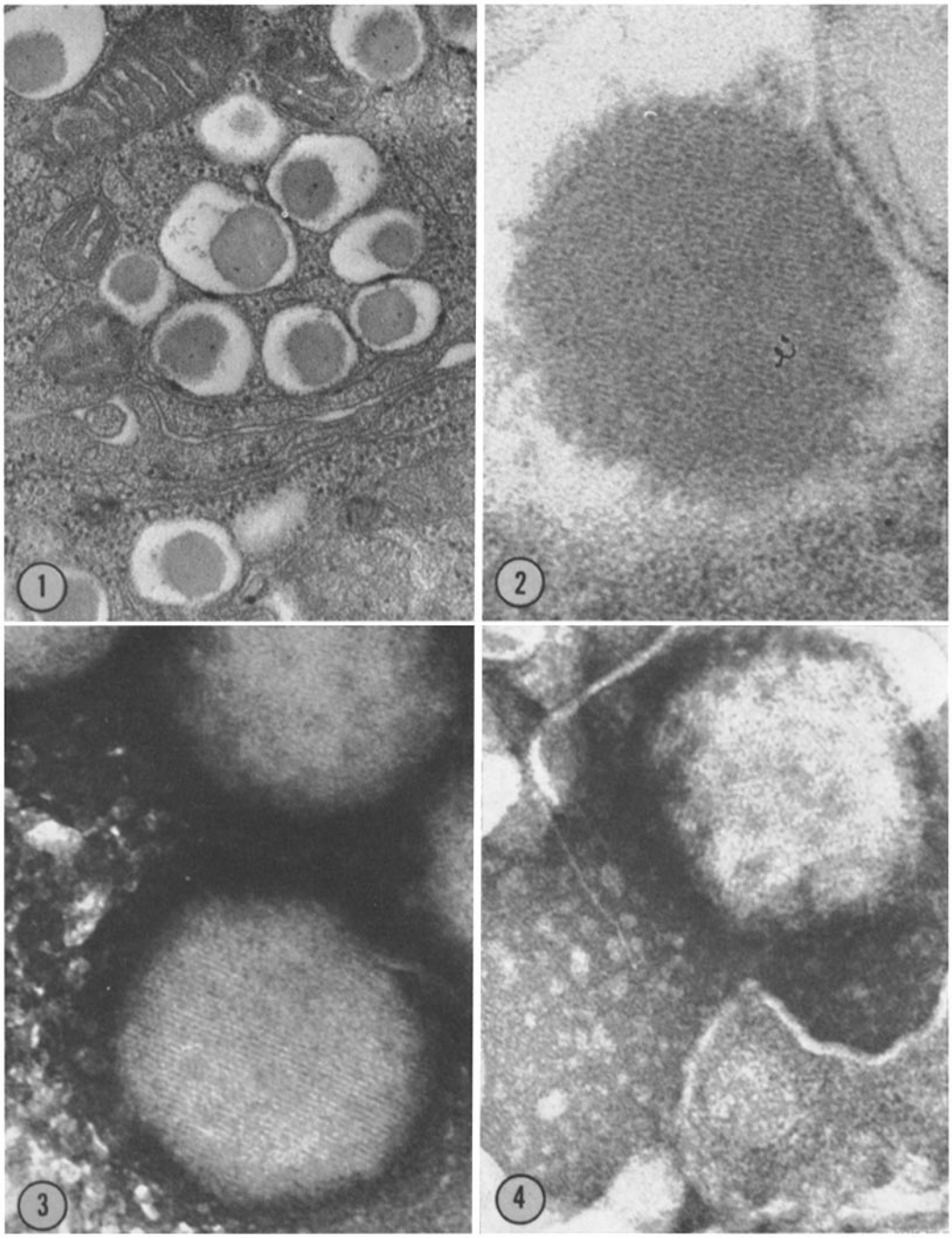


FIGURE 1 Thin-section profiles of beta granules within an islet cell. The angular profile of the granules and the large space between the granule and its limiting membrane are characteristic patterns of beta granules of the rat. × 48,000.

FIGURE 2 Thin-section profile of beta granule within the intact cell, showing ca. 50-A periodicity. × 247,000.

FIGURE 3 Negatively stained beta granule with periodicity of 50 A in one plane and with an angular orientation. × 200,000.

FIGURE 4 Negatively stained beta granule with 50-A periodicity. Part of the membrane of the granule is present. × 190,000.

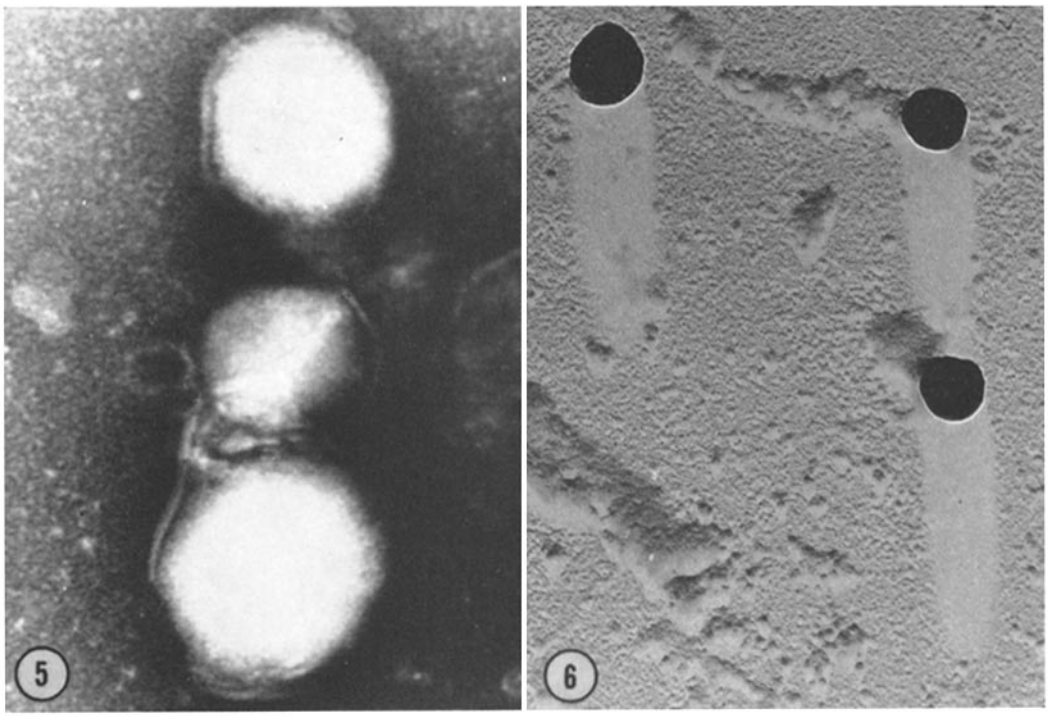

FIGURE 5 Negatively stained beta granules with partially intact membranes. × 132,000.
FIGURE 6 Granules shadowed with platinum at an angle of 10°. × 48,000.

acetate and lead citrate. This procedure made possible a comparison of the appearance of the whole granules with that of thin-section profiles.

The various preparations were observed in a Siemens 1-A or Philips 200-B electron microscope at magnifications of 10,000–50,000.

## RESULTS

### *Negatively Stained Preparations*

Examination of thin sections of beta granules from rat islets showed the granules to possess an angular configuration (Fig. 1), and at high magnifications a periodicity with a line-to-line interval of approximately 50 A was observed (Fig. 2). The mean diameter of the beta granules in thin sections was 180 mμ (24 observations).

It was not possible to identify each granule in the negatively stained preparations in terms of its being an alpha, beta, or delta granule. However, the angularity of certain granules and the occurrence of a 50-A periodicity allowed identification of certain granules as beta granules (compare Figs. 2–4). The 50-A periodicity was seen in granules subjected only to phosphotungstic acid solution and drying, and was an infrequent observation.

Occasionally membranes partially or completely surrounded the granule. The membrane appeared to be that of the granule and not membranous material also present in the preparation (Fig. 5). Granules without membranes did not appear to be morphologically different from granules with intact membranes. The fact that most of the granules in the negatively stained preparations were not surrounded by a membrane, whereas most of the granules in the thin-sectioned pellets of the isolated secretory granule fraction were membrane bounded, suggests that the membranes are ruptured during preparation of the negatively stained material.

### *Shadowed Preparations*

Since 73% of the cell population of the rat islet is composed of beta cells (Carpenter, 1966), and since the majority of the granules observed in shadowed preparations had a crystalline appear-

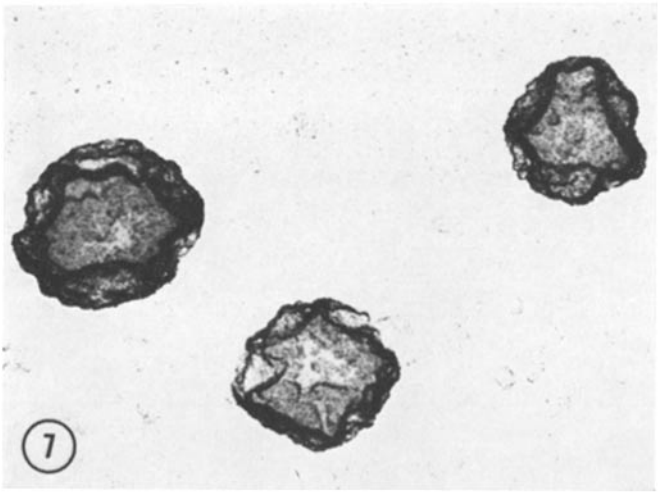

FIGURE 7 Rotationally shadowed granules, indicating heterogeneity of shape. × 112,000.

ance, it was assumed that these granules were beta granules.

The shadow lengths and over-all appearance of the granules, whether fixed in glutaraldehyde, subjected to osmium vapors, or allowed to dry without further treatment, were similar. The mean diameter of the granules was 205 mμ (30 observations); the length of the shadow of the granules indicated that their height was approximately equal to their width (Fig. 6). In rotationally shadowed preparations, the pattern of the shadow indicated a heterogeneity of shape. True spheres and perfect polygons were not observed; instead, the granules appeared to have highly irregular edges and surfaces (Fig. 7). Stereophotomicrographs of rotationally shadowed granules accentuated this irregularity of shape, and suggested a possible lamellar organization.

## DISCUSSION

Periodicity in beta granules observed in thin sections has previously been reported in beta granules of the congo eel, tiger salamander, and the dog (Sato, Herman, and Fitzgerald, 1966). McGavran, as reported by Lacy (1967), observed periodicity in the beta granule of a human islet. It is possible that such periodicity is present in beta granules of all species if sections are made thin enough for obtainment of the resolution required, and if sufficient micrographs are taken at high magnification to record the periodicity.

The fact that the periodicity was observed infrequently both in negatively stained preparations and in thin sections may be related to the orientation of the granule in the electron beam: the periodicity might be observed only when it runs in a plane directly at right angles to the beam. In addition, a proportion of the beta granules contain an amorphous material which may not be crystalline in nature: these might not be expected to exhibit periodicity.

The periodicity observed in negatively stained granules not subjected to the usual fixation procedures was the same as that observed in thin sections of granules in intact beta cells of islets fixed in glutaraldehyde and osmium tetroxide solutions. Thus, the periodicity observed in granules prepared by the latter procedure cannot be considered an artefact induced by fixation. Its presence suggests that the matrix of the beta granule may be composed of a crystalline material.

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