SERIAL RECONSTRUCTION OF THE CHARACTERISTIC GRANULE OF THE LANGERHANS CELL

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ABSTRACT

Three-dimensional models of individual granules in the same Langerhans cell were made after analyzing serial sections of human epidermis in the electron microscope. These models revealed that the granule is made up of a flattened or curved orthogonal net of particles which is bounded externally by a limiting membrane and which may be disc-shaped, cup-shaped, or combinations of both shapes. This variety of shapes accounts for the many configurations of the granule seen in individual electron micrographs. Usually, the granule has a vesicular portion at, or near one margin. This demonstration of the three-dimensional structure of the granule establishes the inaccuracy of previously used descriptive terms, the granule should be called simply the "Langerhans cell granule."

The Langerhans cell is found in the stratum Malpighi of the epidermis. With the light microscope this dendritic cell is best identified by staining with gold chloride, the same reaction used in the original description of the cell by Langerhans 100 years ago. More recently, Birbeck et al. (1) and others (2–7) examined the ultrastructure of the Langerhans cell with the electron microscope, and found a unique granule in its cytoplasm. Other ultrastructural features distinguish this cell from keratinizing epidermal cells. There are no tonofilaments in its cytoplasm and no desmosomes at its surface membrane. These features, however, are not specific and are shared with melanocytes in normal skin. The appearance of the Langerhans cell granule, therefore, is the most precise means of identification of the Langerhans cell.

Although the profiles of the Langerhans cell granule in cross-section are readily identified as rod-shaped structures made up of parallel membranes enclosing a central density (1–7), the precise three-dimensional configuration of the granule has never been described. The granule was first described as "disc-shaped" (1), the rod-shaped configurations representing cross-sections of discs. Later investigators, however, on the basis of two-dimensional profiles, have referred to the granule as "rod-shaped," "flask-shaped," and "tennis racket-shaped" (5–7). Thus, the three-dimensional structure of the Langerhans cell granule is uncertain.

In the present study, serial sections of individual granules were examined so that three-dimensional models could be reconstructed. Such reconstructions show that the granule is a paracrystalline network bounded externally by a limiting membrane which may assume a variety of shapes, from disc-shape to cup-shape. All of the various configurations of the granule seen by us, as well as those previously reported, can be explained on the basis of the proposed models.

MATERIALS AND METHODS

Biopsies of skin, 3 mm in diameter, were taken without anesthesia from the normal forearms of three human volunteers. Two biopsies were taken in a similar manner from the depigmented area of a patient with vitiligo in which Langerhans cells and granules are abundant (1). Each specimen was fixed in cold osmium tetroxide buffered with *s*-collidine,

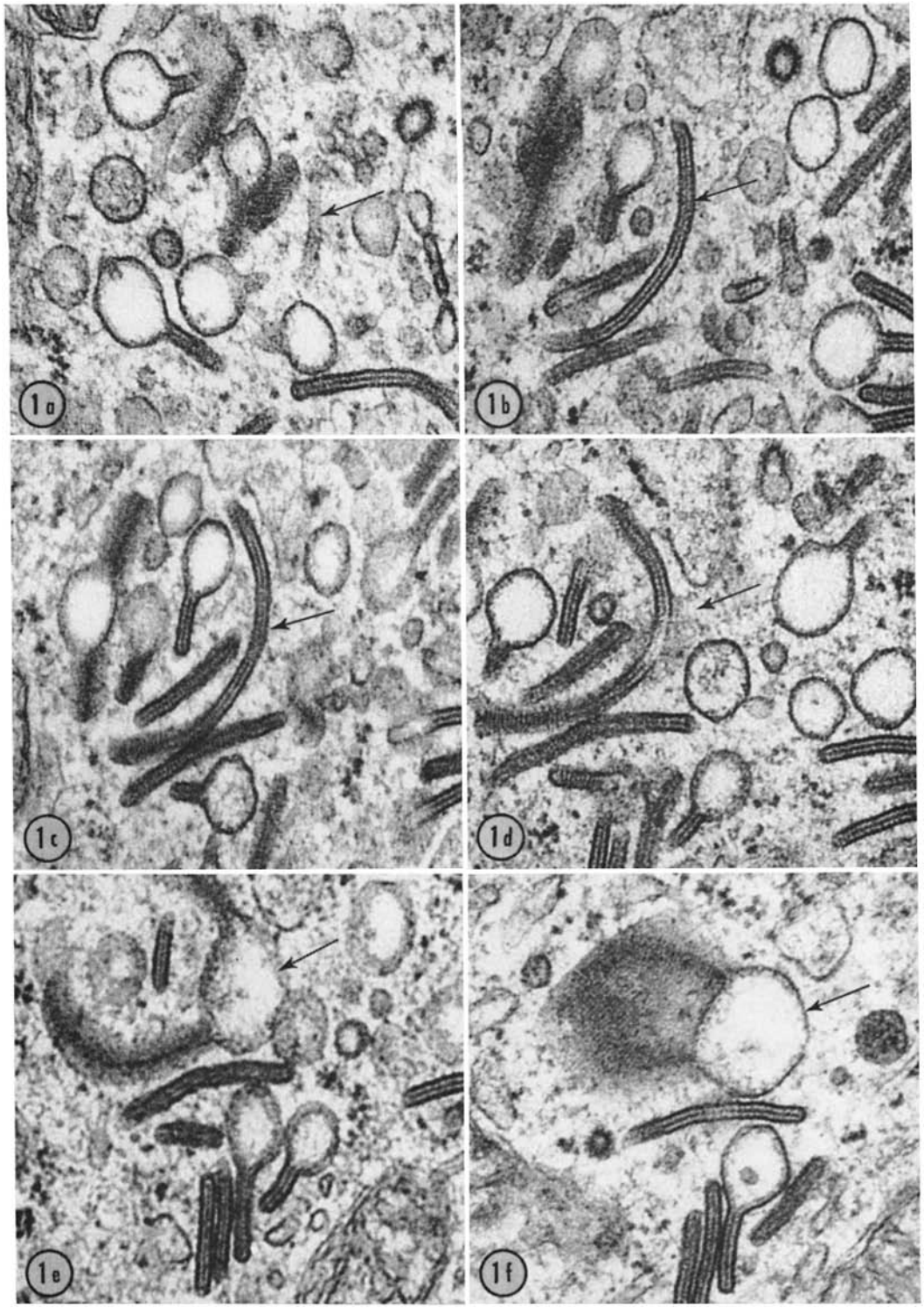

FIGURE 1 Six serial sections from the same Langerhans cell. The curved granule identified by the arrow in each of the micrographs (*a–f*) has been reconstructed in Fig. 3. Only the tip of the granule is seen in *a*. The curved portion is seen in *b* and *c*. In the midportion of the curve in the next section (*d*) the upper border of the vesicle is seen. The vesicle is cut through its midportion in *e* and *f*, while the tangential sectioning of the lower border of the curve of the granule can be seen in *f*. Adjacent granules can be followed in this series as well. × 70,000.

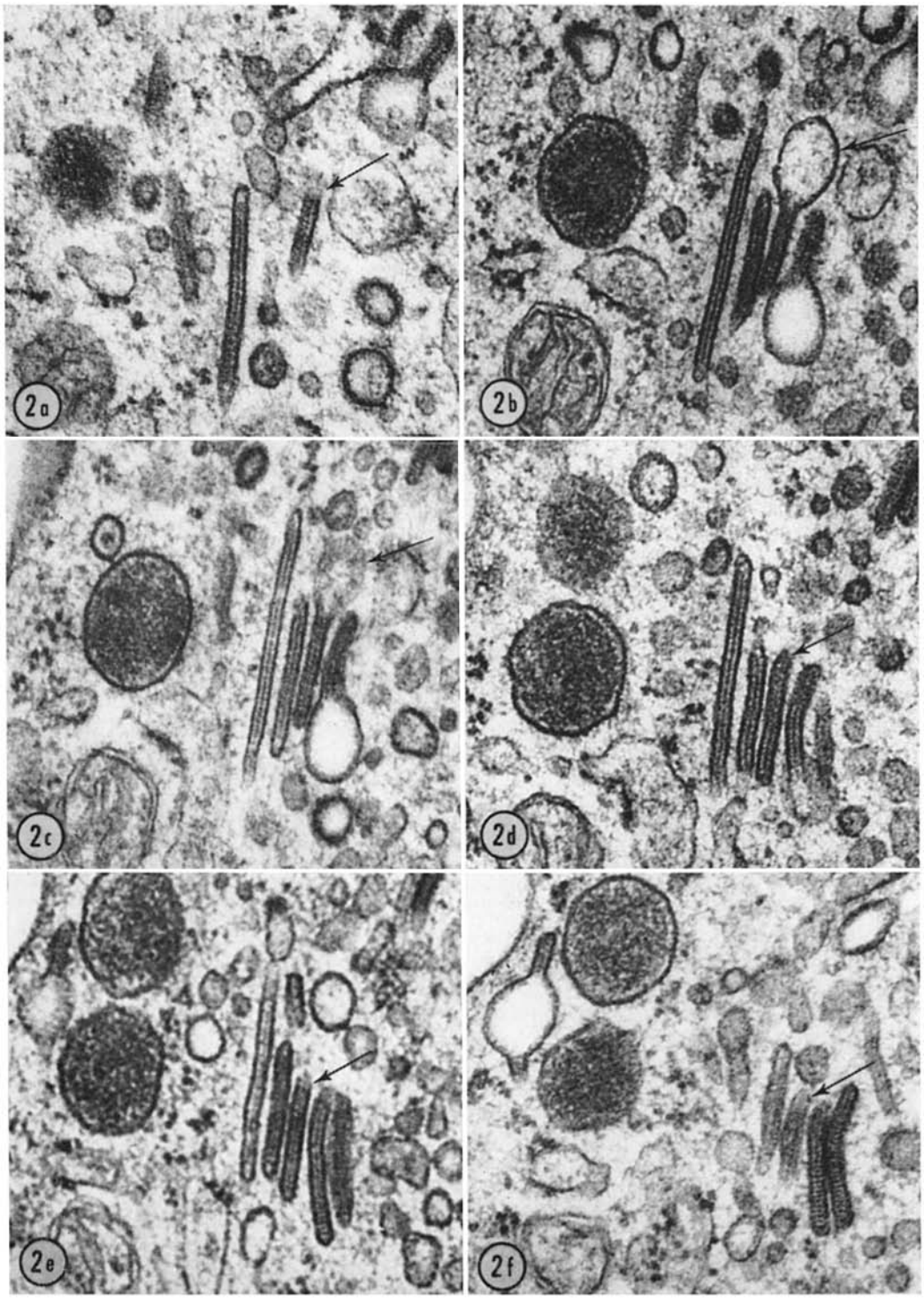

FIGURE 2 Another portion of the Langerhans cell illustrated in Fig. 1. Six serial sections demonstrate a group of four disc-shaped Langerhans cell granules. An arrow identifies one single granule through the micrographs *a–f*. Two adjacent spherical microbodies can be followed through these sections. × 70,000.

according to the procedure previously outlined by Odland (8). After dehydration, the tissue was embedded in Epon. 1-μ-thick sections were used for the purpose of choosing areas of epidermis for thin sections. Selected blocks were sectioned serially on a Reichert ultramicrotome and double-stained with lead citrate and uranyl acetate. The tissue was then examined in RCA 2-A and RCA 3-G electron microscopes.

We made reconstructions of Langerhans cell granules by tracing on paper the appearance of the granule through six serial sections. Models were built from these drawings with the use of Artcraft™ Plastilina.[1] A section thickness averaging 700 A was estimated by interference color at the time of thin sectioning. Subsequent Plastilina models were made with section thicknesses of 600–800 A. It was found that a slight variation in section thickness did not alter the basic concept of the model, but only varied its proportions slightly. The final drawings of Fig. 3 were made from a model which is based on an arbitrarily assumed value of 700 A as the section thickness. The drawing of the internal paracrystalline network in Fig. 5 was based on a 600-A-section thickness so that the number of 90-A particles needed could be simplified.

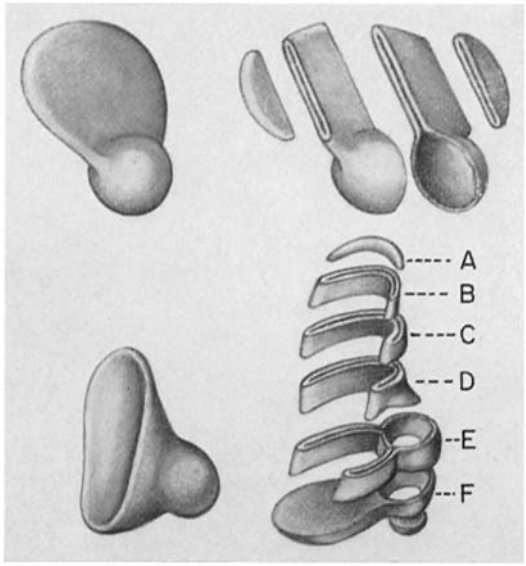

FIGURE 3 Models showing reconstructions of a disc-shaped granule (above) and a curved granule (below). The six separate portions (*A–F*) of the latter granule correspond to the 700-A-thick sections seen in the micrographs of Fig. 1 *a–f*.

RESULTS

The two-dimensional structure of the Langerhans cell granule is shown in Figs. 1 and 2. The granule appears as a rod-shaped profile with a central linear density (Fig. 1 *f*). The internal structure of the granule is made up of a flattened or curved sheet of particles in a paracrystalline array (Fig. 1 *f*), and is bounded by a limiting membrane. Some rod-shaped profiles are continuous with a vesicular portion at, or near one end (Fig. 1 *c*); occasionally, the vesicular portion appears in the middle of the profile (Fig. 1 *c*). In addition, many vesicles unassociated with rod-shaped profiles are seen.

When serial sections of the same Langerhans cell are analyzed (Figs. 1, 2), three-dimensional models of individual granules can be reconstructed. The disc-shaped granule is the more common form and is shown in the upper model of Fig. 3. The arbitrary separation of the granule into four segments in this model shows how rod shapes, both alone and associated with vesicular portions, could occur in cross-section. This model corresponds to any of the four parallel granules which have been serially sectioned in Fig. 2. The lower model in Fig. 3 represents reconstruction from Fig. 1 of a curved or cup-shaped granule. The separated portions of the model are labeled *A–F* to correspond to the electron micrographs seen in Fig. 1 *a–f*, and are drawn to approximate a 700-A-section thickness. Thus, the Langerhans cell granules appear to be disc-shaped, cup-shaped, or combinations of these shapes. They invariably consist, however, of an internal paracrystalline structure bounded by a limiting membrane, which frequently forms a vesicular portion at one edge.

Because orientation of these granules is random within the Langerhans cell, it is uncommon to obtain sections cut tangential to the paracrystalline sheet. However, when such sections occur (Figs. 1 *f* and 4), particles arranged in an orthogonal net are seen. The particles are spaced about 90 A apart. Such a periodicity coincides with that of the centrally located particles seen in profile when the paracrystalline sheet is cross-sectioned.

The three-dimensional structure of the granule can also be used to explain why the majority of the rod-shaped profiles have a linear density forming the central core rather than a regularly spaced periodicity. Only when an axis of the orthogonal net occurs parallel to the plane of section will the periodicity be seen (Fig. 5 *A*). Since sections

[1] M. Grumbacher, Inc., New York.

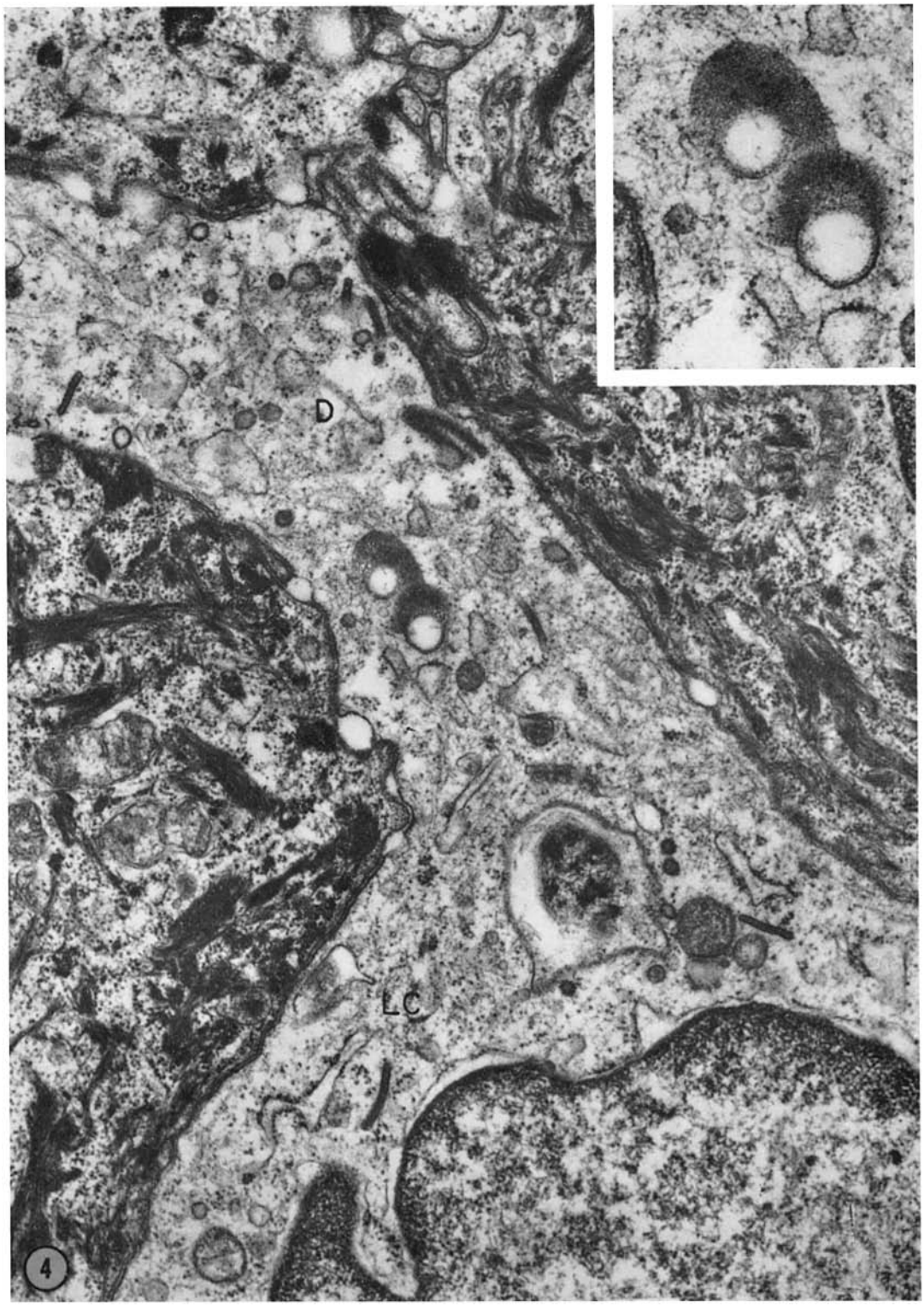

FIGURE 4 Langerhans cell in mid-epidermis (*LC*) showing extension of dendritic process (*D*). Two disc-shaped granules can be seen which have been cut tangentially. × 16,000. *Insert:* At higher magnification, the internal paracrystalline structure can be seen. × 60,000.

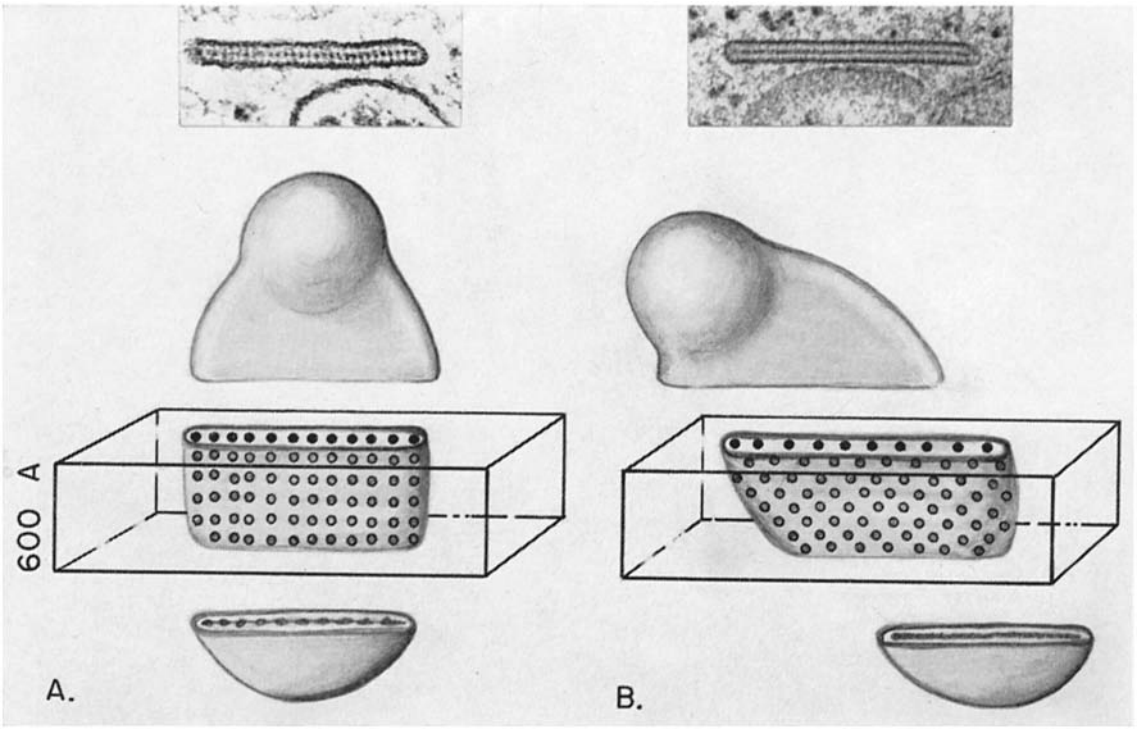

FIGURE 5 Models and comparative electron micrographs of granules explain the two types of internal structures found. The less common type (*A*) has the paracrystalline structure aligned showing interrupted periodicity of 90 A. The more common type (*B*) has the paracrystalline structure not aligned and, therefore, appearing as a continuous line. Electron micrograph inserts, × 130,000.

through the granule will be random, the majority of them will not coincide with either axis of the orthogonal net (Fig. 5 *B*). The internal structure of granules so sectioned will appear as a single dense line.

In random electron micrographs of the Langerhans cell granule, diverse configurations are seen. We can, however, section the reconstructed models in various planes in order to explain all of the various profiles published in the literature. Fig. 6 shows examples of granule profiles taken from electron micrographs, with corresponding models sectioned so as to explain how such profiles could be obtained from the proposed models.

DISCUSSION

Although other authors have postulated a disc-shaped structure for the characteristic granule of the Langerhans cell (1, 2), such a structure had never been proved until the present study. It is apparent that the adjectives "rod-shaped," "tennis racket-shaped," and "flask-shaped" are inaccurate unless clearly applied to the two-dimensional profiles of the granule. In addition, "disc-shaped" as a descriptive term is no longer adequate, since it has been shown that the granule may assume a variety of shapes.

No single descriptive modifying term can encompass the full range of shapes assumed by the granule. Therefore, a more adequate term would be simply the "Langerhans cell granule." This term would imply the variety of shapes that may be encountered.

Since the Langerhans cell granule assumes such diverse configurations, the question of the significance of the shapes arises. Does the granule progressively change from a simple vesicle to a vesicle associated with a disc-shaped body to a vesicle associated with a cup-shaped body? This question cannot be answered by observation of the fine structure of dead cells. It does appear that the smallest granules are usually disc-shaped, whereas

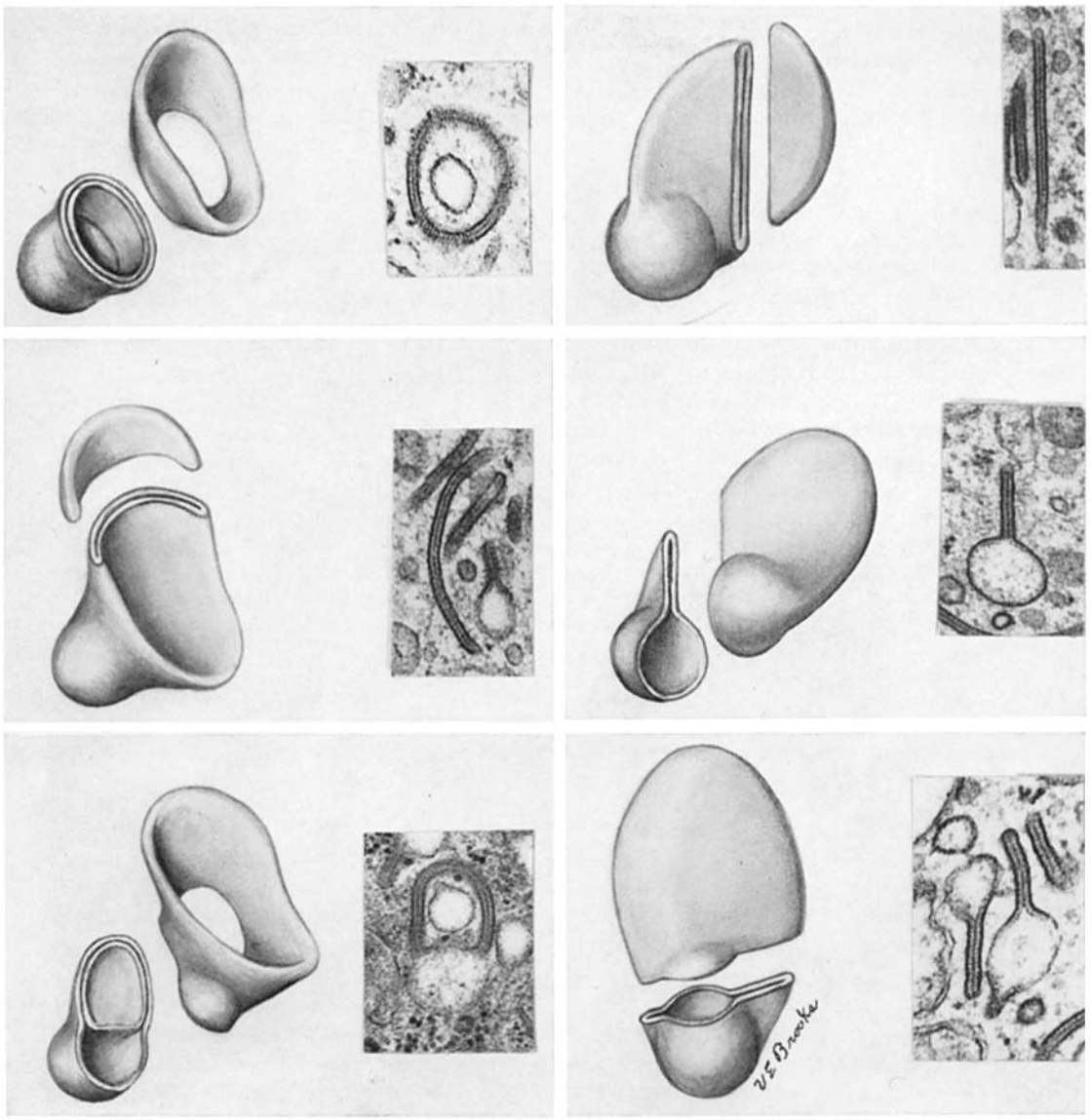

FIGURE 6 Models of the Langerhans cell granule cut in various planes explain the findings in the corresponding two-dimensional electron micrographs.

the larger ones may be either cup-shaped or disc-shaped. The "cupping" of the granule may not be an inevitable step in development, but may be the result of cytoplasmic forces acting on the granule. The reason that the larger granules are more frequently cupped than the smaller ones may relate to the mechanical properties of the granules. The central portion of a large sheet of material having a given pliancy is cupped more easily than that of a smaller sheet of the same pliancy. The possibility that the cupping is created during tissue preparation must also be considered.

The function of the Langerhans cell and granule is not known.

Recently, the specificity of the Langerhans cell granule has been cast in doubt. Cells appearing much like macrophages and containing cytoplasmic organelles identical to the Langerhans

cell granule have been identified in a bone lesion of eosinophilic granuloma (9) and a lung lesion of histiocytosis X (10). The significance of these findings awaits further investigation.

Addendum

Since submitting this manuscript, we have seen an article by Schroeder and Theilade (11) which shows three consecutive sections of a Langerhans cell in gingival epithelium. Their text refers to "rodshaped bodies opening into a vesicle," but the micrographs are compatible with our model of a disc-shaped granule (Fig. 3, upper). In addition, an article by Wolff (12) has appeared which mentions cup-shaped as well as disc-shaped forms, but does not illustrate serial sections. Wolff offers a different model of the internal structure of the paracrystalline net.

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