DEMONSTRATION OF THE LANGERHANS GRANULE BY LANTHANUM

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The term Langerhans granule has been introduced recently as a designation for a characteristic structure in the Langerhans cell of the epidermis and in the infiltrating histiocyte in histiocytosis X (1, 2). This unusual structure is found within the cytoplasm or attached to the plasma membrane, is in the form of a plate or disc, and is composed of two dense membranes separated by a central core of moderate density. Its dimensions vary, and the central core may have a repeating period. No function is known for this granule, which appears to originate from the plasma membrane or from vacuoles of the smooth endoplasmic reticulum in the region of the Golgi zone.

A new method for defining the extracellular space in ultrathin sections by means of a lanthanum complex has been described by Revel and Karnovsky (3). With this technique, they were able to demonstrate in intercellular junctions hexagonal arrays that were not evident with conventional methods of preparation for electron microscopy (3, 4). Since the Langerhans granule is frequently continuous with the extracellular space, the probability that it too could be demonstrated with lanthanum and that additional information could be obtained about its structure seemed likely. This report describes our findings with this technique.

MATERIALS AND METHODS

Granuloma tissue obtained in the operating room from a 4 yr-old girl with a solitary eosinophilic granuloma of the skull was immediately fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. After 6 hr fixation it was washed and stored in the buffer. The tissue was later postfixed for 2 hr in an OsO_4-collidine-lanthanum solution at room temperature as described by Revel and Karnovsky (3), dehydrated in graded alcohols, and embedded in Araldite. Other tissue was postfixed in 1% osmium tetroxide and embedded in Epon 812. Thin sections were cut with a diamond knife, stained briefly in an aqueous solution of uranyl acetate, and photographed in a Siemens Elmiskop 1A.

RESULTS

Typical Langerhans granules were readily demonstrated by the conventional methods of preparation for electron microscopy and were identical with those described before (5). They measured up to 1μ in length and between 330 and 420 A in width. The granules were present throughout the cytoplasm or were continuous with the plasma membrane and appeared as linear profiles consisting of two limiting membranes separated by a central zone of moderate density, there being an intervening intermediate zone of relative electron lucency between the central zone and each membrane (Fig. 1). A saccular dilatation was often present at one end of the granule, and a row of particulate densities was closely applied to the limiting membrane. The central core was homogeneous or exhibited a linear period, depending on the plane of section.

With the lanthanum technique, the lanthanum complex was present in the extracellular space as fine particulate material or as crystalline arrays. The amount of this material varied depending on the extent of the extracellular space or the depth of section in the block. The plasma membranes of many cells were surrounded by the lanthanum, and these cells appeared in relief against the dense background. When the lanthanum tracer penetrated the Langerhans granule, the granule showed

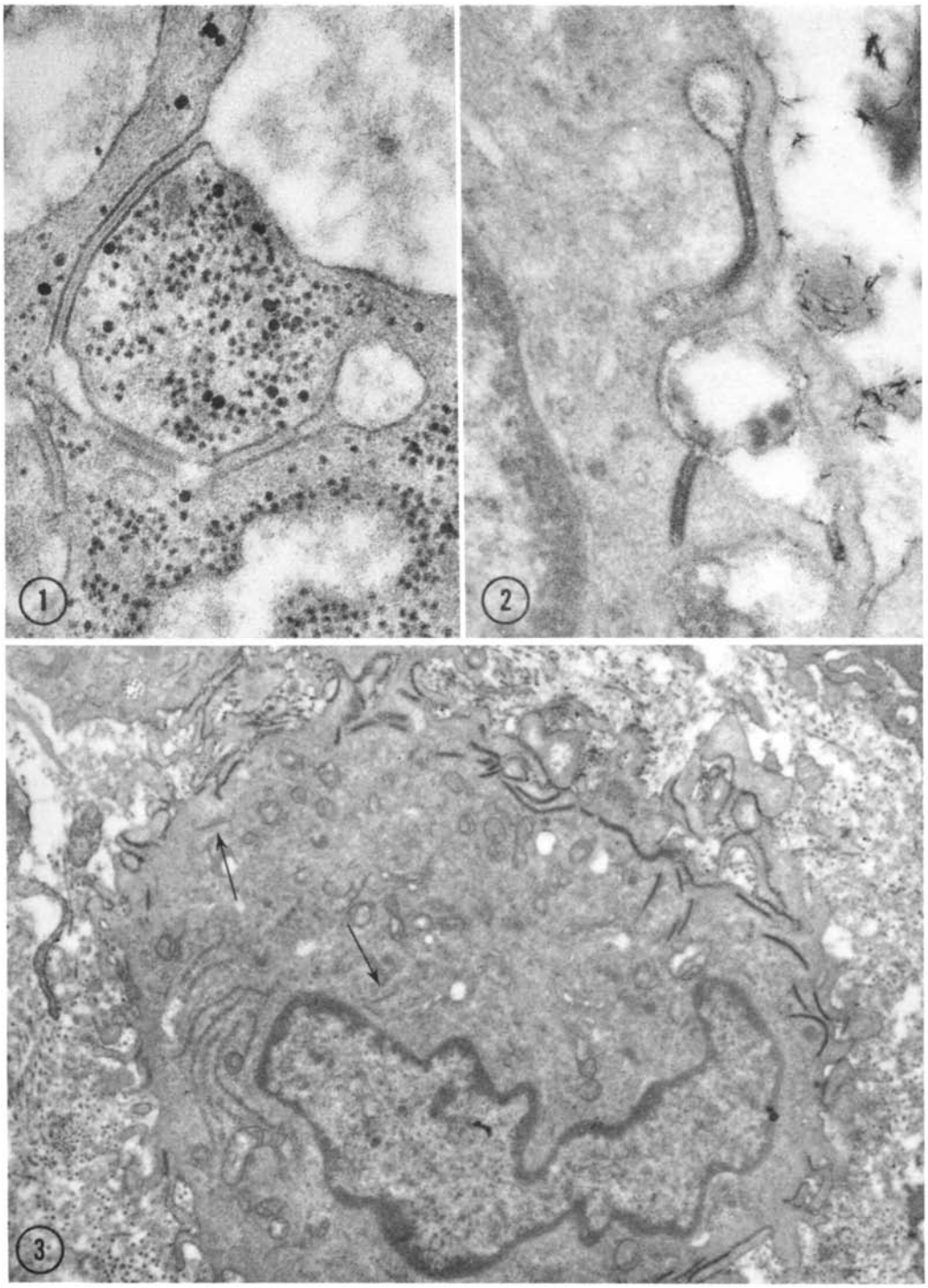

FIGURE 1 Langerhans granule in continuity with the plasma membrane. The appearances of the central core and the limiting membrane vary according to the plane of section. A row of particulate densities is evident beneath the limiting membrane when the central core is prominent. The granule terminates in a saccular dilatation. × 60,000.

FIGURE 2 Compared with Fig. 1, this figure shows the increase in density of these granules which is produced by the particulate lanthanum tracer. Crystalline arrays of lanthanum are present in the extracellular space. × 60,000.

FIGURE 3 A cell in a collagenous matrix. The lanthanum tracer has penetrated the granules continuous with or near the plasma membrane. Several granules unmarked by the lanthanum are present deeper in the cell (arrows). × 11,500.

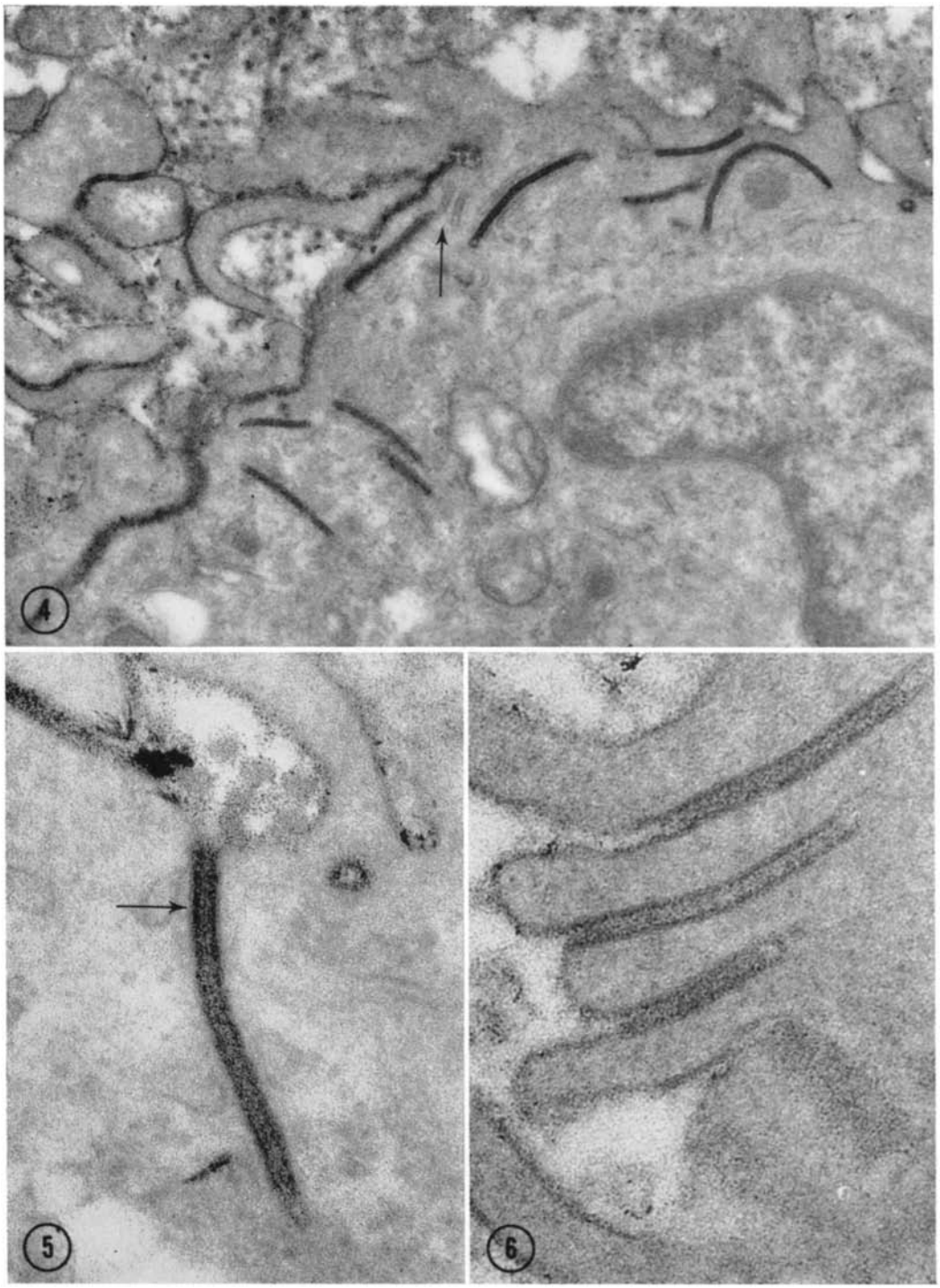

FIGURE 4 The lanthanum complex is uniformly distributed in the granules near the plasma membrane. A portion of one granule and an adjacent granule have not been penetrated by the lanthanum (arrow). × 34,500.

FIGURE 5 A fine central core and two prominent zones of tracer are present in the superficial portion of the granule. Less tracer is evident in the deeper part of the granule. The arrow indicates the limiting membrane of the granule. There is a narrow clear zone between the limiting membrane and the tracer. ×120,000.

FIGURE 6 The tracer is uniformly distributed in the central portion of these granules seen in continuity with the plasma membrane. The row of particulate densities beneath the limiting membrane may be due to the normal structure of the granule or the lanthanum tracer. × 120,000.

increased density and became readily evident (Figs. 2, 3). The granules close to the plasma membrane or continuous with it contained the lanthanum; those in the central portion of the cell were always free of lanthanum (Figs. 3, 4). The lanthanum was dispersed in the central dense core and its adjacent electron-lucent zones; sometimes this dispersion showed a gradient of density, and then more lanthanum was present in the superficial portion than in deeper portions of the granule (Fig. 5). The particulate granularity persisted in tangential sections of the granule without demonstrating any additional structure. Occasionally, a dense core measuring 40–45 A in diameter was present in the center of the granule (Figs. 2, 5), but no consistent structural pattern or periodicity was evident in the central part of the granule. Frequently, particulate densities along the inner aspect of the limiting membrane persisted in spite of a diffuse penetration of the tracer into the granule (Fig. 6). This punctate appearance was obliterated in areas of dense lanthanum penetration; however, a narrow clear zone separated the lanthanum from the limiting membrane (Fig. 5). The tracer was specific for the granule; it was not observed to penetrate smooth- or rough-surfaced endoplasmic reticulum or other organelle.

DISCUSSION

The lanthanum tracer used in this study may have reached the Langerhans granule by diffusion from the extracellular space or by selective binding to some component of its structure. In some situations lanthanum is known to bind to cell membranes (6, 7), but with the method used in this study it is regarded as a marker for the extracellular space (3). The observation that many granules were not "stained" by the lanthanum suggests that the granules which contained lanthanum obtained it by the contiguous spread of the tracer from the extracellular space to the organelle. Only the superficial granules contained lanthanum; exposure to the tracer may have been insufficient to effect a tracer penetration of granules in the depth of the cell. We have not excluded the very likely possibility that there may be two populations of granules, one penetrated by the lanthanum because it is associated with the extracellular space, and the other unavailable to lanthanum because it is sequestered within the cytoplasm. Whether the granules originate from the plasma membrane and move into the cell (2) or develop within the cell and migrate to the cell surface (8) cannot be determined from our material.

Except for some instances in which the lanthanum demonstrated a central core in the granule, the density of the tracer obscured the detail of the granule. This central core was finer than the usual central axis of the Langerhans granule (5) and may represent a narrow central canal as depicted by Wolff (8). Although this central core was penetrated by the tracer, we were unable to demonstrate penetration beyond the row of particulate densities beneath the limiting membrane, for in properly oriented sections a clear zone was present between the lanthanum and the limiting membrane.

SUMMARY

Langerhans granule–containing cells from an eosinophilic granuloma of bone were studied after the introduction of a lanthanum tracer during fixation. The lanthanum provided selective demonstration of the granules in continuity with the extracellular space, and it defined a narrow central core (or canal) in the granule. It was not possible to demonstrate penetration of the tracer beyond the row of particulate densities beneath the limiting membrane.

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