LANGERHANS GRANULES IN FETAL KERATINOCYTES

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Langerhans granules, as reconstructed in three dimensions and interpreted by Sagebiel and Reed (13) and Wolff (17), are discs that in profile look like rods bounded by limiting membranes 50–60 A wide and terminated in vesicular formations. Internally, the rod-shaped portions of the disc demonstrate paracrystalline nets or lattices with a periodicity of 90 A. The presence of these granules has been considered the primary criterion by which dendritic cells have been identified as Langerhans cells.

Although Langerhans granules are most commonly seen in dendritic cells in the epidermis (3–5), they have been found in cells in the dermis (18), in the epithelia of the uterine cervix (7) and of the gingiva (14), and in three forms of histiocytosis X granulomata (1, 8, 15).

Langerhans granules have now been found inside the keratinocytes of fetal epidermis.

MATERIALS AND METHODS

Specimens of scalp skin from a 76 day fetus and from an adult rhesus monkey were cut into blocks (1–2 mm^3), fixed for 2 hr in 1% OsO_4 buffered with veronal acetate (10), dehydrated through an ascending series of ethanols to propylene oxide and 1:1 propylene oxide:Araldite, and then embedded in Araldite (9). Sections for electron microscopy were cut on a Porter-Blum MT-2 microtome, stained with lead citrate (12) and uranyl acetate (16), and viewed on uncoated grids in a Philips 200 electron microscope operating at 60 kv.

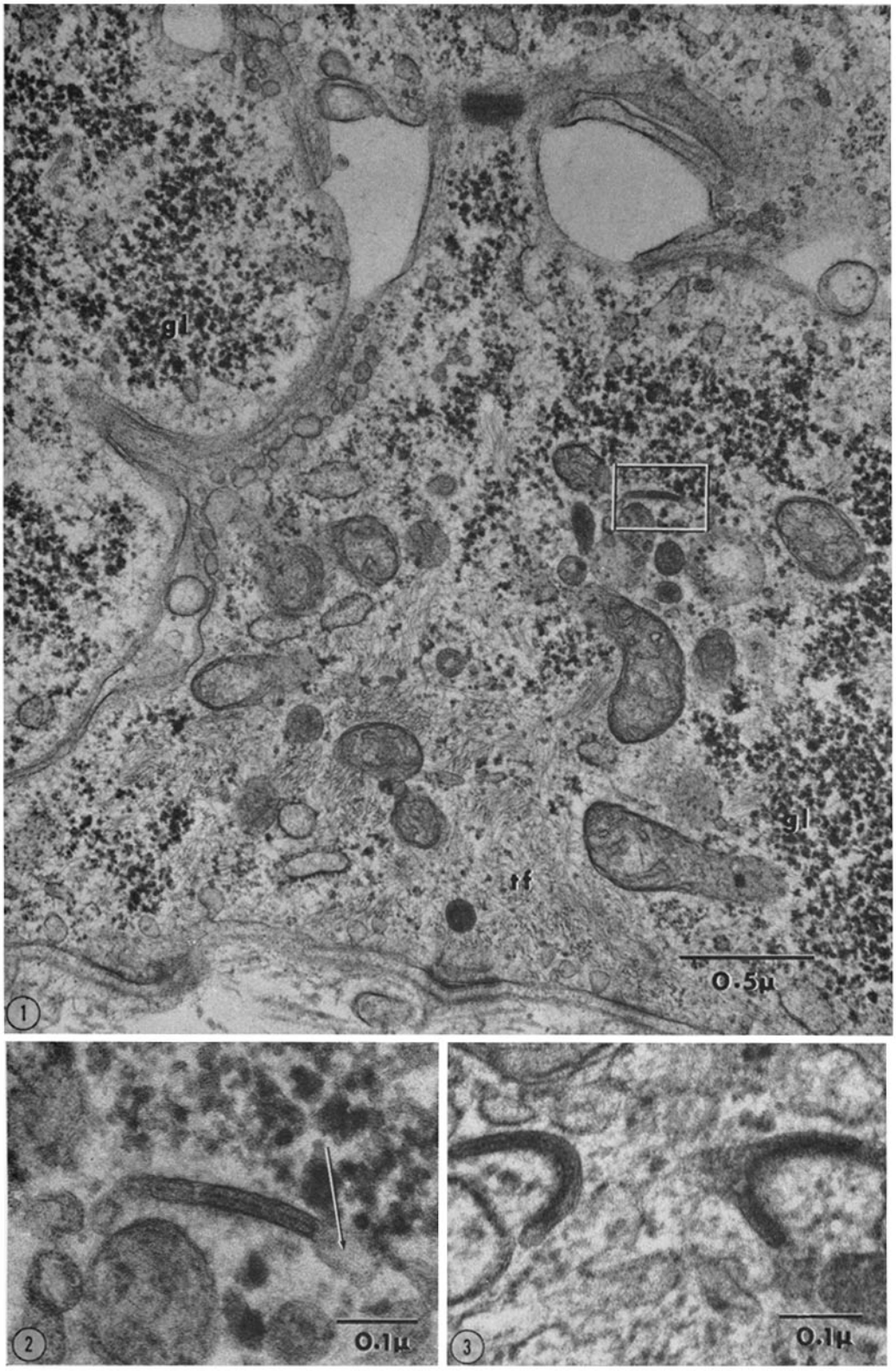
RESULTS AND DISCUSSION

Langerhans granules, identical with those found in dendritic Langerhans cells, are present in some keratinocytes in the germinative layer of 76 day fetal epidermis (Fig. 1) and in cells of the outer root sheath of differentiating hair follicles. Seen

FIGURE 1 A portion of a keratinocyte in the basal layer of 76 day fetal epidermis. Hemidesmosomes are present at the basal surface abutting against the basal lamina, and a desmosome at the top of the field maintains attachment to the more superficially located cell. A Langerhans granule (*inset*) is free in the cytoplasm. Large aggregations of glycogen particles (*gl*) and tonofilaments (*tf*) are also present. × 40,300.

FIGURE 2 A higher magnification of the Langerhans granule shown in Fig. 1. The rod-shaped portion is identical to that of typical Langerhans granules, with outer limiting membranes 50–60 A wide and an inner electron-opaque core; it ends in a terminal vesicle (arrow). × 126,750.

FIGURE 3 Two Langerhans granules from a keratinocyte in the outer root sheath of a differentiating hair follicle. The granule at the left is typically rod-shaped, has an inner core, and ends in a small terminal vesicle; the other has a central vesicle with two rod-shaped ends. × 126,750.



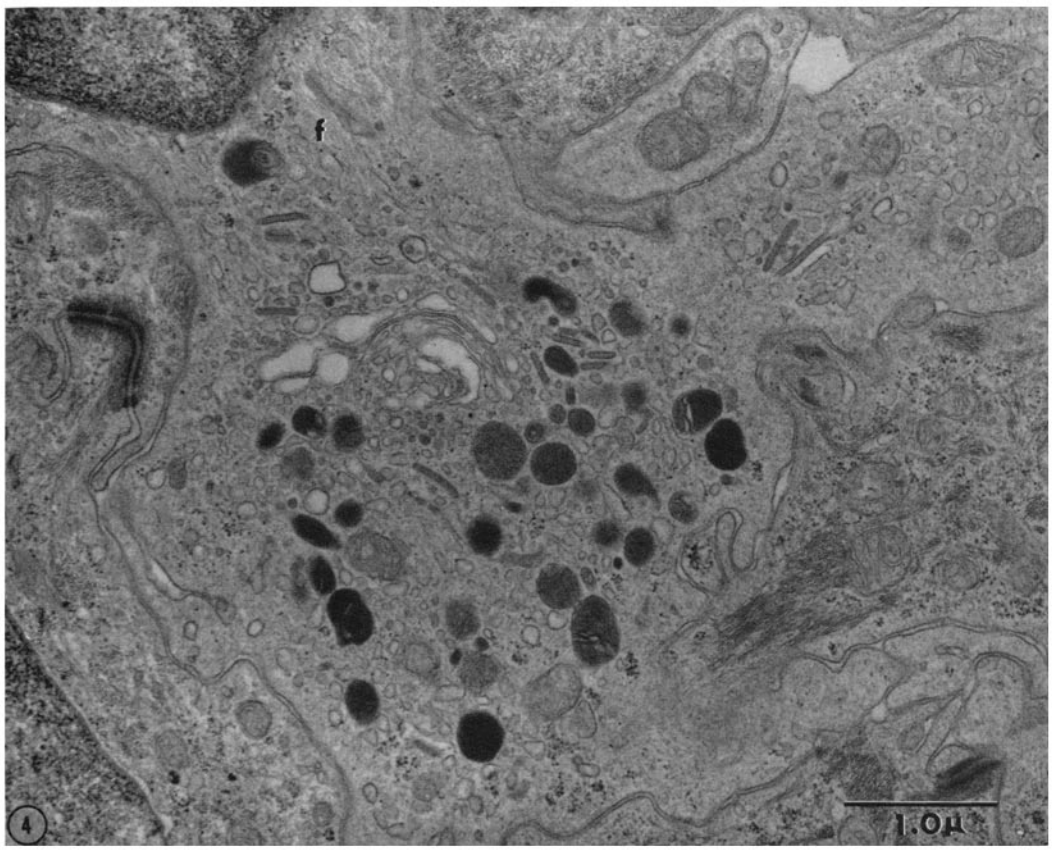


FIGURE 4 A typical Langerhans cell in adult rhesus epidermis. No desmosomal attachments are seen at the cell surface. Several Langerhans granules are visible, and numerous lipid-like droplets are also present in the cytoplasm. The Golgi zone is well-developed and a few cytoplasmic filaments (*f*) are also present. × 20,800.

in cross-section, the rod-shaped portions of the granules consist of outer-limiting unit membranes 50–60 A wide and inner electron-opaque cores that have a periodicity of about 80–90 A (Figs. 2, 3). Although the profiles are predominantly rod-shaped, some granules end in a terminal vesicle that gives the structure its unique appearance (Fig. 2). Less frequently, the granules are arched convexly with two rod-shaped terminations and a central vesicle (Fig. 3).

Such granules are found inside characteristic fetal keratinocytes. These cells maintain desmosomal attachments with adjacent keratinocytes and have prominent hemidesmosomes where they rest against the basal lamina; their cytoplasm contains numerous tonofilaments and tonofibrils (Fig. 1). Fetal keratinocytes also contain large quantities of glycogen particles and extensive Golgi zones.

In the epidermis of adult rhesus monkeys Langerhans cells with numerous typical granules do not have desmosomal attachments at their surfaces (Fig. 4). Although often seen in the basal layer, the cells rarely abut against the basal lamina from which they are usually separated by slender keratinocyte processes. The granules of adult Langerhans cells are identical with those seen in the fetal keratinocytes. Langerhans cells found in adult skin also contain numerous cytoplasmic filaments and large lipid-like droplets (Fig. 4). The keratinocytes in the basal layer of adult rhesus monkey epidermis are morphologically similar to keratinocytes in other species.

These observations show that what we now assume to be characteristic Langerhans granules occur not only in dendritic Langerhans cells but also in fetal keratinocytes. Since such granules

occur inside keratinocytes, one is faced with the question of how they got there. The granules might be transferred into keratinocytes from other cells or they could form within the keratinocytes. Another, less likely, alternative is that fetal Langerhans cells look like keratinocytes.

There is no evidence that these granules are transferred from other cells, but other particles, for example melanosomes and premelanosomes, are synthesized by melanocytes and later transferred to keratinocytes (2, 6, 11). If, on the other hand, these granules are not transferred from other cells, we must assume either that cells other than those identified as Langerhans cells can produce them or that those fetal keratinocytes that contain the granules are differentiating into Langerhans cells. The accepted description of Langerhans cells makes it clear that these cells are not attached to adjacent cells by way of desmosomes. Thus, if one accepts such a definition, any cell with Langerhans granules and no desmosomes is a Langerhans cell. The present observations, however, cast doubt on such a definition, and we must now question whether all other cells that contain Langerhans granules necessarily belong to a specific type of cells. Since cells containing particles identifiable as Langerhans granules have been reported in several different tissues, we conclude that the granules may not be peculiar to Langerhans cells.

It is questionable that the fetal keratinocytes which contain Langerhans granules are transforming into Langerhans cells. Yet, the ontogenetic appearance and germ layer origin of Langerhans cells has not yet been determined, and to date we have not seen any cell in a 75 day old fetal rhesus epidermis that can be defined as a Langerhans cell.

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REFERENCES

1. BASSET, F., C. NEZELOF, R. MALLET, and J. TURIAF. 1965. Nouvelle mise en évidence, par la microscopie électronique, de particules d'allure virale dans une seconde forme clinique de l'histiocytose X, le granulome éosinophile de l'os. *C. R. Hebd. Seances Acad Sci.* Paris. **261:** 5719.
2. BIRBECK, M. S. C., and N. A. BARNICOT. 1959. Electron microscope studies on pigment formation in human hair follicles. *In* Pigment Cell Biology. M. Gordon, editor. Academic Press Inc., New York. 549.
3. BIRBECK, M. S. C., A. S. BREATHNACH, and J. D. EVERALL. 1961. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. *J. Invest. Dermatol.* **37:**51.
4. BREATHNACH, A. S. 1964. Observations on cytoplasmic organelles in Langerhans cells of human epidermis. *J. Anat.* **98:**265.
5. BREATHNACH, A. S. 1965. The cell of Langerhans. *Int. Rev. Cytol.* **18:**1.
6. CRUIKSHANK, C. N. D., and S. A. HARCOURT. 1964. Pigment donation in vitro. *J. Invest. Dermatol.* **42:**183.
7. HACKEMANN, M., C. GRUBB, and K. R. HILL. 1968. The ultrastructure of normal squamous epithelium of the human cervix uteri. *J. Ultrastruct. Res.* **22:**443.
8. HASHIMOTO, K., and W. M. TARNOWSKI. 1967. Ultrastructural studies of racket-bodies in epidermal Langerhans cells and in histiocytosis-X. *Fed. Proc.* **26:**370 (abstr.).
9. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9:**409.
10. PALADE, G. E. 1952. A study of fixation for electron microscopy. *J. Exp. Med.* **95:**285.
11. PARAKKAL, P. F. 1967. Transfer of premelanosomes into the keratinizing cells of albino hair follicle. *J. Cell Biol.* **35:**473.
12. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17:**208.
13. SAGEBIEL, R. W., and T. H. REED. 1968. Serial reconstruction of the characteristic granule of the Langerhans cell. *J. Cell Biol.* **36:**595.
14. SCHROEDER, H. E., and J. THEILADE. 1966. Electron microscopy of normal human gingival epithelium. *J. Periodontal. Res.* **1:**95.
15. TURIAF, J., and F. BASSET. 1965. Un cas d'histiocytose "X" pulmonaire avec présence de particules de nature probablement virale dans les lésions granulomateuses pulmonaires examinées au microscope électronique. *Bull. Soc. Med. Hop.* Paris. **116:**1197.
16. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4:**475.

17. WOLFF, K. 1967. The fine structure of the Langerhans cell granule. *J. Cell Biol.* **35**:468.

18. ZELICKSON, A. S. 1965. The Langerhans cell. *J. Invest. Dermatol.* **44**:201.