

Some New Aspects of Langerhans Cells in the Human Epidermis: Light and Electron Microscopic Observations on the Swelling Sites Seen in the Process Terminals of the Dendritic Cells Described by Langerhans in 1868

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In 1868, Langerhans discovered the dendritic cells now generally called Langerhans cells (LCs) in the human epidermis using the gold impregnation method. He pointed out that the terminals of the upward running processes of the cells end with button-like swellings under the cornified layer. In 1951, Ferreira-Marques classified the shapes of the terminals into 7 types, named the largest one *Organum trompiforme* and reckoned it to be a sense receptor. However, the swellings of the process terminals seem to have been entirely forgotten along with the negation of the nerve cell theory of LCs. The author of this study recovered some of the actual forgotten terminals, the button-shaped (*knopfförmig*) swellings of the process terminals of the cells, beneath the cornified layer of a healthy volunteer epidermis using the methylene blue staining method. Furthermore, the ultrastructures of the swellings were captured for the first time using the Thin-Section-Reembedding and Ultrathin-Sectioning (TRUS) technique named by the author. Investigation of the swellings by electron microscopy showed that there were a number of Birbeck granules and vacuoles of various sizes together with elements of cytoskeletons in the cytoplasm. Possibly this shows that LCs play an essential role in the differentiation of the epidermis at the process terminals.

Key words: electron microscopy; epidermis; Langerhans cells; methylene blue

In 1868, Langerhans described and illustrated the dendritic cells in the human epidermis now generally designated as Langerhans cells (LCs), and he gave his view of the process terminals of the cells as follows: *Sie enden mit einer leichten aber deutlichen knopfförmigen Anschwellung unmittelbar unter der Grenze zwischen Rete und Hornschicht*; that is, they end with a slight but distinct button-shaped swelling directly under the border between the rete and horny layers (Langerhans, 1868). In 1951, Ferreira-Marques reported his widely pursued studies of the terminals of upward running processes of LCs (Ferreira-Marques, 1951). He depicted and classified the shapes of the terminals into 7 types

and named each one, especially the largest one, *Organum trompiforme*. This seems to be the same as the *knopfförmige Anschwellung* which Langerhans described in his original paper. Although both Langerhans and Ferreira-Marques identified the dendritic cells in the human epidermis using the gold impregnation method, the author in this study observed the same characteristic features expressed as *knopfförmig* or *trompiform* beneath the horny layer of a healthy volunteer epidermis including the hair canal with light and electron microscopes using methylene blue staining and reembedding methods.

This study could contribute to a precise understanding of the structure of the LCs and also

Abbreviations: EM, electron microscopy; LC, Langerhans cell; LM, light microscopy; TRUS, Thin-Section-Reembedding and Ultrathin-Sectioning

Table 1. Types of fixative solutions and number of materials

Fixative solution	Number of materials		
	Fixation via physiological saline	Direct fixation	Total
2.5% Glutaraldehyde + 0.05 mol/L cacodylate buffer	3 (2)		
2.5% Glutaraldehyde + 0.1 mol/L cacodylate buffer	5 (3)	5 (2)	
2.5% Glutaraldehyde + 0.05 mol/L phosphate buffer	3 (2)		
2.5% Glutaraldehyde + 0.1 mol/L phosphate buffer	3 (2)		
2.5% Glutaraldehyde + Kindaly 2	3 (2)		
Total	17 (11)	5 (2)	22 (13)

(), number of finished materials; Kindaly 2, acetate dialysate for hemodialysis (Fuso Pharmaceutical Indust., Ltd.).

could suggest the LCs' function. Special attention has been paid to the terminal swellings beneath the cornified layer of the epidermis and to those beneath the same layer of hair canals.

Materials and Methods

A skin specimen sized 5 mm × 15 mm was biopsied under local anesthesia from the lateral side of the left forearm of a healthy volunteer (64-year-old male) who gave informed consent. The biopsy specimen was immediately divided in 2, a large and a small piece. The large one was soaked in physiological saline at the dermatological biopsy-operation room at this Faculty. After 20 min, the specimen was dissected into 5 small pieces in the laboratory, then these small pieces were steeped in 5 types of fixative solution as shown in Table 1. The small specimen was soaked directly into any one of the fixatives as a control in the biopsy-operation room.

The fixative material was 2.5% glutaraldehyde in all fixative solutions, buffered with 0.05 mol/L and 0.1 mol/L cacodylate and phosphate, controlled at pH 7.4. In addition, Kindaly 2 (Fuso Pharmaceutical Indust., Ltd., Osaka, Japan), acetate dialysate for hemodialysis, was used tentatively to evaluate its effect. (The mitotic LCs in the prophase were detected only in the sections fixed in this fixative solution. The data will be published elsewhere.)

All specimens in the fixative were preserved at 4°C. After 1 h fixation, each specimen was

dissected again into smaller pieces (less than 2 mm³) (Table 1). The continual fixation for 24 h, postfixation with 1% OsO₄ for 2 h and dehydration were performed in the usual manner. Then they were embedded in Epon 812 with inverted polyethylene capsules (TAAB Lab. Equip. Ltd., Reading, Berks., United Kingdom) on the slide glass. Polymerization of the epoxy resin was carried out at 37°C, 45°C and 60°C, respectively for 24 h. A polymerized Epon containing skin specimen in a capsule can be easily detached on an 80°C hotplate by hand. The above procedures are Step 1 in Fig. 1.

Step 2 is as follows: The Epon block taken out of the capsule was trimmed largely along the outline of the specimen and was cut into 1.5–2.0 µm slices with glass knives on a Sorvall ultramicrotome MT-1. All sections in this study were cut perpendicular to the surface. Four serial sections each were taken on a slide and dried on the hotplate at 80°C.

In Step 3, after the sections on the slide were stained with a 1% methylene blue alkaline solution of 50% alcohol, the most important sections for electron microscopy (EM) were selected by light microscope and these were noted by marking on the underside of the slide.

In Step 4, the marked sections were re-embedded in epoxy resin with inverted polyethylene capsules on the slide and were polymerized at 45°C and then at 60°C, each for 24 h. The polymerized epoxy resin containing the section for EM in the polyethylene capsules was easily removable by hand on the 80°C hotplate. The sections for EM were affixed to the

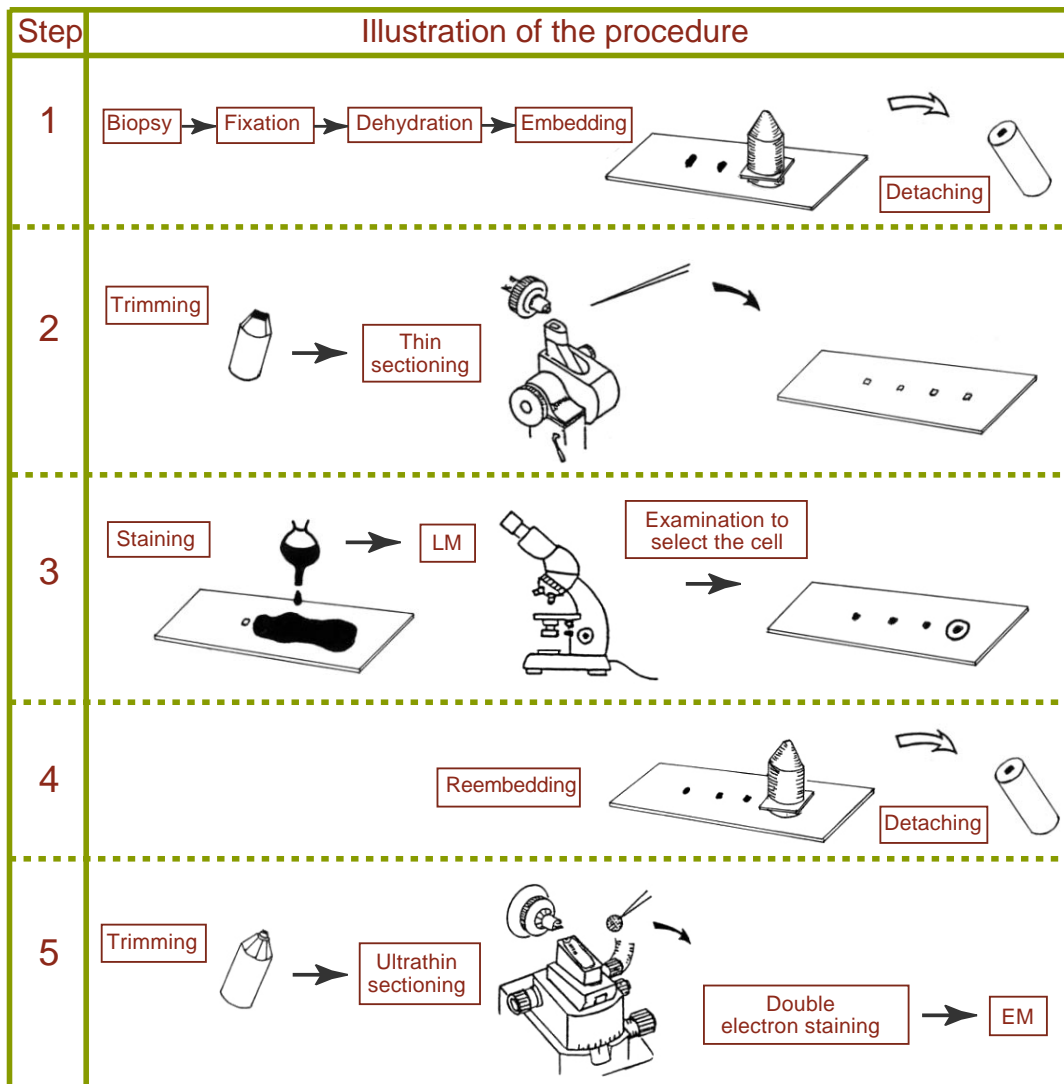


Fig. 1. The procedure of the Thin-Section-Reembedding and Ultrathin-Sectioning (TRUS) technique. **Step 1:** From biopsy to specimen embedding in Epon 812. **Step 2:** Thin sectioning (1.5–2.0 μm thick). **Step 3:** Staining with methylene blue and light microscopic examination followed by selection of the objects. **Step 4:** Reembedding, the same as Step 1. **Step 5:** Ultrathin-sectioning (60–70 nm thick) and electron microscopic examination. EM, electron microscopy; LM, light microscopy.

top of each epoxy resin block. Thereafter, it was necessary to check the sections by using a light microscope for their preservation in handling until this step.

In Step 5, the final step, the well-prepared Epon blocks were trimmed for EM under the dissecting microscope. The trimmed Epon blocks were cut as thick as 60–70 nm with a

diamond knife on the Sorvall MT-7000 ultramicrotome. Then, it was necessary that the surface of the block moves in a strictly tangential direction to the knife edge. The ultrathin sections were placed serially on the copper grid, stained with uranyl acetate and lead citrate, and observed with a Hitachi electron microscope H-800 or H-500, at 75 or 80 kV, respectively.

Results

A total of 11,340 thin sections from 13 of 22 specimen-blocks were observed with a light microscope (Table 1). The observations were carried out with 7 points of view in the epidermis: i) the cells coming into the basal layer; ii) the division of LCs; iii) the apposition of the cells hav-

ing darkstained nuclei and pale stained ones; iv) LCs with processes approaching the granular or the cornified layer; v) LCs in the granular or the subgranular layer; vi) melanocytes, LCs or Merkel cells in the basal layer and nerves coming from the dermis and vii) the others.

Of these points, particularly the 4th point, a feature of the swelling of the process terminal of the clear cell was observed for the first time beneath the cornified layer of the epidermis

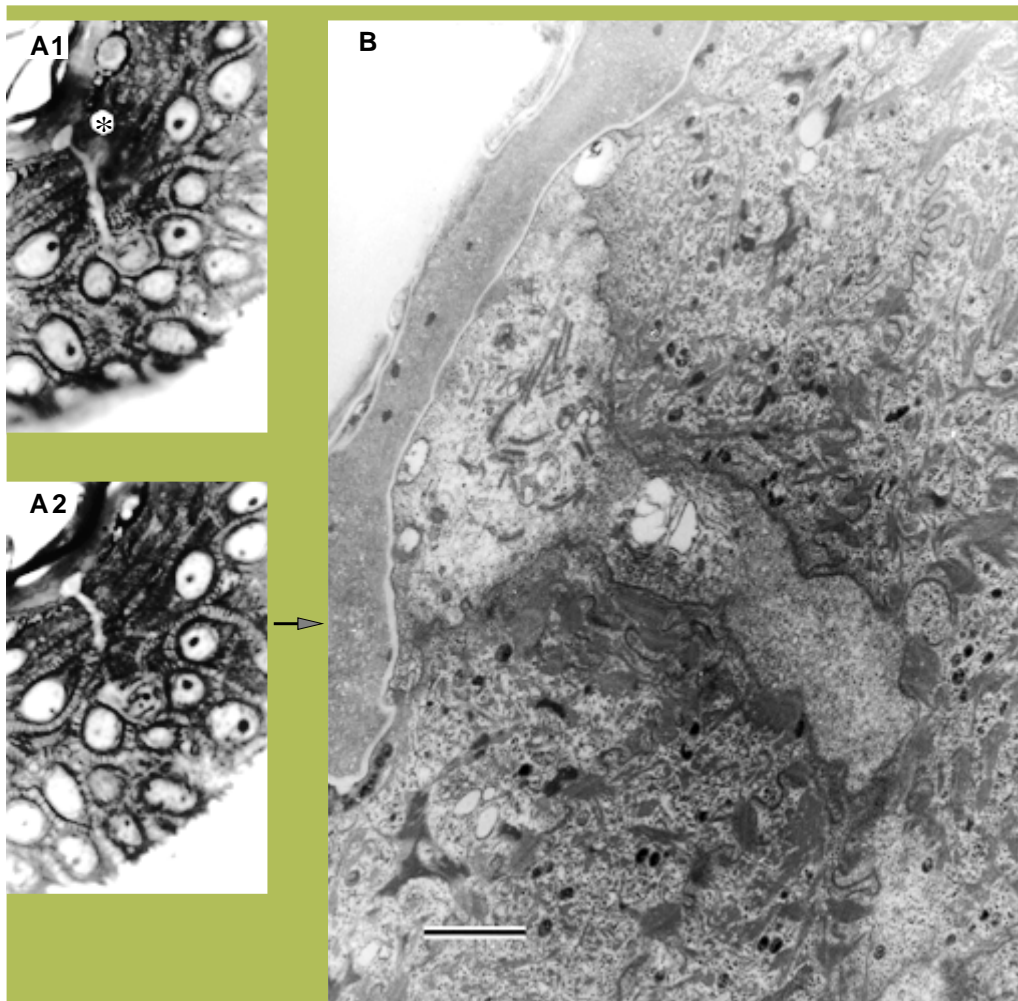


Fig. 2. Illustrations of a Langerhans cell (LC) with a process. The terminal swelling (button) makes contact directly beneath the cornified layer of the epidermis. **A1 and 2:** Views of serial thin sections of the cell. The continuity of the process to the cell body can be confirmed from these 2 pictures. $\times 750$ (oil). *, Undulated part of the section, inadequate to ultrathin sectioning. **B:** The ultra-structure of the process terminal swelling (button) taken from a thin section of the area seen in A2. In the button, a number of Birbeck granules and a few vacuoles of various sizes are seen. The outline of the button is rugged, probably due to the exocytosis of those organelles, while the process of the cell has few organelles. $\times 13,125$ (bar = 1 μm).

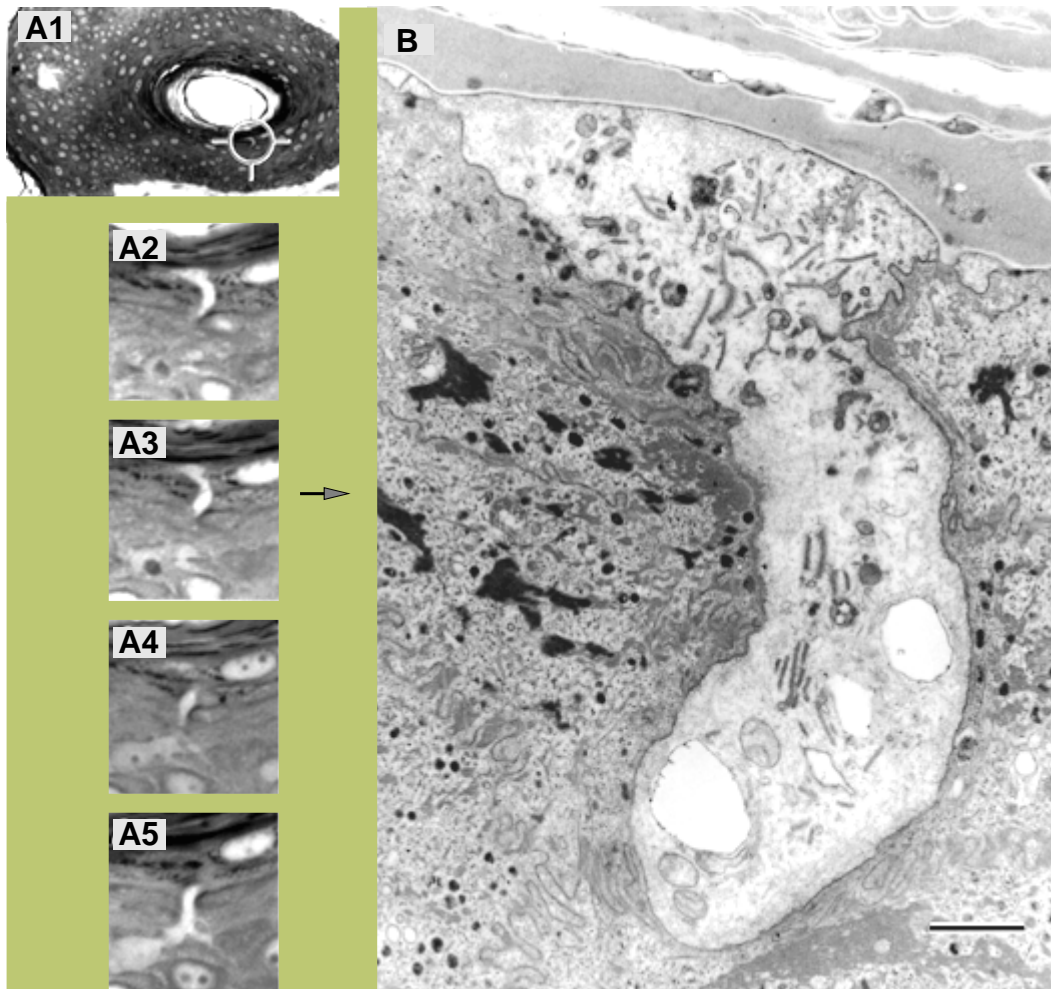


Fig. 3. A process terminal swelling (button) of a Langerhans cell (LC) at the site of the wayout of a hair canal. The button also makes contact directly beneath the cornified layer of the canal. **A1–5:** Serial sections of the above mentioned site. **A1**, $\times 150$; **A2–5**, $\times 750$. A picnotic dendritic clear cell is seen in photographs A2 through A4, but the connection between the process and the cell body is not confirmed. **B:** An electron micrograph of the process terminal from the area seen in section A3. The outline of the terminal swelling (button) is also rugged, and the internal cytoplasm clearly contains plenty of Birbeck granules of various shapes. Some large vacuoles and Birbeck granules are seen in the process near the button. $\times 12,075$ (bar = 1 μm).

(Fig. 2). After this was detected, the same features were found in succession.

The characteristics of light microscopic features

Stainability

By 1% methylene blue staining, the LCs including their processes and terminal swellings (but-

tons) were found clearly in contrast with the adjacent densely stained keratinocytes.

Morphology

In the perpendicular sections, the shape of the button seems to be the side view of a trumpet facing the opening edge to the horny layer. The size of the buttons varied (Figs. 2–5).

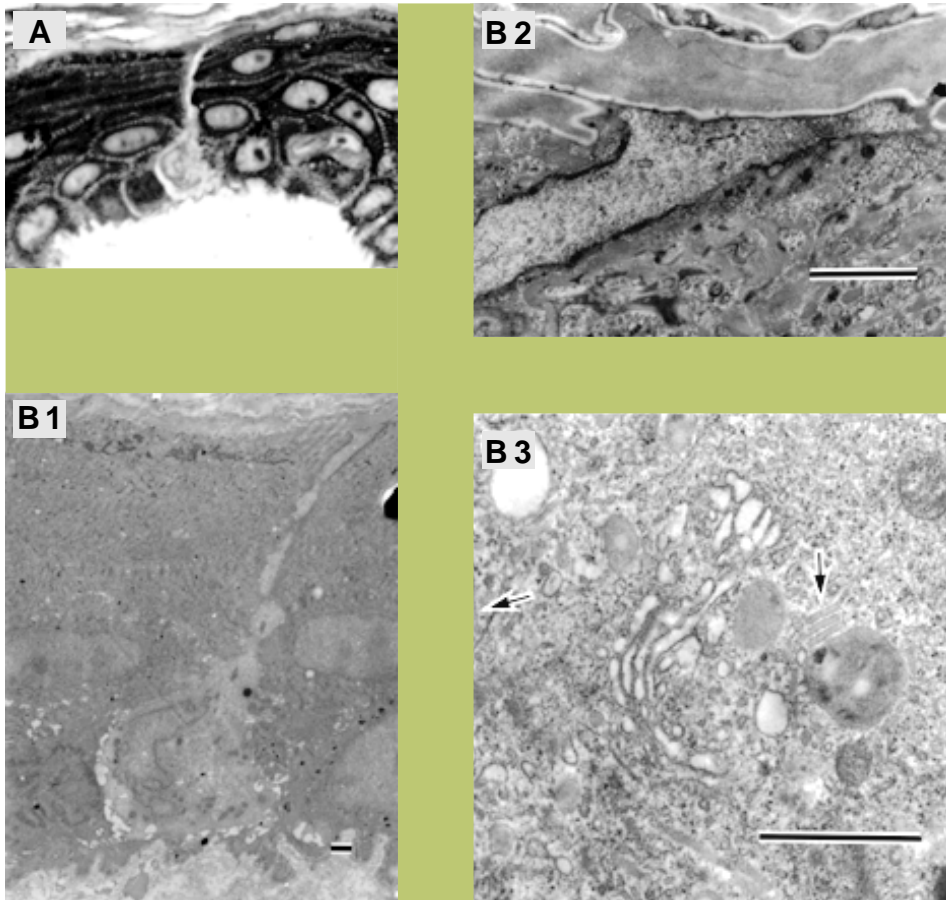


Fig. 4. A Langerhans cell (LC) with a long process approaching to the cornified layer of the epidermis. **A:** The body and the process are clearly recognized between the well-stained keratinocytes. The cell body is in the basal layer. $\times 750$ (oil). **B1:** An electron microscopic view of the whole cell. $\times 2,700$. **B2:** The terminal of the process contacts firmly with the horny layer, but the swelling is not clear in this micrograph. $\times 13,500$. **B3:** The Golgi region of the cell. Birbeck granules are found (arrows). $\times 21,000$ (bar = 1 μm).

The location of the LCs with the button

Most LCs with buttons were in the spinous layer of the epidermis, but rarely in the basal layer extending the process to the cornified layer and thereby forming the button (Fig. 4).

The connection between the button and the cell body of the LC

In most cases, the connection of the button with the LC body was affirmed by serial sectioning, but a few could not be confirmed by any means (Fig. 5).

The characteristics of electron microscopic features

Electron density

The electron density of the LCs including buttons corresponds well to the “clear” image of light microscopy (LM), but sometimes the cytoplasm of the keratinocytes adjoining the LC processes in the granular layer appear to become dense (Figs. 2 and 3). In some cases, the cytoplasm of the button and its process appear dense, and therefore contrary to the LM image, in the electron micrograph (Figs. 5B1 and B2).

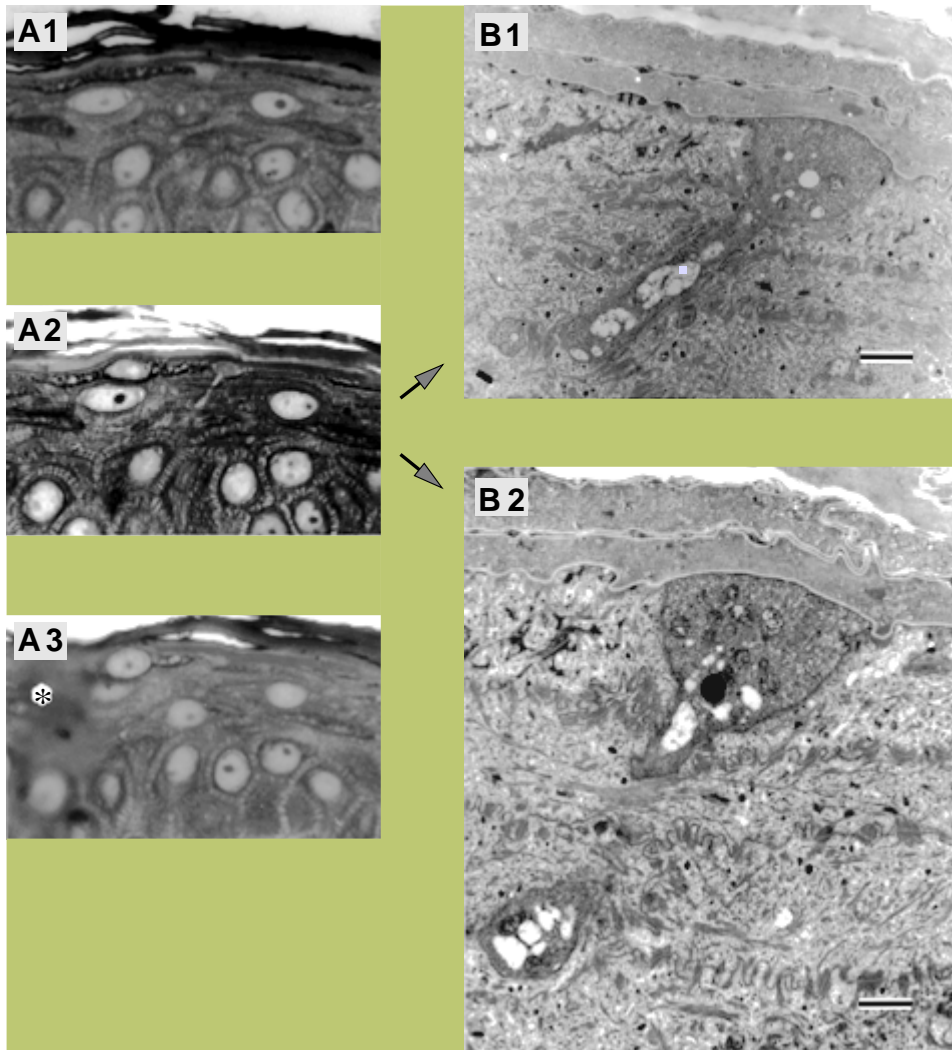


Fig. 5. The process terminal swellings in a round fashion and contacts with the horny layer, but the connection between the process and the cell body can not be confirmed. **A1–3:** Serial thin section. $\times 750$ (A2 oil). A2 shows the process and terminal button, but the connection between the process and the cell body can not be found. **B1 and 2:** In the button, some mitochondria and vacuoles are seen, but Birbeck granules are not observed. The outlines of these buttons are not rugged. $\times 6,750$ (bar = 1 μm).

Morphology

The whole appearance of the button corresponds well to the image of LM. However, it is necessary to make a careful observation, because the features at the start and the end of ultrathin-sectioning differed somewhat from each other (Figs. 5B1 and B2).

Birbeck granules and vacuoles

On one occasion, plenty of Birbeck granules (Birbeck et al., 1961) and many vacuoles of various sizes were observed in the process terminal (button) and, on another occasion, no Birbeck granules were observed at all. (Figs. 2 and 3 versus Figs. 4 and 5).

Discussion

It is very important to start with light microscopic observation for the study of skin specimens where the view of every section changes panoramically. For a detailed examination of a specific cell or tissue in the skin, the section containing the object should first be selected by LM, then the proper object for the electron microscopic study can be carefully investigated on the ultrathin sections taken from the section viewed by LM. The author accomplished the purpose by the Thin-Section-Reembedding and Ultrathin-Sectioning (TRUS) technique (Fig. 1). This TRUS technique is simple, and moreover, trustworthy for anyone. It was used throughout the author's studies. The rate of error was less than 1%. This technique was instructed to the author from Researcher N. Shindo (Shindo et al., 1984) at the Clinical Research Institute, National Medical Center, Tokyo, Japan, and the author drew a picture of the detailed manner of this procedure in Fig. 1. The technique was called the TRUS technique by the author for brevity. In this study, because the "No Cover Glass (NCG)" object lens could not be employed in the light microscopic examination, the micrographs, except for the "oil immersion" views, were taken from the sections covered with a dry coverglass on the slide. The oil immersion micrographs were taken from the sections affixed on the surfaces of the reembedded Epon blocks. Then, a drop of water was put between the section (Epon block surface) and the coverglass in order that the section surface would not get oil on it. The hardness of the Epon used in this study was relatively hard. The ratio of mixture A to mixture B was 3 to 7, according to Luft's method (1961). This Epon hardness seems to be the key to success.

As was stated above, in some cases a great many Birbeck granules were found in the buttons of LCs, but in the other cases were not found at all. These different results could be due to the different stage of interactions between LCs and the adjacent keratinocytes; one would be in a progressive stage of exocytosis of

Birbeck granules, while another may be in the end state of this function. Nevertheless, the exact role of the process terminals (button) and the nature of Birbeck granules remains for further study. The observations of the process terminals (having buttons) of intraepidermal LCs were achieved first by Langerhans, and succeeded by Ferreira-Marques' elaborate examination. Both Langerhans and Ferreira-Marques identified the cells by using the gold impregnation method in the human epidermis. The present author used the methylene blue staining method and identified the cells easily and precisely according to numerous studies by other workers (Breathnach, 1965; Wolff, 1972): dendritic cell shapes, a clear cytoplasm (tonofilaments free), no desmosome, indented nuclei, etc. The methylene blue staining method has the advantage of detailed description of the sections on the slide. To the large swelling of the process terminals Ferreira-Margues gave the name *trompiform*; the author thinks that the word *knopfförmig* (that is "button-shaped") by Langerhans and the word *trompiform* (that is "trumpet-shaped") by Ferreira-Marques are identical entities, and possibly produced from the exocytosis of Birbeck granules. Since Ferreira-Margues' time, the swellings of the process terminals of dendritic cells in the epidermis have been observed with the ATPase staining method of Bradshaw and colleagues (1963) in the human epidermis and were expressed as "buttons" or "caps", but their significance was not discussed at all.

This study describes the first time that electron microscopic examination of Langerhans' "button" has ever been done. Langerhans, who investigated this button in 1868 with a light microscope of the 19th century and recorded his findings as an important matter, was a man of insight. Ferreira-Marques who took notice of these process terminals was also a man of great ability. According to the formation of "buttons" of the process terminals of the intraepidermal LCs and their ultrastructure in the author's findings, it may be assumed that LCs play an essential role in the differentiation of the human epidermis at the process terminals.

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