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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, controled 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 mm^3) (Table 1). The continual fixation for 24 h, postfixation with 1% OsO_4 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 reembedded 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

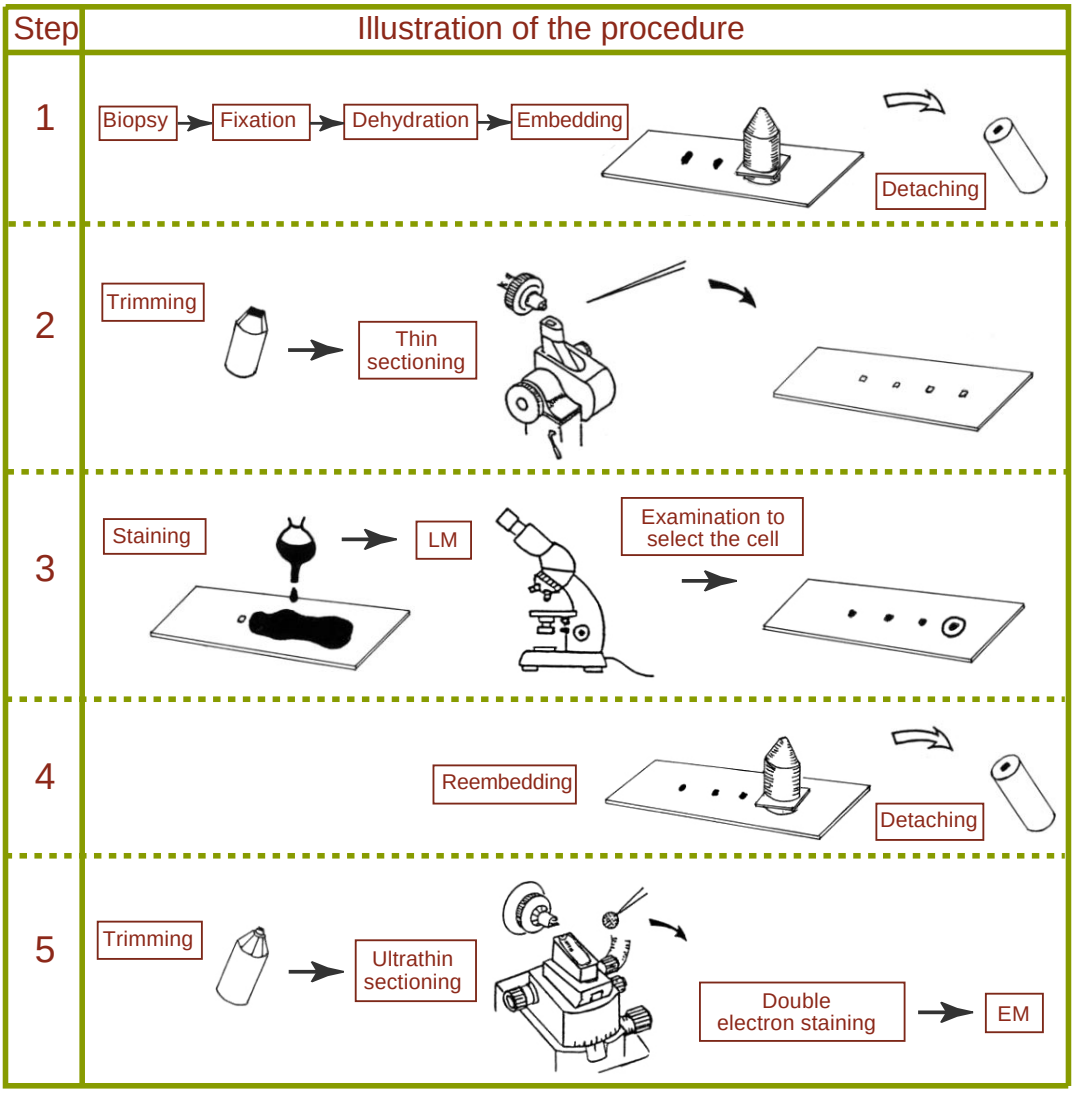

Fig. 1. The procedure of the Thin-Section-Reembedding and Ultrathin-Sectioning (TRUS) technique. **Step 1:** From biopsy to specimen embedding in Epon 812. **Step 2:** Thin sectioning (1.5–2.0 µm thick). **Step 3:** Staining with methylene blue and light microscopic examination followed by selection 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.