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Mitotic Langerhans Cells in the Normal Human Epidermis: Light and Electron Microscopic Observations in All Mitotic Phases

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In three experiments of the normal human epidermis under different biopsy and fixative buffer conditions, Langerhans cells (LCs) in all mitotic phases were found with the Thin-Section-Reembedding and Ultrathin-Sectioning (TRUS) technique; two in the prophase, five in the metaphase, two in the anaphase and two in the telophase. The serial light microscopic examination showed many characteristic features, namely the shapes of mitotic LCs in each section were quite different in one cell as well as between individual cells, even in the same mitotic phase. No round mitotic LCs were seen. Two LCs in the prophase were detected in the specimens prefixed in a fixative dissolved in dialysate (Kindaly 2) instead of buffer solution. Birbeck granules were witnessed in all phases.

Key words: electron microscopy; epidermis; human; Langerhans cells; mitosis

It has been considered difficult to observe mitotic Langerhans cells (LCs) in the normal human epidermis, and so far only one electron microscopic observation of the mitotic LC (in the anaphase) has been reported by Konrad and Hönigsmann (1973). In the present study, comprising three experiments under different biopsy and fixative buffer conditions, 11 LCs in various mitotic phases were observed with the Thin-Section-Reembedding and Ultrathin-Sectioning (TRUS) technique (Oota, 1999). In all phases, the mitotic LCs showed markedly characteristic features. These features of all phases in the human epidermis were obtained for the first time by light and electron microscopy.

Materials and Methods

Skin biopsies (5 mm × 15 mm) were taken 3 times, the 2nd one year after the 1st and the 3rd two months after the 2nd, from the forearm of a 63-year-old healthy male volunteer, who had given informed consent. The 1st and the 2nd biopsy specimens were obtained under lidocaine anesthesia without epinephrine, and the 3rd biopsy specimen was excised under anesthesia with epinephrine. The biopsy specimens were treated in the same manner every time: immersed in physiological saline immediately after operating at the dermatological biopsy-operating room in this Faculty. After 20 min, the specimen was divided into 3 to 5 equal parts and prefixed in the fixative solutions shown in Table 1. Here Kindaly 2 (Fuso Pharmaceutical Industry, Ltd., Osaka, Japan), acetic dialysate solution for hemodialysis, was tried as a physiological vehicle instead of the buffer. The components and osmolarity of the chemical fixatives in Experiments 1 and 2 are shown in Table 2. After 1 h, each specimen was dissected into small pieces and the fixation was continued for 24 h at 4˚C. Subsequent procedures were previously described as the TRUS technique (Oota, 1999). Although in the technique the so-called "inverted gelatin capsule technique" (Robbins and Gonatas, 1964; Miyauchi and Hashimoto, 1987) was used twice, the polyethylene capsules (TAAB Lab. Equip. Ltd., Berks., United Kingdom)

Abbreviations: CB, cacodylate buffer; LC, Langerhans cell; M, mol/L; PB, phosphate buffer; TRUS, Thin-Section-Reembedding and Ultrathin-Sectioning

Table 1. Primary fixative solutions and the number of sectioned blocks and of mitotic LCs found in the materials prefixed with the fixatives

	Primary fixative solutions	No. of sectioned blocks	No. of mitotic LCs	Observed mitotic phase
Experiment 1	2.5% GA in 0.05 M CB	3	0	
	2.5% GA in 0.1 M CB	4	4	Met, Met, Tel, Tel
	2.5% GA in 0.15 M CB	4	2	Met, Ana
Experiment 2	2.5% GA in 0.05 M PB	1	0	
	2.5% GA in 0.1 M PB	3	0	
	2.5% GA in 0.15 M PB	2	0	
	2.5% GA in Kindaly 2	4	1	Pro
Experiment 3	2.5% GA in 0.05 M CB	2	1	Ana
	2.5% GA in 0.1 M CB	2	0	
	2.5% GA in 0.05 M PB	2	0	
	2.5% GA in 0.1 M PB	2	1	Met
	2.5% GA in Kindaly 2	2	2	Pro, Met

Ana, anaphase; CB, cacodylate buffer; GA, glutaraldehyde; LC, Langerhans cell; M, mol/L; Met, metaphase; PB, phosphate buffer; Pro, prophase; Tel, telophase.

were employed instead of gelatin capsules. All thin-sections in the 3 experiments were cut parallel to the surface of the epidermis. The oil immersion pictures were taken from the sections on slides; a drop of water was poured between the section and a cover glass for use of the section for electron microscopy. The ultrathin sections were placed on a copper grid, stained with uranyl acetate and lead citrate, and observed with a Hitachi electron microscope, H 500, at 80 kV.

Results

In the 1st experiment, 6 mitotic LCs (Fig. 1: Rows 3, 4, 5, 8, 10 and 11) were found in the materials prefixed with the fixatives in 0.1 M and 0.15 M cacodylate buffer (CB) (Table 1). In the 2nd experiment, only 1 LC in the prophase (Fig. 1: Row 1) was observed in the materials prefixed in Kindaly 2, which is commonly used for artificial kidneys, and none in the materials prefixed with the fixatives in phosphate buffer (PB) (Table 1). Why no mitotic LCs were observed in these materials is ob-

Table 2. Components and osmolarity of chemical fixatives

	Fixative solution	Osmolarity of buffer (vehicle) (mOsm)	Total osmolarity (mOsm)
Experiment 1	2.5% GA in 0.05 M CB	103	345
	2.5% GA in 0.1 M CB	202	430
	2.5% GA in 0.15 M CB	285	510
Experiment 2	2.5% GA in 0.05 M PB	94	373
	2.5% GA in 0.1 M PB	203	470
	2.5% GA in 0.15 M PB	296	566
	2.5% GA in Kindaly 2	255	484

CB, cacodylate buffer; GA, glutaraldehyde; LC, Langerhans cell; M, mol/L; PB, phosphate buffer.

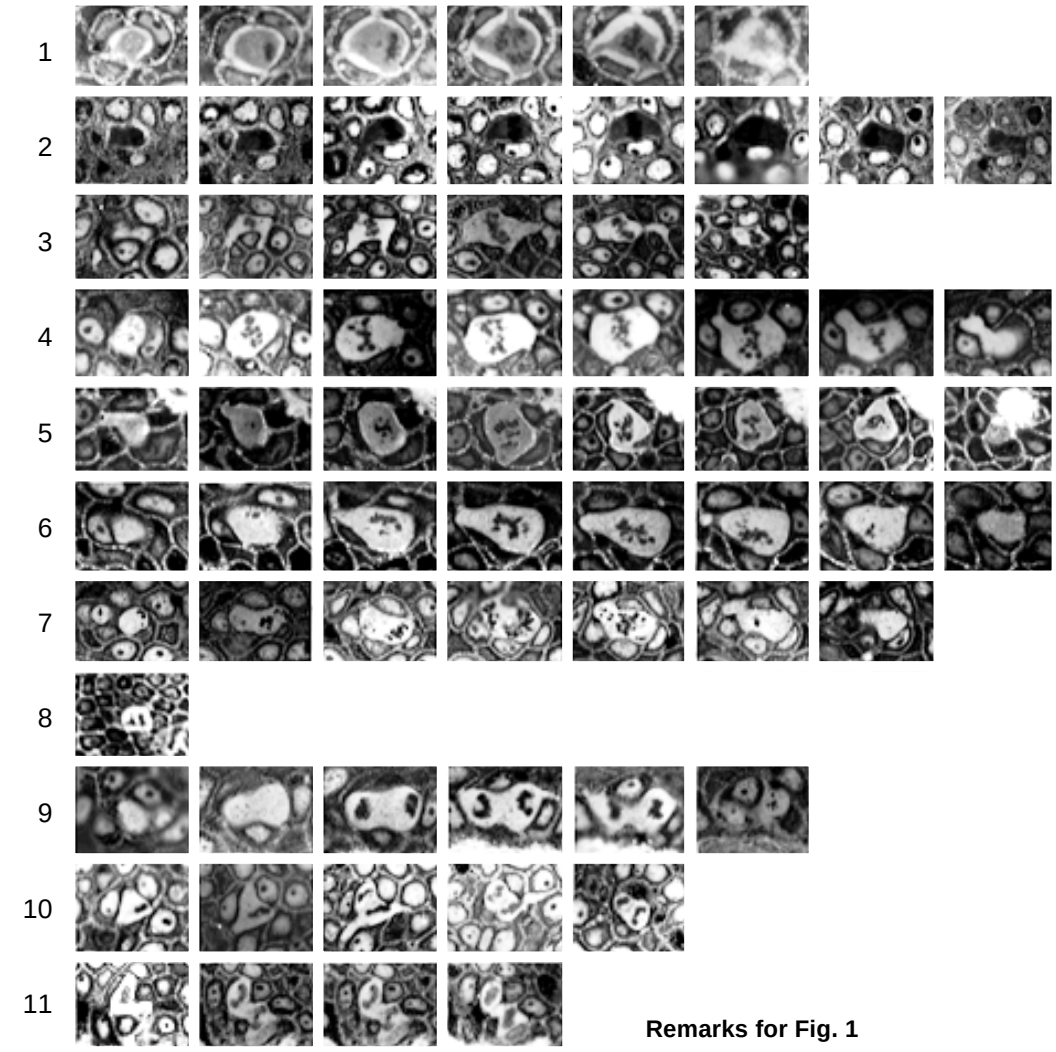

Fig. 1. Light microscopic pictures of mitotic Langerhans cells (LCs). Rows 1 and 2, in the prophase; Rows 3 to 7, in the metaphase; Rows 8 and 9, in the anaphase; and Rows 10 and 11, in the telophase. All pictures, except Row 8, were photographed by oil immersion, × 510. Row 8 is the first mitotic LC encountered by chance, × 255. CB, cacodylate buffer; PB, phosphate buffer.

Remarks for Fig. 1

Row No.	Mitotic phase	Buffer*	Experiment No.
1	Prophase	Kindaly 2	2nd experiment
2	Prophase	Kindaly 2	3rd experiment
3	Metaphase	0.1 M CB	1st experiment
4	Metaphase	0.1 M CB	1st experiment
5	Metaphase	0.15 M CB	1st experiment
6	Metaphase	0.1 M PB	3rd experiment
7	Metaphase	Kindaly 2	3rd experiment
8	Anaphase	0.15 M CB	1st experiment
9	Anaphase	0.05 M CB	3rd experiment
10	Telophase	0.1 M CB	1st experiment
11	Telophase	0.1 M CB	1st experiment

*Buffer or vehicle used in fixative solution.