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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.

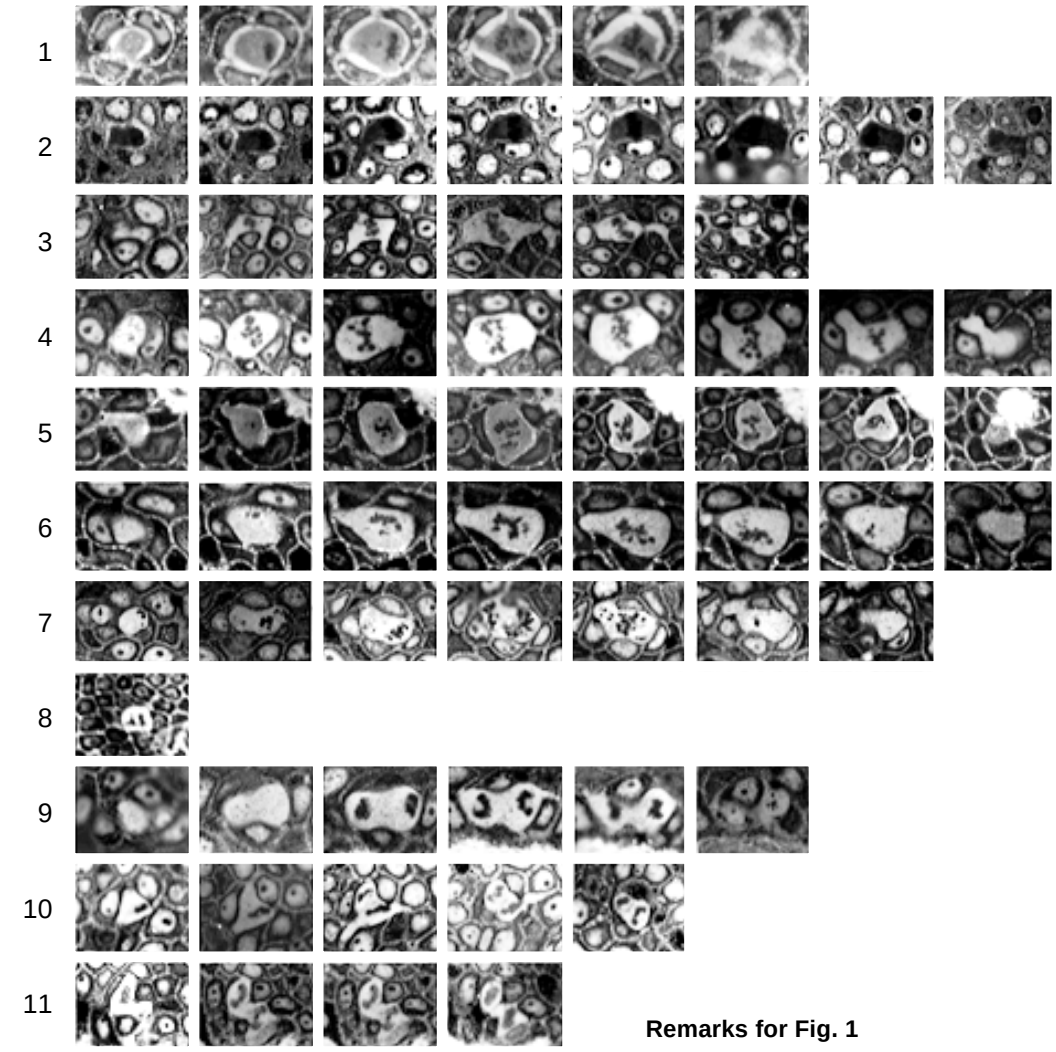

Fig. 1. Light microscopic pictures of mitotic Langerhans cells (LCs). Rows 1 and 2, in the prophase; Rows 3 to 7, in the metaphase; Rows 8 and 9, in the anaphase; and Rows 10 and 11, in the telophase. All pictures, except Row 8, were photographed by oil immersion, × 510. Row 8 is the first mitotic LC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

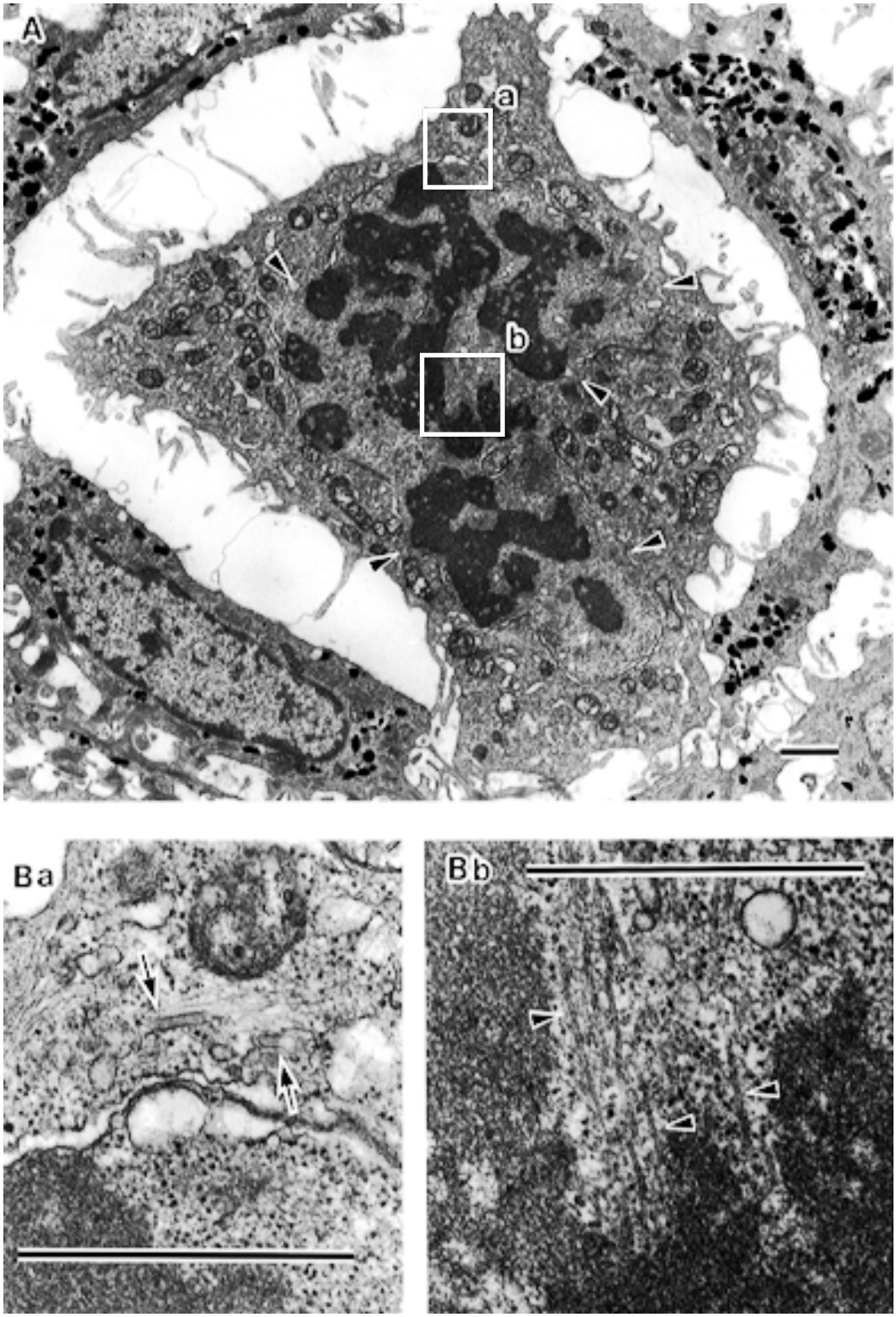

Fig. 2. Legends on p. 205 (bottom).

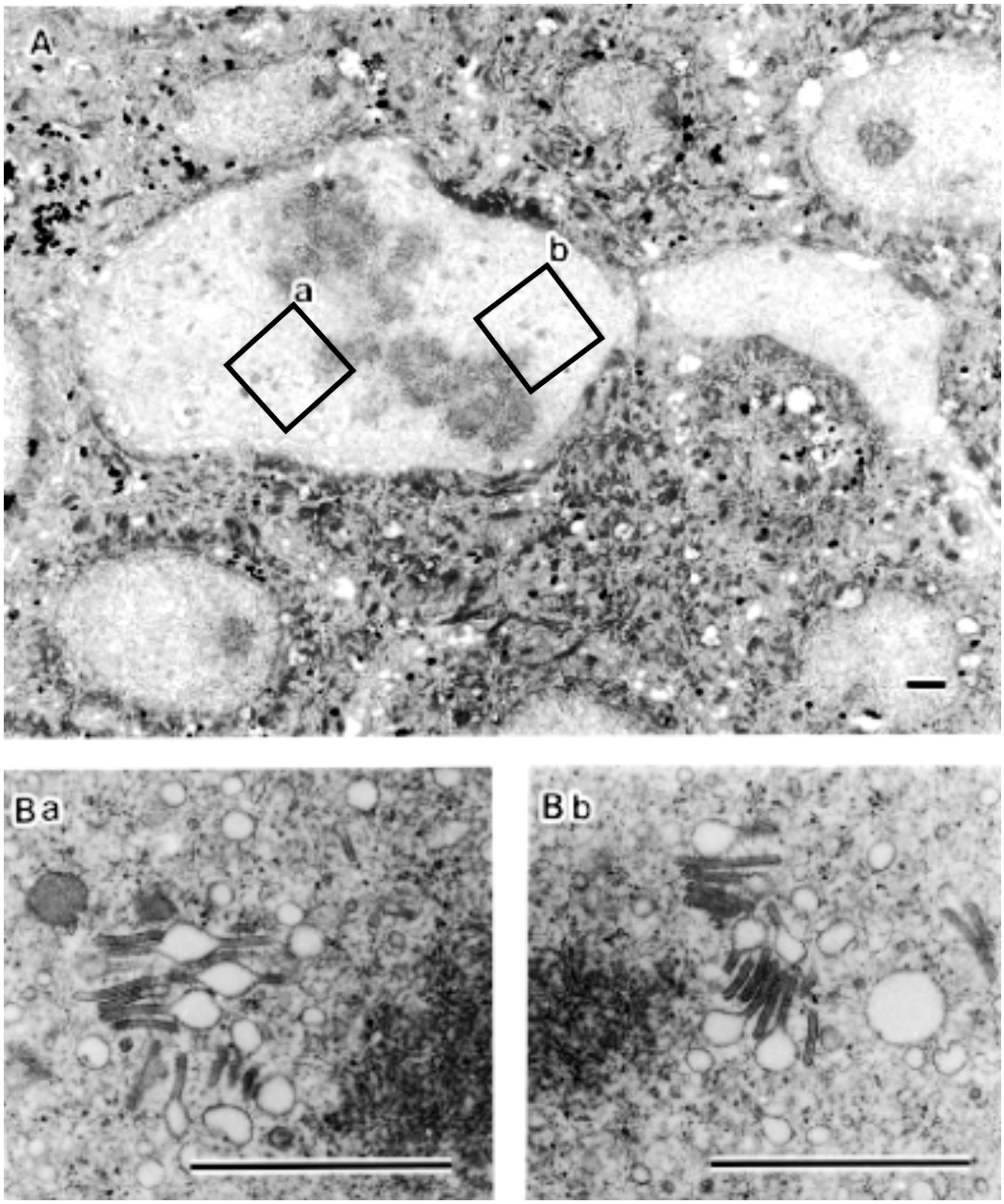

Fig. 3. A: A Langerhans cell (LC) in the metaphase (Fig. 1: Row 3-No. 5), × 5,400. **Ba and b:** High magnification photographs of the rectangular areas in **A**. Parallel-arranged LC granules (Birbeck granules) and many vesicular structures of various sizes are visible at both polar regions, × 36,000. Scale bar = 1 μm.

Fig. 2 (p. 204) A: An electron micrograph of a Langerhans cell (LC) in the prophase (Fig. 1: Row 1-No. 4), × 9,000. In places, the nuclear envelope disappears (arrowheads) and, spindle microtubules pass towards chromosomes which have begun to condense (**Bb:** arrowheads), × 52,500. Birbeck granules are seen (**Ba:** arrows), × 52,500. Scale bar = 1 μm.

scure. So, in the 3rd experiment, the addition of epinephrine as a local anesthetic during the skin biopsy was tried, and CB, PB and Kindaly 2 were again used for fixative vehicle. At this time, 4 mitotic LCs (Fig. 1: Rows 2, 6, 7 and 9) were found in the materials prefixed with the fixatives in 0.05 M CB, 0.1 M PB and Kindaly 2 (Table 1).

In these experiments, features observed with a light microscope showed several characteristics (Fig. 1): i) Row 8 was the 1st mitotic LC encountered by chance during the test for the effect of buffer osmolarity to the cell structures, and so the serial sections and high magnification views were not taken; ii) the cytoplasm of mitotic LCs, after the metaphase to the telophase, was clearer than that of epidermal mature LCs and highly contrasted with the neighboring keratinocytes and the dark-stained chromosomes, whereas the cytoplasm of LCs in the prophase was stained at the same level as neighboring cells, or more intensely, and so the contrast with the condensing chromatin was low (Rows 1 and 2); iii) the serial sections indicated that each mitotic LC possessed several, short or long, slender or podgy, cytoplasmic processes; iv) so in the same way, 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. From the serial sections of each row, the three-dimensional features possessing process(es) could be imaged; and v) no round mitotic LCs were seen.

Electron microscopy showed many characteristics as follows.

Figure 2 is an electron micrograph of one of the LCs in the prophase observed in the material prefixed with the fixative in Kindaly 2 solution. The intercellular space between this cell and the neighboring keratinocytes has been markedly widened possibly due to a fixative artifact. This might have happened based on peculiar situations involving neighboring keratinocytes. However, the wide bases of the processes of this LC still remain (Fig. 2A). In places, the nuclear envelope disappeared (Fig. 2A), and spindle microtubules passed towards chromosomes which had begun to condense (Fig. 2Bb). LC granules (Birbeck granules) were seen (Fig. 2Ba).

Figure 3 is one of the LCs in the metaphase observed in the material prefixed with the fixative in 0.1 M CB. In the row of this cell, every section showed considerably different features. The cell of Fig. 3A showed a relatively long and podgy cytoplasmic process, and the cell seemed to be just like a tadpole. Chromosomes were aligned on the metaphase plate. No centriole was caught in the plane of this section, but at the sites regarded as the neighborhood of the poles, parallel-arranged Birbeck granules and many vesicular structures of various sizes were visible (Figs. 3Ba and b).

Figure 4 is one of the LCs in the anaphase. This picture was observed in the material prefixed with the fixative in 0.05 M CB. This cell possesses large (P) and small (p) cytoplasmic processes (Fig. 4A). In Figs. 4Ba and b, newly opposed segments of the nuclear envelope appeared on the partially fused chromatin mass. Spindle microtubules and the chromatin mass formed junctions at points still devoid of the nuclear envelope. Birbeck granules were sparsely seen.

Figure 5 is one of the 2 telophase LCs. This picture was obtained from the material prefixed with the fixative in 0.1 M CB. Reproduced daughter LCs seemed to be separated by the invasion of the neighboring keratinocyte between the daughter cells (Fig. 5A, double arrows). Although the nuclei of both daughter LCs showed still young forms (Fig. 5A), their function seemed to have become markedly active (Figs. 5Ba and b).

Through these observations, in all mitotic phases of LCs, Birbeck granules were witnessed in the cytoplasm.

Fig. 4 (p. 207) A: A Langerhans cell (LC) in the anaphase (Fig. 1: Row 9-No. 4), × 6,000. **Ba and b:** High magnification photographs of the rectangular areas in **A**, × 30,000. Newly opposed segments of the nuclear envelope have appeared on the partially fused chromatin mass. Spindle microtubules (**Ba:** arrowheads) and chromatin mass form junctions at points still devoid of nuclear envelope. In this section, orthogonal arranged centrioles are seen at one side of poles (**Bb:** C). Birbeck granules are sparsely seen in the cytoplasm (**Ba and b:** arrows). Scale bar = 1 μm.

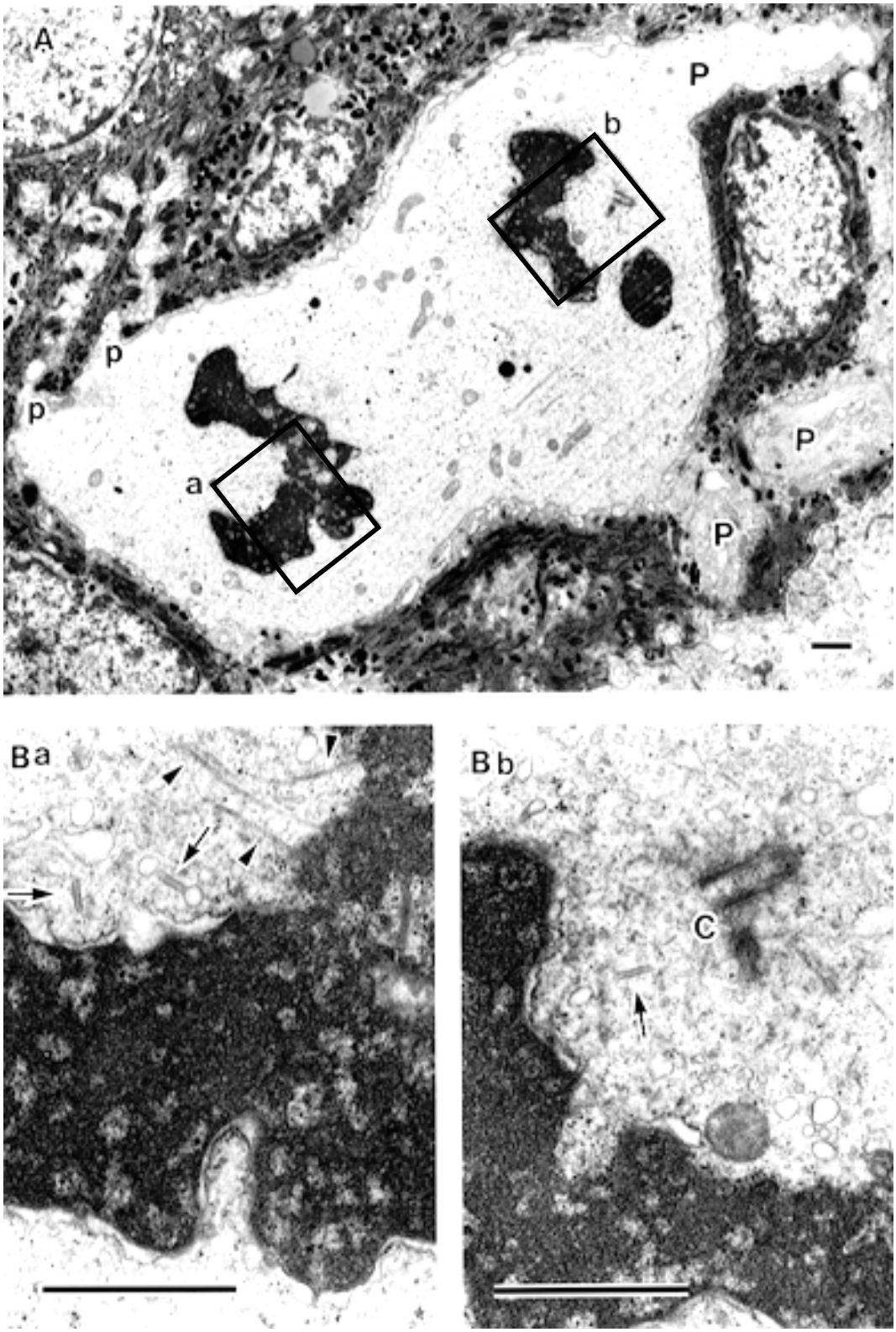

Fig. 4. **Legends on p. 206 (bottom).**

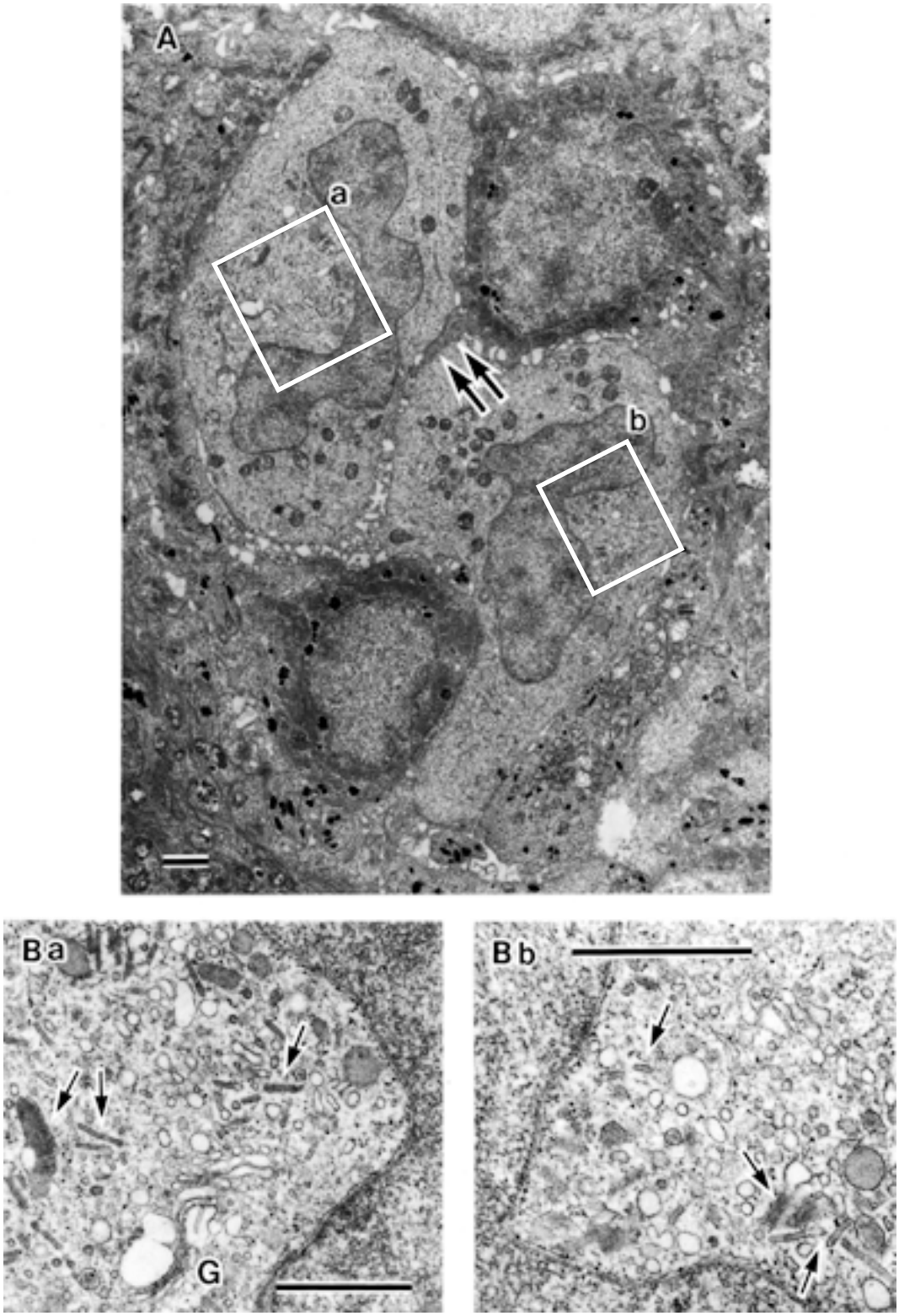

Fig. 5. Legends on p. 209 (bottom).

Discussion

The features of these mitotic LCs in the normal human epidermis are the first ones observed both by light and electron microscopy. They seem to show actual evidence for the reproduction of LCs in the normal human epidermis. In 1979, 2 groups of investigators (Frelinger et al., 1979; Katz et al., 1979) using bone marrow chimeric animals showed that LCs in the epidermis originated from bone marrow, and this concept has since been widely accepted. Thereafter, however, Czernieleski et al. (1985) using flow cytometry showed that human epidermal LCs were a cycling cell population in the normal (physiological) epidermis, and cited the study by Konrad and Hönigsmann (1973) as morphological evidence. The present observations confirm their view. Czernielewski and Demarchez (1987) further studied the self-reproducing capacity of LCs in the human skin grafted onto the nude mouse. Thereafter, Miyauchi and Hashimoto (1989) using the ATPase staining technique presented the mitotic activities of epidermal LCs in the normal mouse skin. Compared with these studies, the present study shows the actual features of mitotic LCs in close proximity to the natural state of the human epidermis. In this study, the relation between the mitosis of LCs and the kind or osmolarity of the fixative buffer (vehicle) could not be clarified, and the relation between the mitosis of LCs and epinephrine in the local anesthetic is also indistinct. However, it is very interesting that the mitotic LCs in the prophase were detected only in the materials which had been prefixed in the fixative dissolved in Kindaly 2 . Why so? This problem remains for further study.

As a whole, it must be one of the most important observations that Bibeck granules have been witnessed in all mitotic phases of human LCs.

By the TRUS technique, it was possible for the first time to carry out a wide survey of the human epidermis, to find a large amount of mitotic LCs at the light microscopic level and to view fine details of the same cells at the electron microscopic level. Thus, anyone hereafter can observe mitotic LCs both in the normal and pathological human epidermis, and consequently, the nature, kinetics and function of LCs can be more clearly understood.

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Fig. 5 (p. 208) A: A Langerhans cell (LC) in the telophase (Fig. 1: Row 11-No. 2), × 7,000. Double arrows indicate the process of the neighboring keratinocyte invading between the daughter LCs. **Ba and b:** High magnification photographs of the rectangular areas in **A**, × 21,000 and × 28,000, respectively. In the Golgi area, a lot of vesicles and Birbeck granules are observed (G, Golgi apparatus; arrows, Birbeck granules). These show clearly the reopening of the cell function as soon as cell division is completed. Scale bar =1 μm.

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