

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),  $\times 6,000$ . **Ba and b:** High magnification photographs of the rectangular areas in **A**,  $\times 30,000$ . 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  $\mu\text{m}$ .

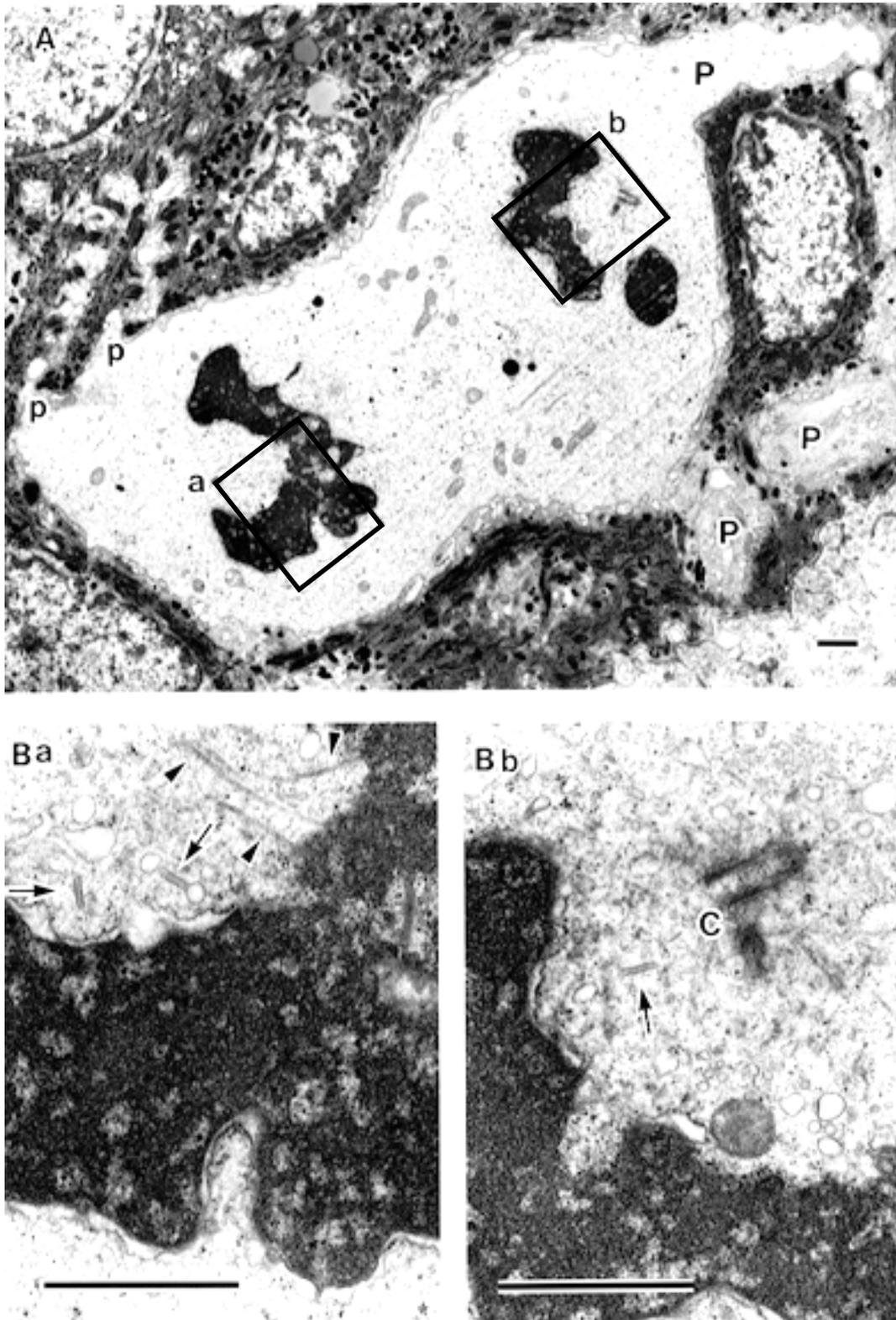


Fig. 4. Legends on p. 206 (bottom).