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LANGERHANS CELLS IN MOUSE EPIDERMIS

To the Editor:

We have read with great interest the article by K. W. Baker and J. E. J. Habowsky on "EDTA Separation and ATPase Langerhans Cell Staining in the Mouse Epidermis" (J Invest Dermatol 80:104–107, 1983). In their study the authors point out that the technique used by Mackenzie and Squier [1] ensures a good definition of cell bodies and dendrites of Langerhans cells (LC). Their Fig 4, however, shows a

FIG 1. Epidermal sheets stained for ATPase activity. Many Langerhans cells are visible with thin and ramified dendrites (× 160).

shrunken aspect of LC cell bodies. Moreover, under the electron microscope—though with a different technique from the one recommended by Mackenzie and Squier—they remarked they "have been unable to observe intracellular Langerhans granules ... an observation which suggests that Trismal buffer, at pH 7.3, is relatively unsuitable for the optimum preservation of epidermal ultrastructure. . . ."

May we report our own experience on the same subject. We used, although with the guinea pig, the method of Mackenzie and Squier for the study of LC with a 5-min incubation period in ATP, as these authors suggest.

The images obtained under the light microscope are very fine (Fig 1): the cells are clearly marked, unshrunken, and many of the dendrites are thin and ramified. We continued our study using electron microscopy, still following Mackenzie and Squier (method of Hirsch and Fedorko), and found a regular though discontinuous labeling (lead sulfide) around a number of cells, with a clearer cytoplasm than that of the neighboring keratinocyte. In the cytoplasm of these clearer cells we could observe no Langerhans granules, but scattered unorganized membranous structures. We then proceeded to the following modifications: (1) adjunction of a 5% calcium chloride solution (0.2 ml per 100 ml) in the Trismal buffer and the cacodylate-buffered formaldehyde, (2) reduction from 20 min to 2 min of the treatment duration in dilute ammonium sulfide at room temperature, (3) fixation of the fragments after revelation of the membranous ATPase activity in Takahashi medium [2].

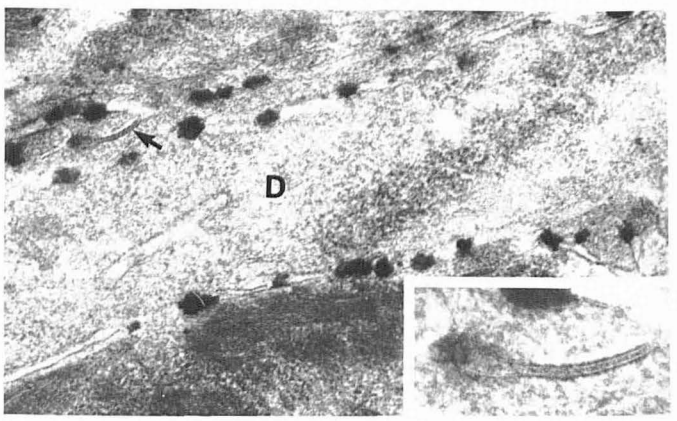

FIG 2. A dendrite whose limits are labeled by lead sulfide. It contains a Langerhans granule (*arrow*) (× 30,000). *Inset*, A higher magnification of the Langerhans granule (× 100,000).

Electron microscopy then showed not only the membranous ATPase reaction product but also well-preserved intracellular organelles, in particular Langerhans granules (Fig 2). Throughout our study, however, we used Trismal buffer! We therefore think that even though Trismal buffer may not be the most adequate medium, other media, in particular the one employed for the fixation, can influence the quality of the results in electron microscopy.

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REPLY

We would like to thank Dr. Hanau, Dr. Fabre, and Dr. Stampf for their comments concerning the adenosine triphosphatase (ATPase) staining of epidermal sheets.

As pointed out in our recent article (J Invest Dermatol 80:104–107, 1983), the epidermis of the mouse differs from that of the guinea pig in a number of respects, most significantly, in the vertical disposition