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ULTRASTRUCTURAL LOCALIZATION OF NUCLEOSIDE TRIPHOSPHATASE IN LANGERHANS CELLS*

KLAUS WOLFF, M.D.† AND R. K. WINKELMANN, M.D., PH.D.‡

Dendritic cells of guinea pig epidermis contain histochemically demonstrable nucleoside triphosphatase and aminopeptidase (1), and there is strong evidence that these dopa-negative cells represent Langerhans cells (1, 2). As electron microscopy has provided new ultrastructural criteria for the identification of Langerhans cells (3–6), it appeared desirable to combine enzyme-histochemical and electron-microscopic methods in further investigations on these cells. The present study was undertaken (1) to demonstrate nucleoside triphosphatase in intraepidermal dendritic cells at the ultrastructural level in order to obtain more information concerning the intracellular localization of the enzyme, (2) to determine whether the dendritic nucleoside triphosphatase-positive cells of guinea pig epidermis are in fact Langerhans cells, and (3) to determine whether the enzyme is demonstrated exclusively in Langerhans cells or whether melanocytes also exhibit this enzymatic reaction.

MATERIAL AND METHODS

Pure red and pure white guinea pigs were used in this experiment. The ears of the animals were depilated chemically. While the animals were under ether anesthesia, the ears were removed with strong scissors. Thin slices of epidermis were made under the dissecting microscope, cuts being made parallel to the surface with a razor blade. These tissue specimens measured less than 3 mm in surface diameter and consisted of pure epidermis at the periphery. The specimens were immediately immersed in a freshly prepared solution of 4% paraformaldehyde in 0.08 M sodium cacodylate buffer containing 0.2 M sucrose, pH 7.3, and fixed at 0 to 4° C for 5 hours. They were then rinsed several times and left overnight in 0.08 M sodium cacodylate buffer, pH 7.3, containing 0.2 M sucrose at 0 to 4° C and incubated in a medium for the demonstration of nucleoside triphosphatase with ATP as substrate (Wachstein and Meisel) (7) at 37° C for 30 minutes. After incubation, the slices of tissue were rinsed in sucrose-cacodylate buffer for 5 to 10 minutes, post-fixed for 2 hours in 2% osmic acid (buffered with barbital acetate buffer), rapidly dehydrated, and embedded in Epon 812. Sections for electron microscopy were cut from the periphery of the specimens on a Porter Blum microtome, stained with uranyl acetate, and examined in an RCA EMU 3E electron microscope.

The light-microscopic control slices of epidermis were fixed as described above, incubated, rinsed, and developed in dilute ammonium sulfide. Fifteen micron sections were cut in a cryostat. Control specimens were also fixed in osmic acid or treated with 20% trichloracetic acid before incubation or were incubated in a medium from which the substrate had been omitted. No histochemical reactions were observed in these controls.

RESULTS

The procedure of fixation just described yielded a preservation of structural detail inferior to that usually obtained with osmium fixatives or glutaraldehyde but satisfactory enough to permit a correlation of enzymatic activity and fine structure. Langerhans cells were identified by the following criteria (3–5): relatively clear cytoplasm, polylobulated nucleus, typical rod-shaped Langerhans-cell profiles, and absence of tonofilaments, desmosomes, premelanosomes, and melanosomes.

Electron-dense material representing the histochemical reaction product, lead phosphate, and indicating nucleoside-triphosphatase activity was found to be present in Langerhans cells. These deposits were observed to be localized within or at the surface of the cytomembranes of these cells (Figs. 1 to 3). The electron-dense material on the dendritic processes outlined very clearly the Langerhans cells within the mosaic of keratinocytes, even at low magnifications (Fig. 4).

The enzyme-histochemical reaction product in most cases also appeared to fill the intercellular space between these cells and adjacent keratinocytes and occasionally coated the surface of the latter cells (Figs. 2 and 3). The electron-dense material was never present between

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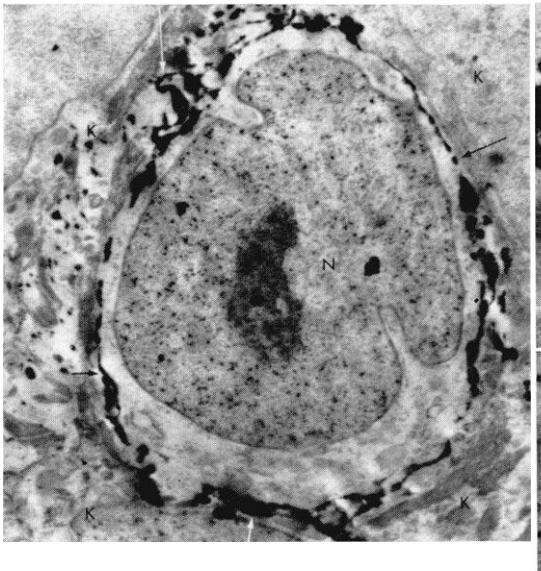
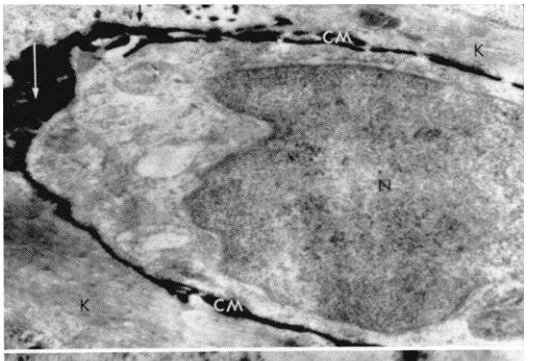
* From the Section of Dermatology,‡ Mayo Clinic and Mayo Foundation and the Mayo Graduate School of Medicine,† University of Minnesota, Rochester, Minnesota.





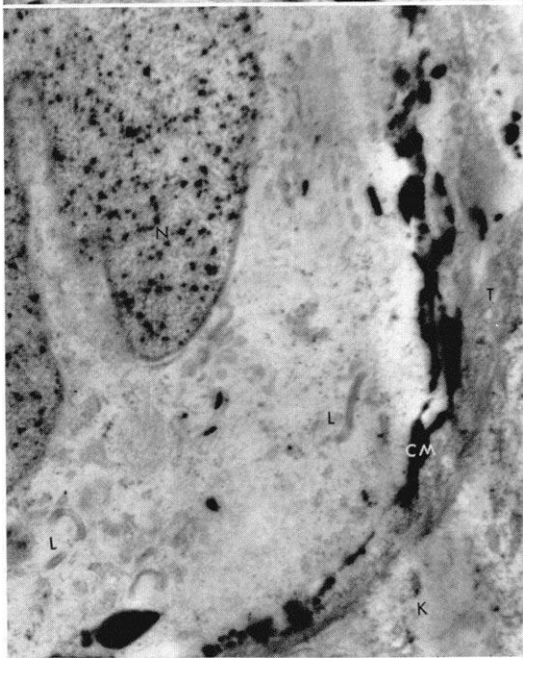


FIG. 1. Langerhans cell. Electron-dense material signifying nucleoside-triphosphatase activity is seen on cell membrane (*small arrows*). In some areas it is also found in intercellular space between Langerhans cell and adjacent keratinocytes (K) and occasionally appears to coat cytomembrane of latter (*large arrow*). Note lobulation of Langerhans cell nucleus (N). (× 11,592.)

FIG. 2. Langerhans cell with intense nucleoside triphosphatase in cytomembrane (CM) and intracellular space (*arrows*). N = nucleus of Langerhans cell. K = keratinocytes. (× 11,592.)

FIG. 3. Langerhans cell with nucleoside triphosphatase in its cytomembrane (CM). Some electron-dense material also coats surface of adjacent keratinocyte (K). L = Langerhans-cell granules, N = nucleus, and T = tonofilaments of keratinocyte. Electron-dense material in nucleus is probably artifact (× 25,536.)

the interfaces of two neighboring epidermal cells or within their limiting membranes (Fig. 4).

No reaction product was detected within the cytoplasm or the endoplasmic organelles of Langerhans cells (Figs. 1 to 3). The Langerhans-cell granule and other membrane-limited bodies were ATPase negative, except in one instance. All recognizable Langerhans cells showed the typical cytomembrane activity, and there were no differences in this reaction in red and so-called albino guinea pigs. Langerhans cells occasionally found in the basal layer exhibited an identical enzymatic response. Recognizable melanocytes failed to show nucleoside-triphosphatase activity (Fig. 4).

During the course of our study of several hundred Langerhans cells, only three cells have been observed that might represent instances of enzyme activity in cells with melanin. These cells were dendritic, lacked desmosomes and tonofilaments, and contained membrane-nucleoside triphosphatase. Langerhans-cell profiles were not seen within their cytoplasm, but granules that could have represented fully melanized melanosomes were present. These organelles, however, were closely packed as if they were contained within a membrane-limited spherical intracytoplasmatic structure. The membrane enveloping these melanosomes was clearly discernible in one cell and also exhibited a nucleoside-triphosphatase reaction.

COMMENT

At present very little is known about the electron-microscopic localization of histochemically demonstrable enzymes of the skin as only a

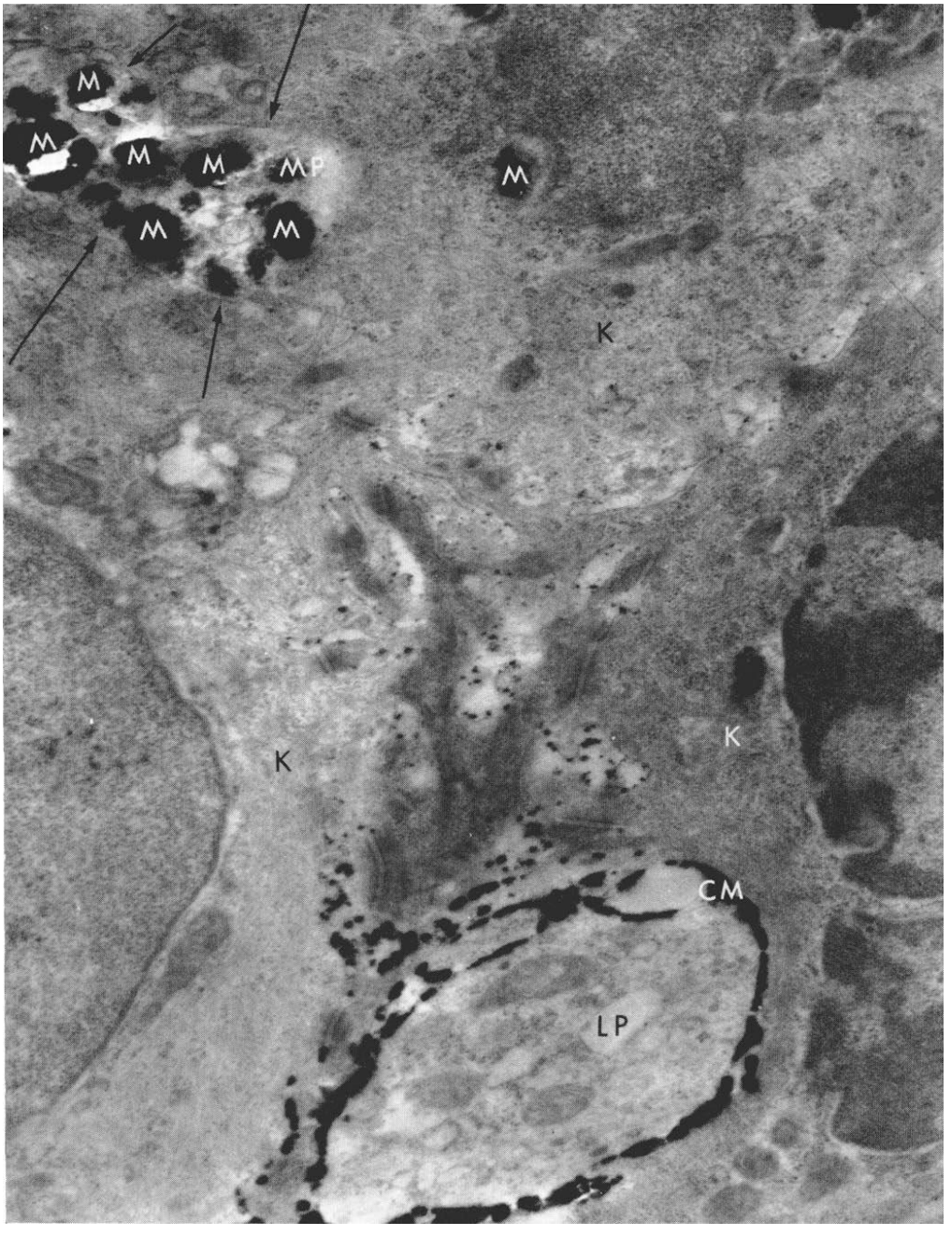


FIG. 4. Process of Langerhans cell (LP) sharply outlined by product of nucleoside triphosphatase activity in its limiting membrane (CM). Minor granular deposits of the enzymatic reaction product are also present within the cytoplasm of the adjacent keratinocyte in close vicinity of the active Langerhans cell process (LP). This probably represents an artifact due to diffusion of either the enzyme or the histochemical reaction product. Cell membranes of keratinocytes (K) and of a melanocyte process (MP) lack nucleoside triphosphatase. Arrows: Negative cell membrane of melanocyte process. M = Melanosomes. (× 22,800).

few studies have been carried out in this field. Acid phosphatase has been demonstrated ultrastructurally within human epidermis (8, 9) and nucleoside triphosphatase has been studied in hamster melanomas with the electron microscope (10). The electron histochemistry of skin is in its earliest developmental stage, and the epidermis represents an especially difficult technical problem. A compromise has to be reached between a sufficient preservation of ultrastructural detail and enzymatic activity. The introduction of various aldehydes, especially glutaraldehyde and hydroxyadipaldehyde, as fixatives for electron histochemistry has resulted in highly improved technics for the ultrastructural localization of enzymes in various tissues (11). Unfortunately, in our hands, glutaraldehyde inhibited nucleoside-triphosphatase activity of Langerhans cells, while the enzymatic reaction was adequately preserved in other structures of the skin. This again emphasizes the heterogeneity of nucleoside phosphatases of skin as demonstrated with ATP as substrate (12). In the search for a fixative which would not inhibit this enzymatic system and at the same time would preserve the cellular detail adequately, we chose paraformaldehyde which yielded, relatively, the best results.

In this study no attempts were made to delineate the type of enzymatic reaction observed within Langerhans cells more in detail, as this has been done extensively at the light-microscopic level (12–14). As far as the ultrastructural localization of the enzymatic reaction is concerned, it was surprising to find activity almost exclusively in the cytomembranes of these cells. Under the light microscope, Langerhans cells display nucleoside triphosphatase at the site of their cell membrane and within their cytoplasm, the cytoplasmatic reaction showing both a diffuse and a granular appearance. Our failure to demonstrate the enzyme in cytoplasmatic organelles of Langerhans cells does not exclude the possibility of such a localization. Blocks of tissue were incubated in this study and it is probable that the incubation medium containing the substrate failed to diffuse through the cytomembrane and to reach the intracellular compartments.

The negative controls carried out at the light-microscopic and ultramicroscopic levels and previous studies (1, 2, 12–14) clearly show that the electron-dense material of the Langerhans-cell cytomembranes represents true enzymatic activity and not a non-enzymatic staining effect. At the present time it is difficult to decide whether the deposits in the intracellular spaces between Langerhans cells and keratinocytes indicate a true localization or diffusion of the enzyme.

It is difficult to interpret the nucleoside-triphosphatase activity of Langerhans cells in regard to cellular function. The pronounced enzymatic reactions of these cells, such as nucleoside-triphosphatase or aminopeptidase (1), suggest very strongly that Langerhans cells possess an active metabolism which at least in regard to these hydrolases appears to be different from that of epidermal cells. Nucleoside triphosphatases have been observed in cytomembranes of cells in other tissues and have been interpreted to control transmembranous transport mechanisms (15–18). Although speculative, it would seem quite attractive to ascribe a similar function to the nucleoside triphosphatase of the Langerhans cell. The presence of such a transfer system in the cell membrane of these cells and the fact that they constitute a very numerous and constant cell population within the epidermis (2) would relate these cells closely to the keratinocytes, indicating possible intercellular interactions, "a Langerhans-epidermal cell unit."

Considerable practical importance can be ascribed to the presence of nucleoside triphosphatase in Langerhans cells. The histochemical technic for the demonstration of this enzyme has been used as a method to visualize Langerhans cells in sections of guinea pig epidermis (1) and to assess this cell population quantitatively under the light microscope (2). Our present observations demonstrate two significant facts: (1) They represent final evidence that the dendritic nucleoside triphosphatase-positive cells seen under the light microscope are in fact Langerhans cells and that apparently all Langerhans cells are demonstrated with this enzyme-histochemical technic; (2) they provide further evidence that in guinea pig epidermis histochemically demonstrable nucleoside triphosphatase appears to be an exclusive property of Langerhans cells, as melanocytes fail to give this enzymatic reaction. These findings support the results of previous light-microscopic studies (1, 2), and the validity of quantitative assessments of Langerhans cells based on their enzymatic reactions (2).

The nature of the three dendritic cells containing nucleoside triphosphatase and melanosomes is, however, not quite clear. The presence of melanosomes could indicate that they were melanocytes containing nucleoside triphosphatase, and thus showing a different enzymatic behavior than the vast majority of this cell population. On the other hand, the arrangement of these melanosomes in closely packed aggregates, enveloped by a limiting membrane, could also suggest that they represented Langerhans cells having phagocytized melanin as recently described (19). The absence of rod-shaped Langerhans-cell profiles could be due to either the fact that none were present in the particular plane of the section or that they were not sufficiently preserved by the fixative used. Possibly, but not likely, these cells might represent a "transition form" between melanocytes and Langerhans cells (5), exhibiting an enzyme typical for a Langerhans cells and signs of melanin formation.

SUMMARY

Nucleoside triphosphatase has been demonstrated with the electron microscope in guinea pig epidermis, exclusively localized in the cytomembranes of Langerhans cells, while keratinocytes and melanocytes failed to exhibit an enzymatic reaction. This confirms the light-microscopic localization of this enzyme and demonstrates that quantitative assessments of nucleoside triphosphatase-positive cells in epidermal sheets are highly representative of the Langerhans-cell population. The close relationship of the Langerhans cells and epidermal cells and the enzyme reactivity at their common cell membranes suggest the existence of a Langerhans cell-epidermal cell unit.

REFERENCES

1. Wolff, K.: Zur Enzymaktivität in den Langerhansschen Zellen. Arch. Klin. Exp. Derm., *218:* 446, 1964.
2. Wolff, K. and Winkelmann, R. K.: Quantitative studies on the Langerhans cell population of guinea pig epidermis. J. Invest. Derm. (In press.)
3. Birbeck, M. S., Breathnach, A. S. and Everall, J. D.: An electron microscope study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. J. Invest. Derm., *37:* 51, 1961.
4. Breathnach, A. S.: Observations on cytoplasmatic organelles in Langerhans cells of human epidermis. J. Anat., *98:* 265, 1964.
5. Zelickson, A. S.: The Langerhans cell. J. Invest. Derm., *44:* 201, 1965.
6. Snell, R. S.: An electron microscopic study of the dendritic cells in the basal cell layer of guinea-pig epidermis. Z. Zellforsch., *66:* 457, 1965.
7. Wachstein, M. and Meisel, Elizabeth: Histochemistry of hepatic phosphatases at a physiologic pH: With special reference to the demonstration of bile canaliculi. Amer. J. Clin. Path., *27:* 13, 1957.
8. Eisen, A. Z., Arndt, K. A. and Clark, W. H., Jr.: The ultrastructural localization of acid phosphatase in human epidermis. J. Invest. Derm., *43:* 319, 1964.
9. Olson, R. L. and Nordquist, R. E.: Ultramicroscopic localization of acid phosphatase in human epidermis. J. Invest. Derm., *46:* 431, 1966.
10. Nakai, T.: Electron microscopic study of "adenosine triphosphatase" activity in malignant melanoma cells of the Syrian golden hamster. J. Invest. Derm., *44:* 264, 1965.
11. Sabatini, D. D., Bensch, K. and Barrnett, R. J.: Cytochemistry and electron microscopy: The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol., *17:* 19, 1963.
12. Wolff, K.: Über die Adenosintriphosphataseaktivität der menschlichen Haut: Eine histochemische Studie. Arch. Klin. Exp. Derm., *218:* 254, 1964.
13. Cormane, R. H. and Kalsbeek, G. L.: ATP-hydrolyzing enzymes in normal human skin. Dermatologica, *127:* 381, 1963.
14. Ishikawa, H. and Klingmüller, G.: Phosphatdarstellung nach Adenosintriphosphat-Inkubation an dendritischen Zellelementen: Beitrag zur Histogenese der epidermalen neuralen Zellen und des Naevuszellnaevus. Arch. Klin. Exp. Derm., *220:* 191, 1964.
15. Torack, R. M. and Barrnett, R. J.: Nucleoside phosphatase activity in membranous fine structures of neurons and glia. J. Histochem., *11:* 763, 1963.
16. Marchesi, V. T. and Barrnett, R. J.: The demonstration of enzymatic activity in pinocytic vesicles of blood capillaries with the electron microscope. J. Cell Biol., *17:* 547, 1963.
17. Post, R. L., Merritt, C. R., Kinsolving, C. R. and Albright, C. D.: Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. Biol. Chem., *235:* 1796, 1960.
18. Bartoszewicz, W. and Barrnett, R. J.: Fine structural localization of nucleoside phosphatase activity in the urinary bladder of the toad. J. Ultrastruct. Res., *10:* 599, 1964.
19. Breathnach, A. S. and Wyllie, L. M.: Melanin in Langerhans cells. J. Invest. Derm., *45:* 401, 1965.