

## **Direct Evidence for the Cytomembrane Derivation of Birbeck Granules: The Membrane-sandwich Effect**

JACEK BARTOSIK, ANDERS ANDERSSON, STURE AXELSSON,  
BENGT FALCK and ANITA RINGBERG<sup>1</sup>

*Department of Histology and <sup>1</sup>Department of Plastic Surgery,  
University of Lund, Lund, Sweden*

Bartosik J, Andersson A, Axelsson S, Falck B, Ringberg A. Direct evidence for the cytomembrane derivation of Birbeck granules: The membrane-sandwich effect. *Acta Derm Venereol* (Stockh) 1985; 65: 157-160.

Digitonin, which is known to cause extensive damage to cytomembranes in general, was found to have a most remarkable effect on epidermal Langerhans' cells. Thus, it generates a membrane-sandwiching process resulting in the formation of large discs which except for the differences in size have the same morphology as ordinary Birbeck granules. This demonstrates that the cytomembrane of the Langerhans' cell has the inherent ability to superimpose upon itself, leaving little doubt that the normal Birbeck granules derive from the cytomembrane. *Key words: Langerhans' cells; Birbeck granules; Digitonin effect; Electron microscopy.* (Received October 10, 1984.)

A. Andersson, Department of Histology, Biskopsgatan 5, S-223 62 Lund, Sweden.

The occurrence of membrane-limited, disc-shaped granules, often provided with a vesicular swelling, in the epidermal Langerhans' cells (LC) was first reported by Birbeck et al. (1). These authors suggested that the vesicle could represent a formative stage and it has since been commonly believed that the granules arise as vesicles in the Golgi apparatus. Recent studies by Elofsson et al. (2) strongly contradict this view, showing that the vesicular swelling is probably a fixation artifact and demonstrating rather that the granules emanate from the cytomembrane. This origin was first suggested by Hashimoto & Tarnowski (3).

Accidentally, a finding was obtained in this laboratory that may eventually resolve this dispute. In homogenates of epidermal cell suspensions membrane-limited sheets appeared which ultrastructurally in cross sections were similar to Birbeck granules. The finding indicated that a cytomembrane disruption leads to a spontaneous formation of Birbeck

granule-like structures. The present study was undertaken to examine whether a chemical membrane damage would have the same effect and if this effect would selectively occur in the LC.

## MATERIAL AND METHODS

Clinically normal skin was obtained with a dermatome from 3 adult patients during plastic surgery. Punch biopsies (3 mm) were taken without anaesthesia from the volar side of the forearm of 3 healthy adults.

Small pieces of the skin specimens were incubated at 37°C in Krebs-Ringer phosphate buffer solution containing 0.1% digitonin (Serva, Germany) for 10, 20 or 30 min. After incubation the specimens were processed for electron microscopy as previously described (4). At least 60 serial sections were produced from each specimen.

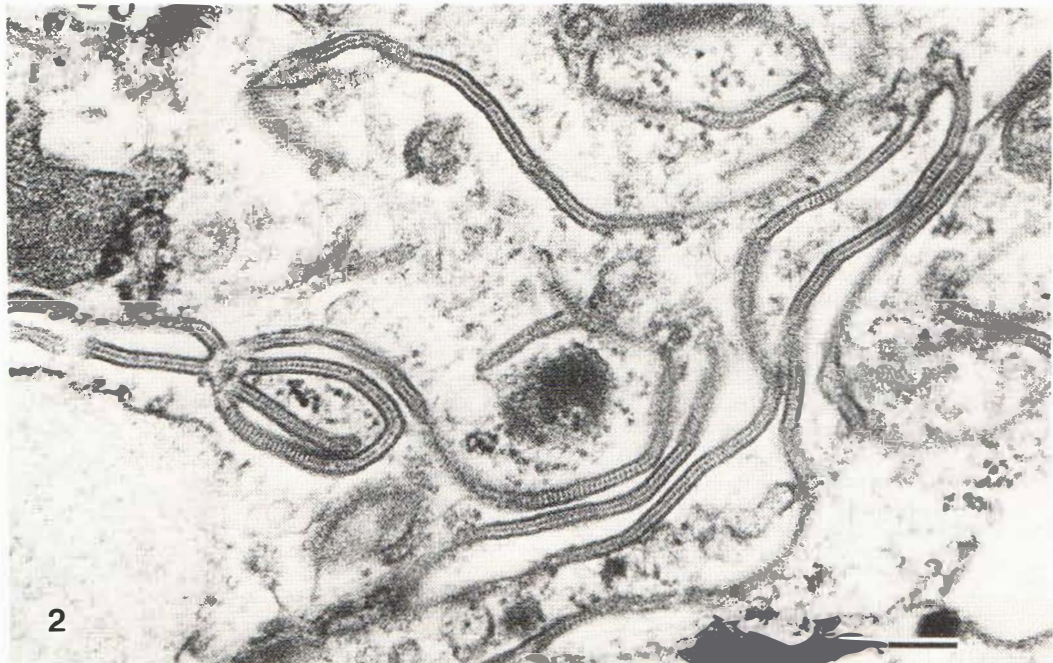
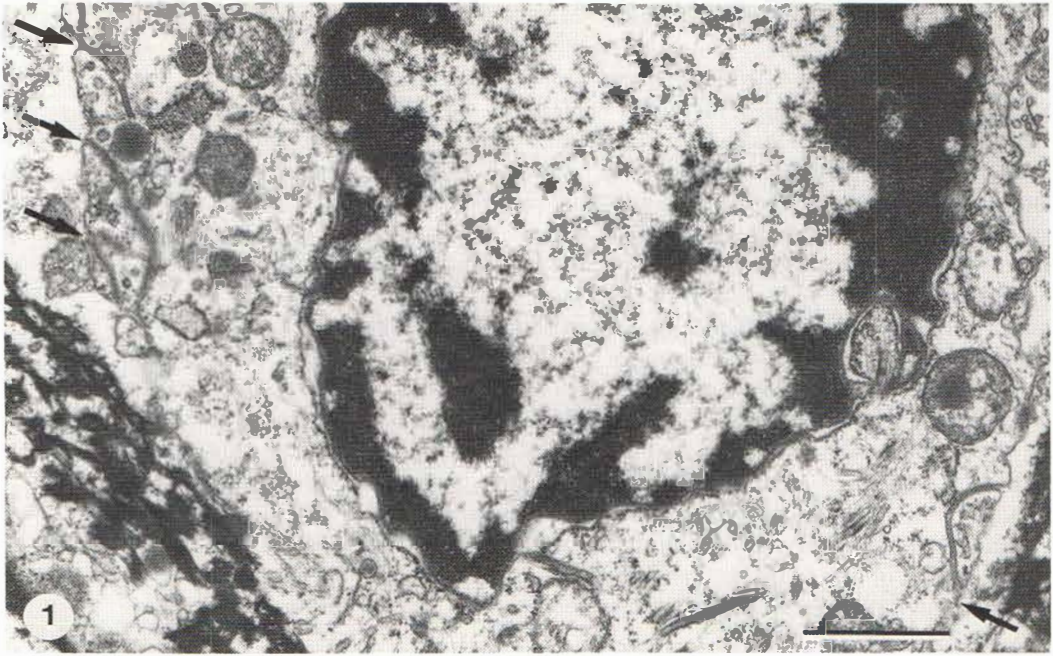
## RESULTS AND COMMENTS

Incubation in 0.1% digitonin caused changes in all epidermal cell types. The degree and extent of the damage varied widely, one extreme being only slight ultrastructural changes, the other extreme being alterations in almost all cell components with only the nuclear envelope and heterochromatin relatively well preserved. Such pictures could be found in one and the same specimen. Moreover, the effects of digitonin tended to be restricted to cells below the outer spinous layer after incubation for 10 and 20 min, whereas the whole living epidermis could be affected after 30 min of incubation. Such variations probably reflect different rates of penetration and were not unexpected. This particular digitonin lot had a very low solubility and formed a soapy suspension in the buffer and the thickness of dermis varied between the individual specimens. In the following discussion only the changes in plasma membranes below the granular layer will be dealt with.

In severely damaged areas the cytomembranes of the keratinocytes and melanocytes had completely disappeared and the intercellular spaces were filled with cytoplasmic material. This membrane-dissolving effect of digitonin is well known. Remarkably, quite another process took place in the LC. Most of the limiting cytomembrane had disappeared and only small remnants of it were preserved in places. Attached to these membrane parts were long (up to 2000 nm), winding, and sometimes branched membrane-limited sheets. At the point of attachment, the cytomembrane invaginated and was continuous with the limiting membrane of the sheets (Fig. 1). Similar structures, including some with circular form, appeared within the cytoplasm (Fig. 2). In cross section their morphology proved to be identical to that of ordinary Birbeck granules; they were surrounded by a triple-layered unit membrane and had a central lamella composed of regularly spaced, seemingly round particles midway between the membrane surface. The membrane, especially its inner leaflet, was more electron dense than is usually seen in granules of normal LCs (Fig. 2). A few membrane-damaged LCs were analyzed in serial sections, combined with tilting of the sections. It then became obvious that a number of membrane sheets were interconnected and that all such groups of sheets were connected to one of the small remaining parts of the cytomembrane. Remarkably, none of the ordinary Birbeck granules present prior to the incubation could be found.

In less damaged epidermal parts the cytomembrane of keratinocytes and melanocytes could appear unperturbed, but a digitonin effect could easily be established in a low power view due to abnormal electron-lucent cytoplasm containing small vacuoles and a disaggregation of the nuclear euchromatin. The LCs in such areas contained a normal number of ordinary Birbeck granules and had a continuous plasma membrane but an unusually high number of "attached granules", that is granules in their formative stage (2). Such profiles are rare in normal LCs, whereas an increased amount is observed in highly activated LCs





*Fig. 1.* An LC after 30 min exposure to digitonin with small remnants of cytomembrane to which are attached Birbeck granule-like structures (arrows). Seemingly detached similar structures are seen deeper in the cell. Scale 1  $\mu\text{m}$ .

*Fig. 2.* Long winding sheets of sandwiched membranes. In cross-sections they display a morphology identical to ordinary Birbeck granules, except for a higher electron density of the limiting membrane. In this cell almost all of the cytomembrane had transformed to double-membrane formations. Some of them are similar to the granules found in LC in histiocytosis X. Scale 0.25  $\mu\text{m}$ .

(2, 5). Thus, a graded response to digitonin can also occur leading only to increased formation of Birbeck granules from the cytomembrane. It could not be established whether these granules were detached and incorporated into the cytoplasm.

Digitonin forms insoluble digitonides with cholesterol and is also a potent detergent; the fact that it causes extensive damage to plasma membranes has long been known. Apparently a series of not yet fully clarified events precede the rapidly occurring solubilization of cytomembranes and the first effect may well be the appearance of discrete ruptures of the cytomembrane (6, 7). However, at present it is futile to speculate about the mechanism leading to the membrane sandwiching of the LCs by digitonin. It also remains enigmatic why sandwiched membranes can survive the presence of digitonin while the cytomembranes of keratinocytes and melanocytes as well as the Birbeck granules formed prior to digitonin exposure do not. One interesting point should be mentioned in this context. The exact prerequisites needed for digitonin to attack and disperse biological membranes are not known. Several factors can be of importance, an obvious one being the availability of unsterified 3- $\beta$ -hydroxysterols (i.e., mainly free cholesterol in mammalian cells) which complex with digitonin. The results obtained in this study indicate an interesting difference in resistance towards digitonin between the inside and the outside of the cytomembrane. From Hashimoto's (3) and our view on the formation of Birbeck granules it follows that the outer leaflet of the cytomembrane will be more protected after the sandwiching process and the former inner leaflet will become the outer surface of the granule membrane exposed to digitonin.

However, the important conclusion of this work is that the effect triggered by digitonin demonstrates that the cytomembrane of the LC has an inherent capacity to form sheets or discs by superimposing upon itself, leaving little doubt that the Birbeck granules in normal cells derive from the cytomembrane.

## REFERENCES

1. Birbeck MS, Breathnach AS, Everall JD. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans' cells) in vitiligo. *J Invest Dermatol* 1961; 37: 51-63.
2. Elofsson R, Andersson A, Falck B, Sjöborg S. Evidence for endocytotic mechanisms in the epidermal Langerhans' cells. *Acta Dermatovener (Stockh)* 1981; Suppl. 99: 29-35.
3. Hashimoto K, Tarnowski WM. Some new aspects of the Langerhans' cell. *Arch Dermatol* 1968; 97: 450-464.
4. Andersson A, Sjöborg S, Elofsson R, Falck B. The epidermal indeterminate cell—a special cell type? *Acta Dermatovener (Stockh)* 1981; Suppl. 99: 41-48.
5. Sjöborg S, Andersson A, Christensen OB. The Langerhans' cell in healed patch tests reactions, before and after oral administration of nickel. *Acta Dermatovener (Stockh)* 1984; Suppl. 111: 1-23.
6. Cook GA, Gattone UH, Evan AP, Harris RA. Structural changes of isolated hepatocytes during treatment with digitonin. *Biochim Biophys Acta* 1983; 763: 356-367.
7. Elias PM, Goerke J, Friend DS, Brown BE. Freeze-fracture identification of sterol-digitonin complexes in cell and liposome membranes. *J Cell Biol* 1978; 78: 577-596.