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THYMIDINE UPTAKE BY HIGH-LEVEL EPIDERMAL CELLS

A Potential Error in Studies of Keratinocyte Proliferation

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Abstract. Evidence is presented that in skin labelled with tritiated thymidine, labelled 'high-level' epidermal cells, presumably Langerhans cells, may add a significant error in keratinocyte proliferation if not recognized. A simple method is described which facilitates recognition of Langerhans cell labelling and, if applied critically, may prove a valuable tool for studying Langerhans cell proliferation and for correcting keratinocyte counts in autoradiographic experiments.

Many studies of epidermal proliferation are based on the autoradiographic identification of keratinocytes labelled with tritiated thymidine in the pre-mitotic period of DNA-synthesis. It is generally believed that in normal epidermis the germinative population of keratinocytes resides in the basal layer. However, there are reports which claim that suprabasal layers are also involved in epidermal regeneration (4) and it has been established that in hyperproliferative conditions, such as psoriasis, keratinocytes are recruited for division from the lower 2–3 cell layers of the epidermis (8).

It has also been shown that the epidermal Langerhans cells constitute a separate pool of proliferating cells and may appear in an immediately suprabasal position. "High-level" labelled cells after injection of tritiated thymidine (2, 5, 6) may thus be mistaken for labelled keratinocytes unless they are specifically identified. This aspect of epidermal labelling has to our knowledge not been taken into account previously in keratinocyte proliferation studies. In all probability, the reasons for ignoring this phenomenon may have been, firstly, the technical difficulties of combining autoradiography with specific methods for identifying Langerhans cells and, secondly, it may have been assumed that this source of error was so small as to be negligible. Nonetheless, by omitting any reference to the labelling of Langerhans cells an error of unknown magnitude is introduced into all studies of keratinocyte proliferation and it seemed worthwhile to develop a simple method which might prove a suitable approach to the study of this problem. This may be of some importance, as it has been shown that the number and distribution of Langerhans cells in the epidermis may vary greatly (1, 3) and it may justifiably be assumed that there is also some variability in their proliferative activity.

MATERIAL AND METHODS

Three albino guinea pigs with a weight of 600–700 g each, received 5–7 injections of a sterile suspension of 10% beryllium oxide in isotonic saline into the skin of the flanks to produce a granulomatous inflammation. Subsequent to these injections considerable reactive epidermal hyperplasia with raised proliferative activity developed, as described in detail elsewhere (7).

Sixty minutes prior to sacrifice of the animals on days 4, 8 and 15, tritiated thymidine (1 μCi/g body weight, spec. act. 26 Ci/mole; The Radiochemical Centre, Amersham, England) was injected intraperitoneally into each animal. The entire area of skin bearing the injection sites was excised from each animal and divided into several specimens. Some of these specimens were then immediately frozen in liquid nitrogen and sectioned on a cryostat at 30 μm. These sections were then subjected to the nucleoside-triphosphatase reaction using the method of Wachstein & Meisel (9) in order to identify Langerhans cells. The remaining 3–5 specimens from each animal were fixed in 10% neutral formalin, embedded in paraffin and sectioned at 5 μm. Autoradiographs were prepared by dipping the sections in Kodak NTB2 nuclear track emulsion and processing in Kodak D19 developer after exposure for 3–5 weeks at 4°C. All autoradiographs were evaluated as follows:

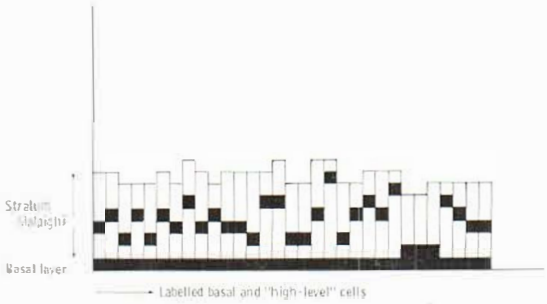

Fig. 1. Diagrammatic representation of the location of 31 randomly 'high-level' labelled cells in relation to their position within the Malpighian layer and to the labelled basal keratinocytes.

1. The 'total epidermal labelling index' was estimated as the proportion of all labelled cells per 100 total basal cells. A total of 1 500–2 000 basal cells were counted for each specimen.

2. In order to determine the exact localization and distribution of each labelled cell, the distance of each from the basement membrane region was plotted graphically and this distance expressed as the number of cell layers between the basal cell layer and the labelled cell.

RESULTS

Considerable acanthosis without papillomatosis developed in the epidermis overlying the granulomas in the 4-day specimens. The total epidermal labelling index at this stage was 15.4%. Most labelled cells seemed to be in the basal cell layer, but a certain number were present at higher levels. After determining the distance of every labelled cell from the basement membrane zone it became evident that 13–14% of all labelled cells were present in the higher epidermal compartments and some were even in the immediately subgranular zone. These high-level cells were distributed randomly within the epidermis (Fig. 1) but sometimes seemed to occur in clusters, as over short distances they would comprise up to 30% of all labelled cells and show a lesser degree of labelling in other areas. Fig. 2 shows the distribution of all labelled cells plotted graphically against their distance from the basement membrane. These 'high-level' labelled cells often possessed a dark kidney-shaped nucleus and a perinuclear halo similar to the 'clear cells' assumed to be melanocytes in the basal layer and thus appeared distinct from the surrounding keratinocytes (Fig. 3 B–C). The overall distribution of these cells reflected the overall distribution of Langerhans cells in these specimens which were present at high levels as NTP-positive dendritic cells in the cryostat sections.

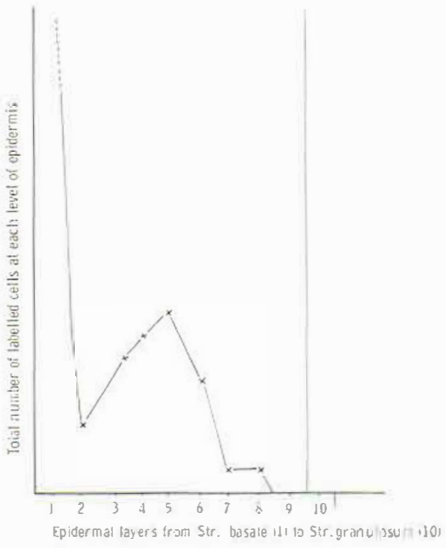

Fig. 2. Curve illustrating the distribution of all labelled cells in acanthotic epidermis as seen at 4 days after injecting beryllium oxide. It is apparent that there are two peaks—the first representing the labelled basal cells, the second peak representing labelled 'high-level' cells.

In normal guinea pig flank skin and in the epidermis affected to a lesser degree by acanthosis (days 8 and 15) such suprabasal labelled cells could not always be easily identified even if serial sections were superimposed, but it appeared that their number was consistently less than 10% of all labelled cells.

DISCUSSION

In this study a considerable number of labelled cells were present within the epidermis but above the basal cell layer in specimens in which there was an experimentally induced acanthosis and a raised labelling index. These cells were distributed randomly within the epidermis and were usually morphologically distinguishable from keratinocytes; they may be assumed to be labelled Langerhans cells. While the overall number of these cells was approximately 10% of all labelled epidermal cells, there was clustering which in places would amount to 30% of all labelled cells in a particular area. The pattern of distribution of these high-level epidermal cells (Fig. 2) in

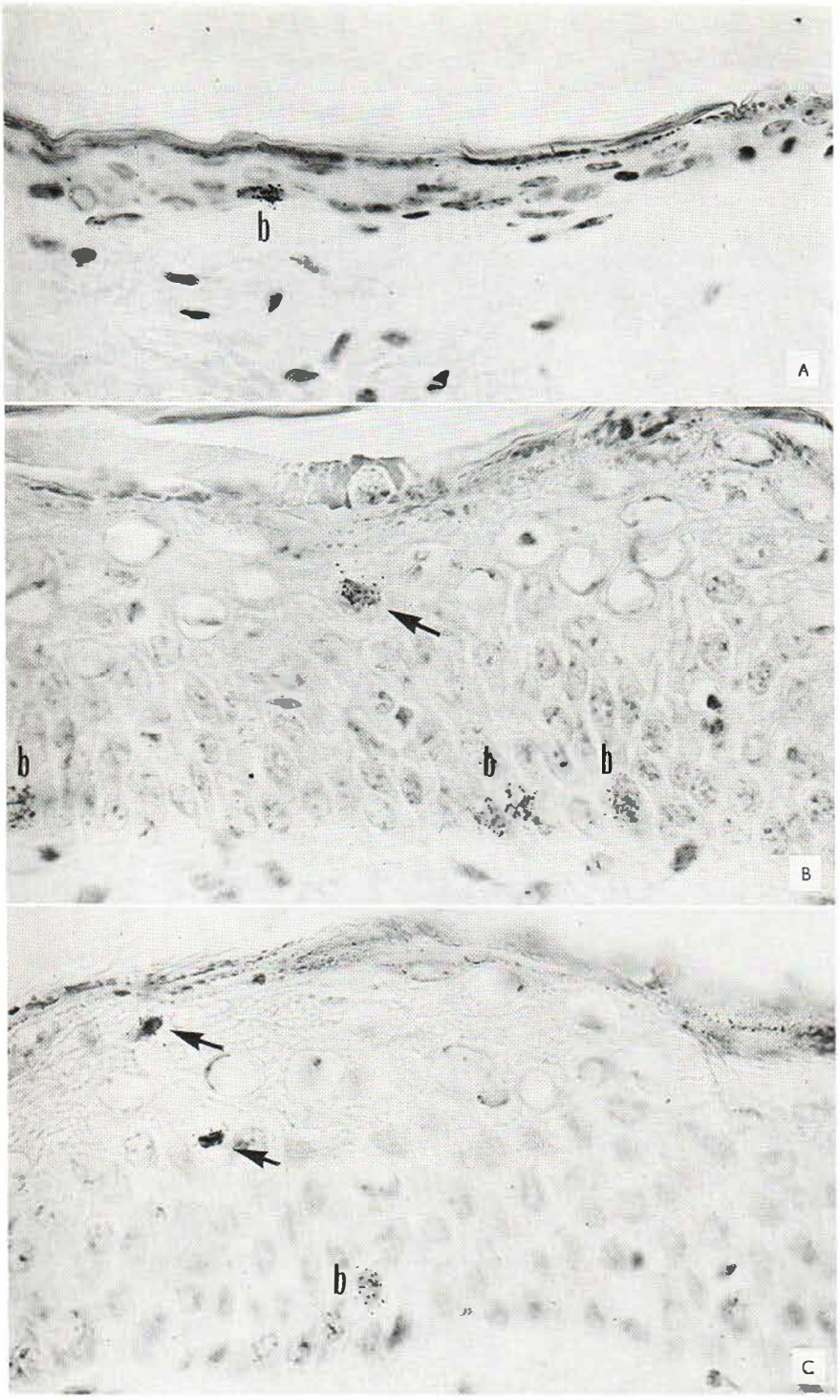

Fig. 3. (A–C) Guinea pig flank skin labelled with tritiated thymidine 60 minutes prior to biopsy. Haematoxylin and eosin, × 240. (A) Normal skin. The Malpighian layer is only 2–4 cell layers thick. One labelled cell (*b*) in basal position. (B, C) Acanthosis as found on day 4 after injection of beryllium oxide. The Malpighian layer is 7 or more cell layers thick. Note the presence of 'high-level' labelled cells (*arrows*) as opposed to labelled cells in the basal layer (*b*).

relation to the labelled cells is distinctly different from that observed in psoriasis and other 'high-output' states of the epidermis where selective recruitment of keratinocytes occurs in the lower 2–3 cell layers of the epidermis (8).

It would appear from our results that labelling of Langerhans cells has been unjustifiably ignored in epidermal cell kinetics. It has been suggested before that the proliferative activity of epidermal Langerhans cells may vary and that a better knowledge of this aspect might promote a better understanding of their proliferative capacity and behaviour.

The shortcomings of this simple approach are evident from the fact that thin, oedematous or papillomatous epidermis may be difficult to evaluate for the presence of such 'high-level' cells. However, if applied critically this approach might, on occasion, be helpful for the study of Langerhans cell kinetics.

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