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RENEWAL RATE OF HUMAN SEBACEOUS GLANDS[1]

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Abstract. Biopsies from various regions of face and back were excised at intervals up to 28 days after intradermal injection of ^{3}H-glycine and ^{3}H-histidine. Post-divisional lipid cell movement was studied with darkfield autoradiography which proved to be the superior technique so far used for the determination of lipid cell migration. Two distinct patterns of lipid cell migration were seen. Either a synchronised mass movement of intense labelling occurred or there was a gradual loss of labelled substance from the sebaceous glands. From the synchronised movement it was possible to determine the speed of cell migration from the basement membrane using a single biopsy. It showed that about 3 newly formed peripheral lipid cell layers were formed per 100 hours. Both patterns of lipid cell migration revealed that the renewal rate of average sized sebaceous lobules in man takes from 21 to 25 days.

The exact rate of human sebaceous gland renewal is still unknown. Mitotic counts (2, 8) and autoradiographical analyses with tritiated thymidine labelling (1, 4, 5) permitted only a limited interpretation of the migratory activity of differentiating lipid cells. The renewal rate for sebaceous glands was originally calculated to be 7.4 days (1). More recently the labelling index was determined as 9–10% for human sebaceous glands taken from various body sites (4). However, a slow cell movement with a replacement time of more than 14 days was found (5).

The sebaceous glands are known to be composed of lobules differing in size and shape (2) (Fig. 1). Especially in view of this complicated structure, functional data concerning renewal time are lacking.

The following experiments were conducted to further clarify this problem and to delineate direction and migratory speed of post-divisional lipid cells in human sebaceous glands.

MATERIALS AND METHODS

Data were obtained by autoradiographical analysis utilizing the incorporation of tritiated glycine and tritiated histidine (The Radiochemical Centre, Amersham; 5μCi of glycine-2-H3 or L-histidine-2,5-H3, suspended in 0.1–0.2 ml of physiological saline) which were injected intradermally between 9 and 11 a.m. A total of 24 biopsies from healthy men, age range 21–30, of face (forehead, perinasal area, cheek, scalp) or upper mid-back were taken 45 min. and 1–28 days later. Autoradiographs were prepared in a routine fashion using

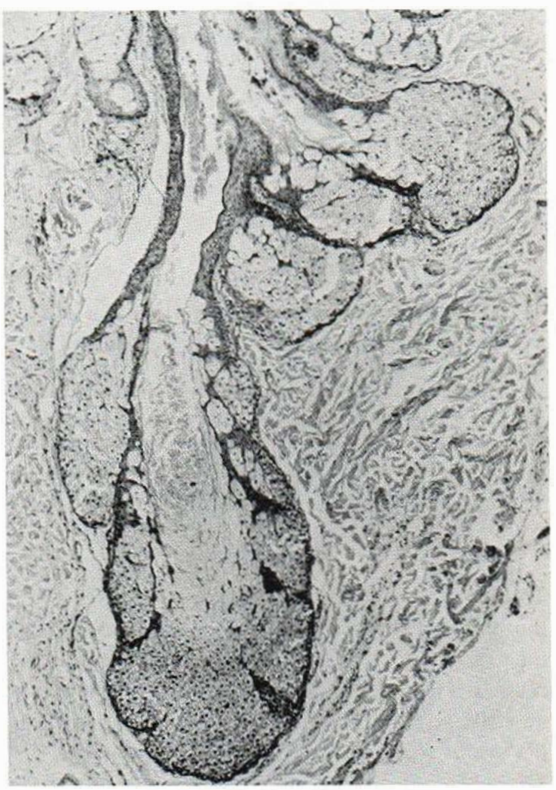

Fig. 1. Large sebaceous follicle from the cheek demonstrating sac-like structures with confluent ducts draining various-sized sebaceous lobules. H + E, ×50.

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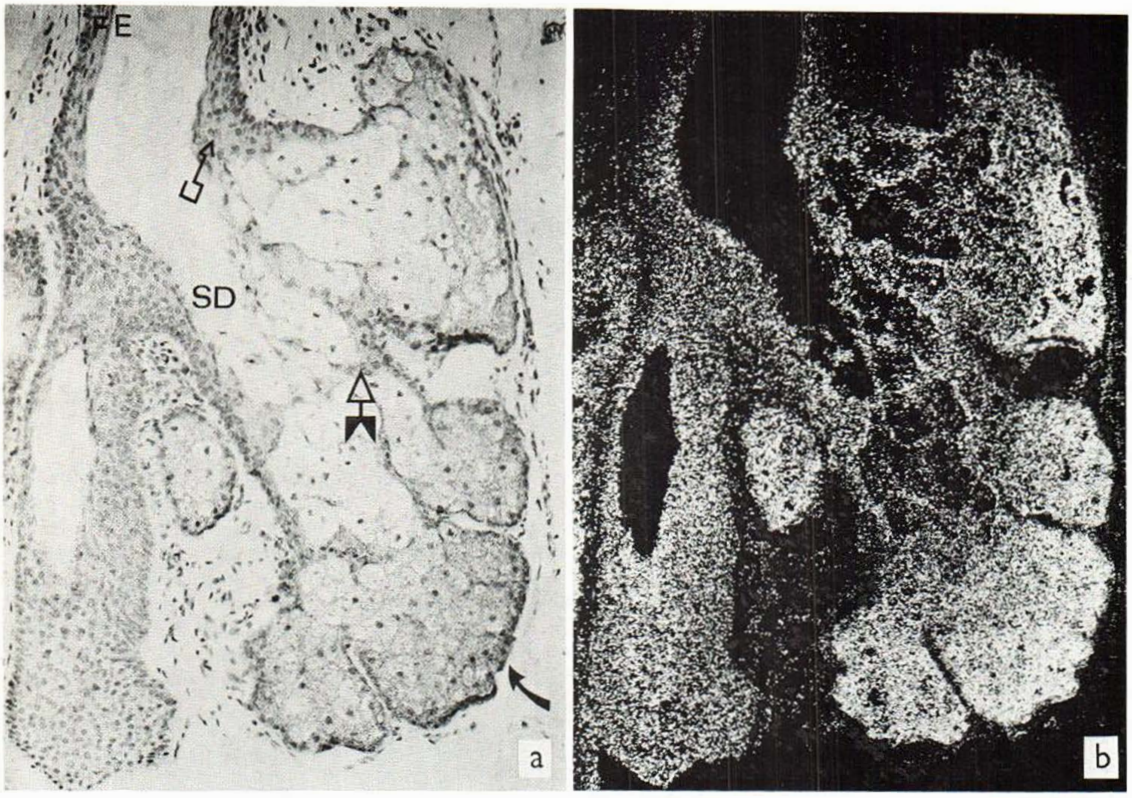

Fig. 2. Silver grain distribution over sebaceous lobuli, sebaceous duct (*SD*) and follicular epithelium (*FE*) by darkfield illumination (Fig. 2*b*) and light microscopy (Fig. 2*a*). Dense labelling over peripheral differentiating lipid cells (↖) is compared with non-differentiated cell pool (⇧) and keratinizing epithelia (⇩). Blood vessels and connective tissue cells are lightly labelled. ^{3}H-histidine, 45 min, ×120.

the dipping method (Kodak NTB II) with an exposure time of 19–21 days (6).

All specimens were analysed with light and darkfield microscopy. *45 min specimens* were analysed to determine the pattern of silver grains following the incorporation of radioactive material into various parts of the sebaceous gland, i.e. the peripheral lobules, sebaceous duct and follicular epithelium. *1–28-day-old specimens* were particularly closely scrutinized for migratory patterns of labelled lipid cells from the proliferative zone on the basement membrane.

RESULTS

The uptake of the two tritiated amino acids within the human sebaceous glands was intense. They thus proved to be useful materials for the study of cellular kinetics in sebaceous glands. There was no striking difference between the two amino acids; the results will therefore be presented together.

45 minutes

Dense labelling was encountered over the entire gland. Nuclei and cytoplasm of all cells were similarly densely labelled with silver grains (Fig. 2*a*). However, uptake was more pronounced in the differentiating cells than in the non-differentiated cell pool (4, 5) or the sebaceous duct (Fig. 2*a*), although the cell density is higher in the latter (5). The follicular epithelium with its granular layer exhibited patterns similar to the already described interfollicular epidermis (6).

The autoradiographs showed various intensities over all cells of epidermal appendages (hair-follicles, sweat-glands), blood vessels and connective tissue cells. Especially the latter contribute to silver grain deposits, which must not be confused with non-specific background when viewed with the sensitive dark field technique (Fig. 2*b*). The silver grains, even in minute quantities, are easily recognized by their bright silvery appearance under the darkfield illumination (Fig. 2*b*).

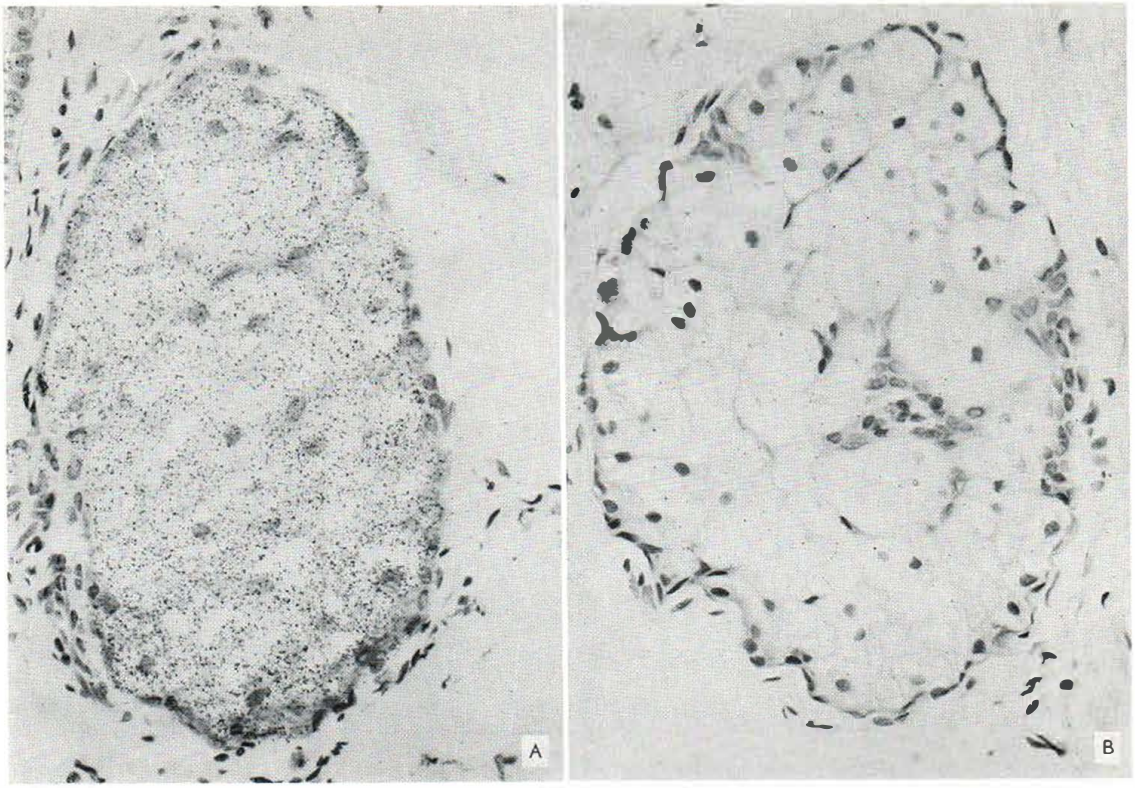

Fig. 3. Gradual thinning out of silver grains with time after injection of ^{3}H-histidine. *A* = 45 min: dense labelling over nuclei and cytoplasm of all cells. Hematoxylin, ×300. *B* = 14 days: only a few silver grains are left. Hematoxylin, ×300.

1–28 days

Within 14–28 days the incorporated tracers disappeared from the sebaceous lobules. No distinct and sharply defined leading edge of a pulse-labelled wave could be seen as in the stratum corneum (7) or in the comedonal kernel of acne vulgaris lesions (6). At the point of rupture of the lipid cell membranes the silver grains were lost from observation.

The information gained from the changing distribution of silver grains at various intervals permitted the determination of the renewal rate of human sebaceous glands. As will be seen below, two distinct patterns were observed concerning the fate of the tritiated amino acids of differentiating lipid cells.

The first pattern showed a steady decrease in silver grains over the sebaceous lobules. The heavy labelling seen in 45 min specimens (Fig. 3*a*) is reduced at 7 days and almost gone by 14 days (Fig. 3*b*). In most of the glands, all silver grains were lost by 20–25 days.

The renewal rate of these glands, therefore, is more than 14 days (since at this time silver grains were still present) and less than 25 days (since silver grains were absent by days 20–25).

The second pattern observed was totally different. In some specimens, mainly from the perinasal and cheek area, the densely labelled lipid cells, located in the mid-portion of the cross-sectioned glands, contrasted sharply to the unlabelled peripheral cells (Fig. 4). The heavily labelled cells formed a distinct mass with a sharply defined trailing edge parallel to the basement membrane (Fig. 4). Strictly vertical sectioning of the lobules was necessary for this observation, since the long time intervals between labelling and excision (more than 10 days) led to a distortion of the cellular arrangements near the bottle-shaped sebaceous duct (Fig. 5).

It is noteworthy that here lipid cells moved in a coordinated fashion and not at random from the basement membrane. This phenomenon has not been seen with previous techniques, using tritiated thymidine autoradiography (1, 5).

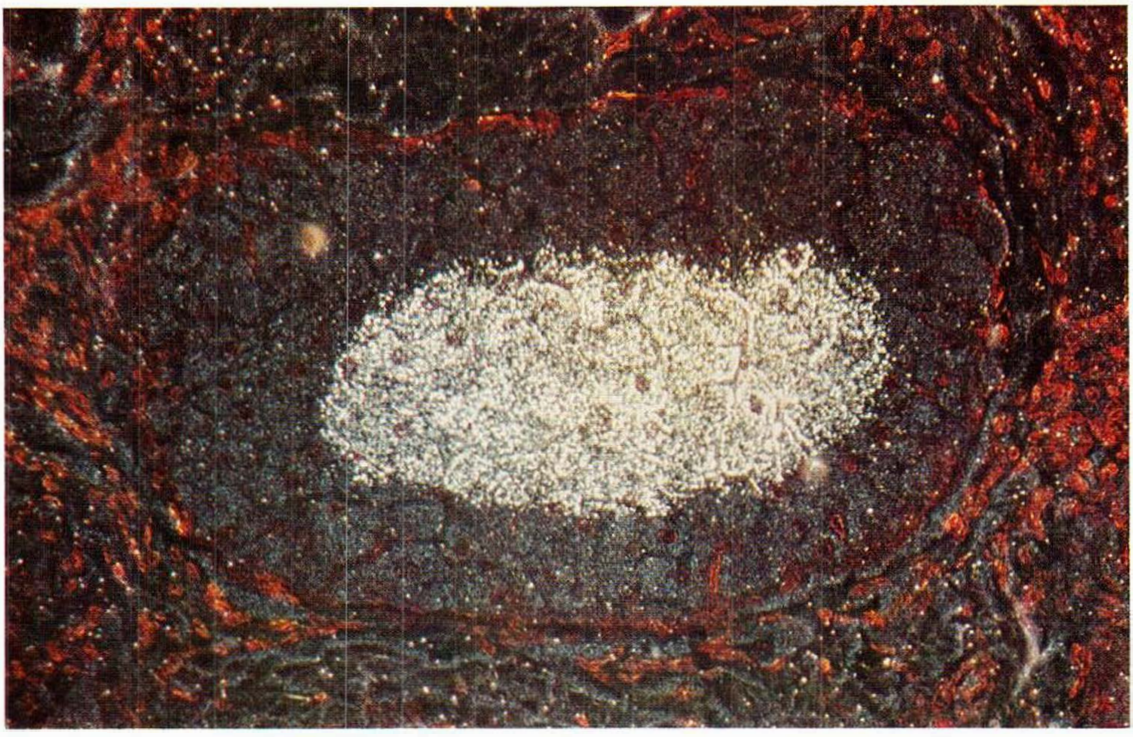

Fig. 4. Horizontal section through a sebaceous gland lobule: 5 days after injection of ^{3}H-glycine. Synchronised cell movement as viewed by darkfield illumination: densely labelled lipid cells contrast sharply to 4 newly formed peripheral cell layers which have almost no labelling. Hematoxylin, × 420.

The speed of migration was assessed from a single biopsy. Approximately 3 cell layers were formed per 100 hours in the periphery of the sebaceous lobules (Table I).

From data given in the table the renewal rate of sebaceous glands can be calculated. For instance, in a gland with 15 lipid cell layers from the basement membrane to the point of cell rupture near the sebaceous duct, 500 hours (or 21 days) are necessary for renewal of the whole lobule. This is in good correlation with the observed entire loss of silver grains (20–25 days) described for sebaceous glands of comparable size (see above). In all sebaceous glands considered for calculation the number of lipid cell layers was close to 15 (Fig. 1).

Table I. *Newly formed lipid cell layers in the periphery of sebaceous lobules per 100 hours in the centrofacial region*

No. of the specimen	Layers free of grains	Days after injection	Newly formed cell layers/ 100 hrs
1	2.0	3	2.8
2	3.0	5	2.5
3	3.5	5	2.8
4	4.0	5	3.3
		Mean	2.9

DISCUSSION

It has been demonstrated here that certain amino acids are abundantly incorporated into sebaceous glands. Therefore, they seem to be more suitable for autoradiographical analysis of the replacement rate than, for instance, tritiated thymidine (1, 4, 5). Further, the presented data are measurements made on single sebaceous glands of known size. The fate of incorporated radioactive material used in the present study makes it possible to distinguish two different patterns of cell migration. First, there was a gradual loss of grains until their final disappearance between 20 and 25 days after labelling. This indicates that the renewal rate of sebaceous lobules falls within this period. Secondly, sharply defined densely labelled lipid cells moved synchronously and cen-

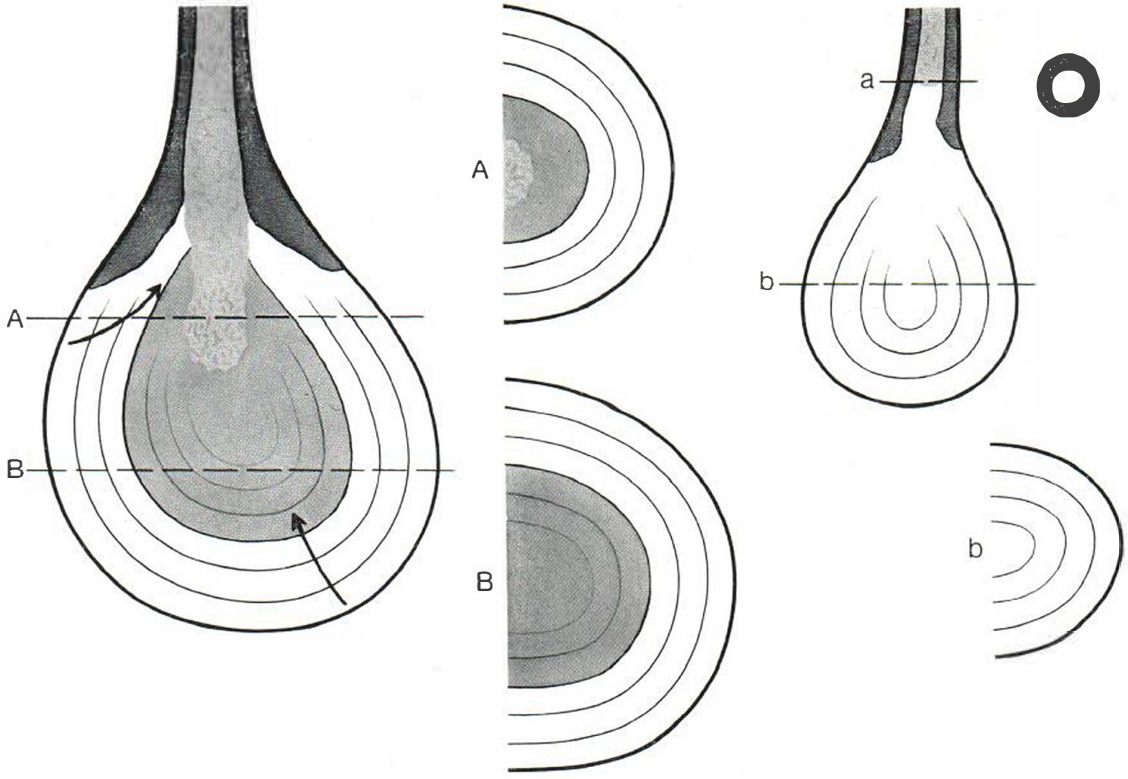

Fig. 5. Vertical and low and high horizontal drawings of two types of human sebaceous glands. In an idealized form sebaceous glands are sacs which empty their contents through relatively narrow ducts. In large glands (*left*) a lipid cell life span ends with cellular disrupture *within* the *gland* (dotted area). In small glands (*right*) cellular disrupture occurs outside the sebaceous lobule, namely *in* the *duct*. Parallel lines indicate upward movement of maturing lipid cells. Central dark core at left represents position of densely labelled lipid cells approx. 7 days after injection of ^{3}H-histidine. Compare with Fig. 4.

tripetally. The newly formed peripheral lipid cells retained only a few silver grains. From this observation alone it is possible to conclude that the movement of lipid cells is synchronised and not random in glands showing this central core.

Furthermore, it is possible to calculate the renewal rate of lipid cells from a single biopsy. It was shown that 2.8–3.3 layers of lipid cells are produced per 100 hours. Thus the turnover time of small sebaceous glands is shorter than that of large glands. It was observed that sebaceous acini with 15 lipid cell layers (basement membrane to area of rupture near the sebaceous duct) require 500 hours to replace all lipid cells. This magnitude correlates well with the time of 20–25 days calculated from the single observation.

The reasons for the two observed migratory patterns are not yet fully explained. A difference in the tracer pool due to local injection of the amino acids seems unlikely. In all specimens under study labelling was uniform over all tissue compartments, namely epidermis and adnexae. Since the centrally located core was mainly seen in the glands from the centrofacial area it may be related to functional peculiarities of these glands and their lipid cell proliferation. Regional variations in sebaceous gland proliferation were shown recently (3, 4), with the highest values typical for the face. Possibly, lipid cells are being produced at a fast rate, leading to a sharply defined trailing edge.

Finally, the proliferative activity of individual glands, even in close proximity within the same site, varies considerably (3). This also is likely to correspond to either the gradual thinning out or dense central labelling as described above.

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