of mycosis fungoides (MF) (6). Our findings suggest that these cells are not Langerhans' cells, which are known to be numerically increased in MF (2, 7), and that indeterminate cells which are related to Langerhans' cells also show absence of lysozyme, AT and ACT. It is possible, however, that the epidermal histiocytes identified in mycosis fungoides may be derived from Langerhans' cells and indeterminate cells by progressive acquisition of "histiocytic features". Immunoelectron microscopic study, simultaneously to label the membrane of Langerhans' cells with OKT6 (3) and the cytoplasm of histiocytes with lysozyme will help to resolve this question.

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Diurnal Fluctuations of Cell Kinetic Parameters in the Epidermis and the Sebaceous Gland of the Hamster Ear

H. Wirth, M. Spannagel and M. Gloor

Department of Dermatology, Ruprecht Karls University of Heidelberg, D-6900 Heidelberg, FRG

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Abstract. By using the colcemid method, the presence of a diurnal variation for the mitosis index was confirmed, showing a definite minimum during the night in the sebaceous gland of the Syrian hamster ear. Furthermore, the number of all labelled cells and the quotient "single-labelled ^{14}C-cells + double-labelled cells/single-labelled ^{3}H-cells" determined with double-labelling autoradiography ([^{3}H] and [^{14}C]thymidine) seem to be subject to rhythmic fluctuations, although statistical support for this observation is lacking. The parallelism of the rhythmic variations in the epidermis and in the sebaceous glands is striking.

Key words: Diurnal rhythms; Epidermis; Sebaceous glands; Mitosis index; Double-labelling autoradiography

The hamster ear model described by Plewig & Luderschmidt (4) has achieved importance in investigations of pharmacologic influence on sebaceous glands. The sebaceous glands of the hamster ear are comparable to those of man with respect to anatomic structure and cell kinetics. Furthermore both are similarly under the control of androgens. The present study was an attempt to determine to what degree diurnal variation has to be taken into account when performing cell kinetic investigations.

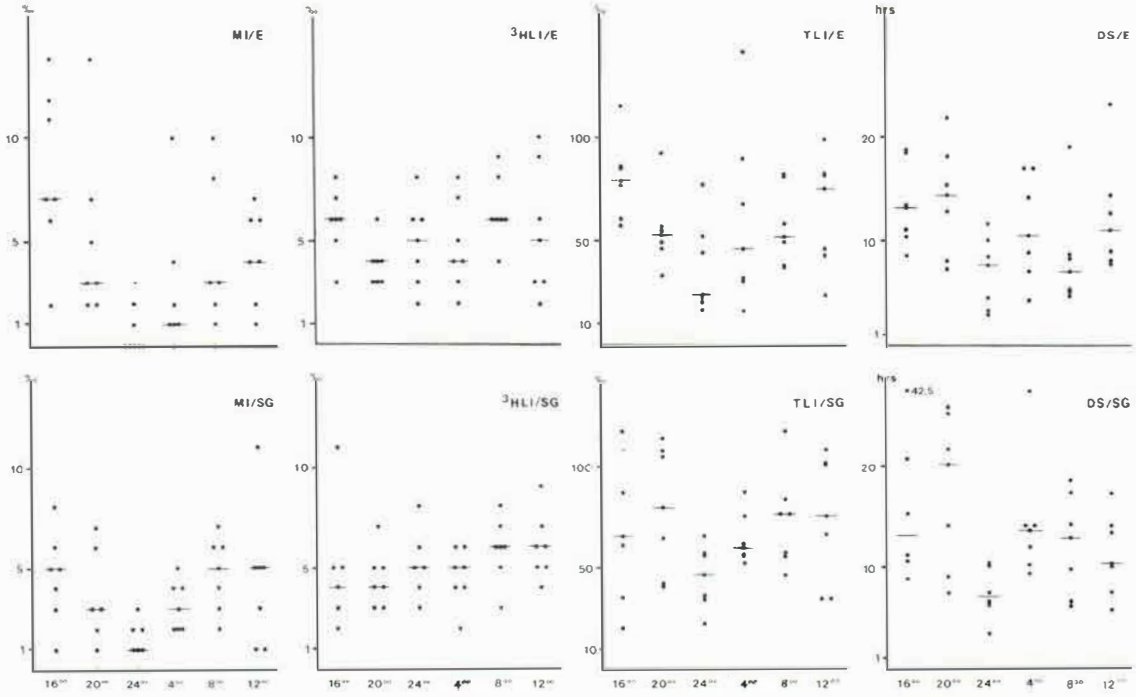

Fig. 1. Diurnal dependency of various parameters of cell kinetics in the basal cell layer of the epidermis (E) and the sebaceous gland (SG) in the Syrian hamster ear. MI: mitosis index (Colcemid method), 3HLI: single-labelled ^{3}H-cells (double-labelling autoradiography), TLI: all labelled cells (double-labelling autoradiography), DS: quotient "single-labelled ^{14}C-cells + double-labelled cells/single ^{3}H-labelled cells" (= duration of S-phase; double-labelling autoradiography).

MATERIALS AND METHODS

Eighty-four male Syrian hamsters were investigated (Breed: Han: Aura; Zentralinstitut für Versuchstiere, Hannover, FRG). The animals ranged in weight between 100 and 121 g. The animals were delivered on Aug. 7, 1981 and then adapted to standard conditions for 18 days (standard diet, water ad libitum, room temperature 22°C, natural day-night rhythm).

The animals were divided into two groups of 42 each. In one group the mitosis rate over a period of 24 hours was analysed with the colchicine method. Seven animals were killed with a blow on the neck at 4 p.m., 8 p.m., 12 p.m., 4 a.m., 8 a.m., and 12 a.m., respectively. Two hours previously the animals had been given 0.36 mg Colcemid i.p. (Colcemid®; Fluka AG, Neu Ulm, FRG; Tradename of Ciba-Geigy AG, Basle, Switzerland), diluted with 1.1 ml normal saline solution. All investigations were carried out on Aug. 25–26, 1981. Directly after killing the animals the right ear was removed and fixed. The histologic preparation and Giemsa staining were performed in the usual manner. The number of mitoses blocked in metaphase (nuclear pyknoses) was counted in the epidermis and sebaceous glands. In each case the number of nuclear pyknoses was related to 1000 cells in contact with the basal lamina.

For the dosage of colchicine (Colcemid®) we applied the principles set forth by Schaaf (5). Our numerous preliminary investigations, as well as current experiments with colchicine, have all verified the dosage of colchicine we used for the Syrian gold hamster. A higher dosage did not lead to an increase in the number of arrested mitoses. We know of no reports suggesting or documenting a change in colchicine metabolism during the day.

The second group of 42 animals was subjected to in vivo autoradiographic investigations with the double labelling method over a period of 24 hours. Seven animals were killed with a blow to the neck at 4 p.m., 8 p.m., 12 p.m., 4 a.m., 8 a.m., and 12 a.m., respectively. At two hours, 1.5 hours, 1 hour, and 30 min before killing the animals, 30 μCi [^{3}H]thymidine, diluted to 0.5 ml with normal saline solution, was injected i.p. (spec. activity 20 Ci/mmol; NEN Chemicals, Dreieich, FRG). At the time of the last two injections 5 μCi [^{14}C]thymidine (spec. activity 51 mCi/mmol; NEN Chemicals, Dreieich, FRG) diluted to 0.5 ml with normal saline solution was also injected i.p. Immediately after killing the animals the right ear was removed and fixed. Autoradiographic preparation was performed in the usual manner with G 5 photoemulsion (Ilford GmbH, Dietzenbach/Steinberg, FRG). Hemalum staining. In the epidermis and sebaceous glands 1 000 cells in contact with the basal lamina were counted. The proportion of single-labelled ^{3}H-cells, corresponding to the number of cells which leave the S-phase in one hour, was evaluated. Furthermore the sum of single-labelled ^{14}C-

cells and double-labelled cells was calculated. The quotient "single-labelled ^{14}C-cells + double-labelled cells/single-labelled ^{3}H-cells" allows an estimation of the duration of the S-phase.

The statistical analysis of results obtained from animals investigated at various times was made with the Kruskal-Wallis test. The required level of significance was $\alpha = 0.05$.

RESULTS

The results are presented in Fig. 1. The following conclusions are of importance:

1. The various parameters show parallel fluctuations during the day in the epidermis and in the sebaceous glands.

2. With regard to the mitosis index (Colcemid method), the sum of labelled cells (corresponding to the [^{3}H]thymidine labelling index in single labelling [^{3}H]thymidine autoradiography), and the quotient "single labelled ^{14}C- and double-labelled cells/single-labelled ^{3}H-cells" (corresponding to the duration of the S-phase) a clear diurnal dependency with a minimum in the night is seen in the epidermis and the sebaceous glands. The differences found at various times of the day using the Colcemid method were statistically significant ($\alpha = 0.05$ in the sebaceous glands, as well as in the epidermis).

3. The analysis of single-labelled ^{3}H-cells (=number of cells which leave the S-phase in one hour) did not reveal any clear diurnal variation.

DISCUSSION

Human sebaceous gland secretion is known to be dependent on circadian rhythms (1). However, since sebaceous gland secretion is dependent on a variety of parameters, these investigations do not allow of the assumption that all cell kinetic parameters in the sebaceous gland are subject to circadian rhythms. Investigations by Hamilton (2) in the mouse indicate that such diurnal dependency could exist. Here the [^{3}H]thymidine labelling index in the sebaceous bland was compared in the morning and at night. The values were strikingly lower during the night. Measurements of the [^{3}H]thymidine labelling index in the Swiss-albino mouse by Laurence et al. (3) make it clear that large diurnal variations exist, without however demonstrating a definite minimum or maximum. It is interesting that both Hamilton (2) and Laurence et al. (3) found a parallel diurnal effect on the [^{3}H]thymidine labelling index in the epidermis and the sebacous gland.

The present results supply for the first time statistical evidence that with the Colcemid method the measured values in the sebacous glands are subject to diurnal variation. In this regard it is also probable that the number of all labelled cells and the quotient 'single-labelled ^{14}C-cells + double-labelled cells/single labelled ^{3}H-cells" (corresponding to the duration of the S-phase) are subject to circadian rhythms. The present results impressively support the observation of Hamilton (2) and Laurence et al. (3) that the epidermis and the sebaceous glands show a parallel diurnal variation.

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Immunofluorescence Studies on Complement Components in Lichen amyloidosus

Shigenori Sudo, Ryoichi Igarashi and Masaaki Morohashi

Department of Dermatology, Toyama Medical and Pharmaceutical University, Toyama, Japan

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Abstract. Immunofluorescence studies were carried out in 7 cases of lichen amyloidosus, chiefly to detect deposition of complement components in the cutaneous lesions. Examination of skin biopsy specimens revealed deposition of Clq, C3, C9 and IgM in all the patients studied. Comple-