On Circadian Rhythms in Human Epidermal Cell Proliferation

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The ascertainment of consistent diurnal variations in human epidermal cell proliferation may have important implications for the treatment of many skin diseases. For the evaluation of diurnal rhythms in the growth of human epidermis, skin biopsies were taken every 4th hour for 48 h from each of two persons under synchronized living conditions. The epidermal cell proliferation was assessed by the fraction of cells in S and in G2-M phase as determined by measurements of the DNA content in the individual cells in single-cell suspensions. The existence of diurnally consistent body functions in the test persons was verified by monitoring the excretion of cortisol by urine. The fraction of cells in G2-M phase indicated circadian rhythmicity for the first 32 h of the test period. No regular variation according to time of day could be established in the fraction of cells in S-phase. ***Key words: Diurnal rhythms; Skin; Cell kinetics; Flow cytometry.***

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For human skin diurnal variations have been established for sweating, cutaneous blood flow and also for the epidermal content of cyclic nucleotides. In rodents, circadian rhythms of epidermal cell proliferation are well known, while in Man the results reported on this topic have been partly conflicting (1, 2).

An evaluation of diurnal variations in the epidermal growth is complicated in Man because of the need for numerous biopsies, and due to interindividual and regional variations, the risk of perturbation of the cell proliferation by the sampling procedure and problems of standardizing the human life style (2). This study was designed to avoid most of these pitfalls. Particular care was taken to synchronize the living conditions of the persons studied.

MATERIALS AND METHODS

Two females designated A and B, both 41 years old, participated in the study. The skin sampling was performed as follows. All living conditions were synchronized for the two individuals from 24 h prior to the first sampling. They thus shared meals and mental and physical activities and resting periods.

Biopsies

From each individual a total of 13 skin biopsies were obtained, using a pair of surgical scissors. Biopsies were taken every 4th hour from the inner surface of the upper arms no less than 2 cm apart. No local anesthesia was used. From person A biopsies number 1–6 were taken from the left arm and number 7–13 from the right arm. From person B, biopsies with uneven numbers were taken from the left arm and the remainder from the right arm.

Urine sampling

At each biopsy time the urine from the previous 4 h was sampled to measure the cortisol excretion by a standard method (nanomoles/ 4 h). In order to check the consistency of the diurnal excretion of cortisol, this part of the trial was repeated 2 months after the initial study. This time the test persons were living apart, but they still synchronized their resting periods.

Flow cytometric DNA analysis

The skin samples, which measured ½–¾ cm, were prepared for and analysed in the flow cytometer, a FACS IV cell sorter (Becton Dickinson FACS Systems, Sunnyvale, Calif., USA) as previously described (3). When the epidermis was peeled of the dermis, each epidermal sheet was divided in two parts (*a* and *b*) for double determination of the proliferative characteristics. The epidermis was disintegrated into a nuclear suspension by three steps: 1) incubation with dithiothreitol, 2) whirling in a buffer (pH 7.4) with the non-ionic detergent Nonidet P 40, EGTA, RNase and spermine, and 3) whirling after addition of citric acid to a final concentration of 1% (pH 2.4). The yield of suspended nuclei has been calculated to approximately 70% of the number of epidermal cells in the sample (3). In the present study, 52 suspensions of isolated nuclei from epidermal cells were obtained. Before the final measuring procedure the nuclei were stained with propidium iodide. As a rule 100,000 nuclei were measured from each sample.

The time-consuming analyses in the flow cytometer had to be performed in four separate assays. The *a* and *b* samples from each of the persons were thus analysed separately. The cell cycle analysis was performed by statistical deconvolution of the fluorescence histograms into fractions of cells with G1, S and G2-M phase DNA content by means of a maximum likelihood estimation procedure (4). The coefficent of variation for the G1 peak of the histograms ranged from 3.61% to 5.35‰.

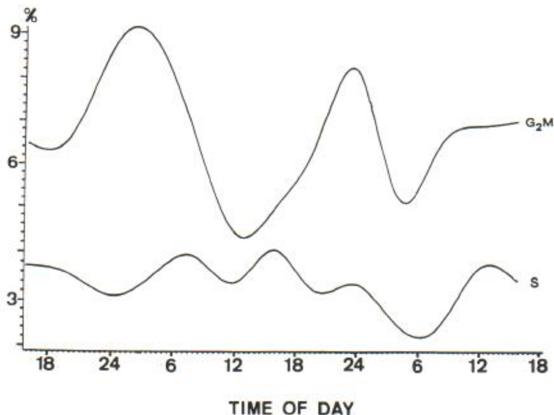

Fig. 1. Course of the fractions of cells in S and G2-M phase in human epidermis, according to time of day. The smoothed curves are based on the averaged values for measurements *a* and *b* for both test persons. Absolute peaks for the G2-M fraction occur at midnight. No trend toward any consistent diurnal pattern in the S fraction is seen.

Statistics

The results were analysed by analyses of variance. For evaluation of diurnal variations in the course of the S and G2-M fractions, the cortisol excretion each of these parameters as functions of time was described according to harmonic functions.

RESULTS

Smoothed plots of the mean values of the S and G2-M fractions at each test point are drawn in Fig. 1. The value for the S fraction varied between 1.6 and 6.6 and for the G2-M fraction between 2.1 and 13.3. The results of the two measurements for the two individuals have been averaged, as two-way analyses of variance did not disclose systematic differences either between the individuals or between measurements *a* and *b*. In the G2-M fraction a diurnal rhythm is strongly suggested by absolute peaks around midnight (average peak value 8.4%, i.e. 8.4% of the cells had a DNA content of $4n$ at midnight against an average of 5.7 at noon). No consistent pattern is seen in the S-fraction value, having average values of 3.3% at midnight and 3.8% at noon. The rhythmicity in the G2-M fraction is blurred after approx. 32 h.

Based on the results from the initial 32 h the course of the G2-M fraction can be drawn as a classic one-harmonic function of the time of day. In Fig. 2 the curve describing the one-harmonic function of the G2-M fraction extrapolated to 48 h from start is further compared with the curve describing the cortisol excretion as a two-harmonic function of time. According to this graph, approx. 6 h elapsed between cortisol excretion peaks and G2-M troughs.

The excretion by urine per 4 h of cortisol shows a pronounced diurnal rhythmicity common for the two individuals. The rhythmicity seen in the first sampling period could be reproduced in the second, 2 months later. The peak values for the cortisol excretion occur at 6–8 a.m. and the troughs at midnight (average values 37.1 and 8.1 µmol/4 h respectively).

The oscillations in the cortisol excretion and in the G2-M fraction are significantly different from 0 ($p < 0.05$).

CONCLUSIONS AND DISCUSSION

The purpose of this study was to evaluate diurnal rhythmicity in the growth of human skin, using a design avoiding the pitfalls which usually blemish the credibility of results of such studies on cell kinetics in Man. Diurnal rhythms in body functions may be controlled by behavioural and environmental conditions. The test persons in the present study therefore had their living conditions synchronized prior to and during the skin sampling period. The biopsies were taken in a well-defined area without use of local anesthesia, in order to neutralize regional variations and avoid chemicals, which might interfere. Furthermore, the biopsies were taken by separate schedules

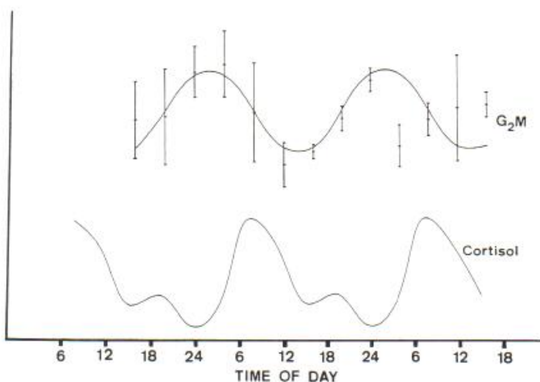

Fig. 2. Course of the G2-M fraction given as a one-harmonic function of time, compared with the course of the cortisol excretion by urine given as a two-harmonic function of time. The simulated curve for the G2-M fraction is extrapolated over the entire 48 h span, while the fits are based on the values obtained from the first nine biopies. The 95% confidence intervals of the G2-M measurements are indicated. The troughs in G2-M are seen approx. 6 h later than the cortisol peaks.

for each individual. This was done in order to avoid interpreting perturbation from the biopsy trauma as a false common diurnal rhythmicity.

The drawback of the study design was the limited number of test persons. Nonetheless, the results supported the concept of diurnal variability in epidermal cell kinetics, since peaks around midnight were seen in the G2-M fraction. This is, as also the trough at noon, consistent with the results of Scheving (5) and Fisher (6) who both studied mitotic indices. In these experiments each individual provided only a few skin samples. In spite of the very different investigational conditions and measuring procedures used, these three experiments are in basic agreement. For the S fraction the oscillations were small and without any trend in diurnal rhythmicity analogous to that seen in the study by Thorud & Volden (7).

For the keratinizing epithelium of the hamster cheek pouch, Møller et al. (8) recently demonstrated that methotrexate injections given to the animals in the resting period had an increased cytokinetic effect, compared with injections given in the active period, while the toxicity in terms of animal mortality decreased. Thus it might be possible to optimize treatment schedules in dermatology using chronotherapy. However, much work remains before it can be established whether methotrexate should be administered to humans in the early morning and podophyllin derivatives in the evening.

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