SPECIFIC VARIATION IN THE NUMBER OF CELLS WITH X-CHROMATIN IN SHORT-TERM CULTURES OF THE BUCCAL EPITHELIUM FROM WOMEN TREATED WITH HORMONES IN DIFFERENT PHASES OF THE INDIVIDUAL DIURNAL BIORHYTHM

T. A. Zaletaeva, V. K. Kanyuka,
O. V. Golovchanskii, and Yu. E. Ershikova

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Changes in the number of cells with X-chromatin in the buccal epithelium of four clinically healthy women were studied at intervals of 2-4 h over a period of 29 h in vivo and in 3-hourly cultures with and without hormone (ACTH, insulin, thyroxine). The presence of individual diurnal fluctuations in the number of cells with X-chromatin (changes in the X-heterochromatin index – X-HCI), both in vivo and in vitro, within the range from 44 to 350 ‰ was confirmed Individual differences in the mean diurnal values of X-HCI ($X\text{-}HCI_{mean}$) and variation in $X\text{-}HCI_{mean}$ in cultures with and without hormones were discovered. Stereotyped "mirror-image" changes were found in the number of cells differing in their type of chromatin condensation during treatment with hormones in different phases of the individual biorhythm. Incubation of cells with minimal X-HCI values in vivo with hormones led to an increase in the number of cells with X-chromatin (from 50 to 240‰ for example), whereas incubation of cells with maximal X-HCI values in vivo led to a decrease (from 190 to 70‰, for example). Changes in the number of the different cell fractions correlated with one another and had the character of a systemic response. It is suggested that the specific response to hormone treatment is preceded by a nonspecific stereotyped response of the tissue which, on the one hand, is determined by the state of the biological substrate at the time of treatment (the phase of the individual biorhythm) and, on the other hand, leads to systemic changes in the population structure of the cell fractions that may perhaps facilitate identification of the stimulus and an adequate response to it. The stereotyped response is accompanied by a change in the degree of condensation of nuclear chromatin and, in particular, of X-chromatin, and these processes are reversible.

KEY WORDS: X-chromatin; Z-heterochromatin index; degree of condensation of chromatin; phases of the individual biorhythm; hormones; stereotyped systemic response of tissue cells.

The morphology of somatic cell nuclei is characterized by a definite degree of condensation and decondensation of chromatin and its specific spatial organization (the heterochromatin pattern of the nucleus). The reversibility of condensation and decondensation of the chromatin of interphase nuclei [5, 7, 8] and the possibility of induction of these processes, detectable even in metaphase chromosomes [12], indicate on the one hand that this phenomenon is universal as a mechanism of regulation of gene activity [5, 8, 12], and on the other hand, that methods of objective recording of the response of cells and their genetic apparatus to all manner of factors can be developed.

The hypothesis that cycles of condensation and decondensation of chromatin in the interphase nucleus play a role in the differential variation of the metabolic potential of the cell was postulated in 1948 [8]. It has now been shown that most processes determining the metabolic activity of the tissues of the body and adaptive behavior are also cyclic, and that the commonest duration of the period is close to 24 h [9, 11]. If it is assumed

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during the investigation of mechanisms of adaptation at the tissue level that cells with a similar type of condensation of their nuclear chromatin will be in a comparatively similar physiological state, the level of the metabolic potential of the tissue at different times of the 24-hour period and in different physiological states of the body can be judged from variation in the proportion of these cells in the system.

The method of recording physiological changes in the tissues relative to the proportion of cells with X-chromatin is widely used in experimental and clinical research [1, 2, 6, 10]. However, most cytogeneticists have been forced to interpret these and similar findings with great caution as a result of contradictions and disagreements in the experimental data [3, 6, 10]. Some workers have attempted to resolve these contradictions [6, 10]. Inter alia, they have studied the diurnal dynamics of buccal epithelial cells differing in the heterochromatization of their nuclei in observations in vivo [3, 4], in which a critical analysis was made by quantitative estimation of cells with X-chromatin (calculation of the sex chromatin index) and a new method was suggested (calculation of the X-heterochromatin index – X-HCI). By means of the present method it was possible for the first time to obtain satisfactorily reproducible results and to establish significant diurnal fluctuations of the proportion of cells with X-chromatin and the proportion of cells with compactly condensed nuclear chromatin (C-HCI) from 30 to 460‰.

In the present investigation the above method was used to analyze the systemic response of the buccal epithelial cells and their genetic apparatus to hormones in experiments in vitro, by taking cells from one donor and transferring them to short-term cultures at different successive times of the 24-hour period and under standard stereotyped external conditions. The state of heterochromatization of the cell nuclei was compared in vivo after incubation for 3 h with or without the hormone. Parallel observations were made on several individuals over a period of 1.5 days.

EXPERIMENTAL METHOD

Buccal epithelium from four clinically healthy women aged 25-45 years was used in the experiments. The program for the day was 8 h of sleep and 16 h of waking, with three meals at fixed times of the day. A standard stereotype of external conditions was maintained. Scrapings were taken from the whole of the surface of the mucous membrane of the cheek during the period of adaptation after intervals of 5, 7, 4, and 7 h. Buccal epithelium was obtained from both cheeks alternately at intervals of 2-4 h for 29 h and stained with carbol-fuchsin (control 1). Meanwhile some cells (7×10^3) were placed in medium 199 without hormone (control 2) and the same number of cells in medium 199 with hormone (experiment) in a final concentration of: insulin 3×10^{-2} IU/ml, ACTH 10^{-4} IU/ml, and thyroxine 10^{-7} IU/ml, The cells were incubated at 37°C for 3 h and films were stained with carbol-fuchsin. In each film the structure of the nuclei was analyzed in at least 1000 cells. Structureless nuclei, nuclei with a fine chromatin pattern but without X-chromatin, nuclei with a fine chromatin pattern with X-chromatin, and nuclei with compactly condensed chromatin (C-chromatin) were counted. The proportions of all four types of condensation of chromatin were calculated in the same way as the proportion of the total number of cells analyzed which contained X-chromatin was calculated (X-HCI).

It was considered that successive analysis of cells at different times of the 24-hour period in the same individual (cells of the same genotype) would enable the error of calculation of the proportion of cells (±r) to be taken as the mean static deviation from the absolute value of the proportion of cells in the analogous physiological state as expected at a particular time of day:

$$r = \pm\sqrt{\frac{r = \pm\sqrt{\%\text{ of cells with X-chromatin} \times \%\text{ of cells without X-chromatin}}}{\text{total number of cells analyzed}}}$$

Synchronous and heterochronous indices of X-HCI were compared in the experimental and both control series individually by means of the Fisher–Student criterion. Individual mean diurnal values of X-HCI were calculated in the experimental and control series ($\text{X-HCI}_{\text{mean}}$). To analyze the character of changes in the proportion of cells with X-chromatin after treatment with the hormone in different phases of the individual biorhythm of physiological activity of the tissue, Wilcoxon's nonparametric criterion of signs was used.

EXPERIMENTAL RESULTS

According to the experimental method, cells of the upper layer of the buccal epithelium which, as a rule, have completed their life cycle were absent from the films and cultures. Cells of the middle layers of epithelium had a varied nuclear structure: cells with a fine chromatin pattern typical of the interphase nuclei of this epithelium, cells with and without sex chromatin; cells with a dense chromatin pattern, in which the chromatin

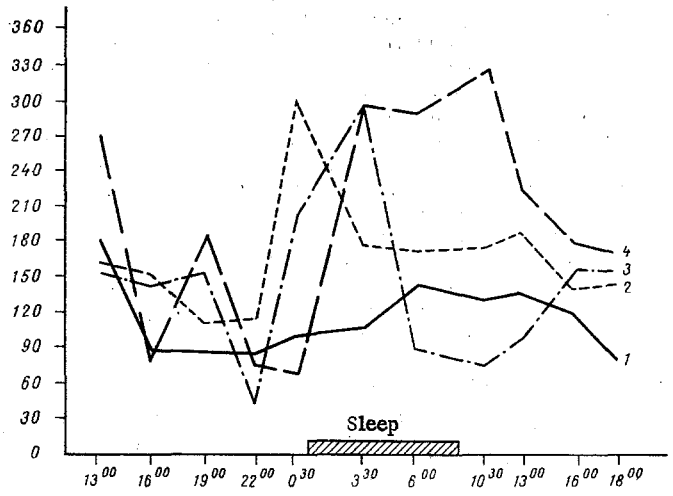

Fig. 1. Changes in number of cells with X-chromatin in buccal epithelium of subjects during parallel observation over 24-hour period. Here and in Fig. 2: abscissa, time of day (in h); ordinate, X-HCI (in ‰). 1) Subject Z., 2) subject V., 3) subject S., 4) subject E.

was concentrated in compact toroid formations (from 15 to 50 masses). Additional investigations (vital staining, examination of living and fixed material in phase contrast, autoradiography, time-lapse microfilming) revealed the viability of the cells mentioned above.

At different times of the 24-hour period the relative proportions of cells differing in heterochromatization of the nucleus were found to change. For instance, diurnal individual variations in the proportion of cells with X-chromatin and with compactly condensed chromatin ranged from 44 to 333 ‰, confirming previous observations [3, 4] on the presence of circadian rhythms of the proportion of cells with X-chromatin (Fig. 1). Statistically significant differences in extremal and mean-daily values of X-HCI and C-HCI were discovered in the course of the 24-hour period in all subjects both in vivo and in vitro ($P \leq 0.01$). The distribution of the extremal and mean-daily values of X-HCI over the 24-hour period was shown to differ individually.

On the basis of these and previous [3, 4] data it was concluded that single investigations of the proportion of cells with X-chromatin in a group of subjects followed by averaging of the results leads to obliteration of differences due to phase variations in individual biorhythms during the 24-hour period. The values of the mean group indices of the physiological state of the tissue in homogeneous groups now investigated and of other similar indices depend on the numerical ratio between people differing in the phase of their biorhythm. This is probably one of the main reasons for disagreement among results obtained by different workers for the X-chromatin level in the buccal epithelium of people belonging to analogous groups. On this basis, it seemed purposeless to continue to investigate the state of the body by means of the sex chromatin index when estimated once only in an individual in the course of the 24-hour period, followed by averaging in the groups of subjects to be compared.

Comparison of the values of X-HCI in controls 1 and 2 showed that transferring the cells into incubation medium in different phases of the individual biorhythm was accompanied by specific changes in the number of cells with X-chromatin: in one phase of the biorhythm the X-HCI index fell during incubation of the cells for 3 h, whereas in other phases it rose. Mean-daily values for the X-HCI index in culture were 15-66‰ lower than in vivo.

Differences in the mean daily values of X-HCI in control 2 and in the experimental series were found to differ individually.

The buccal epithelium of all subjects (people with different genotypes) in vitro gave a stereotyped response to hormones (ACTH, insulin, thyroxine) if treated with them in analogous phases of the biorhythm: at points characterized by minimal values of X-HCI in vivo a statistically significant increase in the number of cells with

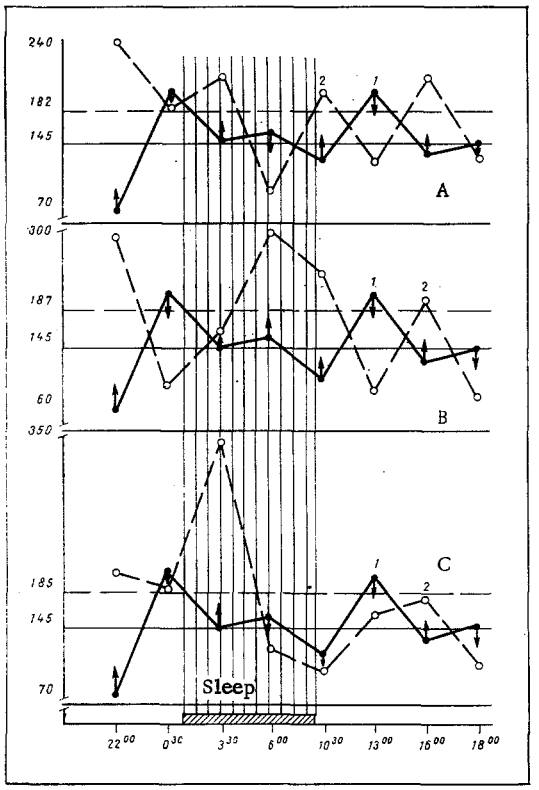

Fig. 2. Variation in number of cells with X-chromatin during incubation for 3 h with hormone at different phases of biorhythm of X-HCI in subject S. 1) X-HCI index in culture without hormone (control 2); 2) X-HCI index in culture with hormone (experiment): A) ACTH, B) thyroxine, C) insulin. Broken line represents $X\text{-}HCI_{mean}$ with hormone, continuous line $X\text{-}HCI_{mean}$ without hormone; arrows denote direction of reaction of change in number of cells with X-chromatin during incubation with hormone.

X-chromatin was recorded, whereas at points with maximal values of X-HCI in vivo a decrease was observed (Fig. 2). The reality of this stereotyped tissue response to stimuli of different nature was subjected to statistical evaluation. The number of maximal and minimal values of X-HCI was determined in vivo for an individual in the course of the experiment (29 h) and the percentage of points at which a significant fall or rise in the number of cells with X-chromatin in the culture was observed during treatment with the hormones was calculated. The coefficient of correlation between the character of the cell response to hormone in vitro and the number of cells with X-chromatin in the cell system in vivo before incubation was 0.92. A change in the level of cells with X-chromatin in culture with hormone in accordance with the established stereotype was recorded, for instance, in the case of subject E. at 91.6% of extremal points of the biorhythm of the X-HCI index, in subject S. at 80%, in subject V. at 77.7%, and in subject Z. at 46.6% of points. At some points the reaction appeared to be delaying in character (delay of the stereotyped response by 3 h). (Subject Z. took an active part in the observations and it may be that some of the differences in the reaction of her buccal epithelial cells are due to unavoidable disturbances of the program of observations.)

Besides changes in the number of fractions of cells with X-chromatin, changes were also observed in the number of other cell fractions of the buccal epithelium: the proportion of cells with compact heterochromatization of the nucleus and the proportion of cells with fine chromatization of the nucleus without X-chromatin. In each phase of the biorhythm in vivo and under the influence of hormones in vitro changes in the numbers of all the above-mentioned cell fractions correlated with each other and had the character of a systemic tissue

reaction reflecting automatic processes of adjustment of the level and character of the metabolic activity of the tissue. Such behavior of cells can be regarded as a reflection of adaptive mechanisms of changes in function of the genetic apparatus of cells in functional cell systems. The generalized stereotyped response of the cell system to treatment with hormones, characterized by a change in the number of the different cell fractions, must in all probability be classed as an automatic systemic tissue reaction, inducing or preparing the biological substrate for specific identification and response to factors differing in their chemical and physical nature. It can thus be concluded from these observations that the specific response to hormones is preceded by a nonspecific stereotyped tissue reaction which, on the one hand, is determined by the state of the biological substrate at the moment of treatment (the phase of the individual biorhythm of metabolic activity) and, on the other hand, leads to a systemic change in the population structure of the cell fractions responsible for the state of preparedness (competence) to recognize the stimulus and to respond adequately to it.

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