3. L. V. Kolesnikova and G. I. Nepomnyashchikh, in: Human Adaptation in Various Climato-Geographic and Industrial Conditions [in Russian], Vol. 5, Novosibirsk (1981), pp. 114-116.
4. L. M. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Heart [in Russian], Novosibirsk (1981).
5. V. S. Paukov and V. A. Frolov, Elements in a Theory of Pathology of the Heart [in Russian], Moscow (1982).
6. D. S. Sarkisov, Essays on the Structural Basis of Hemeostasis [in Russian], Moscow (1977).
7. V. A. Frolov and L. V. Efimov, Byull. Éksp. Biol. Med., No. 10, 20 (1982).
8. R. C. Aloia and E. T. Pengelley, Comp. Biochem. Physiol., 38A, 517 (1971).
9. R. W. Bullard and G. E. Funkhouser, Am. J. Physiol., 203, 266 (1962).
10. R. F. Burlington, W. D. Bowers, R. C. Daum, et al., Cryobiology, 9, 224 (1972).
11. A. R. Dawe and P. R. Morrison, Am. Heart J., 49, 367 (1955).
12. T. H. Rosenquist, Cryobiology, 7, 14 (1970).
13. L. A. Wells, Am. J. Physiol., 221, 1517 (1971).
14. M. L. Zimny, M. Sherman, and C. C. Romano, Cryobiology, 4, 317 (1968).

ANALYSIS OF THE CIRCADIAN RHYTHM OF MITOTIC ACTIVITY AND EPIDERMAL G_2-CHALONE CONTENT IN THE ESOPHAGEAL AND LINGUAL EPITHELIUM

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KEY WORDS: chalone; mitosis; rhythm.

The proliferative activity of cells of different tissues is known to vary during the 24-h period. A circadian rhythm of cell proliferation is also characteristic of the esophageal and lingual epithelium [2, 6, 11]. At the same time it has been shown that endogenous inhibitors of proliferation (chalones [7]) play an essential role in the regulation of tissue homeostasis. Epidermal chalones participate in control over proliferative processes in the epithelium of the esophagus and tongue which, according to N. G. Khlopin's classification, belong to the epidermal tissues. It has been shown, for instance, that this epithelium is sensitive to the action of chalones isolated from the epidermis [15, 16], and that they are involved in its synthesis [10, 16].

In the investigation described below changes in the content of epidermal G_2 chalone and mitotic activity in the esophageal and lingual epithelium during the 24-h period and correlation between the fluctuations of these parameters were studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 180-200 g, obtained from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR, and kept under conditions of natural illumination. The esophageal epithelium was studied at the time of the vernal, and lingual epithelium at the time of the autumnal equinox (in Leningrad). The content of epidermal G_2-chalone and the mitotic activity in the esophageal epithelium were determined after intervals of 3 h, and in the lingual epithelium at intervals of 4 h during the 24-h period (three animals were used at each point of the experiment). These parameters were analyzed in the same animals. Mitotic activity was estimated by calculating the mitotic index (MI) in promille in 5000 cells counted in histological sections of the esophagus and 4000 cells counted in sections of the tongue, stained with hematoxylin and eosin. The location of epidermal G_2 chalone in the esophageal and lingual epithelium was established with the aid of a monospecific immune serum by the indirect Coons' method [8]. Pieces of tissue for this purpose were frozen in liquid nitrogen, after which cryostat sections 6 μ thick were cut.

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Changes in the epidermal G_2-chalone content in the epithelium during the 24-h period were determined by a quantitative immunofluorescence method according to fluctuations in the intensity of fluorescence measured with the LYUMAM I-3 luminescence microscope, the FMEL-1A microfluorometric attachment with FEU-39A photoelectronic multiplier, the UBPV-1 dc source, and U5-9 amplifier. The voltage of the dc source was 1500 V and the input resistance to the amplifier 10^6 Ω. The intensity of fluorescence was measured in units of measurement of the voltage induced by fluctuations in strength of the current as a result of a change in the photic flux recorded by the photoelectronic multiplier. For more accurate measurement of the voltage fluctuations, a V7-26 voltmeter connected in parallel with the voltmeter of the matching unit of the U5-9 amplifier was used. As the standard of intensity of fluorescence, ZhS 19 uranium glass plates 1.5 and 0.03 mm thick were used. The field diaphragm of the microscope was shut down almost to the maximum to exclude fading of neighboring areas of the epithelium during photometry. Its position was unchanged throughout the investigation. Measurements were made with an objective giving a magnification of 10 times, by means of a probe 0.5 mm in diameter, so that the diameter of the area subjected to photometry was 50 μ. In all sections the background intensity of fluorescence of the tissues located near the epithelium was determined. Background fluorescence was not significant and was the same in all sections. Consequently, fluctuations of the intensity of fluorescence in the epithelium depended only on the quantity of test antigen contained in it. Differences between the data were analyzed by the Fisher–Student test and the level of significance was $P \leq 0.05$.

EXPERIMENTAL RESULTS

The results of investigation of the esophageal epithelium are given in Fig. 1. Mitotic activity of the cells of this epithelium reached a maximum at 4 a.m. In the course of the day the level of mitoses fell gradually to reach a minimum by 4 p.m. ($P = 0.03$). The concentration of epidermal G_2 chalone in the epithelium (cells of the stratum basale and stratum spinosum) was raised from 7 a.m. to 10 p.m. (to a maximum at 1 p.m.). It was lowest at 1 and 3 a.m. ($P = 0.05$). The phase diagram shows that the increase in the number of mitoses in the esophageal epithelium was preceded by a fall in concentration of the G_2-inhibitor. The peak of the number of mitoses was followed by an increase in the G_2-chalone content in the epithelium, which occupied much of the time of the passive phase of the mitotic rhythm. Correlation analysis of the data for MI and the G_2-chalone concentration in the two layers of epithelium gave significant coefficients of negative correlation on comparison of results separated by 3-hourly intervals during the 24-h period ($r = -0.53$ and $r = -0.57$). With longer intervals (6 and 9 h), and also when data for the same time of the investigation were compared, the coefficients of correlation were low ($r = -0.10$ and $r = -0.37$). It will be clear from Fig. 1 that the curves of the change in G_2-chalone concentration in the stratum basale and stratum spinosum of the esophageal epithelium were identical in character. At the same time, judging from the intensity of fluorescence, the concentration of G_2-chalone in the stratum basale and stratum spinosum was greater than in the higher layers.

During the investigation of the lingual epithelium, areas of epithelium including cells of the stratum basale and stratum spinosum were subjected to photometry. Epithelium covering the ventral and dorsal aspects of the tongue was examined separately. The curves showing changes in concentration of the inhibitor thus obtained completely repeated each other, but its concentration in the epithelium of the ventral aspect was lower than in the epithelium of the dorsal aspect. It will be clear from Fig. 2 that mitotic activity on the ventral aspect of the tongue reached a maximum at 6 a.m. and a minimum at 10 p. m. ($P < 0.001$). A low concentration of G_2-chalone was observed in the epithelium 4 h before the rise of MI in its circadian rhythm. The subsequent fall of mitotic activity took place at a time of high concentration of inhibitor, which reached a maximum immediately after the peak of mitoses ($P < 0.001$). A second, rather smaller rise in the G_2-chalone concentration in the lingual epithelium was obobserved at 10 p.m., when mitotic activity was minimal.

In both cases, investigation of the rat epithelium revealed a circadian rhythm of mitotic activity with a maximum in the morning, in agreement with data in the literature [4, 5]. Both in the esophagus and in the tongue, peaks of mitoses were preceded by a fall in their G_2-chalone concentration. The fall of its concentration was evidently the cause of the rise in mitotic activity. The increase in the G_2-chalone concentration correlated with the decrease in the number of mitoses in the rhythm. The data given above indicate that besides a circadian rhythm of changes in proliferation, there is also a circadian rhythm of chalone synthesis in the tissues. Circadian fluctuations in G_2-chalone production in Ehrlich's ascites tumor were discovered previously [3, 13].

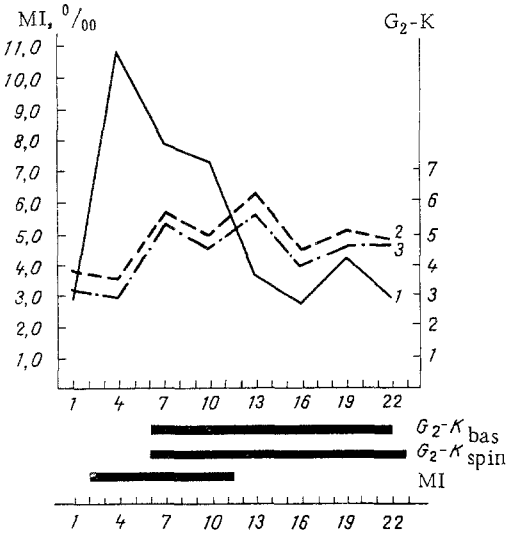

Fig. 1

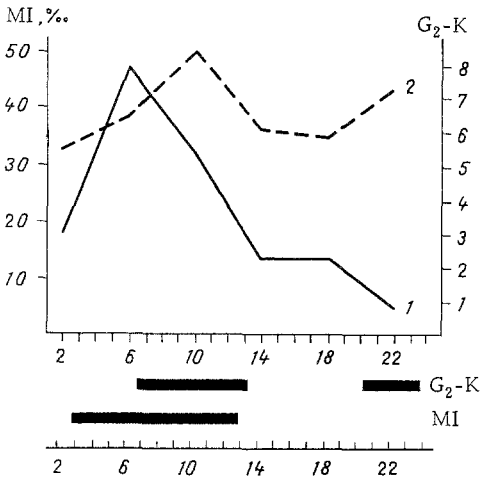

Fig. 2

Fig. 1. Changes in cell division and epidermal G_2-chalone concentration in rat esophageal epithelium during the 24-h period. Abscissa, clock time; ordinate: on left — MI (in promille), on right — G_2-chalone content (conventional units). 1) MI, 2) G_2-chalone content in stratum basale, 3) in stratum spinosum. Below — phase diagram of circadian rhythms of cell division and epidermal G_2-chalone concentration in esophageal epithelium of rats: abscissa, clock time (G_2-K_{bas} denotes G_2-chalone in stratum basale, G_2-K_{spin} denotes G_2-chalone in stratum spinosum); continuous lines show active phases of rhythms.

Fig. 2. Changes in MI and epidermal G_2-chalone concentration in rat lingual epithelium during the 24-h period. Below — phase diagram of circadian rhythms of cell division and epidermal G_2-chalone concentration in rat lingual epithelium. Remainder of legend as to Fig. 1.

In the present investigation the technique of quantitative immunofluorescence revealed reciprocal relations between the level of mitotic activity and the G_2-chalone concentration in the esophageal and lingual epithelium during the 24-h period. The inverse character of the rhythms of mitotic activity and epidermal G_2-chalone concentration in the esophageal epithelium also was observed when the quantitative immunodiffusion method was used [9], although the rhythms of changes of the two parameters themselves differed from those obtained in the present study. It can accordingly be concluded that epidermal G_2-chalone plays an essential role in the maintenance and control of the circadian rhythm of changes in mitotic activity of epithelial cells of epidermal type. The results thus confirm the views of several workers [1, 2, 14] that the circadian rhythm of mitosis is formed with the participation of chalones.

The results are evidence that changes in the G_2-chalone concentration in different cell layers of the epithelium and in different parts of the epithelium (ventral and dorsal aspects of the tongue) are similar in character during the 24-h period. It can be tentatively suggested that the concentration of the inhibitor, which is probably associated with changes in its synthesis, is subjected during the 24-h period to definite influences at the level of the whole organism and, in particular, from its endocrine system.

LITERATURE CITED

1. A. I. Antokhin, V. P. Barysheva, Yu. A. Romanov, et al., in: Synthesis and Mechanism of Action of Physiologically Active Substances [in Russian], Odessa (1976), p. 249.
2. A. I. Antokhin, M. V. Bogoeva, V. P. Rybakov, et al., in: Chalones: Their Importance and Role in Normal and Pathological Processes [in Russian], Moscow (1981), p. 81.
3. M. V. Bogoeva, A. I. Antokhin, and Yu. A. Romanov, Byull. Éksp. Biol. Med., No. 3, 343 (1981).
4. G. A. Vinogradova, Tr. Mosk. Med. Stomatol. Inst., No. 11, 10 (1967).
5. V. N. Dobrokhotov and A. G. Kurdyumova, Byull. Éksp. Biol. Med., No. 8, 81 (1962).

6. S. A. Ketlinskii, Arkh. Anat., 67, No. 11, 76 (1974).
7. S. A. Ketlinskii, Arkh. Anat., 80, No. 2, 29 (1981).
8. S. A. Ketlinskii and A. S. Simbirtsev, Arkh. Anat., 81, 58 (1981).
9. V. B. Okulov, in: Chalones: Importance and Role in Normal and Pathological Processes [in Russian], Moscow (1981).
10. V. B. Okulov and S. A. Ketlinskii, Arkh. Anat., No. 2, 84 (1977).
11. Yu. A. Romanov, I. K. Rakhmatullina, and M. N. Zaikina, in: Biology of Cell Reproduction [in Russian], Moscow (1972), p. 7.
12. V. P. Rybakov, Yu. A. Romanov, and A. V. Timofeev, Tsitologiya, No. 4, 401 (1979).
13. S. S. Filippovich and V. B. Zakharov, in: Mitochondrial Processes in the Temporal Organization of Vital Activity [in Russian], Pushchino (1978), p. 75.
14. A. I. Antokhin (A. I. Antochin) and Yu. A. Romanov (J. A. Romanov), in: Deutsch-Sowjetischen Symposiums "Chronobiologie—Chronomedizin," Vorträge, Berlin (1981), p. 543.
15. W. S. Bullough and E. B. Laurence, Exp. Cell Res., 33, 176 (1964).
16. E. B. Laurence, Natl. Cancer Inst. Monogr., 38, 37 (1973).

PHAGOCYTOSIS OF BACTERIA BY POLYMORPHS IN SUSPENSION OR ADHERENT TO A SURFACE

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The results of investigation of phagocytic activity of polymorphonuclear leukocytes (polymorphs) essentially complement other laboratory and clinical data and are now widely used to assess the condition of a patient and the prognosis of his disease [1, 4, 7]. There is no doubt that the closer the conditions of performance of the *in vitro* test to conditions actually created in the inflammatory focus, the more fully and precisely does the information thus obtained reflect the pathogenesis of the infectious process. Many different versions of the method of studying phagocytosis *in vitro* have been described. These versions differ in many respects, and this is reflected in the end result of analysis. Only those distinguishing features of the method which can affect passive or active movements of polymorphs will be discussed. Soviet workers usually allow the mixture of leukocytes and microorganisms to stand in a tube placed in an incubator [2, 3, 5, 6]. It is stated in one Western publication that if the test is carried out in this way the number of ingested bacteria is very small [15], and in order to increase the uptake of bacteria the tubes in the incubator are placed in an apparatus which spins them at a speed of 8-20 rpm [10, 12, 13]. In both these versions of the test the conditions created *in vitro* differ sharply from the situation in an inflammatory focus. In particular, so important a factor of the phagocytic reaction as chemotaxis cannot play its proper role, because polymorphs, which are capable of active movement only along a surface, and not of "swimming" either remain suspended in the fluid or are impelled by external sources in different directions.

In this investigation the level of ingestion of bacteria in the two versions of the technique described above was compared with their ingestion in a third version, which the present writers proposed, which provides for the possibility of chemotaxic movements of polymorphs.

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