PARENCHYMAL-STROMAL RELATIONS IN THE MYOCARDIUM OF HETEROTHERMAL ANIMALS DURING SEASONAL BIORHYTHMS

G. I. Nepomnyashchikh, L. V. Kolesnikova,
L. M. Nepomnyashchikh, and V. P. Tumanov

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KEY WORDS: seasonal biorhythms; myocardium of red-cheeked susliks; parenchymal-stromal relations; morphometry and stereology.

To study the seasonal variation in the onset and development of cardiovascular diseases it is necessary to investigate the mechanisms of adaptive modifications of the myocardium to various functional loads [4, 6], connected with seasonal factors. In recent years an attempt has been made to determine the seasonal characteristics of myocardial structure and metabolism in warm-blooded animals [5] and seasonal changes in myocardial contractility have been found to correlate with some parameters of metabolism in the injured heart [7]. Sudden fluctuations of cardiac function observed in heterothermal animals and established in the course of evolution [2, 11] are particularly interesting from this aspect. However, morphological studies of the myocardium of these animals at the tissue level are few [1, 3] and greater attention has been paid to the ultrastructural study of cardiomyocyte organelles [8, 10, 12, 14]. There have been only isolated investigations of the structural organization of the myocardium of red-cheeked susliks based on quantitative morphological methods [3].

The object of the present investigation was a morphometric and stereologic analysis of the tissue organization of the myocardium of the red-cheeked suslik in different seasons of the year in order to determine the effect of seasonal biorhythms on interaction between the muscular and connective tissues of the heart.

EXPERIMENTAL METHOD

The hearts of 20 red-cheeked susliks *Citellus erythrogenys*, caught in summer in Novosibirsk Region, were studied. The animals were kept in the animal house until the fall. At the end of September they were transferred to a special room where they were kept in individual cages throughout the period of hibernation. The ambient air temperature in the room was 3-5°C.

Depending on the conditions of keeping the animals were divided into the following groups: 1) active animals before hibernation (n = 6); 2 and 3) animals in a state of hibernation for 3 (n = 5) and 6 (n = ?) months; 4 and 5) animals active for 1 day (n = 3) and 14 days (n = 3) after awakening. The body temperature of the active animals was 37°C and of those in hibernation 8°C. In the course of the investigation susliks were decapitated: at the end of August in group 1, at the end of December in group 2, at the end of March in group 3, and at the beginning of April in groups 4 and 5.

After decapitation of the animals the heart was removed from the thorax and cooled quickly in a special chamber until it had completely stopped beating, when it was weighed. Specimens of papillary muscle were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4 solution, dehydrated, and embedded in Epon-Araldite. Semithin sections (0.5-1 μ) were cut on an LKB Ultrotome and stained with azure II. The morphometric investigation of the myocardium was carried out under a final magnification of 1030 times. The diameter of the cardiomyo-

Laboratory of Connective Tissue Pathology, Department of Pathomorphology and Morphometry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Laboratory of Histochemistry and Autoradiography, Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 95-99, October, 1983. Original article submitted March 25, 1983.

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TABLE 1. Results of Morphometric and Stereologic Investigation of Hearts of Red-Cheeked Susliks in Different Seasons of the Year (M ± m)

Parameter	Fall	Winter	Spring		
	active animal	animals in state of hibernation	animals in state of hibernation	1 day after reawakening	14 days after reawakening
Morphometric characteristics of heart and muscle fibers					
Weight of heart, mg	1658,3±114,3	1400,0±54,7	1167,0±33,3*	1333,0±33,3	1230,0±66,3*
Weight of left ventricle, mg	1189,5±88,1	985,4±34,7	823,3±49,4*	966,7±11,7	900,0±56,5*
Diameter of cardiomyocytes, μ	13,30±0,36	11,25±0,61*	10,92±0,04***	11,98±0,20*	12,92±0,03
Stereologic characteristics of myocardial parenchyma and stroma					
Relative volume (V_{V_i}, t), mm^3/cm^3, of:					
cardiomyocytes	855,0±11,4	883,0±10,9	860,2±6,4	879,2±5,9	866,0±7,8
sarcoplasm	831,6±8,9	860,0±11,8	839,8±5,4	858,4±6,2	844,3±6,8
nuclei	23,4±1,6	22,9±0,9	20,4±1,3	20,4±1,3	21,7±1,3
connective tissue	145,0±12,6	117,0±10,9	139,8±6,4	120,8±5,9	134,0±7,8
capillaries	61,7±4,2	32,7±1,7**	52,9±1,9	49,2±3,8	63,4±3,4
endothelial cells	14,3±1,0	19,0±0,3*	13,7±0,9	11,1±0,7	13,0±1,2
connective-tissue cells	5,6±1,0	6,0±0,8	8,9±1,2	8,5±0,7	6,3±0,9
fibers and ground substances	63,4±16,7	59,3±11,5	64,3±5,7	52,0±4,8	51,3±8,9
Surface density (S_{V_i}, t), m^2/cm^3, of:					
cardiomyocytes	0,148±0,009	0,132±0,002	0,148±0,009	0,131±0,004	0,138±0,007
nuclei	0,018±0,001	0,013±0,0008*	0,012±0,0008*	0,013±0,002	0,014±0,002
capillaries	0,035±0,001	0,032±0,005	0,033±0,004	0,037±0,001	0,038±0,001
connective-tissue cells	0,004±0,0007	0,005±0,001	0,008±0,0009*	0,008±0,002	0,007±0,001
Ratio of surface density of structures to their relative volume (S_{V_i}/V_{V_i}), m^2/cm^3, of:					
cardiomyocytes	0,174±0,019	0,149±0,001	0,172±0,012	0,152±0,005	0,159±0,008
nuclei	0,769±0,081	0,551±0,059	0,575±0,016	0,614±0,048	0,645±0,068
capillaries	0,591±0,073	0,963±0,133	0,632±0,094	0,762±0,089	0,600±0,026
connective-tissue cells	0,714±0,056	0,833±0,127	0,899±0,045	0,941±0,239	1,111±0,285
Ratio of bulk density of structures to relative volume of cardiomyocytes ($V_{V_i}/V_{V_{CM}}$), of:					
stroma to parenchyma	0,170±0,014	0,133±0,014	0,162±0,009	0,137±0,008	0,155±0,010
nuclei to cardiomyocytes	0,028±0,002	0,027±0,001	0,024±0,001	0,024±0,0005	0,026±0,002
connective-tissue cells to cardiomyocytes	0,007±0,001	0,007±0,001	0,010±0,001	0,010±0,0009	0,007±0,001
fibers and ground substance to cardiomyocytes	0,074±0,014	0,067±0,014	0,075±0,007	0,059±0,006	0,059±0,011
Ratio of densities of capillaries and cardiomyocytes					
bulk density of capillaries to bulk density of cardiomyocytes (number)	0,072±0,009	0,037±0,002*	0,061±0,002	0,056±0,005	0,073±0,004
surface density of capillaries to bulk density of cardiomyocytes, m^2/cm^3	0,041±0,003	0,036±0,006	0,038±0,004	0,042±0,001	0,044±0,001
surface density of capillaries to surface density of cardiomyocytes (number)	0,253±0,035	0,239±0,036	0,224±0,023	0,282±0,014	0,277±0,014

Legend. *P < 0.05, **P < 0.01, ***P < 0.001.

cytes was determined by means of a screw-operated MOV-1-15 ocular micrometer. An ocular test grid with short segments was used for stereologic analysis (n = 36; P = 72).

During the investigation of muscle tissue the volume and surface area of the cardiomyocytes and their nuclei and the relative volume of the sarcoplasm were determined. The parameters for the interstitial connective tissue were the bulk and surface density of the connective-tissue cells and the total relative volume of the fibers and ground substance. The microcirculation was evaluated by determining the relative volume of the endothelial cells and capillary lumen and the relative area of the inner surface of the capillaries. On the basis of these primary data, volume and surface-volume ratios were determined for the principal tissue components. The significance of differences between means was determined by Student's t test at the P < 0.05 level of significance.

EXPERIMENTAL RESULTS

Stereologic analysis of the myocardium of the red-cheeked susliks during different seasons of the year (Table 1) showed very small fluctuations in the relative volume of the cardiomyocytes, associated mainly with changes in bulk density of the sarcoplasm. In active animals before hibernation the bulk density of the sarcoplasm was 83.2% of the tissue volume.

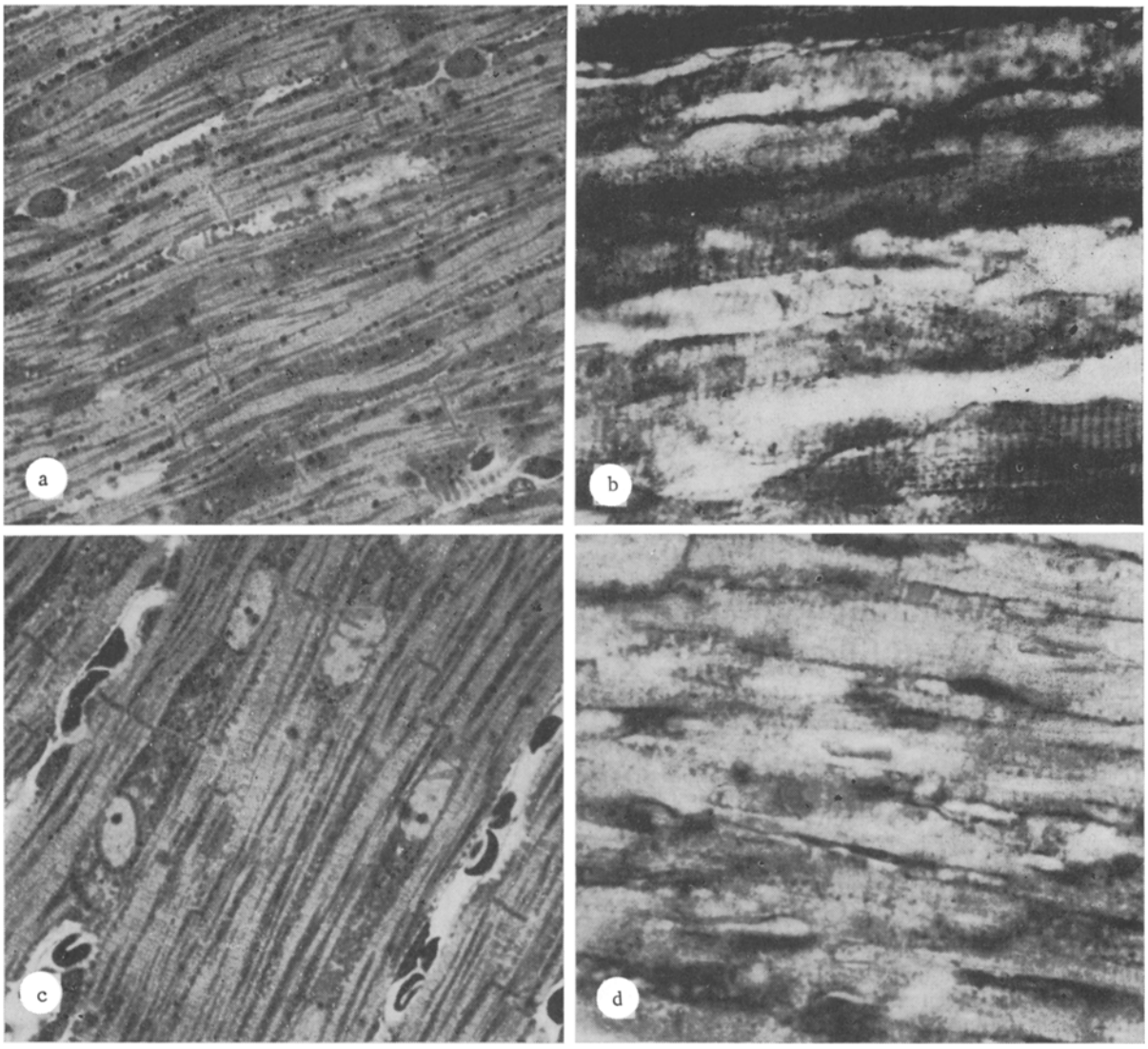

Fig. 1. Myocardium of red-cheeked susliks in different seasons of the year. a) Dense packing of organelles and numerous lipid drops, b) increased glycogen content in cardiomyocytes of animals during hibernation, c) loose packing of organelles and absence of lipid drops, d) moderate glycogen content in cardiomyocytes of active animals in the fall. a, c) Semithin sections, stained with azure II; b, d) PAS reaction, 1250×.

After 3 months of hibernation the relative volume of the sarcoplasm was increased by 3.4%. Under the light-optical microscope the myocardium of these animals (Fig. 1) showed a more compact arrangement of the organelles, and more numerous lipid drops and glycogen granules in the cytoplasm of the cardiomyocytes. In animals after 6 months in a state of hibernation, in which a significant decrease in the weight of the heart, the weight of the left ventricle, and the diameter of the cardiomyocytes was found, deviation of values of the bulk density of the sarcoplasm from this parameter in the active animals was minimal. On the first day of activity after reawakening the bulk density of the sarcoplasm increased again by 3.2%, but on the 14th day in the active state it was only 1.5% higher than initially. Changes in the surface density and surface-volume ratio of the cardiomyocytes in hibernating and active animals were not significant.

The relative volume of the cardiomyocyte nuclei was 2.7% of the volume of the cell and did not change significantly in the animals of any group. A decrease in surface density of the nuclei was found in the cardiomyocytes in animals hibernating for 3 and 6 months compared with that in active susliks before hibernation ($P < 0.05$), possibly in connection with a decrease in the nucleo-cytoplasmic flow of information during hypothermia.

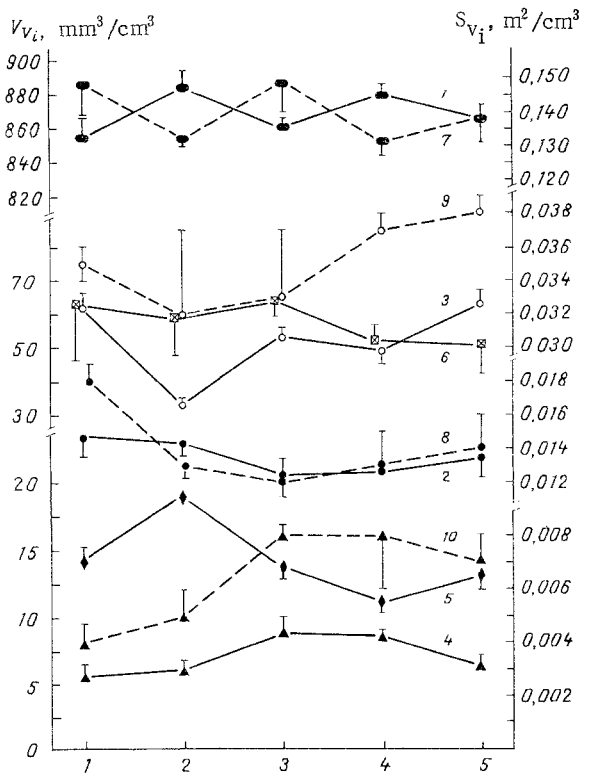

Fig. 2. Results of measurement of primary stereologic parameters of myocardial tissue structures of red-cheeked suslik in different seasons of the year. Abscissa, group of animals; ordinate: on left — bulk density, on right — surface density. 1, 7) Cardiomyocytes; 2, 8) cardiomyocyte nuclei; 3, 9) capillaries; 4, 10) connective-tissue cells; 5) endothelial cells; 6) fibers and ground substance of connective tissue.

The relative volume of the myocardial stroma of the red-cheeked suslik was stable in all the animals studied but the ratio between the components of the stroma varied significantly. For instance, if the total relative volume of the stroma was taken to be 100 in each group, the bulk density of the capillaries in the animals of group 1 was 42.6%, and in animals in a state of hibernation for 3 months it fell to 27.9%. At this same time a significant increase in the bulk density of the endothelial cells was observed. These changes in the microcirculatory system were evidently caused by a decrease in the velocity of the blood flow in hibernating animals [9, 13]. After 6 months of hibernation the bulk density of the capillary system was 37.8%, and reached 47.3% of the total volume of the stroma in susliks active for 14 days after reawakening. It is an interesting fact that the surface density of the capillaries was maintained at the same level in active and hibernating animals. The surface to volume ratio of the capillaries likewise did not change significantly, but a tendency was observed for this parameter to increase in animals hibernating for 3 months as a result of a disproportionate change in the bulk and surface densities of the capillaries.

The volume ratio of the capillaries and cardiomyocytes was significantly lowered only in animals in a state of hibernation for 3 months. It is important to note that the ratio of the surface density of the capillaries to the bulk density of the cardiomyocytes, and also the ratio of the surface density of the microcirculatory system and sarcolemma did not change significantly in active or hibernating animals, although for susliks hibernating for 3 and 6 months, the value of these parameters was characteristically rather less.

Stereologic analysis of the myocardium of the red-cheeked susliks showed a tendency for the relative volume of the connective-tissue cells to increase after 6 months of hibernation, together with a significant increase in their surface density. A somewhat higher percentage content of fibers and ground substance in the stroma of the organ was observed in animals in a state of hibernation for 3 and 6 months (50.7 and 46.0%, respectively), compared with this parameter in the active animals (43.7% in group 1, 43.0 and 38.3% in groups 4 and 5).

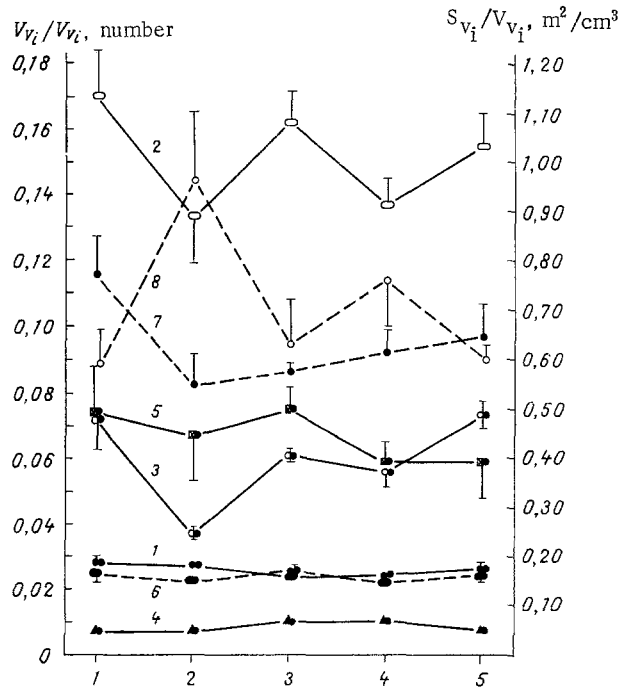

Fig. 3. Results of calculation of secondary stereologic parameters of myocardial tissue structures of red-cheeked suslik in different seasons of the year. Abscissa, groups of animals; ordinate: on left — volume ratio, on right — surface to volume ratio. 1) Nucleus/cytoplasm of cardiomyocytes; 2) stroma/parenchyma; 3) capillaries/cardiomyocytes; 4) connective-tissue cells/cardiomyocytes; 5) fibers and ground substance of connective tissue/cardiomyocytes; 6) cardiomyocytes; 7) cardiomyocyte nuclei; 8) capillaries.

The morphometric and stereologic investigation of the tissue organization of the myocardium of these heterothermal animals in different seasons of the year (Figs. 2 and 3) showed that the ratio of parenchyma to stroma did not change significantly in the active and hibernating animals. Stability of the parenchymal-stromal ratio was accompanied by considerable structural changes in the internal architectonics of the muscular and connective tissues.

In animals hibernating for 3 months the surface density of the cardiomyocyte nuclei and relative volume of the capillary system are reduced and the bulk density of the endothelial cells increased. These changes (together with tendencies for the ratio of surface density of the capillaries to relative volume of the cardiomyocytes to diminish) can evidently be attributed to the character of myocardial function in animals in a state of hibernation, i.e., under conditions of hypothermia, depressed basal metabolism, and utilization of only endogenous sources of energy.

The structural organization of the myocardium in susliks toward the end of hibernation and after reawakening, incidentally, does not differ significantly — the structural substrate is prepared for an increase in the functional load due to the transition of the animals into the active state. Evidently this transition to a different type of metabolism in hibernating animals [2], in which no serious imbalance takes place in the parenchymal-stromal relations, and which in turn does not require any additional expenditure of energy for restoring the structural substrate, was evidently developed in the course of evolution and consolidated by natural selection.

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LITERATURE CITED

1. L. N. Belov and L. A. Semenova, in: Hibernation and Seasonal Rhythms of Physiological Functions [in Russian], Novosibirsk (1971), pp. 56-68.
2. N. I. Kalabukhov, Hibernation in Animals [in Russian], Moscow—Leningrad (1936).

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

I. G. Torshina and A. S. Simbirtsev

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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.

Department of Biology, Medical-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. Laboratory of Experimental Histology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 99-102, October, 1983. Original article submitted February 18, 1983.

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