

---

## METHODS

---

# Histological Method for Evaluation of the Efficiency of Enerlit-Clima

D. V. Gol'dshtein, E. V. Vikhlyantseva, N. K. Sakharova,  
E. I. Maevskii, A. G. Pogorelov, and M. L. Uchitel'

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 8, pp. 233-234, August, 2004  
Original article submitted April 8, 2004

---

We propose a method of evaluation of antilimacteric efficiency of a drug by its effect on the estrous cycle. The study was carried out on 9-month-old mice with retained, but notably reduced reproductive function. Analysis of the cell components of the estrous cycle was carried out on histological preparations of vaginal smears.

---

**Key Words:** *climacteric; estrous cycle; estrus; SHK mice*

Creation of drugs correcting the course of the climacteric period, the onset of which is characterized by disorders in the estrous cycle (EC), is an important problem of modern medicine. The solution of this problem involves the development of adequate methods for preclinical studies of prospective drugs [2]. Laboratory mice are a very perspective object for the studies of the correction of reproductive disorders [1, 3,4]. Short EC (4-5 days) makes them a convenient model for studies of factors regulating the course of the climacteric period.

Female mice reproduce the progeny most actively at the age of 2-6 months, while by 8-9 months their reproductive capacity decreases. EC consists of 4 main stages. The indicator of the female status is the ratio of the mean duration of the estrus period to the duration of the total EC. Each stage is characterized by a certain ratio of different types of cells in the vagina. Therefore analysis of histological preparation of vaginal smears helped to identify the stage of EC.

## MATERIALS AND METHODS

Female SHK mice were kept 10 per cage under standard conditions (20±2°C, PK-121-2 fodder, Informkorm

Firm) and had free access to water. Adult (5-6 months) and old (9-10 months) animals received 0.1 ml water suspension of Enerlit-Clima nutritive (25 mg/kg) twice a day through a gastric tube. Age-matched controls received water. The experiment was performed for at least 5 weeks (7 estrous cycles).

EC stage in animals was evaluated by changes in cell composition of vaginal smears. To this end, 0.9% NaCl was pipetted into the vagina, after which the fluid was sucked into the pipette. A droplet of the sample applied on the slide was dried at 40°C and fixed in a 3:1 mixture of absolute ethanol and glacial acetic acid, after which the preparation was washed in 70° water-ethanol mixture and stained with Azur-eosin by the method of Romanowskii—Giemsa.

The preparations were dehydrated in two portions of acetone, acetone—xylene mixture (1:1), and two portions of xylene, and embedded in Canadian balm. Histological preparations were examined under a Peraval Interphako light microscope (Zeiss) fitted with a WAT-505ex (Watec) digital camera. The image was visualized on an IBM PC monitor by means of VideoIn software.

## RESULTS

Estrus phase can be easily identified by vaginal smears. The smears collected during this phase contain mainly

---

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino. **Address for correspondence:** pogorelov\_a@mail.ru, pogorelov@iteb.ru. Pogorelov A. G.

**TABLE 1.** Morphological Characteristics of EC in Mice of Different Age Orally Treated with Enerlit-Clima Water Suspension or Water (Control)\*

| Parameter          | Age            |                   |                |                   |
|--------------------|----------------|-------------------|----------------|-------------------|
|                    | 5-6 months     |                   | 9-10 months    |                   |
|                    | control (n=12) | experiment (n=11) | control (n=10) | experiment (n=10) |
| EC, days           | 8.4±2.2        | 7.2±0.5           | 8.3±1.1        | 8.2±1.3           |
| Estrus stage, days | 2.8±0.7        | 2.7±0.6           | 1.5±0.5        | 2.7±0.5           |
| Estrus/EC          | 0.33           | 0.38              | 0.18           | 0.33              |

**Note.** *n*: number of animals in the group. \*The data are presented as the mean and standard deviation (mean±s.d.).

anuclear epithelial cells collected in groups. The duration of estrus in older mice was appreciably shorter (Table 1), which was due to climacteric changes in EC of these animals. Long-term treatment with Enerlit-Clima rejuvenated the body and normalized the duration of the estrus stage.

More intricate effect of the preparation was observed during the entire EC. Its duration is known to increase with age, but treatment led to an “opposite” effect in adult females aged 6-7 months: EC characteristic of young animals (7.2 days) was restored and

stabilized. On the other hand, Enerlit-Clima did not change the EC of animals of the oldest age group.

## REFERENCES

1. *From Modern Fundamental Biology to New Scientifically-Based Technologies* [in Russian], Pushchino (2001).
2. *Manual of Experimental (Preclinical) Studies of New Pharmacological Substances* [in Russian], Moscow (2000).
3. Y. Gao and R. V. Short, *J. Reprod. Fertil.*, **101**, No. 2, 477-481 (1994).
4. H. V. Ratajczak, P. T. Thomas, J. Gerhart, and R. B. Sothorn, *In Vivo*, **9**, No. 4, 299-304 (1995).