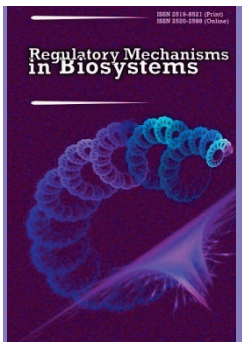

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Treatment of burns using polyethylene-glycol-based drugs: Dynamics of regeneration at the biochemical, cytological, histological, and organism levels of organization

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Every year, up to 11 million burns are recorded. They are first among all traumas, leading to over 300,000 deaths around the globe every year. Burns caused by fire are also one of the main causes of deaths and disability-adjusted life years in countries with low and average levels of income. This research analyzed an experimental modeling of burn treatment using anti-burn drugs. Laboratory guinea pigs were traumatized with 2–3 degree burns with the burnt area of 15–20% of the skin surface. We compared the therapeutic efficacies of the experimental drug based on polyethylene glycol and the officinal medicinal drug – the ointment Pantestin. We assessed pathophysiological and pathomorphological changes over the process of burns, microbial landscape on the skin and in the microbiome of the internal environment of the guinea pigs. The most effective drug was the experimental anti-burn ointment based on polyethylene glycol. On the third day of the experiment, the Pantestin drug ensured the survival of 14.7% of the experimental animals compared with 57.1% survival using the ointment of the experimental drug and 100% death of the control animals that received no anti-burn therapy. The dominant bacterial pathogens that induce pathogenesis of the burn process are purulent-necrotic and toxicogenic ubiquitous prokaryotes *Pseudomonas aeruginosa*, hemolytic capsular variant of *Escherichia coli*, and *Staphylococcus aureus*. From the burn wound, various prokaryotic microflora were isolated, and since day three after the infliction of the burn, in microbiome of the large intestine, no more indigenous bioindicators of the macroorganism's physiological wellbeing – *Aerococcus viridans* and *Mycobacterium vaccae*, were isolated against the background of rapid decrease in isolation of lactobacteria, bifidobacteria, and saccharolytic yeasts.

Keywords: metabolism; pathomorphology; microbiome; burn-related stress; guinea pigs; *Mycobacterium vaccae*.

Introduction

In the natural environment, mammals are in the conditions of uncertainty and variability of various abiogenic and biogenic stress factors that vary in severity. Some of those effects can cause morphofunctional metabolic disorders in the macroorganism of such intensity that force majeure circumstances of the stress factor will bring the vital functions on the verge of incompatibility with biological existence, or lead to loss of potential ability to adequately react to physical, mechanical, or microbial challenges to the external or internal environment.

During ontogenesis, the macroorganism of mammals is subject to permanent polyetiological stress impact of external and internal environments. Combined action of damaging stress factors activates starting from the neonatal period and does not stop until the terminal stage of the macroorganism's existence as a genetically determined biomodel that is able to adapt, vertically transfer genome, and undergo Darwinist evolution at the population level (Greenhalgh et al., 2007). Various environmental factors impact the general biological functions of the macroorganism, provoking a response, and also act in associate combinations during life, i.e. polyethiological stress is an integral component of existance of living creatures. If an influence of external or internal factors is too intensive, the adaptative-compensatory mechanism of a macroorganism enters the stage of maladjustment, with irreversible pathophysiological consequences and death, or ruination of the organs' functional activity (Peck, 2011; Gortazar et al., 2015; Zazharskyi et al., 2020; Popov et al., 2021).

First of all, a macroorganism reacts to all effects of the environment through the skin tissues, i.e. changes in the skin and mucous membranes. First of all, those are external barriers that separate the internal environment of a macroorganism from the outer world (Thabet et al., 2008). Vitality of an organism without integrity and functional competence of those anatomic-physiological barriers is impossible. Besides the anatomic-physiological integrity of the skin tissues, a principally important role (Peck, 2011) is played by the indigenous resident microflora of the skin and mucous memebranes of open cavity organs – the gastrointestinal tract, respiratory system, and urogenital organs.

The skin is quite a large and complex organ that separates the internal environment from the external one and plays a leading role in supporting and regulating the genetically determined homeostasis of the macroorganism. Other than mechanical protection from physical-chemical damages and microbial aggression, thermal regulation, sensory, metabolic, and excretory functions, the skin – together with the general biological non-specific factors of immune reaction of the mucous membranes – is the first

link in the cell-mediated recognition of alien genetic materials and immune protection. Various types of damage to this barrier – mechanical, physiccal-chemical, radiational, or microbial – can lead to heavy consequences for the functioning of the entire organism. One of the most dangerous types of traumatic impact on the skin and macroorganism is burns. The impact of open flame on living objects is incompatible with the vitality of biological systems. Fire comprises heated gases formed during fast chain isothermal chemical reaction of oxidation of a combustible substrate. Fire has characteristics of low-temperature plasma, contains charged ions and solid unburned, and also heated particles. It catastrophically ruins the structure of living tissue, with fatal consequences for the microorganism. Direct contact with an open flame inflicts severe, extreme, and heavy degeneration-necrotic changes, mediated by local and general intoxication and toxemia, suppression of and non-physiological changes in metabolic processes against the background of disintegration of neutrophilic relations, with fatal disorders in the regulatory activity of the nervous system, inhibition of the functions of the immune reactions of antigen-recognizing lymphoid organs, and non-specific immune-biological reaction at local and organism levels. The impact of flame is accompanied by decreased efficiency of anti-infection protection, with induction of purulent-necrotic septic complications (Church et al., 2006; D'Avignon et al., 2010; Sheppard et al., 2011).

On the outer surface, and in the sweat and sebaceous glands, there are various microorganisms – transitory, conditionaly pathogenic, including resident. At first, after infliction of a burn, the local saprophytic and conditionally pathogenic microflora in deep layers of the affected skin continues to live. Emergence of a burn scar and detritus of the tissues create conditions for those microorganisms to intensively breed and transition into the exponential stage of development. Therefore, a burn must be immediately treated with ointments with broad range of antimicrobial action and wound-healing abilities (Branski et al., 2009; Kennedy et al., 2010; Hall-Stoodley et al., 2012; Kallstrom, 2014).

The tissue damage in the region of burn is a nutritional substrate for the development of invasive mixed infection of the wound. Injury of the skin tissues and regional hypermetabolic syndrome causes a significant microbial infection of the wound field, which performs a role of "entrance gate for infection" with development of complex of symptoms of general immunosupression as a result of generation of immunodepressive and toxic substances by the tissues of the burn wound. Microbial contamination of burn wounds in the first hours of infectious process is usually low, accounting for only 10^{1-2} CFU/g of the tissue, but later on, there the number of microorganisms can increase rapidly, reaching 10^{3-4} CFU/g already on the second-third day, and on the fifth-sixth days accounting for 10^{5-6} CFU/g, and in severe cases even up to 10^{7-8} CFU/g of the tissue. This is a critical number of microorganisms, indicating a dysfunctional state of the regional non-specific resistance. Uncontrolled increase in microbial loading intensifies the invasion of deep layers of injured region by microorganisms, inhibits demarcation processes, and complicates the development of the septic-pyemic process (Thabet et al., 2008; Rowley-Conwy, 2010; Sheppard et al., 2011; Rafla & Tredget, 2011).

Thermal trauma negatively impacts not only the qualitative composition of the microflora of the burnt region, stimulating breeding of selective variants of conditionally pathogenic and purulent-necrotic microbiota, but also the general microbial landscape of the resident microbiom. Acute exhausting stress of thermal trauma entails metabolic changes in the macroorganism and reproduction of microorganisms that always exist in the normal living conditions. Such reference prokacryotes of biological well-being are *Aerococcus viridans* and *Mycobacterium vaccae* (Thabet et al., 2008; Biben et al., 2019; Kassich et al., 2019). Subject to non-physiological changes, there occurs intensification, i.e. triggering of the genetic potential of pathogeneity, initiation of genetic modifiers of pathogenic factors, with increase in clones that are able to aggressively colonize and destructively compete for existance, both inside species and among species. This promotes increase in the number of clones of conditionally pathogenic prokaryotes. Decrease and even complete loss of physiologically beneficial prokaryotes are prognostically and physiologically unfavorable indicators that require correction by respective microbial drugs along with the main course of treatment of the burn and general damages to the macroorganism. Local treatment of a burn wound infection against the background of general physiological effect on metabolism with recovery of the resident microbiome is a relevant issue in modern veterinary and humane medicine, microbiology, immunology, and biotechnology (Branski et al., 2009; Rafla & Tredget, 2011; Kallstrom, 2014).

Development of new methods of experimental modeling of skin burns of experimental animals in order to study peculiarities of tissue repair and introduction of effective ways of therapy is a highly relevant research direction. Experimental studies of the dynamics of development of burns, processes of wound healing, and pre-clinical trials of new drugs cannot be carried out on cellular cultures, since burn causes metabolic and immunological changes in the whole organism. Furthermore, a dangerous complication of large deep burns is the burn pathophysiology, which develops as a resut of ruination of the tissues and causes a significant mortality rate. It occurs due to disorders of the central hemodynamics and microcirculatory disorders as a result of initial hypovolemia, stress reaction, and mass release of cytokines (Davenport et al., 2019).

A number of studies (Foubert et al., 2018; Ajit et al., 2023) suggest that using populations of autological cells of fatty tissue improves results in various pre-clinical models of thermal burn. However, those studies were confined to a relatively small degree of damage, accounting for ~2% of the total body surface area (TBSA), with no complications associated with large burns (for example, systemic inflammation and the need in fluid resuscitation). Intending to apply this approach to a clinical trial that would include those complications, other authors used a pre-clinical model that more resembles a patient with a large thermal burn who requires skin transplantation (Foubert et al., 2018; Ajit et al., 2023).

The objective of the study was to characterize the pathogenesis and microbiome of the organism of guinea pigs subject to burns, describe the reparative-recovery cellular and tissue processes in the damaged region based on histological changes and metabolic transformations according to the results of screening blood parameters.

Materials and methods

Modelling of early burn infection in the laboratory animals. The study was performed at the Scientific Research Laboratory of the Department of Infectious Diseases of the Dnipro State Agrarian-Economic University and the Research Center of Safety and Ecological Control of Reasources the Biosafety-Center Agroindustrial Complex. During the studies, the authors adhered to the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), considering the General Ethical Principals of Experiments on Animals, adopted at the National Congress of Bioethics (Kyiv, 2001), the requirements of the Law of Ukraine On Protection of Animals from Abuse (Kyiv, 2006), the requirements of the Directive of the EU 2010/63/EU as of September 22, 2010. The program of studies was approved by the local ethic committee of the Faculty of Veterinary Medicine of the Dnipro State Agrarian-Economic University (Protocol No. 2 as of March 12, 2023).

For the study, we used an experimental drug, whose activity was compared to the officinal ointment Pantestyn, manufactured by Darnytsia (Ukraine), which was chosen as the most widely used drug to treat patients with burns.

The content of Panthestin is as follows: dexpantenol (D-pantenol) 50 mg, myramistyn 5 mg; additional compounds: propylene glycol, polyethylene glycol (macrogol 400), poloxomer, cetyl alcohol, sterile alcohol, and purified water.

The experimental drug consists of ionol – 25.0 g/L, dimethylsulfoxide – 37.5 g/L, polyethylene glycol PEG 400 – 230.0 g/L, PEG 1,500 – 540.0 g/L with the drug form of soluble preparation of gel-like consistency.

For 14 days, the animals were quarantined (according to the sanitary rules of the "Structure and Maintenance of Experimental Biological Clinics", Order of the Ministry of Healthcare of Ukraine No. 755 as of August 12, 1997) with further adherence to the standard water-fodder diet with free access to water and food, taking into account the norms of maintenance (amendments as of December 04, 1997 of the Order of the Ministry of Healthcare of Ukraine No. 163 as of March 10, 1996 On the Daily Norms of Feeding Laboratory Animals and Primary Producers). For the experiment, we used the model of contact-caused thermal burn in the mo-

dification of V. V. Minukhin et al. and the model of infected burn wound. The device for modeling burn injury was applied to the skin region on the side of the body, which was previously trimmed and underwent depilation and anesthetized using local infiltrational anesthesia (0.5% Novocain solution). The diameter of the contact plate was 25 mm, and the exposure lasted for 5 seconds. As a result of this procedure, the guinea pigs were subjected to a third-degree burn wound, which was confirmed by the subsequent histological studies of the affected skin loci. The size of the burn trauma was measured using the Maye-Rubnera formula and this area equaled around 10–15% of the general body surface, on average 79.1 ± 4.9 mm^2.

The study was conducted on 28 guinea pigs – randomized non-pedigree males with the live weight of 210–230 g. Four groups of animals (7 animals in each group)were formed:

– Group I – experimental (burn and treatment using the experimental ointment);

– Group II – experimental (burn and treatment with the Pantestin ointment);

– Group III – control (intact animals – clinically healthy, with no burn and treatment, C+);

– Group IV – control (burn with no treatment, C –).

The experimental and control drugs were applied to the wound for 28 days.

The control of treatment efficacy – weighing, studying the dynamics of changes of morpho-biochemical and histological parameters of the guinea pigs after inflicting burns – was carried out in the following dynamics: prior to burn, every day for the first week, and also on days 14, 21, and 28 of the experiment.

To assess the condition of the wound surface, we studied the periods when the wound was cleared from purulent-necrotic masses, time of emergence of granulation, and complete epithelization of the wound surface.

Morpho-biochemical blood assays. Assays of biochemical blood parameters were performed using the photometers Microlab-200 (International Microlab, Shenzhen, China, 2021) and Vitalab Eclipse (Merck, Netherlands, 2011) with the software after the reaction took place using respective diagnostical test kits manufactured by Lachema (Erba Lachema, Karásek, Czech Republic, 2021).

Screening of metabolic changes in the physiological condition of the macroorganism of the experimental guinea pigs subject to extreme stress factor and in the control was conducted using the standardized buiochemical methods in manual and instrumental regimes: total protein was measured using the biuret method, protein blood fractions according to the reaction with bromocresol green (albumin fraction), content of globulins and protein coefficient by estimation, content of aspartate and alamin aminotransferase by the Reitman–Frenkel method, content of creatinine by the Popper's method, and content of uric acid by the reaction with Folin-Ciocalta reagent.

The number of erythrocytes and leukocytes in the blood was identified by estimating the formed elements in the grid of the Goryaev's chamber. Concentration of hemoglobin was identified using the hemoglobincyanide method. Leukogram was estimated in smears of blood, prepared by Pappenheim's method.

In the blood serum samples, we identified: urea – enzymatically using urease with the Berthelot's reaction, glucose – enzymatically with glucose oxidase, followed by Trinder's reaction, total calcium – by reaction with arsenazo III, inorganic phosphorus – by reaction with ammonium molybdate, creatinine – kinetically by rates of increase in intensity of staining in reaction with picric acid, uric acid – by the urease test. The studies were conducted using an automatic biochemical analyzer Miura-200 (I.S.E. Srl, Milan, Italy, 2020). When measuring the biochemical parameters, we used ready-to-use reagents manufactured by Dialab (Wiener Neudorf, Austria, 2020), Spinreakt (Girona, Spain, 2019), and Cormay (Lublin, Poland, 2020).

Using an authomatic analyzer Mindray BS-230Vet (Mindray, China, 2016), in the blood serum we also determined the activity of amino transferases (alanine aminotransferase, ALT, and aspartate aminotransferase, AST) by the kinetic method, based on the Warburg optical test (using the Spinreakt reagents (Girona, Spain)), and also alkaline phosphatase by the rates of formation of 4-nitrophenol (Cormay, Warsaw, Poland).

Content of lipoproteids was examined using the turbidimetric method of Burstein-Samaille measuring the absorption of the solution on an Ulab 102 spectrophotometer (Haimlaborreaktiv Ltd, Brovary, Ukraine, 2016).

Cultural-bacteriological studies of the microbial landscape of the wound surface and microbiome of the internal environment. Bacteriological isolation of the gut microbiota and the skin tissues were performed using the generally accepted methods of indication and identification of sanitary-demonstrative and conditionally pathogenic microflora, pathogens of lactic-acid fermentation and yeasts, and also indigenous microbial prokaryotes – indicators of the physiological wellbeing of macroorganisms, such as *S. viridans* and *M. vaccae*. The pure cultures of isolated microorganisms were identified using Bergey's Manual of Systematic Bacteriology (2009).

The field culture *S. viridans* was isolated using inoculation of biomaterial (suspension of the guinea pigs' feces) onto indicatory medium of the following composition: KJ – 30.0 g, soluble starch – 10.0 g, meat-peptide agar – 30.0 g, water – 1,000.0 mL (Zazharskyi et al., 2021). Sterilization was carried out through an autoclave for 30 min at 121 ºC. In positive cases, the intensively blue-stained colonies with distinct morpho-tinctorial properties were first inoculated on enriched regular meat-peptone broth (MPB) based on Hottinger agar and cultivated at 37–38 ºC for 2–3 days, then transfered to simple media (Tkachenko et al., 2016). The morpho-tinctorial properties and bacterial purity of the isolated culture was studied by staining the smears according to Gramm and Romanowsky-Giemsa, and the pathogenicity was identified by subcutaneous infection with 1.0 cm^3 of daily broth culture of 4 white mice of 18–20 g live weight.

Bacterial control of presence of *M. vaccae* in feces of the guinea pigs was performed using the Gon method with 15% sulfuric acid with the exposure of no less than 30 min and three-times rinsing with sterile isotonic solution (Zazharskyi et al., 2020). The suspension from supernatant was inoculated onto the Lowenstein-Jensen media into test tubes with rubber caps. The cultivation lasted for a month at 37–38 ºC. The smears were stained using the Ziehl-Neelsen method. Non-pathogenicity of the isolate was determined in biosamples of 4 white mice of 18–20 g live weight with intra-abdominal infection with 10 mg of microbacteria culture (Zazharskyi, 2019).

To isolate sanitary-indicative, indigenous, and conditionally pathogenic microorganisms, we made inoculations of 0.1 cm^3 of liquid biomaterial onto differentiation-diagnostic media (meat-peptone broth, meat-peptone agar, meat-liver-peptone broth, 5% blood meat-peptone agar, yolk-saline meat-peptone agar, Hugh-Liefson medium, Endo's medium, Blaurock medium, MRS-4 medium, Saburo's agar) (Prajapati et al., 2023). The inoculations were incubated in a stationary thermostat at 37–38 °C for 24–48 h. The quantitative characteristics of the isolated cultures were determined using the Lindsay method (Shelkova & Prokopets, 2008).

Planimetric method. Planimetrics of the wound surface was conducted taking into account the general area of the injury (mm^2). The rates of wound healing were assessed using average-rate decrease of the wound surface (mm^2 per day) and reduction of the wound area (percentage a day) using the L. M. Popova's test, which is based on measuring the wound area in dynamics (Cherniakova, 2017). A sterile sheet of cellophane was applied to the wound, and its contrours were traced using a marker. Then, the cellophane with countour was put on millimeter paper and the area of the wound was identified by estimating the number of square millimeters inside the contour.

Histological studies. The material for the study was burnt skin parts of the guinea pigs in dynamics (prior and after burn – days 7, 14, 21, and 28), fixated in 10% aqueous solution of formalin for 24 h. To obtain histological preparations, the organs were engulfed in paraffin according to the generally accepted methods (Liu & Xu, 2011). From the paraffin blocks, on a sledge microtome MC-2 (Medlife, Kharkiv, Ukraine, 2002), we prepared 7–10 μm thick histological sections for visual preparations, with their further staining with hematoxylin and eosin according to the generally adopted methods (Liu & Xu, 2011). Microscopic studies of the histological preparations were carried out using a light microscope Micromed XS-3330 (Ningbo Shengheng Optics & Electronics Co, Yuyao, China, 2020). The histopreparations and their separate regions were photographed using a Micromed MDC-500 camera (Ningbo Shengheng Optics & Electronics Co, Yuyao, China, 2019).

Statistical analysis of results. The study results were processed using the software BioStat LE (AnalystSoft Inc., Walnut, USA, 2019) and Medcalc (MedCalc Software Ltd, Ostend, Belgium, 2016). Statistical analysis was performed using the standard methods of variation statistics. The samplings were compared using the Tukey Test prior to the analysis (ANOVA) with the Bonferroni Correction. The data in the tables are presented as average ± standard deviation (x ± SD).

Results

Morpho-biochemical studies of blood. The burn infliction was extremely distressing for the animals: this was a strong psycho-physiological trauma and above-threshold pathological impact on the guinea pigs. The animals were whimpering, trembling for a long time, refused juicy fodders, and were in an alarmed state and stupor for the first day. Right after the burn, the guinea pigs of the first and second groups were treated with ointments: animals of the first group received the experimental drug, while those of the second received the Panthestin ointment. Animals of the fourth group were not treated. First, in the region of the burn, there was reddening of the skin in the zone of contact with flame. Then, during the first hour, there emerged edema and the reddening spread. During the experiment, the number of guinea pigs who died in the first, second, and fouth groups increased (Table 1). Causes of death were associated with post-traumatic shock and burn intoxication. On the second day after the burn, survival of the guinea pigs in group I (100.0%) was higher than in the animals of group II (57.1%) and group IV (28.6%). On day 7 of the experiment and until the end, survival in group I remained at the level of 42.9% (Table 1). The dynamics of body mass of the animals in the period of study indicated inhibition of the development of the guinea pigs subject to burn-related stress (Table 2).

Table 1
Survival of animals during the monitoring (n = 7)

Group of animals	Period of monitoring, days/survival, %						
	1	2	3	7	14	21	28
I (experimental drug)	7/100.0	7/100.0	4/57.1	3/42.9	3/42.9	3/42.9	3/42.9
II (Panthestin)	7/100.0	4/57.1	1/14.3	0/0.0	0/0.0	0/0.0	0/0.0
III (C+)	7/100.0	7/100.0	7/100.0	7/100.0	7/100.0	7/100.0	7/100.0
IV (C–)	7/100.0	2/28.6	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0

Table 2
Dynamics of body mass of guinea pigs during the monitoring (x ± SD, n = 7)

Group of animals	Period of monitoring, days/					
	prior to burn	3	7	14	21	28
I (experimental drug)	225.3 ± 21.1^{a}	228.4 ± 24.3^{a}	236.7 ± 24.2^{a}	251.4 ± 21.8^{ab}	284.7 ± 26.6^{b}	298.7 ± 25.9^{b}
II (Pantestyn)	232.4 ± 18.6^{a}	226.2 ± 21.8^{a}	219.3 ± 21.7^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
III (C+)	241.1 ± 23.4^{a}	247.5 ± 22.3^{a}	251.2 ± 28.4^{a}	272.6 ± 23.5^{ab}	295.2 ± 24.7^{b}	304.7 ± 28.1^{b}
IV (C–)	231.6 ± 24.2^{a}	198.1 ± 23.4^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}

Note: different letters indicate samplings in a line, which were significantly ($P < 0.05$) different from another according to the Tukey test.

Table 3
Morpho-biochemical changes in blood of guinea pigs after burn when treated with the experimental drug (x ± SD; n = 7 for day 3; n = 3 for 14–28 days)

Parameters	Physiological norm	Period of monitoring, days					
		prior to burn	3	7	14	21	28
Total protein, g/L	42.0–68.0	48.3 ± 3.5^{a}	64.1 ± 5.4^{b}	61.2 ± 5.8^{b}	55.6 ± 6.2^{b}	58.4 ± 4.9^{b}	58.1 ± 5.2^{b}
Albumins, g/L	27.0–37.0	34.1 ± 3.6^{a}	41.2 ± 3.3^{b}	35.0 ± 3.9^{ab}	32.7 ± 2.8^{ac}	28.4 ± 3.1^{c}	27.3 ± 3.4^{c}
Globulins, g/L	14.0–25.0	14.6 ± 2.1^{a}	24.2 ± 2.6^{b}	18.7 ± 2.4^{c}	15.3 ± 2.2^{ac}	14.8 ± 2.7^{a}	14.0 ± 2.3^{a}
Protein coefficient, times	1.2–2.5	2.42 ± 0.34^{a}	1.71 ± 0.13^{b}	1.96 ± 0.39^{ab}	2.14 ± 0.27^{ab}	2.03 ± 0.28^{ab}	1.97 ± 0.12^{ab}
Urea, mmol/L	2.0–9.8	4.93 ± 0.76^{a}	7.04 ± 0.42^{b}	6.59 ± 0.86^{ab}	6.13 ± 0.65^{ab}	5.81 ± 0.74^{ab}	5.68 ± 0.43^{a}
Uric nitrogen, mg/L	7.0–13.0	9.42 ± 1.16^{a}	13.44 ± 1.03^{b}	12.08 ± 1.54^{ab}	10.91 ± 1.33^{ab}	10.46 ± 1.45^{ab}	10.63 ± 1.26^{ab}
Creatinine, μmol/L	53.0–194.0	78.4 ± 6.5^{a}	99.2 ± 12.3^{b}	95.4 ± 11.6^{b}	91.4 ± 9.6^{b}	90.5 ± 8.9^{b}	90.0 ± 9.1^{ab}
Aspartate aminotransferase (AST), U/L	64.0–142.0	85.7 ± 9.1^{a}	133.6 ± 11.4^{b}	125.1 ± 10.3^{b}	100.2 ± 10.9^{ab}	90.1 ± 12.2^{a}	84.0 ± 9.7^{a}
Alanine aminotransferase (ALT), U/L	59.0–99.0	64.3 ± 5.8^{a}	95.1 ± 8.7^{b}	76.4 ± 6.9^{a}	70.2 ± 8.3^{a}	61.4 ± 7.5^{a}	55.1 ± 6.6^{a}
AST/ALT, times	1.1–1.8	1.32 ± 0.24^{a}	1.44 ± 0.31^{a}	1.65 ± 0.23^{a}	1.43 ± 0.37^{a}	1.51 ± 0.33^{a}	1.50 ± 0.26^{a}
Alkaline phosphatase, U/L	192–302	263 ± 32^{a}	275 ± 24^{a}	267 ± 29^{a}	248 ± 26^{ab}	231 ± 23^{ab}	200 ± 19^{b}
Amylase	0.0–3159.0	2335 ± 127^{a}	2061 ± 165^{b}	1100 ± 97^{c}	750 ± 67^{d}	671 ± 62^{d}	634 ± 71^{d}
Total bilirubin, μmol/L	0.0–15.0	2.34 ± 0.31^{a}	0.86 ± 0.13^{b}	1.94 ± 0.27^{a}	2.02 ± 0.16^{a}	2.16 ± 0.24^{a}	2.14 ± 0.25^{a}
Glucose, mmol/L	5.0–16.0	6.22 ± 0.86^{a}	5.18 ± 0.64^{ab}	5.44 ± 0.71^{ab}	4.53 ± 0.57^{b}	4.25 ± 0.63^{b}	4.26 ± 0.52^{b}
Ca, mmol/L	1.8–2.5	2.38 ± 0.32^{a}	2.24 ± 0.27^{a}	2.43 ± 0.46^{a}	2.11 ± 0.39^{a}	2.15 ± 0.22^{a}	2.07 ± 0.24^{a}
P, mmol/L	2.8–4.2	3.04 ± 0.27^{a}	4.06 ± 0.52^{b}	3.21 ± 0.44^{ab}	3.02 ± 0.68^{ab}	3.22 ± 0.43^{ab}	3.24 ± 0.37^{ab}
Ca/P, times	0.6–1.2	0.80 ± 0.14^{ab}	0.62 ± 0.16^{ab}	0.84 ± 0.11^{b}	0.73 ± 0.17^{ab}	0.75 ± 0.13^{ab}	0.61 ± 0.11^{a}
Cholesterol, mmol/L	0.5–2.0	1.06 ± 0.12^{a}	1.14 ± 0.13^{a}	1.05 ± 0.13^{a}	1.13 ± 0.12^{a}	1.04 ± 0.12^{a}	1.06 ± 0.13^{a}
Gamma-glutamyl transpeptidase (GGT), U/L	16.0–40.0	37.1 ± 4.7^{a}	14.8 ± 3.6^{b}	25.4 ± 3.8^{c}	30.3 ± 4.2^{ac}	32.7 ± 4.3^{ac}	32.9 ± 4.6^{ac}
Hemoglobin, g/L	110–150	133.2 ± 14.6^{ab}	147.1 ± 12.9^{a}	138.4 ± 15.7^{ab}	124.6 ± 13.4^{b}	124.3 ± 11.8^{b}	120.8 ± 14.3^{b}
Hematocrit, %	30.0–45.0	39.7 ± 4.1^{a}	42.4 ± 5.8^{a}	39.0 ± 5.4^{a}	40.1 ± 6.3^{a}	39.6 ± 5.7^{a}	39.2 ± 5.9^{a}
Erythrocytes, 10^{12}/L	4.5–6.0	5.52 ± 0.94^{a}	5.21 ± 0.73^{a}	5.74 ± 0.86^{a}	5.97 ± 0.98^{a}	5.93 ± 0.74^{a}	5.91 ± 0.85^{a}
Mean corpuscular volume (MCV), 10^{-15} L	65.0–75.0	72.2 ± 9.4^{a}	81.5 ± 10.1^{a}	73.1 ± 8.9^{a}	70.8 ± 7.8^{a}	70.6 ± 9.3^{a}	69.2 ± 8.3^{a}
Mean corpuscular hemoglobin (MCH), 10^{-12} g	23.0–26.0	24.2 ± 3.8^{a}	28.3 ± 4.2^{a}	25.2 ± 3.7^{a}	24.9 ± 2.6^{a}	24.5 ± 3.9^{a}	25.3 ± 3.7^{a}
Mean corpuscular hemoglobin content (MCHC), %	30.0–40.0	33.5 ± 4.1^{a}	34.7 ± 4.4^{a}	31.8 ± 3.6^{a}	31.6 ± 4.2^{a}	31.1 ± 4.1^{a}	31.3 ± 3.4^{a}
Color parameter, U	0.8–1.2	0.92 ± 0.14^{a}	1.03 ± 0.12^{a}	0.94 ± 0.16^{a}	0.93 ± 0.14^{a}	0.93 ± 0.15^{a}	0.92 ± 0.16^{a}
Erythrocyte settlement rate (ESR), mm/h	0.9–2.5	1.13 ± 0.26^{a}	3.01 ± 0.43^{b}	1.25 ± 0.24^{a}	1.04 ± 0.31^{a}	1.16 ± 0.34^{a}	1.03 ± 0.22^{a}
Platelets, 10^{9}/L	200–250	212.4 ± 31.2^{a}	255.6 ± 36.4^{a}	248.3 ± 33.7^{a}	210.8 ± 29.3^{a}	230.2 ± 34.6^{a}	227.2 ± 32.8^{a}
Leukocytes, 10^{9}/L	4.5–7.0	4.92 ± 0.64^{a}	7.56 ± 0.93^{b}	6.71 ± 0.76^{b}	4.03 ± 0.88^{a}	3.94 ± 0.77^{a}	4.14 ± 0.91^{a}
Basophils, %	0.0–1.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Eosinophils, %	0.0–2.0	1.02 ± 0.16^{a}	2.14 ± 0.21^{b}	1.16 ± 0.24^{a}	1.08 ± 0.13^{a}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
Myelocytes, %	0.0–0.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Young, %	0.0–0.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Band, %	1.0–2.5	1.06 ± 0.12^{a}	2.18 ± 0.46^{b}	2.02 ± 0.34^{b}	1.17 ± 0.15^{a}	1.09 ± 0.16^{a}	1.06 ± 0.14^{a}
Segmented-nucleus, %	19.0–25.0	44.3 ± 5.6^{a}	19.6 ± 3.9^{b}	25.8 ± 4.7^{b}	43.2 ± 5.4^{a}	41.4 ± 5.2^{a}	40.6 ± 5.9^{a}
Lymphocytes, %	50.0–75.0	55.6 ± 4.7^{a}	71.1 ± 6.8^{b}	60.3 ± 6.7^{ab}	57.4 ± 6.1^{a}	55.7 ± 5.2^{a}	57.1 ± 5.1^{a}
Monocytes, %	1.0–4.0	2.04 ± 0.31^{a}	1.12 ± 0.26^{b}	4.07 ± 0.73^{c}	3.03 ± 0.68^{ac}	2.06 ± 0.33^{a}	2.04 ± 0.47^{a}

Note: different letters within one line indicate samplings that are significantly ($P < 0.05$) different one from another according to the results of the Tukey test; physiological norm – results of the studies by Dang et al. (2008).

Three days after the infliction of the burns, there was seen an insignificant 1.4% body-mass gain in guinea pigs of group I, whereas body mass of animals of groups II and IV decrease in compared with intact animals by 8.6% and 20.0% ($P < 0.05$). On the 7th day, the body mass of group II animals was 5.8% lower than group III animals. From days 14 and 28 of the experiment, there was seen increase in body mass of group I animals which had been treated with the experimental drug (from 251.4 to 298.7 g).

The morphological and biochemical blood parameters of guinea pigs treated with the experimental drug are presented in Table 3. Increases in protein concentrations were observed in group I after applying the experimental drug on days 3 and 7, compared with the animals prior to burn, measuring 32.7% ($P < 0.05$) and 26.7% ($P < 0.05$) due to globulins by 65.6% ($P < 0.01$) and 28.1% ($P < 0.05$). From days 14 and 28 of the experiment, the concentration of protein in the group of guinea pigs treated with the experimental drug was within the physiological norm, exceeding such in animals of the clinical control (C+) by 10.1 and 9.8 g/L, respectively. Also, at the moment of recovery (day 28 of the experiment), we observed a 20.0% ($P < 0.05$) decrease in the urea concentration in blood plasma of group I guinea pigs compared with the 3rd day of burn.

Transferases are localized in cell hyaloplasm and mitochondria, and are quite sensitive indicators of liver damage. In blood of the guinea pigs treated with the experimental drug, there were seen 47.9 and 39.4 U/L and 30.8 and 11.7 U/L increases in AST and ALT levels on days 3 and 7, respectively.

Concentrations of urea, uric nitrogen, and creatinine in plasm are important indicators of the kidneys' function. The kidneys accumulate the end product of creatine metabolism – creatinine, which is synthesized in those organs from aminoacids such as arginine, glycine, and methionine. On the 3rd day of treatment, experimental group I animals had 42.9%, 42.6%, and 26.5% increases in the concentrations of urea, uric nitrogen, and creatinine, respectively, compared with the the levels before the injury. On days 21–28 of the study, those parameters returned to the level of the physiological norm.

Total bilirubin is a product of heme metabolism and a component of blood hemoglobin, which is present in erythrocytes and is responsible for delivering oxygen to the tissues. After breakdown of hemoglobin, bilirubin is released from erythrocytes and travels to the liver, where is is metabolized and released from the organism with bile. On day 3 of burn treatment using the experimental drug, we observed a 65.2% ($P < 0.01$) decrease in bilirubin concentration, which was related to intoxication of the organism.

Gamma-glutamyl transferase (GGT) is an enzyme, the main activity of which takes place in the kidneys, liver, and pancreas. This sensitive parameter reacts to slowed bile movement in the liver and bile ducts, and therefore is used in diagnostics as a marker of liver diseases. A 60.1% ($P < 0.01$) decline was confirmed in GGT activity on the 3rd day after treating the burns with the experimental drug compared with the intact animals (C+).

We should note 10.4% and 3.4% increases in the hemoglobin level on the 3rd and 7th days of treatment. Application of the tested drug had a positive effect on the guinea pigs' hemopoiesis: on days 7–28 of treatment, the number of erythrocytes was 3.6–7.3% higher than in the intact group, and the number of platelets was 20.3% ,16.9%, and 8.3% higher on days 3, 7, 21, and 28, respectively. Exerting hemostatic and anti-inflammatory action, platelets can play a substantial role in inhibiting burn pathogenesis. The number of leukocytes in the blood in group I on the 3rd and 7th days increased by 53.1% and 36.7%, mostly due to lymphocytes, indicating a pathological inflammatory process in the animals during that period. The level of band neutrophils on the 3rd and 7th days was 2.1 and 2.0 times higher than before the burn, whereas segmented-nucleus neutrophils declined by 55.8% and 41.8%, suggesting inhibition of the hematopoiesis function. The leukogram indicated that during the experiment, the number of basophils and eosinophils in group I was within the clinical norm, meaning absence of allergic reactions in the guinea pigs.

The tested drug increased the ability of the organism to stimulate the functional activity of the immune system and enhanced the resilience of the animals to burn-related stress. The greatest intensity of immune reactions was observed on days 7 and 14 of treatment, with the highest parameters of monocytes – 2.0 and 1.5 times greater than in the intact (C+) animals, indicating heightened levels of phagocytosis.

Cultural-bacteriological studies of the microbial landscape of the wound surface and microbiome of the internal environment. Cultural-bacteriological studies of the biomaterial of wound exudates and separated tissue detritis, and also washes from skin regions and fecal suspensions revealed the microbial landscapes in the norm, the process of development of burn pathological state, and during regenerative-repair adaptogenesis. During the quarantine preparation of animals to the experiment, in the general sample of feces from all groups of guinea pigs, on the indicatory media, typical indigenous prokaryotes were isolated – indicators of physiological wellbeing such as *Streptococcus viridans* and *Mycobacterium vaccae*, with characteristics typical of those species. Also, on special differentiation-diagnostic media, from the general fecal suspension in obligatory anaerobic conditions, the chemoorganotrophic eubiotics *Lactobacillus plantarum* and *Bifidobacterium bifidum* were isolated in the amount of 10^3–10^4 CFU/g. On Saburo's agar, among various microflora, we saw colonies of saccharolytic yeasts. While conducting biosampling on white mice, in the general mixture of non-differentiated prokaryotes obtained from a weighed amount of biomaterial from quarantined guinea pigs, on various media we observed no pathological processes over 10 days, i.e. in the general sample of isolated microorganisms, there were no pathogenic variants, indicating that the guinea pigs were completely healthy.

During the experiments with isolating indigenous probiotic prokaryotes *S. viridans* and *M. vaccae* on days 7, 14, and 21, and 28 after infliction of the burn, the results of cultural study were negative. Addition of those bacteria as probiotic additive to improve the general wellbeing of injured animals would be practical. Also, we did not isolate any chemoorganotrophic euribiotics *Lactobacillus plantarum* and *Bifidobacterium bifidum* or saccharolytic yeasts. This confirms the necessity of their introduction into injured animals during post-traumatic rehabilitation.

During the post-burn period, after 3 days of treatment with the tested drug, the wound-healing processes begun, and this was reflected in the composition of microbial contamination of the wound field. The number of microorganisms did not exceed 10^4 CFU/g and ranged $(2.12 \pm 0.38) \times 10^3$ to $(4.34 \pm 0.62) \times 10^3$ CFU/g. The bacterial microflora mostly included Gram-negative prokaryotes: *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*. Of coccus microflora, there dominated *Staphylococcus aureus* and *S. epidermidis*. Pathogenicity was observed for *P. aeruginosa*, capsular variants of hemolytic *E. coli*, and *S. aureus*. Besides the identified pathogens of burn complication, we recorded a large amount of spore-bearing Gram-positive bacilli and large clostridial cells together with streptococci, mostly with short chains of cocci, and Gram-negative small bacilli with rounded ends and coccus-like microorganisms.

On day 3 of the experiment, in the biomaterial from the guinea pigs treated with the Panthestin ointment, bacteriological study found that the general microbial contamination with non-differentiated prokaryotes on the wound surface reached 10^4–10^6 CFU/g, ranging $(4.33 \pm 0.42) \times 10^4$ to $(6.56 \pm 0.57) \times 10^5$ CFU/g, which is critically large and prognostically unfavorable in relation to survival and recovery of the animals. Microscopic study of the superficial layers of the wound surface using imprint smears revealed domination of *P. aeruginosa* and capsular variants of hemolytic *E. coli*. Also, the wound exudates contained Gram-negative and Gram-positive bacilli and cocci. The microflora was morphologicaly diverse, which is typical for open purulent-necrotic damage, but *P. aeruginosa* dominated on the burn injury together with capsular variants of hemolytic *E. coli*. Indigenous prokaryotes of physiological microbiome were absent, and instead of saccharolytic yeasts, *Candida albicans* emerged on the Saburo's agar. Cultural-bacteriological study of the wound exudate and cellular-tissue detritus of the guinea pigs that had not been subjected to therapeutical intervention practically did not differ from microflora of the animals that had been treated with the Panthestin ointment, but the number of pathogenic and conditionally pathogenic microflora increased to 10^6–10^7 CFU/g, which was absolutely incompatible with life and usually ends with 100% death of the animals.

Planimetric characteristics of the wound surface in dynamics. Complete cleaning of the wounds from purulent-necrotic masses in the group of animals that received the experimental drug occurred significantly earlier: after 3.7 days. On the third day of the experiment, the last animal in the Panthestin group died from its injury (Table 4).

Table 4
Planimetric dynamics of healing of burn wound in animals of different experimental groups

Planimetric parameters, units of measurement	Experimental groups of animals	
	I (experimental drug)	II (Panthestin)
Complete cleansing of the wound from purulent-necrotic masses, day	3.7 ± 0.2	did not occur
Formation of granulation tissue, day	3.9 ± 0.4	did not occur
Peripheral epithelization of burn wound, day	6.8 ± 0.3	did not occur
Complete epithelization of the wound surface, day	17.9 ± 0.4	did not occur
Reduction of the wound area per day (Popova's index), %	5.1 ± 0.1	did not occur
Average rate of reduction of the wound surface area, mm^2/day	3.7 ± 0.1	did not occur
Healed area, mm^2	120.4 ± 6.7	did not occur

The index of L. M. Popova was significantly higher in the experimental group that received the tested drug than in the Panthestin group. During the study of wound-healing action of the experimental drug on the model of infected thermal burn in guinea pigs, it was determined that reparative processes during the use of the experimental ointment occurred faster than in the Panthestin group. The appearance of burn injuries in the dynamics of treatment with the experimental drug is presented in Figures 1–7.

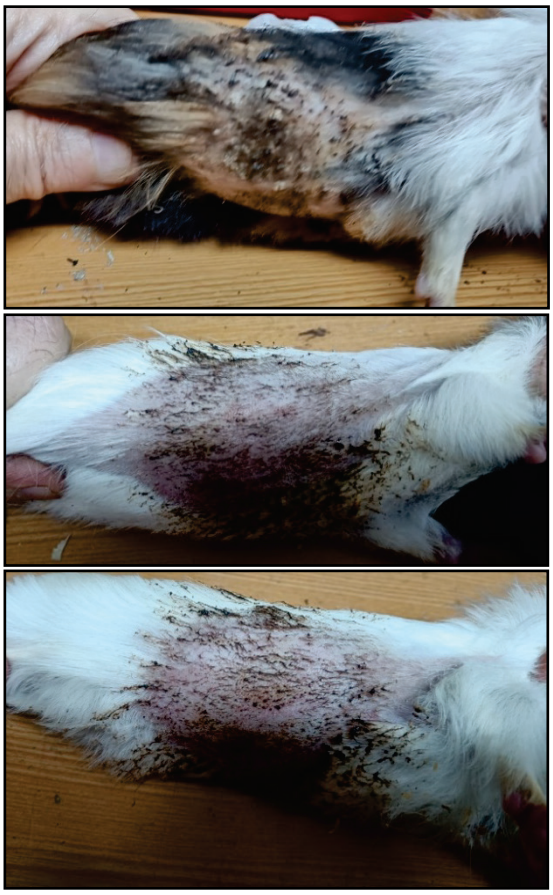

Fig. 1. Appearance of the burn wound, first day of the experiment

The measurement of area of the burn injuries of group I guinea pigs, which had been treated with the tested drug, revealed presence of 12.2–12.6 cm^2 wounds.

On day 3 of the experiment, we saw signs of post-traumatic shock and burn intoxication. In the injured region, a very thin drying crust formed, and when pressed, it released a considerable amount of exudate without purulent admixtures (Fig. 2).

Results of day 5 of the experiment indicated that the general condition of the animals had stabilized and the first shock reactions slowed down. Formation of the granulation tissue on average lasted for 3.9 days. Appetite returned, and psycho-physiological reactions normalized. The prognosis regarding the general state and local progress was positive (Fig. 3).

Fig. 2. Appearance of burn wounds of guinea pigs (experimental drug group, day 3 of the experiment)

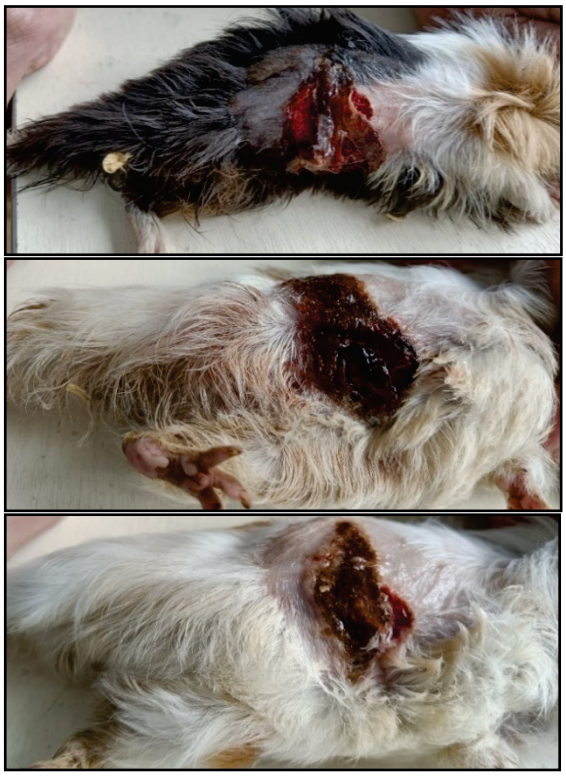

Fig. 3. Appearance of burn wounds of guinea pigs (experimental drug group, day 5 of the experiment)

On day 7 of the experiment, the condition of guinea pigs of group I (treatment with ointment of the tested drug) was satisfactory, they had appetite, and their psycho-emotional reactions were typical of animals

with normal physiological chacaracteristics. The wound surface reduced, and the burn wounds were healing. The average rate of peripheral epithelization of the burn wound was 6.8 days (Fig. 4).

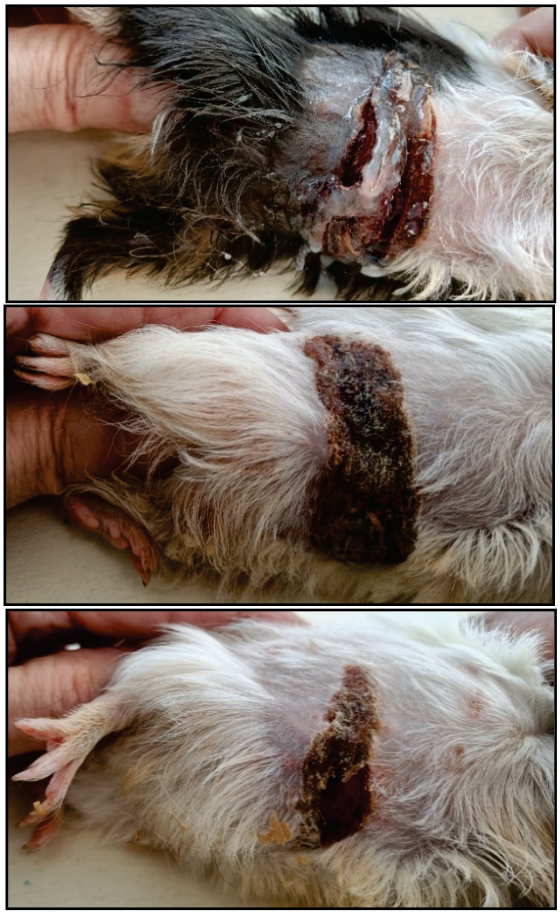

Fig. 4. Burn wounds of guinea pigs
(experimental drug group, day 7 of the experiment)

The results of 14th day of experiment indicated a satisfactory state of guinea pigs of experimental group I; they had appetite, the psycho-emotional reactions were typical of animals in the normal physiological state. The average rate of reduction of the wound surface per day was for 3.8 mm^2 (Fig. 5).

On days 21 and 28 of the experiment, we found no appetite disorders in guinea pigs of the experimental group. Complete epithelization of the wound surface occurred in 17.9 days. The area of healing equaled 120.4 mm^2 (Fig. 6, 7).

Results of the histological studies of skin samples of guinea pigs before and after burn. Prior to the burn, the skin of the guinea pigs had a typical histological structure (Fig. 8). The epidermis comprised malpighian and cornified layers. The malpighian layer was formed of one row of basal-layer epidermocytes, 2–5 rows of spinous and 1–2 rows of grainy cells. The cornified layer was represented by ceratinocytes at various stages of cornification and layer of cornified scales. The dermis contained hair follicules, surrounded by exocrinous glands.

On day 1 after the burn, in the affected skin region, we observed dry necrosis of the upper layer of epidermis. In the basal layer, intercellular edema were seen. Epidermocytes were increased in volume, their cytoplasm was filled with vacuoles with translucent fluid (hydropic dystrophy), the nucleus was shifted towards the periphery, and sometimes it contained vacuoles or was plicated (karyopyknosis). Between the epidermis and dermis, the boundary was smoothed, and venous plethora and stasis were observed. In the dermis, acute inflammatory reaction was expressed, accompanied by sharp increase in vascular permeability, diffusive edema, and disorganization of collagen fibers (fibrinoid swelling), which are typical for degree 2 and 3 burns. Derivatives of the skin (hair follicules and exocrine glands) were plicated and partly ruined (Fig. 9).

Fig. 5. Burn wounds in guinea pigs
(experimental drug group, day 14 of the experiment)

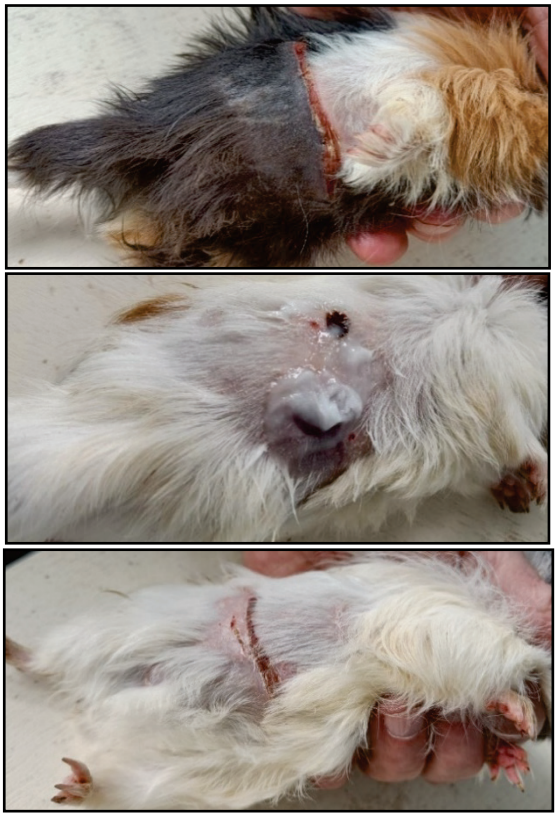

Fig. 6. Burn wounds of guinea pigs
(experimental drug group, day 21 of the experiment)

On day 3 after thermal burn of the skin, intoxication processes increased. The purulent-necrotic crusts were closely associated with the upper layer of epidermis, and their separation was seen in some regions. In the basal layer, there was pronounced spongiosis (intercellular edema) and intracellular edema of epidermocytes (vacuolar dystrophy). In the dermis, the manifestations of acute inflammatory reaction increased, and the main amorphic substance became diffusively swollen, and there appeared leukocytic infiltrates between bundles of collagen fibers and near the blood capillaries. Separation of colaggen fiber bundles followed by their homogenization occurred. Skin appendages – hair folicules and glands were plicated (Fig. 10).

Fig. 7. Burn wounds of guinea pigs (experimental drug group, day 28 of the experiment)

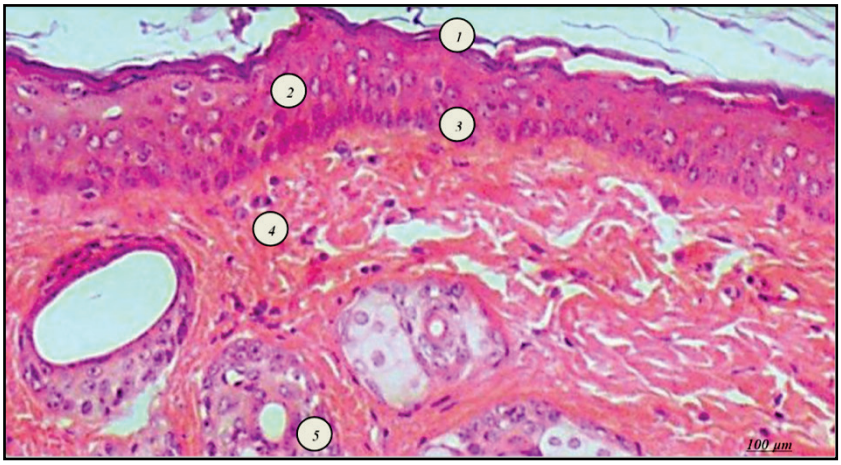

Fig. 8. Light microphotograph of normal *Cavia porcellus* skin (before the burn), staining with hematoxylin and eosin: *1* – stratum corneum of the epidermis; *2* – spiky and granular layers; *3* – the basal layer of the epidermis; *4* – dermis of the skin with bundles of collagen fibers; *5* – hair follicles surrounded by exocrine glands

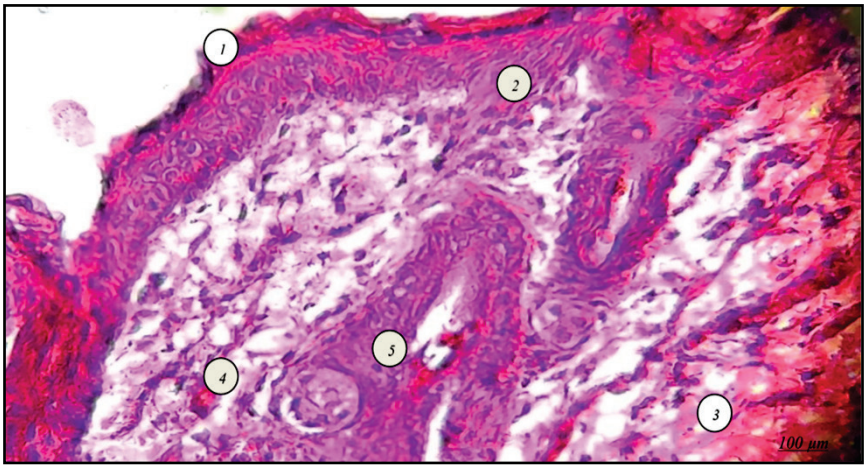

Fig. 9. Light micrograph of the skin of a *Cavia porcellus* affected by a thermal burn (first day), staining with hematoxylin and eosin: *1* – necrosis of the upper layer of the epidermis of the skin (dark color); *2* – vacuolar dystrophy of epidermocytes; *3* – inflammatory swelling of the base of the dermis, swelling of collagen fibers; *4* – expansion of lumens of blood vessels; *5* – damaged skin appendages (hair, glands)

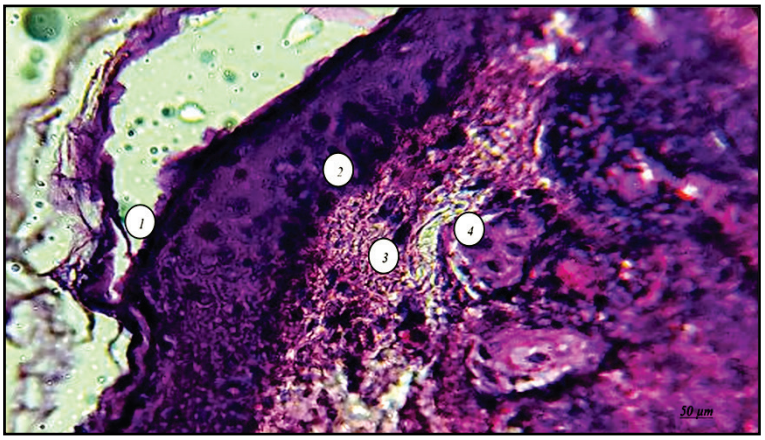

Fig. 10. Light micrograph of the skin of a *Cavia porcellus* on the 3rd day after a thermal burn, staining with hematoxylin and eosin: *1* – detachment of necrotic areas of the epidermis; *2* – vacuolar dystrophy of epidermal cells; *3* – swelling and disorganization of dermis fibers; *4* – appendages of the dermis (hair follicles and exocrine glands)

On day 7 of treating thermal burns, the condition of the skin of guinea pigs treated with the experimental drug significantly improved. We observed recovery of the epidermis as a result of active reproduction of cells of the basal layer, its thickening and non-uniform extension into the upper papillar level of the dermis. In the dermis, there formed sites of reproduction of epidermocytes and dermis cells – fibroblasts; the vessels were enlarged and full-blooded, and skin apprendages were in the state of recovery (Fig. 11). On day 14 after treatment, we observed recovery of the cornified layer of epidermis, which was loosely adjacent to the basal layer. The malpighian layer of epidermis was significantly thickened due to myotic activity of epidermocytes of the basal layer and in some regions was deeply extended into the papillar layer of dermis. The epidermal-dermal boundary was distinct. In the most severely burnt skin regions, the dermis was thinned due to decrease in the number of bundles of collagen fibers and their thickness, the grid of elastic and reticular fibers was scattered-out, and heightened content of cells of the stroma was observed – fibroblasts and fibrocytes, and active formation of hair follicules continued (Fig. 12).

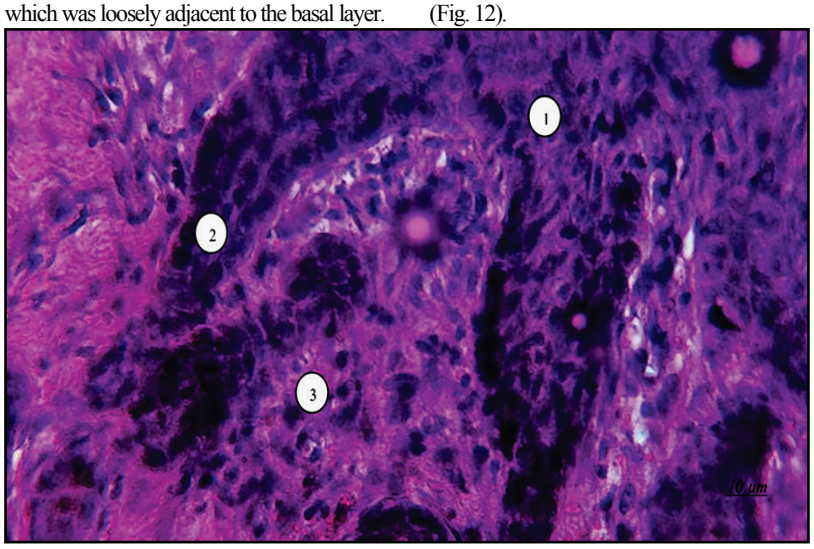

Fig. 11. Light micrograph of the skin of a *Cavia porcellus* on the 7th day after a thermal burn, staining with hematoxylin and eosin: *1* – active reproduction of epidermocytes of the basal layer of the epidermis; *2* – thickening of the epidermis and ingrowth into the dermis; *3* – increased proliferation of dermis cells

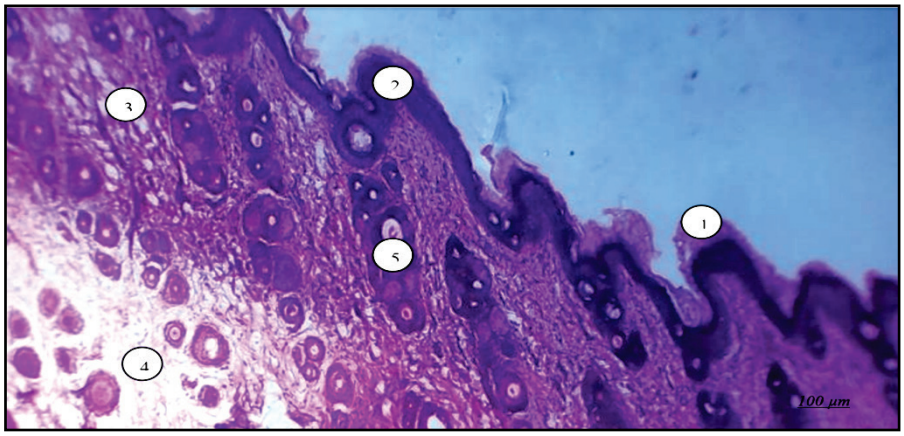

Fig. 12. Light micrograph of the skin of a *Cavia porcellus* on the 14th day after a thermal burn, staining with hematoxylin-eosin: *1* – formation of the stratum corneum of the epidermis; *2* – thickening of the germ layer of the epidermis; *3* – dermis; *4* – sparse networks of connective tissue fibers; *5* – increased epithelization of hair follicles

On day 21 of treatment, the recovery processes in the affected regions of the skin continued. Cornification processes intensified in the epithelium, and the epidermis was significantly thinned compared with the skin of intact animals, the papillae of the dermis were smoothened. In the dermis, we observed an increased number of fibroblasts, especially near bundles of collagen fibers, indicating increased collagen accumulation in the skin. Enhancements occurred in the processes of formation of the skin apprendages – hair follicules and sebaceous glands (Fig. 13).

On day 28 of treatment, we saw complete recovery of the affected regions of the skin. The epidermis was thinned. The epidermal-dermal boundary was clearly expressed, though dermis papillae were smoothened. Thickness of the dermis in the post-burn region was reduced due to decrease in the number of bundles of collagen fibers. Processes of collagenation continued due to presence of dermis cells. Recovery of derivatives of the skin continued (Fig. 14).

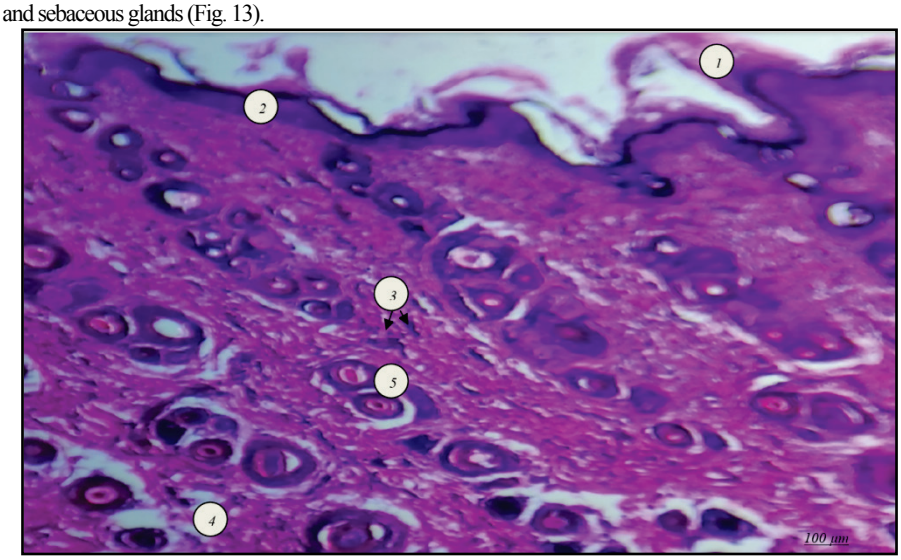

Fig. 13. Light micrograph of the skin of a *Cavia porcellus* on the 21st day after a thermal burn, staining with hematoxylin-eosin: *1* – loose stratum corneum of the epidermis; *2* – thinning of the epidermis with smoothing of the papillae of the dermis; *3* – increased vascularization of the papillary layer of the dermis; *4* – thinned scar tissue due to the thinning of networks of elastic and collagen fibers; *5* – active formation hair follicles surrounded by sebaceous glands

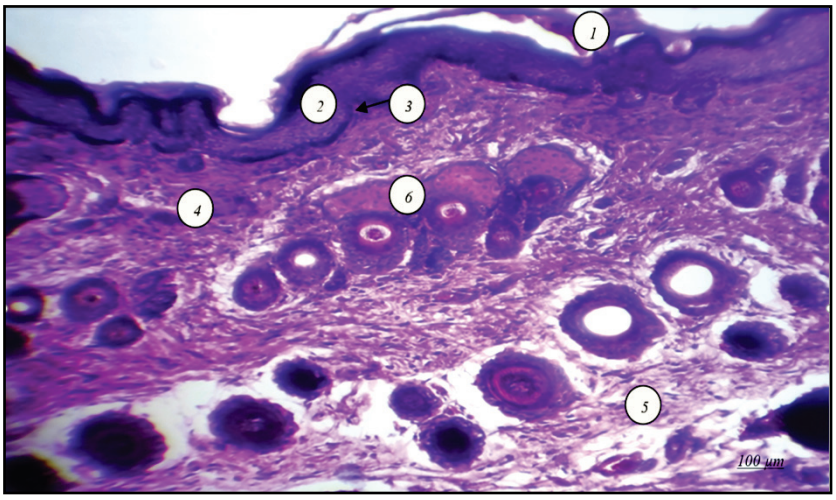

Fig. 14. Light micrograph of the skin of a *Cavia porcellus* on the 28th day after a thermal burn, staining with hematoxylin-eosin: *1* – formed stratum corneum of skin epidermis; *2* – thinned epidermal layer of the skin; *3* – epidermodermal boundary; *4* – an increase in the number of dermal cells and increased vascularization of the papillary layer of the dermis; *5* – thinned mesh layer of the dermis; *6* – active formation of hair follicles and skin glands

Discussion

Studying the pathogenesis of infection pathology of burn-wound surface revealed the presence of histopathological changes and a complex of metabolic symptoms. They accompanied the adaptive reaction of the macroorganism to the extreme thermal stress factor and reproduction of transitory prokaryote microbiota with pathogenic and conditionally pathogenic properties. The competition between transitory and resident microbiota is an extremely relevant scientific practical issue. Infiltration of pathogenic and conditionally pathogenic microorganisms with purulent necrotic functional abilities to thermally injured tissues leads to the development of purulent-septic complications and septic-pyemic shock with lethal outcomes.

Pathogenetic changes and pathophysiological transformations of burn injury at the regional level are based on sequences of pathological disorders, where debut events are rapid development of tissue hypoxy due to release and retention of bound water in the cell, influx of water from the tissue fluid, blood, and lymph as a result of increase in colloid-osmotic pressure and disorders of cellular membrane permeability. At the same time, there occurred total colliquative necrosis (ballooning dystrophic colliquation), causing increase in sizes of epidermis cells, their cytoplasm became filled with a vacuole with transparent fluid, the nucleus transferring onto the cells' periphery, and sometimes vacuoles emerged in it or it wrinkled (rhexis). As a logical consequence of destructive genesis, the cell transforms into a ballooning formation filled with water, in which a bubble-like nucleus floated. In the endl, fatal destruction and death of cell was observed.

Analysis of the results of the experiment revealed that using the original anti-burn drug promoted wound-healing in contrast to treatment with the officinal drug – the Panthestin ointment and the situation without treat-

ment using anti-burn drugs. Treatment of deep burn injuries of the surfaces of the guinea pigs' bodies with the experimental drug led to effective wound cleansing, formation of granulation, and emergence of first marginal and then complete epithelization.

The basis of the drug is important when choosing medical form for curing burns. Use of fat-based ointment Panthestin was relatively ineffective because such drugs have no osmotic activity in the zone of wound and perform no drainage functions, and create the so-called greenhouse gas effect. At the same time, application of the ointment with bandage is possible only during the regenerative phase of burn healing for mechanical protection of granulation on the burn's surface.

The ground-breaking technology for discovering the most effective medical form of anti-burn drug were ointments based on polyethylene glycol (PEG), which was established as a basic element in development of our experimental anti-burn drug. Ointment based on PEG has good adsorbing properties in relation to microorganisms and tissue toxins, which gives the opportunity to discard antibiotic drugs. Such a drug can be used even in case of necrotic tissues, significant exudation, degenerative, inflammatory, and regenerative forms of pathogenesis of the burn process.

When developing the experimental anti-burn ointment, we used various compounds and PEG Chemical properties of the drug's ingredients on which its medical properties are based are as follows:

– ionol, dibutylhydroxytoluene, dibunol, agidol-1 – 2,6 di-tert-butyl-4-methylphenol, lipophenol organic compound of phenols class, which has antioxidant properties due to the ability to neutralize free radicals and interrupt the chain reactions with free radicals. The formula is $C_{15}H_{24}O$. The molecular weight is 220.35 g/mol. The melting temperature is 70 °C. The boiling temperature is 265 °C. The density is 1.05 g/cm^3. It has anti-burn and anti-inflammatory properties;

– dimethyl sulfoxide (DMSO) is an organic compound, $(CH_3)_2SO$. Colorless high boiling-point fluid, it functions as aprotic highly polar solvent, low-toxic. When mixed with water, notable heating occurs; it reacts with iodide methyl, forming sulfoxonium, which is able to interact with sodium hydride. In aqueous solutions (10–15%), it has anti-inflammatory and pain-mitigating properties. Also, 10% concentrations can be cryoprotectants, and in the composition of the ointment, DMSO enhances the ability of active components for transdermal penetration.

– PEG-400 (polyethylene glycol-400). Formula – $C_{2n}H_{4n+2}O_{n+1}$. It is a product of polymerization of ethylene oxide and ethylene glycol. Chemical class – polymers. Colorless or yellowish fluid, significantly hydrophilous, non-toxic, is dissolved in glycerine and glycol, cannot be dissolved in ether and fatty oils. It is a material with the average degree of polymerization and viscosity. Density is 1.1–1.2 g/cm^3. It has properties of preservative, thickener, and creates gel-like structures;

– PEG-1500 (polyethylene glycol-1500). Polyether with the molecular formula $C_{2n}H_{4n+2}O_{n+1}$. It is a product of polymerization of ethylene oxide and ethylene glycol; has an average degree of polymerization. Chemical class – polymers. It is a colorless or yellowish fluid. Depending on molecular mass of polymer, it can be viscous fluid, gel-like, or solid, in the form of scales or dense white mass, compound. It is soluble in water, acetone, alcohols, benzol, glycerine, glycol, and aromatic carbohydrates; harmless and non-toxic compound that promotes mixing of uncombinable components.

The drug promoted deep moistening, alleviated inflammation, increased the rates of formation and recovery of skin cells, wound healing, and also recovery of the mucous membranes. The tested drug moistened and softened the hardened skin regions, removed reddening, and reduced the inflammatory processes. Due to its complex physiological effect on the metabolic processes, the drug carries out functions of regulator of reproductive potential of somatic cells of epidermal origin, adaptogen of cellular metabolism, antioxidant, and inhibitor of free-radical compounds, and their consecutive chain reactions, stimulator of expression of intracellular rhibosomal reproduction of nitrogen-containing compounds, fast-action antidepressant with cryoptectory properties, has adhesive and transdermal properties, intensively stimulates regeneration of the skin epithelium, normalizes the cellular metabolism, boosts mytosis, increases the resilience of collagen fibers, and alleviates inflammation.

The main action of the drug is stimulation of regeneration of damaged areas of the skin and mucous membranes. Therefore, the tested drug is applied to treat and prevent inflammatory processes of various origin on the superficial tissues – degree I and II burns, mechanical damage, irritations caused by toxic compounds, acids or bases, hyporthermia, prophylaxis of purulent-necrotic complications of the wound surface, iatrogenic conditions, pathologies associated with trophic changes in the tissues – trophic ulcers and pressure ulcers (including infected ones), surgical therapy of limbs, it protects the wound surface from anaerobic infections, inhibits the course of post-traumatic infectious process, and enhances the healing phase by stimulating epithelization.

As a result of complex monitoring of clinical condition of the experimental animals and morpho-biochemical assays of blood, it was confirmed that the tested drug promoted effective treatment of the guinea pigs due to its ability to affect the intensity of morpho-biochemical processes in the animals during the experimental modeling of burns.

The most important parameters of physiological condition of macroorganisms are numerical parameters of total protein in the blood, concentrations of albumins and globulins, their ratios, and other parameters (Gotsulya et al., 2020). Proteins are significant for providing physiological blood functions – viscosity, support of volume of vascular course, and retaining erythrocytes, leukocytes, platelets in the process of their movement. Significant increase in concentration of total protein with further gradual decrease, but all the same maintaining high concentration, indicates serious metabolic changes related to consequences of lethal burn, acidosis, nephropathy, loss of the tissue fluid and electrolytes, excessive loading on hematopoietic function of the bone marrow and synergic hyperfunction of hepatocytes together with tissue macrophages.

An informative indicator of stimulation of synthesis of macroergic compounds is increase in concentration of creatinine in blood plasma. Creatinine concentration in the guinea pigs increased from 78.4 ± 6.5 to 99.2 ± 12.3 μmol/L and later gradually decreased to 90.0 ± 9.8 μmol/L. According to the reports by Schattner et al. (2006) and Salomons & Wyss (2007), creatinine is a nitrogen-containing carbonic acid $C_4H_9N_3O_2$ that is present in cellular eukaryotes and mediates the energy metabolism with participation of mitochondrial enzymatic processes. Recovery of creatinine concentration requires amino acid glycerin, arginine, methionine, and transferase enzymes. Creatinine is formed from creatinine phosphate with participation of creatinine kinase. The phosphocreatinine kinase system functions in cytoplasm as an intracellular system of transmission of chemical energy in the form of ATF and its further regeneration in the mitochondrial system. Also, phosphocreatinine activates glycosis and neutralizes acid products of metabolism, which reduce blood pH. Increase in creatinine concentration is accompanied by washing of calcium from the bones and malfunctioning of the kidneys.

The open surface of a burn wound is a very convenient substrate for prokaryote invasion. During the first week, there occurred massive microbial infection (of endogenous and exogenous origins) of damaged tissues of the burnt regions. Microbial infection with exponential reproduction of wound microbiota was induced by destruction of the superficial skin barrier and pathophysiological impairments in the zone of damage, caused by hypermetabolic syndrome. According to Boomer et al. (2011), Hotchkiss et al. (2013), Zazharska et al. (2021), the organism's general post-burn suppression of the functional activity of lymphoid components of the immune system and biomechanisms of non-specific reaction towards non-syngenic factors is a consequence of release of immune-depressing compounds by the burn wound. At the same time, there emerges a necessity to differentiate septic dynamic disorders from routine post-burn pain with hyperdynamic, hyperthermal, and hypermetabolic symptoms, which are typical for pathophysiology of degree II and III burns. Greenhalgh (2017) and Mira et al. (2017) emphasized that laboratory study of hemoculture can be negative, and the highest parameters of hyperthermia do not correspond to the severity degree of the course of wound injury. Regional signs of microbial inhabitation of the wound surface are accompanied by black or brown eschar, change in color of burnt surface (as a result of microthrombosis of the capillaries), fast separation of eschar, and significant expansion of the wound surface until emergence of ulcer along the full depth of the lesion (i.e. secondary necrosis). Then, hyperthermia and hemorrhage develop, damagie to the capillaries around and under the eschar, cyanosis, focal necrosis on the surface of healthy tissues, and, as a drastic finale of severe pathogenesis of the burn process – tertiary necrosis of the

burn wound. Microbial association in burn injuries is extremely diverse. Its composition depends on many biotic and abiotic factors. This is especially true for burn injuries, especially with a large affected surface, low level of immunoreactivity and metabolic activity of the macroorganism, non-physical intervention into pathogenesis of rehabilitation of the injured locus.

According to Barbut et al. (2013), Huttner et al. (2013), and Alhede et al. (2014), parasitocoenotic associations of microorganisms in burn injuries are not stable compositions of random microbionts. Microbial populations are labile, have a tendency towards rotation processes, involving species that are most invasive and adapted to a particular infectious situation in the circulation. Species composition and number of microbiota of wound injury affect the pathogenetic processes of recovery of damaged tissue functions. When Gram-negative microorganisms prevail in the wound injury, especially *P. aeruginosa* or hemolytic capsular variants of *E. coli*, reparative processes are characterized by mostly necrotic phenomena with significant accumulation of fibrin and suppression of protective leukocytic reactions. When Gram-positive non-spore-forming staphylococci *S. aureus* dominate in a wound, there develops a dirty complication – purulent inflammation or purulent-necrotic processes with deep infiltrations of the granular tissue and formation of abscesses.

Based on the studies by Johnson et al. (2000), Keen (2010), Wolf & Arnoldo (2012), Biben et al. (2023), it was determined that the dominant prokaryotes in the induction of pathogenic changes in burn wounds in monoinfection are the pathogenic prokaryotes *S. aureus* and *P. aeruginosa*, which are bioactive, polyresistant to antibiotic drugs, and have high vegetative potential, but more often those are various associations with *E. coli*, *Proteus* spp., *Klebsiella* spp., *Streptococcus* spp., and with complete elution of resident indicatory probiotic microbionts, particularly *S. viridans* and *M. vaccae*. At the same time, during the first week, most often it is possible to isolate the Gram-positive prokaryotes *S. aureus* and *S. epidermidis*, and then Gram-negative microflora such as *E. coli*, *Proteus* spp., *Klebsiella* spp. starts to dominate, and also the most important bacterial pathogen – *P. aeruginosa* (Hussien et al., 2012; Norbury et al., 2016).

As noted by Huttner et al. (2013), Nikokar et al. (2013), Alhede et al. (2014), Azzopardi et al. (2014), Sklyarov et al. (2020), the relevant medical-biological and veterinary-sanitary problem is the widely acquired polyresistance to antibiotic drugs, leading to methodological problems with antimicrobial wound therapy, eradication of polyresistant wound infection pathogens with broad vegetative potential, especially the field strains *P. aeruginosa* and *S. aureus*, against which ß-lactam antibiotics, aminoglycosides, and fluoroquinolones lose their antimicrobial efficacy.

Epidemiological strains of *P. aeruginosa* are common ubiquitous microorganisms with progressing increase in polyresistance to antibiotics. Experimental studies confirmed that the basic mechanisms of growing resistance to antibiotics are supression of production of chromosomal ß-lactamases of class C, as a result of which field variants of *P. aeruginosa* potentially can simultaneously have a large number of mechanisms of antibiotic resistance to ß-lactam antibiotic compounds.

A very important medical-biological problem is antibiotic resistance of field strains of *S. aureus* that makes treatment based on antimicrobial action of drugs of penicillins group ineffective. In the second half of the twentieth century, there was a rise in the spread of pathogen strains capable of producing β-lactamase. Then, there was isolated a methicillin-resistant variant and *Staphylococcus* infection became practically uncontrolled by routine antimicrobial drugs. This encourages using antimicrobial compounds of non-biological origin to cure burn injuries, for example, our experimental anti-burn drug.

During the experimental studies of microflora of wound injury treated using the experimental drug, the critical level of microbial contamination was reduced to the physiologically allowable quantitative parameters of 10^3–10^4 CFU/g on days 3–4 of treatment. At the same time, when using the Panthestin ointment, the level of microbial infection accounted for an incomparably larger value – 10^5–10^6 CFU/g. In animals that had not been subjected to therapeutic treatment, all the period of their struggle for life was accompanied by intensive reproduction of various microorganisms with the leading role of *P. aeruginosa*, hemolytic capsular variant of *E. coli*, and *S. aureus*. According to the results of our experiment, we may state that use of the experimental anti-burn drug promoted earlier and efficient healing of the burn wound, compared with the officinal ointment Panthestin based on more intensive cell-mediated processes of cleansing the wound surface, formation of active granulations, and development of marginal epithelization and fast completion of complete epithelization.

Conclusions

Further studies will be aimed at improving the complex approach to treating severe burns at the level of macroorganism and regional application of the experimental drug enriched with essential amino acids and microelements. Other than increase in the therapeutic effect of the drug as a result of improving its chemical composition, methods of its usage will be improved (in the form of aerosol or application onto the wound surface) with the purpose of prolongation and smoothening of the action of the drug's active compounds towards the pathogenesis of burn process and stimulation of epithelization in order to enhance repair of the damaged tissues. Also, great attention will be paid to restoring indigenous microbiota in the internal environment of macroorganism as a native dynamic microbial body, which plays an important role in stimulation of physiological functions of the lymphoid system.

The authors declare that there is no conflict of interest.

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