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FUNCTIONAL CHANGES IN SKIN MAST CELLS DURING SURGICAL WOUND HEALING IN RATS AFTER THE INFLUENCE OF CHRONIC SOCIAL STRESS

Actuality. *The problem of social stress, as well as the epidemiology of injuries, is currently the most pressing in the world, while the skin, as an external protective layer, is the first to respond to their effects by disrupting structural homeostasis. At the same time, there is evidence that mast cells, as resident, are a local link in the interaction between the neuroendocrine and immune systems of regulation of tissues of the internal environment of the body in normal and pathological conditions. The role of mast cells in control and regulation of internal environment tissues is intensively researched. The question of stressful conditions' impact on mast cells remains relevant.*

The purpose of the work was to characterize changes of functional parameters of mast cells during reparative processes under the influence of chronic social stress: the degree of degranulation and cell size.

Material and methods. *Study involved 50 Wistar male rats. Control group comprised 20 rats. We modeled chronic social stress on animals of the 2nd group, which were more susceptible to stress biologically active substancesed on the open-field test results, (n=30) by the three-week social isolation and prolonged psychoemotional impact. Stress was confirmed in an open field test, which was performed to all animals before and after modeling of chronic social stress. On the day of injury and on days 1, 3, 7, 14, and 30 of wound healing, a skin flap sized 1*1 cm was removed from the interscapular area. Samples were excised so that both the wound healing site and undamaged tissue were in them. Mast cells' size and degranulation degree were counted in sections, selectively stained with toluidine blue.*

Research results. *The process of wound healing in the rat skin was characterized by certain homeostatic and reparative features of the dynamics of the ratio of degranulation types of mast cells and their sizes as physiological regulators. In the control, we noted the maximum degranulation during inflammation and the absence of degranulation in the remodeling stage. In the experiment, even before the injury, a significant increase in the number of mast cells and their significant degranulation according to type 2 was noted, while the decrease in the number of mast cells during the stages of inflammation and proliferation was compensated by their high functional activity: an increase in degranulation mainly according to type 3 and an increase in their size, - in the process of prolonging the stages wound healing, which was accompanied by an increase in the number of mast cells and their functional activity for 30 days.*

Conclusion. *Chronic social stress disrupted the normal course of the wound process, contributing to its chronicity, in which cellular events characteristic of each stage of reparative regeneration were cross-observed in the wound healing process. Received data gives the prospect for therapeutic modification of the wound process with a targeted effect on the mast cells' activity.*

Key words: *chronic social stress, cutaneous wound healing, types of mast cells degranulation, quantitative and functional dynamics of mast cells in remodulation, stages of reparative regeneration.*

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ФУНКЦІОНАЛЬНІ ЗМІНИ ТУЧНИХ КЛІТИН ШКІРИ ПІД ЧАС ЗАГОЄННЯ ХІРУРГІЧНИХ РАН ЩУРІВ ПІСЛЯ ВПЛИВУ ХРОНІЧНОГО СОЦІАЛЬНОГО СТРЕСУ

Актуальність. *Проблема соціального стресу, як і епідеміології травматизму, наразі є найактуальнішою у світі, а шкіра, як зовнішній захисний шар, першою реагує на їх вплив порушенням структурного гомеостазу. Водночас є дані, що тучні клітини, як резидентні, є локальною ланкою у взаємодії нейроендокринної та імунної систем під час регуляції тканин внутрішнього середовища організму в нормі й у разі патології. Нині інтенсивно досліджується роль тучних клітин у контролі та регуляції тканин внутрішнього середовища. Актуальним залишається питання про вплив стресових факторів на тучні клітини.*

Мета дослідження *– охарактеризувати зміни функціональних параметрів тучних клітин під час репаративних процесів під впливом хронічного соціального стресу: ступеня дегрануляції та розміру клітин.*

Матеріал і методи. *У дослідженні взяли участь 50 самців щурів Вістар. Контрольну групу становили 20 щурів. Хронічний соціальний стрес моделювали на тваринах 2-ї групи (n=30), які були більш сприйнятливими до стресових впливів за результатами тесту «відкрите поле» шляхом тритижневої соціальної ізоляції та психоемоційного впливу. Стрес був підтверджений у тесті відкритого поля, що проводили всім тваринам до та після моделювання хронічного соціального стресу. У день нанесення рани й на 1, 3, 7, 14, 30-у добу загоєння рани з міжлопаткової ділянки висікали шкірний клапоть розміром 1*1 см. Зразки вирізали так, щоб у них потрапляло як місце загоєння рани, так і непошкоджена тканина. Розмір тучних клітин і ступінь дегрануляції підраховували в зрізах, селективно забарвлених толуїдиновим синім.*

Результати дослідження. *Процес загоєння ран шкіри щурів характеризувався певними гомеостатичними й репаративними особливостями динаміки співвідношення дегрануляційних типів тучних клітин і їх розмірів як фізіологічних регуляторів. У контролі ми відзначали максимальну дегрануляцію під час запалення та відсутність дегрануляції в стадії ремоделювання. В експерименті ще до травми зазначено достовірне збільшення кількості тучних клітин і їх значну дегрануляцію за другим типом, тоді як зменшення кількості тучних клітин на стадіях запалення та проліферації компенсувалося їх високою функціональною активністю: посиленням дегрануляції переважно за типом 3 та збільшенням їх розмірів – у процесі пролонгації етапів загоєння ран, що супроводжувалося збільшенням кількості тучних клітин і їх функціональної активності протягом 30 днів.*

Висновок. *Хронічний соціальний стрес порушував нормальний перебіг ранового процесу, сприяючи його хронізації, за якої клітинні події, характерні для кожного етапу репаративної регенерації, перехресно спостерігалися в процесі загоєння рани. Отримані дані є перспективними для терапевтичної модифікації ранового процесу із цілеспрямованим впливом на активність тучних клітин.*

Ключові слова: *хронічний соціальний стрес, загоєння ран шкіри, дегрануляційні типи тучних клітин, кількісна й функціональна динаміка тучних клітин у ремодуляції, стадії репаративної регенерації.*

Introduction. It has been shown that chronic stress affects cutaneous wound healing through the overstimulation of mast cells and post-ganglionic sympathetic nerve fiber interaction. Catecholamines released by post-ganglionic sympathetic nerve fibers stimulate mast cell degranulation, which promotes an early anti-inflammatory response, compromising the inflammation and the skin healing process (Romana-Souza, 2023, p. 578104).

Modern interest in the structural and functional studies of mast cells is intensively increasing due to the recent expansion of new experimental data obtained about their exceptional multifunctionality (Komi et al., 2020, p. 298–312; Theoharides, 2020, p. 388–392). Although mast cells are of myeloid origin, related to granular leu migrate (Trautmann et al., 2000, p. 100–106) and are resi kocytes, they do not dent in tissues with a pronounced organ specificity. They are located in groups around blood capillaries, lymphoid capillaries, nerve trunks in the loose connective tissue, therefore they are ubiquitous, but with a predominant location in the dermis of the skin and in the lamina propria of the mucous membranes, i.e. in organs in contact with the external environment. Mast cells constantly synthesize and accumulate in granules various biologically active substances that regulate the homeostasis of surrounding tissues by constantly releasing specific mediators under physiological conditions or by simultaneous degranulation to varying degrees in case of destructive or receptor disorders in tissues (Bhuiyan et al., 2021, p. 2184–2197; Li et al., 2022, p. 2105152).

Based on the diversity of synthesized biologically active substances and the compact arrangement of cells in loose connective tissue, where their number normally takes up to 10%, as well as taking into account their organ specificity, these islets of mast cells are rightly considered relatively autonomous peripheral sections of the endocrine system. This opinion is confirmed by a number of studies on direct and reverse reactions to changes in the synthesis of mast cells' biologically active substances mediators in the central organs of the endocrine system (Gupta & Gupta, 2017, p. 103–251; Olejniczak et al., 2022, p. 153–163; Theoharis, 2017, p. 751–759; Watson et al., 2016, p. 134).

As cells of the innate immune system, mast cells through various mediators affect the functional activity of cells in tissues, including immunocompetent cells of innate and adaptive immunity (Oskeritzian, 2012, p. 23–28; Theoharides, 2020, p. 388–392).

Taking into account the data that mast cells, being resident, are a local link in the interaction between the neuroendocrine and immune systems of tissue regulation of the body's internal environment in normal and pathological conditions, the purpose of our research was to describe the impact of chronic social stressful situations on them.

The specific **purpose of this study** was to characterize changes of functional parameters of mast cells during reparative processes under the influence of chronic social stress: the degree of degranulation and cell size.

Material and methods. Manipulations with animals were performed in compliance with regulated norms and rules for the treatment of laboratory animals: principles of bioethics, legislation and requirements in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Research and Scientific Purposes (Strasbourg, France, 1986), The Law of Ukraine "On protection of animals from cruel treatment". All procedures performed were approved by the Bioethics committee of Zaporizhzhia State Medical and Pharmaceutical University.

The research was performed on 50 white male Wistar rats, aged 12–13 months, weighting 390–430 g at the time of picking, and they were divided into 2 groups. The 1st group, control, comprised 20 rats (4 rats for each time point of wound healing). We modeled chronic social stress on animals of the 2nd group, which were more susceptible to stress biologically active substancesed on the open-field test results, n=30, (6 rats for each time point of wound healing) by the three-week social isolation and prolonged psychoemotional impact. Stress was confirmed in an open field test, which was performed to all animals before and after modeling of chronic social stress. For morphological assessment of the skin, a skin flap on the back in the interscapular region about 1cm*1cm in size was excised on the day of wounding and on days 1, 3, 7, 14, 30 of wound healing. Samples were excised so that both the wound healing site and undamaged tissue were in them.

Skin samples including the boundaries of the wound surface were fixed in 10% neutral formalin solution and followed standard histological methods. Serial sections (5 μm thick) were made using a Thermo Scientific HM 325 microtome (Thermo Fisher Scientific, USA) and selectively stained by acidified toluidine blue. Cytomorphometric studies were performed directly on histological specimens using a Carl Zeiss Primo Star iLED (ZEISS, Germany) microscope. We prepared microphotographs by Carl Zeiss Primo Star iLED microscope and an Axio CamERc5s camera (ZEISS, Germany), that were analyzed by the ZEISS ZEN 2011 (ZEISS, Germany) microscopy program (calculation of the cells size). Cell size was expressed in μm. The degree of mast cells' degranulation was studied by the ratio of mast cells' types with subsequent calculation of the degranulation index (DI).

The following types of mast cells have been identified: 1) with dense and diffuse arrangement of granules in the cytoplasm of the cell; 2) – partially degranulated cells with signs of violation of the integrity of the plasmalemma (blebbing); 3) – deformed, having the appearance of bluish spots, completely degranulated cells, often with the absence of a nucleus or its remnants (fig. 1).

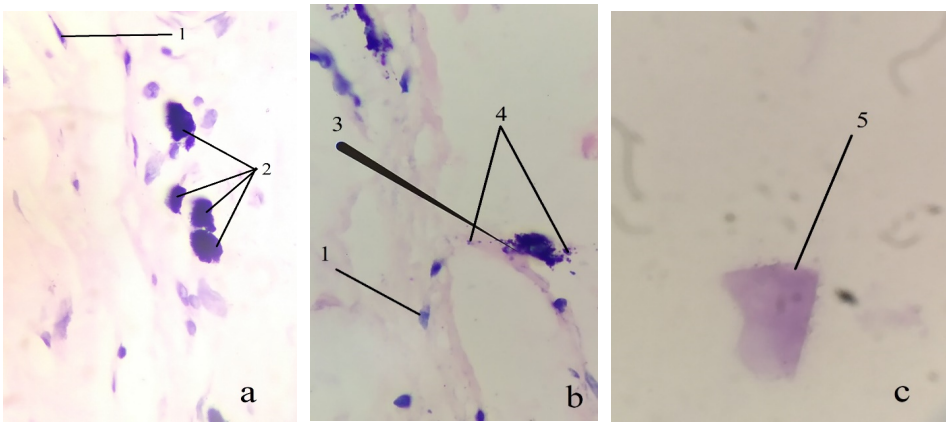

Fig. 1. Degranulation types 1(a), 2(b), 3(c) of mast cells: fibroblast (1), mast cells without degranulation (2), mast cells with partial degranulation (3), granules in extracellular substance (4), degranulated, deformed mast cells (5)

DI was calculated by the formula:

DI mast cells=D/(ND+D)*100%, where

D – number of degranulated cells in the sum of 2nd and 3rd mast cells types,

ND – number of non-derganulated cells-type 1 mast cells.

Statistical analysis and presentation of experimental results were performed using the standard package of statistical programs IBM SPSS Statistics version 26 (IBM corp., Armonk, NY, USA). Normality of quantitative indicators' distribution was checked by Kolmogorov-Smirnov single-sample test. Mann-Whitney test for independent samples with normal distribution was used for the open-field data. Each value is expressed as mean ± standard error of the mean. One-way ANOVA was used to test for overall differences in the extent of days of wound healing process; where appropriate. Correlation analysis of the dependence of cell size on their degranulation type and their significance for each study period was calculated according to Pearson and expressed as mean ± standard deviation. A difference considered statistically significant at $P<0.05$.

Results. In intact, before wounding, histological skin samples of control animals, the amount of type 1 mast cells was in the majority (93.3%), and type 2 mast cells (fig. 2) was in the minimum values (6.7%). During the inflammatory phase, there was a functional redistribution of mast cells. Thus, on the 1st day of wound healing, the number of type 1 mast cells significantly decreased, with a corresponding increase in type 2 cells with

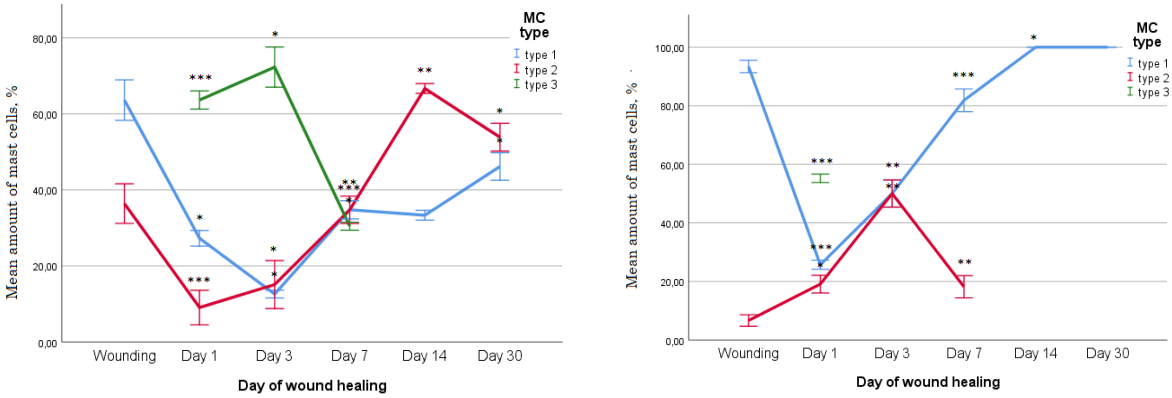

Fig. 2. The ratio of mast cell types (1, 2, 3) in the skin of rats during the healing of a surgical wound in control animals and after exposure to chronic social stress, %

* – differences compared to previous day of wound healing are significant at $P<0.05$.

** – differences compared to previous day of wound healing are significant at $P<0.01$.

*** – differences compared to previous day of wound healing are significant at $P<0.001$.

the appearance of the majority of type 3 cells. By the 3rd day of healing, the activity of mast cells' degranulation decreased: cells of type 1 and 2 already accounted for 50% with the absence of type 3 mast cells. In the proliferative phase, there was a continuing dynamic of the decrease in degranulation activity. So, on the 7th day of wound healing, 81.8% (P<0.001) of mast cells were already type 1, and just 18.2% (P<0.01) were type 2. On day 14, all observed mast cells were type 1 (P<0.05). At the remodeling stage (day 30) all mast cells were type 1.

In the histological preparations of the experimental group of animals, the functional parameters of the mast cells were contrastingly different from the control. Thus, even in intact skin samples, the proportion of type 2 mast cells increased compared to control and amounted to 36.4%. Inflammatory and proliferative phases of wound healing were accompanied by contrasting functional degranulation shifts, different from control. So, on the 1st and 3rd days, the number of mast cells of the 2nd type was – 9.1% and 15.1%, respectively, and the 3rd type was 63.6% and 72.3%, respectively, which significantly differed from values in similar preparations from control animals. Mast cells' degranulation during the proliferative phase somewhat decreased, but it still remained above the control values. So, on the 7th day, the number of type 2 mast cells 34.8%, type 3 – 30.4%, and on the 14th day, all 66.7% of degranulating mast cells were only type 2. At the remodeling stage degranulation of the majority of mast cells by type 2 was still observed (53.9%); the remaining were 1 type.

The functional activity of mast cells was also expressed by us in the form of a degranulation index. The dynamics of the degranulation index of mast cells is shown in fig. 3, from which it follows that the initial values in the control group of animals of this indicator are minimal, and the highest – on the first day at the stage of inflammation after wounding. Further, the degranulation index rapidly decreased and reaching zero values, already at day 14 since all mast cells in this period belonged to type 1.

In the experimental group of animals, degranulation index before wounding was already significant, amounting to 36.4 ± 0.81 % and reached high values on days 1 and 3 of the inflammatory stage. In the proliferation stage, degranulation index decreased, but remained at a high level, on the 7th and 14th days of observation. At the stage of remodeling, degranulation index continued to decrease, but remained high.

Variations in the size of mast cells in the animals of this experiment are presented in fig. 4. The average size of mast cells of type 1 in intact rat skin samples was 5.1 ± 0.30 μm, while their partial degranulation and transformation into cells of type 2 was accompanied by a decrease in their size to 3.5 ± 0.09 μm. At the stages of the reparative process in the control group of animals, the size of type 1 mast cells for the most part varied in the initial sizes (5.1–6.6 μm), but with a pronounced dip to 3.9 ± 0.60 μm (P < 0.001) at the end of proliferation (day 14). Type 2 cells at these stages showed similar variation with type 1 cells with a demonstrative decrease in size of up to 4.4 ± 0.1 μm, but already at the beginning of the proliferation stage.

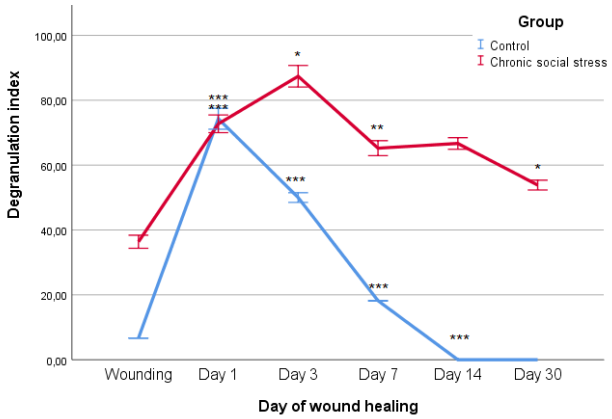

Fig. 3. The index of mast cell degranulation in the skin of rats during the healing of a surgical wound in control animals and after exposure to chronic social stress, %

* – differences compared to previous day of wound healing are significant at P<0.05.
** – differences compared to previous day of wound healing are significant at P<0.01.
*** – differences compared to previous day of wound healing are significant at P<0.001.

In control animals, completely degranulated, type 3 mast cells were observed only in the form of bluish spots only on the first day of the acute phase of inflammation, the cell size of which increased on average to 10.2 ± 0.1 μm, but without gross deformation of the cell and often with the presence of nuclear remnants.

The average size of mast cells in the experimental animals was mostly larger than in the control. Thus, type 1 mast cells in intact (initial) skin samples were 7.5 ± 0.57 μm, while 5.1 ± 0.32 μm in the control (P<0.01), with a significant decrease on the first day of inflammation (6.8 ± 0.72 μm, P<0.05) and with a gradual downward trend towards the end of the inflammation stage (5.7 ± 0.47 μm) with a sharp drop to the beginning of the proliferative stage (7 days) to 4.5 ± 0.51 (P<0.05) and then with a sharp rise towards its end (14 day) – up to 7.9 ± 0.83 μm (P<0.01) and with a return to most of the average sizes for type 1 mast cells by day 30 – 5.9 ± 0.96 (P<0.01) μm.

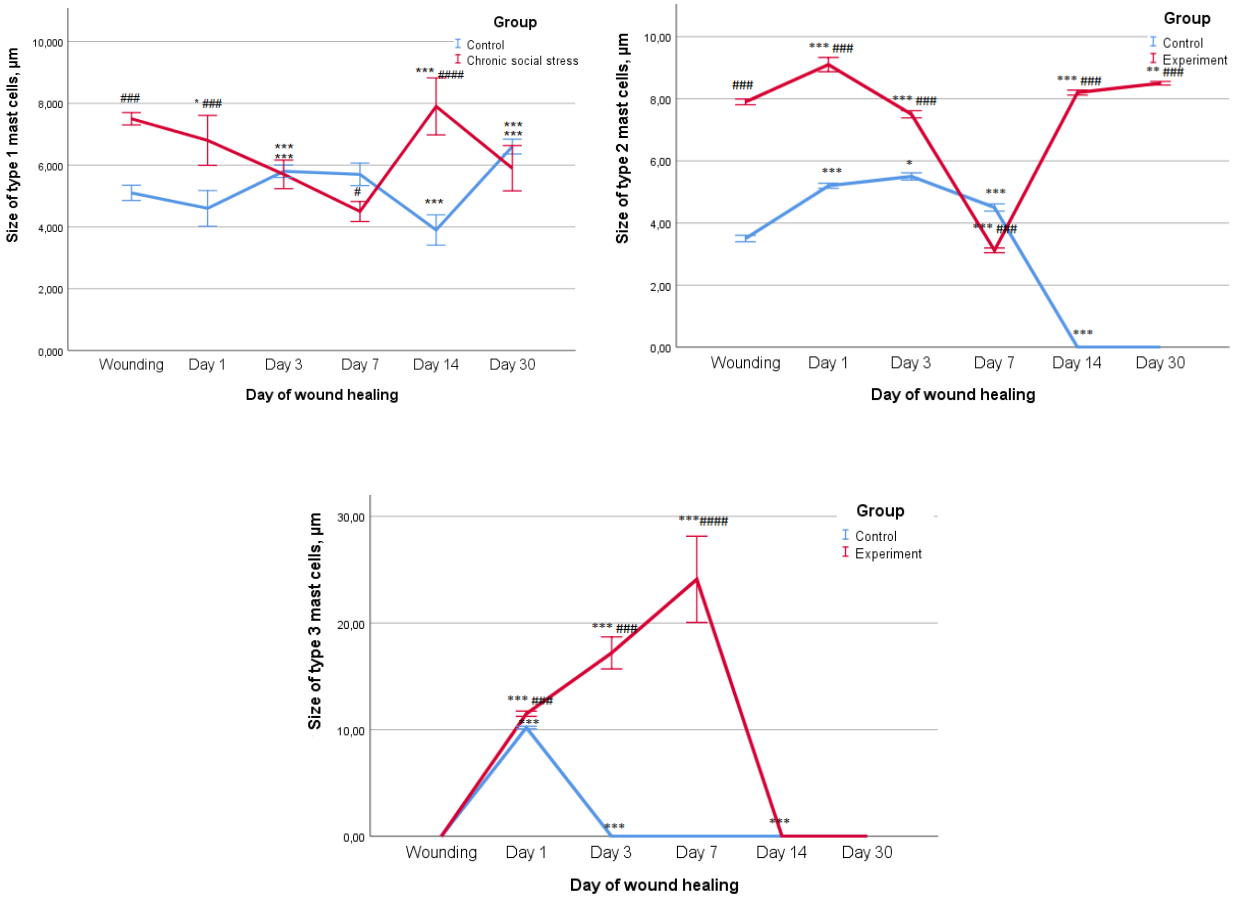

Fig. 4. Sizes of mast cells by days of the reparative process and types of degranulation (1, 2, 3) in the control and experimental groups in the skin of rats, µm

* – differences compared to previous day of wound healing are significant at P<0.05.
** – differences compared to previous day of wound healing are significant at P<0.01.
*** – differences compared to previous day of wound healing are significant at P<0.001.
# – differences compared to control group are significant at P<0.05.
– differences compared to control group are significant at P<0.001.

Partially degranulated mast cells of the 2nd type were even larger before injury – 7.9 ± 0.08 µm, as well as the entire period of inflammation – 9.1 ± 0.22 µm and 7.5 ± 0.11 µm per 1 and 3 days, respectively (P<0.01), with a sharp decrease in the index to 3.1 ± 0.08 µm (P<0.01) at the beginning of the proliferative stage (7th day) followed by an equally sharp increase in size (8.2 ± 0.07 and 8.5 ± 0.05 µm, P<0.01) in the subsequent stages of regeneration (14 and 30 days).

The sizes of type 3 mast cells were typically large, significantly exceeding those in the control at all stages of detection, but the largest were at the end of inflammation (day 3) and at the beginning of the proliferative stage (day 7): 17.2 ± 1.31 µm and 24.1 ± 3.57 µm respectively (P<0.01). Their general morphology was typical – in the form of bluish spots, often with a rough deformation of the edges of the cell.

When studying variations in the mast cells' size, their average values in intact histological samples of control animals before injury were taken as the initial ones, since it was assumed that they correspond to their physiological homeostatic state.

As can be seen from fig. 4, mast cells of 2 type of were detected in control animals on the day of injury, most of which were cells of type 1 (93.4%), the size of which was 5.1 ± 0.31 µm, mast cells type 2 was 6.7%, partial degranulation of which was accompanied by a decrease in size to 3.5 ± 0.09 µm.

Analysis of the results of fig. 4 shows that an increase in the functional activity of mast cells during reparative processes is usually accompanied by an increase in their size. This is typical for both control and experimental groups of animals. It should be noted that the trend towards an increase in the size of the mast cells during

their functional load was contrastingly expressed in the experimental group of animals. In animals of this group, the size of mast cells of the 1st and 2nd types before injury exceeded those in intact histological preparations. Attention is drawn to the appearance of type 3 mast cells with the greatest violation of structural homeostasis – inflammation, accompanied by huge cell sizes, which continues even in the proliferative period of wound healing.

An analysis of the sizes of mast cells types at the stages of reparative regeneration revealed its different direction and amplitude of changes in both experimental and control groups of animals. Thus, in individual phases, maximum and minimum values of the size of the mast cells were observed (for example, the minimum values of the mast cells of the 1st type on the 14th day in the control – 3.9 ± 0.67 μm and on the 7th day of the 2nd type in control, and 3.1 ± 0.08 μm in experiment). The sizes of cells of types 1 and 2 with the amplitude of fluctuations are within the range of histological values for immunological cells of innate immunity, while type 3 mast cells significantly exceed these limits and have a size of 24.1 ± 3.52 μm, as on the 7th day in the experimental group of animals.

The functional dependence of the size of the mast cells, depending on their belonging to the types of degranulation (1, 2, 3) is presented in table 1. So, before wounding (intact period), the coefficient of correlation between the analyzed values was negative and amounted to -0.98 (P<0.001), while on the first day of the inflammatory process this indicator changed sign to positive, remaining high r=0.91 (P<0.001), but by the end of this period (day 3), there was a tendency to change the direction of the relationship r=-0.18, which reached significant values by the beginning of the proliferative stage (r=-0.85, P<0.05). At the remaining observation periods (days 14 and 30), no correlation analysis was performed, since all mast cells were of type 1.

In experimental animals, the direction of the relationship between the size of the mast cells and their types was only positive, and already in the initial histological sections of the skin it was average and amounted to 0.54. At the inflammation stage (days 1 and 3), the relationship was high, approaching functional (0.98 and 0.92, respectively, P<0.01). In the proliferative stage of wound healing, the analyzed indicator remained high 0.82 (P<0.01), but by its end (day 14) it dropped sharply to 0.31, going beyond the significance limits, however, at the remodeling stage, the relationship increased again, approaching to functional (0.93, P<0.01).

Discussion. The results obtained convincingly confirm the literature data on the main function of mast cells in the loose connective tissue and their relationship with the main regulatory system – the neuro-immuno-endocrine system (Bhuiyan et al., 2021, p. 2184–2197). It is on their functional state that the metabolism of cells, tissues, organs and their systems depends due to the circulation of the humoral internal environment: blood plasma – tissue fluid – lymph.

At the same time, an adequate explanation of the dynamics of the obtained results can be logically made only with a comprehensive assessment of the histological dynamics of mast cells in loose connective tissue. Thus, in loose connective tissue, mast cells' precursors constantly repopulate from the bone marrow, differentiating into the corresponding autonomous type of mast cells, where they perform a set of functions and end their cell cycle with apoptosis (Yukihiko et al., 2006, p. 387–405). At the same time, the quantitative shifts of mast cells in the process of healing of surgical wounds were closely related to their functional values: synthesis and constant release of biologically active substances by degranulation.

In this regard, the physiological processes of mast cells in the control of this study can be logically represented by the following interpretation. So, in our previous studies in the same experiment, in control animals, the initial (before wounding) amount of mast cells was found to be 0.95 ± 0.09 per 0.01 mm^2 (Makyeyeva et al., 2021, p. 34–41), which corresponded to the data of other authors (Bayat et al., 2008, p. 931–938). As shown in Figure 2, most of them belonged to type 1 and had an average size of 5.1 ± 0.30 μm, which we took as the initial size for mast cells as cells of this histological series. A small amount of type 2

Table 1

Correlation relationship between sizes of mast cells (μm) and their degranulation types (1, 2, 3) in the control and experimental groups of animals, r ± SD

Group	Days of experiment					
	0	1	3	7	14	30
Control (n=20)	-0.98 ± 0.0001***	0.91 ± 0.0034***	-0.18 ± 0.0201	-0.85 ± 0.0974*	-	-
Experiment (n=30)	0.57 ± 0.0103***	0.98 ± 0.0001**	0.92 ± 0.0031**	0,82 ± 0.0052**	0.31 ± 0.0211	0.93 ± 0.0033**

* – differences are significant at P<0.05
** – differences are significant at P<0.01.
*** – differences are significant at P<0.001.
– all mast cells were type1.

mast cells with partial degranulation appear, probably, for local non-extreme physiological needs, without additional synthesis of biologically active substances. Therefore, their size decreased to 3.5 ± 0.09 μm.

Analysis of the results of Figure 4 shows that wounding, as a source of structural homeostasis disturbance, led to an increase in the functional activity of mast cells, aimed at reparative regeneration. In control animals, this activation was characterized by a moderate increase in the amount of mast cells (Figure 3) and their degranulation (fig. 2), which was especially pronounced in the acute phase of inflammation with the appearance of type 2 mast cells (19.1%), and on the first day, even type 3 mast cells up to 55,2%.

Continued further significant increase in the amount of mast cells in the proliferative phase (on days 7 and 14) up to 4.38 ± 0.20 per 0.01 mm^2 and 4.61 ± 0.07 per 0.01 mm^2, respectively, occurred with simultaneous decrease in degranulation activity, which can be explained as a change in reparative tasks. So, only at the beginning of the proliferative period, a low (18.2%) degranulation type 2 mast cells was recorded, while by its end (on the 14th day) and during the reparation period on the 30th day, all mast cells were of type 1. According to the amplitude of the indicators, during this period (30 days), the main reparative regenerative processes at the site of the surgical wound have biologically active substancesically ended. It can be considered that the drainage and anti-infective function of mast cells in the inflammatory phase, which requires an increased and rapid functional stress of these cells, has changed to a reconstructive repair function in the remaining periods of wound healing, which requires a strictly dosed and qualitatively diverse other type of biologically active substances, functionally different from those in inflammatory stage (Bayat et al., 2008, p. 34–41).

It should be noted that during these periods, granulation tissue is formed in the area of the wound, its revascularization, re-epithelialization and scarring which require fine correction of stradiospecific and differentiating tissue histogenesis in loose connective tissue by immune factors, which will be the subject of our future research. The functioning of mast cells at all stages of the study of the wound process in the control occurred in physiological dimensional parameters of 5.1–6.6 μm. This also indicates that type 2 mast cells degranulation was accompanied by additional adequate synthesis of biologically active substances and their exocytosis with preservation of all cytoskeletal structures.

The probable reasons for the detection of mast cells' sizes on average 3.9 ± 0.60 μm on the 14th day of observation will be discussed below.

In the control group of animals during cytomorphometric studies, the exception is type 3 mast cells, which occurred on the 1st day of the inflammatory reaction after wounding, their size reached 10.2 ± 0.11 μm After complete degranulation and disorganization of the cytoskeleton, they were probably subject to apoptosis.

Social stress, like its other variants, is a damaging factor that, through the hypothalamic-pituitary-adrenal (adrenocorticotropic) axis, strains the regulation of the functioning of both systemic structures (neuro-immune-endocrine) and local regulatory ones, including the mast cells (Bhuiyan et al., 2021, p. 2184–2197). Negative stress factors are manifested through hormone-like mediators, for example, cortisol, various chemokines and other humoral factors, primarily by changing the migration orientation of the cells of the tissues of the internal environment, which include Mast cells, and with an increase in their dose-dependent effects, negative factors, in the future, appear modulation of the proliferative activity of their precursors, up to apoptosis. From these positions it is possible to explain the contrasting quantitative and functional shifts in the parameters of mast cells in the group of experimental animals.

So, already in intact skin samples (before wounding), a multiple increase in quantity of mast cells was observed in the experimental group of animals, which, on average, amounted to 9.47 ± 0.23 per 0.01 mm^2 and 0.95 ± 0.03 per 0.01 mm^2 in the control ($p<0.001$) (Makyeyeva et al., 2021, p. 34–41), a significant number of which (36.4%) (fig. 2) belonged to the 2nd type, also with a significant increase in size in on average up to 7.9 ± 0.08 μm (fig. 4) probably due to compensatory synthesis of biologically active substances and activation of mast cells in general.

If we take into account that mature mast cells do not proliferate in loose connective tissues (Yukihiko et al., 2006, pp. 387–405) then the multiple increase in the number of mast cells after exposure to chronic social stress before wounding can only be explained by their migration from other organs, as well as the migration of their precursors from the bone marrow. At the same time, scientists consider the migratory redistribution of cells between tissues under stress as an important component of the stress stage of the general adaptation syndrome according to Selye (Stefanski et al., 2003, p. 17–24).

Associated with general and regional disorders in the body of experimental animals under the influence of chronic social stress, the application of a surgical wound is also a stress factor that together with the first one additively negatively affected the entire course of the reparative process. Thus, the maximum degranulation according to type 3 up to the 7th day of observation with

the phenomena of karyorrhexis, disorganization of the form as morphological signs of the beginning of apoptosis testifies to the increased tension of the functioning of the mast cells. At the same time, an excessive increase in size to 24.1 ± 3.54 μm on the 3rd day of the experiment probably occurred due to the disorganization of the cytoskeleton.

During these observation periods (days 1–7) and in the subsequent ones (days 14–30), the proportions of type 2 mast cells were recorded in an adequate percentage and, for the most part, they were increased in size compared to control values. This fact convincingly testifies to intense synthesis of biologically active substances in mast cells. In this regard, the decrease in the amount of mast cells from their control values (Makyeyeva et al, 2021, p. 34–41) can be explained by their increased consumption for the restoration of the loose connective tissues structure, that is, with their increased utilization in the inflammatory and proliferative phases of the wound process, which was accompanied by stressful disorganization of migratory and proliferative reactions. At the same time, a multiple increase in mast cells on the 30th day after the surgical wound (5.68 ± 0.76 per 0.01 mm^2), more than half of which belonged to type 2, is probably already an evidence of compensatory reparative reactions to the chronic prolongation of regenerative processes.

It can be considered that this increase in the functional activity of mast cells occurred along with the stage of resolution of the stress reaction of the general adaptation syndrome of the body according to Selye. In the effect of inhibition of mast cells' migration at this stage, their biologically active substances as well as cytokines of their microcellular environment, primarily macrophages and fibroblasts with their subsequent transforming forms – myofibroblasts can also take part. Thus, it was found that the mediator of mast cells protease4 (chymase) promotes the recruitment of circulating leukocytes, including mast cells and, probably, their precursors to the site of the wound process (Gupta & Gupta, 2017, p. 103–251).

As a conclusion of this section, it can be considered that chronic social stress disrupted the normal course of the wound process, contributing to its chronicity, in which cellular events characteristic of each stage of reparative regeneration were cross-observed in the wound healing process.

The formulated conclusion is confirmed by the data of the analysis of the degranulation index (fig. 3). So, if in the control the degranulation index had background values (6.66 ± 0.11%), then in the inflammatory stage (1, 3 days) its level was very high and medium (74.3 ± 1.33% and 50.0 ± 0.62%, respectively), but already at the beginning at the proliferative stage (on the 7th day), the degranulation index was already minimal (18.2 ± 0.25%), and on the 14th day it was 0. This dynamics also confirms our conclusion about the paradigm shift of cellular and humoral processes in the wound, which we described above: drainage prevailed and preparatory regeneration processes in the inflammatory stage, and reconstructive, remodulative processes prevailed in the proliferative one.

Continuing the analysis of the data, it should be noted that in the experimental group the normal course of the wound healing process was sharply disrupted, which was already taking place under the influence of stress damage to skin tissues, as evidenced by significant levels of degranulation index (36.4 ± 0.81) before the wound was applied. Degranulation index, remaining high throughout the experiment, makes it possible to draw a convincing conclusion about the chronicity of reparative processes in the experimental group.

This conclusion is also confirmed by the data of the correlation analysis of the mast cells' size and their belonging to the degranulation type (table). So, in the control, the relationship between these indicators was high, but negative or absent in its majority. And only on the first day of the inflammatory process was highly positive. From this dynamics it follows that normally in the experimental group, both physiological and reparative processes of homeostasis occur mainly with the participation of type 1 mast cells, the consumption of which was continuously replenished from the bone marrow by their predecessors. And only stress loads on the recovery processes on the 1st day of healing induced their degranulation activity, which ultimately led to a high positive correlation coefficient.

Other values of correlation coefficient were between the type of cells and their size in the experimental group of animals. In contrast to controls, correlation coefficient was mostly positive with a tendency to reach significance (on days 1 and 7) or was high reaching functional values. Therefore, the data of the correlation analysis once again argue for the high intension of the mast cells, leading to the prolongation of the recovery processes in the surgical wound in the experimental group of animals. The high synthetic activity of mast cells in the experiment explains the failure to achieve the level of significance of correlation coefficient on the above days of observation, since the sizes of type 1 cells were high, leveling out the desired dependence (7.5 ± 0,56 μm and 7.9 ± 0.87 μm on the 1st and 14th days, respectively).

We have demonstrated the informativeness of the cytomorphometric method of mast cells analysis by analyzing the amplitude values along the course of reparative processes. Thus, regular amplitude dips in the size of the mast cells were found at different stages of observation. In the control, we noted this decrease of mast

cells at the beginning of the proliferative phase (4.4 ± 0.19 μm), and an even greater decrease in the size of mast cells was at its end – on day 14 to 3.9 ± 0.62 μm. In the experimental group, the beginning of the formation of dimensional dips shifted to the inflammatory phase: on the 3rd day for type 1 mast cells – 5.7 ± 0.43 μm, for type 2 mast cells 7.5 ± 0.17 μm, and reached its maximum depth on the 7th day after surgery 4.5 ± 0.51 and 3.1 ± 0.08 μm for mast cells type 1 and 2, respectively.

It is noteworthy that these cytomorphometric changes in the control coincided with a decrease in degranulation activity at the border between the phases of proliferation and remodeling, and in the experiment – by the end of the inflammatory phase and the beginning of the proliferative phase. As we noted above, during these periods of the wound process, there was a change in the functional tasks of the mast cells and their cellular environment. In the literature available to us, we did not find similar cytomorphometric studies of mast cells during the wound process. In this regard, in the absence of other comparative provisions, it is possible to logically explain the detected cytomorphometric failures of mast cells at these stages of the wound process only from the position of migration of new postproliferative precursors from the bone marrow into the wound area. These post-proliferative cells have minimal size (3.1–3.5 μm), since they are just beginning their synthetic activity and further differentiation. Cytologists know that the stem cells of their post-proliferative ool have a minimum size in the histological series equal to 3–5 microns (Hirofumi & Schnittger, 2010, p. 66–74). The reasons for the decrease in size are that rapidly occurring successive mitoses (3–10 or more times) the cytoplasm does not have time to recover to its tissue level, which the cell reaches with further differentiation. We have also documented this in a number of publications. So, in recent publications, we analyzed the dynamics of cytomorphometric parameters in the blood of rats against the background of fecal peritonitis (Frolov et al., 2021, p. 365–375). We found a sharp decrease in peripheral blood recirculation of precisely small classes of lymphocytes, which were deposited in the lesions of mucous membranes.

Conclusion. Mast cells are a key regulatory factor in the wound healing process. Degranulation of mast cells for subsequent stimulation of inflammatory and reparation processes by released biologically active substances is an integral part of the compensatory-adaptive reaction of tissues during the wound process. During normal wound healing, on the 1st day mast cells degranulate predominantly to type 3, in the acute inflammatory period to type 2, and at the stages of proliferation and remodeling, type 1 mast cells predominate.

Chronic social stress alters the dynamics and functional activity of mast cells during surgical wound healing. The number of mast cells, their size and degree of degranulation increases to type 3 during the 1st week after trauma and to type 2 from the 7th to the 30th day.

Thus, the number and functional activity (degranulation and size) of mast cells can serve as a marker of the intensity of inflammatory and regenerative processes, as well as the degree of repair efficiency in wound remodeling.

Further fundamental research is needed on the regulatory properties of the mast cell secretome during wound healing under conditions of the relevance of social stress and the frequency of skin trauma for further analysis of the molecular mechanisms of regenerative processes that ensure the completeness of tissue remodeling through the search for appropriate pharmacological agents.

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Aliyeva O. – conclusions, proofreading of the text and analysis of literary sources.

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