Theoretical and Experimental Cryobiology

https://doi.org/10.15407/cryo35.02.115
UDC: 615.24:612.12: 591.128: 612.592

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EFFECT OF CHRONIC COLD STRESS AND DALARGIN ON BLOOD PARAMETERS IN GUINEA PIGS

This study investigates the effects of the synthetic neuropeptide dalargin on hematological parameters and blood electrolyte homeostasis in guinea pigs under conditions of chronic cold stress (CCS) at 4 °C over a five-day period. It was found that CCS led to a reduction in platelet count and disruption of the leukocyte profile, including an increase in band neutrophils accompanied by a decrease in segmented neutrophils, lymphocytes, and monocytes, as well as a decline in the adaptation index. Simultaneously, elevated concentrations of calcium, potassium, and chloride were observed, indicating disturbances in electrolyte balance and ionic homeostasis. Dalargin administration prevented these changes, promoting the restoration of physiological leukocyte ratios, increasing platelet counts, and normalizing key plasma electrolyte concentrations. Moreover, an increase in the adaptation index was recorded, indicating activation of the hematopoietic system and mobilization of the organism's adaptive potential. These findings support the potential of dalargin as an effective agent for maintaining blood system integrity, electrolyte balance, and adaptive capacity under CCS conditions.

Key words: *adaptation, cold stress, cryobiology, hypothermia, dalargin, electrolyte balance, hematological parameters.*

The study of adaptation mechanisms in homoiotherms exposed to low temperatures remains one of the key areas in modern physiology and cryobiology. Chronic cold stress (CCS), as one of the most prolonged and multifactorial types of stress exposure, induces systemic changes in the body, including disruptions in energy metabolism, imbalance in antioxidant defence, and reduced functional activity of cells [7, 8, 29, 36—38]. In response to CCS, complex adaptive mechanisms are activated to maintain homeostatic equilibrium. However, excessive or prolonged strain on these mechanisms may lead to functional exhaustion of major regulatory systems, accompanied by decreased immune reactivity, metabolic destabilization, and increased susceptibility to pathological conditions of various etiologies [7, 8, 21, 29, 32, 36].

One of the most sensitive indicators of stress-induced disturbances is the state of peripheral blood. The morphofunctional characteristics of highly specialized blood cells, particularly erythrocytes and leukocytes, serve as reliable markers reflecting the level of nonspecific resistance of the organism, the severity of pathological changes, and its functional reserves for activating compensatory-adaptive responses to stressogenic factors [14].

An equally important criterion is electrolyte homeostasis, which plays a crucial role in ensuring normal cell function. The major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, P) are involved in regulating osmotic pressure, maintaining transmembrane potential, activating enzymes, facilitating muscle contraction, and transmitting nerve impulses [4, 6, 11, 13, 15, 27, 28]. Specifically, sodium and

Reference: Myrnyi VH, Moisieieva NM. Effect of chronic cold stress and dalargin on blood parameters in guinea pigs. *Probl Cryobiol Cryomed*. 2025; 35(3): 115—22. https://doi.org/10.15407/cryo35.02.115

potassium maintain the electrochemical gradient necessary for neuromuscular conductivity and metabolite transport; calcium is essential for muscle contractility, enzymatic system activation, and blood coagulation; magnesium stabilizes nerve conduction and reduces cellular excitability; and phosphates participate in energy metabolism, acid-base regulation, and the structural integrity of cell membranes [25, 31].

A comprehensive assessment of changes in the blood system and electrolyte balance under conditions of chronic cold stress (CCS) enables a holistic characterization of the organism's adaptive responses and the effectiveness of pharmacological agents capable of modulating these processes. It is well established that one of the key components of the adaptive response to extreme environmental factors is neuropeptides—particularly those of the opioid family — which are involved in modulating immune, endocrine, and nervous system regulation [9, 10, 16].

In this context, dalargin, being a synthetic analogue of Leu-enkephalin, draws particular interest due to its pronounced protective, membrane-stabilizing, and anti-stress effects. Previous studies have demonstrated its ability to reduce metabolic and structural disturbances following exposure to low temperatures [9, 10]. However, the impact of dalargin on hematological parameters and electrolyte balance specifically under conditions of chronic cold stress has not yet been investigated.

The research object was to determine the effect of the synthetic Leu-enkephalin analogue (dalargin) on electrolyte balance and hematological indices in guinea pigs following chronic cold stress.

MATERIALS AND METHODS

The study was conducted on sexually mature male guinea pigs of the Dunkin-Hartley strain, aged 5—6 months, with an average body weight of 600—800 g. The animals were housed under standard vivarium conditions at the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Kharkiv).

All animal experiments were approved by the Institute's Bioethics Committee (Protocol No. 5 of November 22, 2022) and carried out in accordance with the provisions of the Law of Ukraine «On the Protection of Animals Against Cruelty». All procedures involving animals complied with the guidelines and recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Experimental Design was as follows:

The animals were divided into the following groups:

- **Intact group** — animals not subjected to CCS and maintained at a temperature of 18°C.
- **Control group** — animals administered 0.5 ml of physiological saline subcutaneously 30 minutes prior to CCS induction.
- **Dalargin group** — animals administered dalargin (JSC «Biolik», Ukraine) at a dose of 100 μg/kg subcutaneously 30 min prior to CCS induction.

The data on «intact + dalargin» group is not included in this article, as preliminary studies showed that administration of the drug under physiological conditions did not produce significant changes in the parameters presented.

CCS was modelled using the method of A. Namimatsu et al. [19], adapted to laboratory conditions by modifying the temperature regime to ensure reproducibility of the stress response while maintaining ethical standards for animal care. The animals were alternately exposed to temperatures of 18 °C and 4 °C — initially for one hour, followed by a five-day cycle of 12 hours at 4 °C and 12 hours at 18 °C. Stress exposure was terminated on the sixth day, and blood samples were collected via jugular vein puncture.

Body temperature of the guinea pigs following hypothermia induction was measured using an electronic thermometer inserted to a depth of 2 cm. The temperature decreased by 1—3 °C from the baseline of 38 °C.

The concentrations of K^+, P, Cl^-, Ca^{2+}, and Na^+ ions in blood serum were determined using a spectrophotometric method with reagent kits from «Filisit-Diagnostyka» (Ukraine). Results were expressed in mmol/L.

Hematological parameters were measured using the veterinary automatic hematology analyzer «URIT–2900 Vet Plus» (URIT Medical Electronic Co., Ltd, China), following the manufacturer's instructions.

To assess the immune response to CCS and dalargin administration, the study calculated an adaptation index — a marker of both specific and nonspecific immune activity. This index was determined by the ratio: percentage of lymphocytes/percentage of segmented neutrophils [14].

For statistical Analysis the experimental data were processed using the software packages «Statistica 10» (StatSoft, USA) and «Excel» (Microsoft, USA). Statistical significance of differences between independent groups was determined using the nonparametric Mann—Whitney test at $p < 0.05$.

RESULTS AND DISCUSSION

In the initial phase of the study, we examined the dynamics of electrolyte balance indicators based on the concentrations of Na^+, K^+, Cl^-, Ca^{2+}, and P. As shown in Table 1, CCS led to a significant increase in the concentrations of K^+, Cl^-, and Ca^{2+} in guinea pig blood — on average by 1.3 times compared to intact values. Meanwhile, the concentrations of Na^+ and P under CCS conditions did not differ significantly from those in the intact group.

These findings are consistent with the study by S. Mu et al. [18], which demonstrated that exposure to ice water resulted in a twofold increase in serum Ca^{2+} levels, accompanied by elevated concentrations of total phosphorus and Mg^{2+}. Such changes may indicate disturbances in electrolyte balance, poten-tially increasing the risk of functional disorders under cold stress conditions. Considering this, monitoring electrolyte levels is of critical importance.

Administration of dalargin prior to CCS modeling altered the dynamics of electrolyte metabolism (Table 1). Specifically, a significant increase in K^+ concentration by 1.2 times and a decrease in P concentration by 2.1 times were observed compared to the intact group. Meanwhile, the concentrations of Na^+, Cl^-, and Ca^{2+} ions did not differ significantly from those in the intact control.

In the second phase of the study, the effect of dalargin on hematological parameters in guinea pigs following CCS was investigated (Table 2). The number of erythrocytes, hemoglobin content, and hematocrit values did not show significant changes across groups (Table 2). However, notable alterations under CCS conditions were observed in platelet counts (see Table 1). Specifically, platelet numbers decreased significantly by an average of 1.1 times compared to the intact group, which aligns with findings by A. Teleglow et al. [34] and S. Van Poucke et al. [36]. This reduction may be attributed to platelet margination induced by hypothermia, involving changes in cell morphology, reduced blood flow, and increased expression of adhesion molecules [36].

The result of total leukocyte counts, and their subtypes showed that the overall leukocyte number did not change significantly under CCS conditions. However, specific leukocyte populations underwent notable shifts in response to CCS, indicating a restructuring of the immune response.

A marked increase in the percentage of band neutrophils was observed under CCS, suggesting that the bone marrow was producing more immature neutrophils in response to stress. Conversely, the percentage of segmented neutrophils decreased by 1.3 times compared to intact values, which aligns with the maturation dynamics of neutrophilic cell types.

The percentage of monocytes in peripheral blood also significantly decreased following CCS, both in comparison to the dalargin-treated group and the intact group. This may reflect a weakening of the phagocytic arm of innate immunity.

The most pronounced changes were observed in the lymphocyte population: lymphocyte counts decreased by 2.1 times under CCS compared to the intact group. This points to potential impairment of adaptive immunity and may result from glucocorticoid-induced thymic atrophy or T-cell apoptosis — phenomena commonly associated with stress conditions.

It is well established that typical markers of stress-induced immune response include atrophy of central immunogenic organs (particularly the thymus), granulocytosis, suppression of the lymphoid lineage, and altered activity of hematopoietic stem cells [5, 23, 30]. These stem cells are essential for regenerating immunocompetent cells and play a critical role in restoring immune system homeostasis following stress exposure.

Table 1. **Content of serum electrolytes in homeothermic animals after CCS and Dalargin administration ($n = 12$)**

Serum Electrolytes (mmol/L)	Intact Group	CCS	CCS + + Dalargin
Na^+	133 ± 0.8	135 ± 1.4	132 ± 0.9
K^+	4.16 ± 0.21	5.8 ± 0.1 *	4.9 ± 0.05 #
Cl^-	95 ± 1.6	120.6 ± 2.3 *	100 ± 0.7 #
Ca^{2+}	2.2 ± 2.3	2.9 ± 0.1 *	2.3 ± 0.08 #
P	1.99 ± 0.05	2.14 ± 0.1	0.94 ± 0.01 *,#

Notes: * — significant difference compared to intact group ($p < 0.05$); # — significant difference compared to CCS group ($p < 0.05$).

Following dalargin administration under CCS conditions, a distinct pattern of hematological changes was observed. Notably, platelet counts increased significantly — by 1.3 and 1.4 times compared to the intact and control groups, respectively (see Table 2)—alongside an elevation in erythrocyte numbers, accompanied by a decrease in mean hemoglobin concentration per erythrocyte. This effect may be attributed to the protective action of dalargin, particularly its ability to modulate stress-induced vascular damage and hematopoiesis.

Regarding the leukocyte profile, substantial quantitative and qualitative changes were detected compared to animals exposed to CCS without treatment. Total leukocyte counts increased significantly, while the percentage of band neutrophils remained comparable to intact levels. This may indicate normalization of specific immune system components and restoration of nonspecific immune efficiency under dalargin treatment. Supporting this, the percentage of segmented neutrophils increased by 1.4 times relative to the intact group and by 1.7 times compared to the CCS control group (see Table 2). Such a response is interpreted as a boost of the organism's resistance under dalargin influence, reflecting its immune modulatory properties.

The percentage of lymphocytes in the blood did not differ significantly from intact values (Table 2). Thus, dalargin contributes to restoring the balance between humoral and cell branches of the immune system, mitigating the severity of CCS-induced disturbances.

The effect of CCS on the number of Foa-Kurloff cells — marker macrophage elements that appear in the blood in response to stress stimuli and signal immune activation — is presented in Table 2. It was found that dalargin administration led to a significant twofold increase in the number of these cells compared to both the control and intact groups. These results indicate enhanced phagocytic activity and overall immune system activation under CCS conditions influenced by the synthetic neuropeptide dalargin.

This finding aligns with the observed increase in the percentage of segmented neutrophils in peripheral blood following CCS and dalargin admi-

Table 2. **Hematological Parameters in Guinea Pigs after CCS and Dalargin Administration (*n* = 12)**

Erythrocyte Parameters			
Blood Parameter	Intact	CCS + NaCl	CCS + Dalargin
Hemoglobin (g/L)	158.2 ± 0.5	157.2 ± 0.6	155.6 ± 1.28
Erythrocytes ($\times 10^{12}$/L)	5.86 ± 0.03	5.83 ± 0.01	6.12 ± 0.1
Hematocrit (%)	45.8 ± 0.17	45.9 ± 0.1	48.6 ± 0.74
Mean Corpuscular Volume (MCV)	78.6 ± 0.26	77.12 ± 0.41	81.8 ± 0.58
Mean Hemoglobin Concentration in Erythrocytes	402.4 ± 1.53	405.6 ± 0.76	339 ± 3.34 *
Platelets ($\times 10^9$/L)	439.3 ± 3.1	413.8 ± 1.9 **	564.2 ± 6.9 *
Leukocyte Parameters			
Leukocytes ($\times 10^9$/L)	9.8 ± 0.06	11.5 ± 0.3	15.5 ± 0.4 *
Leukocyte Types (%)			
Cell Type	Intact	CCS + NaCl	CCS + Dalargin
Band Neutrophils	3.3 ± 0.01	9.4 ± 0.7*,**	5.1 ± 0.5
Segmented Neutrophils	30.6 ± 1.4	25.4 ± 0.6*,**	43.1 ± 3.8 *
Lymphocytes	53.1 ± 2.0	24.9 ± 0.9*,**	39.3 ± 0.8 *
Monocytes	4.4 ± 0.9	2.79 ± 0.2*, **	4.1 ± 0.6
Foa-Kurloff Cells ($\times 10^9$/L)	1.5 ± 0.01	2.91 ± 0.2 *, **	5.8 ± 0.3 *
Lymphocyte-to-Segmented Neutrophil Ratio	1.44 ± 0.12	0.8 ± 0.05 *,**	1.14 ± 0.08

Notes:* — significant difference compared to intact group ($p < 0.05$); ** — significant difference compared to CCS group ($p < 0.05$).

nistration, pointing to the activation of nonspecific immunity. Supporting this, the adaptation index — calculated as the ratio of lymphocytes to segmented neutrophils — was significantly reduced to 0.8 ± ± 0.05 in animals exposed to CCS, falling below unity. Such a shift reflects predominant activation of the nonspecific immune branch (neutrophils) alongside suppression of adaptive immunity (lymphocytes), potentially indicating immune destabilization or functional exhaustion.

In contrast, animals treated with dalargin showed no significant difference in adaptation index compared to the intact group (see Table 2), which — as previously noted — suggests a restored balance between the major branches of the immune system.

Analysis of the study results revealed that CCS induces a series of changes in ionic homeostasis, indicating activation of the organism's adaptive mechanisms (see Table 2). In particular, elevated concentrations of K^+ and Cl^- were recorded in blood serum, which may be associated with impaired function of membrane ion pumps triggered by stress-activated signalling cascades [5, 12] (4, 11). The increase in Cl^- concentration, in turn, reflects shifts in acid-base balance and the activation of osmoregulatory and compensatory processes in response to stress [15, 27].

Such disturbances in ion metabolism may lead to a range of adverse effects. They can reduce the efficiency of homeostatic regulatory systems, impair cellular functional activity, and influence immune responses — particularly phagocytosis, which is sensitive to K^+ and Ca^{2+} levels [20, 39]. Furthermore, the reduced ability to maintain electrolyte balance under CCS may be accompanied by coagulation disorders, linked to fluctuations in ion concentrations, especially K^+, Ca^{2+}, and Mg^{2+} [33, 35].

The observed changes indicate that CCS is a significant factor in disrupting ionic balance, which can diminish the organism's adaptive capacity and resilience to prolonged extreme exposures. Notably, phosphorus levels — critical for energy metabolism and ATP synthesis — remained stable under CCS conditions (see Table 1).

Dalargin administration under CCS had a pronounced effect on ionic homeostasis, particularly contributing to the normalization of Cl^- and Ca^{2+} concentrations in the blood. One possible explanation for this outcome is the elevation of stress hormone levels triggered by CCS, which activates signaling cascades — a finding consistent with previous studies [17].

The observed decrease in phosphorus concentration following dalargin administration deserves special attention, as it may reflect the compound's involvement in regulating energy and mineral metabolism under stress. Similar effects of neuropeptides on phosphate-calcium metabolism have been described by H. Park et al. [22] and S. Amar et al. [2], suggesting a potential role for dalargin in enhancing phosphate utilization for ATP synthesis — a key energy carrier essential for restoring disrupted homeostasis under CCS [3, 24].

Moreover, considering phosphorus role in blood buffering systems and maintaining cellular membrane integrity — as noted by I. Shaw et al. [31] and Y. Posor et al. [25] (—its reduction may signal the activation of adaptive cellular mechanisms. This could also be linked to the proliferation and mobilization of hematopoietic stem and progenitor cells (HSPCs), as demonstrated in the study by M. Adamiak et al. [1].

Thus, the decrease in phosphorus levels, combined with the increase in leukocyte populations in the blood of dalargin-treated animals, may indicate activation of the organism's metabolic response. This finding aligns with studies by M.Z. Ratajczak et al. [26] and X. Zhang et al. [40], which showed that elevated ATP production under stress conditions stimulates leukopoiesis and promotes an increase in immune cell numbers in peripheral blood. Specifying the findings, particular attention should be paid to changes in the platelet component. Under CCS conditions, a reduction in platelet count was observed, likely due to temperature-induced apoptosis during rewarming to normothermia [36]. In contrast, dalargin administration resulted in a significant increase in platelet numbers, which may indicate activation of the sympathetic nervous system, mobilization of cells from storage sites, and stimulation of hematopoiesis as part of the organism's adaptive response to cold stress.

Thus, dalargin administration under CCS conditions influences both ionic and hematopoietic homeostasis in experimental animals. The results confirm one of dalargin mechanisms of action, *i.e.* its ability to stabilize cell membranes, thereby normalizing the function of ion channels and transporters. This helps maintain the balance of key ions such as K^+, Na^+, and Ca^{2+} between the

intracellular and extracellular media, which is critical for normal cell function and overall homeostasis under CCS.

Studying changes in electrolyte balance and hematological parameters under cold stress and dalargin treatment is essential for understanding mechanisms of adaptive potential correction during prolonged cooling. Future research should explore the dose-dependence and duration of dalargin effects on ionic and hematopoietic homeostasis, its mechanisms of action on membrane ion channels and transport proteins, potential synergistic effects with other neuropeptides, as well as the long-term consequences of correcting electrolyte and hematological balance for the organism's functional state under chronic cold stress.

CONCLUSIONS

Under CCS conditions, compared to intact animals, there were observed a redistribution of leukocyte, characterized by a 2.8-fold increase in band neutrophils, a 1.2-fold decrease in segmented neutrophils, and a 2-fold reduction in lymphocytes, monocytes, and platelets. Dalargin administration significantly increased platelet counts by 1.3 times, normalized the leukocyte profile, and promoted a 1.4-fold rise in segmented neutrophils.

CCS led to an average 1.3-fold rise in blood concentrations of K^+, Ca^{2+}, and Cl^+ compared to the intact group. Dalargin administration signi-ficantly reduced these concentrations and increased phosphorus levels by approximately 2-fold compared to both intact and control values.

REFERENCES

1. Adamiak M, Bujko K, Cymer M, et al. Novel evidence that extracellular nucleotides and purinergic signaling induce innate immunity-mediated mobilization of hematopoietic stem/progenitor cells. Leukemia. 2018; 32: 1920—31.
2. Amar S, Kitabgi P, Vincent JP. Activation of phosphatidylinositol turnover by neurotensin receptors in the human colonic adenocarcinoma cell line HT29. FEBS Lett. 1986; 201(1): 31—6.
3. Brautbar N, Carpenter C, Baczynski R, et al. Impaired energy metabolism in skeletal muscle during phosphate depletion. Kidney Int. 1983; 24(1): 53—7.
4. Cairns SP. Potassium effects on skeletal muscle contraction: are potassium-metabolic interactions required for fatigue? Eur J Appl Physiol. 2023;123(11): 2341—3.
5. Costa MHG, de Soure AM, Cabral JMS, et al. Hematopoietic niche – exploring biomimetic cues to improve the functionality of hematopoietic stem/progenitor cells. Biotechnol J. [Internet]. 2017 Nov 27 [cited 2025 Feb 25]; 13(2): 1700088. Available from: https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/biot.201700088
6. Fonseca C, Garagarza C, Silva G, et al. Hyperkalemia management: a multidisciplinary expert panel's perspective on the role of new potassium binders. Heart Fail Rev. 2024; 30: 271—86.
7. Gao R, Shi L, Guo W, et al. Effects of housing and management systems on the growth, immunity, antioxidation, and related physiological and biochemical indicators of donkeys in cold weather. Animals [Internet]. 2022 Sep 10 [cited 2025 Jul 10]; 12(18): 2405. Available from: https://www.mdpi.com/2076-2615/12/18/2405
8. Gao Y, Liu Y, He J, et al. Effects of heat waves and cold spells on blood parameters: a cohort study of blood donors in Tianjin, China. Environ Health Prev Med [Internet]. 2024 Apr 15 [cited 2025 Jul 10]; 29: 25. Available from: https://ehjournal.biomedcentral.com/articles/10.1186/s12199-024-01091-z
9. Gulevskyy OK, Moisieieva NM, Gorina OL. Effect of leu-enkephalin (dalargin) on apoptosis and necrosis of leukocytes after cold stress. Probl Cryobiol Cryomed. 2022; 32(1): 14—23.
10. Gulevskyy OK, Moisieieva NM, Gorina OL, et al. Preincubation of L929 line fibroblasts with synthetic leu-enkephalin Tyr-D-Ala-Gly-Phe-Leu-Arg preserves their proliferative potential under cold stress. Cytol Genet. 2022; 56(4): 343—50.
11. Gumz ML, Rabinowitz L, Wingo CS. An integrated view of potassium homeostasis. N Engl J Med. 2015; 373(1): 60—72.
12. Hu Y, Liu Y, Li S. Effect of acute cold stress on neuroethology in mice and establishment of its model. Animals [Internet]. 2022 Sep 30 [cited 2025 Jul 9]; 12(19): 2671. Available from: https://www.mdpi.com/2076-2615/12/19/2671
13. Lemieux P, Roudier E, Birot O**.** Angiostatic freeze or angiogenic move? Acute cold stress prevents angiokine secretion from murine myotubes but primes primary endothelial cells for greater migratory capacity. Front Physiol [Internet]. 2022 Oct 14 [cited 2025 Jul 9]; 13: 975652. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2022.975652/full
14. Lomako VV, Pirozhenko LM. Blood leukocytes in young and aged rats after whole body cryostimulation (–120°C). Probl Cryobiol Cryomed. 2021; 31(1): 23—37.
15. Marunaka Y. Physiological roles of chloride ions in bodily and cellular functions. J Physiol Sci. 2023 Nov 15 [cited 2025 Jul 9]; 73(1): 31. Available from: https://link.springer.com/article/10.1186/s12576-022-00833-6
16. Moisieieva N, Gorina O, Akhatova Yu. Effect of dalargin on apoptosis of L929 fibroblasts during cold stress. CryoLetters. 2023; 44(6): 352—9.

17. Moisieieva N, Myrnyi V, Akhatova Y, Gorina O. Correction of hormonal disorders by chronic cold stress using synthetic neuropeptide. In: Collection of Scientific Papers LOGOS (May 24, 2024; Zurich, Switzerland) [Internet]. 2024 Jun 7 [cited 2025 Mar 11]: 121—3. Available from: https://archive.logos-science.com/index.php/conference-proceedings/article/view/1961
18. Mu S, Xia Y, Wu Q, et al. Response of bone metabolism markers to ice swimming in regular practitioners. Front Physiol [Internet]. 2021 Sep 14 [cited 2025 Jul 9]; 12: 731523. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2021.731523/full
19. Namimatsu A, Go K, Hata T. Regulatory effect of neurotropin on nasal mucosal hypersensitivity in guinea pigs caused by SART (intermittent exposure to cold) stress. Jpn J Pharmacol. 1992; 59(3): 371—7.
20. Nunes-Hasler P, Kaba M, Demaurex N. Molecular mechanisms of calcium signaling during phagocytosis. In: Niedergang F, editor. Molecular and Cellular Biology of Phagocytosis [Internet]. Cham: Springer; 2020 Feb 13 [cited 2025 Feb 28]. p. 103–28. Available from: https://link.springer.com/chapter/10.1007/978-3-030-40406-2_7
21. Nunes P., Demaurex N. The role of calcium signaling in phagocytosis. J Leukoc Biol. 2010; 88(1): 57—68.
22. Park HJ, Kim MK, Kim Y, et al. Neuromedin B modulates phosphate-induced vascular calcification. *BMB Rep*. 2021; 54(11): 569—74.
23. Park S, Lee MS, Jung S, et al. Echinacea purpurea protects against restraint stress-induced immunosuppression in BALB/c mice. J Med Food**.** 2018; (3): 261—8.
24. Porter C, Sousse LE, Irick R, et al. Interactions of phosphate metabolism with serious injury, including burns. JBMR Plus. 2017; 1(2): 59—65.
25. Posor Y, Jang W, Haucke V. Phosphoinositides as membrane organizers. Nat Rev Mol Cell Biol. 2022; 19 (12): 797—816.
26. 2Ratajczak MZ, Kucia M. Hematopoiesis and innate immunity: an inseparable couple for good and bad times, bound together by an hormetic relationship. Leukemia. 2021; 36(1): 23—32.
27. Raut SK, Singh K, Sanghvi S, et al. Chloride ions in health and disease. Biosci Rep [Internet]. 2024 May 1 [cited 2025 Mar 5]; 44(5): BSR20240029. Available from: https://portlandpress.com/bioscirep/article/44/5/BSR20240029/234283/Chloride-ions-in-health-and-disease
28. Rodan AR. Potassium: friend or foe*?* Pediatr Nephrol. 2017; 32(7): 1109—21.
29. Saeki K, Obayashi K, Kurumatani N. Platelet count and indoor cold exposure among elderly people: a cross-sectional analysis of the HEIJO-KYO study. J Epidemiol. 2017; 27(12): 562—7.
30. Semenova Ya-MO. [Influence of a moderate cold stress on redistribution of immune system cells]. Men's Health, Gender and Psychosomatic Medicine. 2019; (1): 33—42. Ukrainian.
31. Shaw I, Gregory K**.** Acid-base balance: a review of normal physiology**.** BJA Educ. 2022; (10): 396—401.
32. Shi H, Yao R, Lian S, et al. Regulating glycolysis, the TLR4 signal pathway and expression of RBM3 in mouse liver in response to acute cold exposure. Stress. 2019; 22(3): 366—76.
33. Shrimanker I, Bhattarai S. Electrolytes. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Updated 2023 Jul 24 [cited 2025 Mar 31]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK541123
34. Teleglow A, Romanovski V, Skowron B, et al. The effect of extreme cold on complete blood count and biochemical indicators: a case study. Int J Environ Res Public Health [Internet]. 2021 Dec 29 [cited 2025 Jul 9]; 19(1): 424. Available from: https://www.mdpi.com/1660-4601/19/1/424
35. Timerga A, Kelta E, Kenenisa C, et al. Serum electrolytes disorder and its associated factors among adults admitted with metabolic syndrome in Jimma Medical Center, South West Ethiopia: Facility based crossectional study. PLOS ONE [Internet]. 2020 Nov 11 [cited 2025 Apr 4]; 15(11): e0241486. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0241486
36. Van Poucke S, Stevens K, Marcus AE, et al. Hypothermia: effects on platelet function and hemostasis. Thrombosis. 2014 May 5 [cited 2025 Jul 9]; 12(1): 31. Available from: https://www.hindawi.com/journals/thromb/2014/105828/
37. Xu B, Lang Lmin, Li SZ, et al. Cortisol excess-mediated mitochondrial damage induced hippocampal neuronal apoptosis in mice following cold exposure. Cells [Internet]. 2019 Jun 12 [cited 2025 Jul 9]; 8(6):612. Available from: https://www.mdpi.com/2073-4409/8/6/612
38. Xu B, Zang S, Li SZ, et al. HMGB1-mediated differential response on hippocampal neurotransmitter disorder and neuroinflammation in adolescent male and female mice following cold exposure. Brain Behav Immun. 76: 223—235.
39. Zhang S, Xin Y, Yang Y, et al. The polarization of macrophages regulated by KCNG3 via the activation of ASK1 mediated by potassium ion efflux. Cell Biol Int. [Internet]. 2025 Apr 21 [cited 2025 Jun 9]; 49(7): 810—23. Available from: https://onlinelibrary.wiley.com/doi/10.1002/cbin.70022
40. Zhang X, Zink F, Hezel F, et al. Metabolic substrate utilization in stress-induced immune cells. Intensive Care Med Exp [Internet]. 2020 Dec 18 [cited 2025 Jul 9]; 8(Suppl 1): 28. Available from: https:// pubmed.ncbi.nlm.nih.gov/33336295/

Received 18.03.2025
Accepted for publication 11.09.2025

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ВПЛИВ ХРОНІЧНОГО ХОЛОДОВОГО СТРЕСУ ТА ДАЛАРГІНУ
НА ПОКАЗНИКИ КРОВІ МОРСЬКИХ СВИНОК

У роботі досліджено вплив синтетичного нейропептиду даларгіну на гематологічні показники та електролітний гомеостаз крові морських свинок за умов хронічного холодового стресу (ХХС) при температурі 4 °С впродовж п'яти днів. Установлено, що ХХС супроводжується зменшенням кількості тромбоцитів та порушенням лейкоцитарної формули, зокрема зростанням вмісту паличкоядерних нейтрофілів на тлі зниження відсотка сегментоядерних нейтрофілів, лімфоцитів і моноцитів та зменшення значень індекса адаптації. Одночасно відзначалося підвищення концентрацій кальцію, калію та хлору в крові, що свідчить про порушення електролітного балансу та іонного гомеостазу. Введення даларгіну запобігало таким змінам, що сприяло відновленню фізіологічного співвідношення клітин лейкоцитарної формули, збільшенню кількості тромбоцитів та нормалізації концентрацій ключових електролітів у плазмі крові. Крім того, зареєстровано підвищення індексу адаптації, що свідчить про активацію системи кровотворення та мобілізацію адаптаційного потенціалу організму. Отримані результати підтверджують потенціал даларгіну як ефективного засобу для підтримки системи крові, електролітного балансу та адаптаційних можливостей організму в умовах ХХС.

***Ключові слова*:** адаптація, холодовий стрес, кріобіологія, гіпотермія, даларгін, електролітний баланс, гематологічні показники.