3. K. N. Veremeenko, L. I. Volokhonskaya, A. I. Kizim, and N. F. Meged', *Lab. Delo*, № 1, 9-12 (1975).
4. F. I. Vismont, in: *7th Congress of the Byelorussian I. P. Pavlov Physiological Society (Abstracts of papers)* [in Russian], Vitebsk (1987), pp. 42-43.
5. O. A. Gomazkov and N. V. Komissarova, *Pat. Fiziol.*, № 1, 70-76 (1982).
6. R. A. Zarembskii, N. A. Belyakov, L. K. Shershneva, and S. V. Obolenskii, *Vopr. Med. Khimii*, № 1, 43-47 (1987).
7. *Guidelines on Methods for Defining the Human Prekallikrein-Kallikrein System*, compiled by K. N. Veremeenko *et al.* [in Russian], Kiev (1978).
8. M. M. Minnebaev and F. I. Mukhutdinova, *Byull. Eksp. Biol. Med.*, **105**, № 3, 284-286 (1988).
9. T. S. Paskhina, T. P. Egorova, V. P. Zykova, *et al.*, in: *Modern Methods in Biochemistry* [in Russian], Moscow (1968), pp. 232-261.
10. P. N. Perevozchikov and V. F. Chekanov, *Byull. Eksp. Biol. Med.*, **106**, № 10, 417-419 (1988).
11. M. S. Surovikina, *Ibid.*, **71**, № 5, 123-125 (1971).
12. M. S. Surovikina, *Kardiologiya*, **13**, № 2, 119-123 (1973).
13. V. A. Shaternikov, *Vopr.Med.Khimii*, **12**, №1, 103-106 (1966).
14. G. Yu. Shatillo, *Metabolism of Vasoactive Kinins in the Lymph and Their Significance in the Regulation of Lymph Formation and Transport* (Author's Abstract of Candidate Thesis) [in Russian], Tomsk (1988).
15. J. F. Miller, M. E. Webster, and K. L. Melmon, *Europ. J. Pharmacol.*, **33**, № 1, 53-60 (1975).
16. J. F. O'Brien, R. W. Forsman, M. S. Rohrbach, *et al.*, *Clin. Chem.*, **29**, № 11, 1990-1991 (1983).

# The Mast Cell System and Blood Coagulation in Mountain Dogs with Myocardial Infarction

**V. I. Frolenko, G. A. Zakharov, G. I. Gorokhova, and N. P. Novikova**

UDC 616.127-005.8-092.9-07:[616.155.36+616.151.5

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 6, pp. 580-583, June, 1994
Original article submitted October 11, 1993

A comparative study of the responses of the mast cell and coagulation systems to experimentally induced myocardial infarction in dogs that had been constantly living at a medium altitude (1600 m above sea level) and those constantly living at a low altitude (760 m) revealed less marked morphological and functional changes in these systems in the former ("mountain") dogs, which may be interpreted as an indication of their enhanced nonspecific resistance acquired as a result of long-term adaptation to conditions prevailing at medium altitudes.

**Key Words:** *myocardial infarction; mast cells; blood coagulation; adaptation; medium altitudes*

Studies in which the impact of various extremely adverse factors on the mast cell and coagulation systems was examined, have demonstrated that a relationship exists between the state of mast cells (MC) and alterations in the coagulation system [3,9-11]. This prompted us to investigate the state and role of MC in pathological conditions involving coagulation disorders, given that MC are known to be producers of heparin and other biologically active substances [4,14]. There is evidence that MC can ensure equilibrium of the coagulation-anticoagulation system, for each cubic millimeter of wall of a large human blood vessel contains as many as 1000 to 8000 MC [13]. Patients with myocardial infarction living in a plain region (around sea level) have been shown to have lowered blood levels of free heparin in conjunction with hypoplasia of MC and their reduced functional activity [7,8].

The objective of the present study was to examine the impact of myocardial infarction on the functional state of MC in relation to changes in

Laboratory for the Study of Respiration and Blood Circulation, Institute of Physiology and Experimental Pathology at High Altitudes, Academy of Sciences of the Kyrgyz Republic, Bishkek. (Presented by K. V. Sudakov, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0006-0579$12.50 

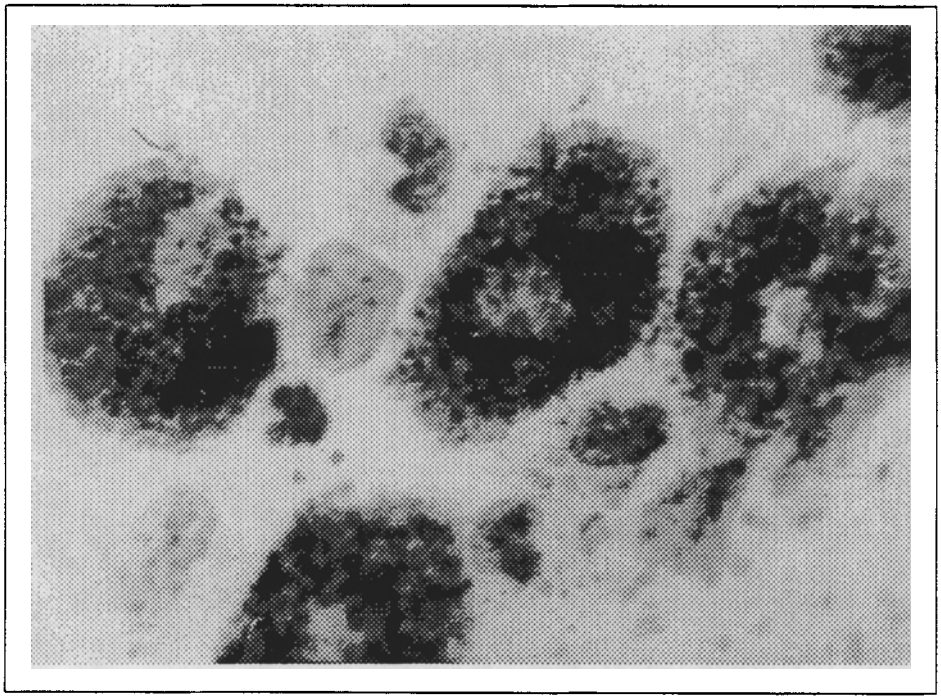

**Fig. 1.** Response of mast cells to myocardial infarction in a medium-altitude dog (first-degree degranulation). Toluidine blue stainining. ×400.

coagulation in dogs that had been constantly living at a medium altitude in comparison with those living at a low altitude.

## MATERIALS AND METHODS

The study was conducted on four groups of mongrel dogs. Groups 1 and 2 consisted, respectively, of intact (control) dogs and those with myocardial infarction (MI) which lived at a low altitude (in the city of Bishkek 760 m above sea level), this being referred to below as low-altitude or plain dogs; groups 3 and 4 comprised intact (control) dogs and those with MI, respectively, living at a medium altitude (near the shore of Lake Issyk Kul 1600 m above sea level) - medium-altitude dogs. MI was produced by applying a ligature at the boundary between the upper and middle thirds of the descending branch of the left coronary artery. The states of MC and coagulation were examined 15 days after the onset of MI. To study MC, full-thickness skin pieces were excised and fixed in 12% neutral formalin and Lillie's fluid. Film preparations of the skin samples were made and stained with toluidine blue at various pH values. MC in intervascular areas were counted in 15 fields of vision (at a magnification of 4000) and the diameters of oval and round cells were measured. The functional activity of the cells was assessed by determining the degree of their maturity (cells <13 μ in diameter were considered as young, those 13-15 μ in diameter as intermediate, and those >15 μ as mature) and their degranulation (first-degree degranulation: only a few granules were lost by the cells; second degree: massive loss of granules by the cells; third degree: completely destroyed cells). The condition of plasma coagulation factors was evaluated by routine biochemical procedures. Platelet adhesion was determined by Multen's method and platelet aggregation by Born's method.

## RESULTS

The low-altitude dogs with MI (group 2) differed significantly from their intact counterparts (group 1) in a number of parameters, as shown in Table 1. They contained fewer MC (by 48%) and their MC were smaller; the numbers of young and mature cells were lower, whereas the number of intermediate cells was higher. The percentage of cells with third-degree degranulation was nearly quadruple that in the intact dogs. These changes, which are indicative of altered functional activity of MC, went along with elevated levels of free heparin as a result of its release from the MC because of their increased degranulation and breakdown, and with intensified primary and secondary coagulation: the percentage of adhered platelets, the index of adhesion, and the platelet aggregation time were increased, as was blood tolerance of heparin, while the prothrombin and recalcification times were shorter (by 7 sec and 13 sec, respectively). Fibrinogen concentration remained within normal limits, but fibrinolytic activity was significantly depressed (Table 1).

A comparative analysis of MC in the two control groups (intact low-altitude dogs and intact

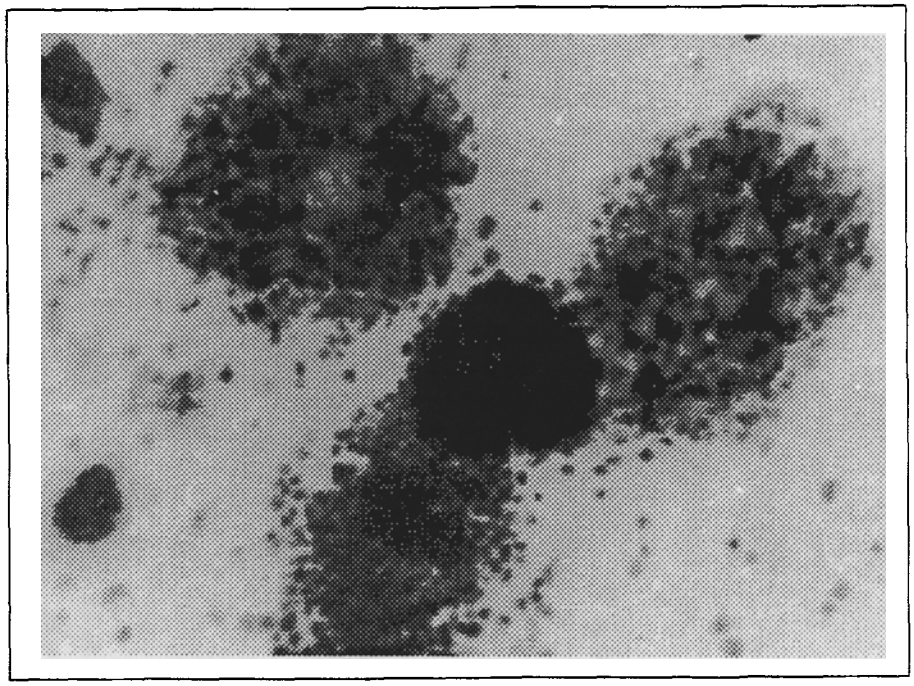

Fig. 2. Response of mast cells to myocardial infarction in alow–altitude dog (second– and third–degree degranulation). Toluidine blue staining. ×400.

medium-altitude dogs) showed that the latter dogs (group 3) contained more MC (by 22%), which were smaller in diameter, and that the proportion of young cells was higher while the contents of intermediate and mature cells were lower than in the intact low-altitude dogs (group 1). The medium-altitude dogs also had a lower percentage of cells showing second- or third-degree degranulation (Table 1). In both groups, MC were uniformly dispersed throughout the connective tissue, but in the medium-altitude dogs, unlike in the plain dwellers, mature cells (>15 μ in diameter) were most frequently seen lying in close proximity to blood vessels, so that the probability of their secretion entering the blood was higher.

Examination of the hemostatic system in the control groups showed a significantly higher blood level of heparin in the medium-altitude dogs. The adhesive properties of their platelets and the heparin tolerance of their blood were reduced (coagulation time was increased by 14 sec as compared to the low-altitude dogs; $p<0.02$). Recalcification and prothrombin times were also longer than in the low-altitude dogs (by 14 sec and 10 sec, respectively; $p<0.01$). Fibrinogen concentration was the same as in the low-altitude controls, but fibrinolytic activity was 26% lower ($p<0.001$). There was therefore a tendency toward hypocoagulation in the presence of reduced fibrinolysis in the medium-altitude dogs as compared to the low-altitude ones.

The modeling of MI in mountain dogs altered the morphofunctional state of their MC. Thus, MC numbers decreased by 18% (Table 1, group 4 vs. group 3), and the percentage of young and mature cells also decreased, whereas that of intermediate cells increased by 177% ($p<0.01$). The decrease in the proportion of young cells was probably associated with their diminished differentiation from precursor cells. The number of cells displaying first-degree degranulation decreased by 21% while that of cells with second- or third-degree degranulation increased by 65% and 83%, respectively. The preserved cells appeared loose and had more vacuoles than those from the control mountain dogs. Lumpy forms of MC and extensive areas studded with metachromatic granules were rarely observed. The staining intensity was markedly decreased.

A comparative analysis of MC in the low-altitude and medium-altitude test groups (groups 2 and 4) showed significantly higher (by 59%) numbers of these cells in the latter group. There were also differences between these groups in the degrees of MC maturity and degranulation. In the medium-altitude dogs, young cells with first-degree degranulation predominated (Table 1 and Fig. 1), whereas intermediate cell forms showing second- or third-degree degranulation preponderated in the low-altitude dogs (Fig. 2). These groups did not differ in the number of mature MC. It should be noted that cells with first-degree degranulation predominated in both control groups and that such cells also constituted a majority of MC in the medium-altitude dogs with MI, whereas cells with third-degree degranulation predominated in their low-altitude counterparts. The direction of changes in the degree of MC maturity was the same in

TABLE 1. Indices of the State of Mast Cells and of Blood Coagulation in Dogs with Experimental Myocardial Infarction

| Parameter | Low-altitude dogs | | Medium-altitude dogs | |
|---|---|---|---|---|
| | 1st (control) group ($n$=10) | 2nd (test) group ($n$=10) | 3rd (control) group ($n$=10) | 4th (test) group ($n$=8) |
| *Mast cells:* | | | | |
| number | 65±4.8 | 39±2.9$^+$ | 79±2.8$^*$ | 62±3.9$^+$ |
| diameter, μ | 15.8±0.8 | 14±0.3$^+$ | 12.8±0.5$^*$ | 12±0.3 |
| degree of maturity, %: | | | | |
| young | 18±2.1 | 11±1.4$^+$ | 71±3.1$^*$ | 54±3.2$^+$ |
| intermediate | 51±3.6 | 80±5.1$^+$ | 13±2.9$^*$ | 36±2.7$^+$ |
| mature | 31±4.1 | 9.0±2.1$^+$ | 16±1.6$^*$ | 10±1.3$^+$ |
| degree of degranulation, %: | | | | |
| I | 63±4.5 | 24±2.4$^+$ | 77±4.1 | 61±3.3$^+$ |
| II | 26±2.8 | 36±3.4$^+$ | 17±2.4$^*$ | 28±2.2$^+$ |
| III | 11±1.1 | 40±3.1$^+$ | 6±2.1 | 11±1.1$^+$ |
| *Blood plasma:* | | | | |
| free heparin, sec | 11±1.1 | 20±2.1$^+$ | 19±1.1$^*$ | 14±2.0$^+$ |
| blood tolerance for heparin, sec | 68±5 | 48±3$^+$ | 82±4$^*$ | 75±10$^*$ |
| prothrombin time, sec | 20±1.0 | 13±0.5$^+$ | 23±1.3 | 23±1.6$^*$ |
| recalcification time, sec | 53±2 | 40±1$^+$ | 67±3$^*$ | 63±6$^*$ |
| antithrombin activity, sec | 28±1 | 24±1.0$^+$ | 38±1$^*$ | 54±3$^+$ |
| fibrinogen, mg% | 313±27 | 281±20 | 314±25 | 363±24$^*$ |
| fibrinolytic activity, % | 38±1 | 16±3$^+$ | 28±2$^*$ | 56±9$^+$ |
| *Platelets:* | | | | |
| number per mm$^3$ | 186±10 | 300±23$^+$ | 180±23 | 210±25$^*$ |
| aggregation time, sec | 235±73 | 500±116 | 572±17$^*$ | 600±0$^*$ |
| disaggregation time, sec | 300±83 | 120±139 | 16±10$^*$ | 0 |
| % adhered cells | 51±2 | 67±5$^+$ | 43±3$^*$ | 35±2$^+$ |
| index of adhesion, arb. units | 1.36±0.1 | 2.6±0.35$^+$ | 1.45±0.1 | 1.1±0.1$^+$ |

Note. The superscript plus sign denotes a significant difference between the control and test groups, while the asterisk denotes a significant difference between the two control and the two test groups.

both test groups, but the low-altitude dogs had considerably decreased numbers of young and mature cells while the medium-altitude dogs contained a significantly increased proportion of intermediate cell forms (as compared to intact dogs) (Table 1). It should also be noted that the mountain dogs had fewer groups and chains of MC than the low-altitude dogs and had cells surrounded by large numbers of granules.

Examination of the hemostatic system in the medium-altitude group with MI (group 4) showed lower percentages of adhered platelets and lower values of the adhesion index than in the other groups. ADP-induced aggregation remained normal. The level of free heparin was 26% lower than in the medium-altitude controls ($p$<0.05) but higher than in the low-altitude controls, i.e., the relative hyperheparinemia persisted. No significant changes were noted in indices of general coagulation activity or in fibrinogen concentration, while antithrombin and fibrinolytic activities were significantly increased (by 16 sec and 2-fold, respectively) as compared to the control dogs (group 3). Thus, 15 days after the onset of MI, the mountain dogs still exhibited signs of hypocoagulation as a consequence of the reduced adhesive properties of their platelets and of the high anticlotting and fibrinolytic potencies of their blood.

A comparison of coagulation changes in the low-altitude and medium-altitude groups with MI (groups 2 and 4) revealed substantial differences between them. The functional activity of platelets increased in the former group and decreased in the latter. The differences between these groups in the parameters characterizing general coagulability also indicated that the medium-altitude dogs, unlike the low-altitude ones, did not develop hypercoagulation as a result of MI (prothrombin and recalcification times did not change, antithrombin and fibrinolytic activities rose) while showing a 29% increase in fibrinogen.

Thus, as the results of this study indicate, the effects of MI in the mountain dogs differed from those in the low-altitude animals. The former dogs had a much better developed MC apparatus than the latter, which was manifested in an increased total number of MC, a predominance of young cell forms, and less intensive degranulation, and this

undoubtedly contributed to the observed differences between these groups in the responses of their hemostatic systems to the experimental MI. The absence of hypercoagulation, the reduced adhesive properties of platelets, the hyperheparinemia, and the enhanced fibrinolysis were all favorable prognostic signs with respect to MI in the mountain dogs.

That the state of the coagulation system is dependent upon that of the MC system under high-altitude conditions is confirmed by the study of Bashkov *et al.* [3], who found that variations in the blood level of heparin depend on the extent to which MC are saturated with heparin and on the degree of their metachromasia and degranulation.

The observed differences between the test groups in the responses of their MC and coagulation systems to MI were consequences of long-term adaptation to conditions prevailing at medium altitudes. Such adaptation has been shown to alter both the morphofunctional state of MC [10] and the coagulation system [6]. The high stability of this system in the mountain dogs recorded in our study appears to have been an expression of their enhanced nonspecific resistance [1] and may be explained by the antistress effect of the long-continued adaptation to medium altitudes - an effect which results from neurohormonal alterations in the hypothalamic-hypophyseal-adrenocortical system, less pronounced activation of the stress-determined lipid peroxidation, enhanced functioning of the antioxidant system, and high antithrombin and antithrombogenic potentials [2,5,6,12].

## REFERENCES


1. N. A. Agadzhanyan, V. A. Isabaeva, and A. I. Elfimov, *Chemoreceptors of Blood Coagulation and High Altitudes* [in Russian], Frunze (1979).
2. M. A. Aliev, V. A. Lemeshenko, L. S. Kostyuchenko, and A. K. Bekbolotova, *The Stress-Protecting Effect of High-Altitude Adaptation* [in Russian], Frunze (1989).
3. G. V. Bashkov and B. A. Umarova, *Byull. Eksp. Biol. Med.*, **112**, No. 11, 494-497 (1991).
4. V. V. Vinogradov and N. F. Vorob'eva, *Mast Cells* [in Russian], Novosibirsk (1973).
5. Dzh. Z. Zakirov, *The Hypophyseal-Adrenal System during Complex Adaptations* [in Russian], Frunze (1979).
6. V. A. Isabaeva, *The Coagulation System and Adaptation to Natural Hypoxia* [in Russian], Leningrad (1983).
7. T. R. Petrova, in: *Histochemistry in Normal and Pathological Morphology* [in Russian], Novosibirsk (1967), pp. 156-158.
8. T. R. Petrova, *Mast Cells of Connective Tissue*, in: *Proceedings of the 3rd All-Union Conference on Connective Tissue* [in Russian], Novosibirsk (1968), pp. 105-108.
9. V. I. Frolenko, V. A. Isabaeva, G. A. Zakharov, *et al.*, *Byull. Eksp. Biol. Med.*, **108**, No. 12, 663-666 (1989).
10. V. I. Frolenko, V. A. Isabaeva, G. A. Zakharov, *et al.*, *Izv. Akad. Nauk Kirgizskoi SSR: Khimiko-Tekhnol. Nauki*, No. 1, 74-78 (1989).
11. V. I. Frolenko, V. A. Isabaeva, G. I. Gorokhova, and G. A. Zakharov, in: *First Congress of Physiologists from Central Asia and Kazakhstan* [in Russian], Part II, Dushanbe (1991), p. 154.
12. T. B. Chernook, *Stress under Conditions of Adaptation to Medium Altitudes* (Author's Abstract of Candidate Thesis) [in Russian], Ashkhabad (1981).
13. S. P. Shchurin, in: *Proceedings of the 3rd All-Union Conference on Connective Tissue* [in Russian], Novosibirsk (1968), pp. 15-19.
14. L. Pearse and K. Thomson, *Physiology*, **372**, 379-393 (1986).