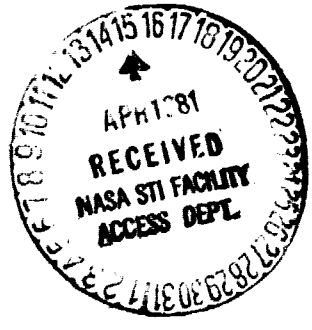
NASA TECHNICAL MEMORANDUM NASA TM-76169

COMPARISON OF EARLY REACTIONS OF THE BLOOD SYSTEM IN RATS TO IMMOBILIZATION, THE ACTION OF HYPOXIA AND THE ADMINISTRATION OF ERYTHROPOIETIN

P. D. Gorizontov, M. I. Fedotova, V. I. Gudim, O. I. Belousova

Translation of "Sopostavlenie rannykh reaktsiysistemy krovi u krys na immobilizatsiyu, vozdeistvie gipoksicheskoy gipoksii i vvedenie eritropoetina", Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya, No. 3, May-Jun 1977, pp 41-49



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D. C. 20546 MAY 1980

STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
NASA TM-76169		

4. Title and Subtitle	5. Report Date
Comparison of Early Reaction of the Blood System in Rats to Immobilization, the Action of Hypoxia and the Administration of Erythropoietin	MAY 1980
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
P. D. Gorizontov, M. I. Fedotova, V. I. Gudim and O. I. Belousova	
	10. Work Unit No.
	11. Contract or Grant No. NASw-3198

9. Performing Organization Name and Address	13. Type of Report and Period Covered
SCITRAN Box 5456 Santa Barbara, CA 93108	Translation

12. Sponsoring Agency Name and Address	14. Sponsoring Agency Code
National Aeronautics and Space Administration Washington, D.C. 20546	

15. Supplementary Notes

Translation of "Sopostavlenie rannykh reaktsiysistemy krovi u krys na immobilizatsiyu, vozdeistvie gipoksicheskoy gipoksii i vvedenie eritropoetina", Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya, No. 3, May-Jun 1977, pp 41-49

16. Abstract

Three series of experiments were simultaneously carried on rats with the purpose of studying the action of different stimulants on the blood system; rats were subjected to immobilization, hypoxic hypoxia and erythropoietin administration. Changes in various cellular forms in the bone marrow, the thymus and the spleen were studied

A unitypical reaction, as in stress, was noted during the first hours; a reduction of the cell count in the lymphoid organs, a reduction of granulocytes and an increase of lymphoid cells in the bone marrow. The differences were chiefly quantitative. This was followed by stimulation of myelo- and erythropoiesis determined by the specific features of the action applied. Nonspecific blood reaction was apparently due to activation of the adaptation mechanisms.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	THIS COPYRIGHTED SOVIET WORK IS REPRODUCED AND SOLD BY NTIS UNDER LICENSE FROM VAAP, THE SOVIET COPYRIGHT AGENCY; NO FURTHER COPYING IS PERMITTED WITHOUT PERMISSION FROM VAAP.

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	12	

COMPARISON OF EARLY REACTIONS OF THE BLOOD SYSTEM IN RATS TO IMMOBILIZATION, THE ACTION OF HYPOXIC HYPOXIA AND THE ADMINISTRATION OF ERYTHROPOIETIN

*/44

P.D. orizontov, M.I. Fedotova, V.I. Gudim, O.I. Belousova

/45

In our research, applying contemporary qualitative methods, we studied the early reaction of the hemogenic organs to such stress stimuli as immobilization and measured electrostimulation. On the basis of this work we concluded the existence of common governing principles for changes developing in the hemogenic system soon after the application of various stressors [3, 4, 9-12]. The use as a stress stimulus of massive bloodletting in rats [2] has shown that changes arising in the first hours in the lymph organs and bone marrow are subject to the same rule governed principle. However, later (in the second 24 hours) instead of generalized granulocyte growth increased erythrocyte hemogenesis characteristic of blood loss was observed. In connection with this the question has arisen of how other stimuli influence the blood system, specifically acting on erythropoiesis, and whether changes will arise under these conditions that are specific to the reaction to the given stressor. With this purpose we compared reactions of the lymph organs and bone marrow over 48 hours after the application of the stimuli of immobilization, hypoxic hypoxia, and intravenous introduction of erythropoietin. Of these factors the first was a typical stressor and the latter two were erythropoiesis stimuli.

Methods. The work was carried out on heterozygous female rats of the Vistar line 160-180 g in mass. Three parallel series of experiments were carried out. In series I (90 rats) the animals were subjected to 6-hour immobilization on their backs. In series II (110) the effect of

---

*Numbers in margin indicate pagination of foreign text.

hypoxic hypoxia was studied. For 9 hours the rats were kept in a decompression chamber in conditions of low (0.5-0.3 technical atmosphere) barometric pressure. In series III (95) the animals were each once given 10 units of domestic erythropoietin [9a].

In 6, 9, 12, 24 and 48 hours from the beginning of each stimulus application the rats were killed and, using the methods described previously [1], the cells of the thymus, spleen and marrow from the femur were counted. The number of cells of various bone marrow growths were calculated. In the peripheral blood the number of leucocytes and the leucocyte formula were determined, and in several cases the number of reticulocytes as well. The experimental data were obtained in repeated tests. Each experimental period consists of the summarized results from 15-18 animals.

Results and discussion. Upon examination of the cell content of the spleen (Fig. 1,a) it is evident that in the first twelve hours identical cell loss occurs in all groups within 45-50% of normal. It is not due to the death of cells in the organ, since on smears there are no figures of decomposition and pyknosis. In the following hours the number of cells in the spleen grows and either achieves the norm or positively exceeds it, as was seen in animals that were given erythropoietin.

In the thymus (Fig. 1, b) the nature of the changes in the number of cells is varied. In rats subjected to immobilization, in 12 hours the number of thymocytes remained within the normal range and only later began to decrease. In 48 hours it was 49% of the original level (the decrease was statistically significant). In series II of experiments in the first 6 - 12 hours a significant decrease in the number of cells took place (by 40 -45%). In this period numerous decomposing cells were found on thymus prints stained

according to Pappenheim's method. In 24 hours there were almost no signs of decomposition and the number of cells increased somewhat, although it remained positively below norm. This permits one to consider that in the condition brought about by hypoxia, the main cause of the decreased cell count is their breakdown. Introduction of erythropoietin did not lead to positive change in the number of cells. Only a tendency to a decreased thymocyte count was noted.

In the bone marrow the number of lymphoid cells (Fig. 2, a) changed unitypically during the observation period in all experimental series. Differences were found only in the degree to which changes were manifested. In the first 12 hours the lymphoid cell count positively increased. This was most significant in duration and degree in rats subjected to immobilization. The cell structure of the lymphoid population was altered at this time. In the bone marrow T-lymphocytes appeared (15). Later the number of lymphoid cells decreased and in 48 hours it was 20 -50% less than the norm /46
(decrease in all cases significant).

The number of mature neutrophil type cells (Fig. 2,b) changed variously in the early experimental series. In experimental series II in 24 hours no positive variance from the norm was found, but in 48 hours the number of rod and segmented neutrophils positively exceeded the norm. With immobilization, in the first 6 - 12 hours the number of these cells decreased, but later began to increase and in 48 hours reached the norm. After introduction of erythropoietin the content of mature neutrophil cells in the bone marrow swiftly and significantly decreased. In 6 hours only 24% remained. Following this a quick revival began and in 48 hours the number reached the lower limits of the norm.

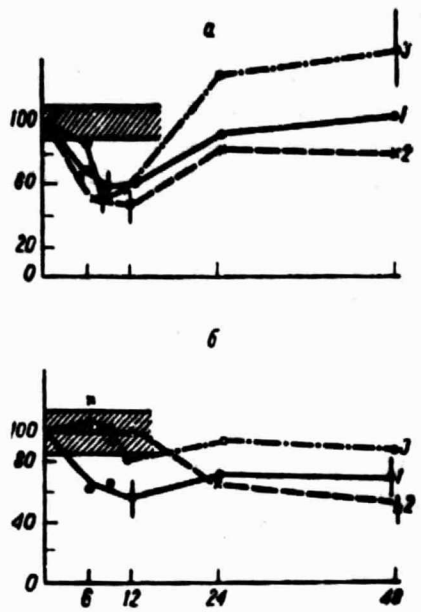


Figure 1. Number of cells in the spleen (a) and thymus (b) in rats with hypoxic hypoxia (1), immobilization (2), administration of erythropoietin (3).

X-axis: time from onset of stimulus (in hours); Y-axis: number of cells in the organ (% to control). Vertical lines: confidence interval. Shaded area: confidence interval of intact animals.

It should be noted that in other experiments in which rats were administered English erythropoietin (standard B) in 3-unit doses identical results were obtained. The neutrophil cell count in the bone marrow decreased in 3 hours by 80% and restoration of the normal level occurred in 3 days. Thus early onset of the stress type reaction was also observed with the English preparation. However, the early effect of the action of erythropoietin was evidently not

examined.

The number of granulocyte type blast cells (myeloblasts, promyelocytes, myelocytes) (Fig 2,c) decreased in the first 12 hours in all three experimental series, especially in animals that had received erythropoietin. In 24 hours the number of these cells regained the norm, and in 48 hours in rats subjected to immobilization the number of blastular neutrophil cells significantly (by approximately 60%) exceeded the original level. The number of erythroid cells (Fig. 2, d) in rats subjected to immobilization fluctuated within normal limits. In experimental series II and III in the first hours a slight decrease in the number of erythroid cells was noted, and then an increase exceeding the norm occurred by 50% in 48 hours.

In the peripheral blood (Fig. 3) changes were unitypical with all stimuli and differed only in the degree of manifestation. In the first hours neutrophilia was observed, particularly with immobilization. Neutrophilia, as can be expected from the experimental data, is promoted not only by the discharge of these cells from the bone marrow, since no correspondence is seen in the degree of loss of mature neutrophil cells from the bone marrow and neutrophilia in the peripheral blood. Thus, with significant loss of neutrophils from the bone marrow after administration of erythropoietin, and with complete absence of any decrease in the number of these cells with hypoxia, identically manifested neutrophilia is observed in the peripheral blood. The number of lymphocytes in the first 12 hours decreased in all experimental series; the decrease in series II was the most weakly manifested.

Discussion of results obtained and conclusions. From /47
the above one may conclude that the early reaction of various lymphoid organs to applied stimuli is unitypical. It is

characterized by a decrease in the cell count. However, the degree of manifestation and the mechanisms lying at the basis of this decrease may vary. The reactions of the spleen to the cited stimuli are practically identical both in the length of the period of development and the degree of manifestation. This permits one to consider that the reasons for the decrease in cell count in the organ are the same. The absence in prints from the organ of figures of decomposing cells, and the speed of the process's development, permit one to conclude that in all cases the main cause of cell loss from the spleen is their migration from the organ. As our previous research has shown, α-receptors of the sympathetic nervous system play a definite role in the mechanism of migration, influencing, evidently, the tonicity of the capsule and the dimensions of the spleen (8, 12). In the /48
thymus a well-manifested dependence was seen between decrease in cell count and the nature of the stimulus. A swift decrease (by 12 hours) in the cell count in the thymus with hypoxia is promoted by cell decomposition; with immobilization decrease in the number of thymocytes takes place largely due to their increased migration, as was shown earlier (13).

A summary view of the early reaction of the hemogenic system, which takes place within 48 hours from the onset of the stress stimulus, allows one to conditionally divide it into two periods. In the first period, lasting about 12 hours, a mobilization, as it were, of accumulated cell reserves occurs. This is manifested in increased migration from the hemogenic organs by mature cells (lymphocytes from the spleen and thymus, and also neutrophils and in several cases erythroid cells from the bone marrow) into the peripheral blood, from whence they reach the regions with the greatest need and given cell elements. At the same time in the bone marrow preparation begins for accelerated renewal and accumulation of corresponding cell types. The number of lymphoid cells

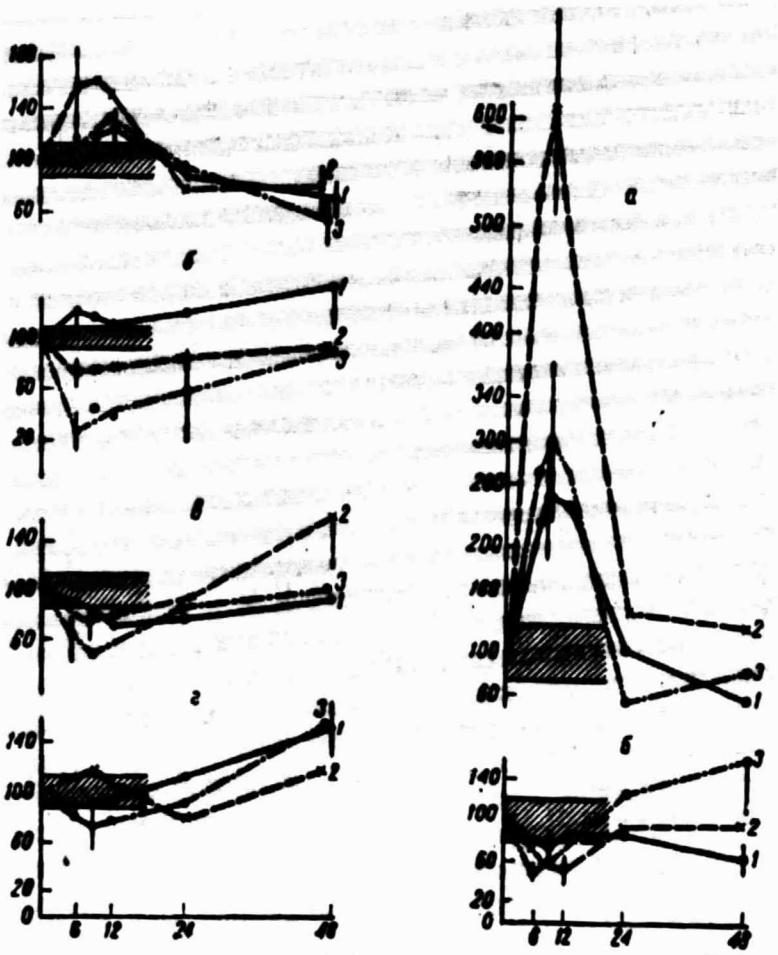


Figure 2. Number of lymphoid (a), mature neutrophil (b), blast cells of granulocyte type (c), and erythroid (d) cells in the bone marrow of the femur of rats with hypoxic hypoxia, immobilization and administration of erythropoietin.

Remaining indices the same as in Fig. 1.

Figure 3. Number of neutrophil cells (a) and lymphocytes (b) in peripheral blood of rats with hypoxic hypoxia, immobilization and administration of erythropoietin.

Remaining indices the same as in Fig. 1.

increases, as well as that of hemopoietic stem cells [14,19], which make up the basis from which under the control of differentiating factors active neutro- and erythopoiesis will begin in the second period. Thus, with increased production of erythropoietin (experiment with hypoxia), in 48 hours we see hyperplasia of erythroid growth in the bone marrow. In the experiment with introduction of exogenous

erythropoietin, when significant loss of neutrophil cells from the bone marrow occurred, increased regeneration of both bone marrow growths took place. In the period from 9 to 48 hours from the moment of introduction of the preparate the overall number of erythroid cells in the bone marrow increased by 100%, and of neutrophils by 70%. In the experiment with immobilization, when in the first hours neutrophil cells were discharged from the bone marrow, hyperplasia of that very cell type was observed. It is interesting that in the period of heightened proliferation and increased number of granulocyte and erythrocyte cells of the bone marrow (depending on the stimulus applied) a positive decrease in the lymphoid cell content took place. A similar occurrence was noted (18) during long-term (7 days) hypoxia in a period of erythropoietin stimulation, when incidentally a significant decrease in the number of lymphocytes was regarded as a stress phenomenon. However, it is difficult to agree with this. The reciprocal relationship in a period of stimulation of any of the cellular growths of the bone marrow between lymphoid cells and cells of the proliferating growth may rather be explained by expenditure of the lymphoid cell pool and their enhanced trephocyte functions (16,17).

Thus all three types of stimuli, regardless of certain variations, caused an early common nonspecific reaction in the blood, characteristic of stress. With this a certain specificity was observed characterizing peculiarities of the stimulus. It seems to us that this situation is of principal importance, in explaining, for instance, the functional mechanisms of hypoxia or erythropoietin. It is difficult to say how the arisal of a common stress reaction in the experiments carried out may be explained. It is not likely that this may be ascribed to any one harmful, for example toxic or physical, factor. Such a suggestion is unlikely, since bleeding, hypoxic hypoxia, erythropoietin, and immobilization caused a unitypical

reaction. Its arisal is evidently promoted by the common regulatory mechanisms providing for adaptation and restoration of homeostasis. These mechanisms are under nervous-endocrine control, which we have shown earlier in studying the reactions of the blood system (5, 7-9). In connection with this, further study of the role of the blood system in processes of damage to and renewal of the organism under various extreme stimuli is of undoubted interest.

REFERENCES

1. Belousova, O.I., M.I. Fedotova. Radiobiol. radioter. 1968, vol. 9, p. 343.

2. Belousova, O.I., M.I. Fedotova. Pat. fiziol., 1971, no. 6, p. 69.

3. Gorizontov, P.D. Arkh. pat., 1973, No. 8, p. 3.

4. Gorizontov, P.D. Vest. AMN SSSR, 1975, No. 8, p. 81.

5. Gorizontov, P.D. Pat. fiziol., 1975, No. 3, p. 34.

6. Gorizontov, P.D. Arkh. pat., 1976, No. 3, p. 3.

7. Gorizontov, P.D., O.I. Belousova, Yu. I. Zimin. Pat. fiziol., 1970, No. 2, p. 38.

8. Gorizontov, P.D., O.I. Belousova, Yu. I. Zimin. Byull. eksper. biol., 1972, No. 12, p. 23.

9. Gorizontov, P.D., O.I. Belousova, Yu. I. Zimin. Byull. eksper. biol., 1974, 11, p. 20.

9a. Gudim, V.I., G.P. Moskaleva, T.I. Koretskaya et al. Probl. gematol., 1971, 10, p. 48.

10. Zimin, Yu. I. Probl. gematol., 1969, 7, p. 19.

11. Zimin, Yu. I. Pat. fiziol., 1969, 3, p. 36.

12. Zimin, Yu. I. Byull. eksper. biol., 1971, 6, p. 21.

13. Zimin, Yu. I. Izv. AN SSSR, Seriya biol., 1973, 4, p. 517.

14. Zimin, Yu. I. Byull. eksper. biol., 1974, 12, p. 17.

15. Zimin, Yu. I., R.M. Khantov. Byull. eksper. biol., 1975, 12, p. 68.

16. Khrushchev, N.G., M.G. Skurskaya. In the book: Limfoidnaya tkan' v vosstanovitelv'nykh i zashchitnykn protsessakh (Lymphoid tissue in processes of renewal and protection). Moscow, 1966, pp. 25-46.

17. Carrel, A. J.A.M.A., 1924, v. 82, p. 225.

18. Joffey, J.M. In the book: Gordon,A. (ed). Regulation of Hematopoiesis. V. 2, New York, 1970, p. 1421.

19. Murphy, M.J., B.J. Lord. Blood. 1973, v. 42, p. 81.