ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XIII Fasc. 2 Szczecin 1983

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Hematology

EFFECT OF PHYSIOLOGICAL FACTORS, STRESS, AND DISEASE ON HEMATOLOGICAL PARAMETERS OF CARP, WITH A PARTICULAR REFERENCE TO LEUKOCYTE PATTERN. I. VARIABILITY OF HEMATOLOGICAL INDICES OF CARP IN RELATION TO AGE AND GONAD MATURITY STAGE

WPŁYW CZYNNIKÓW FIZJOLOGICZNYCH, STRESSOWYCH I CHOROBOWYCH NA PARAMETRY HEMATOLOGICZNE KARPIA ZE SZCZEGÓLNYM UWZGLĘDNIENIEM OBRAZU BIAŁOKRWINKOWEGO. I. ZMIENNOŚĆ WSKAŹNIKÓW HEMATOLOGICZNYCH KARPIA W ZALEŻNOŚCI OD WIEKU I STADIUM DOJRZAŁOŚCI GONAD

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The paper concerns the hematological parameters of carp (*Cyprinus carpio* L.) as related to the age of fish and changes occurring during the spawning period. In blood samples the red and white cell counts, hematocrit, hemoglobin concentration, MCV, MCH, and MCHC were determined, and the leukogram and morphology of blood cells were examined in detail. The results obtained were treated statistically.

INTRODUCTION

The blood of every organism is adjusted to its life requirements, and the changes occurring in it are signalized very early.

Hitherto, the utility of hematological studies in ichthyology has been limited for the lack of accurate information which could define physiological standards for various fish species. However, extensive research conducted all over the world during the recent years has enabled many authors to determine some of the hematological parameters for the teleost fish in artifical and natural habitats. Most of the work concerned the hematological research on salmonids, and some studies only dealt with cyprinids (Field et al., 1943; Dombrowski, 1953; Topf, 1955; Watson, 1963; Chlebeck and Philips, 1969; Summerfelt, 1967; Gardner and Yevich, 1969; Hines and Yashouv, 1970; Ivanova, 1970; Varo, 1970; Houston and De Wilde, 1972; Svobodova, 1973; Svobodova and Janecek, 1973; Golovina, 1975, 1976).

However, the detailed structure of the white blood cells and terminology of their various types still remain controversial. The first studies on the blood morphological elements differentiation were conducted early in this century (Wertzberg, 1911; Meinertz, 1902; Loewenthal, 1927, 1928, 1930; Stolz, 1928; Agnesotti, 1932; Pliszka, 1938). These studies concerned both the elasmobranch and teleost fishes.

Contrary to the hitherto existing opinions on the existence of macrophages in fish blood (Jakowska, 1956; Weinreb and Weinreb, 1969) it is proposed nowadays to adopt different names for phagocyte cells. The blood cells of phagocyte properties should be called monocytes, and the phagocyte cells existing in tissues – macrophages. As a criterion to distinguish between the two, the intensity of phagocyte properties, moderate for monocytes and very intensive for macrophages (Van Furth et al., 1972) is used. Many authors differ also in their opinions on the existence of neutrophilis, eosinophils, and basophils in fish (Watson, 1963; Slicher, 1961; Salvatorelli, 1971; Fijan, 1961; Guarnieri, 1962).

It is evident from the review of the literature that there is a lack of a consistent classification of the fish white blood cells, and there is no uniform nomenclature for them either. The most accurate is the classification worked out by Ivanova (1970) for large material comprising 22 fish species. That author's basic classification criteria were: function of blood cells, their individual development, and morphology. According to this classification, the peripheral blood of carp contains the following cells: hemocytoblasts, myeloblasts, promyelocytes, myelocytes, metamyelocytes, rod and filamented neutrophil, eosinophils, basophils, monocytes, and lymphocytes.

Electron microscope used in the recent years allowed to examine some morphological characters and functions of the white blood cells in greater detail. In spite of using EM techniques, however, some authors still differ in their opinions on structure and affinities of blood cells. Weinreb (1963) considers the structure and development of lymphocytes and thrombocytes in the crucian carp (*Carassius auratus gibelio* Bloch) to be similar. On the other hand, Ferguson (1975) showed those two blood elements to be separate morphologically functionally, and developmentally. This view was also supported by Ellis (1976). The application of electron microscope techniques allowed to detect evident similarities between structures of blood cells of mammals and fish. Based on the analysis of morphological similarities and also immunological properties of these blood cells, it is

proposed to adopt a nomenclature of fish leukocytes identical with that applied for mammals (Ellis, 1977).

The variety of opinions on classification and nomenclature of fish white blood cells concerns also effects of physiological factors on quantitative and qualitative parameters of fish blood.

Dependence of hematological parameters on the fish age was studied by many authors (e.g., Pavlov and Krolik, 1935; Antipova, 1954; Das, 1965; Iuchi, 1973; Dorfman, 1973; Svobodova, 1973; Golovina, 1975, 1976). Results obtained in these works are often controversial.

The effect of sex on quantitative changes in fish blood was studied by many authors. Some authors reported evident quantitative differences between the blood of males and females (Schlicher, 1927; Pavlov and Krolik, 1935; Lange, 1919; Ivlev, 1955; Einszporn-Orecka, 1970; Ezzat et al., 1974; Fourie and Hatting, 1976; Van Vuren and Hatting, 1976, 1978), others, however, did not observe that (Smith, 1930; Glazova, 1967).

Some studies showed changes of hematological parameters to occur during spawning. These changes were observed both in females and males (Drabkina and Telkova, 1949; Pučkov and Fedorova, 1951; Schlicher, 1927; Nusenbaum, 1951), or only in females (Einszporn-Orecka, 1970; Svobodova, 1973; Robertson et al., 1961; Černikova, 1967). Changes in the carp blood occurring during spawning were the subject of a few studies only, with often contradictory results.

The aim of the present work was to study the effects of basic physiological factors, i.e., age of fish and changes occurring during spawning, on the hematological parameters.

It is thought very important to present data on ranges of hematological parameters in the carp, the more so because the variability of metabolism in fish under normal physiological conditions is much more pronounced in fish than in higher vertebrates. Thus, for an objective assessment of disease effects, it is imperative to know hematologic parameters and disregard their possible changes under various normal physiological states.

MATERIALS

Age of fish

The materials studied consisted of 340 individuals of 1–28-months old carp. The fishes came from the same pond stocks at a fish farm. The blood for the assays was obtained immediately after the catch (every sample consisted of 20 fish) every month from March through November, and during the winter in January. During this period the water temperature was measured in pond.

Spawning

The materials consisted of 31 females and 41 males aged 5–6 years. Effects of the physiological changes during spawning on the hematological parameters of spawners were

examined according to Nikolski's method (Opuszyński, 1979), at the following gonad maturity stages:

1. Mature gonad (sexual products mature but not excreted under a slight pressure on the abdomen); the assays were run in the end of May, the blood being obtained from 8 males and 10 females.
2. Spawning (sexual products excreted under a slight pressure on the abdomen); the blood was obtained from 11 males nad7 females in the beginning of June, about 24 hours before spawning.
3. Post-spawning; the blood was collected from 11 males and 8 females 7 days after the spawning.
4. Resting; the hematological assays were carried out four months after spawning; the blood was obtained from 10 females and 10 males.

METHODS

The blood from the fish was collected in the field. The blood was taken from the heart according to the method of Klontz and Smith (1968). The place of needle insertion was thoroughly cleaned of water and mucus. The needle was inserted perpendicular to the fish body surface and slightly aspirated during penetration. The yearlings, marketable fish, and spawners yielded 5–10 cm^3 of blood per individual.

The blood was collected with plastic syringes because the fish blood quickly coagulates on contact with glass (Smith et al., 1952). The blood from the fry was collected with a Pasteur pipette rinsed with heparine (Hattingh, 1975). The pipette content was poured onto a watch glass covered by a thin layer of paraffin (Einszporn-Orecka, 1970). The blood assays were as follows:

1. The red blood cell count. The blood was 1:200 diluted with the Hendricks (1952) solution. The blood cells were counted in Bürker hemocytometer following the generally accepted procedures.
2. The white blood cell count. The blood was 1:100 diluted with the Shaw (1930) solution. The same procedure as for the erythrocytes was followed.
3. The hematocrit value was determined according to Snieszko (1960).
4. The hemoglobin content was estimated according to the Drabkin method reported for purposes of fish hematology by Larsen and Snieszko (1961).
5. The red blood cell parameters were calculated as in Dacia and Levis (1968). The following absolute values were mathematically computed: MCV (mean corpuscle volume), MCH (mean content of hemoglobin), MCHC (mean percentage hemoglobin in an erythrocyte).

All the numerical data were treated statistically, the arithmetic mean and its standard deviation being calculated. The results were tested with Student's t and two-way analysis of variance (Elandt, 1964).

Five blood smears for each fish examined were prepared for the purpose of leukogram

and blood cell morphology. The smears were stained with May-Grünwald-Giemsa (MGG) panchromatic technique applied in hematological studies of fish (Klontz. 1972).

The following blastocyte forms in the carp blood were distinguished: hemocytoblasts, and promyelocytes, as well as leukocyte types: myelocytes, eosinophils, basophils, monocytes, and lymphocytes. The percentages of various kinds of leukocyte types in a leukogram were calculated from 200 white blood cells.

RESULTS

Results of the studies on age effects on hematological parameters of carp blood are presented in Table 1 and Figs. 1–8.

The red blood cell count was found to increase until the age of 14 months and to decrease thereafter. The differerences between various age groups were statistically significant except for those in June and October (Table 1).

The hematocrit value in the youngest age group (fish 1–5-months-old) was lower as compared to older fish, the difference being statistically significant except for the values in October. In the middle age group (7–16 months) an increase in the hematocrit was recorded, and in the oldest group (22–28 months) the parameter decreased with the fish age (Table 1).

The hemoglobin content in the youngest age group was at the lowest (Table 1), the difference from the two older groups being statistically significant. The differences between the middle and oldest age groups were, generally, not significant.

The mean corpuscle volume of red blood cells (MCV) did not change with fish age.

The mean content of hemoglobin in an erythrocyte (MCH) was at its lowest in the youngest age group (particularly before the fourth month of fish life), the difference with other groups being significant.

The mean per cent hemoglobin in an erythrocyte (MCHC) was, similarly to MCH, lower in the fish younger than 4 months. The differences in the other age groups were not significant.

The leukocyte count per 1 mm^3 failed to show any considerable age-dependent differences (Fig. 1).

The blastodermic forms of leukocytes remained in the peripheral blood until the 11th month of fish life, the hemocytoblasts, myeloblasts, and promyelocytes remaining until the 3rd, 5th, and 11th month, respectively, the latter being absent from the carp blood in November and January.

The percentage of neutrophils, generally, increased with age, the differences being statistically significant (Fig. 4).

The percentage of eosinophils: there was an inverse relationship in per cent contents of these blood cells, the percentage of eosinophils in fish blood decreasing with age and the decrease being statistically significant (Fig. 5).

Table 1

The results of the hematological tests of carp peripheral blood during three years of the production cycle

Age of fish (months)	Date of blood collecting	Water temperature (°C)	Erythrocytes n 10^6/mm^3		Hematocrit (per cent)		Hemoglobin (per cent)		Leukocytes (x 10^3/mm^3)	
			SR	SD	SR	SD	SR	SD	SR	SD
1	20.07	22	1.24	0.18	27.10	5.66	5.60	0.96	49.00	7.77
2	22.08	20	1.35a	0.15	28.90a	5.55	5.51a	0.84	42.18a	9.24
3	14.09	14	1.36a	0.13	31.10a	6.89	6.49a	0.75	45.76a	6.91
4	20.10	8	1.37	0.13	31.50a	6.39	5.61a	0.97	51.48a	11.57
5	15.11	6	1.10	0.16	22.70	3.70	5.90	0.99	39.63	4.90
7	12.01	3	1.19	0.16	23.50	3.78	6.80	1.08	39.91	4.30
9	14.03	4	1.71	0.32	36.90	4.34	9.59	1.22	46.81	8.16
10	24.04	8	1.57b	0.24	33.70a	6.45	7.73a	0.99	40.64a	4.63
11	18.05	14	1.52	0.13	34.40	3.55	8.38	0.79	44.43	6.62
12	20.06	18	1.60a	0.19	37.30b	4.00	8.17a	1.26	47.04a	6.13
14	10.08	19	2.34c	3.32	34.50b	3.76	8.04b	0.83	47.25a	6.59
15	24.09	10	1.63b	0.17	36.70b	3.95	8.53b	0.17	52.45b	15.66
16	20.10	9	1.47a	0.15	34.80b	5.12	8.06c	1.02	41.86a	11.28
22	15.04	10	1.40a	0.15	31.70a	5.26	7.15a	0.79	47.83b	6.10
24	20.06	17	1.50a	0.12	34.10a	2.99	7.68a	0.99	50.24a	5.69
26	18.08	20	1.53b	0.11	34.90b	2.69	7.68b	0.81	46.13a	5.89
28	28.10	6	1.38a	0.19	30.60a	6.43	6.89b	0.84	41.33a	12.24

Legend: SR – arithmetic mean, SD – standard deviation, a,b,c – significance of differences of the comparable different age groups of fish, in the same calendar months

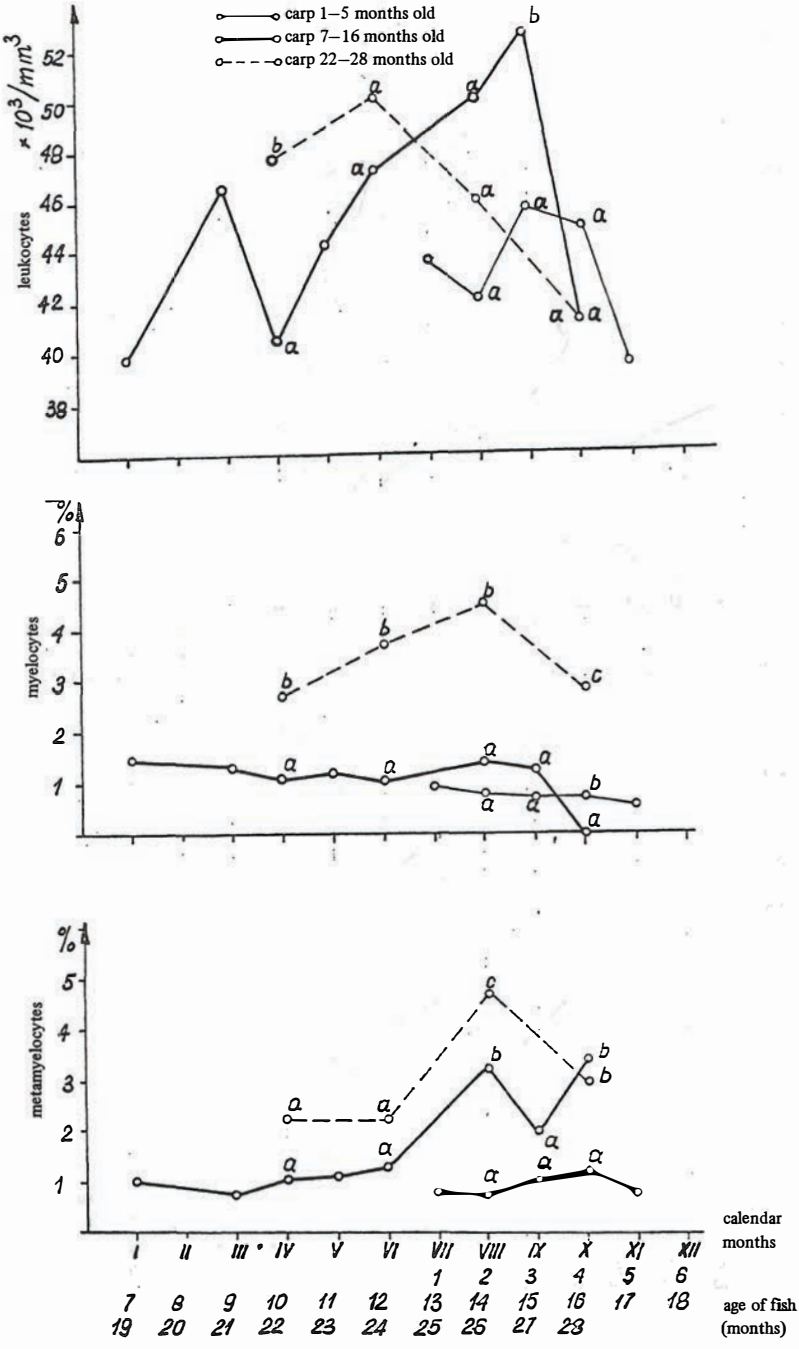

Fig. 1. Age effects on leukocyte count in the carp peripheral blood

Fig. 2, 3. Percentage of myelocytes and metamyelocytes in the carp peripheral blood as affected by age

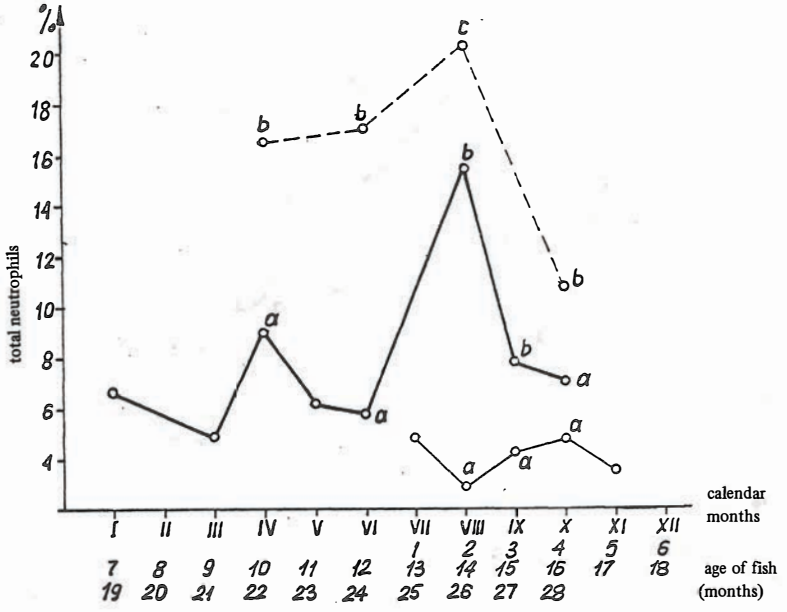

Fig. 4. Percentage of total neutrophils in the carp peripheral blood as affected by age

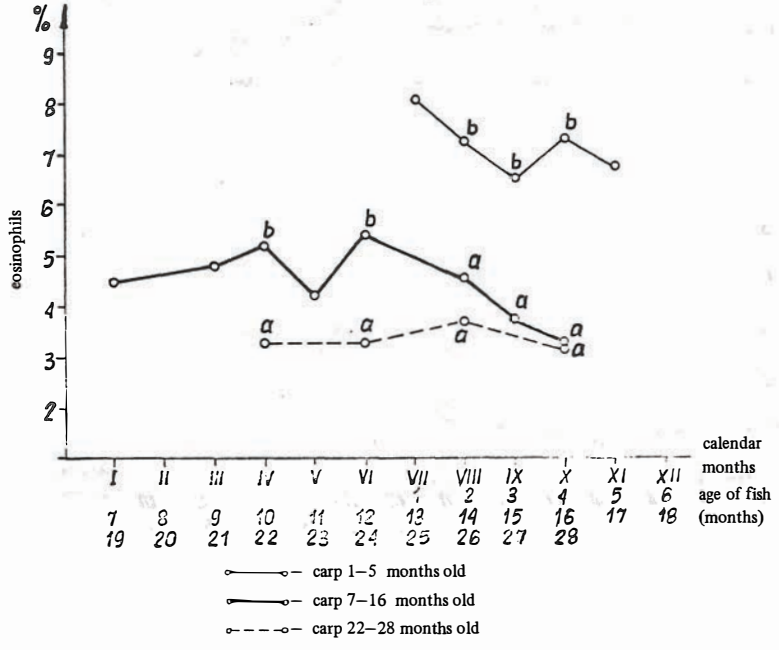

Fig. 5. Percentage of eosinophils in the carp peripheral blood as affected by age

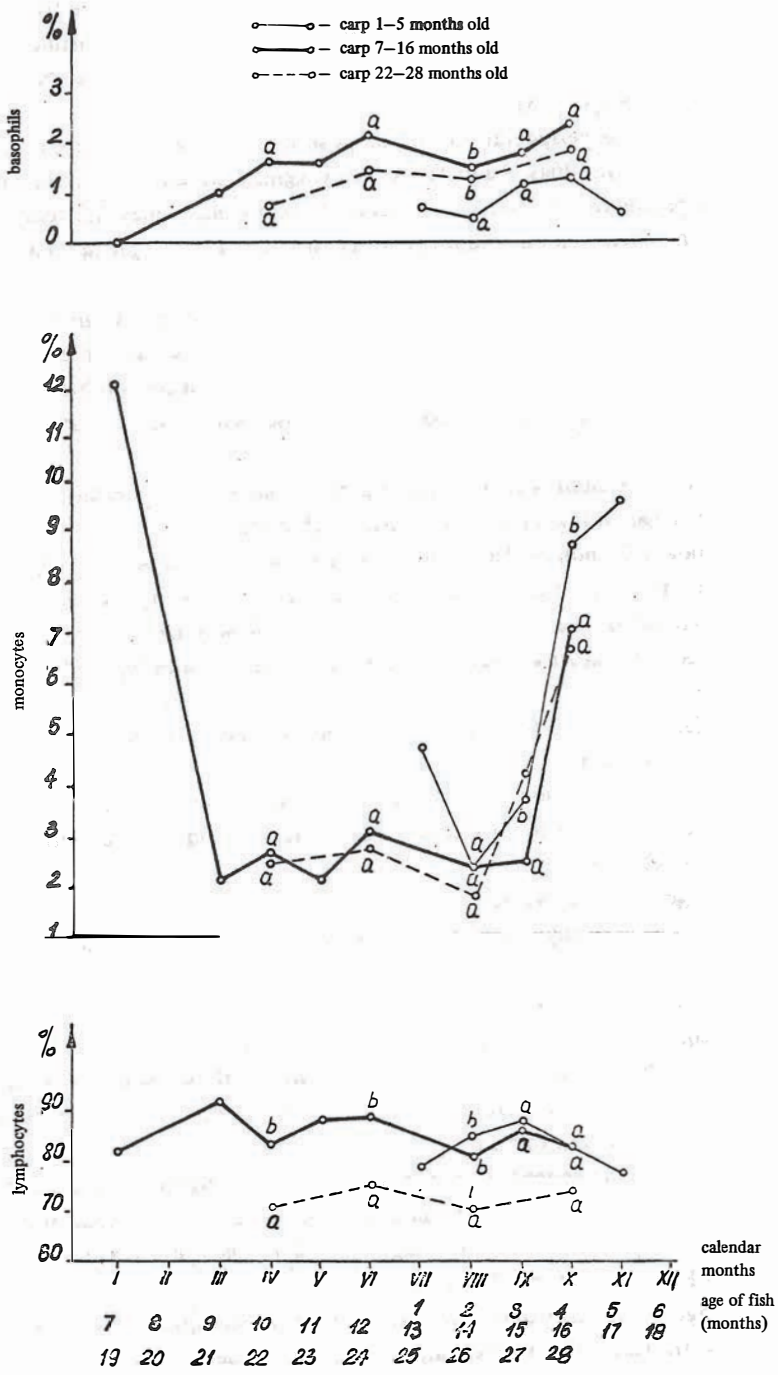

Fig. 6, 7, 8. Percentage of basophils, monocytes, and lymphocytes in the carp peripheral blood as affected by age

The percentage of basophils showed no clear-cut relationship with the fish age (Fig. 6).

The percentage of monocytes and lymphocytes showed no distinct age-related differences. However, an increase in the monocyte percentage could be recorded in autumn and winter (Figs. 7–8).

The changes in the peripheral blood composition versus gonad maturation cycle are presented in Table 2 and Figs. 9 and 10. A highly significant sex stage interaction in most hematological parameters analysed was found. It means that males and females differed significantly in their blood parameters as tested against gonad maturity stage ($p_{sa} < 0{,}01–0{,}001$).

The erythrocyte count is affected highly significantly by sex. At all gonad maturity stages the number of erythrocytes was higher in males than in females (Fig. 9).

The hematocrit value in each of the four gonad maturity stages was higher in males. In both sexes the value increased at stage 2, a decrease being observed at stages 3 and 4 (Fig. 9).

The hemoglobin content was generally higher in males than in females. The effect of gonad maturity stage on this value was significant (Fig. 9).

The red blood cell indices (MCV, MCH, MCHC) were sex-dependent ($p_s < 0.01$); on the other hand, the effect of gonad maturity stage was spurious ($p_a > 0.2$).

The leukocyte count (per 1 mm^3) in the female blood was higher than in males. These differences at gonad maturity stages 2 and 3 were several times higher than at stages 1 and 4 (Fig. 10).

The blastodermic forms of leukocytes in the peripheral blood of carp males and females were not observed.

The percentage of myelocytes in the males blood was higher than in females ($p_s < 0.05$). The decrease in the myelocyte percentage was observed in both sexes at the gonad maturity stage 4 (Table 2).

The percentage of metamyelocytes was higher in males than in females ($p_s < 0.01$). These differences were highly significant at stages 1–3. High increases in metamyelocyte percentage were observed in males at stage 2 and in females at stage 3. In both sexes the percentage decreased at stage 4 (Table 2).

The percentage of rod neutrophils was higher in males than in females at all stages of gonad maturity. The effect of sex on the content of these blood cells was highly significant ($p_s < 0.001$). The percentage of rod neutrophil in both sexes increased gradually from stage 1 to 4 (Table 2).

The percentage of filamented neutrophils showed very highly significant sex-dependent differences (p_s 0.001). At gonad maturity stages 1 and 2 the value was higher in males than in females; however, at stages 3 and 4, the percentage of filamented neutrophils was higher in females (Table 2).

The percentage of all neutrophils was at each gonad maturity stage higher in males than in females. In males, the highest values occurred at stage 2 and in females at stage 3 (Table 2, Fig. 10).

The effect of the spawning period on the percentage concentration of neutrophils in the peripheral blood of males and females of carp (*Cyprinius carpio* L.)

Maturity stage of gonads	Date of blood collecting	Sex	Number of fish	Neutrophils (per cent)									
				myelocytes		metamyelocytes		rod neutrophils		filamented neutrophils		total neutrophils	
				SR	SD	SR	SD	SR	SD	SR	SD	SR	SD
1	24.05	♂	8	4.50	1.95	6.25	2.09	3.05	2.05	3.11	0.78	17.00	2.44
		♀	10	0.70	1.18	1.10	0.83	2.30	1.45	1.10	1.22	5.00	2.36
2	08.06	♂	11	2.45	1.85	18.60	2.30	10.36	2.53	5.80	2.03	37.20	4.46
		♀	7	1.10	0.83	6.80	1.53	4.05	3.75	0.40	0.50	12.30	2.04
3	24.06	♂	11	3.50	1.74	12.10	2.13	8.76	1.84	3.60	1.94	28.50	3.86
		♀	8	2.50	1.75	8.40	1.39	5.35	1.19	6.05	1.90	22.50	4.16
4	07.10	♂	10	0.25	0.64	0.50	1.10	12.00	3.25	5.18	3.12	18.10	3.93
		♀	10	0.00	–	0.85	1.17	7.20	2.95	6.60	2.36	14.70	3.86
mean		♂		2.67		9.40		8.50		4.40		25.50	
		♀		1.07		4.30		4.70		3.50		13.50	
p				$p_s < 0.05$ $p_a > 0.2$ $p_{sa} < 0.01$		$p_s < 0.01$ $p_a > 0.1$ $p_{sa} < 0.001$		$p_s < 0.001$ $p_a < 0.001$ $p_{sa} < 0.001$		$p_s < 0.001$ $p_a > 0.2$ $p_{sa} < 0.01$		$p_s < 0.001$ $p_a < 0.001$ $p_{sa} < 0.001$	

Legend:

SR – arithmetic mean
SD – standard deviation
p – significance of differences
p_{sa} – significance of interaction (of both factors)
p_s – sex-linked significance of differences
p_a – significance of differences dependet of the gonads maturity stage

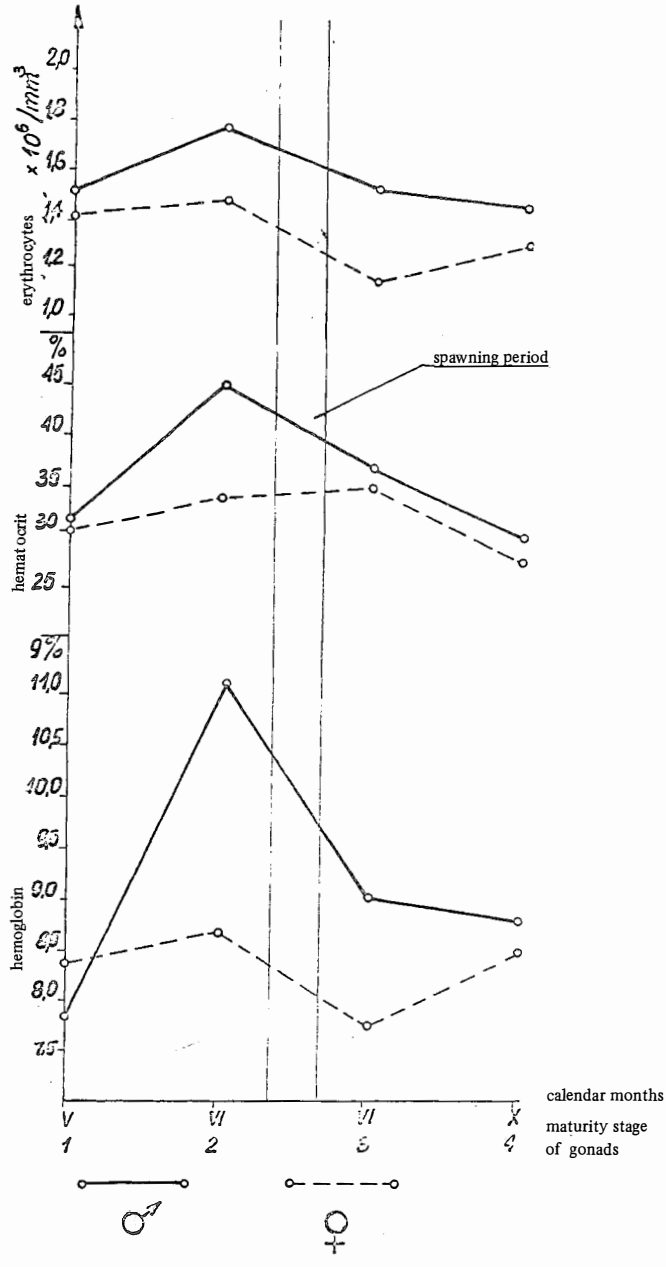

Fig. 9. Effect of spawning time on erythrocyte count, hematocrit, and hemoglobin content of the peripheral blood of carp males and females

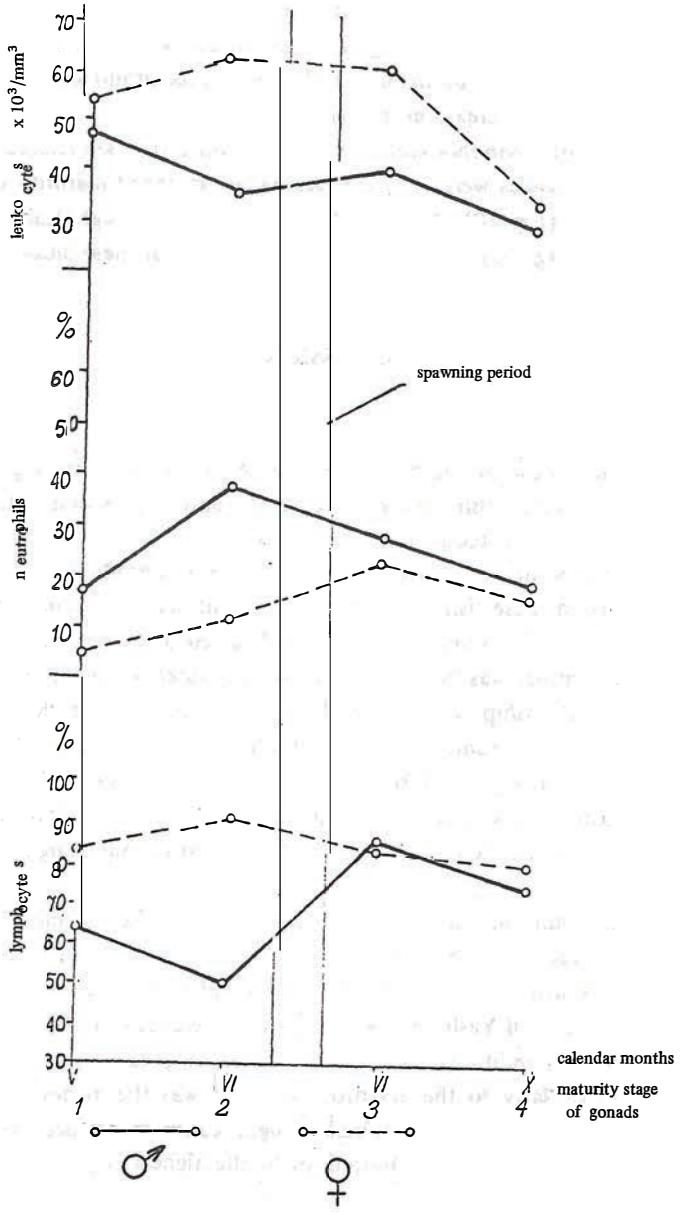

Fig. 10. Effect of spawning time on leukocyte count and neutrophil and lymphocyte per cent composition in the white blood cell pattern of the peripheral blood of carp males and females

The percentage of eosinophils showed significant sex-dependent differencess ($p_s < 0.01$), the parameter being always higher in males.

The percentage of basophils showed no significant sex-related differences ($p_s > 0.2$); however, a significant effect of gonad maturity stage was found ($p_a < 0.05$).

The percentage of monocytes was significantly different between males and females ($p_s < 0.01$). At stage 3 in males an increase in the percentage of monocytes was observed; however, females showed a decrease in this parameter.

The percentage of lymphocytes showed significant sex-related differences ($p_s < 0.001$). The differences were several times higher at gonad maturity stages 1 and 2 than at stages 3 and 4 (Fig. 10). At stage 2 in males a highly significant decrease was recorded, females showing a slight increase in the percentage of these blood cells.

DISCUSSION

I. Age of fish

As shown by the review of the literature, among the factors affecting the results of hematological assays were: fish species, age, and spawning period (Murachi, 1959; Dorfman, 1973; Iuchi, 1973; McCarthy et al., 1973).

The data presented point to the intensive activity of blood-producing organs at the fish age of 9–16 months; in these fish the erythrocyte count was significantly higher than in the fish aged 1–5 and 22–28 months (Table 1). The red blood cell number increase in the fish during the summer was brought about by the water temperature rather than by the fish age. This relationship was described by many authors, e.g., Klontz and Smith (1968); Mc Knight (1966); Summerfelt (1967); Gardner and Yevich (1969); Litzbarski (1974); Fourie and Hattingh (1976); Fourie and Van Vuren (1976). During the autumn-winter season (at low water temperatures of 3–5°C) the erythrocyte count was at its lowest in the carp of different age (Table 1), the relationship being also observed by Pučkov (1962).

The erythrocyte count in the present study was $1.1–2.3 \times 10^6/mm^3$, various other values being reported in the literature: $2.0–2.65 \times 10^6/mm^3$ (Stolz, 1928); $1.31 \times 10^6/mm^3$ (Smith et al., 1952); $1.4 \times 10^6/mm^3$ (Dombrowski, 1953); $1.01 \times 10^6/mm^3$ (Hines and Yashouv, 1970). These differences can be accounted for by different environmental conditions, i.a., different water temperatures.

The hematocrit, similarly to the erythrocyte count was the highest in the fish aged 7–16 months, the difference being particularly significant with respect to the youngest age group (1–5 months). It can be accounted for by the highest erythrocyte count in that group.

The hematocrit value decreased in all age groups during autumnwinter (Table 1), which was probably related to the decrease in temperature during this period (3–5°C), and consequently to a slow-down of metabolic processes, the hemopoietic activity included.

It should be mentioned that the mean volume (MCH) and mean percentage (MCHC) of hemoglobin in an erythrocyte (especially in the youngest carp) increased significantly at this time, which could mean that even though the erythrocyte number dropped, the intensity of oxygen assimilation by these blood cells did not decrease.

The literature data (Snieszko, 1960, Summerfelt, 1967; Houston and De Wilde, 1972) stress the importance of hematocrit value in diagnostic studies owing to its low variability as compared to the erythrocyte count and hemoglobin level; however, based on the present results one cannot confirm the observations mentioned above.

Nevertheless, the significant effect of fish age on the hemoglobin content, MCH, and MCHC can be suggested, the values being at their lowest in the youngest fish (aged 1–5 months). The oxygen and energy requirements of the young fish are very high. Thus it seems that in the earliest period of life the fish employ some additional, unknown mechanisms of oxygen assimilation and distribution, which ensures the normal course of life processes.

There was no clear-cut relationship between the fish age and the leukocyte number. During the autumn-winter period the numbers of these blood cells in all age groups tested were at their lowest, the values increasing in the summer (Table 1, Fig. 1). These results are similar to Schlicher's (1927) data, but contradict the findings of Antipova (1954).

In the present study, the number of leukocytes in the carp aged 1–28 months was 30–50 x 10^3/mm^3 of blood. The carp leukocyte counts in other in other studies were: 90–120 x 10^3/mm^3 (Stolz, 1928); 40–50 x 10^3/mm^3 (the first year of life, Dombrowski, 1953); 26–57 x 10^3/mm^3 (Hines and Yashouv, 1970); 25–80 x 10^3/mm^3 (Kudriacev, 1969). Different environmental conditions, physiological states, and methods of study are probably responsible for the large ranges of and differences in these data. Until recently, thrombocytes had been identified as lymphocytes (due to their similar morphology), the total number of leukocytes being thus artificially overestimated. Ferguson (1976) who used electron microscopy showed morphological and functional differences between the leukocytes and thrombocytes.

The relationship between the carp age and the percentages of blastodermic forms in the blood is very clear, the blood cells existing in the first year of fish life, which evidences a high activity of the hemopoietic organs in this period of life. It is only in the winter that the blastodermic forms were not found in the periopheral blood.

The classification of blastodermic forms of blood cells applied in the present study follows Ivanova (1970). According to this classification, the parental forms for all the blood cells (erythrocytes and leukocytes) are hemocytoblasts. The early forms of all the granulocytes (neutro-, baso- and eosinophils) are myeloblasts. From their fission originate promyelocytes that can be an initial form for neutrophils, eosinophils, and basophils. Subsequently, neutrophils give rise to myelocytes, metamyelocytes, and rod and filamented neutrophils.

Hitherto, the literature has reported different classifications of blastodermic forms of fish blood cells. For some authors, the parental cell for all structural elements of blood was the lymphoidal hemoblast (Jordan and Speidel, 1924; Topf, 1955; Varo, 1970);

others considered the large and small lymphoidal hemoblasts (Catton, 1951), or the large and small hemoblasts (Jakowska, 1956; Weinreb and Weinreb, 1969; Saunders, 1966).

The granulocyte parental cells were called granuloblasts and progranulocytes (Duthie, 1939; Catton, 1951), or progranulocytes and proneutrophil (Jakowska, 1956; Weinreb, 1963; Hines and Yashouv, 1970).

The study presented shows the percentage of neutrophils to increase with the fish age (Fig. 4). The percentage of all neutrophils ranged from 2.9 (in the youngest carp) to 20.3 (in the oldest fish). The results presented differ considerably from some data reported by the literature and concerning this parameter in the cyprinids. Gardner and Yevich (1969) stated that these fish contained no neutrophils and basophils, although Watson (1963) observed the percentage of neutrophils to amount to 1–2, while according to Weinreb and Weinreb (1969) the neutrophil percentage was 5–12. The latter are the most similar to the present results.

The percentage of eosinophils in the carp blood is low and it shows a decreasing tendency with age. However, a relatively high percentage of these blood cells (6.55–8.10) can be observed in the youngest carp as compared with that in the oldest fish (3.15–3.70). The present results were similar to the literature data in terms of the eosinophil percentage in the carp blood. Loewenthal (1928) and Watson (1963) report about 8% and 1–3%, respectively. Golovina (1976) observed an irregular decrease in the percentage of these blood cells with the carp age. In the 1–5 months old fish the eosinophil percentage was 0–12.5, while the carp blood at the age of 7–14 months showed 0–3.6% of eosinophils.

The contents of basophils monocytes, and lymphocytes were not age-related (Figs. 6, 7, 8). There is only a relationship between the monocyte percentage and low temperatures as the percentage of these cells increased markedly in all age groups during autumn-winter.

II. Spawning

The studies presented show the physiological processes related to the reproductive cycle to be very important factors affecting carp hemo- and leukograms.

The present author's own observations and also the studies of Svobodova (1973), Ezzat et al. (1974), Fourie and Hattingh (1976), Siddiqui and Naseem (1979) show metabolic processes connected with the gonad maturation to result in a marked increase in the erythrocyte count, hematocrit value, and hemoglobin content in the blood of males and females of carp (Fig. 9). Increase in these parameters was observed at the spawning stage (stage 2), no increase being recorded at the resting stage (stage 4) as opposed to a study by Svobodova (1973). The present data differed also from the results of Schlicher (1927), Robertson et al. (1961), Černikova (1967), and Einszporn-Orecka (1970) who found decreased erythrocyte counts and hemoglobin contents in the peripheral blood during spawning. In the pre-spawning period of the present study, not only the total number of erythrocytes in the peripheral blood increased, but also immature blood cells were more frequent at this stage than at other times.

At all stages of gonad maturity the erythrocyte count, hematocrit, and hemoglobin content were generally higher in males than in females. On the other hand, the red blood cell indices (MCV, MCH, and MCHC) were higher in females. The data are similar to the results of Einszporn-Orecka (1970), Svobodova (1973), Ezzat et al. (1974), Fourie and Hattingh (1973), and differ from those reported by Schlicher (1927), Robertson et al. (1961), Černikova (1967) who observed higher values of these parameters in males during the spawning period only.

The sex-dependent differences are visible also in changes of the leukocyte count, which was particularly evident during spawning (stage 2). The present study showed the number of white blood cells to increase during spawning in females only. At the resting stage the leukocyte count decreased both in males and in females, parallel to gonad resorption (Fig. 10).

The above results differ from the literature data, e.g., Einszporn-Orecka (1970) observed an increase in the leukocyte count both in males and females during tench (*Tinca tinca*) spawning. This was also found by Ezzat et al. (1974) for *Tilapia zilli*. Similar results were obtained by Černikova (1967) for the lavaret (*Coregonus lavaretus*); however, she did not observe any increase in the leukocyte count of the roach (*Rutilus rutilus*), bream (*Abramis brama*), and burbot (*Lota lota*) during their spawning.

The results of the present study as compared with the literature data show the number of leukocytes in both sexes during spawning to be highly species-dependent.

The present study shows the percentage of all neutrophils to be sex-dependent and also related to the gonad maturity stage (Fig. 10). The situation results mostly from increased contents of immature forms of neutrophils (myelocytes, and particularly metamyelocytes) (Table 2).

A higher percentage of neutrophils in males than in females of *Coregonus macrophthalmus* during spawning was also observed by Cavicchioli and Zavarini (1977).

No changes occurred in the monocyte percentages in males and females during spawning; however, after spawning this parameter increased in males and slightly decreased in females. These results were different from the data reported by Cavicchioli and Zavarini (1977) who found changes in counts of blood cells both in males and females during spawning.

The lymphocyte percentage was highly dependent on physiological processes occurring in the fish during gonad maturation and spawning. It is of interest to note a considerable decrease in the male lymphocyte percentage and a slight decrease of this parameter in females that took place just before spawning (Fig. 10). Similar changes were also observed by Drabkina and Telkova (1949), Antipova (1954), and Cavicchioli and Zavarini (1977).

CONCLUSIONS

1. The age of fish affects the carp hematological parameters, which can be concluded from observations of these parameters in the carp ranging in age from 1 to 28 months.

The young carp peripheral blood (in the first year of life) can contain blastodermic forms of blood cells.

The erythrocyte count and hematocrit were at the lowest in the 1–5-months-old carp, while the highest values were recorded at the age of 6–16 months. The hemoglobin content, mean corpuscle hemoglobin in an erythrocyte (MCH), and the neutrophil (both the developing and mature forms) percentages were lower in the younger than in older fish.

2. Some hematological parameters of the carp varied with water temperature, e.g., the erythrocyte count, hematocrit, and hemoglobin content, which were higher during the summer and lower in winter.
3. The fish sex and physiological changes preceeding spawning and accompanying it exert a marked influence on the carp hematological parameters.

Regardless of the gonad maturity stage, the erythrocyte count, hematocrit, and hemoglobin content were higher in males than in females. On the other hand, the red blood cell indices were higher in gemales than in males. At the reproductive stage (just before spawning), the number of erythrocytes, hematocrit, and hemoglobin level increased in both sexes, and a decrease in the lymphocyte percentage was observed in males. At this time the leukocyte count in females increased, and the myelocyte and metamyelocyte counts increased in males.

At the resting stage the leukocyte count in both sexes decreased.

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WPŁYW CZYNNIKÓW FIZJOLOGICZNYCH STRESSOWYCH I CHOROBOWYCH NA PARAMETRY HEMATOLOGICZNE KARPIA (*CYPRINUS CARPIO* L.) ZE SZCZEGÓLNYM UWZGLĘDNIENIEM OBRAZU BIAŁOKRWINKOWEGO

I. ZMIENNOŚĆ WSKAŹNIKÓW HEMATOLOGICZNYCH KARPIA W ZALEŻNOŚCI OD WIEKU I STADIUM DOJRZAŁOŚCI GONAD

STRESZCZENIE

Badaniami objęto 340 sztuk karpi (*Cyprinus carpio* L.) w wieku 1–28 miesięcy oraz 31 samic i 41 samców w wieku 5–6 lat.

Z analizy leukogramu określono prawidłowy obraz krwinek białych oraz zmiany w tym obrazie w zależności od płci i niektórych czynników fizjologicznych związanych z procesami rozrodu. Wyniki opracowano statystycznie.

Określając jakościowy skład krwi obwodowej karpi wyróżniono formy blastyczne: hemocytoblasty, myeloblasty, promyelocyty oraz rodzaje leukocytów: myelocyty, metamyelocyty, neutrofile pałeczkowate, neutrofile segmentowane, eozynofile, bazofile, monocyty i limfocyty.

Wiek okazał się czynnikiem wpływającym na stan wskaźników hematologicznych u karpia. U ryb młodszych (w pierwszym roku życia) w krwi obwodowej występowały formy blastyczne krwinek. Dominującą formą były neutrofile, których procent wzrastał wraz z wiekiem oraz był zawsze wyższy w okresie letnim. Pozostałe parametry: liczba erytrocytów, wartość hematokrytu, poziom hemoglobiny i MCH były niższe u ryb młodszych niż u starszych.

Niezależnie od stadium dojrzałości gonad u samców 5–6-letnich liczba erytrocytów, wartość hematokrytu, poziom hemoglobiny, procent neutrofilii są wyższe u samic niż u samców. W stadium rozrodu (tuż przed tarłem) u obu płci występuje wzrost liczby erytrocytów, wartości hematokrytu i poziomu hemoglobiny a także u samców spadek procentu limfocytów. W okresie tym u samic zwiększsza się liczba leukocytów. W okresie spoczynkowym u obu płci spada liczba leukocytów.

Antonina SOPIŃSKA

ВЛИЯНИЕ СТРЕССОВЫХ ФИЗИОЛОГИЧЕСКИХ ФАКТОРОВ И БОЛЕЗНЕТВОРНЫХ ФАКТОРОВ НА ГЕМАТОЛОГИЧЕСКИЕ ПАРАМЕТРЫ КРОВИ, В ЧАСТНОСТИ НА КАРТИНУ БЕЛЫХ КРОВЯНЫХ ТЕЛЕЦ. 11. ГЕМАТОЛОГИЧЕСКИЙ ЭФФЕКТ СТРЕССА У КАРПА

Р Е З Ю М Е

Материалом для исследований влияния отлова на гематологические параметры служили 40 особей карпа $К_1$ и 40 особей карпа $К_2$, а материалом для исследований влияния транспорта – 60 особей карпа $К_1$ и 60 особей карпа $К_2$ (по 20 особей для каждого месяца исследований, т.е. июня, июля и августа). Влияние голодания определяли с помощью исследования 160 особей рыбы.

В крови исследовали: количества белых и красных кровяных телец, гематокрытную величину, уровень гемоглобина, МС , МСН, МСНС. Определяли также

лейкограмму, производились морфологические наблюдения белых и красных кровяных телец. Результаты исследований подвергались статистической обработке.

Затрудненные условия отлова, являвшиеся неблагоприятным стрессовым фактором у рыбы, вызвали увеличение объёма красных кровяных телец. (MC), а также гематокрытной величины. Последствием увеличения активности защитных сил организма является повышение количества лейкоцитов, а также процентной доли нейтрофилов и базофилов.

Транспорт - сильнодействующий стрессовый фактор повлиял на увеличение количества эритроцитов, гематокрытной величины и уровня гемоглобина,а также на повышение количества эритроцитов и процентной доли нейтрофилов. Наблюдалось уменьшение процентной доли эозинофилий и лимфоцитов.

Длительное голодание, продолжавшееся в течение 21 недели, вызвало прогрессивную анемию, что проявлялось уменьшением количества эритроцитов, гематокрытной величины и уровня гемоглобина, а также анизоцитоз и полихромазия эритроцитов.

Последствием голодания являлась лейкоцитопения и лимфопения, а также эозинопения. В этих последних тельцах наблюдалась отчётливая деградация.Увеличивалась процентная доля нейтрофилов и базофилов,могла также наблюдаться деградация нейтрофилов.

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Received: 25 January 1983